Prevention and Therapy of Gingivitis

Toothbrush and Mouthwash

PERIODONTOLOGY

Martijn van Leeuwen

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The studies of this thesis were conducted at the department of Periodontology of the Academic Centre for Dentistry Amsterdam (ACTA). ACTA is a joint venture between the Faculty of Dentistry of the University of Amsterdam (UvA) and the Faculty of Dentistry of the Vrije Universiteit (VU) Amsterdam, The Netherlands.

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Marion van Leeuwen Chomet
Caatje van Leeuwen

“Believe you can and you’re halfway there”
Theodore Roosevelt

Voor mijn lieve ouders
Ernst & Marie-Anne

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CHAPTER 1

General introduction

Need for oral hygiene

It is generally accepted that periodontal diseases are caused by bacteria. Bacteria are omnipresent in the oral cavity. When bacterial plaque biofilm is not frequently removed, this leads to the development of oral diseases.[1] Seventy-five years ago, dr. T.S. Smith, a pioneer in the field of oral hygiene prophylaxis, indicated the need for oral self-care: *"Because we have become accustomed to the scientific terms applied to teeth and gums, we lose sight of the fact that teeth are bones protruding through soft tissues where conditions are extremely unfavorable to maintain health."*[2] This quote nicely illustrates that it is almost a miracle that we maintain a healthy dentition throughout life. It also raises the questions of what action is needed to effectively achieve, maintain, and promote oral health and which oral homecare regimens and oral hygiene devices are most suitable for this purpose.[3]

Gingivitis and periodontitis are a continuum of the inflammatory disease process of the periodontal tissues.[4] Though not all patients with gingivitis will progress to periodontitis, the dental profession considers the management of gingivitis a primary prevention strategy for periodontitis. In addition, the management of gingivitis in a reduced periodontium is a secondary prevention strategy for recurrent periodontitis.[5]

Periodontitis is an oral disease that affects more than 50% of the world's adult population.[5, 6] Epidemiologic data have shown that the estimated global prevalence of the severe form of periodontitis that is standardized by age is 11.2%.[7] This severe form of periodontitis is a major cause of tooth loss and subsequently has a negative impact on oral health, quality of life, speech, diet, confidence, and overall well-being.[8] Severe periodontitis is thus a significant issue of concern for public health. Prevention of gingivitis and periodontitis inhibits the occurrence or progression of severe periodontal disease processes in the oral cavity. The dental profession has long recognized the need for prevention strategies, and a call for global action was published by the European Federation of Periodontology.

Historical evidence of oral hygiene

Picking at teeth may be one of humanity's oldest habits. A 1.2 million-year-old hominin jawbone was recently discovered at an excavation site in northern Spain. The jawbone had an interproximal groove with fragments of non-edible wood, which suggests interdental oral hygiene activities.[9] Anthropologic research at Mesopotamian sites in Iraq has found dental care relics, such as toothpicks in gold vanity sets and cases. A vanity set found in the Nigel Temple at Ur is estimated to have been used around 3,000 BC.[10] Dating to the same era, gold-decorated toothpicks used by Sumerians were found in excavations in Mesopotamia. Interpretation of a clay tablet from that period suggests that gingival massage was combined with various medications. This makes

it likely that people of that period also suffered from periodontal diseases.[11-13] Some of the oldest written documents concerning teeth and the oral cavity date back to approximately the same era. Found in the excavations of the Sumerian civilization in the Middle East, these documents in the form of pictographic and cuneiform tablets contain descriptions such as this:

> *If a man's mouth has mouth trouble, thou shalt bray Lelium in well water, introduce salt, alum and vinegar therein, thou shalt leave it under the stars, in the morning, thou shalt wind a linen strip around his forefinger, without a meal thou shalt clean his mouth.*[14]

Over 300 years ago, Antoni Van Leeuwenhoek (1632-1723), inventor of the microscope and father of microbiology, described oral hygiene in relation to the microflora of the oral cavity. In his letter to the Royal Society (1683), he explained his personal oral hygiene habits:

> *I am in the habit of rubbing my teeth with salt in the morning, and then rinsing my mouth with water; and often after eating, to clean my back teeth with a toothpick, as well as rubbing them hard with a cloth, wherefore my teeth back and front remain as clean and white that only a few people of my age (fifty-one) can compare with me. Also when I rub my gums with hard salt, they will not bleed.*[15]

During the same period, the father of modern dentistry, Pierre Fauchard (1678-1761), also recognized the importance of oral health. In *Le Chirurgien Dentiste*, he describes a method of cleaning that involves *"rubbing the teeth from below upwards and from above downwards outside and inside with a little sponge,"* dipped in warm water and brandy, followed by using a toothpick between the teeth.[16]

Modern evidence for oral hygiene

The milestone study ''Experimental Gingivitis in Man,"[17] published in 1965, demonstrated a cause and effect relationship between plaque accumulation and gingival inflammation. It produced a universal principle: bacterial plaque is essential to the initiation of gingivitis and, if unresolved, leads to periodontitis. In this study, Löe and his co-workers conducted a clinical experiment that ultimately had a major impact on the basic paradigms of etiology, pathogenesis, and periodontal disease prevention. This study, in which participants were their own controls, helped to establish that dental plaque biofilm buildup is a primary risk factor for gingivitis. When healthy individuals withdrew oral hygiene efforts for a 21-day period, all study participants predictably developed generalized gingivitis. Once effective plaque removal was recommenced,

the participants returned to low plaque and gingivitis scores that were comparable with their pre-experimental levels. This shows that gingival inflammation is modifiable through optimal control plaque. The outcome of this study underlines the critical importance of dental plaque bacteria in periodontal disease causation. The finding led to advances in the principles of predictable disease prevention and treatment.[18] Adequate removal and control of dental plaque became the basic recommendation in the prevention and treatment of gingivitis and periodontitis.[18] It has since then been emphasized in many studies that plaque-control measures contribute substantially to oral health status.[19]

The maintenance of oral health can be realized by regular, home-based, self-care, oral hygiene practices. These include mechanical means, such as tooth brushing, interdental brushes, woodsticks, dental floss, and rubber interdental cleaners. Additionally, the effect of selected chemical antimicrobial agents on oral tissue health and their effectiveness against oral microorganisms has been studied in vitro, ex vivo, and in vivo.[20-26] These chemical agents are found in dentifrices, gels, mouthwashes, and chewing gums.[20-22, 27-34] In particular, mouthwashes as adjunct to toothbrushing have been shown to contribute to adjuvant effects, such as reducing the accumulation of plaque and the development of gingivitis.[20-26, 35-37] When used as an adjunctive therapy to conventional manual tooth brushing with a fluoridated dentifrice, the use of chemical anti-plaque agents in mouth rinses or incorporated into the fluoridated dentifrice, alone or in combination, offers clear and significant improvements in managing gingival inflammation and preventing plaque accumulation.

Mechanical plaque control

The most common home-care mechanical means of controlling plaque is the use of a toothbrush in combination with a fluoride dentifrice.[38] This is currently the most common universal advice.[19, 39] Twice daily toothbrushing is promoted worldwide and plays an essential role in the prevention of caries and periodontal diseases.[40]

History of the toothbrush

The first human tool for brushing the teeth was most likely the index finger. As far back as 5,000 BC, the Egyptians developed a recipe for a "tooth powder." This consisted of a mixture of ash from ox hooves, myrrh, eggshell fragments, and pumice. It is not known how this powder was used, but it is assumed that it was rubbed onto the teeth with the fingers.[41] As a primitive oral hygiene device, the predecessor of the toothbrush is the chewing sticks of Babylonia, with origins as early as 3,500 BC. Records from

China dating to around 1,600 BC indicate that one end of the stick was chewed until it became brush-like. The other end was pointed and could be used as a toothpick. In Saudi Arabia, such chewing sticks are called "miswaks." Early Mohammedans called their sticks "siwak." The twigs used for these purposes were derived from aromatic trees and thus freshened the breath, in addition to their cleaning action.[42] In ancient Indian medicine, the neem tree was used to create toothbrushes and similar products, and these are still available today.[43] The chewing stick is currently predominantly used in Muslim areas, and its use predates the inception of Islam.[44, 45] It is often mentioned that the prophet Muhammad was an enthusiastic supporter of its use as a cleansing device for the mouth. He developed recommendations and religious rituals for the correct and effective use.[46]

The first bristled "toothbrush," as we know it today, originated in China around 1,000 AD and was brought to Europe by traders. This brush was made from hair from the neck of the Siberian wild boar, fixed to a handle made from bamboo or bone. There is also evidence of ivory handles and bristles made from horse mane hair.[13] Almost one millennia later, the modern industry became involved in dental industry. The English entrepreneur William Addis (1734–1808) began to mass-produce toothbrushes in 1780. In 1770, he had been sent to jail for causing a riot. Addis noticed that the prison floor was swept with a broom and reasoned that the current method to clean teeth with a cloth was highly ineffective and could thus be improved. To develop a prototype based on this supposition, he saved a small animal bone from one of his meals and drilled holes into it. He then obtained some bristles from one of his guards. He tied the bristle filaments in tufts and passed them through the holes in the animal bone. Finally, he sealed the holes with glue. Upon his release from jail, he launched a business to manufacture brushes, among which was a toothbrush. His business evolved into the company "Wisdom," which continues to manufacture toothbrushes today.

By 1840, toothbrushes were being mass-produced in England, France, Germany, and Japan.[47] Nylon bristles were developed in the 1930s by Dupont de Nemours. The first commercially available nylon toothbrush was introduced in 1938 and named "Doctor West's Miracle Toothbrush." Toothbrushes with plastic handles were easy to manufacture and thus more affordable for the public. These commercially available toothbrushes contributed to toothbrushing becoming a common practice in Western society. Since the introduction of the industrialized toothbrush, its design has evolved, and there are now a wide range of manual toothbrushes available. Americans and Europeans were influenced by the disciplined hygiene habits of American soldiers from World War II; and, reflecting an increasing concern with the practice of good oral hygiene, they quickly adopted the nylon toothbrush.[48] In January 2003, the Lemelson-MIT survey queried participants about the invention that they could not

live without. Respondents were asked to rank five items on a list of inventions, including the automobile, personal computer, cellular phone, microwave, and toothbrush. A total of 34% of teens and 42% of adults chose the toothbrush.[49]

Modern toothbrushes

The design of the toothbrush head has evolved over time. Various head designs are now available to the consumer. Most are based on the premise that the majority of people in any population use a simple horizontal brushing action. The bristle and tuft patterns are designed to enhance plaque removal from hard-to-reach areas of the dentition. Proximal areas are of particular interest. Multiple tufts of bristles are used, sometimes angled in different directions. Toothbrush handles have ergonomic designs appropriate for individual hand sizes.[50-52] A systematic review[53] evaluating the efficacy of a single brushing exercise has shown that the plaque score-reduction averages 42%. Based on an indirect comparison with the available evidence, it is concluded that the angled bristle tuft configuration is more effective than the traditional flat trim.[53] Interdental devices, such as interdental brushes, can enhance the mechanical effect on plaque removal for obtaining gingival health.[31, 54, 55]

The American Dental Association perspective on toothbrushes

The merits of daily oral hygiene for oral health are of interest to clinicians. However, patients are also becoming more interested in the effects of their efforts. The American Dental Association (ADA) publishes a public program on its website, www.mouthhealthy.com,[38] wherein consumers can find the association's official recommendations. The ADA also began a seal of acceptance program in 1931. The seal indicates that particular toothbrushes are safe and efficacious for plaque removal and gingivitis reduction. Toothbrushes with the seal of acceptance have shown positive study outcomes when reviewed by the ADA Council on Scientific Affairs.[56] Today, dental care professionals worldwide and US consumers recognize the seal as the gold standard for evaluating the safety and efficacy of dental products. For a healthy mouth and smile, the ADA seal program recommends brushing twice a day with a toothbrush that has soft-bristle filaments, using fluoride toothpaste. The size and shape of the brush should enable it to fit into the mouth and easily reach all areas. In addition, the toothbrush should be replaced every 3-4 months, or sooner if the brush filaments are frayed.[38]

Toothbrush wear

However, the ADA recommendation for toothbrush replacement is not based on scientific evidence. Instead, it is based on the assumption that filaments and tufts do

not retain their optimal shape forever. To examine toothbrush wear, a crossover study compared a new toothbrush to a three-month old toothbrush, revealing that subjects reduced their plaque scores by approximately 34% after one minute of brushing time with a comparable magnitude for both brushes.[57] This outcome is supported by the results of other studies that reached similar conclusions. No significant differences have been observed for used and new brushes with regard to a reduction of plaque scores.[58-61] However, the evidence indicates significant differences with regard to plaque score reduction. For example, a study evaluating the plaque scores of twice-weekly replaced brushes compared to brushes not replaced over a 10-week period reported favorably with respect to unworn brushes. Worn toothbrushes that had not been replaced showed higher plaque scores over time.[62] Another study of young adult participants also showed a significant difference between three-month-old used and brand new toothbrushes.[63] But, this difference was not considered clinically significant. Research has shown that the effective life of a toothbrush can vary, as wear depends on user habits, including frequency and duration of use, brushing force, and brushing technique.[64] The aspects of habit and use complicates the provision of personal and evidence-based advice by dental care professionals to their patients.

Chemical plaque control

Studies of tooth cleaning have suggested that, despite technological innovations in toothbrush design, the level of mechanical oral hygiene practice is often inadequate.[31, 54, 55] A systematic review has found that, in addition to toothbrushing, a standard fluoride dentifrice does not provide an adjuvant effect for the mechanical removal of dental plaque.[65] A recent meta-review appraising the current state of evidence on dentifrices concludes that, compared to a standard sodium fluoride dentifrice, devices containing active chemical ingredients such as triclosan or stannous fluoride have benefits for gingival health and the control of dental plaque.[34] In addition, mouthwashes with chemical components that may be beneficial for oral health have become more customary in recent decades.[36] It is suggested that chemotherapeutic agents in mouthwashes compensate for the difficulty in accessing hard-to-reach-areas, poor manual dexterity, and lack of compliance with regular mechanical plaque removal.[66] Mouthwashes are also appreciated by the public because of their ease of use.[67-70]

History of chemical plaque control

The practice of mouth-rinsing has been common among humans for centuries. Ancient Egyptians were responsible for the earliest known artistic drawings that illustrate an interest in beauty and the importance of hygiene. An unclean body was considered impure. Thus, in addition to washing their bodies in oils, the Egyptians used products to freshen their breath. For instance, they chewed on sodium carbonate and rinsed their mouths with honey and water. To this, a mixture of goose fat, frankincense, cumin, or ocher was added. Surviving recipes explain how to make chewable tablets from dried plant matter such as myrrh, mastic, cypress grass, and lily, which were finely ground, mixed with honey, heated, and then dried in balls.[71] Three millennia later, Hippocrates recommended a mixture of salt, alum, and vinegar.[12] The Jewish solution from the Talmud dates back 1,800 years and recommends using "dough water" and olive oil.[72] The Greek physician Pedanius Dioscorides formulated a mouthwash of a decoct extracted from olive tree leaves, milk, wine, pomegranate peelings, nutgalls, and vinegar. In the Americas, North American and Mesoamerican cultures used derivatives of the *Coptis trifolia* plant as mouthwashes. This is a summary of how ancient mouthwashes were prepared using traditional methods and herbs.[73] The first mouthwash purporting to reduce dental plaque was urine from a child or a newborn baby.[74] In the 18th century, due to its ammonia content, urine was the key active ingredient that rendered the oral cavity free from oral pathogens, especially those organisms that produce sulphur.[12]

Antoni van Leeuwenhoek discovered with his microscope that bacterial organisms resided in the deposits on his own teeth. He found that these organisms were viable and that, upon the action of brandy, they lost their viability. In consequence, van Leeuwenhoek concluded that alcohol has the ability to render the viable organism inactive.[75] More than a century later, in 1879, a mouthwash was developed by dr. Joseph Lawrence and a pharmacist called Jordan Wheat Lambert.[12] This mouthwash was named "Listerine" in honor of Joseph Lister, an English physician and pioneer of antiseptic surgery. Lister is known as the father of modern antiseptics as he developed methods for decontaminating operating theaters with carbolic acid spray. Their mouthwash formulation contained a highly specific mixture of essential oils (EOs) that included thymol, eucalyptol, menthol, and methyl salicylate. It was initially intended as a surgical antiseptic. In the 1880s, W.D. Miller, a dentist trained in microbiology, was the first to suggest using an antimicrobial mouthwash – which, in this case, was made from a Colorado Claro cigar that contained phenolic compounds – to combat gingival inflammation.[76] Miller used this distillation himself with no observed adverse effects. In the last quarter of the 20th century, the use of mouthwashes became common, often following mechanical plaque control.[68]

Modern chemical plaque control

Many chemical anti-plaque agents have been tested in oral hygiene products to improve oral health.[77] Mouthwashes are especially efficient in delivering active substances in the oral cavity, as all surfaces of the dentition come into contact with the mouthwash.[78] Water, as an essential liquefying component of mouthwash, also helps to dissolve the ingredients. Mouthwash formulations with specific chemical agents for plaque control have been shown to provide statistically significant improvements to plaque and gingivitis scores.[79] This is despite their active ingredients being exposed to the oral cavity for only a relatively short period of time (often 20, 30, or 60 seconds of rinsing) before the rinse is expectorated from the mouth.[80] Many mouthwash products have a water-alcohol base, with surfactants and humectants added for cosmetic properties. The general properties of mouthwashes are anti-adhesive, antiseptic, and anti-inflammatory. The effect of anti-adhesive agents results in inhibition of the formation of plaque on the teeth, while having little effect on the microorganisms themselves or the symbiosis in the oral cavity of the host and the oral microbiome. Antiseptic mouthwashes, on the other hand, exert their effect by causing cell death, inhibiting the reproduction and the metabolism of microorganisms.[81] Mouthwashes are formulated so that they effectively penetrate the plaque matrix and gain access to the microorganisms. The anti-inflammatory agents affect the innate immune response that attempts to eliminate foreign bodies and protects against injury or infection.[82]

In the 1940s, the contemporary golden standard in mouthwashes, chlorhexidine (CHX), was developed by Imperial Chemical Industries in England. This was introduced for human use in 1957 as an antiseptic for skin. It has since been widely used in medicine and surgery. Today, CHX is the most thoroughly studied chemotherapeutic agent for oral use. Its use for plaque inhibition was first investigated in 1966 by the Swiss researchers Renggli[83] and Schroeder.[84] Using their experimental gingivitis study design, Löe and colleagues, working in 1970, evaluated the effects of mouthwashes containing CHX on the development of dental plaque and gingivitis.[85] Soon after, they published a study which concluded that the inhibition of dental plaque by CHX had an additional inhibiting effect on the development of dental caries.[86] A comprehensive systematic review of the results of 30 publications revealed that a CHX mouthwash provides significant reduction in plaque and gingivitis scores.[87] This was recently confirmed by a Cochrane systematic review.[88]

Other ingredients in mouthwashes can also have a beneficial effect. For instance, detergents known as soap or surfactants help to lower the surface tension of the fluid. As such, the liquid can more easily spread over large areas. Another detergent, cetylpyridinium chloride (CPC), aids in the solubilization of flavoring agents, as well as providing an antibacterial effect.[89] The incorporation of alcohol/ethanol into

mouthwashes serves several purposes. It is a solvent of active ingredients, has antiseptic properties, and acts as a preservative by enhancing the maintenance of the active ingredient, improving the product shelf life, avoiding contamination, and improving the transport of active ingredients into the dental plaque biofilm.[36] Ethanol is easy to produce and relatively inexpensive, which are important factors, as alcohol-containing mouthwashes can contain up to 26.9% ethanol. Since the commercial interest in mouthwashes has increased, several new products have entered the market. The number of mouthwash variants worldwide has grown from 15 in 1970 to approximately 113 in 2012.[90] As this number increases, questions of efficacy arise.[91]

The American Dental Association perspective on mouthwashes

Mouthwashes with the ADA seal of acceptance are supported by studies that evidence the efficacy of a chemotherapeutic agent in relation to the reduction of gingivitis and the inhibition of plaque formation or reduction of plaque pathogenicity. To evaluate mouthwash products, the ADA recommends that studies are designed according to the Acceptance Program Requirements for Chemotherapeutic Products for Control of Gingivitis.[92] To award the seal of acceptance, the program requires two positive clinical trials lasting for at least six months, with an intermediate evaluation at three months, examining the product's efficacy, the chemical agent's safety, and patient compliance. To date, two antiseptic agents have received the ADA Counsel on Scientific Affairs Seal of Acceptance for controlling plaque and gingivitis, based on long-term clinical studies: CHX (0.12%), found in Peridex™ (3M ESPE, St. Paul, MN),[8] and a fixed combination of EOs, found in Listerine® Antiseptic (Johnson & Johnson Healthcare Products, Division of McNeil-PPC, Inc., Skillman, NJ).[9] Some generic EO mouthwashes have also obtained the ADA seal, though without published clinical studies in support of their efficacy. Their acceptance is based on what is known of Listerine®. Currently, CHX mouthwashes no longer carry the seal of acceptance since the ADA organization decided not to pursue the seal program for prescription products after January 1, 2008.[10]

Research statement

The purpose of this thesis is to investigate the capacity of manual toothbrushes for removing dental plaque and the effectiveness of several mouthwashes in preventing periodontal diseases. The efficacy of toothbrushing is assessed by using dental plaque scores and considering toothbrush wear. Gingival health is evaluated by assessing gingivitis and bleeding scores. Side effects, patient preferences, and microbiological changes related to these interventions are also evaluated. In terms of research design, randomized controlled clinical trials and systematic reviews were used.

The following questions are addressed in this thesis:

Chapter 2	How does a manual toothbrush's capacity to remove plaque change over time?
Chapter 3	What is the effect on gingival health during a 12-month maintenance period?
Chapter 4	What are the effects of mouthwashes containing essential oils compared to those containing chlorhexidine on plaque scores and gingival inflammation?
Chapter 5	What are the effective components of an essential oil mouthwash?
Chapter 6	What is the effect of a 0.07% CPC mouthwash on plaque and gingivitis?

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CHAPTER 2

Toothbrush wear in relation to toothbrushing effectiveness

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Abstract

Objective: To investigate to what extent the degree of toothbrush wear of a 3-month-old manual toothbrushes influence plaque scores.

Material and methods: During a recently published study with a follow-up of 1 year, all participants performed a similar basic home-based oral hygiene regimen. Hence, they were instructed to brush for 2 minutes twice daily according to the Bass method technique and using a standard dentifrice containing sodium fluoride. Toothbrushes were turned in every 3-month, and the degree of wear was scored. The mean plaque score data were additionally analysed and correlated with wear scores of the toothbrushes.

Results: For analysis, for each of 172 individual participants, a set of three identical, 3-month-old used toothbrushes were available. Toothbrush wear varied widely between participants. However, per patient, the 3-month wear status of the three evaluated toothbrushes was strongly correlated (Rho = 0.8, $P < 0.0001$). Participants who returned toothbrushes with extreme wear had significantly higher plaque scores than those who returned toothbrushes with no visible or light wear ($P = 0.01$).

Conclusion: Toothbrush wear per individual patient is fairly consistent. Toothbrushes with extreme wear were less effective than those with no or light wear. Therefore, bristle splaying appears to be a more appropriate measure of brush replacement time then the commonly used toothbrush age. Splaying of the outer tufts beyond the base of the toothbrush is a condition that indicates it is time to change the brush.

Introduction

Toothbrushing is the most widespread mechanical means of personal plaque control in the world[1] and is considered to be an important factor in the long-term maintenance of periodontal health.[2] Effective periodic removal of dental plaque may not only prevent gingivitis, but also resolve it.[3-5]

There is no doubt that using a toothbrush is essential for efficient daily plaque removal.[6] But in order to effectively remove deposits from teeth, it is required that the toothbrush-dentifrice combination possesses some level of abrasiveness. Whatever their specific characteristics, all toothbrushes have one thing in common: they do not last forever. As toothbrushes are over-the-counter products, consumers are given no special instruction when buying them. There are little scientific data to indicate when a toothbrush should be replaced;[7] a wide variation in replacement intervals has been reported, averaging 2.5-6 months.[8-10] Common sense dictates that a brush loses its effectiveness when it wears; the more it is worn, the more it loses its capacity to remove plaque effectively. This is most likely because filament tips that are bent will not adequately disrupt the plaque.

It is difficult to determine exactly when a toothbrush should be replaced. The American Dental Association recommends every 3-4 months or sooner if the bristles become frayed.[11] Toothbrush packaging sometimes includes the manufacturer's advice that the toothbrush should be discarded after 3 months. If a person brushes for 2 minutes, two times a day, 3 months may be equivalent to approximately 500 minutes of brushing per recommended lifetime of a toothbrush.[12] Although surveys among dental professionals show that replacement intervals of 2-3 months are recommended,[13-15] these suggestions do not seem to be based on firm scientific evidence. Interestingly, the lifespan proposed for a toothbrush appears to vary according to the person or organization suggesting it.

The criteria for replacing a toothbrush also differ.[16, 17] It has been hypothesized most recently[18] that plaque removal decreases more due to a toothbrush's wear than to its age. In a study by Rosema *et al.*,[18] the moment advocated for replacement was "when the outer tufts are splayed beyond the base of the toothbrush," as this was the state of wear at which a new brush always performed better than a worn one. This advice, however, was based on analyses of the brushes of only 45 participants.

To establish whether plaque score data would correlate with the wear score of the toothbrushes, and whether this would provide a basis for a recommendation when to replace a toothbrush, an explorative analysis of data obtained from a cohort of 267 participants who participated in a previous study comprising a 1-year period.[19] Clinical assessments were performed every 3 months, and the same type of fresh

manual toothbrushes was provided for each period. Toothbrushes were collected at each subsequent visit and stored for wear analysis.

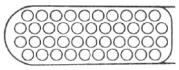
Material and methods

The present study used plaque score data based on the modified Quigley and Hein[20] plaque index[21] (QHPI) obtained from a recent study[19] that was conducted (November 2009-November 2010) at the Department of Periodontology of the Academic Center for Dentistry Amsterdam, the Netherlands. The protocol had been reviewed and approved by the Medical Ethics Committee of the Academic Medical Center (AMC) of Amsterdam (MEC 09⁄195 #09.17.1198) and registered in the Dutch Trial Register (NTR2053). At screening, participants were asked to read and sign the informed consent form and were given a signed copy for their records.

In summary, to qualify for inclusion, the participants had to be ≥18 years of age, to have no systemic disorders, to have a minimum of 5 evaluable teeth per quadrant and to have moderate to advanced gingivitis (≥40% bleeding on marginal probing (BOMP)).[22,23] Exclusion criteria were open caries, Dutch Periodontal Screening Index (DPSI) scores ≥3+,[24,25] orthodontic appliances or removable (partial) dentures and pregnancy.

All participants performed a similar basic oral hygiene regimen of brushing twice daily for 2 minutes with a fluoride-containing dentifrice for the full duration of the study. Table 1 and Figure 1 show detailed product information and instructions for use. Participants were instructed to brush according to the details provided in a written oral hygiene instruction leaflet describing the Bass method technique[26,27] and to brush 2-3 hours before all their appointments.[28] Participants were not allowed to use any other dental product or interdental cleaning aid during the study and/or to undergo dental prophylaxis during routine dental check-ups. At the first visit, participants handed in their used brushes. From that point onwards, each participant was provided with a new identical toothbrush on each subsequent visit (Table 1).

Table 1 Following regimen groups were designed and described using the TIDieR checklist[45]

Basic oral hygiene and ingredients	
Allocated	Brushing twice daily[a] for 2 min with a fluoride-containing dentifrice[b] during the study.
	Dentifrice
	Zendium® classic: sodium fluoride (1100 ppm), aqua, hydrated silica, sorbitol, glycerine, steareth-30, chondrus crispus extract, aroma, titanium dioxide, disodium phosphate, citric acid, sodium benzoate, sodium saccharin, potassium thiocyanate, zinc gluconate, colostrum, lysozyme, lactoferrin, lactoperoxidase, amyloglucosidase, glucose oxidase.
	RDA: 75.
	Toothbrush
	Lactona® IQ soft: 42 tufts, 9.5 mm polished, endrounded, 4 rows, densely concentrated, soft nylon bristles.
	

RDA, radioactive dentin abrasion.
a Lactona®; Europe BV, Bergen op Zoom, The Netherlands.
b Zendium®; Sara Lee, The Hague, The Netherlands.

Figure 1 Lactona® IQ X-Soft

Among the cohort[19] that was followed at 3-month intervals (Figure 2), the effect of the investigated interventions that had been provided at the start of the study on the clinically assessed parameters had worn off at the 4-month evaluation. Given that from that point onwards, no significant differences were found between groups, the

toothbrush wear scores and mean plaque scores were used for all groups combined for this investigation. Out of the original population, only those participants who returned their toothbrush at every occasion after 3 months were included for the analyses.

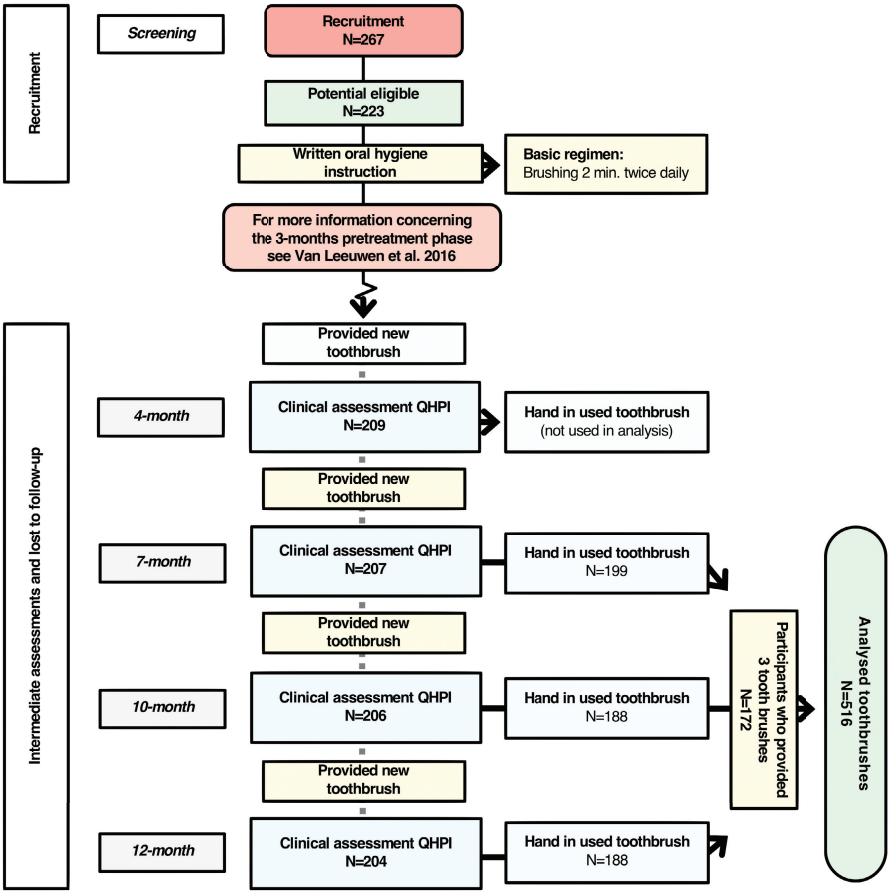


Figure 2 Flow chart depicting measurement moments for data analysis of this study

Wear assessment

In our analysis, the degree of wear of the toothbrushes that had been collected was evaluated on a 5-point scale (Figure 3) according to the method described by Conforti *et al.*[29] The wear ratings were screened independently by three calibrated examiners (GVA, NAMR & SCS). From each time point, all toothbrushes were assessed together in a random order with different sequences for each batch by the three examiners.

Differences concerning the rating of toothbrush wear were resolved by consensus. The interexaminer reproducibility scoring using Cronbach's alpha was calculated.

Wear scores	Description
0 – No wear	No visible signs of wear, inner and outer tufts are intact
1 – Light wear	Outer tufts begin to splay, inner tufts are still intact
2 – Medium wear	Outer tufts are splayed beyond the base of the toothbrush, inner tufts begin to splay
3 – Heavy wear	Outer and inner tufts are splayed
4 – Extreme wear	Outer and inner tufts are splayed v no distinction can be made

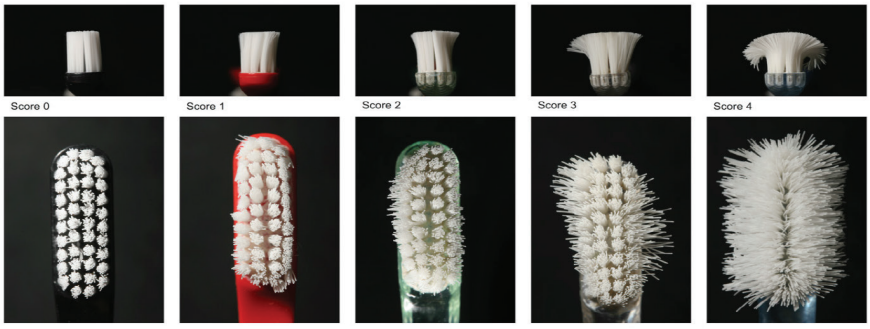


Figure 3 Toothbrush wear scores by category according to the Conforti index[29]

Data analysis

The unit of analysis was the participant. Mean plaque scores per individual, per time point, were used as the main response variable in the analysis to establish whether these were correlated with wear scores. SPSS (SPSS software package for MAC, version 23.0; IBM Corporation, Armonk, NY, USA.) was used to perform the statistical analyses.

The Spearman's Rho correlation coefficient of brush-wear scores was calculated for the toothbrushes used by the same individual for 3 months. These correlations were interpreted according to the suggestions by Evans.[30]

The brush-wear score was assessed per toothbrush, and the plaque score means were calculated for each brush-wear category. These scores were compared using the ANOVA test. Post-testing was performed to determine the origin of observed differences using independent *t*-tests between the wear groups. The *P*-values were corrected for multiple comparisons using the Bonferroni correction and were considered statistically significant if the *P*-values were <0.05.

Results

A complete case analysis of three toothbrushes and corresponding plaque score was available for toothbrushes collected at the designated time points from 172 of the 267 enrolled participants of the original study. Participants from the control I group of the original study only returned for their final assessment, and no intermediate assessment was performed. Therefore, they could not contribute to the present data set (N = 44). Furthermore, there were dropouts (N = 16) and participants that did not return all of their toothbrushes (N = 35). These were excluded from the present study which only assessed those with a complete data set at the 7-month assessment, 10-month assessment and final assessment.

Thus, 516 identical toothbrushes were available for analyses. All toothbrushes were assessed for wear by three independent calibrated examiners who had a high interexaminer reproducibility score (0.95 Cronbach's alpha). Figure 4 shows the number of toothbrushes graded per wear score.

With respect to the influence of the degree of wear after 3 months on plaque removal, there was a significant ($P < 0.0001$) but weak positive correlation (Rho = 0.223). Figure 4 shows that subjects who had toothbrushes with extreme wear (score 4) had significantly higher plaque scores (Plaque index, PI = 1.98) than those with a brush with no visible wear (PI = 1.71) or with light wear (PI = 1.80). Additionally, the scatterplot in Figure 5 shows that there is a wide range within the five wear score groups.

During the experimental period, three toothbrushes were provided per individual. Each brush was used for the same duration and with a similar frequency. This made it possible to analyse the participants' consistency to cause wear to their assigned toothbrushes. The wear status per toothbrush showed a strong to very strong correlation ($P < 0.0001$) with the wear status of the other used toothbrush by the same participant. The Spearman's Rho correlations between the 7-month and 10-month time points were 0.802; between the 7-month and 12-month time points, they were 0.786; and between the 10-month and 12-month time points, they were 0.819.

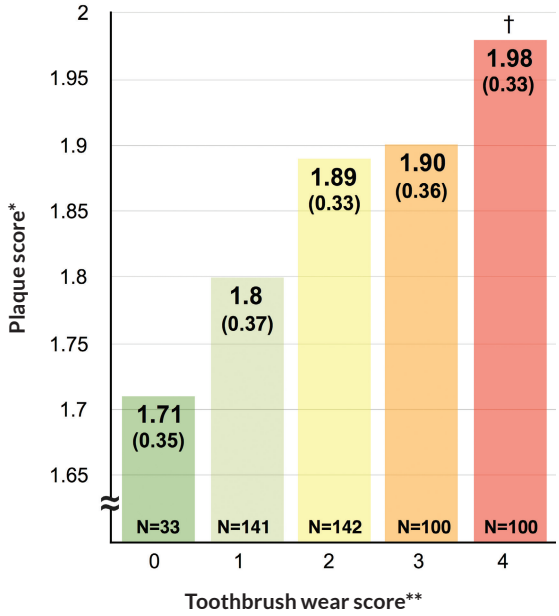


Figure 4 Brush-wear analysis in relation to plaque scores
* Modified Quigley & Hein[20] plaque index (QHPI) according to Paraskevas et al.[21]
** Toothbrush wear score according to Conforti et al.[29]
ANOVA for overall differences between groups p<0.0001
Post hoc comparisons of groups against wear score 0 & 1. (Bonferroni correction)
† Independent t-test between two groups, significant difference versus wear score 0 & 1 (p=0.01)

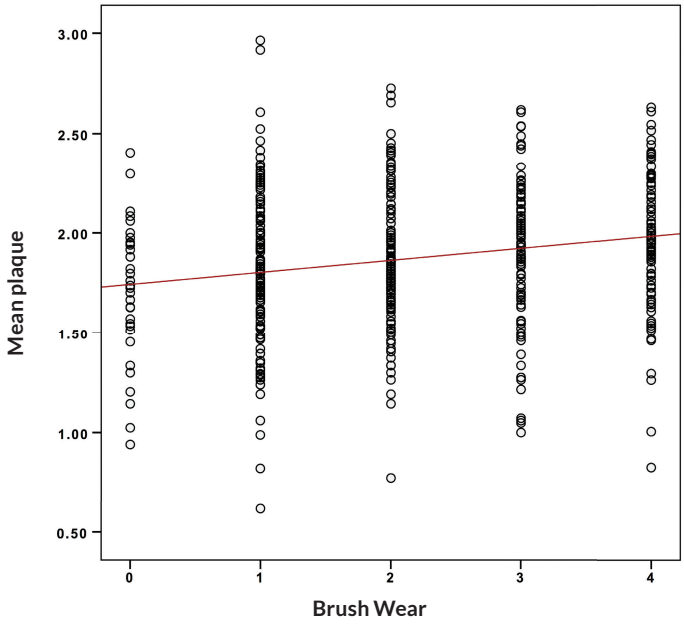


Figure 5 Scatterplot of brush-wear analysis in relation to plaque scores

Discussion

Although individuals were rather consistent in the degree of wear they induced after 3 months, the present study shows that wear varied widely between individuals. With respect to toothbrushing efficacy, it seems that the age of a toothbrush should not be the factor guiding replacement. Instead, the level of wear appeared to be more important. This is consistent with the conclusion of Rosema *et al.*[18] It has also been shown that toothbrush bristles that spread apart take on permanent curvatures.[31]

Variation in the degree of wear is most likely caused by differing toothbrushing forces and techniques amongst individuals.[32] The individual manner of brushing seems to be of more importance than the length of time the brush is in use in the development of wear.[8, 32]

The most obvious aspect of brush wear is bristle splaying whereby the bristles spread apart and take on a permanent curvature. Several methods have been used for the measurement of this phenomenon, including the angle of bending of the outside bristles,[32] increase in brush surface area,[17] subjective rating scales[16, 29, 33] and a qualitative assessment tool whereby mean percentage of bristle splaying in three rows of tufts and brush surface area are calculated.[34] The wear rating used in the present study, as proposed by Conforti *et al.*,[29] although being subjective and qualitative, is a quick means of ranking brushes in various stages of deterioration. Therefore, these methods appear to be suitable not only for research, but also for quality control, the setting of standards, and for substantiation of advertising claims.

Studies comparing manual vs power toothbrushes have shown that in power toothbrush users, bristle splaying was less than among those using a manual brush.[35] Furthermore, also quality issues of optically comparable brushes are apparent with this method of scoring where differences in susceptibility to splaying.[36] Consequently depending on the configuration of the filaments (tufts) and the quality of the bristles, the durability of toothbrushes will vary.

The variability as observed in the present study is consistent with the available literature. McKendrick *et al.*[8] showed that there is substantial variation among individuals to what extent they wear out their brushes. Therefore, they suggested to categorize the individuals into high, medium and low wearers. Most people seem to fall under the low-wear-rate category[37] and, for a given individual, there is remarkable consistency in both the rate of wear among identical brushes and the pattern of wear among brushes having different characteristics.[33]

Splaying is the most visually apparent manifestation of brush wear.[38] Surveys of dental care professionals have found that the majority identify splayed bristles as the main sign of toothbrush wear and recommend replacement when this occurs.[39]

However, individual perceptions differ, and when one person states that a brush is worn out, he or she may be referring to something entirely different from what another person means by the same statement. Individuals respond to questions about brush wear with comments concerning a variety of issues: bristle filaments pulling out, decreased stiffness, reduced cleaning, matted appearance, discoloration and vague descriptions that are difficult to relate to any particular property.[38]

The relationship between the "state-of-wear" of a toothbrush and its plaque-removing effectiveness is a potentially important factor in self-performed oral hygiene since brushes should be discarded before becoming worn out. Unfortunately, there is little objective standard evidence as to: (a) what constitutes a worn-out brush and (b) the degree of loss in plaque removal effectiveness due to brush wear.

It is very likely that the user has little idea of when his/her toothbrush needs replacement. In a study by Hill and Kreifeldt,[40] user's matched their brush against three schematic drawings of worn brushes labelled no wear, some wear and much wear. Whereas only 3% of the users judged their brush to match the "much wear" picture, 14% of 72 returned brushes were judged by the examiners to be in this category. There is either considerable disagreement as to what constitutes the wear category or the user does not easily perceive his own brush as worn.[16]

Previous studies suggest that a toothbrush's cleaning ability decreases as the filaments become worn.[17] Kreifeldt *et al.*[16] explained that tapering will result in reduction in filament diameter, and thus, the brush will become softer and remove less plaque. However, a recent systematic review[41] evaluating the effect of a tapered manual toothbrush compared with a toothbrush with end-rounded filaments was not conclusive. A drawback of the Kreifeldt *et al.*[16] study is that brush wear was produced artificially so that it may not be representative of the type of wear that would have been produced by an individual's personal toothbrushing activities. The strongest evidence points to a progressive loss in efficacy with use. Both in vitro[16] and in vivo[17] results suggest that, whatever the initial shape of a bristle tip (sharp, flat or round) for an evaluated brush, within less than ten percent of the expected user lifetime the different initial geometries all converge towards flat shape. Any change in bristle tip geometry with wear, however, does not appear to significantly affect the abrasivity of the toothbrush. Thereby, both the machine and the human brushing methods demonstrated that end-rounding nylon filaments can be expected to quickly wear flat during normal use.[12]

A study by Turgut *et al.*[42] showed that bristle ends become more rounded in use, which is according to the classification of Silverstone & Featherstone[43] a desirable filament tip with respect to preventing gingival trauma.[16, 44] Different types of commercially available toothpastes influence the deterioration of the bristle tip

morphology. Factors related to the abrasive toothpaste such as type, size and shape of the abrasive particles greatly influence the friction force generated by the toothbrush. [33] Extra soft toothbrushes appeared to be most susceptible to bristle wear.[45]

The American Dental Association (ADA) guidelines on manual toothbrushes[46] suggest that, to claim that one brush is better than the other, there should be a minimum absolute difference of 15% in plaque scores. Although of the level of mean plaque scores in our study was statistically significant between the wear score extremes categories (0 and 4), the maximum observed absolute difference of 13.6% was close, but did not exceed this limit. Given the guidelines from the ADA, in our study, toothbrushes with a brush-wear score of 0 had no clinically relevant benefit over toothbrushes with a brush-wear score of 4. However, the ADA has developed their guidelines around (randomized) controlled clinical trials, whereas the present observational study clearly showed that higher visible wear scores corresponded with higher plaque scores. The observed 13.6% difference in plaque scores deserves further research in order to establish the impact this will have on gingival inflammation in order to establish its clinical relevance.

One possible explanation for the relatively low maximum absolute difference is the study design. To avoid the risk of increased bleeding resulting from toothbrushing,[28] plaque scores were assessed 2-3 hours after brushing. This is contrast to Rosema *et al.*[18] where plaque scores were assessed just before and immediately after brushing. Their study design was more experimental, whereas the present study was designed to evaluate effectiveness in an intervention under more or less ordinary day-to-day circumstances. Likewise, the level of plaque present after brushing is clinically of more relevance than the plaque reduction itself.

On average, the amount of plaque removed by toothbrushes with wear score 4 was significantly different from that removed by brushes with wear score ≤1. It therefore seems prudent to advise patients to replace their toothbrush before it reaches wear score 2, when outer tufts are splayed beyond the base of the toothbrush. This is in accordance with a previous study by Rosema *et al.*[18] but in contrast with older study's[2, 47] who found no significant differences with between new and 3-month-old toothbrushes; however, these studies did not report on wear scores.

A problem associated with toothbrushes is that they are over-the-counter products for which no special instruction is given to the potential users when they purchase such an oral hygiene product. For the consumer, the exact moment at which a toothbrush should be replaced is difficult. Bristle splaying should be advocated as an important indicator for replacing a toothbrush. A simple drawing or picture of a typical worn brush head in which the bristles of the brushing area are splayed could be used to help consumers assess the quality of a toothbrush. If it matches the picture, it is time for the toothbrush to be replaced.[48, 49] But as observed by Hill and Kreifeldt,[40] it seems to be

difficult for user's to judge the state of their own brush by only a picture. A short but concise explanation appears to be an important addition which is a responsibility that could be in the hands of the dental care professional.

Limitations

- The findings of the present study relate to the specific type of toothbrush product used (eg, brand, model, head size and shape, bristle filament diameter and height, number and inclination of bristle tufts and number of bristle rows) as well as to the character of the study population. Other toothbrush designs could have different rates of wear.
- Another limitation is that brushes were used for a restricted period of 3 months. It has been shown that during extended use, bristles become thin near their tips and take on a bent, matted appearance. This is probably the result of abrasive reduction in diameter, fatigue and the gradual accumulation of permanent strain.[16] Both matting and bristle tapering, as components of brush wear, contribute to loss of effectiveness, although matting rather than tapering appears to be the primary cause.[16]
- The wear index described by Conforti *et al.*[29] is an subjective tool.
- Habits such as "chewing" the brush head whilst brushing could also have contributed to the differing appearances of the worn toothbrushes.

Conclusion

Toothbrush wear per individual patient is fairly consistent. Toothbrushes with extreme wear were less effective than those with no or light wear. Therefore, bristle splaying appears to be a more appropriate measure of brush replacement then the commonly used toothbrush age. It is suggested that the threshold at which a brush loses efficacy is when the outer tufts are splayed beyond the base of the toothbrush.

Clinical relevance

Scientific rationale for the study

Advice varies on how frequently a toothbrush should be replaced. There are no data on how consistently an individual causes wear to his or her toothbrush.

Principal findings

After 3 months of use, toothbrush wear per patient was strongly correlated. Toothbrushes with extreme wear were less effective than those with no or light wear.

Practical implications

Equating brush wear (and, presumably, loss of effectiveness) with brush age in use is not justified. Advice on replacing toothbrushes should be based mainly on bristle flaring rather than on a "fixed" period of usage. We recommend that a manual toothbrush should be discarded when its outer tufts are splayed beyond the toothbrush base. Dental professionals should be aware of these differences, both in durability and in cleaning performance, when recommending brushes to their patients.

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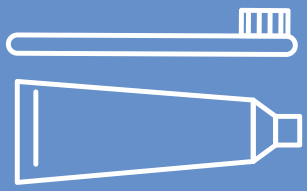

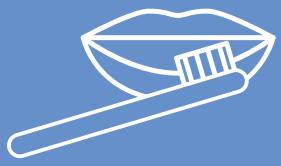

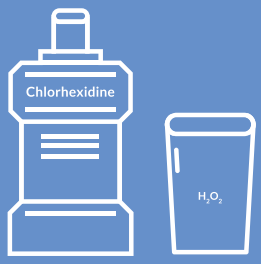
Chlorhexidine
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CHAPTER 3

Effectiveness of various interventions on maintenance of gingival health during 1 year:

A randomized clinical trial

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Abstract

Background: Rinsing with the combined use of an oxygenating-agent (OA) and chlorhexidine (CHX) in addition to mechanical oral hygiene could improve and/or maintain good gingival health over a long period.

Methods: This study had an examiner blinded, randomized, six-group parallel design consisting of two-phases: a 3-week treatment phase and a subsequent 12-month experimental phase. A total of 267 subjects in good general health (≥18 years), without periodontitis, with at least five teeth per quadrant, and with moderate to advanced gingivitis were enrolled. A 3-week treatment phase was initiated to improve gingival health. Subjects were assigned to one of the six groups: two basic oral hygiene groups (Control I & II), one professional oral hygiene instruction group (OHI), one professional prophylaxis group (PP), an OA&CHX rinse group and a group receiving a combination of all regimens (COMBI-group), being OHI + PP + OA&CHX. Dental plaque, gingival bleeding and staining assessments were performed at the start of the treatment phase, at baseline and at 4, 7, 10, and 12 months.

Results: There was a significant reduction in dental plaque-scores for the OA&CHX and COMBI-group (0.51 [SD = 0.37], 0.38 [SD = 0.33] respectively) and a significant reduction in gingivitis scores for the OA&CHX and COMBI group (6.9% [SD = 14.0], 13.4% [SD = 13.4] respectively) from the start of the treatment phase to baseline. No clinically relevant changes were observed for the other four groups. After baseline, bleeding and plaque-scores increased back to a non-significant level between groups, and this level remained throughout the study.

Conclusion: OA&CHX and COMBI-group showed a clinically relevant improvement after the treatment phase in terms of dental plaque and gingival bleeding levels. At the 4-month clinical assessment, there was no longer a significant difference between groups.

Introduction

Epidemiological studies in many countries have suggested a close association between dental plaque and periodontal diseases.[1] Gingivitis is common both in the primary and secondary dentitions of children and also affects most adults.[1] The current theory holds that the gingival lesion is the precursor of periodontitis, but the proportion of gingival lesions converted into periodontitis and the factors causing this conversion have not been well understood.[1] Lang *et al.*[1] reviewed the material presented in longitudinal studies of periodontal disease in humans[2–5] and established that gingival inflammation was a risk factor for tooth loss. Teeth consistently surrounded by inflamed gingiva had a significantly higher risk of being lost than teeth with no or only slight inflammation during a 26-year observational period. Consequently, inflammation of the gingival tissues represents not only the precursor of periodontitis, but also a clinically relevant risk factor for disease progression and tooth loss. Therefore, gingivitis prevention appears to be an important goal to improve the longevity of teeth.[1]

The control of dental plaque is one of the basic approaches to maintain a functional healthy dentition without periodontal diseases.[6] There are different methods of preventing the growth of dental plaque. The use of dentifrice and the use of a toothbrush are an integral part of most oral hygiene regimes.[6] Effective plaque control is difficult, even for the most diligent patients.[6] It has therefore been investigated whether the use of antiseptic agents can boost mechanical removal efforts.[6,7]

The initiation of the present study was based on a gingivitis prevention study model published by Svatun *et al.*,[8,9] whereby during a treatment phase, teeth were scaled and polished to remove all deposits, and the participants received one type of oral hygiene instruction. The purpose of this phase was to motivate the participants to perform an oral hygiene regime capable of achieving a healthy periodontium. The Svatun studies showed that after the treatment phase, the low plaque scores observed at baseline were not maintained during the 6-month period in the control group. Plaque had significantly increased at the 3-month examination and remained at this high level at 6 months.[10] The gingival bleeding followed a similar pattern. After a boost in oral hygiene and oral health, subjects tended to fall back to their original level of oral health, as has been documented in previous work.[11,12] In addition, Stephen *et al.*[10] used a similar protocol to confirm that the initial improvement in the participants' oral health was not maintained by unsupervised brushing in the control group, as gingival bleeding and calculus both increased. The study performed by Rosema *et al.*[13] has shown that rinsing with the combined use of OA and CHX in addition to mechanical oral hygiene during the treatment phase could maintain gingival health over a longer period. The presumption in the present study was that this positive effect on gingival health would

last up to 9 months[13] and possibly up to 12 months. This assumption is based on the fact that a significant correlation has been found between the bleeding index and the plaque index where a healthy gingiva results in less plaque formation.[14–18]

Therefore, the purpose of the present study was to evaluate the long-term effect of various short-term oral hygiene interventions on gingivitis prevention. In other words, can good gingival health obtained within 3 weeks be maintained over a period of 12 months?

Materials and methods

Ethics

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki (update: 18 October 2008, Seoul) and the Medical Research Involving Human Subjects Act (WMO), which approximate Good Clinical Practice (CPMP/ICH/135/95) guidelines. The protocol was reviewed and approved by the Medical Ethics Committee of the Academic Medical Centre (AMC) of Amsterdam (MEC 09/195 #09.17.1198) and registered in the Dutch Trial Register (NTR2053). The study was conducted at the Department of Periodontology of the Academic Centre for Dentistry Amsterdam (ACTA) in The Netherlands.

Participants

For this study, systemically healthy non-dental participants were recruited and screened for suitability. Inclusion criteria were as follows: participants had to be ≥ 18 years of age, have a minimum of five evaluable teeth per quadrant, and have moderate to advanced gingivitis (≥40% bleeding on marginal probing (BOMP)).[19, 20] Exclusion criteria were open caries, pockets of 4–5 mm in combination with gingival recession or pockets of ≥6 mm, as assessed according to the Dutch Periodontal Screening Index (DPSI) scores 3+ and[4, 21, 22] orthodontic appliances or removable (partial) dentures, a history of allergic reactions to erythrosine and/or any of the mouth rinse components, pregnancy, systemic disease or any adverse medical history or long-term medication that might interfere with the response variables. No restriction with respect to smoking status was applied.

Sample size

The American Dental Association (ADA) Acceptance Program Guidelines: Chemotherapeutic Products for Control of Gingivitis[23] states that a sufficient number of participants should be enrolled in a study to ensure that appropriate statistical tests

can be performed. The sample size of 40 participants per group was calculated a priori (PS: Power and Sample Size program)[24] based on a pooled standard deviation (σ) of 0.23 (as taken from the gingivitis scores in a previous 6-month mouth rinse study by Paraskevas *et al.*[25] and a detectable difference (δ) of 0.18 (between groups) with an α= 0.05 to obtain 80% power.

Study design

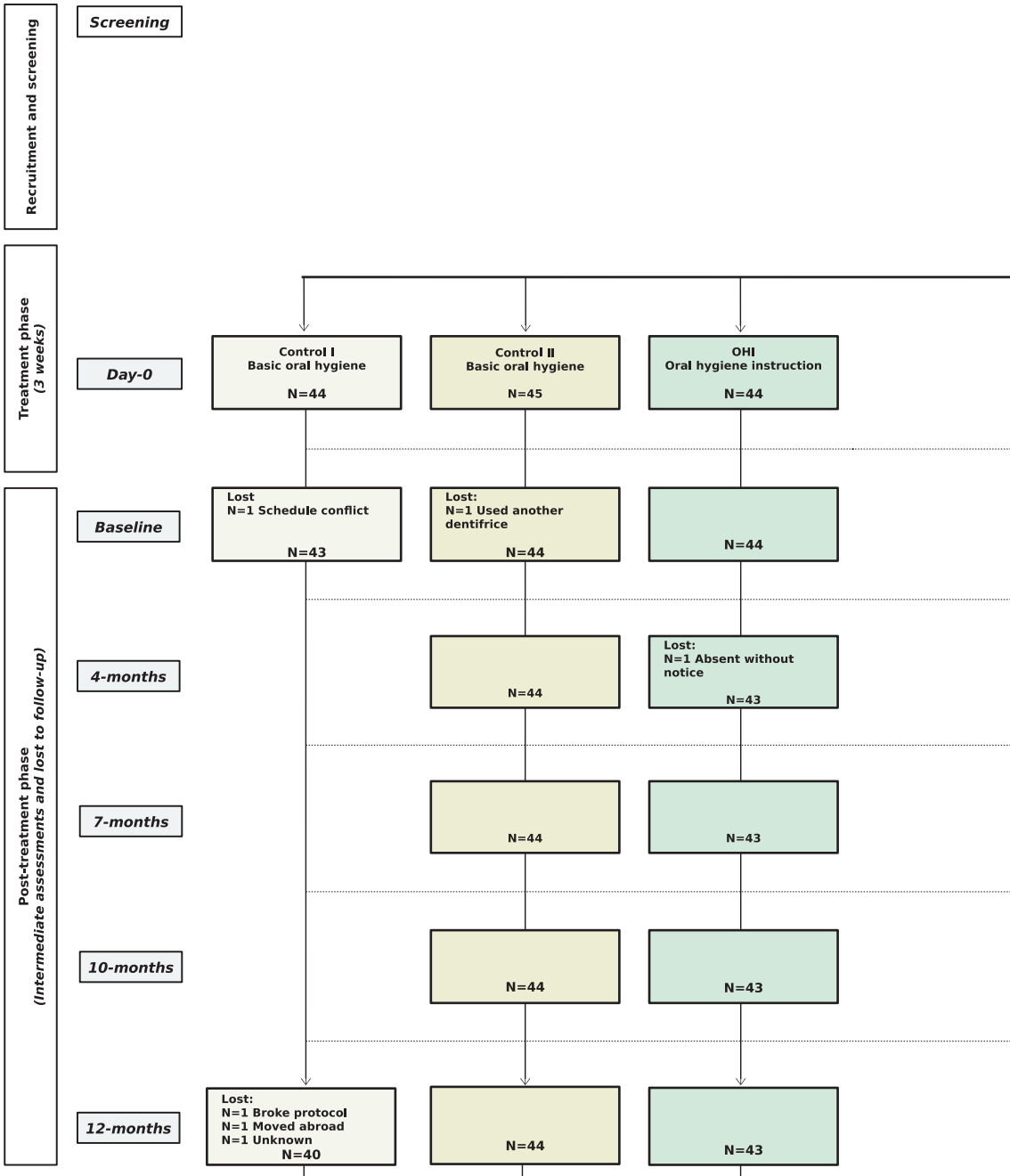
The protocol was a randomized, examiner-blind, parallel study design consisting of six groups and two phases: a 3-week treatment phase and a 12-month experimental phase (Figure 1).

Recruitment

The study was conducted (November 2009–November 2010) at the Department of Periodontology of the Academic Center for Dentistry Amsterdam, The Netherlands. Participants were students recruited from different universities and colleges in and around Amsterdam by e-mail and flyers. The investigator provided students with further detailed information before their enrolment. Subsequently, subjects had time to consider whether they wished to be involved and undergo screening.

Screening

At the screening, participants were asked to read and sign the informed consent, and they received a signed copy for their records. Subjects completed a medical questionnaire to be classified as systemically healthy. Eligibility (inclusion/exclusion) criteria were reviewed. An experienced dental hygienist performed an oral examination and screened subjects for moderate to advanced gingivitis (BOMP > 40%) and for the absence of periodontitis, using the Dutch Periodontal Screening Index[21] (scores ≤ 3-). Participants were considered eligible for the study after meeting all study entrance criteria. Appointments were scheduled for their first appointment, day 0. Furthermore, participants were instructed to brush 2 to 3 h before all of their appointments to avoid the risk of increased bleeding as a result of tooth brushing.[26] Subjects were not allowed to use any other dental products or interdental cleaning aids during the study and/or to undergo a dental prophylaxis during routine dental check-ups.


Recruitment and screening
Screening
Treatment phase
(3 weeks)
Day-0
Control I
Basic oral hygiene
N=44
Control II
Basic oral hygiene
N=45
OHI
Oral hygiene instruction
N=44
Post-treatment phase
(Intermediate assessments and lost to follow-up)
Baseline
Lost
N=1 Schedule conflict
N=43
Lost:
N=1 Used another dentifrice
N=44
N=44
4-months
N=44
Lost:
N=1 Absent without notice
N=43
7-months
N=44
N=43
10-months
N=44
N=43
12-months
Lost:
N=1 Broke protocol
N=1 Moved abroad
N=1 Unknown
N=40
N=44
N=43

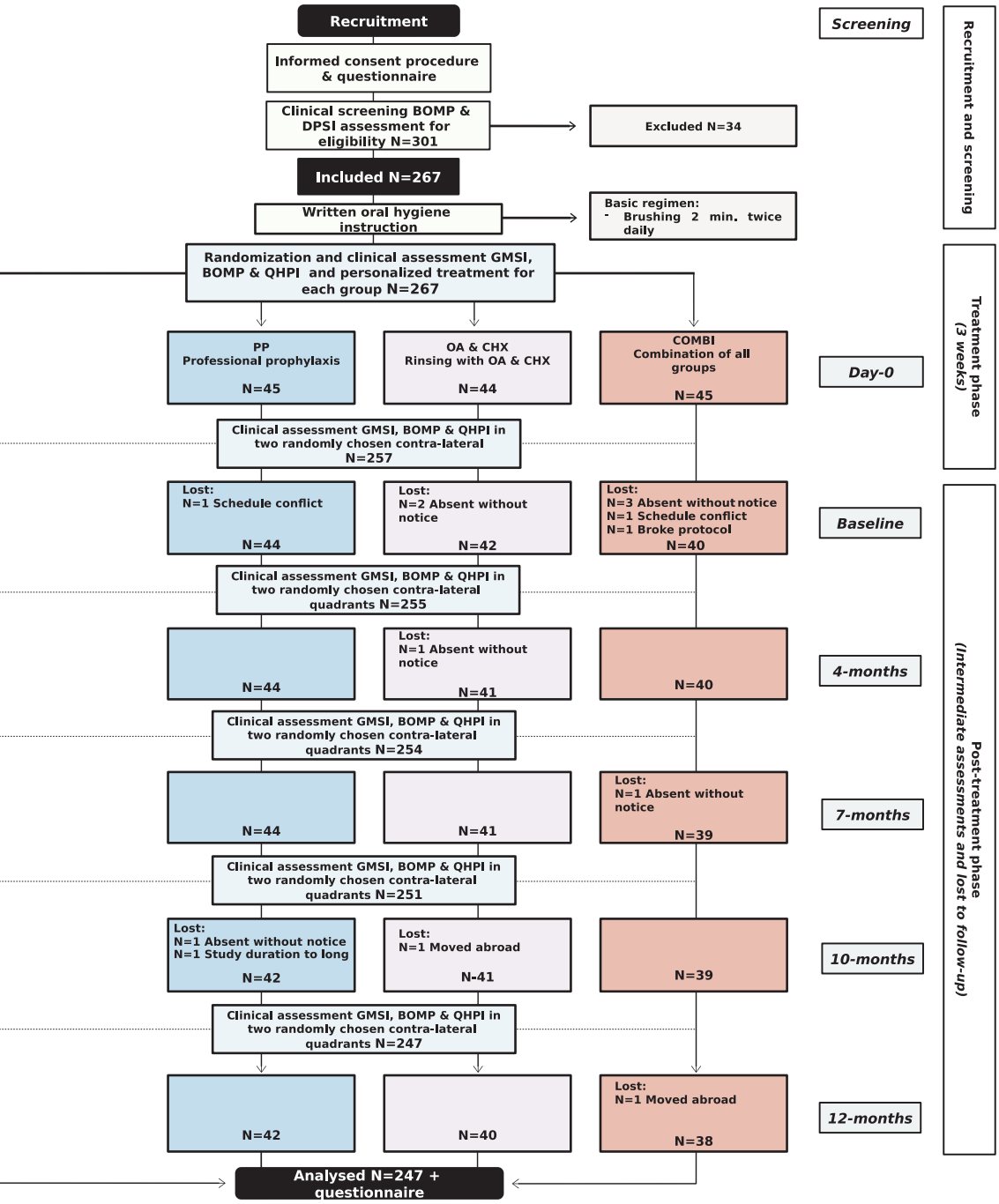


Figure 1 Flowchart depicting subject enrollment and measurements. Control I & II, basic oral hygiene regimen; OHI, oral hygiene instruction; PP, professional prophylaxis; OA&CHX, oxygenating-agent & chlorhexidine; COMBI: OHI, PP and OA&CHX combined

Study procedures

All participants performed a similar basic oral hygiene regimen of brushing twice daily for 2 min with a fluoride-containing dentifrice for the full duration of the study. At day 0 (see Figure 1), participants were instructed to brush according to the details provided in a written oral hygiene instruction leaflet describing the Bass method technique.[27, 28] The participants in control groups I & II were instructed to adhere to this 'basic oral hygiene regimen' only. To minimize the potential Hawthorne effect,[29] the clinical parameters of control I were assessed at day 0, baseline (3 weeks later) and 12 months later. The clinical parameters of all other groups were assessed at day 0, baseline, 4, 7, 10 and 12 months.

In addition to the basic oral hygiene regimen, the oral hygiene group (OHI) verbally received professional individual oral hygiene instruction (at day-0), and the professional prophylaxis group (PP) received a professional oral prophylaxis from an experienced dental hygienist at day 0. Teeth were scaled and polished as described by Slot *et al.*[30] The purpose of this prophylaxis was to render the participants free of plaque, stains and calculus to give them an identical start at baseline. During the treatment phase from day 0 until baseline, the oxygenating-agent (OA) and chlorhexidine (CHX) group (OA&CHX) were instructed to rinse with a combination of the OA Bocasan® (Oral-B Laboratories, Boston, MA, USA) and CHX 0.20% (Corsodyl; GlaxoSmithKline, Zeist, The Netherlands) twice daily for 3 weeks in addition to the basic oral hygiene. In addition to the basic oral hygiene regimen, participants in the COMBI group received all three supplementary preventive interventions: OHI, PP and OA&CHX. Table 1 shows detailed information on the interventions and the product information and usage instructions for each group.

A sufficient product supply was given to the participants for their use until the next visit. As a compliance check, they were asked to register the time of their use of these products on a calendar record chart. In an effort to ensure further compliance, all bottles and tubes of dentifrice were weighed before the products were distributed to the participants. They were then re-weighed when they had been returned.

Any adverse events reported by the subjects during the course of the study were appropriately recorded.

Table 1 Following regimens groups were designed and described using the TIDieR checklist[31]

	Basic oral hygiene and ingredients	Groups	Intervention
Allocated	Brushing twice daily* for 2 min with a fluoride-containing dentifrice† during the study. *Dentifrice:* Zendium® Classic: Sodium Fluoride (1100 ppm), Aqua, Hydrated silica, Sorbitol, Glycerin, Steareth-30, ChondrusCrispus Extract, Aroma, Titanium Dioxide, Disodium Phosphate, Citric Acid, Sodium Benzoate, Sodium Saccharin, Potassium Thiocyanate, Zinc Gluconate, Colostrum, Lysozyme, Lactoferrin, Lactoperoxidase, Amyloglucosidase, Glucose Oxidase. RDA: 75 *Toothbrush:* IQ Lactona® pro soft: 42 tufts, 9.5 mm polished, end-rounded soft bristles	Control I	NA
		Control II	NA
		OHI	Verbal professional individual oral hygiene instruction at day 0
		PP	Professional oral prophylaxis as provided by an experienced dental hygienist at day 0
		OA&CHX	Rinse with a combination of the oxygenating agent Bocasan®‡ and CHX 0.20%§ twice daily for 3 weeks¶
		COMBI	Additionally to the verbal professional individual oral hygiene instruction and professional oral prophylaxis at day 0. Participants rinsed with a combination of the oxygenating agent Bocasan®‡ and CHX 0.20%§ twice daily for 3 weeks¶

Control I & II, basic oral hygiene regimen; OHI, oral hygiene instruction; PP, professional prophylaxis; OA&CHX, oxygenating-agent & chlorhexidine; COMBI: OHI, PP and OA&CHX combined; RDA, radioactive dentin abrasion; NA, not applicable.
*Lactona® Europe B.V. Bergen op Zoom, The Netherlands.
†Zendium®, Sara Lee, De Hague, The Netherlands.
‡Oral-B® Laboratories, Boston, MA, USA.
§Corsodyl®, GlaxoSmithKline, Zeist, The Netherlands.
¶Twice daily rinsing for 2 min with 30 ml of Bocasan® (1.7 gram) followed by a 1-min rinse with 10 ml of chlorhexidine.

Clinical parameters

Partial mouth examinations (half-mouth examinations) were performed in two randomly chosen contra-lateral quadrants (1st and 3rd quadrants or 2nd and 4th quadrants),[32] excluding third molars.[32] Staining was assessed (NLHH) at four sites per tooth, according to the Gründemann Modification of the Stain Index (GMSI), on a scale of 0 to 3.[33] Subsequently, the level of gingival health was assessed (NLHH) at six sites (Mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual) around the selected quadrants by scoring BOMP on a scale of 0 to 2.[19, 20] Dental plaque was also assessed (GVA) at six sites after disclosing with Mira-2-Ton (Hager & Werken GmbH & Co. KG. Duisburg, Germany), and scores were based on the modified Quigley and Hein[34] plaque index (QHPI)[35] with a scale of 0 to 5. Throughout the study, all examinations were performed under the same conditions by one and the same experienced examiner for the separate indices (NLHH & GVA). The examiners were blinded to treatment randomization, and the records of earlier examinations were not available at the time of re-examination. Intra-examiner Spearman's rank correlation coefficient for QHPI scores demonstrated a very strong (0.936) calibration at the 0.01 level.

Randomization

Randomization for group and quadrant selection was performed (by CEB) using true random numbers generated by sampling and processing a source of entropy outside the computer. The source was atmospheric noise, which was sampled and fed into a computer without any buffering mechanisms in the operating system (www.random.org). The study coordinator assigned the participants to their randomly chosen group. Allocation was concealed by using sequentially numbered, opaque, sealed envelopes (SNOSE-method).[36] The participants were not informed about the group allocation. The examiners were blinded with respect to treatment allocation.

Questionnaire

At 12 months, upon completion of the clinical assessments, all participants were asked to complete a questionnaire designed to evaluate their attitudes towards the assigned intervention. The participants answered the questions by placing a vertical mark on a 10-cm-long uncalibrated line (the visual analogue scale, VAS); the left of this line represented the 'negative' extreme, whereas the right represented the 'positive' extreme.[37] As such, a mean score of five would represent an 'average' score, being neither positive nor negative.

Data analyses

The SPSS software package version 22.0 (IBM Corporation, Armonk, NY, USA) was used to perform the statistical analyses. The individual measurements were summarized for each individual and were analysed within their respective group. For each group, the means were calculated for BOMP and QHPI as primary response variables, and GMSI as secondary parameter. GMSI and bleeding scores were calculated based on the percentages of examined sites. The Kolmogorov–Smirnov test was used to verify the normality of data distribution. Normally distributed variables (BOMP) were compared using the ANOVA test. In case of a significant outcome, post-testing to determine the origins of the differences was performed using independent *t*-tests between groups. Paired sample *t*-tests were used to assess differences within groups between day 0 and baseline. Non-normally distributed variables (QHPI and GMSI) were tested with Kruskal–Wallis for comparison among groups. When significant, further tests were performed using the Mann–Whitney *U* test (independent samples) for pairwise comparison between two groups. Within-group change was evaluated using the Wilcoxon signed rank test. The *P*-values were corrected for multiple comparisons using the Bonferroni correction and were considered statistically significant if the *P*-values were ≤0.05.

Results

In total, 301 participants were screened, of which 267 participants were found suitable and were enrolled into the study. Of this number, twenty participants did not complete the 12-month protocol for various reasons unrelated to the study products. Participants who completed the whole protocol were considered in the analysis. For further details, see Figure 1. Baseline demographics were comparable, as shown in Table 2.

Table 2 Study subject demographics by group for those providing a full data set

	Control I	Control II	OHI	PP	OA&CHX	COMBI
n	40	44	43	42	40	38
Male/Female	13/27	11/33	10/33	7/35	6/34	6/32
Smoking/non smoking*	6/34	3/41	2/41	3/39	5/35	5/33
Mean age in years (SD)	21.1 (2.66)	21.4 (2.99)	21.2 (2.91)	21.6 (3.39)	21.9 (3.17)	20.6 (2.19)
Age range	18-33	18-30	18-32	18-34	18-29	18-27

Control I & II, Basic oral hygiene regimen; OHI, Oral Hygiene Instruction; PP, Professional Prophylaxis; OA&CHX, Oxygenating-agent & Chlorhexidine; COMBI, OHI, PP and OA&CHX combined.
* Self reported smoking status.

Clinical results

Gingival bleeding scores

No significant difference (P = 0.927) among the six groups was present at day 0 (Table 3). After the treatment phase at baseline, a significant difference among the six groups (P < 0.0001) was observed. Following the 3-week treatment phase in which participants had been performing their assigned regimen, BOMP scores decreased significantly in the OA&CHX and COMBI groups. The incremental change in the OA&CHX group and the COMBI group was 6.9% (SD = 14.0, P = 0.003) and 9.3% (SD = 11.4, P = 0.0001), respectively. Differences among the groups showed that the OA&CHX group was significantly different from the control II group, whereas the COMBI group intervention was significantly more effective compared with both control groups and the OHI group. However, at the 4-month assessment, the BOMP scores increased back to a nonsignificant level among the groups. This level remained throughout the remainder of the study. See Figure 2 for bleeding on marginal probing over time.

Table 3 Percentage (SD) of bleeding on marginal probing (BOMP) sites for all groups at six time points

	Group	Day 0	Baseline	4 months	7 months	10 months	12 months
Overall mean & percentage bleeding	**Control I**	55.5% (12.1)	56.8% (11.7)	-	-	-	65.3% (13.0)
	Control II	53.3% (16.1)	57.5% (13.7)*	59.1% (13.6)	60.8% (13.3%)	56.1% (14.1)	62.6% (13.7)
	OHI	52.2% (15.1)	54.0% (17.1)†	57.8% (13.6)	57.8% (14.6)	54.5% (15.6)	61.7% (17.3)
	PP	56.1% (14.5)	53.2% (12.8)	59.2% (15.0)	58.4% (14.8)	56.3% (13.7)	62.7% (14.8)
	OA&CHX	54.1% (13.1)	47.2% (13.6)*,‡	58.2% (12.7)	58.9% (12.7)	54.6% (14.3)	62.8% (14.3)
	COMBI	53.0% (17.8)	43.7% (18.6)*,†,‡	57.3% (15.5)	57.1% (15.2)	54.1% (16.6)	60.3% (14.5)
	***P*-value§**	*0.927*	*<0.0001*	*0.963*	*0.794*	*0.947*	*0.784*

Control I & II, basic oral hygiene regimen; OHI, oral hygiene instruction; PP, professional prophylaxis; OA&CHX, oxygenating-agent & chlorhexidine; COMBI: OHI, PP and OA&CHX combined.
* Paired sample *t*-test among groups (Bonferroni correction): significant difference between day-0 and baseline.
Post hoc comparisons of groups against Control I & II (Bonferroni correction):
† Independent *t*-test between two groups, significant difference versus Control I.
‡ Independent *t*-test between two groups: significant difference versus Control II.
§ ANOVA for overall differences between groups.

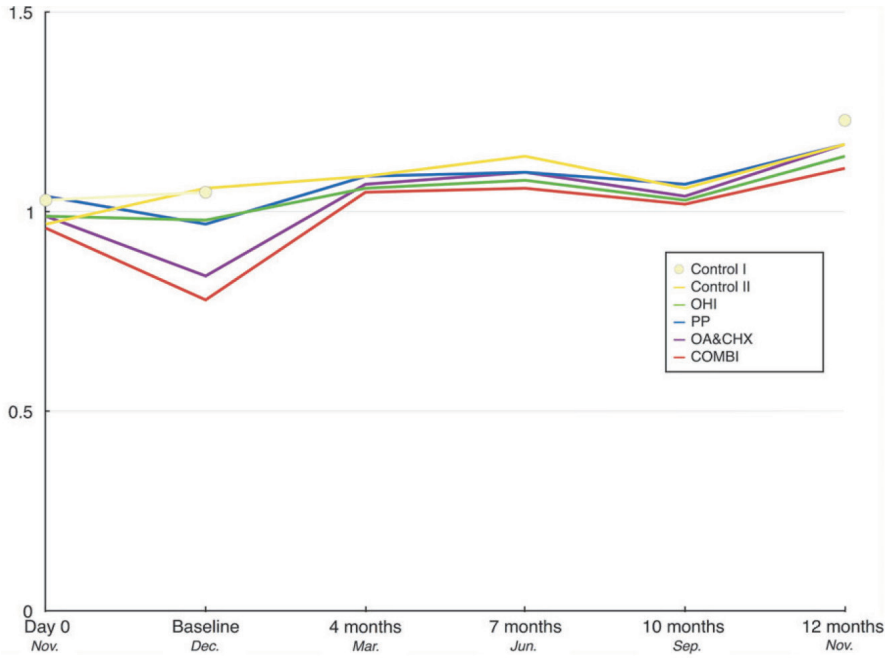


Figure 2 Bleeding on marginal probing over time. Control I & II, basic oral hygiene regimen; OHI, oral hygiene instruction; PP, professional prophylaxis; OA&CHX, oxygenating-agent & chlorhexidine; COMBI: OHI, PP and OA&CHX combined

Dental plaque scores

Among the six groups with different oral hygiene regimens at day 0, no significant difference (P = 0.727) was present (Table 4). Following the treatment phase at the baseline assessment, a significant difference (P < 0.0001) among groups was observed. Within-group analysis showed that after the 3-week treatment phase, plaque scores

decreased significantly for both the OA&CHX and COMBI group (P < 0.0001). Differences among groups showed that the OA&CHX and COMBI interventions were significantly more effective compared to either one of the control groups. At the 4-month assessment, a similar pattern was observed for the bleeding scores, whereby plaque levels increased back to a non-significant difference among groups. They remained thus for the remainder of the study.

Table 4 Mean (SD) plaque Quigley and Hein scores (Q&H) for the six time points

	Group	Day 0	Baseline	4 months	7 months	10 months	12 months
Overall mean	**Control I**	1.63 (0.29)	1.75 (0.25)*	-	-	-	1.79 (0.39)
	Control II	1.58 (0.29)	1.75 (0.26)*	1.86 (0.33)	1.95 (0.36)	2.01 (0.37)	1.82 (0.37)
	OHI	1.67 (0.26)	1.73 (0.24)	1.86 (0.29)	1.92 (0.32)	1.95 (0.42)	1.85 (0.34)
	PP	1.62 (0.25)	1.64 (0.22)	1.72 (0.31)	1.88 (0.29)	1.87 (0.38)	1.77 (0.35)
	OA&CHX	1.62 (0.29)	1.11 (0.39)*,†,‡	1.80 (0.32)	1.89 (0.29)	1.83 (0.32)	1.68 (0.35)
	COMBI	1.58 (0.24)	1.20 (0.33)*,†,‡	1.75 (032)	1.83 (0.30)	1.81 (0.40)	1.73 (0.31)
***P*-value§**		*0.727*	*<0.0001*	*0.200*	*0.845*	*0.071*	*0.138*

Control I & II, basic oral hygiene regimen; OHI, oral hygiene instruction; PP, professional prophylaxis; OA&CHX, oxygenating-agent & chlorhexidine; COMBI: OHI, PP and OA&CHX combined.
* Wilcoxon signed rank test (Bonferroni correction): significant difference between day-0 and baseline.
Post hoc comparisons of groups against Control I & II (Bonferroni correction):
† Mann–Whitney *U* test for pairwise comparison between two groups: significant difference versus Control I.
‡ Mann–Whitney *U* test for pairwise comparison between two groups: significant difference versus Control II.
§ Kruskal–Wallis test for comparison among groups.

Staining

Among the six groups at day 0, no significant difference (P = 0.642) was present (Table 5). Following the treatment phase at baseline, a significant difference among groups (P < 0.0001) was observed. *Post hoc* analyses among groups showed significantly more staining in the COMBI group compared to both control groups. Staining in the OA&CHX group was significantly higher than control group II. Similar to the clinical assessments on bleeding and plaque scores, the percentages of stained sites changed to levels no longer significantly different among the groups at the 4-month visit and all subsequent assessments.

Table 5 Percentage (SD) of staining for all groups at six time points

	Group	Day-0	Baseline	4 months	7 months	10 months	12 months
Percentage staining	**Control I**	4.6% (7.1)	6.4% (7.8)	-	-	-	8.2% (10.1)
	Control II	3.5% (6.2)	5.8% (9.4)	7.4% (10.3)	5.4% (8.0)	7.3% (8.0)	9.8% (9.8)
	OHI	2.7% (4.4)	4.4% (4.7)	6.2% (6.3)	5.4% (6.9)	4.9% (6.7)	7.3% (6.6)
	PP	2.6% (3.1)	3.2% (4.2)	4.0% (6.6)	2.8% (6.8)	4.6% (7.8)	4.7% (7.0)
	OA&CHX	3.5% (5.1)	12.1% (13.7)*,‡	6.0% (6.0)	4.9% (6.8)	4.7% (4.7)	7.5% (8.0)
	COMBI	2.7% (4.8)	17.4% (17.0)*,†,‡	5.5% (8.8)	5.5% (8.5)	6.6% (8.5)	8.2% (9.0)
***P-value*§**		*0.642*	*<0.0001*	*0.153*	*0.096*	*0.141*	*0.092*

For footnote see table 4.

Table 6 Questionnaire responses for the Visual Analogue Scale (scored from 0 to 10). The mean scores are presented for all groups

Paraphrase	Extremes		Mean scores (SD)						*P*-value*
	From	To	Control I	Control II	OHI	PP	OA&CHX	COMBI	
Participation	Not nice	Very nice	7.08 (1.72)	6.65 (1.65)	6.60 (1.49)	6.85 (1.54)	6.79 (1.59)	6.89 (1.59)	*0.774*
Dentifrice	Disgusting	Delicious	6.00 (2.55)	5.63 (2.60)	6.05 (2.24)	5.77 (2.47)	5.69 (2.34)	5.38 (2.58)	*0.849*
	Not enough foaming	Too much foaming	3.69 (1.93)	3.60 (1.76)	3.73 (1.81)	3.73 (1.57)	4.00 (1.45)	3.67 (1.76)	*0.840*
Twice daily brushing	Not enough	Too often	4.14 (1.71)	4.51 (1.00)	4.61 (1.14)	4.35 (1.25)	4.39 (1.02)	4.45 (1.14)	*0.610*
Sensation after brushing	Not clean	Very clean	6.43 (2.40)	5.88 (2.50)	7.32 (2.24)	6.35 (2.49)	6.79 (2.01)	6.15 (2.40)	*0.078*
Toothbrush	Not pleasant	Very pleasant	5.61 (2.32)	6.05 (2.64)	5.63 (2.35)	5.00 (2.51)	4.60 (2.02)	6.15 (2.61)	*0.031*
	Too soft	Too hard	4.30 (1.56)	4.39 (1.44)	4.19 (1.55)	3.86 (1.57)	4.06 (1.85)	4.58 (1.60)	*0.411*
	Not effective	Very effective	5.65 (1.91)	5.96 (1.71)	5.77 (1.73)	4.97 (1.80)	5.42 (1.40)	5.81 (1.90)	*0.125*
Sensation of oral health	Not healthy	Very healthy	6.08 (2.00)	5.92 (1.80)	6.13 (1.69)	5.98 (1.41)	6.21 (1.66)	5.94 (1.50)	*0.969*
Sensation of oral health after study	Not at all	Very much	4.18 (1.78)	4.08 (1.83)	4.61 (1.91)	4.11 (2.01)	4.41 (1.98)	4.74 (2.05)	*0.517*

Paraphrase	Extremes		Mean scores (SD)				*P*-value†
	From	To	OHI	PP	OA&CHX	COMBI	
3-weeks rinsing	Not pleasant	Very pleasant	-	-	4.63 (2.78)	5.22 (5.42)	*0.301*
Taste perception	Very bad	Very good	-	-	3.67 (2.41)	3.61 (2.40)	*0.967*
PP induced perception of oral health	Not at all	Very much	-	5.98 (2.18)	-	7.23 (1.63)	*0.128*
Dental awareness after oral hygiene instruction	Not at all	Very much	7.34 (1.64)	-	-	6.91 (1.72)	*0.906*
Effective cleaning after oral hygiene instruction	Not at all	Very much	6.35 (2.02)	-	-	6.22 (2.10)	*0.543*

Control I & II, basic oral hygiene regimen; OHI, oral hygiene instruction; PP, professional prophylaxis; OA&CHX, oxygenating-agent & chlorhexidine; COMBI: OHI, PP and OA&CHX combined.
* ANOVA for overall differences between groups.
† Independent *t*-test.

Participant compliance, attitudes and adverse events

Table 6 presents data with respect to the questionnaire, which was completed by the participants after their final appointment at 12 months. Although all groups used the same toothbrush, a significant difference among groups was observed concerning

their perceptions of the pleasantness of the toothbrush used (P = 0.031). Exploring the differences among the six groups, the Bonferroni-corrected *t*-tests revealed no significant difference. The amount of mouth rinse and dentifrice used (Table 7) was calculated per participant who returned all used bottles and tubes. No significant difference between groups was observed for the amount of CHX (P = 0.663) and dentifrice (P = 0.353) used. The average amount of dentifrice and mouth rinse used per brushing or rinsing session was approximately 1 ml and 9 ml respectively. Furthermore, no adverse events related to the used products were observed during the study.

Table 7 Amount of mouth rinse and dentifrice used per participant

Groups	n*	Day 0 – 12 months x- used (SD) dentifrice (ml.)	n*	Day 0 – Baseline x- used (SD) mouth rinse (ml.)
Control I	26	687 (331)	-	-
Control II	36	803 (255)	-	-
OHI	37	694 (207)	-	-
PP	31	728 (208)	-	-
OA&CHX	31	748 (284)	40	378 (85)
COMBI	28	773 (274)	37	340 (81)
Average:	189	740 (244)	77	360 (85)
P-value		*0.353*†		*0.663*‡

Control I & II, basic oral hygiene regimen; OHI, oral hygiene instruction; PP, professional prophylaxis; OA&CHX, oxygenating-agent & chlorhexidine; COMBI: OHI, PP and OA&CHX combined.
* Including participants who provided a full data set.
† ANOVA for overall differences between groups.
‡ Independent *t*-test.
x- mean mouth rinse/dentifrice usage.

Discussion

An acceptable criterion for gingival health is difficult to define in practical terms. Perfect gingival health could be defined as the absence of any inflammation as judged by BOMP. This ideal would be impractical, as few people would achieve it and no one will be free of some degree of subclinical inflammation.[8] Most individuals claim to brush their teeth at least twice a day. However, it is clear from both epidemiological and clinical studies that mechanical oral hygiene procedures as generally performed by people, are insufficient for controlling supragingival plaque formation and completely preventing gingivitis, and even if neglected, more severe forms of periodontal disease.[38, 39]

Over the past few decades, several studies[33, 40–45] have proven that CHX has plaque inhibiting potential, which is increased when combined with an OA, such as H_2O_2, peroxymonosulfate or Bocasan®. Dona *et al.*[44] compared the combination of CHX and Bocasan® with CHX alone in a 3-day plaque accumulation model and found significantly lower plaque scores in favour of the combination of CHX and Bocasan®.

Gründemann *et al.*[33] evaluated the combination of CHX and Bocasan® in a 14-day non-brushing protocol. Significant improvements in dental plaque, bleeding scores and tooth staining were observed. A recent systematic review by Van Maanen-Schakel *et al.*[45] provided supportive evidence showing that the ability of CHX to inhibit supragingival plaque is improved when CHX is used in combination with an OA. The combination also reduces commonly observed CHX tooth staining. Rinsing once or twice a day with a CHX solution inhibits plaque formation and can therefore help prevent inflammation of the gums and tooth decay.[6] This antiseptic's mechanism of action involves strongly binding to bacterial cell membranes.[46, 47] On the other hand, CHX readily adsorbs onto surfaces in the mouth, including pellicle-coated teeth. Once adsorbed, unlike some other antiseptics, CHX shows a persistent bacteriostatic action lasting in excess of 12 h.[48] Radio-labelled CHX studies indicate a slow release of the antiseptic from surfaces,[49, 50] which suggests the production of a prolonged antibacterial environment in the mouth.[51]

The present study tested the concept that good gingival health obtained at a treatment phase could be maintained over an extended 12 months period, within a context in which less plaque develops in the absence of gingivitis.[16–18] During the treatment phase, a marked improvement in both rinsing groups occurred; however, at the 4 month assessment the levels increased back to a point at which there were no longer differences between the groups. This finding indicated a relatively rapid loss of the dedication that is required to maintain a high degree of plaque control.[52] As Glavind *et al.*[53] suggested, the maintenance of a high level of oral hygiene in a preventive program can be ascribed to psychological and feedback mechanisms rather than professional prophylaxis and instructions *per se*. This idea is supported by the results in the OHI group, in which a single professional oral hygiene instruction showed no effect. The present results show that, without the use of a complementary preventive aid, improved gingival health tends to fade over time and return to its original values.[11] This model thus seems appropriate to investigate preventive aids after a treatment phase where the gingival health was improved to an optimum level.

The concept of this model has been published by Svatun *et al.*[8, 9] and has proved to be effective in testing oral hygiene aids to suppress plaque accumulation and the development of gingivitis.[41]

In the present study, the increase in both plaque and bleeding scores in the OA&CHX and COMBI-groups demonstrates that the success of the treatment phase could not be maintained during the first 3 months after baseline. Rosema *et al.*[13] used a similar study design that also involved rinsing with OA&CHX in the treatment phase and observed contrasting results to those found in the present study. The reduction of plaque and bleeding levels in their control group obtained at baseline was maintained at

the 6-week, 10-week, 6-month and 9-month assessments. However, their participants received four professional oral instructions over the course of the trial to obtain the optimal effect of the toothbrushes used. Oral hygiene instruction was given initially at day 0, baseline, 6 weeks and at the 10 week assessment. These instruction methods clearly differed from those of the present study, in which the oral hygiene instruction was not repeated.

A systematic review[39] showed that a single oral hygiene instruction describing the use of a mechanical toothbrush in addition to a single professional 'oral prophylaxis' provided at 6 months had a significant, although small, positive effect on the reduction of gingivitis.[39] This observation was not substantiated in the OHI group at baseline in the present study. In retrospect, the dissimilarities between the present study and the Rosema *et al.*[13] study are likely to stem from the repeated oral hygiene instruction in the latter. In Rosema *et al.*, the thorough oral hygiene instruction may have had a lasting effect that exceeded the treatment phase and could therefore be considered an unintended intervention. A systematic review by Needleman *et al.*[54] showed that there appears to be little value in professional polishing without oral hygiene instruction. This conclusion is supported by the outcome in the PP group, which showed no change after the treatment phase.

To appropriately allocate oral health care resources to achieve maximum benefits and minimal side effects for the population, a simple means of preventing gingival inflammation could be beneficial. The combination of OA and CHX has previously been used in a long-term study to prevent gingivitis and obtain optimal periodontal health over a short period. However, CHX has the propensity to induce tooth staining. In terms of tooth staining, a significant increase was observed for both rinsing groups. This increase occurred even though OA, which has been shown[45] to reduce CHX induced staining, was added. However, this reduction appeared to not fully inhibit CHX-induced staining. After the treatment phase when CHX was discontinued, this staining faded over time and returned to values that no longer revealed a significant difference between the groups. A possible clarification is that just a single brush with a manual toothbrush with toothpaste for 1 min removes a significant number of stains.[55] It is therefore most likely that twice daily brushing with a medium abrasive dentifrice (RDA-value 75) for 2 min contributed to the reduction of tooth discoloration over time.

As is apparent from Figure 2, there was a slight fluctuation in the mean gingivitis scores during the study. An explanation for this phenomenon cannot be derived from this study, nor does the literature provide a solution. However, a seasonal effect has been suggested in relation to (acute) gingivitis. British authors have shown that the lowest incidence of gingivitis was found during the summer months and the highest incidence was found in the winter, but they offered no explanation for this seasonal

difference.[56] As early as 1944, Stammers[57] speculated that 'frequent colds' contributed to local predisposing causes, including poor or non-existent oral care. Assuming that colds occur more frequently in the winter, this association with climatic conditions could be noteworthy. In 1960, Wood[58] suggested that there was a marked reduction of blood flow to the skin induced by a cool environment, and a hypothesis has been advanced[58–60] that low temperatures induce gingival vascular peripheral constriction. However, Škach *et al.*[61] failed to show evidence of any correlation between a particular type of weather condition and ulcerative gingivitis, even though 40% of the participants stated that they had recently suffered from a common cold or tonsillitis. Additionally, in the present study, the cold temperatures and reduced resistance in November may have an effect on the development of gingivitis. However, more research is needed to investigate the seasonal effect on naturally occurring gingivitis. Also fluctuations observed in mean scores could (in part) explain the slightly different scoring during the course of the study.

The level of gingival health after the treatment phase was still higher (BOMP 44%) than the inclusion criterion of ≥40% BOMP, whereas the aim was to achieve a healthy periodontium. Although a marked decline was observed after 3 weeks for the groups using CHX, participants still showed moderate gingivitis after treatment phase. Data of the 4 months showed limited residual effects of the treatments. One possible explanation is that patient's compliance may have decreased during such a long period. Another reason may be that the existing gingivitis present at baseline exacerbated during the study.

Therefore, a limitation of this study is that the level of gingivitis was reduced but after the interventions still a reasonable level existed. The outcome of the study could have been be different, when these patients would have had a low level of gingivitis at the end of the intervention phase. Altogether the data emerging from this clinical trial show that the interventions did not result in a considerable reduction of gingivitis nor was the obtained effect sustained during the first 3 months. Participants of the present study were not allowed to use any interdental cleaning aids in order to avoid adding a confounding factor. As is known from previous research young individuals seldom use interdental cleaning aids.[62] However, the fact that no interdental cleaning was allowed during the study may be seen as a limitation. Therefore, the study results may not be generalizable to those who use interdental cleaning aids on a regular basis.

Conclusions

After the treatment phase (i.e. following 3 weeks of interventions), only the groups that used a combination of OA and CHX showed a significant improvement in terms of plaque and bleeding levels. However, neither group maintained the lower levels of plaque and bleeding at the 4-month clinical assessment.

Clinical relevance

Scientific rationale for the study

Can optimum oral health be maintained for a period of 1 year?

Principal findings

The evidence of the present study showed that a single oral hygiene instruction or a professional prophylaxes has no additional effect on plaque and bleeding scores. However, rinsing for 3-weeks with CHX and an OA showed a significant reduction for plaque and bleeding scores, but increased back to approximately original levels at the 4 month assessment.

Practical implications

A single OHI, a single oral prophylaxis and rinsing with CHX and an OA have no or only short term (<4 months) effects.

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CHAPTER 4

Essential oils compared to chlorhexidine with respect to plaque and parameters of gingival inflammation:

A systematic review

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Abstract

Background: The purpose of this review is to systematically evaluate the effects of an essential-oil mouthwash (EOMW) compared to a chlorhexidine mouthwash with respect to plaque and parameters of gingival inflammation.

Methods: PubMed/MEDLINE and Cochrane CENTRAL databases were searched for studies up to and including September 2010 to identify appropriate articles. A comprehensive search was designed, and the articles were independently screened for eligibility by two reviewers. Articles that evaluated the effects of the EOMW compared to chlorhexidine mouthwash were included. Where appropriate, a meta-analysis was performed, and weighted mean differences (WMDs) were calculated.

Results: A total of 390 unique articles were found, of which 19 articles met the eligibility criteria. A meta-analysis of long-term studies (duration ≥4 weeks) showed that the chlorhexidine mouthwash provided significantly better effects regarding plaque control than EOMW (WMD: 0.19; P = 0.0009). No significant difference with respect to reduction of gingival inflammation was found between EOMW and chlorhexidine mouthwash (WMD: 0.03; P = 0.58).

Conclusion: In long-term use, the standardized formulation of EOMW appeared to be a reliable alternative to chlorhexidine mouthwash with respect to parameters of gingival inflammation.

Introduction

Systematic reviews have rapidly gained an important place in aiding clinical decision making in medicine, although dentistry has been somewhat slower to adopt this approach. The objective of a systematic review is to provide a comprehensive and contemporary appraisal of research using transparent methods while aiming to minimize bias. If such conditions are met, there should be greater confidence in the conclusions of the review than in other summaries of clinical evidence.[1]

Mouthrinses have been used for centuries for medicinal and cosmetic purposes, but it is only in recent years that the rationale behind the use of chemical ingredients has been subject to scientific research and clinical trials.[2] One essential-oil mouthwash (EOMW) (Listerine, Johnson & Johnson, Skillman, NJ.) has the longest history of use, dating back to the 19th century. It has been used as a mouthwash for the prevention of dental and periodontal diseases.[2] In a recent systematic review,[3] an antigingivitis potential was established when this EOMW was used as an adjunct to unsupervised oral hygiene compared to a placebo or control. The first official approval of this EOMW dates back to 1987 and was based on clinical studies that satisfied the American Dental Association (ADA) criteria.[4-8] Currently, seven flavors of this EOMW have been approved for the control of supragingival plaque and gingivitis by the ADA.[9] Another mouthrinse product approved by the ADA is chlorhexidine (CHX), which is a cationic bisbiguanide with a very broad antimicrobial spectrum. It was proven many times over as the most effective agent against plaque. It is used as an adjunct to mechanical cleaning procedures as well as used alone. Its effectiveness was also shown for control of gingivitis in long-term studies. The major advantage of CHX over most other compounds lies in its substantivity. It binds to soft and hard tissues in the mouth, enabling it to act over a long period after application of a formulation.[2] Bacterial counts in saliva consistently drop to between 10% and 20% of baseline after single rinses and remain at this level for ≥7 hours[10] and probably >12 hours.[11] Therefore, CHX is used as a positive control in many clinical trials of new mouthrinse formulations and is considered the gold standard. To our knowledge, there is no systematic review available that has evaluated comparisons of EOMW to a CHX mouthwash (CHX-MW).

Therefore, the aim of this review is to gather and evaluate, in a systematic manner, available data on the effect of a standardized EOMW formulation compared to a CHX-MW with respect to plaque, parameters of gingival inflammation, stains, and calculus when the products were used as an adjunct to self-performed, daily, oral hygiene procedures or as a monotherapy.

Materials and methods

Focused Question

For patients with gingivitis, what is the effect of a standardized EOMW compared to a CHX-MW with respect to the clinical parameters of gingival inflammation?

Search Strategy

Two internet sources were used to search for appropriate articles that satisfied the study purpose: the PubMed/MEDLINE and Cochrane Central Register of Controlled Trials (CENTRAL) databases. Both databases were searched for studies conducted during the period up to and including September 2010. This comprehensive search was designed to include any published articles that evaluated the effects of EOMW compared to CHX-MW. Detailed search strategies are shown in Figures 1 and 2.

The eligibility criteria for articles were as follows: randomized controlled clinical trials (RCTs) or controlled clinical trials; trials conducted in humans with subjects ≥16 years of age and in good general health (no systemic disorders); intervention: an EOMW as a standardized formulation of essential-oil technology; comparison: a CHX-MW; mouthwashes either used as a monotherapy or as an adjunct to self-performed daily oral hygiene; parameters mentioned in short-term studies (duration <4 weeks): plaque; parameters mentioned in long-term studies (duration ≥4 weeks): plaque, stain, calculus, bleeding, and gingivitis.

<Intervention AND comparison>

Intervention:
<([text words] Listerine or essential oils or essential oil or Phenol or Phenols
or
[MeSH terms /all subheadings] "Oils, Volatile" or "Phenol" or "Phenols"
or
[Substance Name] "Listerine " or "tartar control listerine")

AND

Comparison:
([text words] chlorhexidine or chlorhexidine phosphanilate or chlorhexidine di-gluconate or chlorhexidine gluconate or zinc-chlorhexidine or chlorhexidine gluconate lidocaine hydrochloride or CHX or CHX formulations
or
[MeSH terms /all subheadings] " chlorhexidine ")>

Figure 1 PubMed/MEDLINE search strategy and terms.

<Intervention AND comparison>

Intervention:
<([text words] Listerine or essential oils or essential oil or Phenol or Phenols
or
[MeSH terms /all subheadings] "Oils, Volatile" or "Phenol" or "Phenols")

AND

Comparison:
([text words] chlorhexidine or chlorhexidine phosphanilate or chlorhexidine di-gluconate or chlorhexidine gluconate or zinc-chlorhexidine or chlorhexidine gluconate lidocaine hydrochloride or CHX or CHX formulations
or
[MeSH terms /all subheadings] " chlorhexidine ")>

Figure 2 Cochrane CENTRAL search strategy and terms.

Screening and Selection

Only articles written in the English language were accepted. Case reports, letters, and narrative or historical reviews were not included in the search. First, the articles were independently screened by title and abstract by two reviewers (GAW and MVL). If the search key words were present in the title, the article was selected. If none of the key words were mentioned in the title, the abstract was read in detail to search for key words. When the abstract was not clear, but the title seemed to be relevant, the article was selected for full-text reading. If no abstract was available, but the title contained the key words, the article was also selected for full-text reading. After selection, full-text articles were read in detail by two reviewers (DES and MVL). Articles that fulfilled all selection criteria were processed for data extraction. Disagreements were resolved by a discussion. If the disagreement persisted, the judgment of a third reviewer (GAW) was decisive. All reference lists of the selected studies were hand searched by two reviewers (DES and MVL) for additional published work that could possibly meet the eligibility criteria of the review.

Assessment of heterogeneity

Factors used to evaluate the heterogeneity of the outcomes of the different studies were as follows: study design and subject characteristics; comparison and regimen; and industry funding.

Quality assessment

Two reviewers (DES and MVL) individually scored the methodologic quality of the included studies. The assessment of methodologic quality was performed by combining the proposed criteria of the RCT checklist of the Dutch Cochrane Center[12] with the quality criteria obtained from the Consolidated Standards of Reporting Trials statement[13] by Moher *et al.*,[14-17] Esposito *et al.*,[18] Needleman *et al.*,[19] the Delphi List,[20] and the Jadad scale.[21] This combination resulted in a quality-criterion list.

Criteria were designed to address external validity, internal validity, and statistical methods. An aspect of the score list was given a plus (+) for an informative description of the item at issue for a study design meeting the quality standard, a minus (-) for an informative description but a study design that did not meet the quality standard, and a question mark (?) for a lack of sufficient information.

When random allocation, defined inclusion and exclusion criteria, masking of patients and examiners, balanced experimental groups, identical treatment between groups except for intervention, and reporting of follow-up criteria were present, the study was classified as having a low risk of bias. Studies that were missing one of these six criteria were considered to have a moderate risk of bias. Studies missing two or more of these criteria were considered to have a high risk of bias. To assess the methodologic quality, the Center for Evidence-Based Medicine (CEBM) Levels of Evidence[22] resource was used. In this system, the level of evidence was scored as follows: score 1a was given to a systematic review (with homogeneity) of RCTs, score 1b was given to individual RCTs with a narrow confidence interval (CI), and score 1b- was given to individual RCTs with a wide CI. According to the CEBM, there are four grades of recommendation (A through D), where grade A denotes consistent level-1 studies.

Data extraction

From the collection of articles that met the inclusion criteria, data were extracted with regard to the effectiveness of EOMW compared to CHX-MW as a monotherapy or as an adjunct to self-performed oral hygiene. Mean values and SDs were extracted for baseline, end, and difference with respect to the parameters of interest (DES and MVL). The authors of this review specifically used only the data concerning the results of essential oils and CHX from the selected articles. Some of the studies provided SEs of the mean. Where possible, the authors calculated the SD based on the sample size (SE = SD/√N). Studies were categorized as non-brushing studies (*de novo* plaque accumulation and experimental gingivitis) and brushing studies (<4- and ≥4-week durations).

Data analyses

With the exception of one article (XIV),[23] only baseline data and end-of-trial assessments were available. Consequently, it was not possible to perform a meta-analysis of the differences because the SDs of the differences were not available and could not be calculated. Therefore, data for baselines and ends of trials were presented separately. An analysis was performed for both time points. A meta-analysis was performed for plaque parameters for studies ≥4 weeks of duration and for the *de novo* plaque accumulation studies. Because the non-brushing studies started with a thorough prophylaxis, the meta-analysis was performed using only available data from

the end-of-trial assessments. Weighted mean differences (WMDs) were calculated with software (Review Manager, version 4.2 for Windows, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark) using a random-effect model. Not all studies were included in the meta-analysis (i.e., cases of non-comparable indices, inappropriate date presentation, or unknown SDs were excluded). Therefore, data were summarized in a descriptive manner.

Results

Search and Selection

The PubMed/MEDLINE and Cochrane CENTRAL searches identified 383 and 66 articles, respectively (Table 1). In total, 390 unique articles were found. Screening of titles and abstracts initially identified 25 full-text articles. The reasons for exclusion of seven papers[24-30] are shown in Table 1. Hand searching of reference lists of selected studies identified one additional article for exclusion (XIX).[31] Ultimately, 19 articles were processed for data extraction.

Table 1 Search and Selection Results

Selection	Results
Search	383 studies from PubMed/MEDLINE, 66 studies from Cochrane CENTRAL, and 59 identical studies
Unique papers	390
Articles excluded by title and abstract	365
Selected articles for full reading	25
Articles excluded after full reading	7: Reasons for exclusion: No Listerine [24*, 25*] No outcome parameter of interest† [26†, 27†, 28‡] Non-eligible subject selection [29§, 30‡]
Articles included after full reading	18
Articles excluded for insufficient data presentation	0
Articles included from reference list	1
Articles included in final selection for data extraction	19

* Essential oils from *Lippia sidoides* were obtained from hydrodistillation of fresh leaves.
† McNeil, Stockholm, Sweden.
‡ Warner Lambert, Morris Plains, NJ.
§ Davis (India), Hyderabad, India.

Assessment of Heterogeneity

Considerable heterogeneity was observed in the study design, evaluation period, oral prophylaxis, intervention, industry funding, comparisons, and regimens used

in the 19 selected articles. Furthermore, the numbers, ages, age ranges, and sex of participants also varied among studies. Table 2[4, 23, 31-47] presents information regarding study characteristics.

Study Design and Subject Characteristics

All studies but one (XVI) were conducted as RCTs. Fourteen studies were double-masked, and five studies were single-masked. Eight studies were performed using a crossover design, whereas 11 studies had parallel designs. In all studies, subjects received an oral prophylaxis before the experiment. The study populations in 12 selected studies were subjects with gingivitis without periodontitis, whereas study I included successfully treated periodontal patients who received professional periodontal maintenance care with a mean probing depth at baseline of 2.43 mm. Six studies (IV, V, VII, XII, XIII, and XVI) provided no specific information about the periodontal status of included subjects. Evaluation periods varied among the selected studies. When intermediate assessments regarding the use of CHX and essential oils were presented, baseline and final evaluations were used in this review.

Comparison and Regimen

Six studies (I, IV, XII, XV, XVII, and XVIII) used the EOMW or CHX-MW as adjuncts to self-performed, daily oral hygiene procedures. The other 13 studies used mouthrinses as a monotherapy with no other oral hygiene procedure permitted during the experimental periods. Two studies (I and VIII) specifically mentioned that a particular version of EOMW (Cool Mint Listerine, Johnson & Johnson) was used. No specific description of the EOMW product was provided in the other 17 studies.

The CHX-MW used in the selected studies included several brands. Peridex was used in six studies (I[*], IV[*], VIII[§], XIII[§], XV[*], and XVII[§]), Corsodyl[|] was used in two studies (X and XIV), and Eburos[||], Hexident[†], Chlorhexamed[‡], and Hibitane[#] were each used in a single study (studies II, III, VII, and XVIII, respectively). In eight studies (V, VI, VIII, IX, XI, XII, XVI, and XIX), the brand name was not specified. Consequently, different concentrations of CHX-MW were used in different studies ranging from 0.09% to 0.2%. The study by Axelsson and Lindhe[47] (XVIII) evaluated two different concentrations of CHX-MW: 0.1% and 0.2%. In 14 studies, the CHX-MW contained alcohol. In one

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* peridex, Zila Pharmaceuticals, Phoenix, AZ.
§ Peridex, Procter & Gamble, Cincinnati, OH.
| Corsodyl, ICI Pharmaceuticals, Macclesfield, Cheshire, UK.
|| Eburos, Betafarma, Cesano -cona, Italy.
† Hexident, Ipex AB, Solna, Sweden.
‡ Chlorhexamed, Procter & Gamble, Schwalback, Germany.
# Hibitane, ICI Pharmaceuticals.

study (V), the CHX rinse was alcohol free, and in three other studies (III, XII, and XVI), it was unclear whether the CHX-MW contained alcohol. Both 0.12% alcohol-free and 0.12% alcohol-containing CHX rinse were used in a study by Eldridge *et al.*[39] (VIII). The rinsing time of essential oils and CHX varied, ranging from 30 to 60 seconds with 10, 15, or 20 ml. The study by Eldridge *et al.*[39] (VIII) is presented in Table 2 in the 15-ml group, in which patients rinsed for precisely 60 seconds with 0.5 oz ± 14 ml. In a study by Haffajee *et al.*,[32] the rinse volume was not mentioned.

Table 2 Overview of the Studies Processed for Data Extraction

Study Number and Reference, Evaluation Period and Design	Subjects (n) at Baseline (end of study), Age in Years (range), Sex of Subjects, and Prophylaxis	Inclusion and Exclusion Criteria	Groups, Regimen, and Super vision	Conclusion
I. Haffajee *et al.*, 2009[32] 3 months RCT, parallel and double-masked	59[Δ](59[Δ]) Mean age: 49[Δ]; (range: ?) Male: 26[Δ] Female: 33[Δ] OP	Good general health. ≥20 years of age. ≥20 natural teeth and ≥4 teeth with pocket depths >4 mm and AL >3 mm before therapy. Perio maintenance.	EOMW; ? ml, 60 seconds CHX 0.12%; ? ml, 60 seconds Twice daily Brushing Unsupervised	The use of antibacterial mouthrinses reduced supragingival plaque levels and affected the composition of the adjacent subgingival biofilm.
II. Pizzo *et al.*, 2008[33] 4 days RCT, crossover and double-masked	15 (15) Mean age: 23.2; (range: 19 to 30) Male: 9 Female: 6 OP	Good general health. ≥22 natural teeth with two scorable surfaces. No recession ≥2 mm, and no other signs of periodontitis. No subject received mouthrinses, gels, or chewing gums containing anti-microbial agents ≤3 months before the trial. Non-perio.	EOMW; 20 ml, 30 seconds CHX 0.12%; 15 ml, 30 seconds Twice daily Non-Brushing Semi-supervised (compliance assessed by measuring the bottle weight at the end of the study)	EOMW rinses may represent effective alternatives to CHX-MW as adjuncts to oral hygiene.
III. Sekino and Ramberg, 2005[34] 2 weeks RCT, crossover and single-masked	21 (?) Mean age: 27; (range: 20 to 42) Male: ? Female: ? OP	Good general health. No sign of destructive periodontal disease. ≥24 teeth (six teeth in each quadrant). No antibiotic treatment ≤3 month before the trial. No regular use of oral antiseptics. Non-perio.	EOMW; 10 ml, 60 seconds CHX 0.1%; 10 ml, 60 seconds Twice daily Non-brushing Unsupervised	The effect of the EOMW on gingivitis was more pronounced than on plaque formation. This indicated that the phenolic compound may have anti-inflammatory effects.
IV. Charles *et al.*, 2004[35] 6 months RCT, parallel and double-masked	70[Δ] (70[Δ]) Mean age: 31.7; (range: 20 to 57) Male: 25[Δ] Female: 45[Δ] OP	≥20 sound, natural teeth. Minimal criteria PI (≥1.95) and GI (≥0.95). Non-perio: ?	EOMW; 20 ml, 30 seconds CHX 0.12%; 15 ml, 30 seconds Twice daily Brushing One of two daily rinses was supervised.	The EOMW and CHX-MW had comparable antiplaque and antigingivitis activities and can have a distinct role in the management of patients with periodontal diseases.

Table 2 (continued) Overview of the Studies Processed for Data Extraction

Study Number and Reference, Evaluation Period and Design	Subjects (n) at Baseline (end of study), Age in Years (range), Sex of Subjects, and Prophylaxis	Inclusion and Exclusion Criteria	Groups, Regimen, and Super vision	Conclusion
V. Rosin *et al.*, 2002[36] 4 days RCT, crossover and double-masked	16 (16) Mean age: 23.4; (range: ?) Male: 6 Female: 10 OP	Good general health. High standard of oral health and gingival health. ≥25 scorable teeth. Non-perio: ?	EOMW; 20 ml, 60 seconds CHX 0.12%; 20 ml, 60 seconds Twice daily Non-brushing Semi-super vised (compliance assessed by measuring the bottles)	Plaque inhibition with the EOMW was essentially the same as with the CH-MW.
VI. Claydon *et al.*, 2001[37] 24 hours RCT, crossover and single-masked	42 (42) Mean age: 33; (range: 20 to 60) Male: 11 Female: 31 OP	Good general health. High standard of oral hygiene and gingival health. Non-perio.	EOMW; 20 ml, 30 seconds CHX 0.09%; 15 ml, 60 seconds Twice daily Non-brushing Semi-super vised (morning rinses)	The EOMW resulted in significantly greater plaque areas compared to the CHX rinses.
VII. Riep *et al.*, 1999[38] 4 days RCT, crossover and double-masked	24 (23$^{\Delta}$) Mean: ?; (range: 20 to 34) Male: 14 Female: 9 OP	Good general health. ≥20 natural teeth with two scorable surfaces. Minimal criteria PI (≥1.95). Non-perio: ?	EOMW; 10 ml, 30 seconds CHX 0.1%; 20 ml, 30 seconds Twice daily Non-brushing Super vised	The plaque reductions seen in the EOMW and CHX-MW groups were statistically significant.
VIII. Eldridge *et al.*, 1998[39] 21 days RCT, parallel and double-masked	32 (?/32) Mean age: 24.5; (range: ?) Male: 24 Female: 8 OP	Good general health. Non-perio.	EOMW; 15$^{\Delta}$ ml, 60 seconds CHX 0.12% (Alc+); 15$^{\Delta}$ ml, 60 seconds CHX 0.12% (Alc-); 15$^{\Delta}$ ml, 60 seconds Twice daily Non-brushing Semi-supervised	Mean plaque scores for both CHX-MW products decreased after 21 days, whereas the mean for the EOMW increased. Bleeding and GI scores for all 3 groups increased, which may have been due to the initially healthy tissues of the participants.
IX. Netuschil *et al.*, 1995[40] 3 days RCT, parallel and double-masked	20Δ (?/20$^{\Delta}$) Mean age: ?; (range: 16 to 31) Male: ? Female: ? OP	All selected teeth displayed clinically sound vestibular enamel surfaces. Non-perio.	EOMW; 10 ml, 60 seconds CHX 0.2%; 10 ml, 60 seconds Twice daily Non-brushing Semi-supervised (compliance assessed by checking remaining solution)	The EOMW showed no difference compared to the control rinse. Because of the strong antibacterial action of CHX during use, only a thin plaque developed. As a clinical consequence, CHX showed retardation of plaque development as reflected by significantly reduced plaque indices.
X. Moran *et al.*, 1995[41] 4 days RCT, crossover and single-masked	15 (15) Mean age: ?; (range: ?) Male: 15 Female: 0 OP	Good general health. High standard of oral hygiene and gingival health. ≥15 anterior teeth. No recession ≥2 mm. Non-perio.	EOMW; 20 ml, 30 seconds CHX 0.2%; 10 ml, 60 seconds Twice daily Non-brushing Supervised	The CHX-MW was significantly more effective than EOMW.

Table 2 (continued) Overview of the Studies Processed for Data Extraction

Study Number and Reference, Evaluation Period and Design	Subjects (n) at Baseline (end of study), Age in Years (range), Sex of Subjects, and Prophylaxis	Inclusion and Exclusion Criteria	Groups, Regimen, and Super vision	Conclusion
XI. Ramberg *et al.*, 1992[42] 4 days RCT, crossover and double-masked	10 (10) Mean: 29.5; (range: 24 to 40) Male: ? Female: ? OP	No third molars. Non-perio.	EOMW; 10 ml, 60 seconds CHX 0.12%; 10 ml, 60 seconds Twice daily Non-brushing Unsupervised	The EOMW was significantly less effective than the CHX-MW.
XII. Brecx *et al.*, 1992[43] 3 weeks RCT, parallel and double-masked	20[Δ] (20[Δ]) Mean age: ?; (range: 20 to 35) Male: ? Female: ? OP	Good general health. Fair, but not optimal oral hygiene. Non-perio: ?	EOMW; 10 ml, 60 seconds CHX 0.2%; 10 ml, 60 seconds Twice daily Brushing Semi-supervised (compliance assessed by measuring the bottles)	When mouthrinses were used to supplement habitual mechanical oral hygiene, CHX remained the most powerful solution.
XIII. Maruniak *et al.*, 1992[44] 2 weeks RCT, parallel and double-masked	44[Δ] (44[Δ]) Mean age: ?; (range: 18 to 55) Male: 21[Δ] Female: 23[Δ] OP	Good general health with preexisting plaque and gingivitis. ≥20 sound natural teeth. Minimal criteria PI (≥1.95) and papillary BI (≥1.95). Non-perio: ?	EOMW; 20 ml, 30 seconds CHX 0.12%; 15 ml, 30 seconds Twice daily Non-brushing Supervised	CHX-MW was superior for reducing plaque and gingivitis compared to EOMW.
XIV. Moran *et al.*, 1991[23] 19 days RCT, crossover and single-masked	15 (15) Mean age: ?; (range: 20 to 28) Male: 7 Female: 8 OP	Good general health. High standard of oral hygiene. ≥22 permanent teeth. No PDs >2 mm. Non-perio.	EOMW; 20 ml, 60 seconds CHX 0.2%; 10 ml, 30 seconds Twice daily Non-brushing Unsupervised	Both CHX-MW and EOMW significantly reduced plaque regrowth; however, the CHX-MW was more effective.
XV. Overholser *et al.*, 1990[4] 6 months RCT, parallel and double-masked	? (82[Δ]) Mean age: ?; (range: 21 to 62) Male: 32[Δ] Female: 50[Δ] OP	Subjects with preexisting plaque and gingivitis. ≥20 sound natural teeth. Minimal criteria PI (≥1.95) and GI (≥1.95). No third molars. Non-perio.	EOMW; 20 ml, 30 seconds CHX 0.12%; 15 ml, 30 seconds Twice daily Brushing Semi-supervised (weekdays)	CHX-MW was more effective in inhibiting plaque formation, and the EOMW and CHX- MW were comparable in inhibiting the development of gingivitis when used as adjuncts to routine oral hygiene after professional prophylaxis.
XVI. Brecx *et al.*, 1990[45] 3 weeks CCT, parallel and double-masked	17[Δ] (17[Δ]) Mean: ?; (range: 20 to 34) Male: ? Female: ? OP	Good general health. Non-perio: ?	EOMW; 10 ml, 60 seconds CHX 0.2%; 10 ml, 60 seconds Twice daily Non-brushing Unsupervised	The CHX-MW was superior to the EOMW in its ability to maintain low plaque scores and gingival health during a 3-week period of no mechanical oral hygiene.

Table 2 (continued) Overview of the Studies Processed for Data Extraction

Study Number and Reference, Evaluation Period and Design	Subjects (n) at Baseline (end of study), Age in Years (range), Sex of Subjects, and Prophylaxis	Inclusion and Exclusion Criteria	Groups, Regimen, and Super vision	Conclusion
XVII. Grossman *et al.*, 1989[46] 6 months RCT, parallel and double-masked	242[Δ] (242[Δ]) Mean age: 37.0[Δ]; (range: ?) Male: 81[Δ] Female: 161[Δ] OP	Subjects with preexisting gingivitis. ≥16 natural teeth (incl. four molars). Non-perio.	EOMW; 15 ml, 30 seconds CHX 0.12%; 20 ml, 30 seconds Brushing Unsupervised	When used unsupervised as a part of regular oral hygiene and professional care, the CHX-MW provided significantly greater plaque and gingivitis reductions when compared to the EOMW.
XVIII. Axelsson and Lindhe, 1987[47] 6 weeks RCT, parallel and double-masked	72[Δ] (66[Δ]) Mean age: ?; (range: 16 to 50) Male: ? Female: ? OP	All subjects had signs of varying degrees of gingivitis. Non-perio.	EOMW; 20 ml, 30 seconds CHX 0.1%; 10 ml, 60 seconds CHX 0.2%; 10 ml, 60 seconds Brushing Twice daily Semi-super vised (weekdays)	CHX-containing mouth rinses are equally or more effective in reducing plaque than the EOMW but not as effective in enhancing gingivitis resolution.
XIX. Siegrist *et al.*, 1986[31] 3 weeks RCT, parallel and single-masked	18[Δ] (17[Δ]) Mean age: ?; (range: 19 to 28) Male: ? Female: ? OP	Good general health. High standard of oral hygiene. Maximal criteria PI (<2.0) and GI (<2.0). Non-perio.	EOMW; 20 ml, 30 seconds CHX 0.12%; 15 ml, 30 seconds Twice daily Non-brushing Semi-supervised (weekdays)	The 0.12% CHX-MW was superior to the EOMW in its ability to maintain optimal gingival health during the entire 3 weeks of mouthrinse use.

OP, professional prophylaxis at baseline; ?, not specified/unclear; Δ, calculated by the authors; PI, plaque index; GI, gingival index; BI, bleeding index; CCT, controlled clinical trial; Alc⁺, alcohol containing; Alc⁻, alcohol free; perio maintenance, history of periodontitis; non-perio, no history of periodontitis.

Industry Funding

Funding was mentioned in 10 articles, including grants from two commercial companies (studies VII¶, XIII¿, and XV¶), a grant from the University of Palermo (study II), and an educational grant (study I⁻). Several other articles received funding from commercial companies (studies IX¥, XII¥, XIII¢, XVI¥, and XVIII┐). Some articles included authors who were employed by various companies (studies VI₪, VII∩, X¢, XI», and XVII┐). Of the studies funded by industry, two studies had affiliations with essential oil mouthwash¡ products,

¶ Warner-Lambert, Freiburg, Germany
¿ Glenbrook Laboratories, a division of Sterling Drug, New York, NY
¥ GABA International, Therwil, Switzerland.
¢ ICI Pharmaceuticals.
┐ Procter & Gamble.
₪ SmithKline Beecham Consumer Healthcare, Weybridge, U.K.
∩ Warner-Lambert.
» Colgate-Palmolive Technology Center, Piscataway, NJ.

¡ Listerine, Johnson & Johnson.

whereas seven other studies had connections with CHX products, and one study was supported with an educational grant.

Quality Assessment

Quality assessment parameters, including external, internal, and statistical validity, are presented in Table 3. Based on a summary of these criteria, the estimated risk of bias was low in 12 of 19 studies. The risk was considered moderate for five studies and high for two studies. One study (IV) received a score of 1b, and the other 18 studies received a score of 1b- because they did not present CIs. All studies consistently had a score of level 1 according to the CEBM,[22] which allowed a grade-A recommendation to emerge from this review. Furthermore, all studies ≥4 weeks of duration also had a low level of potential bias, which suggested that this review presented a high level of evidence.

Study Outcomes

Differences between baseline and end-of-trial scores for parameters of interest are shown in Tables 4 through 8.[48-64] Outcomes are presented for non-brushing and brushing studies. The short-term non-brushing studies are subdivided into *de novo* plaque accumulation and experimental gingivitis. The brushing studies are subdivided into short-term (<4 weeks) and long-term (≥4 weeks) studies.

Within Groups

Only a few included data presented baseline and end-of-trial scores with respect to changes in time within each group (Tables 4 through 8). From studies that did provide data, the general trend was that, with two exceptions (studies I and XVIII), the CHX-MW showed a significant change between baseline and end-of-trial scores for all evaluated parameters.

Table 3 Methodologic Quality Scores of Included Studies

	Study									
Quality criteria	I[32]	II[33]	III[34]	IV[35]	V[36]	VI[37]	VII[38]	VIII[39]	IX[40]	X[41]
Internal validity										
Random allocation	+	+	+	+	+	+	+	+	+	+
Allocation concealment	?	?	?	?	+	?	?	?	?	?
Masked to patient	+	+	?	+	+	-	+	+	+	?
Masked to examiner	+	+	?	+	+	+	+	+	+	?
Masking during statistical analysis	?	?	?	?	+	?	?	?	?	?
Balanced experimental groups	+	+	+	+	+	+	+	+	+	+
Reported loss to follow up	+	+	-	+	+	+	+	+	+	+
Dropouts (n [%])	?	0,(0)	0,(0)	0Δ,(0Δ)	0,(0)	0,(0)	1,(4.17Δ)	0,(0)	0,(0)	0,(0)
Treatment identical except For intervention	+	+	+	+	+	+	+	+	+	+
External validity										
Representative population group	-	+	+	+	+	+	+	+	+	+
Eligibility criteria defined	+	+	+	+	+	+	+	+	+	+
Statistical validity										
Sample-size calculation and power	+	?	?	+	?	?	?	?	?	?
Point estimates	+	+	+	+	-	+	+	-	-	-
Measures of variability presented for the primary outcome parameter	+	+	-	+	-	+	+	-	-	-
Include an intention-to-treat analysis	-	?	?	?	?	?	?	?	?	?
Authors' estimated risk of bias	Low	Low	High	Low	Low	Mod	Low	Low	Low	Mod
Levels of evidence (Center for Evidence-Based Medicine 2009)[22*]	1b-	1b-	1b-	1b-	1b-	1b-	1b-	1b-	1b-	1b-

+, yes, -, no; ?, not specified/unclear; Δ, calculated by the authors; Mod, moderate. ΔA minus sign after 1b denotes a wide or unknown CI.

Table 3 (continued) Methodologic Quality Scores of Included Studies

Quality criteria	XI[42]	XII[43]	XIII[44]	XIV[23]	XV[4]	XVI[45]	XVII[46]	XVIII[47]	XIX[31]
Internal validity									
Random allocation	+	+	+	+	+	-	+	+	+
Allocation concealment	+	?	?	?	+	?	?	?	?
Masked to patient	+	+	+	-	+	+	+	+	+
Masked to examiner	+	+	+	+	+	+	+	+	?
Masking during statistical analysis	+	?	?	?	+	?	?	?	?
Balanced experimental groups	+	+	+	+	+	?	+	+	+
Reported loss to follow up	+	+	+	+	+	+	+	+	+
Dropouts (n [%])	0,(0)	0^{Δ},(0^{Δ})	0,(0)	0,(0)	?	0,(0)	0,(0)	6^{Δ},(12.50^{Δ})	1^{Δ},(5.56^{Δ})
Treatment identical except For intervention	+	+	+	+	+	+	+	+	+
External validity									
Representative population group	+	+	+	+	+	?	+	+	+
Eligibility criteria defined	+	-	+	+	+	-	+	+	+
Statistical validity									
Sample-size calculation and power	?	?	?	?	+	?	?	+	?
Point estimates	+	-	+	+	+	-	+	+	-
Measures of variability presented for the primary outcome parameter	+	-	-	+	+	-	-	+	-
Include an intention-to-treat analysis	?	?	?	?	-	?	?	-	?
Authors' estimated risk of bias	Low	Mod	Low	Mod	Low	High	Low	Low	Mod
Levels of evidence (Center for Evidence-Based Medicine 2009)[22*]	1b-	1b-	1b-	1b-	1b-	1b-	1b-	1b-	1b-

+, yes, -, no; ?, not specified/unclear; Δ, calculated by the authors; Mod, moderate. *A minus sign after 1b denotes a wide or unknown CI.

Table 4 Effects on the Plaque Index (mean ± SD)

Study	Index	Intervention/ group	Baseline*	End	Difference	Significant Baseline-End
Non-brushing *De novo* model						
II[33]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.12%)	– –	1.91 (0.62) 1.21 (0.53)	– –	– –
V[36]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.12%)	– –	• •	– –	– –
VII[38]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.1%)	– –	1.96 (0.35) 1.65 (0.41)	– –	– –
IX[40]	Silness and Löe, 1964[50]	EOMW CHX (0.2%)	– –	? ?	– –	– –
XI[42]	Silness and Löe, 1964[50]	EOMW CHX (0.12%)	– –	0.88 (0.16) 0.53 (0.17)	– –	– –
VI[37]	Shaw and Murray stain index, 1977[51] modified by Addy *et al.*, 1983[52]	EOMW CHX (0.09%)	– –	238.88 (111.68) 204.06 (109.62)	– –	– –
Experimental gingivitis model						
III[34]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.1%)	– –	2.08 1.36	– –	– –
VIII[39]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.12%) AIc+) CHX (0.12%) AIc-)	– – –	4.15 3.83 3.63	– –	– –
XIV[23]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.2%)	– –	4.86 (1.06) 2.72 (1.31)	– –	– –
XIII[44]	Quigley and Hein, 1962[48]	EOMW CHX (0.12%)	– –	2.87 2.20	– –	– –
XVI[45]	Silness and Löe, 1964[50]	EOMW CHX (0.2%)	– –	1.44 •	– –	– –
XIX[31]	Silness and Löe, 1964[50]	EOMW CHX (0.12%)	– –	• 0.51	– –	– –
Brushing Study duration < 4 weeks						
XII[43]	Silness and Löe, 1964[50]	EOMW CHX (0.2%)	• •	• •	? ?	No Yes
Study duration ≥ 4 weeks						
I[32]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.12%)	0.91 (0.61) 1.09 (0.71)	0.84 (0.64) 0.55 (0.43)	-0.07Δ -0.54Δ	No Yes
IV[35]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.12%)	2.50 (0.41Δ) 2.64 (0.42Δ)	1.77 (0.41Δ) 1.71 (0.48Δ)	-0.73Δ -0.93Δ	? ?
XV[4]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.12%)	2.492 (0.27Δ) 2.378 (0.23Δ)	1.048 (0.52Δ) 0.815 (0.51Δ)	-1.444Δ -1.563Δ	? ?
XVII[46]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.12%)	1.48 1.41	1.13 0.76	-0.35Δ -0.65Δ	Yes Yes

Table 4 (Continued) Effects on the Plaque Index (mean ± SD)

Study	Index	Intervention/ group	Baseline*	End	Difference	Significant Baseline-End
XVIII[47]	Quigley and Hein, 1962[48] modified by Turesky *et al.*, 1970[49]	EOMW CHX (0.1%) CHX (0.2%)	1.2 (0.5Δ) 1.2 (0.5Δ) 1.4 (0.5Δ)	0.6 (0.5Δ) 0.5 (0.5Δ) 0.3 (0.4Δ)	-0.6Δ -0.7Δ -1.1Δ	Yes Yes Yes

Significant Baseline-End, significant change between baseline and end of trial; –, not applicable; •, insufficient data presented; ?, not specified/unclear; Alc+, alcohol containing; Alc- alcohol free; Δ, calculated by the authors.
*Professional prophylaxis at baseline rendering zero visible plaque.

Table 5 Effects on the Gingival Index (mean ± SD)

Study	Index	Intervention/ group	Baseline	End	Difference	Significant Baseline-End
Non-brushing Experimental gingivitis model						
III[34]	Löe, 1967[54]	EOMW CHX (0.1%)	0.43 0.47	• •	? ?	? ?
VIII[39]	Löe and Silness, 1963[53]	EOMW CHX (0.12%) Alc+) CHX (0.12%) Alc-)	• • •	• • •	? ? ?	? ? ?
XIV[23]	Löe and Silness, 1963[53]	EOMW CHX (0.2%)	0.19 (0.13) 0.20 (0.14)	0.37 (0.16) 0.31 (0.16)	+0.18 (0.24) +0.11 (0.15)	? ?
XVI[45]	Löe and Silness, 1963[53]	EOMW CHX (0.2%)	• •	• 0.48	? ?	? ?
XIX[31]	Löe and Silness, 1963[53]	EOMW CHX (0.12%)	• •	• •	? ?	? ?
Brushing Study duration ≥ 4 weeks						
I[32]	Löe and Silness, 1963[53]	EOMW CHX (0.12%)	0.78 (0.36) 0.81 (0.39)	0.65 (0.42) 0.56 (0.43)	-0.13Δ -0.25Δ	No Yes
IV[35]	Löe and Silness, 1963[53]	EOMW CHX (0.12%)	1.31 (0.23Δ) 1.35 (0.24Δ)	1.04 (0.17Δ) 0.99 (0.18Δ)	-0.27Δ -0.36Δ	? ?
XV[4]	Modified gingival index[55]	EOMW CHX (0.12%)	2.234 (0.14Δ) 2.281 (0.20Δ)	0.748 (0.41Δ) 0.810 (0.42Δ)	-1.486Δ -1.471Δ	? ?
XVII[46]	Löe, 1967[54]	EOMW CHX (0.12%)	0.5227 0.5332	0.3308 0.2514	-0.1919Δ -0.2818Δ	No Yes
XVIII[47]	Löe and Silness, 1963[53]	EOMW CHX (0.1%) CHX (0.2%)	1.19 (0.34Δ) 1.26 (0.34Δ) 1.18 (0.34Δ)	0.48 (0.29Δ) 0.61 (0.29Δ) 0.65 (0.30Δ)	-0.71Δ -0.65Δ -0.53Δ	Yes Yes Yes

Significant Baseline-End, significant change between baseline and end of trial; •, insufficient data presented; ?, not specified/unclear; Alc+, alcohol containing; Alc-, alcohol free; Δ, calculated by the authors.

Table 6 Effects on the Bleeding Index (mean ± SD)

Study	Index	Intervention/ group	Baseline	End	Difference	Significant Baseline-End
Non-brushing Experimental gingivitis model						
III[34]	Bleeding aspect of the Löe index, 1967[54]	EOMW CHX (0.1%)	? ?	10.7% 13.5%	? ?	? ?
VIII[39]	Bleeding on probing	EOMW CHX (0.12%) Alc$^+$) CHX (0.12%) Alc$^-$)	• • •	• • •	? ? ?	? ? ?
XIII[44]	Papillary bleeding score (Loesche, 1979[56])	EOMW CHX (0.12%)	2.71 2.35	2.51 1.94	-0.20$^\Delta$ -0.41$^\Delta$	? ?
XIV[23]	Bleeding aspect of the Löe and Silness index, 1963[53]	EOMW CHX (0.2%)	0.93 (1.39) 0.80 (1.10)	1.27 (1.33) 1.00 (1.25)	+0.34 (2.26) +0.20 (1.32)	? ?
XIX[31]	Bleeding aspect of the Löe and Silness index, 1963[53]	EOMW CHX (0.12%)	• •	36% •	? ?	? ?
Brushing Study duration ≥ 4 weeks						
I[32]	Bleeding on probing	EOMW CHX (0.12%)	15.37% (9.21) 20.16% (14.47)	17.87% (11.82) 18.65% (15.05)	+2.5$^\Delta$ -1.5$^\Delta$	No No
IV[35]	Bleeding aspect of the Löe and Silness index, 1963[53]	EOMW CHX (0.12%)	33.29% 35.60%	12.72% 11.01%	-20.57% -24.59%	? ?
XV[4]	Interdental bleeding index (Caton and Polson, 1985[57])	EOMW CHX (0.12%)	0.71 (0.31$^\Delta$) 0.72 (0.36$^\Delta$)	0.29 (0.27$^\Delta$) 0.25 (0.29$^\Delta$)	-0.42$^\Delta$ -0.47$^\Delta$	Yes Yes
XVII[46]	Bleeding aspect of the Löe index, 1967[54]	EOMW CHX (0.12%)	0.1225 0.1273	0.0678 0.0493	-0.0547$^\Delta$ -0.0780$^\Delta$	? ?

Significant Baseline-End, significant change between baseline and end of trial; •, insufficient data presented; ?, not specified/ unclear. Alc$^+$, alcohol containing; Alc$^-$, alcohol free; Δ, calculated by the authors.

Between Groups

Differences between the EOMW and CHX-MW are presented in a descriptive manner in Table 9.

Plaque scores

In the seven studies that evaluated *de novo* plaque accumulation, five studies (II, V, VI, X, and XI) provided statistical data, of which four studies (II, VI, X, and XI) showed that a CHX-MW was more effective than the EOMW with respect to plaque scores. The studies (III, VIII, XIII, XIV, XVI, and XIX) that used the experimental gingivitis model all provided statistical data that a CHX-MW was more effective than the EOMW with respect to plaque scores. In the five long-term brushing studies, four studies (I, IV, XV and XVII) provided statistical data, of which three studies (I, XV, and XVII) showed that a CHX-MW was more effective for plaque inhibition.

Table 7 Effects on the Stain Index (mean ± SD)

Study	Index	Intervention/ group	Baseline	End	Difference	Significant Baseline-End
Non-brushing Experimental gingivitis model						
XIV[23]	Shaw and Murray, 1977[51] modified by Addy *et al.*, 1983[52]	EOMW CHX (0.2%)	0[Δ] 0[Δ]	0.06 (0.05) 0.04 (0.05)	+0.06 (0.05)[Δ] +0.04 (0.05)[Δ]	Yes Yes
	Self-developed stain index (Moran *et al.*,1991[23])	EOMW CHX (0.2%)	0[Δ] 0[Δ]	1.33 (0.72) 1.47 (0.52)	+1.33 (0.72)[Δ] +1.47 (0.52)[Δ]	Yes Yes
XIX[31]	Meckel stain index described by Lang *et al.*, 1982[58]	EOMW CHX (0.12%)	0[Δ] 0[Δ]	35.63 56.86	+35.63 +56.86	? ?
	Discoloration index system[59]	EOMW CHX (0.12%)	0[Δ] 0[Δ]	0.93 1.28	+0.93 +1.28	? ?
Brushing Study duration ≥ 4 weeks						
IV[35]	Lobene extrinsic tooth-stain index, 1968[60]	EOMW CHX (0.12%)	0.29 0.30	0.33 2.08	+0.04[Δ] +1.78[Δ]	? ?
XV[4]	Lobene extrinsic tooth-stain index, 1968[60]	EOMW CHX (0.12%)	0.07 (0.15[Δ]) 0.11 (0.21[Δ])	0.13 (0.24[Δ]) 1.45 (1.27[Δ])	+0.06[Δ] +1.34[Δ]	? Yes
XVII[46]	Self-developed stain index (Grossman *et al.*, 1989[46])	EOMW CHX (0.12%)	3.34 2.94	3.48 5.15	+0.14[Δ] +2.21[Δ]	Yes Yes
XVIII[47]	Lobene extrinsic tooth-stain index, 1968[60]	EOMW CHX (0.1%) CHX (0.2%)	0.13 (0.44[Δ]) 0.13 (0.44[Δ]) 0.00 (0[Δ])	0.09 (0.24[Δ]) 0.10 (0.29[Δ]) 0.14 (0.30[Δ])	-0.04[Δ] -0.03[Δ] +0.14[Δ]	No No No

Significant Baseline-End, significant change between baseline and end of trial; ?, not specified/unclear; Δ, calculated by the authors.

Table 8 Effects on the Calculus Index (mean ± SD)

Study	Index	Intervention/ group	Baseline	End	Difference	Significant Baseline-End
Brushing Study duration ≥ 4 weeks						
IV[35]	Volpe-Manhold calculus index, 1965[61-64]	EOMW CHX (0.12%)	0.30 0.26	0.24 0.45	-0.06[Δ] +0.19[Δ]	? ?
XV[4]	Volpe-Manhold calculus index, 1965[61-64]	EOMW CHX (0.12%)	0.19 (0.33[Δ]) 0.21 (0.31[Δ])	0.14 (0.22[Δ]) 0.36 (0.37[Δ])	-0.05[Δ] +0.15[Δ]	? Yes

Significant Baseline-End, significant change between baseline and end of trial; Δ, calculated by the authors; ?, not specified/unclear.

Gingivitis scores

Five studies (III, VIII, XIV, XVI, and XIX) used the experimental gingivitis model. Two (XVI and XIX) of four studies that provided statistical data reported that CHX-MW was more effective than EOMW with respect to the gingival index (GI). Two other studies (VIII and XIV) showed no differences. The CHX-MW was found to be more effective than the EOMW in only one (XVII) of the long-term brushing studies, whereas the other

four studies (I, IV, XV, and XVIII) did not show a difference between the two products with respect to GI.

Bleeding scores

With respect to bleeding scores, only one (XIX) of five short-term experimental gingivitis studies that provided statistical analyses showed a significant effect in favor of a CHX-MW. The four other studies (III, VIII, XIII, and XIV) did not detect a significant difference. Three (I, IV, and XV) of four long-term brushing studies also showed no difference between the EOMW and CHX-MW with respect to bleeding.

Stain and calculus scores

Five long-term brushing studies (IV, XII, XV, XVII, and XVIII) evaluated stain development, of which three studies (IV, XV, and XVII) showed that rinsing with CHX resulted in more stain. In two studies (IV and XV) in which calculus scores were also assessed, more calculus formation was found with CHX-MW compared to EOMW.

Meta-analysis

A meta-analysis was performed to compare the effects of the EOMW and CHX-MW as monotherapies or as adjuncts to self-performed daily oral hygiene procedures. A summary is presented in Table 10. Data from study XVIII concerning the EOMW were used twice, once each for the comparison of the EOMW to a 0.1% and 0.2% CHX-MW. The non- brushing designs (*de novo* plaque) evaluating plaque scores at the end of the trial (Quigley and Hein[48] modified by Turesky *et al.*[49]) showed a significant effect in favor of a CHX-MW with a WMD of 0.46 (P = 0.01). In long-term studies that included self-performed, daily oral hygiene procedures, the WMD for plaque scores was 0.19 (P = 0.0009).

However, the long-term studies that allowed a meta-analysis of GI (Löe and Silness[53]) did not show a significant difference between the two products with a WMD of -0.03 (P = 0.58). The WMD for staining (Lobene extrinsic tooth stain index[60]) in studies with durations ≥4 weeks was -0.42, which was not statistically significant (P = 0.12).

Table 9 Summary of Significant Differences in Favor of the EOMW Compared to a CHX-MW as an Adjunct to Daily Brushing or Rinsing Alone

Study	PI	GI	BI	SI	CI	Comparison
Non-brushing *De novo* model						
IX[40]	?	NA	NA	NA	NA	0.2% CHX
X[41]	–	NA	NA	NA	NA	0.2% CHX
II[33]	–	NA	NA	NA	NA	0.12% CHX
V[36]	O	NA	NA	NA	NA	0.12% CHX
XI[42]	–	NA	NA	NA	NA	0.12% CHX
VII[38]	?	NA	NA	NA	NA	0.1% CHX
VI[37]	–	NA	NA	NA	NA	0.09% CHX
Experimental gingivitis model						
XIV[23]	–	O	O	+	n	0.2% CHX
XVI[45]	–	–	n	n	n	0.2% CHX
VIII[39]	? ?	O O	O O	n n	n n	0.12% CHX (Alc$^+$) 0.12% CHX (Alc$^-$)
XIII[44]-	–	n	O	n	n	0.12% CHX
III[34]	–	?	O	n	n	0.1% CHX
XIX[31]	–	–	–	O	n	0.12% CHX
Brushing Study duration < 4 weeks						
XII[43]	–	NA	NA	NA	NA	0.2% CHX
Study duration ≥ 4 weeks						
I[32]	–	O	O	n	n	0.12% CHX
IV[35]	O	O	O	+	+	0.12% CHX
XV[4]	–	O	O	+	+	0.12% CHX
XVII[46]	–	–	–	+	n	0.12% CHX
XVIII[47]	? ?	O O	n n	O O	n n	0.2% CHX 0.1% CHX

PI, plaque index; GI, gingival index; BI, bleeding index; SI, stain index; CI, calculus index; ?, not specified/unclear; NA, not applicable; -, comparison was significant more effective; O, no difference; +, intervention was significantly more effective; n, no data available; Alc$^+$, alcohol containing; Alc$^-$, alcohol free.

Discussion

The effective control of supragingival plaque is a critical factor for preventing and treating periodontal disease.[65-67] However, most adults do not properly control dental plaque because of problems with motivation and compliance.[68-70] The adjunctive use of antimicrobial mouthrinses has been shown to be of value in inhibiting or reducing supragingival plaque formation. Therefore, mouthrinses are recommended when mechanical oral hygiene is difficult, compromised, or impossible.[33, 68, 71-73] In most countries, there is a variety of mouthwash formulas available for the general public.[74]

Table 10 Meta-Analysis Comparing EOMW and CHX-MW as Monotherapies or as Adjuncts to Self-Performed Oral Hygiene Procedures

Model	Index	Included study		WMD (random)	95% CI	Test for Overall Effect (*P*-value)	Test for Heterogeneity (I^2 value [%])	Test for Heterogeneity (*P*-value)
De novo	Plaque index[48,49]	II[33] VII[38]	End	0.46	0.09, 0.84	0.01	62.5	*0.10*
≥ 4 weeks	Plaque index[48,49]	I[32] IV[35] XV[4] XVII[47*]	Base	-0.05	-0.20, 0.09	0.48	56.0	*0.06*
			End	0.19	0.08, 0.30	0.0009	0	*0.53*
≥ 4 weeks	Gingival index[53]	I[32] IV[35] XVIII[47*]	Base	-0.04	-0.12, 0.04	0.37	0	*0.96*
			End	-0.03	-0.16, 0.09	0.58	62.0	*0.05*
≥ 4 weeks	Stain index[60]	XV[4] XVIII[47*]	Base	0.01	-0.10, 0.11	0.86	33.3	*0.22*
			End	-0.42	-0.94, 0.10	0.12	94.7	*<0.000001*

CI, Confidence Interval, *EOMW data were used twice, once each for 0.1% and 0.2% CHX.

Evaluation Period

The clinical evaluation of chemical agents included short-term studies (durations of 4 days to 2 weeks) used to investigate antiplaque effects. Intermediate length trials (durations of 2 weeks to 2 months) evaluated both antiplaque and antigingivitis efficacy.[75] Clinical trials using experimental gingivitis models[76] were frequently used as a short-term model to evaluate the antiplaque and antigingivitis efficacy of mouthrinses containing antimicrobial agents[77] and were accepted as a valid model to determine and compare the efficacy of antiseptic mouthrinses.[23] However, this model allows the estimation of the effect of the mouthrinse without the influence of mechanical plaque control.[77] Therefore, it is not an accurate reflection of the patient's actual habitual use of the product.[75] The ADA requires long-term studies (≥6 months) for a seal of acceptance, with an intermediate evaluation at 3 months to evaluate the efficacy and safety of chemical agents and patient compliance.[78] Because mouthrinses are also used and prescribed for short periods, their short-term efficacy is also of interest.[79] Therefore, besides experimental gingivitis studies, studies with an evaluation period ≥4 weeks were also included in this review with respect to gingivitis.[71] This is in accordance with the ADA requirements concerning adjunctive dental therapies for the reduction of plaque and gingivitis.[80]

Effect Size

This review is part of a series of reviews[3, 71, 81-85] that have addressed the efficacy of various chemical agents in oral health care products for patients with gingivitis. These include the use of stannous-fluoride, essential oils, cetylpyridinium chloride (CPC), hexetidine, hydrogen peroxide (H_2O_2), triclosan, and CHX. Addy *et al.*[86] also evaluated

the effect of delmopinol. The review of hexetidine and hydrogen peroxide did not provide sufficient data to calculate WMD. The two reviews that addressed stannous-fluoride and triclosan included a meta-analysis of these chemical agents incorporated in a dentifrice. The WMDs compared to a control product in terms of GI were 0.21 (95% CI: 0.14 to 0.27) and 0.24 (95% CI: 0.13 to 0.35), respectively. Haps *et al.*[71] evaluated the effect of a CPC mouthrinse. Their meta-analysis revealed a WMD of 0.15 (95% CI: 0.24 to 0.47) with respect to GI.[54] In a meta-analysis of a 0.2% delmopinol mouthrinse, Addy *et al.*[86] established a WMD of 0.10 (95% CI: 0.06 to 0.14) with respect to the modified GI.[55] In the light of these results, the largest effect has been established for essential oils in mouthrinses. The WMD reported by Stoeken *et al.*[3] was 0.32 (95% CI: 0.15 to 0.46). However, the test for heterogeneity was also significant, suggesting that the exact measure of the outcome should be interpreted cautiously. A recent review by Van Strydonck *et al.*[85] on CHX established a WMD of 0.31 (95% CI: 0.40 to 0.22) for GI,[53,54] which is similar to the effect observed with essential oils; these data also tested positive for heterogeneity. For plaque,[48] the WMDs were 0.83 (95% CI: 0.53 to 1.13)[3] and 0.67 (95% CI: 0.53 to 0.87)[85] for essential oils and CHX, respectively. Thus, for this parameter, it can also be concluded that the effect of essential oils was the largest; however, the test for heterogeneity was significant in both studies. Heterogeneity in the meta-analysis reflected different behaviors of the study populations with respect to the study product as well as differences in study designs and all other factors that may influence outcomes. In such a circumstance, we should be cautious when interpreting WMD as the exact measure for the effect. The observations of Stoeken *et al.*[3] with respect to EOMW and of Van Strydonck *et al.*[85] with respect to CHX-MW were the main reasons for the present review, which presents a direct comparison of CHX-MW and EOMW. This present review found that the CHX-MW was more effective in terms of plaque scores; however, a difference is not established with respect to parameters of gingival inflammation.

Anti-Inflammation

It is generally accepted that there is a correlation between plaque scores and parameters of gingival inflammation.[87] However, this does not agree with the observations in the present review. The CHX-MW was found to be more effective with respect to plaque scores but failed to show a similar difference in parameters of gingival inflammation. The most likely explanation for this observation is that the CHX-MW acts through an antiplaque effect on the level of gingival inflammation, whereas the effect of the EOMW occurs more predominantly through an anti-inflammatory process. This presumption is in agreement with in vitro observations of Dewhirst,[88] who observed that phenolic compounds have anti-inflammatory and prostaglandin synthetase-inhibiting activity. In

a neutrophil chemotaxis assay, Azuma *et al.*[89] demonstrated that phenolic compounds act as scavengers of free oxygen radicals and, hence, affect leukocyte activity. Further, in an in vitro study, Firatli *et al.*[90] showed that the antioxidative effect of EOMW expressed as the percentage inhibition of spontaneous oxidation was greater than that of CHX and CPC. Hence, the anti-inflammatory potential of essential oils may explain the absence of a pronounced effect on plaque in conjunction with a significant effect on gingival inflammation.[34]

Periodontal Inflammation

The goal of antiplaque and antigingivitis agents is to decrease gingival inflammation so that destructive periodontal disease will not develop. The evidence demonstrates that mouthrinses containing CHX or essential oils reduce the level of gingival inflammation. It is not clear what level of reduction is necessary to decrease or prevent periodontal disease. However, gingival inflammation is a necessary, but insufficient, condition for the initiation and progression of periodontal disease.[75] Still, there are limitations of this review, which predominantly addresses the effect of the two mouthwashes in subjects with gingivitis.

Formulations

The proper formulation of active agents in mouthrinses is important for maintaining bioavailability and, in some cases, improving substantivity. Thus, different formulations of the same active agents may have different levels of efficacy.[75] The authors of this review chose the fixed and controlled formula of EOMW (Listerine, Johnson & Johnson) as representative of essential oil-based mouthwashes; this brand also has an ADA seal. After full-text reading, two articles by Botelho *et al.*[24, 25] were excluded because they provided data on essential oils other than the EOMW selected for this review. These authors demonstrated that the essential oil Lippia sidoides-based mouthrinse was relatively safe and effective in reducing the plaque index, gingival index, and gingival bleeding index scores. Compared to 0.12% CHX-MW, no statistical significant difference in the observed effect was established. Therefore, the data of the studies by Botelho *et al.*[24, 25] are in support of the findings for the EOMW and CHX-MW determined in this systematic review.

Safety of Alcohol-Containing Mouthwashes

Alcohol is used in mouthwashes as a solvent for other ingredients and as a preservative of the preparation. For years, different formulas of mouthwashes have been used; however, the question of whether the alcohol content is a threat for health is raised at regular intervals. The high quantity of alcohol in EOMW combined with the fact that

these rinses are kept in contact with the oral mucosa for much more time than alcoholic drinks could induce a harmful effect from a local mechanism.[74]

Over the last 3 decades, ≥10 case-control studies have been published assessing the possible relationship between alcohol-containing mouthrinses and oral cancer.[91] Epidemiologic findings on mouthwashes and oral cancer were not consistent across the various studies, populations, and strata of major risk factors considered, including smokers and non-smokers.[92] More specifically, the pattern of risk is not different with reference to alcohol-containing mouthwashes and other types of non-alcohol containing mouthwashes. This absence of an association is also consistent with our knowledge of the dose-risk relationship between alcohol consumption and risk of upper digestive tract cancers, which show no excess risk for low doses of ethanol.[91, 93] A review by Silverman and Wilder[94] concluded that abundant clinical data have demonstrated the safety of alcohol-containing mouthrinses and failed to find any evidence for a relationship with increased risk of developing oral cancer, xerostomia, burning, or irritation. There have been some reports of alcoholics drinking alcohol-containing mouthwashes. These non-beverage alcoholics may cause symptoms such as severe gastritis.[95]

Staining and Calculus

Stains are generally recognized as an esthetic problem. They may interfere with patient compliance in long-term treatment regimes. Staining is not currently a recognized side effect of EOMW, although few, if any, studies have actively recorded this parameter. Mandel[96] alluded in a review to the possibility of tooth staining by EOMW but offered no evidence. However, in an experimental gingivitis study,[23] greater extrinsic staining was observed with EOMW compared to the control rinse. In the latter study[23] and a study by Addy *et al*.,[27] the masked nature of scoring left little doubt that increased staining did occur with EOMW. The design of both of these studies, in which normal toothbrushing was suspended, makes it difficult to extrapolate the findings to normal home usage of EOMW.[27] Even so, these short-term results do not seem to translate into long-term actual use. One (XVII) of the selected four long-term studies (IV, XV, XVII, and XVIII) reported a significant increase in staining for the EOMW; however, the magnitude of this increase (0.14) was negligible compared to the increase observed with a CHX-MW (2.21). Results for staining in the present review were as expected for the CHX-MW.[97] The lack of significance of WMD (0.42) (Table 10) may be due to the wide CI and the observed heterogeneity. In Table 9, all but one (XVIII) of the four studies (IV, XV, XVII, and XVIII) shows significantly more staining with the CHX-MW compared to the EOMW, suggesting that the CHX-MW has a pronounced effect on extrinsic tooth stain.

Also, calculus scores seemed higher with CHX-MW compared to EOMW, which is confusing in the context of the higher plaque control with CHX-MW. The explanation for this enhanced supragingival calculus formation has been provided by Addy and Moran[98] who suggested that this side effect of CHX is due to the precipitation of salivary proteins on the tooth surface, pellicle thickness, and/or the increased precipitation or inorganic salts on or in the pellicle layer.

Costs

Before any preventive measure is implemented, even one as conceptually simple as the control of plaque, a decision has to be made about its benefits and disadvantages. The costs of implementing the measures and any side effects that are seen with the use of a mouthwash are important considerations in this respect. Over a period of 1 year, the costs of twice daily rinsing with the EOMW would be $220 for an individual according to the regimens of use recommended by the manufacturer Twice daily rinsing with CHX would cost approximately $234. This is comparable to the cost of two to three extensive visits to a dental hygienist in The Netherlands. The dental professional has to consider the benefits of both daily rinsing and a professional prophylaxis and weigh the advantages of one against the other.[3]

Conclusions

This review demonstrates that, compared to EOMW, CHX-MW provided better results for plaque. For the long-term control of gingival inflammation, the standardized essential-oil formulation is not different from CHX. Furthermore, CHX caused considerably more staining and calculus.

Considering the potential benefits in the light of the observed side effects, EOMW appears to be a reliable alternative to CHX-MW with respect to gingival inflammation in those cases where the dental professional has judged that long-term anti-inflammatory oral care may be beneficial. However, for indications where plaque control is the main focus such as post-surgery wound-healing, a CHX-MW remains the first choice. Further research could study the potential anti-inflammatory effect of essential oils in greater depth.

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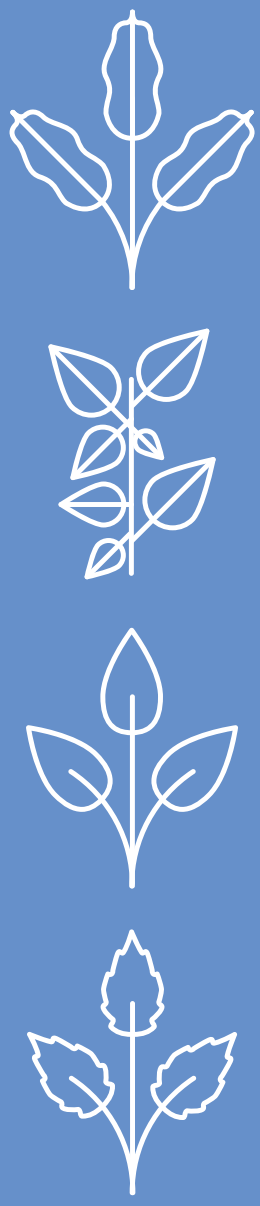

CHAPTER 5

The effect of an essential-oils mouthrinse as compared to a vehicle solution on plaque and gingival inflammation:

A systematic review and meta-analysis

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Abstract

Objective: The purpose of this review was to systematically evaluate the effects of an alcohol vehicle solution (V-Sol) compared with an essential-oils mouthwash (EOMW) and if available with a water-based control (WC) on plaque, gingival inflammation parameters and extrinsic tooth staining.

Materials and Methods: The PubMed-MEDLINE, Cochrane-CENTRAL and EMBASE databases were searched. Where appropriate, a meta-analysis was performed, and difference of means (DIFFM) as calculated.

Results: In total, 971 unique papers were found of which five met the eligibility criteria. The DIFFM of the meta-analysis of four 6-month studies showed that the EOMW provided significantly better plaque control (DIFFM = 0.39, $P < 0.00001$) and gingival inflammation reduction as measured by the Löe and Silness Index (DIFFM = 0.36, $P = 0.00001$) as compared to the V-Sol. Regarding extrinsic tooth staining, a small but significant difference (DIFFM = 0.08, $P = 0.03$) was observed.

Conclusion: Limited data, but with a low risk of bias, were available to assess the potential benefit of the alcohol-containing V-Sol. 'High'- and 'moderate'-quality data were available for the analysis of plaque and gingivitis, respectively. Within these limitations, EOMW appears to provide a significant oral health benefit during the 6 months of use. The data retrieved for this review suggest that the essential oils produce an effect on plaque and gingivitis that extends beyond the V-Sol. Furthermore, the V-Sol proved to be no different from a WC.

Introduction

Dental plaque is a key factor in the aetiology of gingival inflammation. Gingivitis represents a risk factor for periodontal attachment loss and tooth loss.[1] Therefore, it is important to encourage patients to perform accurate oral hygiene procedures aimed at removing dental plaque and preventing gingival inflammation. Antiseptic chemical agent use may supplement oral hygiene programmes and compensate for hard-to-reach areas as well as inadequate skill, poor motivation and lack of compliance.[2] The antiplaque potential of multiple antimicrobial agents has been assessed. These agents include stannous fluoride,[3] essential oils,[4] cetylpyridinium chloride (CPC),[5] hexetidine,[6] hydrogen peroxide (H_2O_2)[7] triclosan[2] and chlorhexidine (CHX).[8] Among these agents, chlorhexidine is considered to be the gold standard.[8, 9]

A previous systematic review Stoeken *et al.*[4] established that a standardized formulation of an essential-oils mouthwash (EOMW) was significantly more effective with regard to plaque and gingivitis reduction than a control mouthwash. In a recent systematic review of Van Leeuwen *et al.*,[10] the effects of CHX and the EOMW were compared on plaque and gingival inflammation parameters. CHX mouthwash provided significantly better plaque control than the EOMW, but no significant difference in the reduction of gingival inflammation was observed.

Essential-oils mouthwash was initially marked and commonly known as Listerine®, with a fixed formula containing the essential oils thymol (0.06%), eucalyptol (0.09%), menthol (0.04%) and methyl salicylate (0.05%) with either a 21.6 or 26.9% hydro-alcohol as a vehicle solution.[11] Alcohol is in general used to both dissolve and stabilize certain active ingredients and to improve the product's shelf life.[12] Alcohol also adds to the flavour and provides a 'strong taste perception' to the mouthwash. It has been suggested that not only the essential oils but also the alcohol vehicle solution contributes to the antibacterial effect.[2]

Previous systematic reviews regarding the evaluation of essential oils in a fixed formula compared this to a placebo, a (5%) hydro-alcohol control[4] or as a positive control CHX mouthwash.[4, 10] To our knowledge, no systematic review available has evaluated the effect of the alcoholcontaining vehicle solution of 'over-the-counter' available EOMW on plaque and gingivitis. Therefore, the aim of this review was to evaluate to what extent an alcohol-containing (21.6–26.9%) vehicle solution as compared to an standardized essential-oils mouthwash affected plaque, gingival inflammation parameters and extrinsic tooth staining in patients with gingivitis. The hypothesis is that there is no significant difference between a fixed essential-oils mouthwash and its vehicle solution nor between the vehicle solution and a water-based control.

Materials and methods

This systematic review was conducted in accordance with the guidelines for the Transparent Reporting of Systematic Reviews and Meta-Analyses (PRISMA-statement).[13]

Search strategy

Three electronic Internet databases were used to search for appropriate papers that satisfied the study purpose: the National Library of Medicine's (Washington, D.C.) PubMed-MEDLINE, the Cochrane Central Register of Controlled Trials, and the Excerpta Medica Database (EMBASE). The databases were searched for studies conducted during the period up to and including September 2013. This comprehensive search was designed to include any published paper that evaluated the effects of an essential-oils (a standardized essential-oils mouthwash formulation – Listerine® Antiseptic) mouthwash compared with its alcohol vehicle solution. For the detailed search strategies, see Figure 1. In addition, the manufacturer (Johnson & Johnson, Skillman, NJ, USA) was contacted for unpublished data.

The eligibility criteria for suitable articles were as follows:

- Randomized controlled clinical trials (RCTs) or controlled clinical trials (CCTs)
- Conducted in humans
  - ≥18 years of age
  - Good general health (no systemic disorders);
- Mouthrinses were used as an adjunct to self-performed daily mechanical oral hygiene
- Treatment
  - Standardized essential-oils mouthwash formulation (EOMW)
- Comparison
  - Vehicle solution from a fixed formula of essential oils containing between 21.6 and 26.9% hydro-alcohol (V-Sol)
  - When available a water-based control (WC)

The parameters of interest were retrieved from the following study types:

- Short-term studies (duration ≤4 weeks)[7]
  - Plaque scores;
- Intermediate length studies (>4 weeks to <6 months)[7]
  - Primary outcome, plaque and gingivitis scores
  - Secondary outcome, extrinsic tooth staining.

- Long-term studies (duration ≥6 months)[7]
  - Primary outcome, plaque and gingivitis scores
  - Secondary outcome, extrinsic tooth staining.

Screening and selection

Only papers with the English and Dutch language were accepted. Case reports, letters and narrative or historical reviews were not included in the selection. Two reviewers (GAW and MVL) independently screened the papers by title and abstract to select studies that potentially met the inclusion criteria. If the search keywords were present in the title, the paper was selected. If none of the keywords were mentioned in the title, the abstract was read in detail to search for keywords. After selection, full-text papers were read in detail by two reviewers (DES and MVL). Papers that fulfilled all of the selection criteria were processed for data extraction. Disagreements concerning eligibility were resolved by consensus, or if a disagreement persisted, by arbitration through a third reviewer (GAW). All reference lists of the selected studies were hand-searched by two reviewers (DES and MVL) for additional published work that could possibly meet the eligibility criteria of this study. In addition, the manufacturer of Listerine® was contacted for unpublished data.

{<ingredient> AND <vehicle>}

{<ingredient: phenol OR phenols OR oils, volatile [Mesh] OR tartar control Listerine [Substance Name] OR LISTERINE OR essential oils OR essential oil OR phenol OR phenols [text words]>

AND

<vehicle: mouthwashes [Mesh] OR mouthwashes OR mouthwash OR mouthwash* OR mouthrinses OR mouthrinse [text words]>}

Figure 1 The search strategy was customized according to the search requirements of the individual databases. The following terms were used in the PubMed-MEDLINE, Cochrane Central Register of Controlled Trials and EMBASE search strategy

Assessment of heterogeneity

The following factors were used to evaluate the heterogeneity of the outcomes of the different studies: the study design, evaluation periods, the subject characteristics, comparison and regimen, and industry funding.

Quality assessment

The methodological quality of the included studies was independently scored by two reviewers (DES and MVL). For the criteria listed, see Appendix S2. In short, a study was considered to have a 'low risk' of bias when 'random allocation, defined inclusion/exclusion, blinding to patient and examiner, balanced experimental groups, report of follow-up criteria and an identical treatment between groups except for intervention'

were present. Studies that met five of these six criteria were considered to have a potential 'moderate risk' of bias, and the absence of two or more of these six criteria was considered to represent a potential 'high risk' of bias, as proposed by Van der Weijden.[14]

Data extraction

From the collection of papers that met the inclusion criteria, data were extracted with regard to the EOMW compared with the V-Sol as an adjunct to self-performed oral hygiene. When feasible, the mean values and standard deviations (SD) were extracted by two reviewers (DES and MVL) concerning data from baseline, end-trail and specific increments with respect to the parameters of interest. When present in the selected papers, the authors of this review specifically used the data concerning the results of an EOMW and its V-Sol as well as a WC. When intermediate assessments regarding the use of EOMW and the V-Sol were presented, the baseline and final evaluations were used in this review. If applicable, any other data were neglected. Some of the studies provided standard errors (SE) of the mean. When possible, the authors calculated the standard deviation based on the sample size ($SE = SD/\sqrt{N}$).

Data analysis

The Cochrane Collaboration's statistical guidelines were followed to determine the choice of summary statistics and estimates of the overall effect.[15] Regarding plaque and gingivitis scores, studies II[16], IV[17] and V[18] provided baseline data and end-trial assessments, while study I[19] and III[20] only presented end data.

Considering the above, a meta-analysis was performed using only the available data from the end-of-trial assessments. Differences of means (DIFFM) were calculated with Review Manager (version 4.2 for Windows, The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark) using a random-effect model or a fixed-effect model in case there are <4 studies included.[8] Not all studies could be included in the meta-analysis (i.e. cases of non-comparable indices, instances of inappropriate data presentation or unknown SDs were excluded). Heterogeneity was tested by chi-squared test and the I^2 statistic. A chi-squared test resulting in a $P < 0.1$ was considered an indication of significant statistical heterogeneity. As a rough guide for assessing the possible magnitude of inconsistency across studies, I^2 statistic of 0–40% was interpreted as not be important, and above 40% moderate to considerable heterogeneity may be present.

Grading the 'body of evidence'

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) system, as proposed by the GRADE working group,[21,22] was used to grade the evidence

that emerged from this review. Two reviewers (DES and MVL) rated the quality of the evidence and strength of recommendations. Any disagreement between the two reviewers was resolved after additional discussion, and if a disagreement persisted, the judgement of a third reviewer (GAW) was decisive.

Results

Search and selection

The PubMed-MEDLINE, Cochrane-CENTRAL and EMBASE searches identified 940, 159 and 224 papers, respectively (Figure 2). In total, 971 unique papers were found. The screening of titles and abstracts initially identified 25 full-text articles. Fifteen papers were excluded because the alcohol containing control rinse had a low hydro-alcohol concentration of 0.02%[23, 24] or 5%,[25–37] and in one study, the concentration of the hydro-alcohol was not provided.[38] In addition, in one study, the control rinse was either a chlorhexidine or sanguinarine rinse,[39] and in two another, a water-based control was used.[40, 41] Furthermore, in two studies, the participants were periodontitis patients.[42, 43] By hand-searching the reference lists of the selected studies, one additional paper was identified[20] (study III), which was found in Gordon *et al.*[16] (study II). Additionally, the manufacturer (Johnson & Johnson, Skillman, NJ, USA) provided two reports with unpublished data, studies IV and V. Eventually, five studies[16–20] were processed for data extraction, which displayed variations in the study design, participant age range, evaluation period, oral prophylaxis, treatment, industry funding involvement, comparisons and regimens. Furthermore, the gender distribution of the participants varied between the studies or was unknown. Detailed information regarding the study characteristics is provided in Appendix S1.

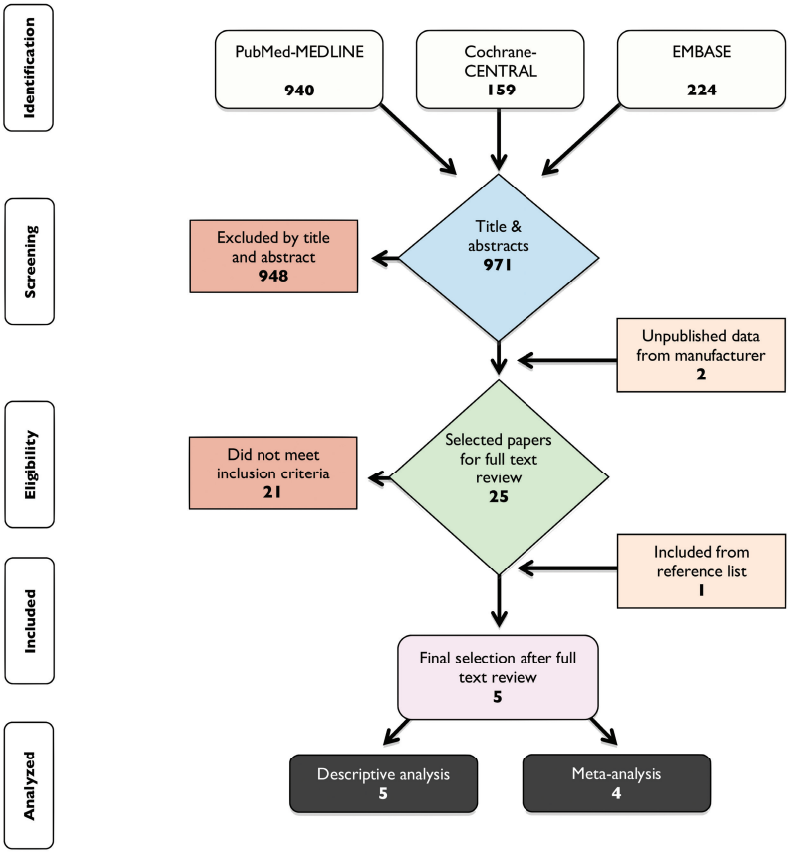

Figure 2 Flowchart of the search and selection process, including the results

Study design and subject characteristics

The study populations of all selected studies were participants with gingivitis but without periodontitis. All of the studies were conducted as randomized controlled clinical trials and were double-blind. Participants in studies I, II, IV and V underwent oral prophylaxis before the experiment, while no oral prophylaxis was provided in study III. The evaluation periods varied between 3 weeks and 6 months among the selected brushing studies. The participants in the short-term brushing study I were scored in the upper jaw where one quadrant (#1) was not brushed, while the other (#2) was brushed. As only the upper left quadrant was brushed in addition to the rinsing, data obtained from this quadrant were used. In study II (originally a 9-month study), most of the participants did not participate in the last assessment. Therefore, end scores at 6 months were used.

Comparison and regimen

The V-Sol with a hydro-alcohol concentration of 26.9% was used in five studies, while in one study (I), a 22% concentration was used. In two studies (II and III), additional groups rinsed with WC. The rinsing regime, performed twice daily for all five studies, included 30-second rinses with 20 ml of the EOMW, V-Sol and WC. In all studies, participants received a toothbrush and dentifrice and in addition to rinsing continued their usual oral hygiene. Participants in study I also received dental floss, while in study III, it was explicitly mentioned that flossing was allowed.

Quality assessment

Quality assessment parameters, including external, internal and statistical validity, are presented in Appendix S2. Based on a summary of these criteria, the estimated risk of bias was low. However, four of the five studies were either funded by the manufacturer or involved contributing authors who were employed by the same manufacturer.

Study outcomes

Differences between the baseline and end scores for parameters of interest within groups are shown in Appendix S3 (A–C). Outcomes are presented for brushing studies. Table 1 summarizes the differences between the V-Sol, EOMW and WC in a descriptive manner.

Table 1 A descriptive analysis of the comparison of an essential-oils mouthwash, alcohol-containing vehicle solution or water control as an adjunct to daily brushing

Treatment	Study #	Plaque scores	Gingival index	Staining scores	Comparison
EOMW	I[19]	o	■	■	V-Sol
	II[16¶]	+	o	?	V-Sol
	III[20]	+	+	o	V-Sol
	IV[17¶‡]	+	o	o	V-Sol
	V[18¶‡]	+	+	–	V-Sol
V-Sol	II[16¶]	o	o	?	WC
	III[20]	o	o	o	WC

+, in favour of treatment; –, Treatment significantly less effective; o, no difference; ■, no data available; ?, not reported/unclear; EOMW, Listerine® mouthwash; V-Sol, alcohol-containing vehicle solution (21.6%–26.9%); WC, water control and ¶professional prophylaxis at baseline, rendering the panellist with zero visible plaque.
‡ See reference list

Meta-analysis

Meta-analyses were performed to compare the effect of the V-Sol and essential oils as adjunct to self-performed daily oral hygiene. A summary is shown in Table 2A, B. The 6-month brushing studies that evaluated the plaque scores at the end of the trial

[Quigley & Hein[44] modified by Turesky[45]] showed a significant effect in favour of the EOMW when compared to the V-Sol, with a difference in means of 0.39 [95% CI = (0.30; 0.47), P < 0.00001]. There was also a significant effect in favour of the EOMW for gingivitis reduction according to the Löe and Silness Index,[46] with a DIFFM of 0.36 [95% CI = (0.26; 0.62), P = 0.0001], and the Modified Gingival Index,[47] with a DIFFM of 0.17 [95% CI = (0.08; 0.25), P < 0.001]. Regarding extrinsic tooth staining Lobene extrinsic tooth stain index [48], a small but significant difference, with a DIFFM of -0.08 [95% CI = (-0.16; -0.01), P = 0.03], was shown between the rinses. When the V-Sol was compared to WC, no significant difference was found for either the plaque scores or the gingivitis reduction, DIFFM = 0.04 [95% CI = (-0.09; 0.18), P = 0.51] and DIFFM = 0.03 [95% CI = (-0.06; 0.13), P = 0.51], respectively. The majority of meta-analysis showed non-important heterogeneity (I^2-value: 0–9%).

Table 2 Meta-analysis: A comparison of essential-oils mouthwash, alcohol-containing vehicle solution or water control as an adjunct to daily brushing. (A) An alcohol-containing vehicle solution compared with an essential-oils mouthwash as adjunct in brushing studies (random or fixed effect where appropriate) and (B) alcohol-containing vehicle solution compared with a water control

(A)					Test for Overall Effect		Test for Heterogeneity	
Model	Index	Study #		DIFFM	95% CI	*P-value*	I^2 value	*P-value*
				(random effect)				
6-month brushing	Quigley and Hein [44] modified by Turesky[45]	II[16] III[20] IV[17‡] V[18‡]	End	0.39	(0.30; 0.47)	*<0.00001*	0%	*0.41*
				(fixed effect)				
	Gingival index Löe and Silness[46]	II[16] III[20]	End	0.36	(0.26; 0.62)	*0.00001*	92%	*0.0004*
	Lobene modified gingival index[47]	IV[17‡] V[18‡]	End	0.17	(0.08; 0.25)	*<0.0001*	0%	*0.92*
	Lobene extrinsic tooth stain index [48]	III[20] IV[17‡] V[18‡]	Base	0.02	(-0.06; 0.10)	*0.65*	0%	*0.7*
			End	-0.08	(-0.16; -0.01)	*0.03*	9%	*0.33*
(B)					Test for Overall Effect		Test for Heterogeneity	
Model	Index	Study #		DIFFM	95% CI	*P-value*	I^2 value	*P-value*
				(fixed effect)				
6-month brushing	Quigley and Hein [44] modified by Turesky[45]	II[16] III[20]	End	0.04	(-0.09; 0.18)	*0.51*	0%	*0.94*
	Gingival index Löe and Silness[46]	II[16] III[20]	End	0.03	(-0.06; 0.13)	*0.51*	0%	*0.46*

DIFFM, difference of means; CI, Confidence Interval. ‡ See reference list

Grading the body of evidence

Table 3 shows a summary of the various aspects that were used to rate the quality of the evidence and the strength of the recommendations according to GRADE.[22] The

study results were generalizable. The strength of the recommendation appeared to be dependent on the consistency of the outcome parameter. Consequently, the plaque score data were 'strong', the evidence for gingivitis was 'moderate', while the evidence of the side effect of tooth staining was 'weak'.

Table 3 GRADE evidence profile assessing the strength of the recommendation that an alcohol vehicle control is different from an essential-oils mouthwash

GRADE	Plaque score	Gingivitis index	Stain index
Risk of bias	Low	Low	Low
Consistency	Consistent	Moderately consistent	Inconsistent
Directness	Generalizable	Generalizable	Generalizable
Precision	Precise	Precise	Precise
Publication bias	Possible	Possible	Possible
Strength recommendation	**Strong**	**Moderate**	**Strong**

Discussion

The available evidence emerging from this review shows that the reduction in plaque and gingivitis between the V-Sol and WC was not significantly different, whereas the vehicle solution was significantly less effective when compared to the EOMW. These findings suggest that active agents in the EOMW formulation effectively contribute to plaque reduction and gingival inflammation. Subsequently, the antiseptic effect of the hydro-alcohol solution seems negligible. Therefore, the essential oils should be considered as the active ingredient.[49,50] The apparent ineffectiveness of the hydro-alcohol solution as an antiplaque agent and inhibitor of dental plaque bacteria conflicts with the well-established high sensitivity of bacteria to hydro-alcohol and its use as an effective preservative at 10–12%.[50] One possible explanation for this disparity is that bacterial biofilms have greater resistance than dispersed bacteria.[49–52]

The clinical evaluation of the mouthrinses included one 3-week brushing study and four studies with a duration of 6 months. The brushing model was only used to evaluate the plaque scores of these products. The limitation the 3-week brushing study[53] is that the long-term efficacy of the product, which would more accurately reflect the patient's actual use of the mouthrinse, cannot be evaluated. Subsequently, 6-month brushing studies have been used to evaluate the efficacy of mouthrinses,[9,54] as required according to the guidelines of the American Dental Association (ADA).[55]

This systematic review included only papers that provided data concerning the mouthwash when used as an adjunct to self-performed oral hygiene. Given that mouthrinses can be used and prescribed for short duration, data about their efficacy over shorter periods are of interest.[58] Consequently, studies with an evaluation period of <4 weeks were

included for the evaluation of plaque scores in this review. However, in concordance with ADA requirements, it was not intended to extract gingival inflammation data from short-term studies (<4 weeks). Concerning adjunctive devices for controlling plaque and gingivitis, the ADA requires an evaluation period of ≥4 weeks.[59] Therefore, selected studies with the duration of 4 weeks or more were considered for extraction of both plaque and gingivitis data. All but one included study had the duration of 6 months, while Preus *et al.*[19] evaluated the product over 3 weeks.

Regarding heterogeneity, the composition of the used controls needs to be considered. Testing the efficacy of the original essential products should be carried out against its alcohol vehicle as the negative control. The alcohol content of the VC solution in four studies (II, III, IV and V) was identical to the commercial product with a hydro-alcohol concentration of 26.9%. The exception was Preus *et al.*[19] in which the hydroalcohol solution was made from 96% ethanol diluted with water to a final concentration of 22%. Table 1 shows that in all but the study by Preus *et al.*,[19] the essential-oil product was significantly more effective regarding plaque scores than the vehicle control. An explanation for this disparate finding could be due to the model, the limited evaluation time of 3 weeks and/or the composition of the hydro-alcohol control solution.

As incremental data were sparsely presented and in some instances lacking (SDs), the best way to perform a meta-analysis was to use baseline and end-trail data separately. Where appropriate and feasible, separate meta-analyses were performed.

Limitations

- A rigorous search was conducted across various electronic databases. Titles and abstracts were screened by two reviewers in an effort to locate all relevant papers. Despite these efforts, some papers may have been missed. The search eventually yielded five studies with a high quality of evidence.
- The formal testing for publication bias proposed by Egger *et al.*[56] could not be used because fewer than 10 studies were included in the meta-analysis.[15]
- Furthermore, a sensitivity analysis, intended to examine the effects of random sequence generation, allocation concealment and blind outcome assessment on the overall estimates of the effects, could not be conducted because of the limited number of included studies.
- The studies were considered as being double blind. However, some of the participants may have unravelled their group assignment by recognizing the EOMW taste as opposed to those, who rinsed with the V-Sol or WC.[57]
- The number of studies that was included in this systematic review was limited because only those with a true control V-Sol that contained a hydro-alcohol concentration between 21.6 and 26.9% were selected.

- As suggested by the Cochrane handbook, unpublished data were searched. The manufacturer provided two unpublished papers. Including data of unpublished studies can in itself introduce bias. For instance, they may be of lower methodological quality than published studies or may be an unrepresentative sample of all unpublished studies.[15] The methodological quality of the unpublished studies was assessed (Appendix S2), and those studies were considered to have a low estimated risk of bias. Moreover, this systematic review concerned one specific product of one manufacturer. Therefore, underrepresentation is no item of concern.

Conclusion

Limited data, but with a low risk of bias, were available to address the potential benefit of the alcohol-containing V-Sol on plaque and gingivitis scores. 'High'- and 'moderate'-quality data separately were available for the analysis. Within these limitations, EOMW appears to provide a significant oral health benefit during the 6 months of use. The data retrieved for this review suggest that the essential oils have an effect on plaque and gingivitis parameters that extends beyond the V-Sol. Furthermore, the V-Sol proved to be no different from WC.

Practical implications

- This systematic review is applicable for patients with gingivitis
- The alcohol containing vehicle solution of the essential oil mouthwash alone does not contribute to the efficacy in reducing plaque scores and gingivitis when compared to a water control.
- The essential oils themselves effectively contribute to the reduction of plaque and gingival inflammation.

Acknowledgements
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Supporting information

Appendix S1 Overview of the studies processed for data extraction

Study Number and Reference, Evaluation Period and Design	# of Participants at Baseline (end of study), Age in Years (range), Gender and Funding	Inclusion & Exclusion criteria	Groups, regimen and supervision	Authors' own Conclusion
I Preus *et al.* (2013)[19] 3 weeks RCT, Parallel, split-mouth and double-blind	30[A] (30[A]) Mean: 25.0 Range: 19-38 ♂: 19[A] ♀: 26[A] Self funded	Good general health. At least three if the following teeth in each quadrant of the maxilla: The canine, 1st & 2nd bicuspid, 1st molar. No antibiotic or anti-inflammatory drugs and non-smoking. Non-periodontal participants	EOMW V-Sol (22%) Twice daily Not-supervised Brushing (2nd quadrant)	EOMW had no statistically significant effect on plaque formation as compared to its V-Sol.
II Gordon *et al.* (1985)[16] 6 Months RCT, Parallel and double-blind	144 (127[A]) Mean: ? Range: 18-54 ♂: ? ♀: ? Grant from Warner-Lambert Company	Good general health. ≥ 20 natural teeth. Minimal criteria: plaque and gingival index. Grossly carious, fully crowned or restored, and orthodontically bonded teeth were not included in tooth count. No antibiotic or anti-inflammatory drugs. Non-periodontal participants	EOMW V-Sol (26.9%) WC Twice daily Semi-supervised (weekdays) Brushing	EOMW significantly reduced the development of plaque at 1, 3 and 6 months compared to its V-Sol or WC.
III Lamster *et al.* (1983)[20] 6 Months RCT, Parallel and double-blind	145 (129) Mean: 26.9[A] Range: 18-54 ♂: ? ♀: ? Grant from Warner-Lambert company	Good general health. ≥ 20 natural teeth. Minimal criteria: plaque and gingival index. Grossly carious, fully crowned or restored, or orthodontically bonded teeth, abutments and third molars were not included in the tooth count. No antibiotics or anti-inflammatory therapy. Use of oral contraceptives was permitted. Non-periodontal participants	EOMW V-Sol (26.9%) WC Twice daily Semi-supervised (weekdays) Brushing	EOMW when used twice daily for 30 seconds is effective in controlling plaque accumulation and gingivitis in a population that did not receive a prophylaxis.
IV‡ Charles *et al.* (1992)[17] 6 Months RCT, Parallel and double-blind	130[A] (115[A]) Mean: 30.5[A] Range: ? ♂: 30[A] ♀: 85[A] Some authors were employees of the Warner-Lambert Company	Good general health. ≥ 20 natural teeth. Minimal criteria: plaque and gingival index. Grossly carious, fully crowned or restored, or orthodontically bonded teeth, abutment and third molars were not included in tooth count. No antibiotic or anti-inflammatory therapy. Non-periodontal participants	EOMW V-Sol (26.9%) Twice daily Semi-supervised (weekdays) Brushing	The EOWM was not significantly different from the V-Sol for gingivitis at 6 months, calling into question the reliability of these data and suggesting that no definitive conclusions should be based on this review.

Appendix S1 (continued) Overview of the studies processed for data extraction

Study Number and Reference, Evaluation Period and Design	# of Participants at Baseline (end of study), Age in Years (range), Gender and Funding	Inclusion & Exclusion criteria	Groups, regimen and supervision	Authors' own Conclusion
V‡ Hovliaras *et al.* (1993)[18] 6 Months RCT, Parallel and double-blind	156Δ (133Δ) Mean: 31.8Δ Range: ? ♂: 44Δ ♀: 89Δ Some authors were employees of the Warner-Lambert Company	Good general health. ≥ 20 natural teeth. Minimal criteria: plaque and gingival index. Grossly carious, fully crowned or restored, or orthodontically bonded teeth were not included in the tooth count. No antibiotic or anti-inflammatory drugs. Non periodontal participants'	EOMW V-Sol (26.9%) Twice daily Semi-supervised (weekdays) Brushing	EOMW was found to be effective in inhibiting the development of supragingival plaque, gingivitis, and bleeding on probing following a professional prophylaxis compared to the V-Sol.

EOMW, Listerine® mouthwash; V-Sol: alcohol-containing vehicle solution, concentration in parenthesis; WC, water control; ?, not specified/unclear; **Δ**, calculated by the authors

Appendix S2 Methodological quality scores of the included studies

	Quality criteria	Study #				
		I[19]	II[16]	III[20]	IV[17]	V[18]
Internal validity	Random allocation*	+	+	+	+	+
	Allocation concealment	+	?	?	?	?
	Defined inclusion/exclusion criteria*	+	+	+	+	+
	Blinded to patient*	+	+	+	+	+
	Blinded to examiner*	+	+	+	+	+
	Blinding during statistical analysis	?	?	?	?	?
	Balanced experimental groups*	+	+	+	+	+
	Reported loss to follow-up*	+	+	+	+	+
	Number of drop-outs	# 0	# 17Δ	# 16Δ	# 15Δ	# 23Δ
	Percent of drop-outs	0 %	11.81%Δ	11.03%Δ	11.54%Δ	14.74%Δ
	Treatment identical, except for intervention*	+	+	+	+	+
External validity	Representative population group	-	+	+	+	+
	Eligibility criteria defined	+	+	+	+	+
Statistical validity	Independent research	+	-	-	-	-
	Sample size calculation and power	+	+	?	+	+
	Point estimates presented for the primary outcome §	+	+	+	+	+
	Measures of variability presented for the primary outcome	+	+	+	+	+
	Intention-to-treat analysis	+	?	?	?	-
	Authors' estimated risk of bias	Low	Low	Low	Low	Low

As proposed by Van der Weijden,[14] a "+" was given for an informative description of the item at issue for a study design that met the quality standard; when this standard was not met, a "-" was given. For missing or insufficient information, a "?" was denoted, **Δ**: calculated by the authors

Appendix S3a Extracted data of the selected studies by plaque scores

Model	Study #	Index	Treatment/ groups	Baseline	End	Difference	Significant Baseline-end
3 weeks brushing	I[19¶]	Plaque index, 1967[60]	V-Sol	?	0.13 (0.13)	?	?
			EOMW	?	0.05 (0.06)	?	?
6 months brushing	II[16¶]	Quigley & Hein, 1962[44] modified by Turesky, 1970[45]	V-Sol	2.13 (0.34Δ)	2.22 (0.47Δ)	+0.24Δ	?
			EOMW	2.06 (0.33Δ)	1.84 (0.40Δ)	-0.07Δ	?
			WC	2.09 (0.37Δ)	2.27 (0.43Δ)	+0.25Δ	?
	III[20]	Quigley & Hein, 1962[44] modified by Turesky, 1970[45]	V-Sol	?	2.436 (0.441Δ)	-0.136Δ	?
			EOMW	?	1.929 (0.416Δ)	-0.283Δ	?
			WC	?	2.480 (0.433Δ)	-0.171Δ	?
	IV[17¶]	Quigley & Hein, 1962[44] modified by Turesky, 1970[45]	V-Sol	2.43 (0.31Δ)	1.79 (0.54Δ)	-0.64Δ	?
			EOMW	2.40 (0.22Δ)	1.50 (0.52Δ)	-0.90Δ	?
	V[18¶]	Quigley & Hein, 1962[44] modified by Turesky, 1970[45]	V-Sol	2.74 (0.44Δ)	2.39 (0.44Δ)	-0.35Δ	?
			EOMW	2.77 (0.51Δ)	2.03 (0.44Δ)	-0.74Δ	?

EOMW, Listerine® mouthwash; V-Sol: alcohol-containing vehicle solution; WC, water control; ¶ Professional prophylaxis at baseline, rendering the panelist with zero visible plaque, **Δ**: calculated by the authors, and ?: not reported/unclear.

Appendix S3b Extracted data of the selected studies by gingivitis scores

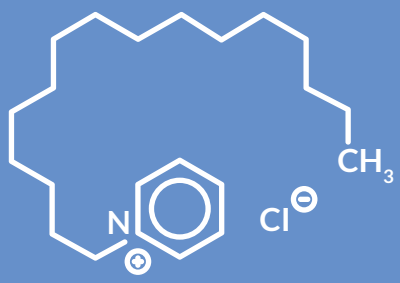
Model	Study #	Index	Treatment/ groups	Baseline	End	Difference	Significant Baseline-end
6 months brushing	II[16 ¶]	Gingival Index Löe & Silness, 1963[46]	V-Sol	1.33 (0.34Δ)	1.37 (0.47Δ)	-0.09Δ	?
			EOMW	1.39 (0.33Δ)	1.31 (0.46Δ)	-0.23Δ	?
			WC	1.38 (0.25)	1.46 (0.37)	-0.12Δ	?
	III[20]	Gingival Index Löe & Silness, 1963[46]	V-Sol	?	1.655 (0.265Δ)	-0.545Δ	?
			EOMW	?	1.197 (0.255Δ)	-0.885Δ	?
			WC	?	1.668 (0.262Δ)	-0.649Δ	?
	IV[17 ¶]	Lobene Modified Gingival Index[47]	V-Sol	2.23 (0.15Δ)	1.40 (0.46Δ)	-0.83Δ	?
			EOMW	2.22 (0.15Δ)	1.24 (0.45Δ)	-0.98Δ	?
	V[18 ¶]	Lobene Modified Gingival Index[47]	V-Sol	2.24 (0.27Δ)	2.03 (0.27Δ)	-0.21Δ	?
			EOMW	2.25 (0.29Δ)	1.86 (0.29Δ)	-0.39Δ	?

EOMW, Listerine® mouthwash; V-Sol, alcohol-containing vehicle solution; WC, water control, ¶ Professional prophylaxis at baseline, rendering the panelist with zero visible plaque; **Δ**: calculated by the authors; and ?, not reported/unclear.

Appendix S3c Extracted data of the selected studies by extrinsic tooth staining

Model	Study #	Index	Treatment/ groups	Baseline	End	Difference	Significant Baseline-end
6 months brushing	II[16¶]	Lobene extrinsic tooth stain index, 1968[48] modified by Lamster, 1983[20]	V-Sol	?	?	?	No
			EOMW	?	?	?	No
			WC	?	?	?	No
	II[20]	Lobene extrinsic tooth stain index, 1968[48]	V-Sol	0.50 (0.522$^{\Delta}$)	0.60 (0.726$^{\Delta}$)	+0.10$^{\Delta}$	?
			EOMW	0.46 (0.376$^{\Delta}$)	0.53 (0.510◊$^{\Delta}$)	+0.07$^{\Delta}$	?
			WC	0.42 (0.485$^{\Delta}$)	0.55 (0.479$^{\Delta}$)	+0.13$^{\Delta}$	?
	IV[17¶]	Lobene extrinsic tooth stain index, 1968[48]	V-Sol	0.19 (0.31$^{\Delta}$)	0.12 (0.15$^{\Delta}$)	-0.07$^{\Delta}$	?
			EOMW	0.16 (0.22$^{\Delta}$)	0.20 (0.29$^{\Delta}$)	+0.04$^{\Delta}$	?
	V[18¶]	Lobene extrinsic tooth stain index, 1968[48]	V-Sol	0.06 (0.27$^{\Delta}$)	0.03 (0.18$^{\Delta}$)	-0.03$^{\Delta}$	?
			EOMW	0.13 (0.81$^{\Delta}$)	0.21 (0.73$^{\Delta}$)	+0.08$^{\Delta}$	?

EOMW, Listerine® mouthwash; V-Sol, alcohol-containing vehicle solution; WC, water control, ¶ Professional prophylaxis at baseline, rendering the panelist with zero visible plaque; **Δ**, calculated by the authors; and ?, not reported/unclear.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CHAPTER 6

Long-term efficacy of a 0.07% cetylpyridinium chloride mouth rinse in relation to plaque and gingivitis:

A 6-month randomized, vehicle-controlled clinical trial

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Abstract

Objective: To evaluate the effectiveness of 0.07% cetylpyridinium chloride (CPC) mouth rinse for reduction of gingival inflammation and inhibition of plaque compared to a vehicle control (VC) mouth rinse over a 6-month period.

Materials and Methods: Participants (n = 62) used their randomly assigned product as adjunct to toothbrushing. Bleeding, plaque and staining scores were assessed at baseline, 3 and 6 months. Plaque and saliva samples were taken at each assessment monitoring possible shifts in the composition of the microbiota.

Results: A significant difference (P = 0.002) in favour of the CPC mouth rinse, with respect to plaque scores, was found. Bleeding scores at 6 months were not significantly different (P = 0.089). However, when correcting for baseline values, a tendency towards a significant difference in bleeding scores at end trail was observed in favour of the CPC mouth rinse (P = 0.061). Regarding staining at 3 and 6 months, a small but significant difference (8.6% and 10.4%, respectively) (P < 0.0001) was observed with lower scores for the VC group. There was a significant reduction in total anaerobic count in the CPC group at 6 months (P < 0.05). The ratio of aerobes/anaerobes was markedly increased at 3 months, especially in the CPC group. No further differences were observed between groups at 6 months.

Conclusions: The use of 0.07% CPC mouth rinse was significantly more effective in reducing plaque scores than the vehicle control. Bleeding scores were not different at 6 months. The test product was well accepted and did not cause any serious clinical side effects or negatively affected the microbiota.

Introduction

Micro-organisms in the oral cavity grow in complex biofilms on hard and soft tissues. Dental plaque, however, is a multispecies biofilm of microorganisms that grows on hard tissues only. The efficient removal of dental plaque is essential for maintaining oral health, as plaque has long been identified as a critical factor in the aetiology of caries, gingival inflammation and chronic periodontitis.[1–3] Toothbrushing is generally accepted as the most efficient oral hygiene method of cleaning one's teeth.

However, a recent systematic review assessing the efficacy in dental plaque removal showed that following a single brushing exercise, the plaque reduction is 42% on average.[4] Patients' efforts, however, are often compromised by the presence of hard-to-reach areas as well as inadequate skill, poor motivation and lack of compliance. Consequently, the use of antimicrobial mouth rinses has been proposed as adjuncts to mechanical oral hygiene regimens and is considered a mean to enhance plaque removal.[5, 6] Mouthrinsing was first described as an oral hygiene measure in Chinese medicine in 2,700 BC.[7]

Cetylpyridinium chloride (CPC) is a cationic quaternary ammonium compound that is a common ingredient in over-the-counter mouth rinses.[7–10] Schroeder *et al.*[8] first described the plaque-inhibiting effect of CPC, which exhibits antimicrobial activity against gram-positive bacteria and has a fungicidal effect, particularly on yeast.[11, 12] Cetylpyridinium chloride binds to the phosphate groups of lipids in cell walls of bacteria. It penetrates the cell and causes membrane damage[13] that leads to leakage of cell components, disruption of bacterial metabolism, inhibition of cell growth and finally cell death.[14–16] Because of its surface-active properties, CPC exerts a prolonged effect in the oral cavity by binding to glycoproteins that cover the teeth and oral mucosa.[17] The use of CPC-containing mouth rinses has shown to be safe and does not disturb the balance of the oral microbiota.[11] A shift in indigenous bacteria from facultative gram-positive streptococci, in particular, to anaerobic gram-negative anaerobic bacteria does not occur.[12]

In a systematic review,[18] CPC-containing mouth rinses were shown to provide a modest but significant additional benefit in reducing plaque and gingival inflammation when used as an adjunct to either supervised or unsupervised oral hygiene measures. A recent 4-day *de novo* plaque accumulation model[19] showed that a 0.05% CPC rinse was able to reduce plaque formation. In another 3-day crossover *'de novo'* plaque accumulation model,[20] 0.07% CPC was found to be more effective than a placebo rinse. According to the guidelines of the American Dental Association (ADA),[21] long-term studies are needed to make claims concerning the effect on gingivitis. The purpose of the present study was therefore to evaluate, over a 6-month period, the effectiveness

of a 0.07% CPC mouth rinse with respect to inhibition of plaque formation and gingival inflammation compared to a vehicle control (VC) mouth rinse and to monitor possible shifts in the composition of the microbiota, adverse effects and tooth staining.

Materials and methods

Ethics

The study followed instructions based on the Helsinki principles. The protocol was approved by the Medical Ethics Committee of the Academic Medical Centre (AMC) of Amsterdam under registration number MEC 09/098 no. 09.17.0873 and registered at the Dutch Trial Register (NTR1855). The study was scheduled and executed from June to December 2009 at the department of periodontology at the Academic Center for Dentistry Amsterdam, the Netherlands, with a minimum of 60 participants. Recruitment of the participants was performed by e-mail and flyers. Before enrolment, further detailed information was provided at the screening visit by the investigator. The voluntary participants were requested to give their written informed consent, asked to fill out a medical questionnaire prior the start of the study and verified for willingness to comply with the objectives of the study.

Participants

In total, 81 systemically healthy participants were recruited being non-dental students from universities and colleges in and near Amsterdam. Inclusion criteria were ≥18 years of age with at least 20 teeth (minimum of five evaluable teeth per quadrant) and moderate gingivitis with ≥40% bleeding on marginal probing (BOMP).[22, 23] Exclusion criteria were open caries, pockets of 4–5 mm in combination with gingival recession or pockets of ≥6 mm as assessed according the Dutch Periodontal Screening Index (DPSI) scores 3+ and 4.[24, 25] In addition, orthodontic appliances or removable (partial) dentures, a history of allergic reaction to erythrosine and/or CPC, use of antibiotics in the preceding 3 months, pregnancy and any adverse medical history or long-term medication might interfere with the response variables. In addition, the eligible participants did not use a mouth rinse as part of their daily oral hygiene procedure.

Study design

This was a 6-month, randomized, parallel, double-blinded, placebo-controlled study (see Figure 1). At baseline, participants were assessed for microbiological and clinical parameters. Subsequently, the dentition was stained for plaque with a suitable dye, for example 0.5% erythrosine disclosing solution (ACTA, Amsterdam, The Netherlands),

and the participants received professional oral prophylaxis for a maximum of 30 min performed by experienced dental hygienists. Teeth were scaled and polished to be free of plaque, stain and calculus in order to give the participants an identical start as described by Slot *et al.*[26]

All teeth in two randomly selected contra-lateral quadrants (one upper and one lower quadrant) were clinically examined except for the third molars.[27] Randomization for group and quadrant selection was performed using true random numbers, which were generated by sampling and by processing a source of entropy outside the computer. The source was atmospheric noise, which was sampled and fed into a computer without any buffering mechanisms in the operating system (www.random.org). Allocation concealment was accomplished using the sequentially numbered, opaque, sealed envelopes (SNOSE) method.[28] The opposing contra-lateral quadrants were used for microbiological sampling. Mouth rinses were identically packed and could only be identified by corresponding subject numbers. Subsequently, every subject received a unique trial number and was randomly assigned to either the CPC group or VC group.

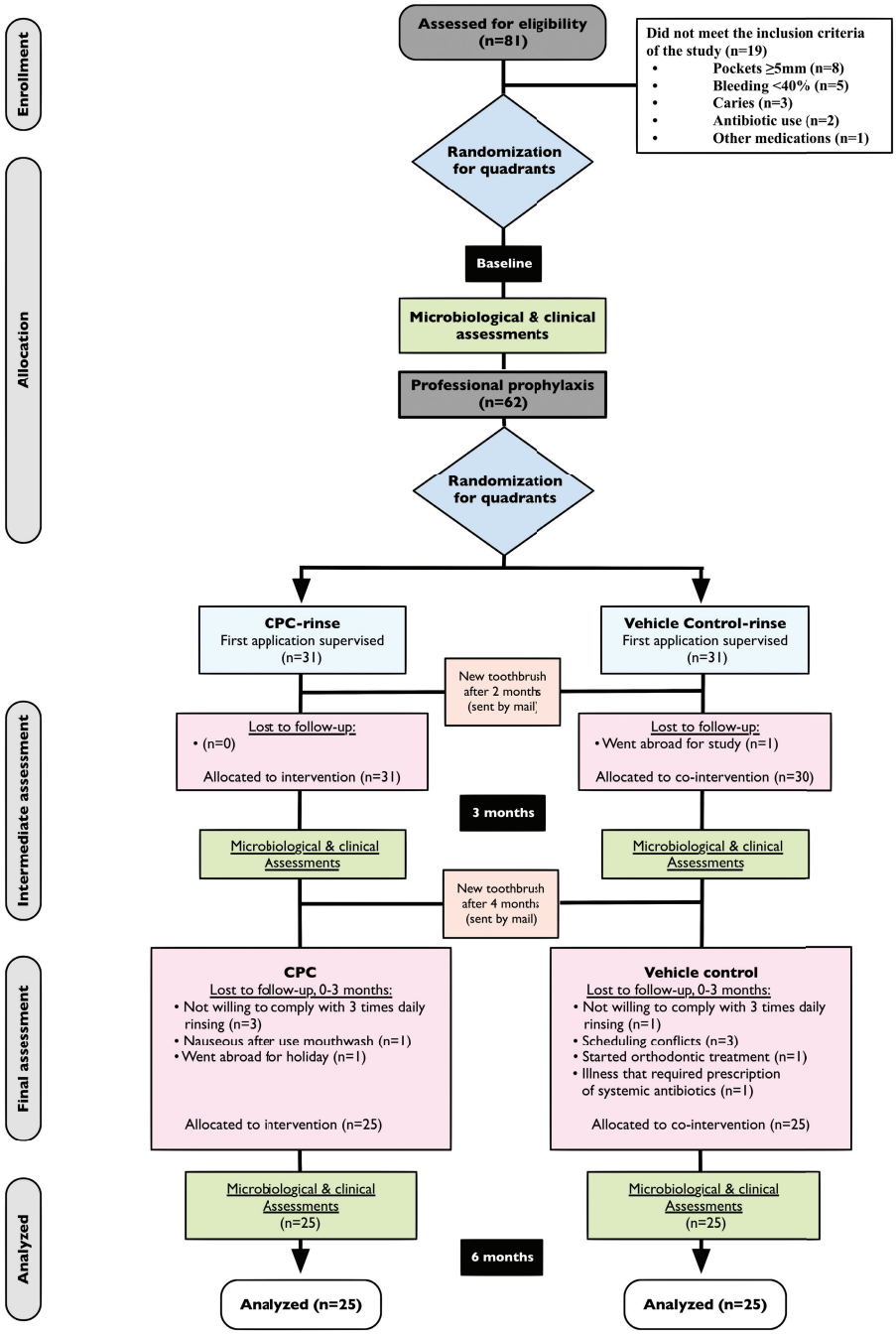

Figure 1 Flowchart depicting subject enrolment and measurements

Study products and regimen

The test product was an experimental 0.07% CPC mouth rinse, and the VC mouth rinse was identical to the test product, however, without the 0.07% cetylpyridinium chloride. The bioavailability of this 0.07% CPC product, according to disc retention assay, was approximately 100%.[29, 30] The test and control rinses were identically packed by Dentaid (Cerdanyola, Barcelona, Spain), with the same colour and could only be identified by the corresponding participant numbers. The randomization key was held by the principal investigator and the sponsor and was not available to the participants and the examiner. All participants received their assigned products immediately after the professional prophylaxis as well as a demonstration and verbal instruction by the study coordinator (CEB). The participants were then asked to rinse under supervision for the first time with their allocated product. In addition, detailed instruction form was provided that explained how to use of the products. Participants first brushed with a standard toothbrush (VITIS Encias, Dentaid®) and one brush length of dentifrice [Aquafresh (GlaxoSmithKline, Zeist, The Netherlands) containing sodium fluoride without additional chemical plaque inhibitors]. Furthermore, participants were instructed to brush three times daily for 2 min after followed by rinsing with their assigned mouth rinse (15 ml) for 30 s with as recommended by the manufacturer after breakfast, after lunch and before bedtime. Mouth rinse, toothbrushes and dentifrice were supplied throughout the study, to last up till the next appointment. To check for compliance, all bottles were weighed before the products were distributed to the participants; they were re-weighed when they were returned.

Clinical parameters

After baseline measurements, participants returned after 3 and 6 months. Subjects were instructed to brush between 2 and 3 h prior to each appointment to avoid the risk of increased bleeding as a result of tooth brushing.[31, 32] All partial mouth examinations were performed in two randomly chosen contra-lateral quadrants.[27] The same experienced examiner (PAV) recorded scores using the same conditions, in the following order. As the primary outcome variable, gingival condition was assessed at 6 sites around the selected teeth (Mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and distolingual) by scoring BOMP on a scale of 0–2.[22, 23] As the secondary outcome, plaque was assessed at six sites after disclosing with (Mira-2-Ton®; Hager & Werken GmbH & Co. KG., Duisburg, Germany) and based on a modified Quigley & Hein[33] plaque index as described by Paraskevas *et al.*[34] on a scale of 0–5. Tooth stain was scored for all selected teeth at four sites from the buccal aspect according to the Gründemann Modification of the Stain Index (GMSI) on a scale of 0–3.[35, 36]

Microbiological parameters

To monitor the composition of supragingival plaque during the experimental period, qualitative and quantitative analyses of the dental plaque were performed. As suggested by Heijnsbroek *et al.*,[37] before the clinical assessments, at 3 months and at 6 months, supragingival plaque was collected from the buccal sites of the first and second (pre)molars from both the upper and lower jaws in contra-lateral quadrants, which were the opposing areas in relation to those used for clinical assessment. Plaque from the preselected sites was dried with compressed air before a sample was obtained using a sterile Teflon Ash (Neos 425/5; KerrHawe, Bioggio, Switzerland). Plaque samples were pooled and transferred to a vial containing 2 ml of sterile reduced transport fluid (RTF).[38] In addition, a 1-ml sample of unstimulated saliva was obtained and mixed with 1 ml of sterile RTF. All samples were kept at 4°C until transport to the laboratory, where they were vortexed for 60 sec and prepared in 10-fold dilutions in sterile saline. Aliquots of 100 µl were transferred to selective and non-selective plates, where they were spread equally and incubated. For total aerobic counts, non-selective blood agar plates (Oxoid no. 2, Basingstoke, UK) were incubated at 37°C under 5% CO_2 for 5 days; for total anaerobic counts, non-selective blood agar plates (Oxoid) supplemented with hemin (5 mg 1^{-1}) and menadione (1 mg l^{-1}) were incubated at 37°C under 80% N_2, 10% CO_2 and 10% H_2 in the presence of regenerated palladium catalyst for up to 14 days. Selective plates for total streptococci (Mitis salivarius aga; BBL, Cockeysville, MD, USA) and lactobacilli species (Rogosa agar; Oxoid) were incubated anaerobically for 5 days. *Candida* spp. was isolated on Sabouraud agar (BBL). Confirmation of the identity of streptococci, lactobacilli and *Candida* spp. was performed using Gram staining.

Questionnaire

At the 6-month assessment, after completion of the microbiological and clinical assessments, all participants were asked to complete a questionnaire designed to evaluate their attitudes towards the assigned mouth rinses. To assess these subjective items, each participant marked the severity of each symptom on a 10-cm-long visual analogue scale (VAS) with the negative and the positive on the left and right.

Sample size

The American Dental Association (ADA) Acceptance Program Guidelines: Chemotherapeutic Products for Control of Gingivitis[21] does not propose a minimum number of participants, but just states that a sufficient number of participants should be enroled. Therefore, the sample size was calculated a priori [PS: Power and Sample Size program[39]] based on a pooled standard deviation (σ) of 0.3 [as taken from plaque scores in a previous 6-month mouth rinse study by Paraskevas *et al.*[40]], as well as a

detectable difference (δ) of 0.25 (between groups) with an α = 0.05 to obtain 80% power. This calculation indicated that 24 subjects in each group would be sufficient. The study was initiated with 31 participants in each group to allow for dropouts.

Data analysis

For each participant, the mean values for each group were calculated. Subject-based data of the CPC and VC groups were compared with regard to plaque and bleeding indices using a univariate analysis, with measurements at 6 months as dependent variables and baseline scores as covariate.[41] For posttest and explorative analysis, nonparametric tests were used. The Wilcoxon test was used to test for differences within each regimen over time, whereas the Mann–Whitney *U* test was used for evaluation between regimens. For GMSI, overall scores were tested using Kruskal–Wallis tests to compare scores between regimens at each assessment. A *t*-test was used to evaluate the VAS scores of the questionnaire data. Chisquared and binomial tests were used for questions concerning binomial choices. Values of $P < 0.05$ were defined as statistically significant. Microbiological outcomes were compared between groups using a Friedman test (nonparametric repeated-measures analysis).

Based on total colony-forming units (CFU) values for anaerobic and aerobic micro-organisms, a ratio was calculated with total anaerobic CFU in the denominator to indicate the proportion of aerobic bacteria. Reduction of anaerobes was considered a beneficial change. All data were analysed 'intention-to-treat'. It involved data of all randomly assigned participants who provided a full data set.[42]

Results

In total, 81 participants were screened, of which 62 participants were enrolled into the study. Twelve participants did not complete the 6-month protocol for various reasons (for further details, see Figure 1). Baseline demographics were comparable, as shown in Table 1.

Table 1 Study subject demographics by group

	CPC group	VC group	*P-value*
n	25	25	-
Male ♂	5	7	*P =0.508**
Female ♀	20	18	
Mean age in years (SD)	22.5 (3.20)	21.1 (2.32)	*P = 0.083***
Age range	19–30	18–27	–

CPC, cetylpyridinium chloride; VC, vehicle control; SD, standard deviation.
* Chi-square analysis. ¨Independent *t*-test analysis.

Clinical results

For plaque, the mean baseline scores were 1.58 for the CPC group and 1.77 for the VC group (P = 0.082). At 3 months, the scores were 1.55 for the CPC group and 1.95 for the VC group (P = 0.002). At 6 months, the scores were 1.28 for the CPC group and 1.68 for the VC group (P = 0.001). A univariate analysis, with the baseline as the covariate and 6-month scores as dependent variables, showed a difference (P = 0.002) between groups in favour of the CPC mouth rinse with respect to plaque scores (Table 2a).

With respect to gingivitis, baseline scores were 1.14 for the CPC group and 1.16 for the VC group (P = 0.854). At 6 months, the scores were 1.03 for the CPC group and 1.14 for the VC group (P = 0.089). Univariate analysis with the baseline as the covariate and 6-month scores as dependent variables showed no significant differences between groups (Table 2b). The mean percentage of sites showing staining at baseline was 2.6% for the CPC group and 3.4% for the VC group (P = 0.958).

With regard to staining, both groups after having received a professional prophylaxis started the study with equally clean teeth. At 3-month staining, this was 10.1% for the CPC group and 1.5% for the VC group (P = 0.0001), and at 6 months, it was 13.3% for the CPC group and 2.3% for the VC group (P = 0.0001, Table 2c). In case of toothstaining, the intensity of the stain was primarily score I (Table 2d).

Microbiological results

As presented in Table 3, the mean total aerobic and anaerobic counts were affected by the CPC and VC mouth rinses; there was a 2- to 3-fold significant decrease in the total anaerobic count in the CPC group (P < 0.05), but not in the VC group at 6 months relative to baseline counts. A significant increase in both the CPC (P < 0.05) and VC (P < 0.001) groups for total aerobic counts was noted at 3 months, although these differences were not observed at 6 months (Table 3). The ratio between the total cultivable aerobic and anaerobic counts changed in both the CPC and VC participants, although at 3 months the increase was more pronounced in the CPC group (5.3 compared to 3.4). At 6 months, both ratios were comparable and still higher (4.3) than baseline values. Mean levels of total streptococci did not change during the 6-month period in the CPC group. In the VC group, a slight increase in total streptococci was observed at 3 months and at 6 months relative to the baseline counts. Among the VC participants, mean levels of lactobacilli decreased during the experimental period within ±1 log, whereas the prevalence of lactobacillipositive participants decreased from 76% at baseline to 60% at 6 months. No significant changes in lactobacilli counts were observed in the CPC group during the 6-month period. The number of participants positive for *Candida* spp. decreased in both groups, whereas absolute counts in culture-positive participants decreased slightly in the CPC group (P < 0.05). No significant changes in *Candida* spp. counts were noted in the saliva from both groups.

Table 2 (a) Mean (SD) plaque Quigley & Hein scores (Q&H) values and percentage (SD) plaque site for both the CPC (N=25) and VC (N=25) groups at the three assessments
(b) Mean (SD) Bleeding on Marginal Probing (BOMP) values and percentage (SD) bleeding sites for both the CPC and VC groups at the three assessments
(c) Mean (SD) percentages of sites showing staining according to the Gründemann Modification of the Stain Index (GMSI) for both the CPC and VC groups at the three assessments
(d) Description of the Lobene tooth stain intensity scale in percentage of sites for both the CPC and VC groups at the three assessments

(a)	**Plaque** (Q&H)		Baseline	3-months	6-months	*P-value***
	CPC group	Mean scores	1.58 (0.39)	1.55 (0.45)	1.28 (0.37)	
		Percentage scores	72.2 (12.7)	68.2 (13.8)	63.0 (13.5)	
						0.002
	VC group	Mean scores	1.77 (0.40)	1.95 (0.38)	1.68 (0.37)	
		Percentage scores	78.1 (11.6)	81.1 (10.6)	75.3 (12.1)	
Mean difference			0.19 (0.54)	0.40 (0.41)	0.40 (0.42)	
P-value*			0.082	0.002	0.001	
(b)	**Gingivitis** (BOMP)		Baseline	3-months	6-months	*P-value***
	CPC group	Mean scores	1.14 (0.31)	1.20 (0.20)	1.03 (0.26)	
		Percentage scores	64.3 (15.5)	65.9 (9.5)	58.9 (14.2)	
						0.061
	VC group	Mean scores	1.16 (0.31)	1.12 (0.23)	1.14 (0.29)	
		Percentage scores	64.2 (14.0)	62.5 (10.6)	65.6 (14.2)	
Mean difference			0.02 (0.47)	0.08 (0.31)	0.11 (0.39)	
P-value*			0.854	0.281	0.089	
(c)	**Staining** (GSMI)		Baseline	3-months	6-months	*P-value***
	CPC group	Percentage scores	2.6% (4)	10.1% (9)	13.3% (8)	
						< 0.0001
	VC group	Percentage scores	3.4% (8)	1.5% (2)	2.3% (10)	
Mean difference			0.8% (9)	8.6% (9)	11% (11)	
P-value*			0.958	< 0.0001	< 0.0001	
(d)	**Staining** (GSMI) Lobene intensity		Baseline, %	3-months, %	6-months, %	
	CPC group	Score 0	97.4	89.9	86.7	
		Score 1	2.3	8.1	8.7	
		Score 2	0.3	2.1	4.2	
		Score 3	0	0	0.4	
	VC group	Score 0	96.6	98.5	97.7	
		Score 1	2.5	1.5	2.3	
		Score 2	0.9	0	0.1	
		Score 3	0	0	0	

CPC, Cetylpyridinium chloride; VC: Vehicle Control; *Mann–Whitney *U* test used for post-testing; **univariate analyses with mean baseline data as covariate and 6-month data as dependent variables.

Table 3 Microbiological data and statistical analysis with respect to total CFU per ml (SD) and streptococci, lactobacilli and Candida in particular and prevalence at baseline, 3 and 6 months derived from supragingival plaque and saliva

	Baseline		3-months		6-months		
	Prevalence (%)	Cells/ml (SD)	Prevalence (%)	Cells/ml (SD)	Prevalence (%)	Cells/ml (SD)	*P-value*[*]
Total cfu $Ø_2$							
CPC group	100	$4.7x10^8$ ($8.4x10^8$)	100	$3.6x10^8$ ($2.9x10^8$)	100	$1.5x10^8$ ($1.4x10^8$)	*<0.05*
VC group	100	$3.0x10^8$ ($2.8x10^8$)	100	$6.8x10^8$ ($8.1x10^8$)	100	$1.6x10^8$ ($1.7x10^8$)	*ns*
Total cfu O_2							
CPC group	100	$9.0x10^8$ ($1.4x10^9$)	100	$1.9x10^9$ ($1.9x10^9$)	100	$6.5x10^8$ ($7.3x10^8$)	*ns*
VC group	100	$5.7x10^8$ ($6.0x10^8$)	100	$2.3x10^9$ ($1.8x10^9$)	100	$6.9x10^8$ ($6.2x10^8$)	*ns*
Ratio CPC O_2 / $Ø_2$		1.9		5.3		4.3	
Ratio VC O_2 / $Ø_2$		1.9		3.4		4.3	
Streptococci (cfu)							
CPC group	100	$1.2x10^7$ ($1.6 x10^7$)	100	$2.4x10^7$ ($2.9x10^7$)	100	$4.4x10^7$ ($6.8x10^7$)	*ns*
VC group	100	$9.9x10^6$ ($1.7 x10^7$)	100	$4.1x10^7$ ($6.3x10^7$)	100	$6.5x10^7$ ($8.7x10^7$)	*<0.01*
Lactobaci lli (cfu)							
CPC group	76	$6.9x10^2$ ($1.4x10^3$)	64	$7.3x10^3$ ($1.2x10^4$)	60	$2.1x10^4$ ($5.0x10^4$)	*ns*
VC group	76	$3.4x10^4$ ($8.2x10^4$)	64	$1.2x10^4$ ($2.4x10^4$)	60	$4.2x10^3$ ($1.3x10^4$)	*ns*
Candida							
CPC group	68	$8.8x10^3$ ($1.8x10^4$)	48	$9.0x10^3$ ($1.1x10^4$)	52	$6.0x10^3$ ($8.4x10^3$)	*<0.05*
VC group	68	$8.2x10^3$ ($1.2x10^4$)	40	$2.1x10^4$ ($1.7x10^4$)	40	$1.8x10^4$ ($1.5x10^4$)	*ns*
Saliva							
CPC group	76	$1.1x10^3$ ($1.6x10^3$)	60	$4.7x10^2$ ($5.5x10^2$)	56	$3.7x10^2$ ($3.0x10^2$)	*ns*
VC group	68	$1.0x10^3$ ($1.5x10^3$)	64	$3.8x10^2$ ($5.3x10^2$)	52	$4.2x10^2$ ($6.7x10^2$)	*ns*

CPC, cetylpyridinium chloride (n = 25); VC, vehicle control (n = 25); $Ø_2$, anaerobic; O_2, aerobic; SD, standard deviation; ns, not significant; *Friedman test (nonparametric repeated-measures analysis) baseline-6-months.

Participant attitudes and adverse events

The amount of used mouth rinse was calculated per participant. No significant differences were observed between the CPC and VC groups regarding the amount of mouth rinse used during the first or the second part of the study. However, an analysis of these data revealed that participants' had used less than the prescribed amount of mouth rinse over the 6-month period. Table 4 provides additional details.

Table 5 presents the data with respect to the questionnaire, which was completed by the participants after their 6-month appointment. No significant differences were observed concerning any of the addressed items.

After visit 1, two participants reported staining as an adverse event. After completion of the study when the product allocation was revealed, these participants were shown to have used the CPC rinse. One subject in the CPC group complained about nausea and discontinued participation in the study.[43]

Table 4 Mean amount of mouth rinse product used per participant in ml

	Baseline–3 months	3–6 months	*P-value*[*]
CPC group (n = 25)	3210	3540	*0.043*
VC group (n = 25)	3390	3520	*0.243*
P-value[**]	0.312	0.879	

*paired samples *t*-test; **independent *t*-test.

Table 5 Questionnaire responses for the visual analogue scale (scored from 0 to 10) The mean scores are presented for the CPC group and VC group

Paraphrase	Extremes		Mean scores (SD)		*P-value*[*]
	From	To	CPC group (n=25)	VC group (n=25)	
Sensitive mucosa and/or teeth	Not at all	Very much	2.79 (2.64)	2.60 (2.51)	*0.798*
Burning sensation	Not at all	Very much	2.71 (2.69)	3.00 (2.83)	*0.710*
Experience dry mouth	Not at all	Very much	2.16 (2.37)	2.88 (2.81)	*0.328*
Experience numbness	Not at all	Very much	1.53 (2.00)	1.54 (2.31)	*0.984*
Staining of teeth	Not at all	Very much	1.58 (1.99)	2.67 (3.17)	*0.153*
Taste perception	Very bad	Very good	6.70 (1.44)	5.88 (1.84)	*0.084*
Duration of taste	Very short	Very long	5.38 (1.99)	5.60 (1.73)	*0.667*
Opinion regarding rinsing time	Very short	Very long	5.16 (1.51)	5.03 (1.74)	*0.776*
Alteration of taste	Negatively changed	Positively changed	4.74 (0.81)	4.29 (0.80)	*0.058*

CPC, Cetylpyridinium chloride; VC, Vehicle Control; SD, standard deviation; *independent *t*-test.

Discussion

In the present study, rinsing with the CPC mouth rinse, three times daily, significantly reduced the level of dental plaque scores (by approximately 24%) relative to the VC product at 3 and 6 months. With respect to gingivitis, no significant difference was found between groups at 6 months. However, the overall analysis correcting for baseline scores revealed a trend towards a significant effect in favour of the CPC group (P = 0.061). The magnitude of this effect is limited (0.11 on a 2-point bleeding score). To be clinically important, a substantial change in outcome would be needed. With regard to the negative side effect of staining, a significant difference was observed at 3 and 6 months, with the CPC group displaying more staining, although patients did not complain of this in the questionnaires.

Other studies

These results are similar to those of previous studies. Versteeg *et al.*[20] showed that the 0.07% CPC mouth rinse, which was identical to the present test product, was capable of reducing plaque formation by approximately 47%. Recently, Costa *et al.*[44] showed

a clear beneficial effect of the adjunctive use of the experimental 0.07% mouth rinse when compared with a placebo. Garcia *et al.*[19] tested a lower concentration 0.05% CPC mouth rinse and found 25% plaque inhibition in a *de novo* plaque formation model. However, Rioboo *et al.*[45] evaluated a 0.05% CPC mouth rinse over a 4-week study and failed to establish a difference between the test and control products with respect to gingivitis, although they reported a trend for differences in plaque scores. Haps *et al.*[18] systematically evaluated the effects of CPC-containing mouth rinses when used as adjuncts to either supervised or unsupervised oral hygiene regimens in a systematic review (SR) and showed, based on a meta-analysis, a small but significant additional benefit of CPC in reduction of plaque and gingival index scores.

Compliance

Compliance in the present study was measured by the average amount of mouth rinse used during the 6-month period for both groups. No significant differences between the CPC and VC groups were observed with respect to the amount of mouth rinse used during either the first or second part of the study. Presumably, the participants rinsed 3 times daily only for 50% of the study duration. On the other occasions, participants may have possibly rinsed only twice daily. The cause of this lack of compliance may be the inconvenience associated with for instance bringing the toothbrush, dentifrice and bottle of mouth rinse with them to work for the afternoon oral hygiene procedures. This lack of compliance was also shown in two other studies,[46, 47] which noted that only 30 to 50 per cent of patients were highly compliant with the suggested oral hygiene procedures up to a period as short as 30 days after receiving instructions. Obviously, if patient compliance is lacking, effects of a daily antimicrobial rinse regimen will be suboptimal.[48] The practicability of a mouth rinse should therefore match with a person's long-term compliance, otherwise the value of such a mouth rinse is negligible.

Bleeding scores

The non-significant trend on gingival bleeding scores in the present study is not in support of a recent systematic review,[18] which showed a significant effect of CPC on gingivitis. The reason for this is unclear. The higher CPC concentration found in two[49, 50] of the included experiments in the meta-analysis of the SR[18] used a higher concentration (0.1%) which may have contributed to the enhanced effect, although the participants only rinsed once a day. This resulted in these two studies in a total delivery of 15 mg CPC per day as compared to the intended 31 mg in the present study. Other factors that may explain the differences among the present study and the outcome of the SR are differences in formulations (e.g. presence or absence of alcohol) or the lack of compliance to the three times daily usage (Table 4). Also, as suggested by Addy

et al.,[51] studies attempting to assess the effect of mouth rinses on plaque formation are hampered not only by the number of components in the formulation but also by the mechanical action of the toothbrush. Additionally, varying compliance may have resulted in different outcomes. In general, the results from this study show that CPC rinsing has a clear tendency towards an effect on gingivitis, but this effect is small. This will need a larger study population to provide significance. In addition, the present study only compered the CPC formulation to a vehicle control. When designing another study, a group using a positive or benchmark control should be considered. The present study used clinical surrogate outcome measurements being plaque, bleeding and staining scores. And therefore it is impossible to draw conclusions based on hard outcomes like tooth loss. The question how many more teeth will be maintained, if patients use the rinsing solution three times a day for many years, remains unclear. However this was not the aim of the current study and will need a different methodological approach when a study will be designed for answering this question.

Bioavailability

The FDA subcommittee states that CPC bioavailability is indicative of a product's performance as *'it readily defines the amount of drug available for deposition at the site of action'*.[52] Consequently, the FDA subcommittee recommends CPC bioavailability ranging from 72% to 77%. However, the bioavailability of most CPC formulations has not been properly reported.[19] It has been shown that a possible interaction between the active agents and the excipients within the formulation can influence CPC bioavailability in a specific product. When used immediately after brushing with toothpaste, the activity of the mouth rinse could be inhibited by the toothpaste formulation.[11, 53] Because the positively charged hydrophilic region of CPC is critical for its antimicrobial activity, mouth rinse formulations should not contain ingredients that diminish or compete with the activity of this cationic group. When the formulation is improperly prepared, inactivation of CPC is likely to occur as a result of chemical reactions such as complexing, micelle formation or other sources of deactivation. Therefore, is it recommended that the bioavailability of CPC in each formulation should be determined to minimize such a possibility.[43] For the present study as in a previous study,[20] a CPC rinse with approximately 100% bioavailability (according to Dentaid International, Barcelona, Spain) was used.

Safety and adverse effects

The safety of CPC has been extensively evaluated and confirmed, based on data collected from animal and pharmacokinetic studies, via assessment of adverse events in randomized, placebo-controlled clinical trials[54–58] and from post-market spontaneous

adverse event data reported to the manufacturer and the FDA. In the present study, the participants reported no serious adverse effects, and there was no difference in taste perception between the CPC rinse and its true *placebo*. Compared to the VC, the CPC rinse resulted in a clinically small (10.4%) but significant increase in tooth stain scores (Table 2c); however, this was not an item which came out as a significant difference in the patient perception questionnaire. In fact, patients in the VC group self-reported more tooth staining than patients in the CPC group (Table 5). Staining following the use of CPC mouth rinse is a known side effect according to the systematic review of Haps *et al.*[18]

Microbiological monitoring

The culture technique was used for microbiological analysis in this study to provide an open test system that enables the determination of total aerobic and anaerobic bacterial counts. To compare potential changes in oral microbiota, microbiological parameters were established at baseline before SRP. Subsequently, microbiological assessments were performed at 3 months and after termination of the test period at 6 months.

During the experimental period of 6 months, no negative shifts in microbiota were observed in the dental plaque or saliva. An interesting observation was the increase in the ratio of total aerobic to total anaerobic counts, which occurred for both the CPC mouth rinse and VC mouth rinse. However, this shift was most pronounced in the CPC group at 3 months. Clinically, this is a relevant environmental shift towards a more beneficial microbiota. However, this could be also be due to the SRP preformed after baseline measurements. Still, the CPC seems to have an additional effect during the first 3 months (ratio aerobe/anaerobe, Table 3). This parameter can be interpreted as a determinant of improved plaque quality.[59] According to the regulations of the ADA Acceptance Program Guidelines: Chemotherapeutic Products for Control of Gingivitis,[21] products should be evaluated for both clinical and microbiological parameters. The requirements include qualitative microbial plaque improvement and safety of the product in terms of emergence of opportunistic pathogens. The oral microbiota should be monitored in participants during the study for the development of opportunistic and pathogenic organisms. Evidence must be provided that the oral microbiota has not been adversely affected. Which organisms should be monitored in relation to the safety of a novel mouth rinse product is not specifically defined, however. For this study, *Candida* spp. were selected as indicators of potential overgrowth arising from bacterial inhibition by CPC.

A decrease was observed in the number of *Candida*-positive plaque samples among the CPC participants whereby the difference in the *Candida*-positive plaque between

baseline and 6 months was statically significant ($P < 0.05$), and no candida overgrowth occurred. The decrease in the amounts of candida is interesting, and it might also be clinically relevant. Patients suffering of recurrent candida infections may benefit from CPC mouth rinse, as alternative for prophylactic candida medications. This could be a topic for further research. On the basis of these observations, no apparent changes in microbiology occurred that would indicate increased risk for opportunistic infections. This is in agreement with a 6-month study on the microbiological effects of CPC (0.07%), which showed that the subgingival microbiota was not significantly affected.[60]

A limitation of the performed study is that microbiota and clinical assessments were obtained from contra-lateral quadrants in order not to affect the clinical assessment of plaque by removing it before sampling Therefore, the microbiological data might not correlate directly with the clinical data. However, a study performed by Bentley[27] showed that partial assessments (half-mouth) were similar in magnitude to those derived from full-mouth examinations. Therefore, it seems legitimate to obtain assessments from different sites with the intention not to influence proper sampling and scoring.

Conclusions

The results of this clinical trial showed that the use of a 0.07% CPC mouth rinse was significantly more effective in reducing plaque scores than the use of the VC product. No significant differences between the CPC and the VC groups with respect to bleeding scores were observed at 6 months. The test product was well accepted and did not cause any serious adverse clinical side effects or negatively affected the oral microbiota.

Clinical relevance

Scientific rationale for the study

CPC-containing mouth rinses were shown to provide a modest but significant additional benefit in reducing plaque and gingival inflammation. To assess the effect on parameters of gingivitis, long-term studies were needed. Therefore, the 0.07% CPC mouth rinse was evaluated over a 6-month period.

Principle findings

The 0.07% CPC mouth rinse was significantly more effective in reducing plaque scores than the use of the vehicle control. No serious clinical side effect of the CPC mouth rinse was reported nor it changed the oral microbiota composition.

Practical implications

Rinsing twice daily with a 0.07% CPC mouth rinse delivers therapeutic benefits by inhibiting plaque accumulation. Thereby it decreased the amount of candida in the CPC group, which can be considered as clinically relevance. Patients suffering of recurrent candida infections may benefit from a CPC mouth rinse, as alternative for prophylactic candida medications.

Limitation

Due to the methodological approach and chosen surrogate outcome parameters of the present study, it was impossible to draw conclusions based on hard outcomes like tooth loss.

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CHAPTER 7

General discussion, summary, and conclusions

This thesis has investigated the capacity of manual toothbrushes to remove dental plaque and examine the effectiveness of several mouthwashes for preventing periodontal diseases. It is generally accepted that mechanical approaches such as toothbrushing are essential to control dental biofilms. The toothbrush is the most important and frequently employed tool in everyday dental self-care, and its benefits for oral hygiene and clinical efficacy are undisputed. However, to maintain oral hygiene, daily use and a correct brushing technique are important.[1] The ideal toothbrush design is user-friendly, able to remove plaque effectively, and has no deleterious effect on soft or hard tissues.[2] Aspects such as size, degree of filament stiffness, filament design, durability, and extra features can also play a role in efficacy. Nonetheless, consumers tend to select manual toothbrushes primarily based on nonscientific criteria, such as brand, cost, color, and shape.[3] Manufacturers also use attractive packaging to interest buyers.[4]

No standard recommendation has been provided for the number of times per day consumers should brush their teeth. Based on existing research, it is reasonable to recommend meticulous mechanical removal of plaque by toothbrushing and interdental cleaning aids. At least once every 24 hours appears to be adequate to prevent the onset of gingivitis and the development of interdental caries.[5] The ADA advocates brushing twice per day, for at least two minutes.[6] In addition to daily toothbrushing and interdental oral hygiene aids, the use of mouthwash may be helpful.[5] The consensus holds that mouthwashes with antiplaque agents should be used as an adjunct to mechanical cleaning, rather than as an alternative to oral hygiene.[7]

Toothbrush

The purpose of the study presented in **Chapter 2** is to correlate the wear of a toothbrush with its plaque-score reduction ability. In the study, the toothbrushes had been in use for three months, and participants were instructed to brush their teeth for two minutes, twice daily, according to the Bass-method technique, while using a standard dentifrice containing sodium fluoride. These toothbrushes' degree of wear was scored. Each participant contributed three toothbrushes.

Toothbrush wear varied widely among the participants. The three-month wear status of the three evaluated toothbrushes was strongly correlated, and the wear per individual was fairly consistent. Participants who returned toothbrushes with extreme wear had significantly higher plaque scores than those who returned toothbrushes with no visible wear or light wear. The age of the toothbrush did not appear to be the decisive criterion in plaque removal efficiency. Rather, the level of toothbrush wear was the determining factor with regard to loss of efficacy. Thus, replacement advice should

relate to the stage of wear, rather than toothbrush age. Wear is evaluated through visible signs, recognized as bending, splaying, or matting of brush head filaments.

This finding is partly in conflict with the general recommendation of the ADA because they recommend replacing the toothbrush every 3-4 months. In addition, there is a recommendation to replace the brush sooner if the bristles become frayed.[8] The latter aligns with Rosema *et al.* 2013, who describe critical wear as when *"the outer tufts are splayed and have lost tuft definition,"* the *"inner tufts are becoming less distinct,"* and the *"definition between inner and outer tufts is lost."*[9] Based on their observations they suggest that an image depicting brush head wear should be added to the consumer packaging of toothbrushes to help consumers assess the quality of their toothbrush.[10, 11] However, the value of this would be questionable, as toothbrush packages are usually not stored after opening. Therefore, it is crucial that information on this topic is provided to patients by dental care professionals. Evidence from the late 1970s suggests that the cleaning efficiency of a toothbrush increases slightly after a short period of use.[12] However, this observation may be limited to toothbrushes and bristles produced 40 or more years ago. The post-processing of filaments may have had an impact on the effectiveness. With use, bristle filaments would wear to a round-ended shape, as processed end-rounding was not common at the time. Today, the degree of filament end-rounding among commercially available toothbrushes varies substantially, and the proportion of filaments that are end-rounded is unknown.[3, 13, 14] Nevertheless, the effective life of a toothbrush varies according to user habits, such as frequency and duration of use, brushing force, and brushing technique, which are all factors that affect wear status.[15]

Optimal oral health

Adequate control of dental plaque biofilm is critical for the maintenance of gingival health. It remains unclear whether there are health benefits to professional mechanical plaque removal, oral hygiene instructions, or a combination of the two.[16-20] Repeated oral hygiene instructions appear to have similar benefits to repeated professional mechanical plaque removal.[20] A recent Cochrane systematic review came to the following conclusion: high-certainty evidence indicates that, over a period of 2-3 years, following scale and polish treatments in adults without severe periodontitis is associated with little to no difference in the level of gingivitis, probing depths, and oral health-related quality of life, compared to those who have not received scheduled scale and polish treatments. According to low-certainty evidence, little to no difference is expected over two years with respect to plaque score levels. However, based on high-certainty evidence, routine scaling and polishing does reduce calculus levels compared to no routine scale and polish, over 2-3 years of follow-up. In this respect,

six-monthly treatments reduced calculus more so than 12-monthly treatments. The clinical importance of these reductions is uncertain.[21]

The effectiveness of self-performed mechanical plaque removal by adults with gingivitis using a manual toothbrush was evaluated in relation to the level of plaque and gingivitis. It was observed in this review, based on studies over a six-month period, that the quality of self-performed mechanical plaque removal was not sufficient and there was definite room for improvement. It was concluded that a single oral hygiene instruction during which the use of a mechanical toothbrush is described, with the addition of a single professional "oral prophylaxis," had a significant favorable reducing effect on the level of gingivitis.[17]

Moreover, rinsing with a combination of an oxygenating-agent (OA) and CHX, in addition to mechanical oral hygiene, could improve and/or maintain gingival health over a long period. Clinical studies that have investigated the potential synergistic effect of OA in addition to a CHX mouthwash have suggested that this specific combination can be effectively used to reduce dental plaque scores.[22-24] A systematic review evaluating this combination found moderate evidence that CHX and OA reduce tooth staining without interfering with plaque growth inhibition.[25]

Chapter 3 evaluates the long-term effects of six different oral hygiene interventions on periodontal diseases during a three-week treatment phase and a subsequent 12-month follow-up phase. Participants were assigned to one of six groups: two basic oral hygiene groups (Control I & II), one professional oral hygiene instruction (OHI) group, one professional prophylaxis (PP) group, an OA and CHX rinse group, and a group receiving a combination of all regimens (COMBI-group). Dental plaque, gingival bleeding, and staining assessments were performed at the start of the treatment phase, at baseline, and every three months. The results indicated that a single OHI or a PP had no additional effect on plaque or bleeding scores. During the treatment phase, a significant improvement in dental plaque and gingival bleeding scores was observed in both rinsing groups. Assessments during the 12 months after the treatment phase revealed that the levels increased to a point at which differences were no longer observed between the groups throughout the study. This finding indicated a relatively rapid loss of the dedication required to maintain a high degree of plaque control.[26] In consequence, improvements in gingival health faded over time and returned to their original values.[27] This study design serves as a model with which to investigate oral hygiene products after a treatment phase in which gingival health improves to an optimum level, such that the potential to prevent deterioration is assessed. Applying this model could contribute to cost-effective and time-saving clinical research.

Mouthwashes

To enhance the effect of mechanical approaches to oral hygiene, several chemical antimicrobial agents have been incorporated into oral care products, such as dentifrices and mouthwashes.[28] Mouthwashes are the most frequently tested products. They are generally divided into products that provide cosmetic or therapeutic effects. The cosmetic products have compositions that provide a fresh and invigorating feeling, for instance, while therapeutic mouthwashes contain active ingredients to improve oral health. A mouthwash is an ideal vehicle for antiplaque and anti-gingivitis ingredients to help prevent periodontal diseases.[7, 29, 30] Different types of therapeutic mouthwash can maintain or improve oral health through anti-adhesive properties that prevent the initial step of biofilm formation, antiseptic, or anti-inflammatory properties.[31] The most commonly studied active agent in mouthwash formulation is CHX. With numerous studies in support of its effectiveness, CHX is considered the gold standard chemical antiplaque and anti-gingivitis agent. Its well-established beneficial properties result in that it is commonly used as a positive control in many clinical trials (**Chapter 4**).

Various negative side effects are associated with the use of CHX mouthwash. Staining is the most common complaint, as CHX can lead to a brown or black discoloration of the teeth and of the lingual mucosa. Additionally, an increased formation of supragingival calculus has been reported. Discoloration of some restorative materials and taste disturbances have also been reported.[32] Some evidence suggests that a combination of CHX and an OA, such as hydrogen peroxide, slightly inhibits the staining of teeth and significantly inhibits plaque growth.[25] A recent systematic review evaluated the effect of an anti-discoloration system added to the mouthwash that purportedly reduces staining while maintaining chlorhexidine efficacy.[33] The review concluded that there is moderate evidence that, for non-brushing situations, the prescription of a product containing an anti-discoloration system could reduce the CHX side effect of dental staining. No negative effects on the clinical parameters of plaque, bleeding, and gingival-index-scores were found for anti-discoloration system in brushing and non-brushing studies. Thus, the practical implication is that a product containing an anti-discoloration system reduces a negative side-effect that may consequently improve patient-compliance.[33] The patients should also be made aware that the staining effect is increased when other products also known to cause staining – such as tea, coffee, wine, and cigarettes – are consumed at the same time.[34-36] Moreover, CHX, which by itself tastes bitter, can cause a transient taste disturbance that has been found to reduce the perceived intensity of the taste of salt.[37] A rare reported side effect is parotid swelling,[38] though it has been suggested that this may not only be related to CHX mouthwash. Instead, it may be the consequence of the rinsing action itself, as a similar observation has been described for a hexetidine mouthwash.[38]

Mouthwashes containing EOs were available decades before CHX mouthwashes entered the market. Having fewer side effects, they are thus considered suitable for long-term use. When used as an adjunct to mechanical oral hygiene, EO mouthwashes provide additional benefits with regard to the reduction of plaque and gingivitis, as compared to placebo or control products.[39-41] The systematic review presented in Chapter 4 summarizes and evaluates the available data on the effects of a standardized EO mouthwash formulation, compared to a CHX mouthwash. The parameters evaluated included plaque, gingival inflammation, dental staining, and calculus, in situations where the products are used as an adjunct to self-performed daily oral hygiene procedures or solely as a monotherapy. Striking results were obtained in a 25-day non-brushing clinical trial. It showed that, on teeth that harbored plaque before initiation of 0.12% CHX use, significantly more calculus accumulated than on teeth that were plaque-free at the start of the study.[42] This seeming paradox of a higher accumulation of calculus among CHX users does not correspond with the other property of CHX, which is the reduction of plaque. This is, in effect, the major component of dental calculus and an initiator of calculus formation. In those participants who, prior to the commencement of CHX use, did not have plaque-free tooth surfaces, the presence of plaque on their teeth may have been the cause of an early induction of calculus accumulation.[43] The observation of an increased level of calculus accumulation for CHX users is of interest for future research that evaluates mineralization in the oral cavity. For EO products, local side effects have been reported, such as extrinsic tooth stain and calculus formation. Although this conflicts with the findings of the narrative review by Martin Addy, the magnitude of the observed increase is negligible compared to the increase observed for CHX.[32, 43]

In Chapter 4, the meta-analysis of long-term studies indicates that CHX mouthwash provides significantly better plaque control than EO mouthwash. However, no significant difference with respect to reductions in gingival inflammation was found for the EO and CHX mouthwashes. A correlation between plaque scores and parameters of gingival inflammation is generally accepted, though this is not supported by these results.[44] The most likely explanation for this observation is that CHX acts through an antiplaque effect on the level of gingival inflammation, whereas the effects of EOs occur through an anti-inflammatory process. This presumption aligns with observations that EOs have antimicrobial and anti-inflammatory activities.[45, 46] For most types of mouthwash, a water or a water-alcohol base is used to solubilize various ingredients that are added to the product. Among these are flavoring agents, surfactants, humectants, and active antiplaque and anti-gingivitis ingredients.[46] Alcohol is also added to improve the transport of active ingredients into the dental plaque biofilm and to provide preservative power. Some dental professionals have suggested that the

beneficial effect of an EO mouthwash results from the relatively high concentration of alcohol, ranging from 21.6% to 26.9%. There has also been concern that alcohol in mouthwash is converted to acetaldehyde in the oral cavity, which may result in DNA damage and lead to mutations. Quantitative analysis of mouthwash use and oral malignancy[48] has revealed no statistically significant associations between mouthwash use and an increased risk of oral cancer. Neither was an association found between the use of a mouthwash containing alcohol and oral cancer risk. Despite this, the demand for alcohol-free mouthwashes has increased over recent years.[49] The question then emerges as to whether the inclusion or exclusion of alcohol affects the efficacy of the mouthwash. In a meta-analysis by Serrano and colleagues, 10 studies evaluating EOs included nine mouthwash products that contained alcohol and one that did not.[50] No major mean difference was observed between alcohol mouthwashes and the alcohol-free mouthwash. In another study, however, in which 0.12% and 0.2% CHX were compared, a sub-analysis of 0.12% CHX with and without alcohol compared to 0.2% CHX with alcohol was performed, and the data indicated that the nonalcohol product was slightly less effective than the product that contained alcohol. [50]

Chapter 5 evaluates the effect of the alcohol-containing base of a fixed formula of an EO mouthwash. The vehicle solution was systematically compared with an EO mouthwash and a water-based control on plaque, gingival inflammation parameters, and extrinsic tooth staining. The alcohol-containing vehicle solution of the EO mouthwash alone did not contribute to the efficacy of reducing plaque scores and gingivitis when compared to a water control. The EOs themselves, therefore, effectively contributed to improving oral health. This outcome is in line with two recent clinical trials evaluating EO mouthwash with and without alcohol, which found no significant differences in plaque and gingivitis reduction.[52, 53] Theoretically, the accumulated effects of rinsing with a mouthwash product with a high percentage of alcohol and the ingestion of alcohol can predispose users towards oral or pharyngeal carcinoma. However, the contributory effects of alcohol in mouthwashes are unclear. Most national regulatory organizations – including the US Food and Drug Administration – consider them not proven.[48, 54, 55]

An alternative to EOs is cetylpyridinium chloride (CPC), a basic ingredient in many mouthwashes. This alternative was evaluated in a systematic review that concluded that CPC provides a small but significant additional benefit for reducing plaque accumulation and gingival inflammation.[56] A four-day *de novo* plaque accumulation model was used to evaluate a new formula.[57] The results indicated that the 0.05% CPC rinse reduced plaque formation. In another three-day crossover *de novo* plaque accumulation model, 0.07% CPC was found to be more effective than a placebo rinse.

According to the guidelines of the ADA, long-term studies are needed to make claims concerning the effect on gingivitis.[58, 59]

Chapter 6 evaluates the effectiveness of a 0.07% CPC mouthwash in the reduction of gingival inflammation and inhibition of plaque, compared to a placebo mouthwash over a six-month period. The use of a 0.07% CPC mouth rinse was significantly more effective for reducing plaque scores than the vehicle control. Bleeding scores were not different at six months. The new product was well accepted and did not cause any serious clinical side effects or negatively affect the microbiota.

A recent systematic review evaluated the efficacy of EOs compared to CPC as adjuncts to mechanical plaque control for the reduction of plaque and gingivitis.[60] It concluded that, in patients with gingivitis, EO-containing mouthwashes are more efficacious for the reduction of plaque and gingival inflammation than mechanical plaque control alone, mechanical plaque control in combination with mouthwashes with CPC, or a placebo. The expected benefits may be clinically relevant and possibly observed in the interproximal area.[60] The authors state that mouthwashes containing EOs should be considered the first choice for daily use as adjuvants to self-performed mechanical plaque control. At present, based on the data presented in this thesis, EO mouthwashes can be considered the gold standard for daily home use. However, the strong taste of EOs could be a limitation for some patients.

Principal findings

- Toothbrush age is not the critical parameter for efficient plaque removal. Rather, the wear rate is the determining factor.
- Rinsing for three weeks with chlorhexidine and an oxygenating-agent leads to a significant reduction in plaque and bleeding scores.
- In long-term use, the standardized formula of an essential oil mouthwash is a reliable alternative to chlorhexidine mouthwash, with respect to the parameters of gingival inflammation.
- The alcohol-containing vehicle solution of the essential oil mouthwash alone does not contribute to efficacy in reducing plaque scores. Subsequently, the essential oils themselves effectively contribute to the reduction of plaque and gingival inflammation.
- Rinsing twice daily with a 0.07% cetylpyridinium chloride mouthwash delivers therapeutic benefits by inhibiting plaque accumulation.

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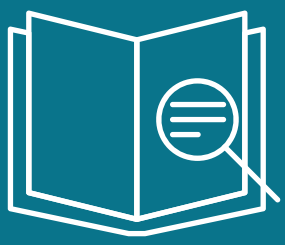


CHAPTER 8

Nederlandse samenvatting voor leken

Tandplaque is een dun, kleverig laagje dat zich gedurende de dag op de tanden en kiezen afzet. Het bestaat hoofdzakelijk uit opgeloste voedselresten, speeksel en micro-organismen, en wordt beschouwd als de belangrijkste veroorzaker van ontstoken tandvlees (gingivitis). Oppervlakkige tandvleesontsteking kan zich uitbreiden naar de diepere gelegen steunweefsels, waarbij ook het bot rondom de tanden en kiezen verloren gaat (parodontitis). Dit proefschrift evalueert diverse aspecten van de dagelijkse mondverzorging en in welke mate deze kunnen bijdragen aan het verminderen van tandplaque en tandvleesontsteking. In een breder perspectief draagt dit proefschrift bij aan het verkrijgen en behouden van een gezonde mond.

Tandenpoetsen is voor velen een dagelijks terugkerende gewoonte die van jongs af aan wordt aangeleerd, met in eerste instantie het oog op het voorkomen van tandbederf. Het algemeen tandheelkundig basisadvies is tweemaal per dag gedurende twee minuten met een fluoride tandpasta poetsen. Het blijkt echter lastig te zijn om met een tandenborstel goed tussen de tanden en kiezen te reinigen. Om deze reden wordt dan ook aangeraden om de tussenruimte te reinigen met speciale hulpmiddelen, zoals flossdraad, tandenstokers, plastic-rubber reinigers en ragers. Echter, dit is een handeling die voor velen lastig is of tijdelijk onmogelijk na een chirurgische ingreep. Het aanvullend gebruik van een mondspoelmiddel op tandenpoetsen kan in bepaalde gevallen een uitkomst zijn.

Als gevolg van het tandenpoetsen met een borstel treedt slijtage op van de borstelharen. Het gebruikelijke advies is daarom om de tandenborstel elke drie maanden te vervangen. In **Hoofdstuk 2** van dit proefschrift wordt gekeken naar het effect van de mate van slijtage van een handtandenborstel op de verwijdering van tandplaque. In een recent gepubliceerde studie met een follow-up van één jaar (zie **Hoofdstuk 3**) voerden alle deelnemers het basismondhygiëne-advies uit. Tijdens elke driemaandelijkse evaluatie werd de tandenborstel vervangen voor een nieuw exemplaar en werd de plaquescore geanalyseerd. Deze werd vervolgens gecorreleerd met slijtage van de tandenborstels. Van 172 deelnemers was er een set van drie maanden oude, gebruikte tandenborstels beschikbaar. Uit analyse bleek een grote variatie in de mate van borstelslijtage tussen de deelnemers, en dat dit per deelnemer wel (vrij) consistent is. Deelnemers die hun tandenborstel met ernstige slijtage inleverden, vertoonden significant hogere plaquescores dan diegene met tandenborstels zonder zichtbare of met een lichte slijtage. De mate van borstelslijtage lijkt daarom van groter belang als reden om een tandenborstel te vervangen dan de gebruiksduur.

Uit een eerder wetenschappelijk onderzoek is gebleken dat het gecombineerd (na elkaar) gebruiken van een waterstofperoxide (H_2O_2) en chloorhexidine (CHX) spoelmiddel naast het dagelijks tandenpoetsen een langdurig positief effect zou hebben op de mondgezondheid. Hier wordt verder op ingegaan in **Hoofdstuk 3**. Een behandelfase van drie weken werd gestart om de gezondheid van het tandvlees te verbeteren. De totale onderzoeksgroep van 276 personen met een goede gezondheid werd volgens het lot in zes groepen verdeeld. Twee controlegroepen kregen geen specifieke behandeling, maar kwamen met verschillende intervallen terug voor controle. Een groep kreeg één professionele poetsinstructie en een groep kreeg één professionele gebitsreiniging. Een andere groep spoelde gedurende drie weken met een combinatie van H_2O_2 en CHX. De zesde groep kreeg een professionele poetsinstructie en een professionele gebitsreiniging en daarbij ook het spoel regime van drie weken. Aan het begin en eind van de drieweekse behandelfase, en na vier, zeven, tien en twaalf maanden werd de hoeveelheid tandplaque, mate van tandvleesontsteking en tandaanslag gescoord. Aan het einde van de drieweekse behandelfase werd alleen een significante vermindering waargenomen van de hoeveelheid tandplaque en tandvleesontsteking voor de twee groepen die spoelden. Voor de andere vier groepen werden geen klinisch relevante veranderingen waargenomen. Bij alle groepen, ongeacht wel of geen succesvolle behandelfase, waren bij de daaropvolgende observatiemetingen de hoeveelheid tandplaque en mate van tandvleesontsteking weer op hetzelfde niveau als aan het begin van de studie. Dit onderzoek laat zien dat een enkele mondhygiëne instructie of een enkele professionele gebitsreiniging geen positief effect heeft op de hoeveelheid tandplaque of de mate van tandvleesontsteking. Daarbij is gebleken dat drie weken spoelen met H_2O_2 en CHX wel degelijk een significant positief effect heeft, maar dit was na vier maanden niet meer waarneembaar.

Ondanks dat CHX een positieve werking heeft op het verminderen van tandplaqueaccumulatie en tandvleesontsteking, zijn er ook nadelen bij gebruik op de lange termijn. De bekendste negatieve bijwerking van CHX is smaakverandering en de bruine of zwarte verkleuring van de tanden en slijmvliezen, met een verhoogde vorming van tandsteen. Hoewel een gecombineerd gebruik met H_2O_2 een positieve bijdrage levert aan het verminderen van de hoeveelheid aanslag, leidt dit niet altijd tot een afdoende resultaat. Lang voordat spoelmiddelen met CHX op de markt kwamen, waren al spoelmiddelen met essentiële oliën (EO) die opgelost zijn in alcohol verkrijgbaar. Deze laatste hebben minder bijwerkingen en zijn daarom wel geschikt als aanvulling op de dagelijkse mondhygiëne. Listerine® is al ruim honderd jaar op de markt. Dit is wellicht het meest bekende spoelmiddel op basis van EO en heeft

een vaste samenstelling. Het feit dat een product langdurig op de markt is en een constante formulering heeft, maakt het uitermate geschikt om wetenschappelijke literatuur met elkaar te vergelijken.

In Hoofdstuk 4 wordt de beschikbare wetenschappelijke literatuur waarin CHX- en EO-spoelmiddelen (met een vaste formule (Listerine®)) met elkaar worden vergeleken samengevat in een systematisch review. In de geneeskunde hebben systematische reviews een belangrijke plaats gekregen bij de klinische besluitvorming en de wens om *evidence based* te werken. Het doel is om zo objectief mogelijk alle beschikbare wetenschappelijk literatuur te gebruiken om tot een weloverwogen wetenschappelijke uitspraak te komen. De tandheelkunde heeft deze trend in de laatste twee decennia opgepakt. Op basis van negentien studies met in totaal 826 proefpersonen kon een uitgebreide analyse worden uitgevoerd. Hieruit is gebleken dat CHX significant effectiever was dan EO ten aanzien van de mate van tandplaque-accumulatie. Niettemin werd in lange-termijnstudies (≥4 weken) geen verschil gevonden in de mate van tandvleesontsteking. Daarmee lijkt een EO-spoelmiddel met een vaste formule (Listerine®) een alternatief voor CHX om tandvleesontsteking te reduceren.

In een spoelmiddel met EO wordt alcohol vaak gebruikt als oplosmiddel en om de houdbaarheid van het product te verbeteren. Gesuggereerd wordt dat niet alleen de EO, maar juist ook de alcohol bijdraagt aan het positieve effect van het spoelmiddel. Dit was de aanleiding tot een tweede systematisch review (Hoofdstuk 5). Het doel hiervan was om het effect van EO te vergelijken met een placebomondspoeling met een identieke hoeveelheid alcohol, temeer het additionele effect van EO op zich te kunnen evalueren. De zoekresultaten leverden vijf geschikte studies op die allen lieten zien dat een placebomondspoeling minder effectief was bij tandplaque-accumulatie dan EO-mondspoeling. In twee van de vier lange-termijnstudies werd eenzelfde observatie waargenomen voor de mate van tandvleesontsteking.

Indien mogelijk werd ook de vergelijking tussen de placebomondspoeling en water meegenomen. Hierbij werd echter geen significant verschil gevonden in zowel de mate van tandplaque-accumulatie als de mate van tandvleesontsteking. Kortom, het verschil tussen de vermindering van tandplaque en tandvleesontsteking bij de placebo en de watercontrole was niet significant, terwijl het placebospoelmiddel significant minder effectief was als dit werd vergeleken met EO. Deze bevindingen ondersteunen de claim dat de EO zelf in Listerine® de effectieve bijdrage leveren aan de reductie van plaque en tandvleesontsteking, en niet de alcohol. De werking van de alcohol lijkt daarmee verwaarloosbaar en derhalve dienen de EO te worden

aangeduid als het werkzame bestanddeel. Bovendien blijken EO-spoelmiddelen een positieve bijdrage te leveren aan de mondgezondheid als deze worden gebruikt als aanvulling op de dagelijkse mondverzorging.

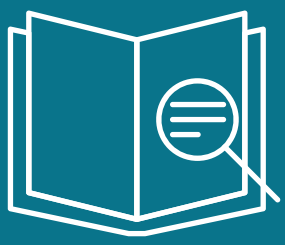
Desondanks kunnen er diverse redenen zijn om een alcoholvrij spoelmiddel te verkiezen boven een spoelmiddel met alcohol, waaronder religie, verslavingsverleden en leeftijd. Cetylpyridiniumchloride (CPC) is dan een ander alternatief. In **Hoofdstuk 6** wordt een alcoholvrij spoelmiddel met 0,07 procent CPC vergeleken met een placebo. In dit onderzoek werd ook het effect op plaquegroei en de remming van tandvleesontsteking onderzocht. In totaal voldeden 62 proefpersonen aan de onderzoekscriteria. De proefpersonen waren willekeurig toegewezen aan een van de twee groepen. Aan het begin van het onderzoek, na drie maanden en na zes maanden werden de hoeveelheid tandplaque en mate van tandvleesontsteking en aanslag gescoord. Om te beoordelen of er geen ongewenste microbiële verschuiving plaatsvond, werden ook plaque- en speekselmonsters afgenomen. Na analyse van de verzamelde data werd een verschil gevonden ten gunste van de CPC-mondspoeling met betrekking tot de mate van tandplaque. Met betrekking tot de mate van tandvleesontsteking werd na zes maanden geen verschil gevonden. Wat betreft de hoeveelheid tandaanslag na drie en zes maanden werd een kleine, maar significante toename gevonden voor het CPC-spoelmiddel ten opzichte van de placebo.

Conclusie

Optimale mondhygiëne is een kritische factor in de preventie van cariës en gingivitis en bij de behandeling van parodontitis. De wetenschappelijke literatuur en de dagelijkse praktijk laten echter zien dat het voor de meeste volwassenen een moeilijke opgave is om hun mond adequaat te reinigen. Motivatie en handigheid spelen hierbij een rol. Met dit in gedachte is het gebruik van een antibacterieel spoelmiddel mogelijk een nuttige aanvulling op de dagelijkse mondverzorging. Mondspoelmiddelen worden aanbevolen als normale mondhygiëne onvoldoende effect sorteert of als mechanische reiniging moeilijk, gecompromitteerd of zelf onmogelijk is.

Belangrijkste bevindingen

- De mate van borstelslijtage in plaats van de gebruiksduur is de bepalende factor een tandenborstel te vervangen.
- Gedurende drie weken spoelen met een combinatie van waterstofperoxide en chloorhexidine geeft een vermindering van tandplaque en tandvleesontsteking.
- Het langdurig spoelen met etherische oliën in een vaste formule lijkt een effectief alternatief voor chloorhexidine om tandvleesontsteking tegen te gaan.
- Een spoelmiddel met louter alcohol draagt niet bij aan de reductie van plaque en tandvleesontsteking.
- Essentiële oliën leveren een effectieve bijdrage aan de reductie van plaque en tandvleesontsteking.
- Tweemaal daags spoelen met een 0,07 procent cetylpyridiniumchloride-mondspoeling vermindert plaque-accumulatie.



CHAPTER 9

Résumé français pour les laïcs

La plaque dentaire est une fine couche collante qui se dépose sur les dents et les molaires pendant la journée. Principalement constituée de résidus alimentaires dissous, de salive et de micro-organismes, cette plaque est considérée comme la principale cause d'inflammation des gencives (gingivite). La gingivite superficielle peut se propager aux tissus de soutien situés plus profondément, ce qui entraîne la perte de l'os autour des dents et des molaires (parodontite). Cette thèse évalue divers aspects des soins bucco-dentaires quotidiens et dans quelle mesure ces soins peuvent contribuer à la réduction de la plaque dentaire et de la gingivite. Dans une perspective plus large, cette thèse contribue à assainir la bouche et à la maintenir saine.

Pour beaucoup de gens, le brossage des dents est un rituel quotidien appris dès le plus jeune âge, dans le but principal de prévenir la carie dentaire. En médecine dentaire, le conseil de base est de vous brosser les dents deux fois par jour pendant deux minutes avec un dentifrice au fluor. Cependant, nettoyer correctement les espaces situés entre les dents et les molaires avec une brosse à dents se révèle une tâche ardue. Pour cette raison, il est recommandé de nettoyer l'espace avec des objets spéciaux tels que du fil dentaire, des cure-dents, des nettoyants en caoutchouc plastique et des brosses. Toutefois, c'est un geste que beaucoup de gens trouvent difficile ou temporairement impossible après une intervention chirurgicale. Dans certains cas, l'utilisation supplémentaire d'un rince-bouche après s'être brossé les dents peut être une solution.

À la suite du brossage des dents avec une brosse, les poils commencent à présenter des traces d'usure. Il est généralement préconisé de remplacer la brosse à dents tous les trois mois. Le **Chapitre 2** de cette thèse explore l'effet du degré d'usure d'une brosse à dents manuelle sur l'élimination de la plaque dentaire. Dans une étude récemment publiée avec un suivi d'un an (voir **Chapitre 3**), tous les participants se sont pliés aux conseils de base en hygiène buccale. Lors de chaque évaluation trimestrielle, la brosse à dents a été remplacée par une nouvelle et l'étendue de la plaque a été analysée. Ce résultat a ensuite été corrélé avec l'usure des brosses à dents. Sur 172 participants, un ensemble de brosses à dents usagées de trois mois était disponible. L'analyse a révélé une grande variation du degré d'usure des brosses entre les participants, cela étant (assez) constant pour tous les participants. Lorsqu'ils ont rendu leur brosse à dents, les participants qui présentaient une usure sévère ont affiché des niveaux de plaque nettement plus élevés que ceux dont la brosse à dents ne présentait qu'une usure légère, voire invisible. Plutôt que la période d'utilisation, le degré d'usure de la brosse semble donc être une raison plus importante justifiant le remplacement d'une brosse à dents.

Des recherches scientifiques antérieures ont montré que l'utilisation combinée (séquentielle) d'un liquide de rinçage au peroxyde d'hydrogène (H_2O_2) et à la chlorhexidine (CHX) en plus du brossage quotidien des dents aurait un effet positif à long terme sur la santé bucco-dentaire. Nous en discuterons plus en détail au **Chapitre 3**. Une phase de traitement de trois semaines a été lancée pour améliorer la santé des gencives. Le groupe de recherche total, composé de 276 personnes en bonne santé, a été divisé en six groupes selon le lot. Deux groupes témoins n'ont reçu aucun traitement spécifique, mais sont revenus à différents intervalles afin de subir un contrôle. Un groupe a reçu des instructions de brossage professionnel et un groupe a bénéficié d'un nettoyage dentaire professionnel. Un autre groupe a été invité à rincer la bouche pendant trois semaines avec une combinaison de H_2O_2 et de CHX. Le sixième groupe a reçu des instructions de brossage professionnel et un nettoyage professionnel des dents ainsi qu'un programme de rinçage étalé sur trois semaines. Au début et à la fin de la phase de traitement de trois semaines, et après quatre, sept, dix et douze mois, la quantité de plaque dentaire, le degré de gingivite et le tartre ont été évalués. À la fin de la phase de traitement de trois semaines, n'a été observée qu'une réduction significative de la quantité de plaque dentaire et de gingivite auprès des deux groupes qui se sont rincés les dents. Aucun changement cliniquement significatif n'a été observé chez les quatre autres groupes. Dans tous les groupes, indépendamment de la réussite ou non de la phase de traitement, la quantité de plaque dentaire et le degré de gingivite étaient revenus au même niveau qu'au début de l'étude dans les mesures d'observation suivantes. Cette recherche montre qu'une seule instruction d'hygiène buccale ou un seul nettoyage dentaire professionnel n'a aucun effet positif sur la quantité de plaque dentaire ou le degré de gingivite. Il a été constaté que trois semaines de rinçage avec du H_2O_2 et CHX avaient permis un effet positif significatif, mais cet effet n'était plus visible au bout de quatre mois.

Malgré le fait que le CHX ait un effet positif sur la réduction de l'accumulation de plaque dentaire et des maladies des gencives, l'utilisation à long terme comporte également des inconvénients. Parmi les effets secondaires négatifs du CHX, le plus connu est le changement de goût et la décoloration brune ou noire des dents et des muqueuses, avec une augmentation de la formation de tartre. Bien que l'utilisation combinée avec H_2O_2 contribue positivement à réduire la quantité de plaque, cela ne permet pas toujours d'obtenir un résultat satisfaisant. Bien avant la commercialisation des agents de rinçage au CHX, des agents de rinçage aux huiles essentielles (HE) dissous dans l'alcool étaient déjà disponibles. Ayant moins d'effets secondaires, ils conviennent donc comme complément à l'hygiène buccale quotidienne. Listerine® est sur le marché depuis plus de cent ans. Il s'agit peut-être de l'agent de rinçage à base d'huiles essentielles le

plus connu et sa composition est fixe. Le fait qu'un produit soit commercialisé depuis longtemps et ait une formulation constante le rend extrêmement approprié à des comparaisons de littératures scientifiques entre elles.

Au Chapitre 4, nous résumons dans une revue systématique la littérature scientifique disponible comparant les agents de rinçage CHX et HE (avec une formule fixe (Listerine®)). En médecine, les revues systématiques occupent une place importante dans la prise de décision clinique et le désir *de* travailler de manière factuelle. L'objectif est d'utiliser toute la documentation scientifique disponible de la manière la plus objective possible pour parvenir à une déclaration scientifique mûrement réfléchie. La dentisterie a repris cette tendance au cours des vingt dernières années. Sur la base de dix-neuf études portant sur un total de 826 sujets, une analyse complète a pu être réalisée. Cette analyse a montré que le CHX était significativement plus efficace que l'HE en ce qui concerne l'étendue de l'accumulation de plaque dentaire. Néanmoins, dans les études à long terme (≥4 semaines), aucune différence n'a été constatée dans le degré de maladie des gencives. Dès lors, un produit de rinçage HE avec une formule fixe (Listerine®) constitue une alternative au CHX pour réduire les gingivites.

Dans un agent de rinçage avec HE, l'alcool est souvent utilisé comme solvant, ceci dans le but d'améliorer la durée de conservation du produit. Cela laisse entendre que non seulement l'HE, mais aussi l'alcool contribuent à l'effet positif de l'agent de rinçage. C'était la raison d'une deuxième revue systématique (Chapitre 5). Le but était de comparer l'effet de l'HE d'un bain de bouche placebo avec une quantité identique d'alcool, d'autant plus pour pouvoir évaluer l'effet supplémentaire de l'HE sur lui-même. Les résultats de la recherche ont été suivis de cinq études appropriées, qui ont toutes montré qu'un bain de bouche placebo était moins efficace dans l'accumulation de plaque dentaire qu'un bain de bouche HE. Dans deux des quatre études à long terme, la même observation a été faite pour le degré de maladie des gencives.

Dans la mesure du possible, la comparaison entre le bain de bouche placebo et l'eau a également été prise en compte. Néanmoins, aucune différence significative n'a été constatée dans le degré d'accumulation de plaque dentaire et le degré de maladie des gencives. Pour résumer, la différence entre la réduction de plaque dentaire et l'inflammation des gencives avec le placebo et le contrôle de l'eau n'était pas significative, tandis que le rinçage placebo était significativement moins efficace par rapport à l'HE. Ces résultats viennent étayer l'affirmation selon laquelle l'HE lui-même dans Listerine® contribue efficacement à la réduction de la plaque et des inflammations des gencives, et non l'alcool. L'action de l'alcool semble donc négligeable et il convient

donc de constater que l'HE est le composant actif. De plus, les agents de rinçage à base d'HE semblent apporter une contribution positive à la santé bucco-dentaire lorsqu'ils sont utilisés en complément des soins bucco-dentaires quotidiens.

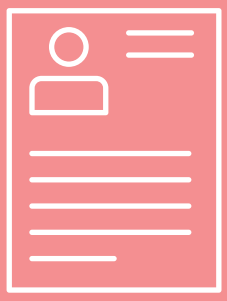
Néanmoins, diverses raisons peuvent expliquer le choix d'un liquide de rinçage sans alcool plutôt qu'un liquide de rinçage à l'alcool, notamment la religion, les antécédents de dépendance et l'âge. Le chlorure de cétylpyridinium (CPC) est alors une autre alternative. Au **Chapitre 6**, un produit de rinçage sans alcool avec 0,07 % de CPC est comparé à un placebo. Cette étude a également examiné l'effet sur la croissance de la plaque et l'inhibition de la maladie des gencives. Au total, 62 sujets répondaient aux critères de recherche. Les sujets ont été assignés de manière aléatoire à l'un des deux groupes. Au début de l'étude, après trois mois et après six mois, la quantité de plaque dentaire et l'étendue de la maladie des gencives et de la plaque dentaire ont été observées. Pour évaluer si un changement microbien indésirable s'est produit ou non, des échantillons de plaque et de salive ont également été prélevés. Après analyse des données recueillies, une différence a été constatée en faveur du rince-bouche CPC en ce qui concerne le degré de plaque dentaire. Aucune différence n'a été constatée après six mois en ce qui concerne le degré de maladie des gencives. En ce qui concerne l'étendue de la plaque dentaire au bout de trois et six mois, une augmentation faible mais significative a été observée pour le liquide de rinçage CPC par rapport au placebo.

Conclusions

Une hygiène buccale optimale est un facteur capital dans la prévention des caries et de la gingivite et dans le traitement de la parodontite. Cependant, la littérature scientifique et la pratique quotidienne révèlent que la plupart des adultes ont bien du mal à se laver la bouche convenablement. La motivation et l'habileté y sont pour beaucoup. Dans cet esprit, l'utilisation d'un liquide de rinçage antibactérien peut être un complément utile aux soins bucco-dentaires quotidiens. Les bains de bouche sont recommandés si l'hygiène buccale normale a un effet insuffisant ou si le nettoyage mécanique est difficile, compromis, voire impossible.

Voici les principales conclusions

- Le degré d'usure de la brosse au lieu de la durée d'utilisation est le facteur déterminant lorsqu'il s'agit de remplacer une brosse à dents.
- Le rinçage avec une combinaison de peroxyde d'hydrogène et de chlorhexidine pendant trois semaines entraîne une réduction de la plaque dentaire et des maladies des gencives.
- Le rinçage prolongé avec des huiles essentielles dans une formule fixe semble être une alternative efficace à la chlorhexidine pour prévenir les maladies des gencives.
- Un produit de rinçage à l'alcool pur ne contribue pas à la réduction de la plaque dentaire et des maladies des gencives.
- Les huiles essentielles contribuent efficacement à la réduction de la plaque dentaire et des maladies des gencives.
- Un rinçage deux fois par jour avec un bain de bouche au chlorure de cétylpyridinium dilué à 0,07 % réduit l'accumulation de plaque.



Curriculum Vitae

Martijn van Leeuwen was born on April 22, 1985 in Naarden, the Netherlands. He and his brother and sister grew up in a close family in Bussum, where he now lives with his wife. In his spare time, he likes doing odd jobs around the house, cooking, and playing sports, especially tennis.

After finishing high school, he studied at InHolland University of Applied Sciences to become a dental hygienist. During his third year, he completed an internship in Nepal at the Kantipur School of Dentistry in Kathmandu. In 2008, he graduated from the four-year Bachelor degree of Dental Hygiene. Almost directly after graduating, he started working in Amersfoort at a periodontology clinic, where he had undertaken an internship in his final year. He became intrigued by periodontology and began researching the fundamentals of oral health at the Department of Periodontology. In 2009, he began writing his first systematic review under the supervision of Fridus van der Weijden and Dagmar Else Slot which is published in 2010. In the same year, he continued his career by studying dentistry at the Academic Center for Dentistry in Amsterdam and conducting research on a voluntary basis.

He graduated cum laude in Dentistry in 2016, after which he took over his father's dental practice. Today, father and son still work together in their new dental clinic.

At the wedding of Martijn and Marion on August 4, 2016, Dagmar announced that Martijn could officially begin his PhD trajectory, of which Fridus and Dagmar were proud to be his supervisors. Now, four years after graduation as a dentist, he will defend his PhD thesis at the Faculty of Dentistry, with the support of his wife and sister.

PhD portfolio

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Position:	Buitenpromovendus
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Promotores:	prof. dr. G.A. van der Weijden
	dr. D.E. Slot

General courses	Year	ECTS
Scientific integrity	2016	2.0
Oral biology course	2016	4.0
English writing and presenting	2017	4.0
Practical Biostatistics	2020	4.0

Specific courses	Presentation	Year	ECTS
International dental summer camp, China, Chengdu	Oral	2014	6.0
Experimental gingivitis models		2015	1.0
Epidemiology & Evidence Based Practice		2016	3.0

(Inter)national conferences	Presentation	Year	ECTS
ISDH, Glasgow, United Kingdom	Poster	2010	2.0
Euro Perio 7, Vienna, Austria	Poster	2012	2.0
NVvP, Ede, the Netherlands		2014	0.5
NVvP, Ede, the Netherlands		2015	0.5
ACTA Paro Lustrum Symposium		2015	0.5
Advisory board Johnson & Johnson Consumer B.V.	Oral	2015	
NVVRT		2016	0.5
EuroPerio 9, Amsterdam, the Netherlands	Poster	2018	2.0

Supervising	Year	ECTS
Luc Laurent and Raoul Schyns, Bachelor student Dentistry, Academic Centre for Dentistry Amsterdam (ACTA), the Netherlands *"Accessing gingival inflammation with an intra oral camera system"*	2018-2019	2.0

Total ECTS	34.5

List of publications

Peer reviewed full-text publications

In this thesis

1. **M.P.C. van Leeuwen**, D.E. Slot, G.A. van der Weijden. Essential oils compared to chlorhexidine with respect to plaque and parameters of gingival inflammation: a systematic review. *Journal of Periodontology*. 2011:82:174-194
2. **M.P.C. van Leeuwen**, G.A. van der Weijden, D.E. Slot. The effect of an essential-oils mouthrinse as compared to a vehicle solution on plaque and gingival inflammation: a systematic review and meta-analysis. *International Journal of Dental Hygiene*. 2014A;12(3):160-167
3. **M.P.C. van Leeuwen**, N.A.M. Rosema, P.A. Versteeg, D.E. Slot, A.J. van Winkelhoff, G.A. van der Weijden. Long-term efficacy of a 0.07% cetylpyridinium chloride mouth rinse in relation to plaque and gingivitis: a 6-month randomized, vehicle-controlled clinical trial. *International Journal of Dental Hygiene*. 2015;13(2):93-103
4. **M.P.C. van Leeuwen**, N.A.M. Rosema, P.A. Versteeg, D.E. Slot, N.L. Hennequin-Hoenderdos, G.A. van der Weijden. Effectiveness of various interventions on maintenance of gingival health during 1 year – a randomized clinical trial. *International Journal of Dental Hygiene*. 2017;15(4):e16-e27
5. **M.P.C. van Leeuwen,** G.A. van der Weijden, D.E. Slot, N.A.M. Rosema. Toothbrush wear in relation to toothbrushing effectiveness. *International Journal of Dental Hygiene*. 2019;17(1):77-84

Other

1. E. van der Sluijs, D.E. Slot, N.L. Hennequin-Hoenderdos, **M.P.C. van Leeuwen**, G.A. van der Weijden. Prebrushing rinse with water on plaque removal: a split-mouth design. *International Journal of Dental Hygiene*. 2017;15(4):345-351

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1. E. van der Sluijs, D.E. Slot, N.L. Hennequin-Hoenderdos, G.A. van der Weijden. A specific brushing sequence and plaque removal efficacy: a randomized split-mouth design. *International Journal of Dental Hygiene*. 2018;16: 85-91.
2. R.S. Keukenmeester, D.E. Slot, N.A.M. Rosema, C. van Loveren, G.A. van der Weijden. Effects of sugar-free chewing gum sweetened with xylitol or maltitol on the development of gingivitis and plaque: a randomized clinical trial. International *Journal of Dental Hygiene*. 2014;12(4):238-244

Other relevant publications and scientific contributions

1. **M.P.C. van Leeuwen**, D.E. Slot, G.A. van der Weijden. (2011). Listerine® vs. Chloorhexidine! NTvT, 16: 26-27.
2. **M.P.C. van Leeuwen**, D.E. Slot, G.A. van der Weijden. (2014) Etherische oliën zijn het effectieve component, Spoelen tegen plaque. Dentista 42-45.
3. **M.P.C. van Leeuwen**, D.E. Slot, G.A. van der Weijden. Evidence on the effect of fluoride, essential oils, and chlorhexidine mouthrinses on dental plaque and gingivitis in patients with and without dental caries. Evidence-Based Dent for the Dent Hyg. 1(2), 2015; 115-117

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Chapter 2

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Chapter 5

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List of frequently used abbreviations

ACTA	Academic Centre for Dentistry Amsterdam
ADA	American Dental Association
AMC	Academic Medical Centre
BOMP	Bleeding On Marginal Probing
BI	Bleeding Index
CEBM	Center for Evidence-Based Medicine
CHX	Chlorhexidine
CFU	Colony-Forming Units
CI	Confidence Interval / Calculus Index
CONSORT	Consolidated Standards Of Reporting Trials
CCT	Controlled Clinical Trial
CPC	Cetylpyridinium Chloride
DPSI	Dutch Periodontal Screening Index
DiffM	Difference of Means
DPSI	Dutch Periodontal Screening Index
EMBASE	Excerpta Medica dataBASE
EO(MW)	Essential Oils (Mouthwash)
GI	Gingival Index
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GMSI	Gründemann Modification of the Stain Index
MA	Meta-Analysis
MEC	Medical Ethics Commission
MEDLINE	Medical Literature Analysis and Retrieval System Online
NVvP	Nederlandse Vereniging voor Parodontologie (Dutch Society of Periodontology)
OHI	Oral hygiene instruction
OA	Oxygenating-agent
PP	Professional prophylaxis
PI	Plaque Index
PS	Power and Sample size
PRISMA	Preferred Reporting Items for Systematic
PubMed	Public Medline database
Q&H/QHPI	Quigley & Hein Plaque Index
RTF	Reduced Transport Fluid
RCT	Randomized Controlled Trial
RDA	Relative Dentin Abrasion
SD	Standard Deviation
SE	Standard Error
SNOSE	Sequentially Numbered, Opaque, Sealed Envelopes
SI	Stain Index
TIDiER	Template for Intervention Description and Replication
VAS	Visual Analogue Scale
VC	Vehicle Control
V-Sol	Vehicle Solution
WC	Water Control

Dankwoord

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Every ending has a new beginning.

Martijn

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PERIODONTOLOGY