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Dr. Mekhala Mukherjee, et al.

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Written by

Dr.Mekhala Mukherjee Ass.Prof.

Dr.Abhijit Chakrabarty Prof & Head.

Dr.Ravi Prakash B S Prof.

Dr.Manoj Kumar Singh Prof.

Dr.Rajashree Ganguly Assoc. Prof

Dr.Dibyendhu Kumar Kundu Assoc. Prof

Dr.Surupa Dutta Ass. Prof.

Dr.Gautam Das Ass. Prof.

Dr.Soumya Roy Chaudhuri Ass. Prof.

Dr.Trishik Basak Ass. Prof.

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INTRODUCTION

In the year 1960, Loe et al established the essential role of dental plaque as the etiological agent responsible for periodontal disease[1].

Periodontal disease is highly prevalent and can affect up to 90% of the world population. Since then control of biofilm accumulation on the teeth has been the key to periodontal disease prevention. Toothbrushes and the use of dental floss and other devices to remove bacterial plaque from the teeth are the most common ways of removing biofilm. Despite its important role in the control of periodontal disease, mechanical plaque control is not properly practiced by most individuals. A systemic review of the effectiveness of self-performed mechanical plaque removal in objects with periodontal disease concluded that it had limitations. So the adjunctive use of chemical plaque control might beneficial.

Antimicrobial and plaque inhibitory agents in mouthwashes or toothpaste, used to inhibit bacterial plaque formation and thus to prevent or resolve chronic gingivitis, can only affect supragingival plaque. Therefore prevention through supragingival plaque control remains the mainstay of controlling gingivitis, and therefore the occurrence or recurrence of periodontitis.

The importance of oral hygiene and the long-term success of therapy for periodontal disease is hampered by the frequent ineffectiveness of mechanical cleaning of specific sites using a toothbrush, and the limited or lack of use of interdental cleaning by any individual. This supports the concept of employing agents to control plaque which requires minimal compliance and skill in their use. This is the concept that underlies chemical supragingival plaque control, but as with the oral hygiene instructions in mechanical methods, it will have to be vastly overprescribed is to be achieved in susceptible individuals. Chemical supragingival plaque control has thus been the subject of extensive research using scientific methodologies for 40 years.

Agents that could inhibit the development or maturation of supragingival plaque have been classified according to possible mechanisms of action[2]: (1) anti-adhesive; (2) antimicrobial; (3) plaque removal; and (4) antipathogenic. The majority of agents used to control supragingival plaque are contained in “oral hygiene” products and available to the general public either directly

"over the counter" or following recommendations/prescriptions by a dental or medical professional. Manufacturers of these products and, for that matter, published literature use a variety of terms to describe the action of these chemical agents, often interchangeably, which has tended to confuse. In an attempt to clarify the various descriptive.

Terms used the European Federation of Periodontology in the 1996 European Workshop on Periodontology recommended definitions for the terminology employed for agents in chemical supragingival plaque control3as follows:

• Antimicrobial agents: Chemicals that have a bacteriostatic or bactericidal effect in vitro that alone cannot be extrapolated to a proven efficacy in vivo against plaque.

• Plaque reducing/inhibitory agents: Chemicals that have only been shown to reduce the quantity and/ or affect the quality of plaque, which may or may not be sufficient to influence gingivitis and/or caries.

• Antiplaque agents: Chemicals that affect plaque sufficient to benefit gingivitis and/or caries4.

• Antigingivitis agents: Chemicals that reduce gingival inflammation without necessarily influencing bacterial plaque (includes anti- inflammatory agents).

HISTORICAL BACKGROUND

The terminology “oral hygiene products” is recent but there is evidence dating back at least 6000 years that formulations and recipes existed to benefit oral and dental health[5].

This concludes that the written Ebers Papyrus from 1500 BC contains recipes for tooth powders and mouth rinses dating back to 4000 BC. A considerable number of formulations can be attributed to the writer and scientist Hippocrates 480 BC.

Alcohol-based mouth rinses were particularly popular with the Romans and included White wine and beer.

Fauchard (1690-1761) in France recommended fresh urine.

The Jewish Talmund, dating back about 1800 years, suggests a cure for gum ailments containing “dough water “and olive oil.

Perhaps the biggest change to toothpaste came with the chemoparasitic theory of tooth decay of W.D. Miller in 1890. Shortly after, and at the beginning of the 20thcentury, various potassium and sodium salts were added to toothpaste as a therapy for periodontal disease.

Chlorhexidine gluconate was developed in the 1940s by Imperial Chemical Industries, England, and marketed in 1954 as an antiseptic for skin wounds.

Use in dentistry was initially for presurgical disinfection of the mouth and in Endodontics. The first definitive study on chlorhexidine was performed by Loe and Schiott (1970)6. This study showed that rinsing for 60 seconds twice per day with 10 ml of a 0.2% (20 mg dose) chlorhexidine gluconate solution in the absence of normal tooth cleaning, inhibited plaque regrowth and the development of gingivitis.

In the 1960s Harald Loe showed that chlorhexidine compound could prevent the build-up of dental plaque.

Aqueous alcohol solutions of 0.2% Chlorhexidine were first made available for mouth rinse products for twice-daily use in Europe in the 1970s.

THE CONCEPT OF CHEMICAL SUPRAGINGIVAL PLAQUE CONTROL

Epidemiologic studies revealed a peculiarly high correlation between supragingival plaque levels and chronic gingivitis, and clinical research led to the proof that plaque was the primary etiologic factor in gingival inflammation. Subgingival plaque, derived from supragingival plaque, is also intimately associated with the advancing lesions of chronic periodontal diseases. On the basis that plaque-induced gingivitis always precedes the occurrence and recurrence of periodontitis, the mainstay of primary and secondary prevention of periodontal diseases is the control of supragingival plaque. Periodontal diseases appear to occur when a pathogenic microbial plaque acts on a susceptible host. What constitutes a pathogenic subgingival plaque has been and continues to be, a much-researched area in periodontology. In the 1996 World Workshop on Periodontology a small number of bacteria were confirmed as true pathogens with a longer list considered as putative pathogens. Much has been learned in the intervening decade, not the least of which is the bacterial diversity of subgingival plaque in health and disease, highlighted in several reviews. The possibility that viruses may be involved has also been postulated. If the latter postulate becomes proven an extension of the classification of chemical agents, including antiviral, will be necessary. Interestingly, some of the antimicrobial agents used in chemical plaque control do have antiviral activity. Susceptibility to periodontal disease is less well understood and, at this time, certainly difficult to predict and quantify, although risk factors have been identified including genetic markers. The relationship of plaque levels to pathogenicity and susceptibility is also poorly understood and therefore, for anyone individual, what constitutes a satisfactory level of oral hygiene cannot be stated. This aside, there is evidence which demonstrates that improving oral hygiene and gingival health, over several decades, noted in developed countries, has been associated with a decreasing incidence of periodontal disease. Additionally, long-term follow-up of treated periodontal disease patients has shown that success is dependent on maintaining plaque levels compatible with gingival health. Supragingival plaque control is thus fundamental to the prevention and management of periodontal diseases and, with appropriate advice and instruction from professionals, is primarily the responsibility of the individual. It could be argued that the heavy reliance on mechanical methods to prevent what are microbially associated diseases is outdated.

Very few hygiene practices against microorganisms used by humans on themselves, in the home, at the workplace, or in the environment rely on mechanical methods alone and some methods are only chemical. The contrary argument must be that the prevention of periodontitis, through the control of gingivitis, would require the discovery of a safe and effective agent. Also, such a preventive agent would have to be applied from an early age to a large proportion of all populations, many of whom would have low or no susceptibility to periodontal disease. These discussions aside, chemical preventive agents, aimed at microbial plaque, have been a feature of periodontal disease management for almost a century. The consensus appears to be that the use of preventive agents should be as adjuncts and not replacements for the more conventional and accepted effective mechanical methods and only then when these appear partially or ineffective alone.

Mechanical tooth cleaning through toothbrushing with toothpaste is arguably the most common and potentially effective form of oral hygiene practiced by people in developed countries; although, per capita in the world, wood sticks are probably more commonly used. Interdental cleaning is a secondary adjunct and would seem particularly important in individuals who, through the presence of disease, can be retrospectively assessed as susceptible. Unfortunately, it is a fact of life that a significant proportion of all individuals fail to practice a high enough standard of plaque removal such that gingivitis is highly prevalent and from an early age, presumably, arises either or both from a failure to comply with the recommendation to regularly clean teeth or lack of dexterity with tooth cleaning habits. Certainly, many individuals remove only around half of the plaque from their teeth even when brushing for 2 minutes. Presumably, this occurs because certain tooth surfaces receive little or no attention during the brushing cycle. The adjunctive use of chemicals would therefore appear a way of overcoming deficiencies in mechanical tooth cleaning habits as practiced by many individuals.

INTRODUCTION TO DENTAL PLAQUE AND ITS FORMATION

Dental Plaque is defined as an organized mass, consisting mainly of microorganisms, that adheres to teeth, prostheses and oral surfaces and is found in the gingival crevice and periodontal pockets. Other components include an organic, polysaccharide-protein matrix consisting of bacterial by-products such as enzymes, food debris, desquamated cells, and inorganic components such as calcium and phosphate.

A direct relationship was often assumed to exist between the total number of accumulated bacteria and the amplitude of the pathogenic effect; biologically relevant differences in the composition of plaque were not usually considered. This bacterial mass termed plaque was shown to produce a variety of irritants, such as acids, endotoxins, and antigens, which, over time, invariably dissolved teeth and destroyed the supporting tissues. Consequently, the need to discriminate among bacterial deposits from different patients or at healthy or diseased sites was not yet recognized in detail. Individuals with extensive periodontal disease were either suspected of having weak resistance to bacterial plaque as a whole or were blamed for inadequate home care. Such a view of dental plaque as biomass is referred to as the non-specific plaque hypothesis. The propensity of inflamed sites to undergo permanent tissue destruction was recognized later to be more specific in nature because not all gingivitis lesions seemed invariable to progress to periodontitis. Most periodontal sites in most subjects do not always show clinical signs of active tissue destruction with loss of connective tissue fiber attachment to the root surface, even though they may constantly be colonized by varying numbers and species of bacteria. Possible pathogens have been suggested among the organisms regularly found at elevated levels in periodontal lesions about those observed under clinically healthy conditions. Longitudinal studies have indicated an increased risk for the periodontal breakdown in sites colonized by some potentially pathogenic organisms. Treatment outcomes were better if these organisms could no longer be detected at follow-up examinations. If periodontal disease is indeed due to a limited number of bacterial species, the continuous and maximal suppression of plaque as a whole may not be the only possibility to prevent or treat periodontitis. Hence, specific elimination or reduction of presumptive pathogenic bacteria from plaque may become a

valid alternative. Treatment may only be necessary for those patients diagnosed as having the specific infection and may be terminated once the pathogenic agents are eliminated. Such a view of periodontitis being caused by specific pathogens is referred to as the specific plaque hypothesis.

Growth and maturation patterns of bacterial plaque have been studied on natural hard oral surfaces, such as enamel and dentin, or artificial surfaces, such as metal or acrylic, using light and electron microscopy and bacterial culture.

The ability to adhere to surfaces is a general property of almost all bacteria. It depends on an intricate, sometimes exquisitely specific, series of interactions between the surface to be colonized, the microbe, and an ambient fluid milieu. Immediately upon immersion of a solid substratum into the fluid media of the oral cavity or upon cleaning of a solid surface in the mouth, hydrophobic and macromolecules begin to adsorb to the surface to form a conditioning film, termed the acquired pellicle. This film is composed of a variety of salivary glycoproteins (mucins) and antibodies. The conditioning film alters the charge and free energy of the surface, which in turn increases the efficiency of bacterial adhesion. Bacteria adhere variably to these coated surfaces. Some possess specific attachment structures such as extracellular polymeric substances and fimbriae, which enable them to attach rapidly upon contact. Other bacteria require prolonged exposure to bind firmly. Behaviors of bacteria change once they become attached to surfaces. This includes active cellular growth of previously starving bacteria and synthesis of new outer membrane components. The bacterial mass increases due to the continued growth of the adhering organisms, adhesion of new bacteria, and synthesis of extracellular polymers. With increasing thickness, diffusion into and out of the biofilm becomes more and more difficult. An oxygen gradient develops as a result of rapid utilization by the superficial bacterial layers and poor diffusion of oxygen through the biofilm matrix. Completely anaerobic conditions eventually emerge in the deeper layers of the deposits. Oxygen is an important ecologic determinant because bacteria vary in their ability to grow and multiply at different levels of oxygen. Diminishing gradients of nutrients supplied by the aqueous phase, i.e. the saliva, are also created.

Reverse gradients of fermentation products develop as a result of bacterial metabolism. Dietary products dissolved in saliva are an important source of nutrients for bacteria in the

supragingival plaque. Once a deepened periodontal pocket is formed, however, the nutritional conditions for bacteria change because the penetration of substances dissolved in saliva into the pocket is very limited. Within the deepened pocket, the major nutritional source for bacterial metabolism comes from the periodontal tissues and blood. Many bacteria found in periodontal pockets produce hydrolytic enzymes with which they can break down complex macromolecules from the host into simple peptides and amino acids. These enzymes may be a major factor in the destructive processes of periodontal tissues. Primary colonization is dominated by facultatively anaerobic Gram-positive cocci. They adsorb onto the pellicle-coated surfaces within a short time after mechanical cleaning. Plaque collected after 24 hours consists mainly of streptococci; S. sanguis is the most prominent of these organisms. In the next phase, Gram-positive rods, which are present in very low numbers initially, gradually increase and eventually outnumber the streptococci. Gram-positive filaments, particularly Actinomyces spp., are the predominating species in this stage of plaque development. Surface receptors on the deposited Gram-positive cocci and rods allow subsequent adherence of Gram-negative organisms with poor ability to attach directly to the pellicle. Veillonella, fusobacteria, and other anaerobic Gram-negative bacteria can attach in this way. The heterogeneity of plaque thus gradually increases and, with time, includes large numbers of Gram-negative organisms. A complex array of interrelated bacterial species is the result of this development. Exchange of nutrients between different species, but also negative interactions, e.g. the production of bacteriocins, play a role in the establishment of a stable bacterial community. Due to the influences of local environmental factors, structurally different types of plaque evolve at different locations.

Protection of the growing plaque from shear forces and local availability of certain nutrients are most important. A distinct composition of mature bacterial deposits can eventually be recognized at specific sites and under specific clinical conditions. Examples are the plaque on a smooth enamel surface versus fissure plaque, or the plaque in shallow and less shallow gingival crevices. Accumulation of plaque along the gingival margin leads to an inflammatory reaction of the soft tissues. The presence of this inflammation has a profound influence on the local ecology. The availability of blood and gingival fluid components promotes the growth of Gram-negative bacterial species with increased periodontopathic potential. Bacterial samples from established gingivitis lesions have increased numbers of these bacteria. Because of the capability enzymatically to digest proteins, many of these organisms do not depend upon a direct

availability of dietary carbohydrates. Such bacteria do not produce extracellular polymers and develop only loosely adherent plaque in the developing periodontal pocket. Cultivation of samples from advanced periodontal lesions reveals a predominance of Gram-negative anaerobic rods. Under the microscope, particularly high numbers of anaerobic uncultivable spirochetes can be demonstrated. In summary, immediately following immersion of hard, non-shedding surfaces into the fluid environment of the oral cavity, adsorption of macromolecules will lead to the formation of a biofilm. Bacterial adhesions to this glycoprotein layer will first involve primary plaque formers, such as Gram-positive facultative cocci and rods. Subsequent colonization on to receptors of these organisms will involve Gram-negative, strictly anaerobic bacteria, while the primary plaque formers also multiply to form colonies. The heterogeneity of the complex biofilm increases with time, as the ecologic conditions gradually change.

The term biofilm describes the relatively undefinable microbial community associated with a tooth surface or any other hard, non-shedding material7. In the lower levels of most biofilms, a dense layer of microbes is bound together in a polysaccharide matrix with other organic and inorganic materials. On top of this layer is a looser layer, which is often highly irregular in appearance and may extend into the surrounding medium. The fluid layer bordering the biofilm may have a rather “stationary” sub-layer and a fluid layer in motion. Nutrient components may penetrate this fluid medium by molecular diffusion. Steep diffusion gradients, especially for oxygen, exist in the more compact lower regions of biofilms. The ubiquity with which anaerobic species are detected from these areas of biofilms provides evidence for these gradients. Accumulation of bacteria on solid surfaces is not an exclusive dental phenomenon. Biofilms are ubiquitous; they form on virtually all surfaces immersed in natural aqueous environments. Biofilms form particularly fast in flow systems where a regular nutrient supply is provided to the bacteria. Rapid formation of visible layers of microorganisms due to extensive bacterial growth accompanied by excretion of copious amounts of extracellular polymers is typical for biofilms. Biofilms effectively protect bacteria from antimicrobial agents. Treatment with antimicrobial substances is often unsuccessful unless the deposits are mechanically removed. Adhesion-mediated infections that develop on permanently or temporarily implanted materials, such as intravascular catheters, vascular prostheses or heart valves, are notoriously resistant to antibiotics and tend to persist until the device is removed. Similar problems are encountered in water conduits, wherein potentially pathogenic bacteria may be protected from

chlorination, or on ship hulls, where biofilms increase frictional resistance and turbulence. In summary, dental plaque as a naturally occurring microbial deposit represents a true biofilm that consists of bacteria in a matrix composed mainly of extracellular bacterial polymers and salivary and/or gingival exudate products.

The supragingival plaque has been examined in several studies by light and electron microscopy to gain information on its internal structure. The introduction of the electron microscope in dental research was a significant development for studies of dental plaque, both because the size of many bacteria approaches the ultimate resolving power of the light microscope, and because the resins used for embedding allowed for sections thinner than the smallest bacterial dimension. The substructure of plaque could therefore be identified.

APPROACHES TO CHEMICAL PLAQUE CONTROL

Mechanical cleaning aims to regularly remove sufficient microorganisms to leave a "healthy plaque" present, which cannot induce gingival inflammation. Chemical agents, on the other hand, could influence plaque quantitatively and qualitatively via several processes. The action of the chemicals could fit into four categories:

1.Anti-adhesive

2.Antimicrobial

3.Plaque removal

4.Antipathogenic

ANTIADHESIVE AGENTS:

Antiadhesive agents would act at the pellicle surface to prevent the initial attachment of the primary plaque-forming bacteria. Such adhesive agents should have to be preventive in their effects, acting most effectively on an initially clean tooth surface. Adhesive agents do exist and are used in the industry, domestically, and in the environment. Such chemicals prevent the attachment and the development of a variety of biofilms and are usually described as antifouling agents. Unfortunately, the chemicals found in such applications are either too toxic for oral use or ineffective against dental bacteria plaques. But still, the concept of antiadhesiveness continues to attract research interest[8]. To date, effective formulations or products with Antiadhesive properties are not available to the general public, although the amine alcohol, delmopinol, which appears to interfere with bacterial matrix formation and fits somewhere between the concepts of antiadhesion and plaque removal, has been shown effective against plaque and gingivitis [9,10].

ANTIMICROBIAL AGENTS:

Antimicrobial agents could inhibit plaque formation through one of the two mechanisms alone or combined. The first is bacteriostatic which includes inhibition of bacterial proliferation.

These agents could exert their effects either at the pellicle-coated tooth surface before the primary plaque formers attach or after attachment but before the division of these bacteria. The second effect is bactericidal where the antimicrobial agent destroys all of the microorganisms either attaching or already attached to the tooth surface. The antimicrobial agents probably exert both bactericidal effects followed by a bacteriostatic action of variable duration.

PLAQUE REMOVAL AGENTS

The idea of employing a chemical agent in a mouth rinse is expected to reach all tooth surfaces effectively which is similar to a toothbrush that removes bacteria from a tooth surface. For this reason, chemical plaque removal agents are also referred to as "the chemical toothbrush".

ANTIPATHOGENIC AGENTS

It is theoretically possible that an agent could affect plaque microorganisms, which might inhibit the expression of the pathogenicity without necessarily destroying the microorganisms. At present such an approach within the oral cavity for either gingivitis or caries is still under research.

VEHICLES FOR THE DELIVERY OF CHEMICAL AGENTS

The carriage of chemical agents into the mouth for supragingival plaque control includes a small but varied range of vehicles [11,12]:

1. Toothpaste
2. Mouth rinses
3. Spray
4. Irrigators
5. Varnishes

TOOTHPASTE: The most commonly used vehicle for the carriage of the plaque control agent is toothpaste. The ingredients present in the toothpaste have a role in either influencing the consistency and stability of the product or its function[13].

The major ingredients are as follows

1. *ABRASIVES:* It affects the consistency of the toothpaste and assists in the control of extrinsic dental staining. Examples include silica, alumina, dicalcium phosphate, and calcium carbonate either alone or more usually today in combination.
2. *DETERGENTS:* It imparts the foaming properties to the product. Moreover, detergents may help dissolve active ingredients and the anionic detergent sodium lauryl sulphate has both antimicrobial and plaque inhibitory properties. Certain toothpaste products cannot employ anionic detergents as they interact with the cationic substances that may be added to the product such as chlorhexidine, or polyvalent metal salts such as strontium, used in the treatment of dentine hypersensitivity.
3. *THICKENERS:* Such as silica and gums, primarily influence the viscosity of the products.
4. *SWEETENERS:* Such as saccharine.
5. *HUMECTANTS:* Such as glycerine and sorbitol to prevent drying out of the paste once the tube has been opened.
6. *FLAVORS:* Herbal flavors are more popular in the Indian subcontinent.
7. *ACTIVES:* Fluorides for caries prevention; triclosan and stannous fluoride for plaque control; pyrophosphates which are anti calculus agents; polyphosphates which are whitening agents; strontium and potassium salts which are desensitizing agents.

MOUTHRINSES:

Despite the ideal nature of toothpaste vehicle, most chemical plaque control agents have been evaluated and later formulated in the mouth rinse vehicle. Mouthrinses vary in their constituents but are usually less complex than toothpaste. They can be simple aqueous solutions with the addition of flavoring, coloring, and preservatives such as sodium benzoate. Anionic detergents are included in some products but cannot be formulated with cationic antiseptics such as cetylpyridinium chloride or chlorhexidine14. Ethyl alcohol is commonly used both to stabilize certain active ingredients and to improve the shelf life of the product. Several concerns have been expressed over alcohol-containing mouth rinses[15]. The proportion of alcohol is usually less than 10% but some rinses have more than 20% alcohol. Some manufacturers are producing alcohol–free mouth rinses.

IDEAL REQUISITES OF MOUTHWASH

- Reduce plaque and gingivitis
- Prevent growth of pathogenic bacteria
- Prevent the development of resistant bacteria
- Compatible with oral tissues
- Not stain teeth/ after taste
- Exhibit good retentive properties.
- Inexpensive and easy to use.

SPRAY: Spray delivery of chemical plaque control agents has the advantage of focusing delivery on the required site. The dose is reduced and for antiseptics such as chlorhexidine, this has the taste advantage.

When correctly applied, chlorhexidine sprays were as effective as mouth rinses for plaque inhibition, although there was no reduction in staining [16,17]. Chlorhexidine sprays were found particularly useful for plaque control in physically and mentally handicapped groups[18].

IRRIGATORS: Irrigators were designed to spray water, under pressure, around the teeth. As such they only removed debris, with little effects on plaque deposits (Frandenson 1986). Antiseptics and other chemical plaque control agents, such as chlorhexidine, have been added to the reservoir of such devices. A variety of dilutions of chlorhexidine has been employed to good effect19but again with the inevitable local side effects.

CHEWING GUM: Chewing gums are used to deliver a variety of agents for oral health benefits. There appear to be significant benefits to dental health through the use of sugar-free chewing gum. They can reduce occlusal plaque deposits[20] but it is unlikely to be beneficial in the prevention of fissure caries. This vehicle has been used to deliver chemical agents such as chlorhexidine and when used as an adjunct to normal tooth brushing, reduced plaque and gingivitis levels have been shown [21,22].

VARNISHES: Varnishes have been employed to deliver antiseptics including chlorhexidine, but the purpose has been to prevent root caries rather than as a reservoir for plaque control throughout the mouth.

CHEMICAL PLAQUE CONTROL AGENTS

Over 4 decades there has been quite intense interest in the use of chemical agents to control supragingival plaque and thereby gingivitis. The number and variation of chemical agents evaluated are quite large but most have antiseptic or antimicrobial actions and success has been extremely variable. It is important to emphasize that formulations based on antimicrobial agents provide a considerably greater preventive than therapeutic action. The most effective agents inhibit the development of plaque and gingivitis but are limited or slow to affect established plaque and gingivitis. Antiadhesive agents would similarly be expected to provide preventive rather than therapeutic effects. Plaque removal agents, on the other hand, would almost certainly provide both preventive and therapeutic actions. Based on knowledge derived from chlorhexidine[23], the most effective plaque inhibitory agents in the antiseptic or antimicrobial group are those showing persistence of action in the mouth measured in hours. Such persistence of action, sometimes termed substantivity[24], appears dependent on several factors:

1. Adsorption and prolonged retention on oral surfaces including importantly, pellicle coated teeth.
2. Maintenance of antimicrobial activity once adsorbed primarily through a bacteriostatic action against the primarily plaque-forming bacteria.
3. Minimal or slow neutralization of antimicrobial activity within the oral environment or slow desorption from surfaces.

Antimicrobial activity of antiseptics *in vitro* is not a reliable predictor of plaque inhibitory activity *in vivo* [25,26]. Early studies on several antiseptics revealed similar antimicrobial effects but there is a large variation in clinical effects. For example, compared to chlorhexidine, the cationic quaternary ammonium compound, cetylpyridinium chloride, has a similar antimicrobial profile *in vitro* and is initially adsorbed in the mouth to a considerably greater extent[27]. The persistence of action of cetylpyridinium chloride is; however, much shorter than chlorhexidine [28,29] and plaque inhibition is considerably less. Several reasons may explain these apparent anomalies, including poor retention of cetylpyridinium chloride within the oral cavity, reduced activity once adsorbed, and neutralization in the oral environment [30], or a combination of these factors. Attempts to improve the efficacy of cetylpyridinium chloride can of course include

increasing the frequency of use, but this is likely to incur compliance problems and side effects. Alternatively, substantivity could be improved by combining antimicrobials or using agents to increase the retention of antimicrobials [31].

GROUPS OF AGENTS USED IN THE CONTROL OF DENTAL PLAQUE AND GINGIVITIS

Group	**Example of agents**	**Action**	**Used now/ Product**
Antibiotic	Penicillin Vancomycin Kanamycin Niddamycin Spiromycin	Antimicrobial	No
Enzymes	Protease Lipase Nuclease Dextranase Mutanase	Plaque removal	No
Bisbiguanie antiseptics	Chlorhedine Alexidine Octenidie	Antimicrobial	Yes Mouthrinse Spray Gel Toothpaste Chewing gum Varnish
Quarternary ammonium compounds	Cetylpyridinium chloride Benzalconium Chloride	Antimicrobial	Yes Mouthrinse
Phenols and essential oils	Thymol Hexylresorcimol Ecalyptol Triclosan	Antimicrobial Anti inflammatory	Yes Mouthrinse Toothpaste
Natural products	Sanguinarine	Antimicrobial	No

Fluorides	Sodium fluoride Sodium monofluorophosphate Stannous fluoride Amine fluoride	Antimicrobial	Yes Toothpaste Mouthrinse Gel
Metal salts	Tin Zinc Copper	Antimicrobial	Yes Toothpaste Mouthrinse Gel
Oxygenating agents	Hydrogen peroxide Sodium peroxiborate Sodium peroxicarbonate	Antimicrobial	Yes Mouthrinse
Detergents	Sodium louryl sulphate	Antimicrobial	Yes Toothpaste Mouth rinse
Amine alcohols	Octapynol Delmopynol	Plaque matrix inhibition	No Yes Toothpaste Mouth rinse

CLASSIFICATION OF CHEMICAL AGENTS ACCORDING TO THEIR ANTIMICROBIAL EFFECT AND SUBSTANTIVITY

1ST Generation - capable of reducing plaque score by 20% -50% and efficacy is limited by poor retention in the mouth

E.g., Antibiotics, Phenols, Quarternary ammonium compounds, Sanguinarine

2nd Generation - more effectively retained by oral tissues and flow released properties provide a reduction in plaque score by70%-90%

E.g., Bisbiguanides

3rd Generation- characterized by an ability to inhibit or disrupt the formation of plaque while having no demonstrable effect on bacteria.

E.g., Amine Alcohols

Kornman-1986

Despite evidence for efficacy in preventing caries and gingivitis or resolving gingivitis, the opinion today is that systemic antimicrobials should not be used either topically or systemically as preventive agents against these diseases. The risk-to-benefit ratio is high and even systemic antimicrobial use in the treatment of adult periodontitis is open to debate. Thus, systemic antimicrobials have their own specific side effects, not all of which can be avoided by topical application. Perhaps of greatest importance is the development of bacterial resistance within human populations, for example, methicillin-resistant Staphylococcus aureus (MRSA), which causes serious and life-threatening wound infections, particularly within hospitalized patients.

Phenols and essential oils

Phenols and essential oils have been used in mouth rinses and lozenges for many years. One mouth rinse formulation dates back more than 100 years and, although not as efficacious as chlorhexidine, has antiplaque activity supported by a number of short- and long-term home use

studies. This mouth rinse product may reduce gingivitis via both a plaque inhibitory action and an anti-inflammatory action possibly due to an anti-oxidative activity. The data from home use studies led the American Dental Association to accept the product as an aid to home oral hygiene measures. When compared directly with chlorhexidine one 6- month study has demonstrated equivalent effects on plaque and gingivitis but without the inherent side effects of chlorhexidine[32]. Nevertheless, the pH of the product is low (pH 4.3) and has been shown in vitro and in situ to cause erosion of dentine and enamel respectively, albeit to a considerably less degree than orange juice[33]. Combining essential oils with cetylpyridinium chloride has been attempted and with promising results from initial studies[34]. The non-ionic antimicrobial triclosan, a trichlora2-hydroxy phenyl ether, is usually considered to belong to the phenol group and has been widely used over many years in a number of medicated products including antiperspirants and soaps. More recently, it has been formulated into toothpaste and mouth rinses and, for the former, has accumulated an impressive amount of literature, some of which is conflicting. In simple solutions, at relatively high concentrations (0.2%) and dose (20 mg twice per day), triclosan has moderate plaque-inhibitory action and antimicrobial substantivity of around 5 hours. The dose-response against plaque of triclosan alone is relatively flat35, although significantly greater benefits are obtained at 20 mg doses twice daily compared to 10 mg doses. In terms of plaque inhibition, a 0.1% triclosan concentration (10 mg dose twice per day) was considerably less effective than a 0.01% chlorhexidine mouth rinse (1 mg twice per day).

The activity of triclosan appears to be enhanced by the addition of zinc citrate or the co-polymer, polyvinyl methyl ether maleic acid. The co-polymer appears to enhance the retention of triclosan whereas zinc is thought to increase the antimicrobial activity. Only triclosan toothpaste with the co-polymer or zinc citrate has shown antiplaque activity in long-term home use studies36. Some home-use studies showed little or no effect for one or other of the products on plaque alone, gingivitis alone or both compared to the control paste or conventional fluoride toothpaste[37,38,39]. Triclosan toothpaste appears to provide greater gingivitis benefits in some studies than plaque reductions and this could be explained by a possible anti-inflammatory action for this agent[40]. More recently, long-term studies have suggested that triclosan-containing toothpaste can reduce the progress of periodontitis, although the effects have been considered small[41]. Mouth rinses containing triclosan and co-polymer are available, with some evidence of adjunctive benefits to oral hygiene and gingival health when used alongside normal tooth

cleaning. This latter study was again interesting with, unusually, no clear Hawthorne effect in the control group. Other studies on the plaque inhibitory properties of a triclosan/copolymer mouth rinse showed effects significantly less than those of an essential oil mouth rinse product[42].

Quaternary ammonium compounds

Benzalkonium chloride and, more particularly, cetylpyridinium chloride are the most studied of this family of antiseptics. Cetylpyridinium chloride is used in a wide variety of antiseptic mouth rinse products, usually at a concentration of 0.05%. At oral pH, these antiseptics are monocationic and adsorb readily and quantitatively, to a greater extent, than chlorhexidine to oral surfaces. The substantivity of cetylpyridinium chloride however appears to be only 3–5 hours due either to loss of activity once adsorbed or rapid desorption. Cetylpyridinium chloride in mouth rinses has some chemical plaque-inhibitory action but evidence for gingivitis benefits is equivocal, particularly when formulations are used alongside toothbrushing with toothpaste. Home use studies, given the large number of rinse products containing this antiseptic, are surprisingly few. Those available, with one exception, failed to demonstrate any adjunctive benefits to toothbrushing with toothpaste. The one exception[43] was peculiar in that there was a lack of the expected Hawthorne effect in the control group and the plaque reduction in the active group, 28%, was as great as seen in no brushing chemical plaque inhibition studies. As will be discussed, it is not unusual to find chemicals that provide modest, even moderate, plaque inhibition in no brushing studies but fail to show effects in adjunctive home use studies. This occurs because the range over which to show a benefit of the chemical is limited by the mechanical oral hygiene practices of the study subjects. Additionally, the plaque inhibitory properties of cetylpyridinium chloride are reduced by toothpaste used before or after the rinse [44,45]. This may explain why a pre-brushing cetylpyridinium mouth rinse offered no adjunctive benefit to mechanical plaque control. The efficacy of cetylpyridinium chloride can be increased by doubling the frequency of rinsing to four times per day (Bonsvoll & Gjermo 1978), but this increases local side effects, including tooth staining, and would probably affect compliance. Mouth rinses combining cetylpyridinium chloride with chlorhexidine are available and compare well with established chlorhexidine products[46]. Whether the cetylpyridinium chloride actually contributes to the activity of the chlorhexidine cannot be assessed. A slow-release system and lozenges have been used to deliver cetylpyridinium chloride but provided no greater plaque inhibition than the cetylpyridinium mouth rinse and significantly less than a chlorhexidine

rinse[47]. Interestingly, in this study, the lozenges produced the most dental staining. There is limited information on quaternary ammonium compounds in toothpaste and very few products are available.

Natural products

Herbs and plant extracts have been used in oral hygiene products for many years if not centuries. Unfortunately, there are little data available and such toothpaste products provide no greater benefits to oral hygiene and gingival health than do conventional fluoride toothpaste. The plant extract sanguinarine has been used in a number of formulations. Zinc salts are also incorporated, which makes it difficult to evaluate the efficacy of sanguinarine alone. Even when it is combined with zinc, however, data are equivocal for benefits. Some positive findings were reported for the combined use of sanguinarine/zinc toothpaste and mouth rinses[48], but the benefit-to-cost ratio must be low. Importantly and very recently, sanguinarine containing mouth rinses has been shown to increase the likelihood of oral precancerous lesions almost ten-fold even after cessation of mouth rinse use. The manufacturer of the most well-known product has replaced sanguinarine in the mouth rinses with an alternative agent. More recently, tea tree oil has been suggested to be of value when topically delivered with positive effects at reducing gingival inflammation[49] (Sookoulis& Hirsch 2004) but as yet no conclusive evidence for effects on plaque accumulation.

Bisbiguanide antiseptics

Chlorhexidine is thus far the most studied and effective antiseptic for plaque inhibition and the prevention of gingivitis. Consequent upon the original publication[50], chlorhexidine arguably represents the nearest that research has come to identify a chemical agent that could be used as a replacement for, rather than an adjunct to, mechanical oral hygiene practices. Other bisbiguanides such as alexidineand octenidine have less or similar activity, respectively, to chlorhexidine but bring with them no improvement in local side effects and have fewer toxicity data available. Chlorhexidine has thus remained the only bisbiguanide used in a number of vehicles and available in commercial products. In view of the importance of this antiseptic within preventive dentistry.

Amine alcohols

This group of compounds does not truly fit into an antimicrobial or antiseptic category; indeed they exhibit minimal effects against microbes. Of these morpholinoethenol derivatives, octopinol was the first to be shown effective as an antiplaque agent but was withdrawn for toxicologic reasons. Delmopinol followed and at 0.1% and 0.2% in mouth rinses was shown to be effective against plaque and gingivitis in short-term no oral hygiene and long-term home use studies [51,52]. Arguably, the short-term no oral hygiene studies showed plaque inhibition closer to chlorhexidine than any other previous agent. Recently, the data from eight studies from seven independent research groups in five European countries using a 0.2% delmopinol mouth rinse as an adjunct to normal oral hygiene practices were subjected to a meta-analysis. Delmopinol, one of the very few chemical plaque-control agents to be subjected to such analyses, was shown to be a significantly effective adjunct for reducing the plaque burden and severity of gingivitis53. The data for gingivitis in several studies met the efficacy criteria for gingivitis reduction of the American Dental Association. The mode of action of delmopinol can be debated but appears to be an interference with plaque matrix formation, reducing the adherence of the primary plaque-forming bacteria of the successional bacteria[54,55]. If correct, delmopinol would closest fit classification as an anti-adhesive agent. Side effects include tooth discoloration, transient numbness of the tongue, and burning sensations in the mouth. Interestingly, the staining was considerably less than with chlorhexidine, rarely reported by study participants and easily removed. In these adjunctive studies, discontinuations were considerably less with delmopinol than chlorhexidine. Rinses containing 0.2% delmopinol are available in some countries.

Enzymes

Enzymes fall into two groups. Those in the first group are not truly antimicrobial agents but more plaque removal agents in that they have the potential to disrupt the early plaque matrix, thereby dislodging bacteria from the tooth surface. In the late 1960s and early 1970s enzymes such as dextranase, mutanase and various proteases were thought to be a major breakthrough in dental plaque control that might prevent the development of both caries and gingivitis. Such agents, unfortunately, had poor substantivity and were not without unpleasant local side effects, notably mucosal erosion. The second group of enzymes employed glucose oxidase and amyloglucosidase to enhance the host defense mechanism. The aim was to catalyse the conversion of endogenous

and exogenous thiocyanate to hypothiocyanite via the salivary lactoperoxydase system. Hypothiocycanite produces inhibitory effects upon oral bacteria, particularly streptococci, by interfering with their metabolism. This approach is a theoretical possibility and the chemical processes can be produced in the laboratory. A toothpaste product containing the enzymes and thiocyanate was produced but equivocal results for benefits to gingivitis were obtained and there are no convincing long-term studies of efficacy.

Fluorides

The caries-preventive benefits for a number of fluoride salts are well established but the fluoride ion has no effect against the development of plaque and gingivitis. Amine fluoride and stannous fluoride provide some plaque-inhibitory activity, particularly when combined; however, the effects appear to be derived from the non-fluoride portion of the molecules. A mouth rinse product containing amine fluoride and stannous fluoride is available and there is some evidence from home use studies of efficacy against plaque and gingivitis [56,57] (Brecx et al. 1990, 1992), but less so than chlorhexidine.

Metal salts

Antimicrobial actions including plaque inhibition by metal salts have been appreciated for many years, with most research interest centered on copper, tin, and zinc. Results have been somewhat contradictory but appear dependent on the metal salt used, its concentration, and frequency of use. Essentially, polyvalent metal salts alone are effective plaque inhibitors at relatively high concentrations when taste and toxicity problems may arise. Stannous fluoride is an exception but is difficult to formulate into oral hygiene products because of stability problems, with hydrolysis occurring in the presence of water. Stable anhydrous gel and toothpaste products are available with evidence of efficacy against plaque and gingivitis[58]. Stannous pyrophosphate at 1% has been added to some stannous fluoride toothpaste to good effect. Indeed, it appears that the concentration of available stannous ions is the most significant factor in determining efficacy. Dental staining, however, occurs with stannous formulations and appears to occur by the same mechanism as for chlorhexidine and other cationic antiseptics, involving interaction with dietary chromogens. Combining metal salts with other antiseptics produces added plaque and gingivitis inhibitory effects, for example, zinc and hexetidine and, as already described, zinc and triclosan.

Copper also causes dental staining but is not available in oral hygiene products. Zinc, at low concentration, has no side effects and is used in a number of toothpaste and mouth rinses; however, alone it has little effect on plaque except at higher concentrations. Zinc salts nevertheless, may be of value at reducing volatile sulfur compounds associated with oral malodor[59] (Rosing et al. 2002).

Oxygenating agents

Oxygenating agents have been used as disinfectants in various disciplines of dentistry, including endodontics and periodontics. Hydrogen peroxide has been employed for supragingival plaque control and more recently has become important as bleach in tooth whitening. Similarly, peroxyborate may be used in the treatment of acute ulcerative gingivitis. Products containing peroxyborate and polycarbonate were, until recently, available in Britain and Europe with evidence of antimicrobial and plaque-inhibitory activity. There are little data from long-term home use studies and such evaluations would seem warranted before conclusions about the true antiplaque activity can be drawn.

Detergents

Detergents, such as sodium lauryl sulfate, are common ingredients in toothpaste and mouth rinse products. Besides other qualities and, for that matter, side effects, detergents such as sodium lauryl sulfate have antimicrobial activity and probably provide most of the modest plaque-inhibitory action of toothpaste. Alone, sodium lauryl sulfate was shown to have moderate substantivity, measured at between 5 and 7 hours, and plaque-inhibitory action similar to triclosan. Detergent-only formulations are not available and no long-term evaluations have been performed.

Salifluor

Salifluor, a salicylanilide with both antibacterial and anti-inflammatory properties, has been studied for its effects of plaque inhibition and retardation of onset of gingivitis[60]. To improve oral retention and to maximize adsorption, Gantrez (PVM/MA) has been incorporated in salifluor toothpaste and mouth rinse formulations. Perhaps surprisingly, salifluor has not been extensively evaluated, since initial 4-day plaque regrowth studies and 14day gingivitis studies have

suggested equivalent efficacy to a 0.12% chlorhexidine mouth rinse. Despite this evidence to suggest the potential value of the chemical as an antiplaque agent, further long-term studies have yet to be carried out.

Acidified sodium chlorite

This agent does not sit well with any particular group listed in Table 36- 1; however, depending on the acid chosen and the conditions of the reaction between the acid and the sodium chlorite, a varied and complex range of reaction products can ensue. Under ideal conditions for antimicrobial benefits sodium chlorite is reacted with a protic acid to produce chlorous acid, which then liberates a range of higher oxidant species but contains minimal amounts of chlorine dioxide. These higher oxidant species have a broad range of antimicrobial action against bacteria, fungi, yeast, and viruses and products are available in the US within the veterinary and food industry, both as a preventive for mastitis in cows and for the preservation of frozen poultry. Experimental mouth rinses have been tested in short-term plaque regrowth studies and salivary bacterial count investigations61. Surprisingly, given that the acid and sodium chlorite are mixed immediately before rinsing, and that the duration of the chemical reaction would be limited to the rinsing time, three experimental formulations were shown to be as good as chlorhexidine against plaque regrowth and showed the same substantivity as chlorhexidine. Although not tested in longer-term studies, side effects, particularly staining and alteration of taste, would appear unlikely with the acidified sodium chlorite mouth rinses. Unfortunately, the low pH of the formulations would be expected to cause some dental erosion and this has been proven in studies in situ. Such erosion, which was found comparable to that of orange juice in situ, would tend to obviate the long-term continuous use of such agents. Acidified sodium chlorite mouth rinses, however, could find application in preventive dentistry similar to those to be described for chlorhexidine. The erosive effects would not, in short- to medium-term use, reach clinically significant levels. To date no commercial products are available.

Other antiseptics

A number of antiseptics/antimicrobial agents have been studied for plaque inhibition. Most have been found to have little or no effect in vivo; a few have been formulated in mouth rinse products including povidone-iodine and hexetidine. Povidone-iodine at 1% has a substantivity of only 60

minutes and lacks appreciable plaque-inhibitory activity or action in acute infections such as acute ulcerative gingivitis[62], for which it is recommended. Povidone-iodine is large without side effects but as a rinse has the potential to affect thyroid function adversely. Hexetidine, a saturated pyrimidine, at 0.1% was shown to have limited plaque-inhibitory action and no evidence for antiplaque activity when used as an adjunct for oral hygiene[63] (Chadwick et al. 1991). The action of hexetidine against plaque appears enhanced by zinc salts but data are derived only from short-term studies. Side effects for hexetidine include tooth staining and mucosal erosion, although both are uncommon. Nevertheless, mucosal erosion is markedly increased in incidence if the concentration is raised to 0.14%. A mouth rinse product containing 0.1% hexetidine is available in some European countries. Recent studies have shown favorable effects on plaque and gingivitis64 and when compared to 0.1% chlorhexidine, less tendency for stain production.

CHLORHEXIDINE

History

Chlorhexidine was developed by Imperial Chemical Industries in England during the 1940's. It was marketed as a general antiseptic in the year 1950. In 1957 chlorhexidine was introduced for human use in Britain as an antiseptic for the skin. Later it was widely used in medicine and surgery. Plaque inhibition was first investigated by Schroeder in 1969. Later, the antiseptic was more widely used in medicine and surgery including obstetrics, gynecology, urology, and presurgical skin preparation for both patient and surgeon. Use in dentistry was initially for presurgical disinfection of the mouth and in endodontics. The first definitive study on chlorhexidine was performed by Löe and Schiott (1970). This study showed that rinsing for 60 seconds twice per day with 10 ml of a 0.2% (20 mg dose) chlorhexidine gluconate solution in the absence of normal tooth cleaning, inhibited plaque regrowth and the development of gingivitis. Numerous studies followed, such that chlorhexidine is one of the most investigated compounds in dentistry.

Forms

Chlorhexidine is available in various forms such as digluconate, acetate and hydrochloride salts which are sparingly soluble in water.

Structure

Chlorhexidine is a bisbiguanide formulation with cationic properties. The molecule is symmetric with two 4, chlorophenyl rings and two biguanide groups connected by a central hexamethylene chain. CHX is a strong base and, at physiologic pH, is a large dicationic molecule [1, 6-di (4-chlorophenyl-diguanido) hexane] with two positive charges distributed over the nitrogen atoms on either side of the hexamethylene bridge. By virtue of its positive charge, CHX has the ability to bind to negatively charged surfaces such as the bacterial cell wall. Since most intraoral surfaces are negatively charged, the drug gets well distributed in the oral cavity and is not easily displaced. Once bound, it can exert its bacteriostatic and bactericidal effects. The substantivity of CHX is given by the fact that once adsorbed to intraoral surfaces, it gets only slowly displaced by calcium ions from saliva. However, the dicationic nature making CHX extremely interactive

with anions is not only relevant to its efficacy and safety but also contributes to the local side effects and difficulties faced with product formulation.

In-vivo experiments using 14C-ring-labeled CHX have shown a correlation between clinical action and CHX retention in the oral cavity. These studies suggest a slow release of the antiseptic from surfaces and this was suggested to produce a prolonged antibacterial milieu in the oral cavity. CHX is available as digluconate, acetate, or hydrochloride salts with digluconate and acetate salts being water-soluble while hydrochloride salt being weakly soluble in water. Most studies and oral formulations and products use the digluconate salt, manufactured as a 20% V/V concentrate.

Introduction

Chlorhexidine (CHX) is an antibacterial used for numerous applications. It is a cationic polybiguanide (bisbiguanide) used primarily as its salts, dihydrochloride, diacetate, and digluconate. Chlorhexidine is one of those drugs which are enlisted/included in the World Health Organization's List of Essential Medicines, a list of the most important drugs needed in a basic health system. At physiologic pH, chlorhexidine salts dissociate and release the positively charged chlorhexidine cation. The bactericidal effect is a result of the binding of this cationic molecule to negatively charged bacterial cell walls. At low concentrations of chlorhexidine, this results in a bacteriostatic effect; however, at relatively higher concentrations, membrane disruption results in cell death. Chlorhexidine is active against Gram-positive and Gram-negative organisms, facultative anaerobes, aerobes, and yeasts. Chlorhexidine is particularly effective against Gram-positive bacteria in concentrations $\geq$ 1 μg/l. Significantly higher concentrations (10 to more than 73 μg/ml) are required for Gram-negative bacteria and fungi[65]. Chlorhexidine is often used as an active ingredient in various mouth rinses in periodontal medicine as one of the most efficient antiplaque agents. It has been shown to have immediate bactericidal action and a prolonged bacteriostatic action due to adsorption onto the pellicle-coated enamel surface66. If it is not deactivated, chlorhexidine lasts longer in the mouth than other mouth rinses, which is partly why it is still considered to be the most preferred antiplaque agent today. The clinical efficacy of chlorhexidine as a component is well documented by many clinical trials summarized by review articles however, continuous use of chlorhexidine for prolonged periods has also been seen to lead to staining of the tissues in the oral cavity, this being one of the most important

limitations of long term use of chlorhexidine[67]. The brownish discoloration of teeth and tongue is due to the disintegration of bacterial membranes, leading to the denaturation of bacterial proteins[68]. At the same time, disulfide ions are reduced to thiols that form dark-colored complexes with ferric (III) ions present in saliva[69].

Prolonged use of chlorhexidine has also been seen to reduce bitter and salty taste sensations, although reversibly. Chlorhexidine is deactivated by forming insoluble salts with anionic compounds, including the anionic surfactants commonly used as detergents in dentifrices and other mouth rinses, anionic thickeners such as carbomers, and anionic emulsifiers such as acrylates/C10-30 alkyl acrylate cross polymers, among many others. For this reason, chlorhexidine mouth rinses should be used with a minimal time gap and either before or after the usage of certain products with which it might have a propensity to interact.

Pharmacodynamics of Chlorhexidine in the oral cavity

After the first report of complete inhibition of plaque formation by chlorhexidine[70], it soon became evident that the effect could not be explained by a general suppression of the oral flora due to the killing off bacteria during mouth rinsing alone. Gjermo et al (1970)[71] showed that several other antibacterial agents with equal or stronger in-vitro activity against salivary bacteria were not able to prevent plaque formation in vivo, with the possible exception of benzalkonium chloride, a quaternary ammonium compound with a strong cationic charge. By use of 14C-ring-labeled chlorhexidine in-vivo, it has been shown that approximately 30% of the agent introduced in a conventional mouth rinse (10 ml 0.2% aqueous solution of chlorhexidine digluconate for one min) was retained in the oral cavity when the amount was swallowed. Since the penetration of any significant amount of chlorhexidine through the oral mucosa is unlikely, it is conceivable that the major part of it is bound to the surface structures in the oral cavity, probably to the glycoproteins covering most oral surface structures. The amount retained during mouth rinsing is dependent on concentration, dosage, and rinsing time. Chlorhexidine-protein interactions have been shown to be electrostatic in nature and thus reversible and pH-dependant. Experiments employing chlorhexidine mouthrinses with low pH supported the notion that carboxyl groups on the surface proteins played an important role in the retention of chlorhexidine from mouthrinses, and showed that retention of chlorhexidine to oral surfaces was inhibited by lowering the pH of the rinsing solutions (pH =3), resulting in a reduced plaque inhibition. Furthermore, it was shown that acidic mouthrinses after a conventional chlorhexidine mouth rinse removed most of the drug retained, in contrast to what was obtained by water after-rinses (65% vs. 25%, respectively), and strongly reduced the plaque-inhibiting capacity of the agent. These studies have given rise to an explanatory model of the pharmacodynamics of chlorhexidine in the oral cavity. The implication of the model is that the instant killing of bacteria in the mouth during rinsing with an antibacterial agent is of less importance than the agent's ability to be retained in the mouth with reversible binding. Thus, oral surfaces can act as a reservoir, releasing molecules of the agent over a prolonged period of time in amounts sufficient to maintain a bacteriostatic environment in the oral cavity and prevent bacteria from normal metabolic activities necessary for multiplication and adherence. This model of explanation had been questioned by Jenkins et al (1988). However, their experiments lack the controls necessary to support their conclusion scientifically. The effects they observed are most likely due to regular disinfection (twice daily) of enamel slabs

worn in the mouth rather than to a bacteriostatic effect obtained by a slow release of previously retained chlorhexidine. Such principles have been shown to work earlier, and are not restricted to agents with substantive properties. It has been suggested that chlorhexidine may be involved in the inhibition of adherence of bacteria to surfaces and to each other by competing with calcium for retention sites and thus may prevent the formation of calcium bridges between bacteria and oral surfaces or between bacteria (Rolla and Melsen 1975). There are indications that high concentrations of calcium may inhibit the effect of chlorhexidine in-vivo, and Skjorland et al (1978) have shown a certain effect upon plaque formation by magnesium ions which do not possess antibacterial activity, supporting the idea that non-specific chemical reactions are working in-vivo. Other observations question whether these non-specific interactions are of importance in bacterial adhesion. However, chlorhexidine may probably also inactivate glucosyltransferase, which is believed to be important in bacterial adhesion[71]. Bonesvoll and Olsen (1974) showed that the influence of plaque-free teeth on the amount of chlorhexidine retained in-vivo was negligible and concluded that the oral mucosa constituted the most important structure for the retention of the agent in the mouth. However, they also showed that a significant amount could be retained in plaque on the teeth. This may have importance when the caries-inhibiting effect of chlorhexidine is explained. In their comparative study, Gjermo et al (1970) found that the only agent showing a significant effect on plaque formation in addition to chlorhexidine was benzalkonium chloride. This formulation belongs to the family of quaternary ammonium compounds which carry a strong positive charge, as do the bisbiguanides. Thus, the quaternary have been shown to be retained in the human oral cavity to an even higher degree than is chlorhexidine. The same authors showed that rinsing twice daily with equimolar concentrations of cetylpyridinium chloride, benzalkonium chloride, and chlorhexidine revealed some plaque inhibitory effect of the quaternary, but not at all comparable with that of the bisbiguanide. However, clearance studies showed that the concentration of the agents in saliva following single mouthrinses was very different for chlorhexidine and the two quaternary compounds. After 4 hrs, the concentrations of the quaternary in the saliva were significantly lower than that of chlorhexidine and probably below bacteriostatic concentrations. Chlorhexidine, on the other hand, could be detected in bacteriostatic concentrations after 8 hrs and was also found in detectable concentrations up to 24 hrs after a single mouth rinse with 10 ml of a 2.2 mmol/L aqueous solution for 1 min. In addition to this, it was also shown that rinsing four times a day with the quaternary ammonium compounds resulted in plaque index figures

similar to those obtained when chlorhexidine was used twice daily. These results clearly demonstrated not only the importance of the initial retention of the agent, but also the significance of the rate of clearance from the binding sites. Besides, other cationic agents-piperazine and octenedine have been suggested for chemical control of plaque. There is reason to believe that these agents may exhibit pharmacodynamic properties in the oral cavity similar to those of the bisbiguanides and the quaternary ammonium salts, and thus constitute interesting future alternatives. However, until now, piperazine and octenedine have not been tested sufficiently to be recommended for clinical use.

Pharmacokinetics of Chlorhexidine in the oral cavity

Extensive research, extending back for more than a decade, has developed chemotherapeutic approaches for the control of microbial plaque and oral infections with both over-the-counter preparations and prescription drugs being available in abundance. Drugs for oral disease therapy have been traditionally administered by localized topical application in the form of mouthwashes, gels and toothpaste, and rarely by systemic means. Studies also have led to the development of slow-release devices capable of rendering the much higher drug concentrations at the disease site for prolonged periods. Pharmacokinetic characteristics often dictate therapeutic outcomes (Goodson 1987). Agents for drug therapy of oral infections vary greatly in those characteristics that relate most directly to pharmacokinetics: potency, permeability, intrinsic efficacy, and substantivity. In addition, regardless of the agent used or the mode of administration, salivary and crevicular fluid flow greatly affect the success of drug therapy for oral diseases.

Drug Characteristics

Several characteristics must be considered in the choice of drugs and agents for use in controlling plaque and oral infection, and selection of an appropriate delivery system. These include toxicity, potency, permeability, intrinsic efficacy and substantivity. Except for the gingiva, dorsum of the tongue, and hard palate, the oral mucous membranes are covered with non-keratinizing epithelia, a tissue efficient in absorbing many topically applied drugs and agents. As a result, drugs destined for topical oral use must have low acute and chronic toxicity. Drug potency is exceedingly important in the determination of drug dose, frequency and route of

administration, and formulation. Some of these- such as antibiotics, sanguinarine, and chlorhexidine- have very high potencies. The more potent agents are preferable because of the relative ease of maintaining effective antibacterial levels. Permeability and solubility characteristics are important determinants of efficacy. Many of the topically applied agents in use- including chlorhexidine, sanguinarine, and cetylpyridinium- are large, highly-charged molecules that are poorly absorbed and exhibit low toxicity. The intrinsic efficacy of a drug is the fraction of the maximum achievable effect that can be obtained, usually reported as a percentage. The maximum intrinsic efficacy of an antibacterial agent would be its ability to inhibit microbial growth completely. None of the agents used in mouth rinses is able to achieve this in clinical application. Evans et al (1977) tested the inhibitory effects of chlorhexidine on the growth of Streptococcus mutants. A high degree of substantivity is one of the most important characteristics of drugs and agents to be used for the control of microbial plaque. The term conveys the idea of a prolonged association between a material and a substrate, an association that is greater or more prolonged than would be expected with simple mechanical deposition. Agents with high substantivity manifest non-specific binding by Van der Waals forces, ionic attraction, hydrophobic attraction or covalent binding to sites other than the primary site of the drug action, such as its receptor. Agents with high substantivity are highly desirable for oral topical application, provided the non-specific binding compartment is in equilibrium with the site of action, a significantly large fraction of the administered dose is bound, and the dissociation constant from the non-specific binding site reservoir is sufficiently high to provide therapeutic levels of free drug or agent. When the reservoir of the non-specifically bound drug is not in equilibrium with the site of action, no beneficial drug effect is seen. For example, a solution of erythrosine dye used as a mouthwash does not reach equilibrium with fluid in periodontal pockets, nor do antibacterial mouth rinses affect the subgingival pocket flora. Agents vary greatly with regard to the fraction of the administered dose that remains in the non-specific binding compartment. For example, approximately 30% of the total amount of chlorhexidine in 10 ml. of a 0.2% rinse held in the mouth for one minute is retained. The association constant of the agent for its binding site needs to be sufficiently high to result in high substantivity, but sufficiently low to permit the release of pharmacologically active concentrations of the agents over time. Chlorhexidine was significantly more effective in suppressing plaque formation and in reducing pre-formed plaque than was the placebo or sanguinarine, even though the minimum inhibitory concentration for 90% of the organisms tested was four times greater for chlorhexidine

than for sanguinarine. Thus, both of these agents demonstrate in-vitro antimicrobial activity and substantivity. Drug concentration in saliva following a rinse can be considered a reflection of the amount of free drug available for diffusion from bound sites. If affinity is high, low levels will appear in the saliva, and disappearance will be rapid. Antibacterial activity persists in saliva for at least 2 hrs following a chlorhexidine rinse, and the half-time of release into saliva is approximately 2 hrs. The first point to be made from this simulation is that individuals may differ substantially in the benefit they receive from daytime rinsing. The clearance of chlorhexidine in a "high retention" subject (T1/2 = 4 hrs) is compared with that in a medium-retention subject (T1/2 =1.75 hrs). With the high-retention subject, antibacterial activity was maintained for approximately eight hours from a single daytime rinsing. With the medium-retention subject, antibacterial activity was maintained for three hours. Although salivary flow rate differences were not evaluated in the study by Bonesvoll (1977), it seems likely that some, if not all, of the differences between rates of subject clearance of chlorhexidine, could be attributed to the salivary flow rate differences. Regardless of the cause of individual differences in chlorhexidine clearance, it is clear that for optimal results, the frequency of rinsing should be adjusted for individual drug clearance. The second point illustrated in this simulation is that a single rinse in the evening will likely maintain antibacterial activity for the entire night-time period, regardless of the retention characteristics of the subject. This is primarily due to the low salivary flow rates that occur during sleep. For this reason, the same principle would be applicable to individuals with xerostomia. The selection of an appropriate delivery system is an important ingredient in the use of antimicrobial agents to control microbial plaque and treat oro-dental infections. Mouth rinses exhibit a characteristic exponential concentration profile. Initial concentrations are generally from 20 to 50 times the MIC. Following expectoration, drug levels fall rapidly to approximately one-tenth of their initial concentration and decline exponentially with halftimes of from 0.5 to 4 hrs. By initial treatment at high concentrations, antibacterial activity can be sustained for periods of several hours, depending on the substantivity characteristics of the agent used and the salivary flow rate. Pharmacokinetic studies with Chlorhexidine gluconate oral rinse indicate approximately 30% of the active ingredient, Chlorhexidine gluconate, is retained in the oral cavity following rinsing. This retained drug is slowly released into the oral fluids. Studies conducted on human subjects and animals demonstrate Chlorhexidine gluconate reached a peak of 0.206 μg/g in humans 30 mins after they ingested a 300-mg dose of the drug. Detectable levels of chlorhexidine gluconate were not

present in the plasma of these subjects 12 hours after the compound was administered. Excretion of chlorhexidine gluconate occurred primarily through the feces (-90%). Less than 1% of the chlorhexidine gluconate by these subjects was excreted in the urine.

Mode of action

Chlorhexidine is a cationic bisbiguanide with a broad spectrum of antibacterial activity, low mammalian toxicity and a strong affinity for binding to skin and mucous membranes (Denton 1991). Chlorhexidine has a wide spectrum of activity encompassing gram-positive and gram-negative bacteria, yeasts, dermatophytes and some lipophilic viruses[72]. Its antimicrobial activity is of the membrane-active type[73]. The antibacterial action of the biguanides has been reviewed by Woodcock (1988) and related to the mechanism of action of chlorhexidine proposed by Russell and Chopra (1990) and Denton (1991). Interestingly, and critically, Chlorhexidine shows different effects at different concentrations; at low concentrations, the agent is bacteriostatic, whereas at higher concentrations the agent is bactericidal. The actual levels at which the bacteriostatic and bactericidal effects manifest themselves vary between bacterial species[74]. The antibacterial action of Chlorhexidine is substantiated on the basis that the bacterial cell membrane is characteristically negatively charged. The cationic chlorhexidine molecule is rapidly attracted to the negatively charged bacterial cell surface, with specific and strong adsorption to phosphate-containing compounds. This alters the integrity of the bacterial cell membrane and chlorhexidine is attracted towards the inner cell membrane. Chlorhexidine binds to phospholipids in the inner membrane, leading to increased permeability of the inner membrane and leakage of low- molecular-weight components, such as potassium ions. At this bacteriostatic (sublethal) stage, the effects of chlorhexidine are reversible; removal of excess chlorhexidine by neutralizers allows the bacterial cell to recover (Denton 1991). This implies that the structural changes to the cytoplasmic membrane caused by low levels of chlorhexidine are minor compared with the gross damage caused by higher concentrations (bactericidal levels) of the agent. Increasing the concentration of chlorhexidine causes progressively greater damage to the membrane [75,76]. As the concentration of chlorhexidine increases, leakage of low molecular weight cytoplasmic components falls, reflecting the coagulation and precipitation of the cytoplasm by the formation of phosphate complexes such as adenosine triphosphate and nucleic acids. Electron micrographs show the cytoplasm of such cells to be chemically precipitated; this bactericidal stage is irreversible. It has been difficult to demonstrate specific binding sites in the

membrane for chlorhexidine, mainly due to the several different effects the agent causes by disrupting the membrane and also due to the paucity of data in this area. Working with alexidine and chlorhexidine, Chawner and Gilbert[82] suggest that there may either be specific binding sites for these molecules in the bacterial membrane or different intramolecular interactions of the two molecules at the membrane and the differences in the end group substitution between the biguanides affecting their ability to produce lipid domains in the cell membrane. Russell and Furr (1986) have suggested that the outer membrane in some mutant Escherichia coli strains may confer some mechanism by which the bacteria are less susceptible to chlorhexidine, wherein the inner membrane does not seem to be involved. The difference in effects of chlorhexidine on the outer and inner membranes suggests some degree of specificity of the action of chlorhexidine on the membrane(s). Addy and Kornman (1986)[83] ascribed chlorhexidine's superior antiplaque activity to its property of persistence (substantivity). Kornman (1986) differentiated antiseptics into first- and second-generation antiplaque agents, depending on whether or not they exhibit the ability to persist within the oral cavity. At physiological pH, chlorhexidine is a large dicationic molecule, (1, 6-di (4-chlorophenyl- diguanido) hexane, with the positive charge distributed over the nitrogen atoms on either side of the Hexamethylene Bridge. Thus, chlorhexidine has the ability to adsorb onto negatively charged surfaces, such as bacterial cell membranes, where it exerts its bacteriostatic and bactericidal effects. Chlorhexidine also binds to the different surfaces within the oral cavity (teeth and mucosa) and also to the pellicle and saliva; for example, after a single rinse with chlorhexidine, the saliva itself exhibits antibacterial activity for up to 5 hours, whereas persistence at the oral surfaces has been shown to suppress salivary bacterial counts for over 12 hours[84]. Thus, although chlorhexidine is able to bind to different anionically charged elements within the oral cavity, it also, importantly, maintains its antibacterial activity for several hours[85]. Given that plaque formation occurs on the tooth surface, paradoxically, the binding of chlorhexidine to the pellicle-covered tooth surface was considered to be small compared with that involved in chlorhexidine- protein interactions at other oral surfaces considered that neither the teeth nor the tongue was of major importance as receptor sites for chlorhexidine in preventing the accumulation of sucrose-enhanced plaque formation. This led to speculation that it was the interaction of chlorhexidine at sites other than the tooth surfaces that were important for chlorhexidine's antiplaque effect. This involved a "reservoir" of chlorhexidine slowly desorbing from all the oral surfaces, resulting in a bacteriostatic milieu in the mouth. Rolla and

Melsen (1975) postulated that chlorhexidine, desorbed from the oral mucosa, might have three mechanisms of plaque inhibition.

1. an influence on pellicle formation by blocking the acidic groups on the salivary glycoproteins, thus reducing the protein adsorption onto the tooth surfaces;
2. an influence on the adsorption of plaque onto the tooth surfaces by binding to the bacterial surface in sublethal amounts; and
3. an influence on the formation of plaque by precipitating the agglutination factors in saliva and displacing calcium from the plaque matrix.

However, Jenkins et al (1988)86 showed that plaque growth on enamel inserts was inhibited equally well by 0.2% chlorhexidine applied topically or by rinsing. They considered, through electron microscopy of the enamel inserts, that chlorhexidine achieved its antiplaque effect as a result of immediate bactericidal action at the time of application, followed by a prolonged bacteriostatic action as a result of chlorhexidine adsorbing to the pellicle-coated enamel surface. Thus, bacterial attachment to the enamel surface is not entirely inhibited, but bacterial growth is delayed by the bacteriostatic effects at the surface. This implied that tooth surface-bound chlorhexidine was of greater importance in preventing plaque formation than was first thought. It may be worth attempting to explain the difference in these findings to that of Waaler and Rolla (1985) this may lie in the findings of Davies (1973), who considered that sucrose enhancement of plaque formation may reduce the effects of chlorhexidine, such that the low “bacteriostatic” levels of chlorhexidine are no longer able to penetrate the cell wall of plaque bacteria grown in the presence of excess sucrose. Based on the work by Jenkins et al (1988), it is worth reappraising the concept of an oral reservoir of chlorhexidine as the basis of the antiplaque activity of this antiseptic. For this oral reservoir to create a “bacteriostatic milieu”, there must be adsorption of chlorhexidine to the oral mucosa and saliva at the time of application, followed by progressive desorption over time, resulting in a reduction in the bacterial challenge to the tooth surface through irreversible adsorption of chlorhexidine to the bacterial cell. The chlorhexidine must move from the mucosa, saliva etc. to the bacterial cell. The methods of analysis of desorbed chlorhexidine do not distinguish between “free” chlorhexidine (if such an entity can occur in a mouth rinse with proteinaceous material), reversibly bound chlorhexidine and irreversibly protein-bound chlorhexidine. So, there is no evidence that chlorhexidine slowly desorbs from the oral surfaces at all; it is always likely to be bound to saliva, epithelial cells, pellicle, bacteria etc.

In addition, unless one believes there is a preferential removal from the saliva (by chlorhexidine) of the bacteria that are able to begin colonizing the tooth surface, one cannot easily explain why the oral reservoir has an antiplaque effect. Although chlorhexidine may reduce the salivary bacterial counts- a single rinse with chlorhexidine can reduce the oral flora by over 90% for several hours, many millions of bacteria present in the saliva and on the oral surfaces are still not affected. As the oral cavity cannot be sterilized, there must be a continual challenge to the tooth surface by bacteria that are able to begin the process of plaque formation. As the salivary-bound chlorhexidine patently has not eradicated putative plaque-forming bacteria, it would seem logical therefore to assume that the process of plaque prevention occurs at the tooth surface itself -by tooth-bound chlorhexidine.

Effect On Initial Plaque Formation

Immediately the following application of Chlorhexidine in the oral cavity in bactericidal concentrations, a substantial amount of the bacteria gets killed. A reduction of the numbers of bacteria in saliva from 50% to 90% has been reported, however, according to Stalfors (1962), this reduction would not be sufficient to prevent plaque formation. Taking the rapid reproduction of bacteria in the oral cavity into consideration, he calculated that 99% of the bacteria had to be killed twice daily to prevent plaque formation. However, due to the substantiveness (retention and sustained release) of Chlorhexidine and related compounds, a bacteriostatic concentration of these drugs may be maintained in the saliva for several hours after the application. Bacteria in a bacteriostatic phase do not multiply, and their metabolic activity is strongly inhibited, probably impairing their ability to produce the substances necessary for adherence. In addition, the presence of strong cationic molecules (bisbiguanides, quaternary ammonium compounds) may interfere with the non-specific adhesion mechanisms by competing with, for instance, calcium ions for the retention site. A selective effect upon some of the bacteria involved in early plaque formation may also play a role in this respect.

Effect Upon Established Plaque

It has been shown that frequent applications (six times per day) of Chlorhexidine mouthrinses (10ml of 0.2% Chlorhexidine) will cause dispersion and elimination of existing plaque. Topical application of strong concentrations of this drug and other antimicrobial agents may have similar

effects. Bonesvoll and Olsen (1974) showed that plaque was present on the teeth when Chlorhexidine was applied as a conventional mouth rinse (10ml of 0.2% Chlorhexidine) would retain a substantial amount of the agent. Plaque-retained Chlorhexidine is probably bound to phosphate groups on bacterial surfaces and to sulfates in thiol groups of surface-bound bacterial enzymes. Chlorhexidine and other cationic substances and metal ions were also found to inhibit acid production in established plaque under experimental conditions in humans. The duration of this effect was found to be dependent on the agent's ability to be retained in the oral cavity and in plaque, and also on their rate of release from the binding sites. In addition to reducing the acidic challenge to the tooth surface per se, the effect may also be that acidophilic and cariogenic organisms such as S mutants will have less favorable conditions for colonization the expected caries-inhibiting effect of Chlorhexidine has support in clinical experiments, but the mechanisms behind the effect are still not known. However, it seems that the presence and amount of S. mutants may successfully be used for screening purposes to select subjects at high risk and to evaluate the effect of anti-bacterial treatment aimed at establishing a noncariogenic oral flora.

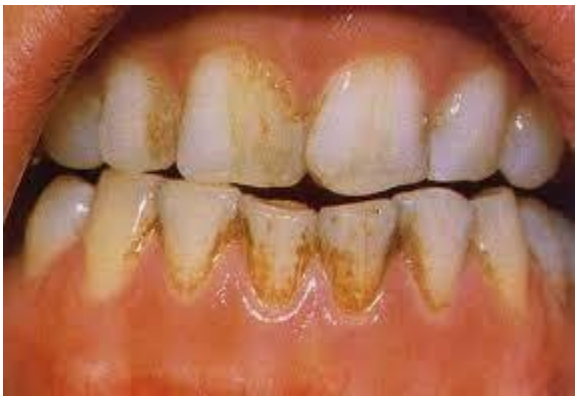
In the nutshell, there are three possible mechanisms suggested for the antiplaque action of chlorhexidine

1. The effective blocking of acidic groups of salivary glycoproteins reduces their adsorption to hydroxyapatite and the formation of acquired pellicle;
2. The ability of bacteria to bind to tooth surfaces may be reduced by the adsorption of chlorhexidine to the extracellular polysaccharides of their capsules or glycocalyces; this mechanism is of particular interest as further studies have demonstrated that when sucrose is added to bacterial suspensions in vitro, the antibacterial effect of chlorhexidine is actually reduced. Production of extracellular polysaccharides increases in the presence of sucrose. A greater proportion of the drug is then absorbed by the cell coatings and less is available to act upon the cellular membrane of the microorganisms to effect the direct killing of them.
3. Chlorhexidine may compete with calcium ions agglutination factors in plaque; laboratory studies have suggested that chlorhexidine can bond with hydroxyapatite. However, it is now considered that it is the affinity of Chlorhexidine for the acidic proteins in the pellicle, plaque, calculus, oral mucosa and on the surfaces of bacterial cell membranes which is of greater clinical significance than its affinity for hydroxyapatite.

Potential adverse effects of Chlorhexidine: Since 1954, chlorhexidine has been used clinically for several purposes. The effect of chlorhexidine on oral mucosa has been tested and its calculus-inhibiting capacity has also been studied by Schroeder (1969). However, no harmful effect on the oral mucosa had been reported. However, later in 1971, Flora and co-workers conducted a study over a 4-month period on the side effects of chlorhexidine. The clinical trial noted that the use of chlorhexidine was associated with problems that merit further consideration. These included oral and other associated side effects.

Adverse effects pertaining to oral cavity

A. Staining: When used as a mouth rinse, Chlorhexidine has a number of local oral side effects[87], the most common being a brownish discoloration of the teeth, some restorative materials, the mucosae and notably, the dorsum of the tongue, decreasing patient compliance. The amount of staining seems to be dependent on the mode of application, concentration, and presence of the potential discoloring agents within the extraneous factors including diet. The mechanisms of the origin of the Chlorhexidine - staining are still debated.



1. Degradation of the chlorhexidine molecule to release parachloraniline: Degradation of the chlorhexidine molecule to release parachloraniline appears to occur on storage or as a result of metabolic processes;
2. Catalysis of Maillard reactions: Non-enzymatic browning reactions catalyzed by chlorhexidine are a theoretical possibility; however, evidence is inconclusive. A series of chemical reactions between sugars and amino acids called the non-enzymatic browning reactions or Maillard reactions lead to the production of metabolic end-products that are

responsible for this prominent side effect. In clinical testing, 56% of oral rinse users exhibited a measurable increase in staining on the facial aspects of anterior teeth, compared to 35% of control users after 6 months;

3. Protein denaturation with metal sulfide formation: Protein denaturation produced by chlorhexidine with the interaction of exposed sulfide radicals with metal ions from food sources are also theoretically considered to be a possible cause for such staining seen with prolonged chlorhexidine usage
4. Precipitation of anionic dietary chromogens: Precipitation of anionic dietary chromogens by cationic antiseptics, including chlorhexidine and numerous polyvalent metal ions as an explanation for the phenomenon of staining is well supported. Thus, the locally bound antiseptics or metal ions on mucosa or teeth can react with polyphenols in dietary substances to produce staining.

B. Bitter taste/taste disturbance (dysgeusia): Aqueous solutions of CHX have a very bitter taste leading to a transient change in taste perception (dysgeusia). Objective testing of the taste sensation has also confirmed a transient effect on the perception of sweet and salt taste with salt taste preferentially being affected;

C. Mucosal desquamation: Desquamation and subsequent, ulcerations and erosions of the oral mucosa in connection with mouth rinses with bisbiguanides have been sporadically reported and have been explained by precipitation of the mucin layer weakening its lubricating effect. A few cases of painful desquamations of the oral mucosa have been reported after chlorhexidine mouth rinses. This side effect though is concentration-dependent. To maintain the dose and thereby the effect, a double volume has to be rinsed. Dilution of the 0.2% formulation to 0.1%, but rinsing with the whole volume to maintain dose, usually alleviates the problem. Erosions are rarely seen with 0.12% rinse products used at 15 ml volume.

In addition to these, certain adverse effects pertaining to the para-oral region are also seen which include:

D. Unilateral or bilateral parotid swelling: Although not a common an adverse effect, this is seen as an extremely rare occurrence with no plausible explanation. The reports of virus infections (parotitis) in connection with chlorhexidine mouth rinses might probably be purely co-incidental, but cannot be completely disregarded. Secretory IgA, which is known to possess antiviral activity, accumulates on the mucous membrane. Possible precipitation of acidic proteins

in the mucin layer coating mucous membrane of the oral cavity may thus interfere with the antiviral mechanisms;

E. Enhanced supragingival calculus formation: This effect may be due to the precipitation of salivary proteins onto the tooth surfaces, thereby increasing the pellicle thickness and/or precipitation of inorganic salts onto the pellicle layer.[40] Zannata et al in 2010 conducted a study on staining and calculus formation after 0.12% chlorhexidine rinses in plaque-free and plaque-covered surfaces in a randomized controlled trial. The presence of plaque was seen to increase as a prominent side effect of chronic usage of 0.12% CHX. These results strengthened the necessity of biofilm disruption prior to the start of CHX mouthrinses in order to reduce these side effects. Certainly, pellicle formed under the influence of chlorhexidine showed an early and highly calcified structure.

Chlorhexidine products

Chlorhexidine has been formulated into a number of products.

Mouth rinses

Aqueous alcohol solutions of 0.2% chlorhexidine were first made available for mouth rinse products for twice-daily use in Europe in the 1970s. A 0.1% mouth rinse product also became available; however, questions were raised over the activity of the 0.1% product and in some countries, the efficacy of this product is less than would be expected from a 0.1% solution. Later, in the US, a 0.12% mouth rinse was manufactured but to maintain the almost optimum 20 mg doses derived from 10 ml of 0.2% rinses, the product was recommended as a 15 ml rinse (18 mg dose). The studies revealed equal efficacy for 0.2% and 0.12% rinses when used at appropriate similar doses. More recently alcohol-free chlorhexidine rinses have become available, some formulated with the inclusion of 0.05% CPC. Such formulations have been shown to possess equivalent effects at inhibiting plaque and gingivitis compared to alcohol-containing chlorhexidine rinses but with better taste acceptability with the non-alcoholic rinse.

Gel

A 1% chlorhexidine gel product is available and can be delivered on a toothbrush or in trays. The distribution of the gel by toothbrush around the mouth appears to be poor and preparations must be delivered to all tooth surfaces to be effective. In trays, the chlorhexidine gel was found to be

particularly effective against plaque and gingivitis in handicapped individuals. The acceptability of this tray delivery system to the recipients and the carers was found to be poor. More recently, 0.2% and 0.12% chlorhexidine gels have become available.

Sprays

Sprays containing 0.1% and 0.2% chlorhexidine are commercially available in some countries. Studies with the 0.2% spray have revealed that small doses of approximately 1–2 mg delivered to all tooth surfaces produce similar plaque inhibition to a rinse with 0.2% mouth rinses. Sprays appear particularly useful for the physically and mentally handicapped groups, being well received by individuals and their carers.

Toothpaste

Chlorhexidine is difficult to formulate into toothpaste for reasons already given and early studies produced variable outcomes for benefits to plaque and gingivitis. More recently, a 1% chlorhexidine toothpaste with and without fluoride was found to be superior to the control product for the prevention of plaque and gingivitis in a 6-month home use study (Yates et al. 1993). Stain scores, however, were markedly increased as was supragingival calculus formation, and the manufacturer did not produce a commercial product. For a short time, a commercial product was available, having been shown to be efficacious for both plaque and gingivitis. Although effective, chlorhexidine products based on toothpaste and sprays produce similar tooth staining to mouth rinses and gels; taste disturbance, mucosal erosion, and parotid swellings tend to be less or have never been reported.

Varnishes

Chlorhexidine varnishes have been used mainly for prophylaxis against root caries rather than an antiplaque depot for chlorhexidine in the mouth.

Slow-release vehicles

A chlorhexidine chip has been produced commercially for placement into periodontal pockets as an adjunct to scaling and root planing.

Clinical uses of chlorhexidine

Despite the excellent plaque inhibitory properties of chlorhexidine, widespread and prolonged use of the agent is limited by local side effects. Moreover, because of the cationic nature of chlorhexidine and therefore its poor penetrability, the antiseptic is of limited value in the therapy of established oral conditions including gingivitis and is much more valuable in the preventive mode. A number of clinical uses, some well researched, have been recommended for chlorhexidine.

As an adjunct to oral hygiene and professional prophylaxis

Oral hygiene instruction is a key factor in the treatment plan for patients with periodontal disease and as part of the maintenance program following treatment. Adequate plaque control by periodontal patients is therefore essential to successful treatment and the prevention of recurrence of the disease. Chlorhexidine should therefore increase the improvement in gingival health through plaque control, particularly following professional prophylaxis to remove existing supra- and immediately subgingival plaque. There is, however, a potential disadvantage of using such an effective chemical plaque-control agent at this stage of the periodontal treatment plan. Thus, following oral hygiene instruction, it is normal, usually by the use of indices, to quantify the improvement in plaque control by patients so instructed and, in particular, the improvement at specifi c sites, which previously had been missed by individual patients. By virtue of the excellent plaque-control effects of chlorhexidine, the response to oral hygiene instruction cannot be accurately assessed since the antiseptic will overshadow any deficiencies in mechanical cleaning. Indeed, as the original research demonstrated, with chlorhexidine mouth rinse patients could maintain close to zero levels of plaque following professional prophylaxis without using any form of mechanical oral hygiene. Nevertheless, chlorhexidine mouth rinse may be of value in maintaining oral hygiene following scaling and root planing when adequate tooth brushing may be compromised by post-treatment soreness or sensitivity.

Postoral surgery including periodontal surgery or root planing

Chlorhexidine may be used post-operatively since it offers the advantage of reducing the bacterial load in the oral cavity and preventing plaque formation at a time when mechanical cleaning may be difficult because of discomfort. In periodontal surgery, periodontal dressings

have largely been replaced by the use of chlorhexidine preparations, in particular mouth rinses, since healing is improved and discomfort reduced. Regimens vary but chlorhexidine should be used immediately post-treatment and for periods of time until the patient can re-institute normal oral hygiene. Depending on the appointment schedule, chlorhexidine could be used throughout the treatment phase and for periods of weeks after completion of the treatment plan. If dressings are used, chlorhexidine is of limited value to the post-operative site since it does not penetrate beneath the periodontal dressings. Although chlorhexidine rinses are probably used after root planing by many clinicians, evidence of therapeutic benefit has only recently been published88. The idea of full-mouth disinfection using chlorhexidine both supra- and subgingivally as an adjunct to scaling and root planing has been assessed by one group in a number of papers since 1995. In the event, few adjunctive benefits could be shown89. It appeared that the more dominant factor was the time over which the non-surgical treatment plan was completed. Thus, root planing performed totally within 24 hours was more effective than root planing completed over more conventional periods of several weeks. Similar clinical research, however showed no difference between root planing completed within 24 hours compared to within several weeks.

For patients with jaw fixation

Oral hygiene is particularly difficult when jaws are immobilized by such methods as intermaxillary fixation. Chlorhexidine mouth rinses have been shown markedly to reduce the bacterial load, which tends to increase during jaw immobilization and to improve plaque control. The more recent trend to use sub-dermal or sub-mucosal plates to stabilize bony fragments probably impedes oral hygiene procedures to a lesser degree, providing there are no oral mucosal lacerations. The influence of these factors on oral hygiene and therefore the role of chlorhexidine formulation has never been investigated.

For oral hygiene and gingival health benefits in the mentally and physically handicapped

Chlorhexidine has been found particularly useful in institutionalized mentally and physically handicapped groups, improving both oral hygiene and gingival health. Spray delivery of 0.2% solutions was found particularly useful and acceptable to patients and care workers [90].

Medically compromised individuals predisposed to oral infections

A number of medical conditions predispose individuals to oral infections, notably candidiasis. Chlorhexidine is effective as an anticandidal agent but is most useful when combined with specific anticandidal drugs, such as nystatin or amphotericin B. Indications for chlorhexidine use combined with anticandidal drugs have been for the prevention of oral and systemic infections in the immunocompromised, including those with blood dyscrasias, those receiving chemotherapy and/or radiotherapy, and notably bone marrow transplant patients[91]. The value of chlorhexidine appears greatest when initiated before oral or systemic complications arise. A chlorhexidine spray was also found to produce symptomatic/psychologic oral care benefits in the terminally ill.

High-risk caries patients

Chlorhexidine rinses or gels can reduce considerably the Streptococcus mutants counts in individuals who are caries prone. Additionally, and interestingly, chlorhexidine appears synergistic with sodium fluoride and combining chlorhexidine and fluoride rinses appears beneficial to such at-risk individuals. Sodium monofluorophosphate on the other hand reduces the effect of chlorhexidine and probably vice versa. A chlorhexidine rinse product with sodium fluoride has recently become available.

Recurrent oral ulceration

Several studies have shown that chlorhexidine mouth rinses and chlorhexidine gels reduce the incidence, duration, and severity of recurrent minor aphthous ulceration[92]. The mechanism of action is unclear but may relate to a reduction in contamination of ulcers by oral bacteria, thereby reducing the natural history of the ulceration. Regimens have included three times daily use of chlorhexidine products for several weeks. Interestingly, one study showed that triclosan rinses reduce the incidence of recurrent mouth ulcers. There have been no controlled studies of chlorhexidine in the management of major aphthous ulceration or other oral erosive or ulcerative conditions, although anecdotally chlorhexidine appears ineffective. Again, this may reflect the low therapeutic potential of this and other antiseptics, and the considerable amount of proteinaceous material associated with these lesions which would both tend to inactivate chlorhexidine and block access to underlying microorganisms. A similar explanation may be propounded for the failure of chlorhexidine mouth rinses in the treatment of acute necrotizing

ulcerative gingivitis (periodontitis): further evidence for the lack of absorption into tissues and biofilms of this cationic antiseptic.

Removable and fixed orthodontic appliance wearers

Plaque control in the early stages of orthodontic appliance therapy may be compromised and chlorhexidine can be prescribed for the first 4–8 weeks. Additionally, chlorhexidine has been shown to reduce the number and severity of traumatic ulcers during the first 4 weeks of fixed orthodontic therapy.

Denture stomatitis

Chlorhexidine has been recommended in the treatment of Candida-associated infections; however, in practice even applying chlorhexidine gel to the fitting surfaces of dentures produces, in many cases, slow and incomplete resolution of the condition. Again, chlorhexidine is less effective in the therapeutic mode and it is more advantageous to treat denture stomatitis with specific anticandidal drugs and then employ chlorhexidine to prevent a recurrence. The denture itself can be usefully sterilized from Candida by soaking in chlorhexidine solutions.

Oral malodo

Rinsing with chlorhexidine as with other antiseptic mouth rinses containing CPC, triclosan, and essential oils have been suggested to be of value in reducing halitosis. Reductions in volatile sulphur compounds and morning malodor have been noted with all these chemicals[93].

Immediate pre-operative chlorhexidine rinsing and irrigation

This technique can be used immediately prior to operative treatment, particularly when air polishing, ultrasonic scaling, or high-speed instruments are to be used. Such pre-operative rinsing markedly reduces the bacterial load and contamination of the operative area, operator, and staff. Additionally, in susceptible patients, irrigation of chlorhexidine around the gingival margin reduces the incidence of bacteremia[94]. This should be seen, however, only as an adjunct to appropriate systemic antimicrobial prophylaxis. Chlorhexidine mouth rinsing now features as an adjunct to antibiotic prophylaxis in the new UK guidelines.

Subgingival irrigation

Numerous antimicrobial agents have been used as subgingival irrigants in the management and treatment of periodontal diseases[95]. Alone, irrigation with antimicrobial agents produces effects little different from using saline and they are of short duration, suggesting that the action is a washing-out effect. Irrigation combined with root planing appears to provide no adjunctive benefits.

POVIDONE – IODINE

The antibacterial properties and uses of iodine-povidone in medicine are well established. The natural element, iodine, has been used for more than 150 years in mucosal antisepsis, in the therapy of skin infections and burns, and in wound management. Yet, only after the introduction of Povidone-iodine in the 1960s, was it possible to employ this highly efficient microbicide to a wide variety of bacterial, fungal and viral infections. Despite its impressive antimicrobial properties, povidone-iodine is not widely used in the prevention and treatment of oral infections in the USA and Europe. Povidone-iodine is water-soluble, does not irritate healthy or diseased oral mucosa, and exhibits no adverse side- effects, such as discoloration of teeth and tongue and change in taste sensation, as seen with chlorhexidine. Blue povidone-iodine stains on starched linen will wash off with soap and water. Other types of povidone-iodine stains can be readily removed with a 5% sodium thiosulfate solution. Povidone-iodine has the potential to induce hyperthyroidism due to excessive incorporation of iodine in the thyroid gland and should therefore be used only for short periods of time. Contraindications are patients with iodine hypersensitivity and thyroid pathosis, as well as pregnant and nursing women in order to protect the infant. For subgingival irrigation, an effective concentration is 10% povidone-iodine applied repeatedly by an endodontic syringe to obtain a contact time of at least 5 min. This is generally performed upon completion of each session of scaling and root planing, but may also be done prior to mechanical debridement to reduce the risk of bacteremia, particularly in medically compromised individuals and in patients with severe gingival inflammation.

In summary, as long as gargling with Povidone-iodine is performed according to the guidelines given in the drug package insert, the urinary excretion of iodine is about 5 mg at most, and the majority of normal subjects will remain euthyroid. However, in certain susceptible subjects, moderate to severe iodine-induced hypothyroidism may develop, particularly when gargling is done for a prolonged period, as in the present case. However, this hypothyroidism resolves spontaneously and rapidly upon discontinuation of the gargling.

SODIUM HYPOCHLORITE

Sodium hypochlorite (household bleach) has been used as a disinfectant for more than 100 years, as an antiseptic for more than 85 years, and as an endodontic irrigant for more than 75 years. Hecker in 1913 used antiform in (concentrated sodium hypochlorite solution) as an epithelial specific solvent in the treatment of periodontal disease. Sodium hypochlorite has many of the properties of an ideal antimicrobial agent, including broad antimicrobial activity, rapid bactericidal action, relative non-toxicity at use concentrations, no color, no staining, ease of access, and very low cost. The active species is undissociated hypochlorous acid (HOCl). Hypochlorite is lethal to most bacteria, fungi and viruses. Activity is reduced by the presence of organic material, heavy metal ions and low pH. Hypochlorite solutions will gradually lose strength so that fresh solutions should be prepared daily, especially if the solution is not stored in closed brown opaque containers. Disadvantages include irritation of mucous membranes when used in high concentrations, bleaching of colored fabrics and corroding effect on some metals. No contraindications exist. A sodium hypochlorite solution for subgingival irrigation can be prepared from household bleach that usually contains 5.25–6.0% of available chlorine. If 1 part bleach is combined with 49 parts water, the resulting solution will contain an appropriate working concentration of about 0.1% or 1000 p.p.m. of available chlorine. In actual use situations, a working bleach solution can be obtained by adding 1 teaspoon (5 ml) household bleach to 250 ml water (approximately 2 large drinking glasses), and deliver the bleach solution subgingivally via a commercial oral irrigator at a high-pressure setting. The lowest concentration of chlorine that reliably inactivates test bacteria in vitro is 0.01%. In experimental biofilms with various endodontic/periodontal pathogens, the highest bactericidal activity was obtained with 2.25% sodium hypochlorite and 10% povidone-iodine followed by 0.2% chlorhexidine. At low concentrations, sodium hypochlorite can be used as a debriding and topical antibacterial agent for wounds and skin ulcers without inhibiting fibroblast activity. Kalkwarf et al. showed histologically that subgingival application of sodium hypochlorite solution might be adequately controlled to provide hemolysis of the soft tissue wall of a periodontal pocket with minimal effect upon adjacent tissues, and may exert no adverse effect upon healing. Kalkwarf et al. recommended the use of subgingival sodium hypochlorite irrigation in the maintenance phase of periodontal therapy. That sodium hypochlorite application might improve periodontal histological healing was suggested by Perova et al. who found markedly better regeneration of

connective tissue at the gingival base of sites that had received an application of 0.1% hypochlorite for 10 min during periodontal surgery than in control sites. However, dilute sodium hypochlorite treatment may not enhance the outcome of pedicle flap surgery for the coverage of gingival recession. Lobene et al. showed that subgingival irrigation with 0.5% sodium hypochlorite (Dakin's solution) caused significantly greater and longer-lasting reduction in plaque and gingivitis than irrigation with water. In localized juvenile periodontitis lesions, gingival curettage with dilute sodium hypochlorite irrigation caused a greater reduction in proportions of subgingival spirochetes than water irrigation. Dilute sodium hypochlorite applied to extracted teeth resulted in more than 80- fold less adherent endotoxin compared to water application. The American Dental Association Council on Dental Therapeutics proposed using dilute sodium hypochlorite as a topical antiseptic, for irrigation of wounds and as a mouth rinse. Considering sodium hypochlorite's significant antimicrobial properties and good safety profile and the promising research data, it seems rational to recommend hypochlorite subgingival irrigation as part of patients' oral self-care regimen.

CONCLUSION

The number and use of oral hygiene products have grown enormously in recent years and, as an example, hundreds of millions of pounds per year are spent on oral hygiene products in the UK and presumably billions worldwide. There can be no doubt that the oral hygiene industries, through their collaboration and research with the dental profession and the promotion of their products have, in no small way, contributed to the improvement in dental health seen in many countries. Claims for efficacy of oral hygiene products, however, are frequently made and it is essential that these are supported by scientific evidence. Without such evidence, the profession and the public may be confused or misled. The dental profession is, however, faced with a large number of oral hygiene products supported by huge quantities of varied promotional literature and media advertising, which makes impossible, in many cases, any valid judgment or assessment of the efficacy or value of individual products to specific patient groups or the public as a whole. Even those with specialized interest, and research experience in specific aspects of oral hygiene product evaluation, must find validation, based on published literature, a daunting task. This is made all the more difficult since what constitutes proof of efficacy is not generally agreed even amongst so-called experts. Few countries of the world have central control over what evidence is required before efficacy claims can be made and there are very few guidelines suggesting requirements for proof of efficacy for oral hygiene products. The scientific evaluation of dental products, and for that matter, preventive and therapeutic agents in medicine as a whole, is a relatively modern concept but today must be the backbone on which to base claims of efficacy. Anecdotal and case reports, uncontrolled studies and data listed as “held on file” by manufacturers, whilst interesting, should not be used as the basis for efficacy claims. Blind, randomized, controlled clinical and laboratory studies must be the methods used today to obtain data on the activity of agents, formulations, and products. Terminology and phraseology in product claims also need to be carefully reviewed and assessed. Perhaps the greatest area for criticism must be the implied claim by the manufacturer and/or the inferences left to be drawn, from promotional material, by the dental profession or public. A classic scenario for which there is precedence can be stated as follows: A is the cause of B, C reduces A, leaving the inference to be drawn that C can control B. Perhaps nowhere is this more apparent than in the use of agents which are known to control plaque, and therefore it can be implied, without evidence, they must

control gingivitis. The now-familiar claim would be “this product reduces plaque, the major cause of gum disease”.

Similarly, creative arithmetic is not infrequently used to give inflated impressions of efficacy. Proportional differences, rather than actual differences, are not infrequently quoted, as are percentages of percentages giving hundreds of percent improvements over another product or control, yet the actual benefit is a fraction of the scoring index used. Finally, “piggyback” claims are not uncommon when a known active ingredient is formulated into a new product and equivalent efficacy to established products is assumed. It would seem reasonable here to repeat the definitions for the terminology for oral hygiene products, agreed at the European Workshop on Periodontology in 1996 which defined certain terms:

• Antimicrobial agents: Chemicals that have a bacteriostatic or bactericidal effect in vitro that alone cannot be extrapolated to a proven efficacy in vivo against plaque.
• Plaque reducing/inhibitory agents: Chemicals that have only been shown to reduce the quantity and/ or affect the quality of plaque, which may or may not be sufficient to influence gingivitis and/or caries.
• Antiplaque agents: Chemicals that have an effect on plaque sufficient to benefit gingivitis and/or caries.
• Antigingivitis agents: Chemicals that reduce gingival inflammation without necessarily influencing bacterial plaque (includes anti-inflammatory agents).

Thus, the fact that antimicrobial agents such as antiseptics kill or inhibit the growth of bacteria does not necessarily mean they will be effective plaque inhibitors. Also, the mere incorporation of a known antiplaque agent into a formulation is not a guarantee of efficacy because inactivation by other ingredients may occur. This section looks at methods that have been used to test oral hygiene products both in the laboratory and the clinic. No one protocol can provide all the answers, and research and development of agents into products is a step-by-step process, hopefully culminating in a body of evidence proving efficacy, beyond doubt, of a final product. Methods in vitro and in vivo will be summarized but animal testing will not be discussed except to acknowledge that the use of animals is still necessary for drug development, in understanding the mode of action of drugs, and particularly, in evaluating safety from a

toxicologic point of view. The evaluation of oral hygiene products on animals, however, particularly for efficacy, must be questioned on a number of scientific and moral grounds. Most laboratory and clinical methods have been developed to test antimicrobial agents but methodologies are available, or present ones could be modified, to study potential antiadhesive and plaque removal chemicals96.

While many of the agents discussed above have significant plaque inhibitory activity and many of the more effective agents share the side effects of producing tooth staining, which limits their long-term use.

Only one group of agents the bisguinadies of which chlorhexidine are the most effective, produce appreciable antiplaque activity and substantivity (oral retentiveness) with antibacterial activity and thus retain active in the mouth for long period after their use.

Practitioners must be aware of their clinical judgment as to which product or group of products and which delivery system is best for their patients. Clinical judgment should be based on the scientific validity of the products selected and their relationship to patients' specific needs.

It is relevant to employ antimicrobial medications to control effectively various types of periodontal disease. Combating periodontal infections is best accomplished by combined mechanical and chemotherapeutic efforts of the dental professional and the patient. It can be concluded that properly used systemic antibiotics and subgingival irrigation with 10% povidone-iodine (dental professionals) and 0.1% sodium hypochlorite (patients) along with oral rinsing with 0.12–0.2% chlorhexidine constitute effective, essentially safe and inexpensive antimicrobial therapies that can readily be incorporated into the current armamentarium for periodontal treatment. An effective anti-carious fluoride treatment should constitute an integrated part of periodontal therapy. Continued research into anti-infective agents to prevent and treat periodontal diseases will undoubtedly lead to even more effective therapies. With the improved knowledge of the periodontopathic microbiota and with various safe and affordable, yet effective, periodontal antimicrobial agents and therapies, the future looks bright for patients at risk of or suffering from destructive periodontal disease.

Terminology concerning oral hygiene products needs to be standardized and defined. Efficacy claims, which are implied, or rely on inferences to be drawn, should be avoided. Studies in vitro can provide supportive data to clinical investigations but cannot stand alone as proof of efficacy in vivo. Research and development of oral hygiene products needs to be step-by-step processed, making available a body of knowledge supporting the efficacy of a final formulation.

Clinical proof should be largely dependent on data from blind, randomized, controlled clinical trials conducted to the Guideline for Good Clinical Practice (GCP).

In reporting clinical trials the clinical significance of the finding should be considered. Statistical significance should not necessarily be taken as proof per se of the benefit of an oral hygiene product to the general public.

Clinical outcome, when possible, should be evaluated against side effects and the cost–benefit ratio should be determined.

Where possible, systematic reviews with meta-analysis need to be conducted to prove the efficacy of agents and products for the control of supragingival plaque.

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