Chapter 1

From urine to chlorhexidine

General introduction
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1.1 From urine to chlorhexidine

Over the centuries people have tried to find a way to keep their own teeth safe and secure onto old age. Ancient man quickly found out that rinsing with the urine of a child freshens the mouth and helps against gum diseases. Over many centuries, rinsing with human urine appears to have been very popular. The first known reference to mouthrinsing with urine for the treatment of gingivitis is in the Chinese medicine, in about 2700 BC. This custom persisted through the Egyptian, Greek and Roman period until the early 18th century (Weinberg 1948). Reflecting on history, mouthrinses have covered a long distance from the previously mentioned human urine, over salt water, mixtures of honey, oil and beer and combinations of herbal products (dill, anise) with alcohol (white wine) to the fresh-smelling and fresh-tasting rinses available today (Mandell 1988, Fishman 1997). The main ingredient of urine is ammonia, but as time went on, different items were added to and subtracted from the rinse to make mouthrinses more palatable to the human taste as well as to increase their potency to control gingival health. Unfortunately, many agents also had the potential to cause local damage to tissues and led to systemic toxicity (Dilling & Hallam 1936). Preparations for gum health however were never evaluated scientifically until the relationship of plaque bacteria to gingivitis was established (Löe et al.1965).

In the 17th century, Antonie Van Leeuwenhoek, commonly known as “the Father of Microbiology”, was the first to describe living organisms in the deposits on teeth. However, more than two centuries elapsed after his observations before Robert Koch (Münch 2003) showed the causal relationship between microbes and infectious diseases. Hereby, the scientific base for antiseptic mouthrinses was created. W.D.Miller (1890), a trained researcher in bacteriology, compared a large series of antisepsics at varying dilutions for their ability to prevent the growth or the killing of bacteria. Miller noted that many antisepsics cannot be used orally since they were injurious to general health, mucous membranes and teeth, or have to be excluded because of bad smell and taste. He pointed out that an antiseptic of any importance would always be accompanied by the greatest difficulties and that “there are places around every dentition which will remain untouched by even the most thorough application of an antiseptic or the antiseptic will reach them in such a diluted condition that it possesses little or no action”. Through this, Miller established many of the fundamentals that
the dental research community followed until today in order to develop effective antibacterial mouthrinses (Mandel 1988).

From the 20th century, various brands came onto the market. The forerunner with respect to general oral hygiene in Germany was Odol. Odol with its antibacterial formula of Salvia and Camomile was marketed by Switzerland’s Karl August Lingner in 1893 and considered as the first antiseptic mouthrinse. The Odol white “side-necked bottle” is still regarded as the most important design in the brand products industry and mostly included in every international exhibition of modern American art. In the 1880’s, the Lambert Pharmaceutical Company developed the alcohol-containing mouthrinse Listerine, which was originally not marketed as a mouthwash, but as a general antiseptic during World War I. Turning the entire product around to fight halitosis after the war, they began to market Listerine as a mouthrinse instead of an antiseptic with the result that sales jumped from just hundreds of thousands of dollars to millions within just a few years. In the same period, the term “plaque” (what we now call “biofilm”), was proposed by Black (1899) and the antimicrobial chlorhexidine (CHX), developed by Imperial Chemical Industries England and initially brought on the market as an antiseptic for skin wounds in 1954, became widely used in medicine as a multipurpose antiseptic. Hereafter, CHX was gradually integrated in dentistry for pre-surgical disinfection of the mouth and in endodontics (Johnson 1995). More specific attempts to use CHX as an oral rinse came with the acknowledgement that plaque was the etiologic agent in gingivitis (Löe et al. 1965). The issue of plaque inhibition and prevention of gingivitis by CHX was initially investigated by Schroeder (1969). One year later the efficacy of CHX has been confirmed by Löe & Schiött (1970) in a pioneering clinical study.

Besides Odol, Listerine and CHX, many mouthrinses are now for sale to the general public. Together with dentifrices, mouthrinses are the most frequently used mode of application of chemical anti-plaque agents. In the last decades, there has been an intense commercial interest in mouthrinses, and mouth care companies, promoting their products on a commercial basis in the grey area between medicine and cosmetic, are still investing in this multibillion dollar business. Many mouthrinses are subject to scepticism because of their limited transitory effects in the oral cavity, which makes further research for other products valuable (Addy 1986, Addy & Moran 1997, Gunsolley 2006). However, in the light of 5000 years of mouthrinse history, the availability of mouthrinses nowadays is certainly easier than, let’s say, running around in order to collect human urine from slaves and travelers!
1.2 Mechanical plaque control: a matter of gingival health and compliance

Gingivitis is a very common, reversible periodontal disease entity limited to the gingiva without loss of periodontal connective tissue attachment nor loss of alveolar bone (Löe et al 1965, Lindhe & Rylander 1975). It is a highly prevalent disease starting at young age in all populations (Stam 1986). Estimates of the general prevalence of adult gingivitis vary from approximately 50 to 100% for dentate subjects. In the USA, 50% of the adult population between 30-90 years of age has gingival bleeding at one or more teeth (Albandar et al. 2002), while among the population aged 70 and over, the prevalence of gingivitis is 85% (Fox et al. 1994). Also in Europe gingivitis is very common (Truin et al. 1985, Bourgeois et al 1997, Hugo-son et al. 2005). Fortunately, most forms of gingivitis remain stable for many years. However, for patients who are or have become susceptible to periodontal breakdown, gingivitis may evolve into periodontitis, which may eventually lead to tooth loss.

Although periodontal diseases are considered a result from the interaction between plaque bacteria and the immune responses of the host, there are to date no safe methods to influence the host response in order to avoid periodontal breakdown. Therefore, the inhibition of plaque formation and the mechanical removal of the biofilm continue to be important issues in the prevention and treatment of periodontal diseases in the 21th century. At present, a high standard of mechanical plaque control still remains an important therapeutic goal in the management of oral health. For a motivated, well-instructed person with the time and skill, manual toothbrushing only should be highly effective to obtain gingival health (Frandsen 1986, Moran 2008). However, the high prevalence of gingivitis suggest that manual cleaning is often insufficient for many individuals. It is well recognized that 50 seconds of manual toothbrushing removes approximately 60% of the plaque around the teeth (Morris et al. 1998). Only 10% of the time is spent on the lingual surfaces (MacGregor & Rugg-Gunn 1979, De la Rosa et al. 1979).

From results of many behavioural toothbrush studies in the past, it can be deducted that manual toothcleaning, i.e. using a toothbrush, is only little influenced by design features of oral hygiene aids, but largely by the compliance and the dexterity of the individual (Stewart & Wolf 1989). Across a wide range of diseases and therapies, non-compliance is estimated for 50% of all patients (Haynes 1979). The more the health recommendations interfere with established life patterns, the less likely they are to be followed (McAlister et al. 1976). Particularly for patients who have what they perceive as a “non-threatening chronic disease”,
compliance with health recommendations appears to be poor (Wilson 1987). For many reasons patients may fail to comply with given instructions because poor dental health belief about the degree of control they have over what happens to them (Duke & Cohen 1975), unwillingness to perform oral self-care (Weinstein et al. 1983), lack of motivation (Alcouffe 1988), stressful life events (Becker et al. 1988), low socio-economic status (Tadesco et al. 1992), poor understanding of recommendations (Berndsen et al. 1993) and unfavorable dental health values (Cammer et al. 1994). Although dental care workers try very hard to educate, motivate and instruct patients to perform an adequate self-care regime, the efforts are not always successful. Even if they manage to establish an initial change in the patient’s behaviour, the tendency to relapse into old habits is a well-known problem (Addy et al. 1987, De La Rosa et al. 1979). In short: the greatest improvement in personal mechanical oral hygiene must be derived from development of a positive attitude towards and motivation for adopting a decent self-care regime. Increased knowledge following oral hygiene behavior interventions and the use of a professional psychologist in the treatment of patients with a history of non-compliance could be of some value.

1.3 Chemical plaque control: a matter of comfort

Chemical plaque control by use of a mouthrinse requires minimal cooperation and skill. Since the active participation of the individual appears to be crucial in the prevention and treatment of periodontal disease, the wide range of factors mentioned above support the use of mouthrinses, as a monotherapy or as an adjunct to regular daily toothbrushing. Furthermore, an adequate mouthrinse, could be of most value to patients in whom the ability to perform adequate oral hygiene procedures is compromised. For patients with intermaxillary fixation or fixed orthodontic treatment, mouthrinsing has been found useful for the prevention of inflammation (Nash & Addy 1979, Stirrups et al. 1981). Long-stay bonehandicaps, particularly those who live in institutions, may benefit of the use of chemical anti-plaque agents (Brayer et al. 1984). Considerable evidence reviewed on several occasions indicates the value of mouthrinses after scaling and rootplaning, periodontal surgery, gingivectomy and extraction (Addy 1986, Addy & Renton-Harper 1996, Lang & Karring 1994, Feres et al. 2009).
1.4. The formulation: a matter of simplicity

Although anti-plaque mouthrinses may differ from each other to a certain extent, there are two dominating factors that are common to all types. The first and major one is the type of the active ingredient (the antiseptic). Examples of antiseptics include chlorhexidine digluconate (CHX), cetylpyridinium chloride (CPC), eucalyptol, hexetidine, methyl salicylate, menthol, benzalkonium chloride, methylparaben, hydrogen peroxide and domiphen bromide. The other one is the need of the consumer: the detergent, the flavouring- & the preservative agents, the sweeteners, the coloring ingredient, fluoride and calcium additives, water and alcohol.

The **detergent**, also called soap or surfactant, is an essential part of the mix: it helps to remove plaque and food debris from the teeth and lowers the surface tension of the liquid. In this way, the liquid can more easily spread over large areas. Detergents like Sodium Lauryl Sulphate (SLS) and Cetylpyridinium Chloride (CPC) aid solubilisation of the flavouring agents and the active ingredients but also provide antibacterial effects.

**Flavouring agents** such as eucalyptol or menthol give a mouthwash its distinctive taste. Most mouthwashes are minty flavoured.

The **preservative** part prolongs the life of the mouthwash and prevents the formation of bacteria and other microbes. An example of this is sodium benzoate.

**Sweeteners** enhance the taste of the mouthwash. Examples of these include sodium saccharine and sucralose.

A **colouring factor** is added for aesthetic reasons, to improve the visual attractiveness of the mouthwash.

**Fluoride and calcium** are often added to water filtration systems as a means of further protection against tooth decay. It also helps to strengthen the teeth.

**Water**, an essential component of the mouthwash, helps to liquefy all of the ingredients.

**Alcohol** is considered an important part of the mouthwash and has a strong anti-bacterial effect. The incorporation of alcohol (ethanol) into mouthrinses serves several purposes: it is a solvent for active ingredients, has antiseptic properties and acts as a preservative by enhancing the maintenance of the active ingredient, improving the shelf life of the product and avoiding contamination. Ethanol is easy to produce and is relatively cheap. Some alcohol-containing mouthrinses contain around 11% ethanol (Corsodyl), others may even contain 21,6% (Listerine). Other mouthrinses are totally free of alcohol (Perio-aid, Paroex).
1.5. The anti-plaque effect: a matter of action

Mouthrinses have various indications such as an anti-plaque, anti-cariogenic, anti-halitosis, anti-hypersensitivity, anti-mucositis or anti-erosive activity. In order to control gingivitis and prevent periodontal breakdown, the focus is mainly on their anti-plaque capacity, since, in the absence of plaque, periodontal disease will not occur (Ash et al. 1964, Löe et al. 1965, Theilade et al. 1966, Löesche & Syed 1978).

The efficacy of anti-plaque agents varies greatly (for review see Kornman 1986a, Addy 1986, Mandell 1988) and is depending on the “substantivity” of the agent. Substantivity is the persistence of action of a chemical agent, which is greater or more prolonged than would be expected with simple mechanical deposition (Kornman 1986a,b, Lang & Brecc 1986). Agents that do not exhibit any significant substantivity (only minutes), such as CPC, Essentials Oils, SLS & monofluorophosphate, are considered antimicrobials of the “first generation”. Although these antiseptics have an antimicrobial efficacy “in vitro”, they are, once expectorated, immediately removed from the mouth allowing plaque to build up again. Devoided of substantivity, their effect ‘in vivo’ is therefore very limited and rinsing the mouth ten times a day to counterbalance this limited effect and have a long-standing anti-plaque effect is not very practical. So, antimicrobial activity “in vitro” could easily lead to the wrong conclusion and is not a reliable predictor of plaque inhibition by an antiseptic ‘in vivo’. The most effective antiseptics belong to the “second generation” and include CHX, aminefluoride, stannous fluoride and triclosan. These anti-microbials are characterized by a high-substantivity – that is retention of 25-30% after a one minute of rinsing – and therefore show a persistence of action in the mouth for many hours. Compared to CHX, CPC for instance has a similar antibacterial profile in vitro (Gjermo et al. 1970), but its persistence ‘in vivo’ and the plaque inhibition is less, due to the limited substantivity of CPC (Schiött et al. 1970).

Unfortunately, antiseptics of the second generation appear to provide a considerably greater preventive than therapeutic action. Most effective antiseptics inhibit the development of dental plaque but are limited or slow in dealing with older established plaque, manifested as a true biofilm (Marshall 1992, Marsh 2005, Vitkov 2005).

The problem with biofilms is that they protect bacteria effectively from antimicrobial agents. While “plaque” is defined as a soft layer of bacterial deposits on tooth surfaces (Nolte 1982), a biofilm is well-organized and consists of a lower dense layer of microbes on the tooth surface with on top a looser fluid layer mainly composed by a matrix of extracellular bacterial polymers and salivary and/or gingival exudate products. As nutrient components may
penetrate into the biofilm by molecular diffusion from the upper layer “in motion” into the lower “stationary” microbial layer, antiseptics can also penetrate through dental biofilms, but the penetration is slower and the bacteria are more resistant to therapy than bacteria in a planctonic environment (Pratten et al. 1998, Hope et al. 2004).

In order to provide clarity in the world of chemical agents, the Council on Dental Therapeutics of the American Dental Association (ADA), established in 1986, developed guidelines for the acceptance of anti-plaque and antgingivitis agents (American Dental Association 1986). These guidelines, revised in 1994 by Imrey et al., describe the clinical, biological, and laboratory studies necessary to evaluate stability, substantivity, specificity, efficacy and safety of an agent. Using these criteria, years of documented research have established that twice daily rinsing for 60 seconds with a 0.2% alcohol-containing CHX mouthrinse is recognized by the ADA and the pharmaceutical industry as the gold standard for chemical supra-gingival plaque control and the positive control against which other anti-plaque agents are assessed (Jones 1997).

1.6. **Chlorhexidine an active molecule (anti-bacterial & anti-plaque)**

CHX is widely marketed under different brand names such as Peridex (USA), Corsodyl, Perioaid (Benelux) and Chlorohex (UK), Curasept (Italy), Chorhexamed (Germany), Savacol (Australia), Suthol (India), Peridont (Venezuela), Oronine (Japan) and Clorexil in some parts of Central America. It is available in three forms: the digluconate-, acetate- and hydrochloride salts. Most studies have used the CHX-digluconate salt because of its high aqueous solubility.

The amount of CHX retained within the mouth helps to reduce the salivary bacterial challenge, which may be useful in situations such as postoperative healing. Besides its antibacterial quality, CHX has a strong anti-plaque effect, ascribed to its great substantivity (Kornman 1986 a,b). Even after a single rinse, persistence at the oral surfaces has shown to suppress salivary counts for over 12 hours (Schiött 1973). This substantivity is the result of the specific di-cationic nature of the CHX molecule, which allows the agent to attach with one cation to different surfaces within the mouth (saliva, teeth, mucosa and the anionic tooth pellicle). These sites are considered as “reservoirs” from which CHX can be desorbed. The other end of the CHX molecule binds to the bacterial cell membrane of the bacteria attempting to colonize the tooth surface.

As a potent antibacterial substance, CHX acts on the cell wall of the micro-organisms by changing their surface structure. The molecule is both bactericidal and bacteriostatic. At the
moment of application, when the concentration of CHX is high, the molecule achieves an immediate “bactericidal” action, causing irreversible damage to the bacterial cell membrane, which reflects in coagulation and precipitation of the cytoplasm. At lower concentrations, the integrity of the cell membrane is not completely damaged, but altered and CHX binds to the inner cell membrane. This leads to increased permeability of the cell membrane with leakage of intracellular components (Hugo & Longworth 1964, 1966). In this stage, bacterial attachment to the enamel surface is not completely inhibited, but a high amount of plaque is prevented because bacteria cannot multiply.

1.7 Chlorhexidine an inter-active molecule

In vitro data have shown that CHX interact with anions such as phosphate, sulphate and carboxyl to form salts of low solubility (Rölla et al. 1970, Kirkegaard et al. 1974, Rölla & Melsen 1975, Bonesvoll 1977, Barkvoll et al. 1988). Therefore, it has been concluded that CHX is not compatible with SLS in an aqueous solution (Barkvoll et al. 1989). The inactivation of the CHX di-cationic antiseptic by interaction with specific anionic dentifrice ingredients, e.g. sodium mono-fluorophosphate and the detergent SLS, has been confirmed in vivo (Barkvoll et al. 1989, Owens et al. 1997, Kolahi & Soolari 2006).

Since CHX appears to affect preferentially the salt taste, interaction of CHX with food makes food taste rather bland and forms the basis of the CHX taste disturbance (Lang et al. 1988).

In the presence of a tooth pellicle, interaction and local precipitation occurring between the tooth-bound CHX and the chromogens found in some food and beverages, may probably be the most plausible explanation for the well-known CHX-associated tooth staining, a phenomenon that is not limited to CHX alone (Addy et al. 1991). The mechanisms are however still being debated (Eriksen et al. 1985, Addy & Moran 1995).
2. General aim of the thesis

The interaction between chlorhexidine (CHX) and Sodium lauryl sulphate (SLS)
A common ingredient found within certain types of dentifrice is the detergent SLS. Several studies performed in the past led to the conclusion that the effectiveness of a CHX rinse was reduced by toothbrushing with an SLS-containing dentifrice. The fact that a CHX mouthrinse and the dentifrice detergent SLS may counteract in the oral cavity is, however, based on research data from earlier non-brushing studies using an SLS-slurry (an SLS-dentifrice/water-rinse) instead of toothbrushing with an SLS-dentifrice. For this reason, the plaque inhibiting effect of a 0.2% CHX mouthrinse under influence of toothbrushing with an SLS-containing dentifrice is studied in a series of three “SLS-brushing/CHX rinsing” interaction studies which are described in chapter 2, 3 and 4.

The inclusion of alcohol in a CHX mouthrinse
The investigation described in chapter 5 evaluates the necessity of alcohol in CHX mouthrinses. As already mentioned in the introduction, the presence of alcohol may have an important role in the formulation of a mouthrinse. The inclusion of alcohol however is still a controversial topic due to possible health risks. Therefore, two commercially available CHX mouthrinses, with (Corsodyl®) and without (Perioaid®) alcohol, were studied and compared for their plaque inhibiting effect.

The effect of a CHX-releasing toothbrush
The experimental manual toothbrush, tested in chapter 6, contains a template within the brush head that releases CHX when brought into contact with oral fluids. The purpose of the study is to test whether toothbrushing with this experimental toothbrush, having a slow-release system of CHX, is safe and more effective in inhibiting plaque and gingival bleeding compared to rinsing with a 0.2% CHX as an adjunct to toothbrushing without dentifrice. A secondary objective of the experimental CHX toothbrush was to assess the amount of stain on the teeth, being considered as a side-effect of CHX that may have a negative impact on compliance.
The results of a systematic review of CHX mouthrinses

A systematic review is considered as a comprehensive and unbiased review process that locates, appraises and synthesizes evidence from the scientific studies to obtain a reliable overview. The literature relating to the use of CHX mouthrinses as anti-plaque & anti-gingivitis agents is immense and many narrative reviews have been brought forward. However, in dental literature, a systematic review on the effect on plaque and gingivitis of CHX, the primary, “gold standard” chemical agent, is lacking. Therefore, a systematic review on this topic was carried out of which the results are presented in chapter 7.
References


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General introduction


