Antimicrobial caries preventive strategies

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Antimicrobial Caries Preventive Strategies

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Antimicrobial Caries Preventive Strategies

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Chapter 1

General Introduction

Caries

Dental caries may only occur when the factors involved in the carious process are concurrently and unfavourably present. The relationship between these factors was presented by Keyes in his diagram (Fig.1.1) [Keyes and Jordan, 1963]. After fermentation of dietary carbohydrates, oral microorganisms produce acids that cause a pH drop and consequently demineralisation of enamel. After the acids are neutralized, the pH rises and remineralisation occurs. Remineralisation is the redeposition of lost mineral (hydroxyapatite) either by the original, dissolved calcium and phosphate ions from the mineral or by ions derived from the saliva. Both de- and remineralisation are dependent on the interplay of buffering saliva, the saturation level of enamel apatite in dental plaque and the presence of fluoride. Whether a carious lesion will develop is dependent on the balance between de- and remineralisation.

Prevention of dental caries aims to protect the dental tissues from dissolving. This can be achieved by making the dental hard tissues less soluble, by removing dental plaque, by obtaining less cariogenic plaque or by reducing the intake of fermentable carbohydrates.

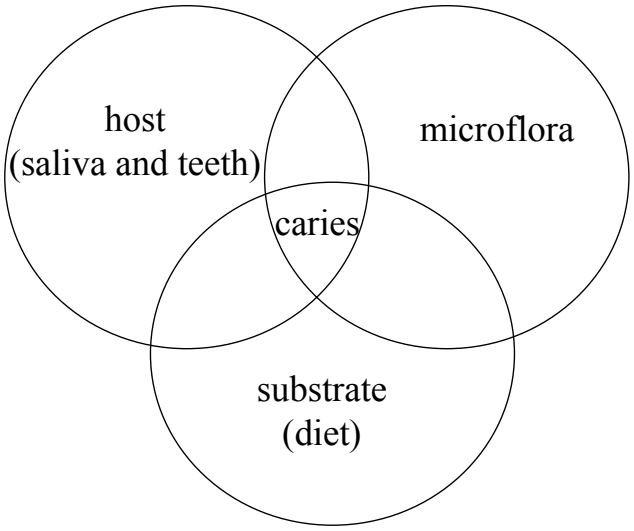

Figure 1.1: Keyes' model (1963)

Caries risk

Caries risk depends on the preventive agents and services which are available to the individual and the extent to which these are effectively used by each of them [König, 2004]. Children and adolescents in low socio-economic communities, immigrant children [Skeie *et al*., 2006] and institutionalised [Montal *et al*., 2006] and hospitalised individuals [Peltola *et al*., 2005] comprise the vulnerable group of patients who require intensive preventive care. Intensive preventive care may include dental health education, oral hygiene instructions, dietary counselling, supervised tooth brushing, school-based prevention programs and diligent, topical use of fluoride and antimicrobial agents, such as amine fluoride / stannous fluoride (AmF/SnF_2) solutions or chlorhexidine (CHX) rinses, gels and varnishes. The fact that preventive measures are often lacking in above-mentioned risk groups is of great concern.

Caries prevalence

The widespread and daily use of fluoride toothpaste in Western society has lead to an enormous decrease in caries prevalence since the 70's [Kalsbeek, 1982; Marthaler *et al*., 1996; von der Fehr and Haugejorden, 1997]. This decrease has come to an end in the mid 90's [Truin *et al*., 1999]. A further decrease in caries incidence was not observed in low prevalence communities [Marthaler, 2004]. Today caries is not eradicated and the statement "80% of the caries in industrialised countries occurs in 20% of the individuals" is frequently cited. Surveys on caries prevalence have actually reported numbers between 60-80% caries occurring in 20-25% of the population [Macek *et al*., 2004] dependent on particular dentitions and age groups. Today, it is reported by dental professionals that caries prevalence might be increasing in children but this is not confirmed by epidemiological data.

A reason for the 20% individuals to have caries could be that these individuals do not have sufficient access to dental health education and services. However, in

industrialised countries this is questionable and it is more likely that these individuals do not comply with caries preventive measures. If so, these individuals need reinforced dental education and support in compliant behaviour. In spite of compliance to preventive measures, some might not benefit sufficiently from their habitual fluoride intake. First, their fluoride use could be increased and second, an extra preventive measure could be beneficial to them. The latter might be the additional use of an antimicrobial mouthrinse next to daily tooth brushing with fluoride toothpaste or omitting the usual water rinse after tooth brushing. Omitting the usual water rinse could enhance the substantivity (*i.e.* the presence of an agent in its active form) of caries preventive agents. In the dental office, professionally applied supportive preventive measures aim to decrease the caries risk. In this respect, the application of dental varnishes containing fluoride and / or antimicrobial compounds in high concentrations could be significant.

Aim

The research objectives of this thesis were chosen as they could contribute to more effective caries prevention to individuals at risk. The **specific aims** were to study 1) the effects of antimicrobial preventive measures on the acidogenicity of dental plaque after daily use of AmF/SnF_2 mouthrinse at home and after single and repeated 40% chlorhexidine varnish applications in the dental office, 2) the retention of fluoride and antimicrobials in plaque and saliva after tooth brushing with and without water rinsing, and 3) the ability of a newly developed diagnostic test to assess a person's individual caries risk.

Caries prevention with antimicrobial agents

Antimicrobial mouthrinses do not replace conventional tooth brushing but could support mechanical plaque removal by 1) reducing existing plaque, 2) preventing formation of new plaque, 3) selectively inhibiting specific bacteria associated with disease or 4) inhibiting the expression of virulence factors [Marsh, 1992]. The daily use of an antimicrobial mouthrinse should aid to maintain the natural balance of the oral microflora and should not favour colonisation of undesirable microorganisms nor result in microbial resistance. Antimicrobials are widely used in the prevention of periodontal disease but their use in caries prevention is less common and their benefit is not consistently proven.

Since 1960, the effect of today's available antimicrobial solutions on oral microorganisms was tested and evaluated. *In vivo* experiments have shown that antimicrobial agents potentially reduce plaque amounts [Axelsson, 1993; Brecx *et al*., 1993; Zimmermann *et al*., 1993; Madlena *et al*., 2004] and numbers of specific microorganisms [Emilson, 1981; Zickert *et al*., 1982; Lindquist *et al*., 1989; Schaeken *et al*., 1991].

Amongst the antimicrobials, chlorhexidine (CHX) is regarded as the gold standard. It was first incorporated into mouthrinse solutions at a concentration of 0.2% in 1970. It was shown that CHX reduced plaque formation, affected the bacterial flora, prevented periodontal diseases and root caries [Loë and Schiøtt, 1970]. Other antimicrobial solutions also claimed to have anti-plaque, anti-gingivitis, anti-calculus, and anti-caries activity. These solutions contain different antimicrobial compounds such as: plant extracts (sanguinarine), metal salts (*e.g.* zinc, stannous, copper), fluorides (*e.g.* AmF/SnF_2 and SnF_2), phenols (triclosan), "essential oils" (*e.g.* thymol, menthol) and enzymes (*e.g.* glucanase, amyloglucosidase/glucoseoxidase). While most of these compounds exhibit a broad spectrum of antimicrobial activity *in vitro*, they may exert a small effect on dental plaque *in vivo* [Donlan and Costerton, 2002]. This is, amongst others, related to a different effect of antimicrobials on bacteria living in suspensions

compared to the effect on bacteria living together in *e.g.* dental plaque [Costerton *et al*., 1978].

A promising antimicrobial agent in caries prevention is AmF/SnF_2. The antimicrobial amine (Am) and stannous (Sn) ions have been combined with fluoride for which the evidence in caries prevention is beyond question [Rølla *et al*., 1991; ten Cate, 2004]. The organic AmF-complex enhances the attachment of F^- to enamel due to its long amine-chains and its slightly acidic pH and consequently, AmF provides a higher F^- concentration on the enamel surface compared to the commonly used inorganic NaF [Mühleman *et al*., 1957]. Additionally, amine has intrinsic anti-glycolytic properties and therefore the antimicrobial effect of AmF is stronger compared to NaF [Breitenmoser, 1975; van Loveren, 2001]. The stannous ion itself has no cariostatic properties but studies [Liang *et al*., 1995; White *et al*., 1995] have shown the antimicrobial activity of the SnF_2-complex *in vivo*. Its effect on the inhibition of plaque formation is stronger compared to other fluoride combinations. AmF/SnF_2 can be effective in low concentrations by slowing down bacterial growth by inhibiting bacterial metabolism e.g. acid production.
The home use of the AmF/SnF_2 mouthrinse containing 0.025% F^- (meridol®, GABA International AG, Münchenstein Switzerland) additionally to daily tooth brushing with fluoride containing toothpaste could have a beneficial effect to the oral health of caries susceptible individuals. Therefore, it is interesting to study the effects of AmF/SnF_2 on caries. The effect of the daily use of AmF/SnF_2 mouthrinse on the bacterial acid production in dental plaque -as a surrogate parameter of caries- was evaluated in **Chapter 2** of this thesis. Plaque samples were collected from interproximal surfaces (plaque stagnation areas) and buccal or smooth surfaces (non-stagnation areas). Because stagnation areas are more susceptible to caries than smooth surfaces, these sites were considered to be at higher risk compared to smooth surfaces (low risk) in the group of participants.

Professional Chlorhexidine Applications

Chlorhexidine (CHX) is a cationic bis-biguanide, soluble in water and alcohol, and it has a broad antimicrobial spectrum. CHX is bactericidal in high concentrations and bacteriostatic in low concentrations. In high concentrations CHX damages irreversibly the membrane of the bacterial cell followed by the precipitation of its cytoplasmic content and denaturation of its proteins [Longworth, 1971]. Because of this bactericidal activity, active membrane transport becomes impossible and the cellular substances leak out of the cell. A significant bacteriostatic effect was found in CHX-solutions diluted up to 0.0002% [Hennessey, 1973]. The positively charged CHX-molecules attach to negatively charged surfaces such as teeth, oral mucosa and microorganisms, *e.g.* mutans streptococci, a highly acidogenic and aciduric species [van Houte, 1980; van der Hoeven and Franken, 1982]. The complexity of plaque as a multi-species biofilm and its relative resistance to antimicrobials because of their incomplete penetration into plaque, influence the *in vivo* efficacy.

Reductions in plaque and bleeding indices after the use of CHX mouthrinses have been reviewed frequently [Hull, 1980; Mandel, 1988; Addy and Moran, 1997]. Antimicrobial mouthrinse solutions with other active antimicrobial compounds than CHX were shown to be less effective and therefore CHX is considered to be the gold standard [Jones, 1997] and often used as a positive control in clinical trials on antimicrobial formulations. Nevertheless, the evidence of CHX as a caries preventive agent is inconclusive [Twetman, 2004].

As caries preventive agent, CHX has been used in several vehicula *e.g.* toothpaste [Jenkins *et al*., 1990], rinsing solutions [Adams and Addy, 1994], gels [Tenovuo *et al*., 1992; Gisselsson *et al*., 1994; Gisselsson *et al*., 2005] and varnishes [Bratthall *et al*., 1995; Petersson *et al*., 1998; Forgie *et al*., 2000; Lunsen *et al*., 2000]. The well-known side effects of CHX like changes in taste and staining of the dentition, both dependent on the concentration and frequency of use, have not favoured its application in caries prevention. In caries prevention CHX gels and varnishes aim to have a prolonged effect and could be applied infrequently. A meta-analysis by van Rijkom *et al*. (1996) reported a caries-inhibiting effect of 46% (95% CI: 35% - 57%) of CHX treatments

not restricted to specific risk groups. Despite the narrow confidence interval, the 46% inhibition should be interpreted critically. Caries prevalence was high in the time span when the data were obtained. Therefore, the conclusions might be applicable to a population at a different risk than the 20% individuals of today. In the analysis, CHX rinse (0.02%), gel (1%) and toothpaste (2%) were considered one formulation; some of the studies included [Zickert *et al*., 1982; Gisselsson *et al*., 1988] had no proper controls and other studies [Luoma *et al*., 1978; Gisselsson *et al*., 1988; Lindquist *et al*., 1989] were included twice.

The application of gels and varnishes aim to kill microorganisms, especially mutans streptococci. The regrowth of mutans streptococci is retarded by the 'colonisation resistance' of dental plaque: the capacity of dental plaque to hinder foreign species to re-enter the plaque community and to colonise the tooth. Beside an initial killing effect, the varnishes have a longer contact time (7.5-15 min) compared to a rinse (2 min) and their substantivity is higher due to a higher concentration [Schaeken *et al*., 1991]. Active ingredients are released from the varnish and after removal of the varnish from teeth and soft tissues into dental plaque [Sandham *et al*., 1988; Schaeken and de Haan, 1989; Sandham *et al*., 1991].

In clinical practice, individuals are being subjected to single and repeated treatments with CHX varnishes containing 1-40%. The effects on the numbers of mutans streptococci [Sandham *et al*., 1988; Ie and Schaeken, 1993; Lunsen *et al*., 2000; Gerardu *et al*., 2003] and on the number of Decayed Missing Filled Surfaces (DMFS) of teeth [Twetman and Petersson, 1999; Forgie *et al*., 2000] were not conclusive with regard to the efficacy of the treatments in caries prevention. In this thesis, the effects of single and repeated CHX applications on microbial acid production are studied. The reductions in mutans streptococci reported in **Chapter 3** were paralleled by a decrease in plaque acidogenicity. Not only reductions in mutans streptococci account for a beneficial effect of CHX in caries prevention, also the outcome that during the suppression of these species the acidogenic properties of plaque are decreased. The decrease in plaque acidogenicity after a single 40% CHX application lasted approximately 3 wks. Multiple treatments could not prevent the regrowth of acidogenic microorganisms, *in casu* mutans streptococci, 3 wks after the

last treatment. Whether a repeated application of 40% CHX decreases the acid production in plaque such that a sustained effect in plaque acidogenicity could be achieved, is studied in **Chapter 4**.

Substantivity of caries preventive agents

The presence of a caries preventive agent in its active form (*i.e.* substantivity) diminishes as the concentration decreases after being diluted by saliva and gradually swallowed. Adsorption onto oral surfaces increases the substantivity. From the oral surfaces, including teeth and soft tissues, the retained fluoride and other caries preventive agents can be released into saliva and subsequently taken up in dental plaque. Fluoride is bound and stored in dental plaque, thereby acting as a 'reservoir' for fluoride [Duckworth and Morgan, 1991]. This could be similar for the retention and release of caries preventive antimicrobials.

An additional and simple measure to enhance the retention of caries preventive agents in the oral reservoir could be to omit the water rinse after daily tooth brushing with fluoride containing toothpaste. Several trials have been undertaken to study the effect of (no) water rinsing after tooth brushing. The results showed that subjects using beakers to rinse with water after tooth brushing had consistently higher caries increments than 'non-beaker' users [Chesters *et al*., 1992; O'Mullane *et al*., 1997; Ashley *et al*., 1999] and that an inverse relationship existed between the amounts of water used for rinsing and the salivary fluoride concentration [Sjögren and Birkhed, 1993]. In their case control study, Sjögren and Birkhed (1993) showed that subjects rinsing with 0.7 ml water compared to 1.5 ml had significantly lower numbers of DMFS and DS (decayed surfaces). They suggested a relation between the caries activity (DS) and the fluoride retention after tooth brushing. In another study [Sjögren *et al*., 1995] they evaluated the number of new decayed filled surfaces (dfs) after 3 years in groups of 4-year old children after a modified toothpaste technique. The modified technique comprised a careful distribution of toothpaste before brushing, no unnecessary spitting during brushing, 1 min rinsing with toothpaste-and-sip-of-water

slurry after brushing. The results indicated that the modified toothpaste technique reduced approximal caries significantly in 7-year old children by an average of 26%. In contrast, Machiulskiene *et al*. (2002) concluded from their study that post-brush rinsing with water does not significantly enhance the caries reducing effect of fluoride toothpaste. Contrariwise their conclusion, they reported larger caries increments on radiographs after 3 years in the water-rinse group compared to the no-rinse group. It remains unclear whether their results support or deny the previously reported findings that water rinsing affects the retention of fluoride.

The duration of the elevated salivary fluoride concentration influences the uptake of fluoride in newly formed dental plaque. In that way, plaque contains a fluoride reservoir that can deliver fluoride to the site of action [Skold-Larsson *et al*., 2000]. Elevated salivary and plaque fluoride levels have been recorded up to 3 hrs [Duckworth and Morgan, 1991; Sjögren and Birkhed, 1994; Campus *et al*., 2003; Issa and Toumba, 2004] and measured at 24 hrs after tooth brushing [Sidi and Wilson, 1991]. From a clinical point of view, it is relevant to assess the retention of preventive agents between two tooth brushing exercises, *i.e.* the commonly used period of 12 hrs. In **Chapter 5** this was studied by measuring the effects of water rinsing or not rinsing after tooth brushing on the retention of fluoride in saliva and plaque 6 hrs after brushing.

Another measure to enhance the substantivity of toothpaste, especially AmF/SnF_2 toothpaste, is the additional use of AmF/SnF_2 mouthrinse after brushing [Banoczy *et al*., 1989; Madlena *et al*., 2004]. Retention and release of AmF/SnF_2 in both products in relation to their efficacy as caries preventive measures are not addressed in the literature. The beneficial effect of retention of AmF/SnF_2 toothpaste and rinse might be twofold as not only fluoride could be stored in the reservoir but also antimicrobial compounds. Furthermore, no clinical studies have been undertaken to elucidate the effect of omitting the water rinse after brushing with antimicrobial toothpaste on plaque acidogenicity. Whether the release of an antimicrobial agent uploads the newly formed dental plaque and affects its acidogenic potential has not been described. In **Chapter 6**, samples of tongue saliva and dental plaque were collected after brushing

with AmF/SnF_2-toothpaste and rinsing with either water or AmF/SnF_2 solution or without rinsing. The effects of these antimicrobial combinations on the lactic acid production in dental plaque and in tongue flora were evaluated.

Caries Risk Assessment

Since caries is not equally distributed in Western populations, several attempts have been made to develop a test to assess caries risk with both high sensitivity and specificity. Most variables that have been included in these tests were related to Keyes' classical caries model: teeth, microorganisms and carbohydrates. However, other variables such as fluoride intake, presence of carious lesions, past caries experience, number of new restorations, dental attitude and compliance, salivary flow and buffer capacity and socio-economic status are also important in caries risk assessment. Among all variables in risk assessment, the recommendation to count specific microorganisms in saliva [Krasse, 1988] has gained considerably large popularity. The results of counting cariogenic mutans streptococci and lactobacilli have been evaluated by studies reporting data on specificity, sensitivity and predictive values [van Houte, 1993]. This review indicates that the use of such data for the prediction of caries in individuals is not possible but is more promising for that of groups. The search for a single test that will take all variables into account would seem meaningless because of the complex interplay of several factors in caries development. For the individual patient no test with sufficient power to predict whether he will or will not develop caries is yet available.

In clinical practice, the experienced naked eye or 'clinical expertise' of the professional often assesses the caries risk of the individual patient. It would be informative for his decision making and for the individual patient to underscore this clinical judgement with a caries risk assessment test. A new semi-quantitive test was developed industrially. This test, ClinPro Cario-L-Pop (CCLP, 3M ESPE, Seefeld, Germany) measures the lactic acid production on the tongue. The bacterial production of lactic acid after carbohydrate fermentation is an indicator of metabolic activity of

caries-causing microorganisms. The concept behind this test is 'the higher the metabolic activity of cariogenic bacteria in a patient's mouth, the higher the potential that these bacteria will cause caries'. In a randomized crossover clinical trial, the variation in risk assessment using the CCLP was evaluated after 4 comparable washout periods. Furthermore, the capacity of the CCLP-test to monitor the effect of different antimicrobial interventions was studied. The test results were compared to tongue and plaque samples that were both analysed by capillary ion electrophoresis for lactic acid contents (**Chapter 7**). The question was whether the CCLP-test could be used to provide the clinician and the patient objective information on the patient's oral metabolic activity and individual potential for caries development.

Chapter 8 provides a general discussion and the final conclusions. In addition, a summary in English and Dutch is given followed by the references, acknowledgements and curriculum vitae.

Chapter 2

Plaque Formation and Lactic Acid Production after Using AmF/SnF_2 Mouthrinse

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Submitted for publication

Abstract

Our aim was to study the effects of 3-wks daily rinsing with AmF/SnF_2 mouthrinse on plaque formation at specific sites and on the acid production in plaque in a randomized clinical trial with 30 participants. Plaque scores were recorded according to the Quigley and Hein index. Plaque samples were collected before and after sucrose rinsing from buccal and interproximal surfaces of upper (pre)molars at 2 baseline visits and on the 2nd and 7th day after the discontinuation of 3-wks daily rinsing. Metabolic acid ions were determined by capillary electrophoresis. The results at baseline showed 1) higher lactic acid concentrations in resting interproximal plaque than in buccal plaque and 2) a higher acid production in response to sucrose challenge in buccal plaque than in interproximal plaque. After 3-week use of AmF/SnF_2 mouthrinse, no significant differences in plaque scores were observed and the alleged effect on the acidogenicity of dental plaque was not significant on the 2nd day after the last mouthrinse. We conclude that 3-week use of AmF/SnF_2 rinse once daily does not result in a reduction of plaque formation or in a reduction of sucrose metabolism in plaque at specific sites after discontinuing the rinse.

2.1 Introduction

Various antimicrobial formulations have been developed to support mechanical plaque removal. Some have been studied extensively such as meridol® mouthrinse (GABA International AG, Münchenstein, Switzerland). This product contains amine fluoride/stannous fluoride as the antimicrobial complex (AmF/SnF_2). Clinical studies on the effects of AmF/SnF_2 products on the amounts of plaque have shown that plaque formation was significantly reduced [Banoczy *et al*., 1989; Brecx *et al*., 1993; Madlena *et al*., 2004; Paraskevas *et al*., 2004]. However, these studies did not evaluate the effects on acid production in plaque that is a measure for the cariogenic properties of plaque. Banoczy *et al*. (1989) reported a decrease in the acid solubility of

enamel after the combined use of AmF/SnF_2 toothpaste and mouthrinse and suggested an increased clinical effect compared to placebo combinations. Damen *et al*. (2002) found that one single AmF/SnF_2 rinse reduced acid production with 37% in sucrose challenged buccal plaque up to 3 hrs after use. However, the observed difference did not reach statistical significance presumably as a result of the low number of participants (n=5). Daily use of an AmF/SnF_2 rinse after brushing with AmF/SnF_2 toothpaste for 1 week reduced the sucrose induced lactic acid production significantly up to 6 hrs in newly formed plaque compared to water rinsing [Gerardu *et al*., 2006]. Our previous studies [Damen *et al*., 2002; Gerardu *et al*., 2006] did not relate the effect of rinsing with AmF/SnF_2 mouthwash on the acid concentrations in plaque to the amounts of plaque formation. We questioned whether extending the daily use of AmF/SnF_2 rinse to 3 weeks would affect plaque formation at specific sites and reduce lactic acid concentrations at these respective sites for a period beyond 6 hrs.

The combination of reduced amounts of plaque and reduced lactic acid production may be supplementary in providing more healthy oral conditions. The effect of antimicrobial agents on acid concentrations at different sites is of interest since plaque differs from site to site. The lower accessibility of the interproximal areas for tooth brushing compared to the free surfaces, the lower salivary flow and clearance [Dibdin *et al*., 1995] typically result in thicker and older plaque that provides different conditions for acid producing microorganisms to thrive in [Wilson and Ashley, 1990]. These site-related variations may result in differences in microbial composition and may well affect the topical effectiveness of antimicrobial mouthrinses.

In this randomized clinical trial we assessed 1) the amount of plaque in lateral parts of the upper jaw and 2) the duration of the effects of an AmF/SnF_2 mouthrinse after 3-wk daily use on lactic acid formation in buccal and interproximal plaque.

2.2 Materials and methods

Subjects

Thirty non-dental Dutch students (15 male, 15 female; mean age ± SD: 28 ± 9 yrs; median 25 yrs) were recruited by an advertisement in their university. Inclusion criteria were: good general and dental health, no use of antibiotics during the last 3 months. Twenty participants were randomly assigned to the test-group by means of a random number list. All participants received 1400 ppm fluoride toothpaste (Prodent® Cool Mint 1450 ppm F^-, Sara Lee, Veenendaal, The Netherlands) to be used 1 week before the first sampling occasion until the end of the study. The Institutional Ethics Committee approved the study protocol (Academic Medical Centre, Amsterdam, The Netherlands, reference MEC 02/085) and the participants signed the informed consent letter.

Study-outline

Before the antimicrobial mouthrinse protocol was started, 2 sets of 6 dental plaque samples each were collected to determine baseline levels. A third and fourth set were collected on the 2^{nd} (=evaluation 1) and 7^{th} day (=evaluation 2) after the discontinuation of the 3-week daily use of either AmF/SnF_2 mouthrinse (meridol®, GABA International AG, Münchenstein, Switzerland) or control water rinse. In each subject one upper quadrant was randomly assigned for plaque sampling at all visits. In the other upper quadrant a photo picture was taken from erythrosine-disclosed plaque in the premolar-molar region. At each visit, six plaque samples were collected: one buccal and one interproximal sample before sucrose rinsing, and 4 samples at 8 (buccal), 10 (interproximal), 18 (buccal) and 20 (interproximal) min after the sucrose challenge, respectively. Buccal plaque was collected from the first 2 upper molars, interproximal plaque from the premolar-molar and molar-molar interspaces. When too little plaque was available for sampling, the next approximating tooth surface was sampled. At the last visit the bottles with the test rinse were collected and weighed for remaining content to check compliance.

Instructions participants

Neither oral hygiene instructions nor professional cleaning were given before the start of the experiment and flossing was not allowed during the study. No dietary restrictions were given. Participants were instructed to rinse every night during 3 wks with AmF/SnF_2 mouthwash or with tap water, both 10 ml, for 30 s following their regular tooth brushing exercises. The sampling visits were scheduled on the 2nd and 7th day after the last rinse and 18 hrs after tooth brushing. Before plaque sampling subjects had to refrain from food and drinks for 2 h.

Plaque index

Staining erythrosine disclosing liquid was applied with a cotton swab on the canine and 3 neighbouring (pre)molars in the assigned upper quadrant on every visit. To avoid disruption of the disclosed plaque, water from the dental unit syringe carefully rinsed off excessive colouring shortly after application. The disclosed plaque was air dried gently. After disclosing, pictures (Minolta camera Dynax 600si, AF100mm f/2.8 Macro lenses, Macro Flash 1200AF) were taken perpendicular to the dental arch on film for colour slides (Fujichrome Sensia II100 RA135 DX). Capi Lux Vak (Amsterdam, The Netherlands) developed the films. All pictures were scanned (HP scanjet 5470c) and filed as bitmap images (1280x1024 pixels, 24 bits colour). Two experienced investigators [van der Weijden *et al*., 2005] scored independently the plaque amounts on the mesiobuccal and buccal surfaces of the 4 stained teeth on these images using the 0-5 scale of the Quigley & Hein index (1962) modified by Turesky *et al*. (1970). The images were scored according to a computer-generated randomization list in one session.

Plaque activity assessment

Buccal plaque samples were collected with a Teflon spoon from upper (pre)molars in the assigned quadrant before and 8 and 18 min after a 2 min 10 ml 10% sucrose challenge. These plaque samples were transferred separately into 50 µl MilliQ-water in precooled Eppendorf vials. Interproximal plaque was collected with unwaxed dental floss (Johnson & Johnson®) before and 10 and 20 min after sucrose challenge. Plaque

was removed from the dental floss by drawing it through a slit cut in the lid of a precooled Eppendorf vial containing 50 µl MilliQ-water. All 6 plaque samples were spun down in an Eppendorf vial at 16,100 x *g* within 1 min in order to stop metabolic activity and kept on ice until further processing within 1 hour.

Processing plaque

Within 1h after sampling, plaque samples were heated for 5 min in 80°C to release acids, cooled in ice for 5 min and centrifuged for 15 min at 16,100 x *g* and 4°C. The supernatants were filtered through 0.22 µm Micro spin filters (Ultrafree-MC 0.22 µm, Millipore, Bedford, Mass., USA) for 4 min at 13,148 x *g* and 4°C, and subsequently stored at -80°C until analyses of acid anions. Plaque pellets were stored at -80°C until the analyses of protein.

Organic acid analyses

Samples were analysed for protein content according to Bradford (1976) and for acid concentrations by capillary ion electrophoresis described by Damen *et al*. (2002).

Statistics

The Statistical Package for the Social Sciences (SPSS version 10.0) was used to perform statistical analyses. The correlation between the data at the 2 baseline visits was calculated with Spearman's regression analysis. The differences between the test and control were analysed with ANOVA with post-hoc Tukey and within the groups General Linear Model Repeated Measures was used. The study design had a power of > 80% to discern a difference in lactic acid concentrations of 0.28 in buccal and interproximal plaque with a within-patient standard deviation of the response variable of 0.37 [Damen *et al*., 2002].

2.3 Results

After the use AmF/SnF_2 mouthrinse, no side effects were reported. One subject did not return for evaluation on the 2nd day after 3-wks rinsing with AmF/SnF_2 (evaluation 1).

Plaque amounts

Table 2.1 shows the mean plaque scores and standard deviations for both observers for the 4 visits. The observers did not detect statistically significant differences in the average plaque scores on the 2 baseline or evaluation visits (One-way ANOVA with post-hoc Tukey).

Table 2.1: Mean plaque scores ± SD (standard deviation) per observer at 2 baseline visits and on the 2nd (evaluation 1) and 7th day (evaluation 2) after 3-wks daily rinsing with AmF/SnF_2 or control water rinse

		Observer 1	Observer 2
Mouthrinse	visit	mean (± sd)	mean (± sd)
water	baseline 1	2.59 (0.95)	2.75 (0.66)
	baseline 2	2.19 (0.93)	2.44 (0.59)
	evaluation 1	2.68 (0.55)	2.72 (0.34)
	evaluation 2	1.86 (1.12)	2.32 (0.89)
AmF/SnF_2	baseline 1	1.86 (0.88)	2.51 (0.89)
	baseline 2	1.86 (0.96)	2.55 (0.57)
	evaluation 1	1.86 (0.72)	2.49 (0.75)
	evaluation 2	1.84 (1.06)	2.18 (0.66)

Acid concentrations

Lactic and acetic acid constituted 76% of all acids. As acetic and minor acid concentrations were found not to vary during the experiment (data not shown), we only report on lactic acid concentrations.

Baseline visits: At the 2 baseline visits, consistent average lactic acid values (n=30) were found in both buccal and interproximal plaque samples, before and after sucrose rinsing. The correlations (r^2) with P-values of the samples per subject taken at the 2 visits are shown in Table 2.2.

Lactic acid concentrations in resting buccal plaque were 2.5 fold lower than in resting interproximal plaque. In buccal plaque, the lactic acid concentrations increased 8-fold after sucrose rinsing while in interproximal plaque this increase was only 2-fold. At 18 min after the sucrose rinse, lactic acid concentrations in buccal plaque were still significantly higher than the resting values ($p<0.01$), while in interproximal plaque the concentrations had decreased at 20 min to approximately resting values ($p>0.5$; Table 2.2).

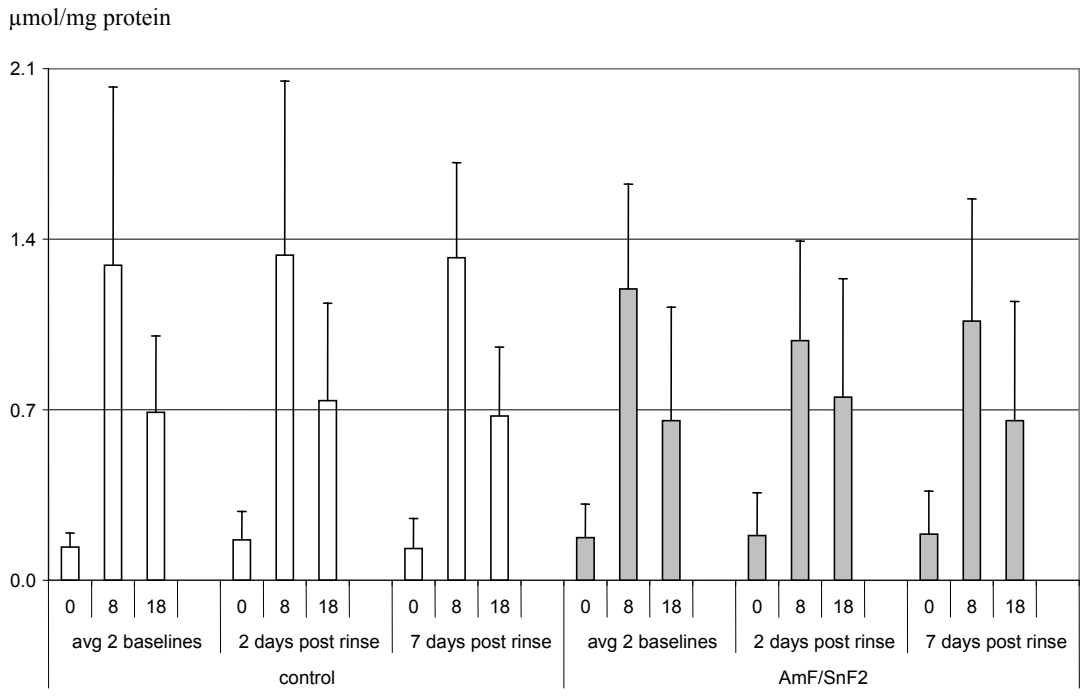
Table 2.2: Average lactic acid concentration (± standard deviation) in buccal and interproximal plaque before and after sucrose rinsing at 2 baseline visits and Spearman's correlation coefficients (r^2) with p-values in parentheses.

sample	pre / post sucrose	lactic acid in µmol/mg protein		
		baseline 1	baseline 2	Spearman's correlation coefficient r^2 (p)
buccal plaque	pre	0.16 (0.13)	0.15 (0.12)	0.01 (0.58)
	8 min post	1.14 (0.48)	1.29 (0.60)	0.29 (0.00)
	18 min post	0.65 (0.45)	0.68 (0.41)	0.35 (0.00)
interproximal plaque	pre	0.43 (0.42)	0.36 (0.38)	0.49 (0.00)
	10 min post	0.80 (0.37)	0.87 (0.50)	0.17 (0.03)
	20 min post	0.52 (0.39)	0.48 (0.37)	0.13 (0.07)

Evaluation visits: No differences were found in lactic acid concentrations in resting buccal plaque and at 8 and 18 min after the sucrose challenge after 3-wk use of post-brush water rinse (n=10) compared with AmF/SnF_2 rinse (n=19) (Fig. 2.1; ANOVA, $p>0.05$).

In interproximal plaque, the average lactic acid concentrations did neither differ significantly in resting plaque nor in sucrose challenged plaque after 8 and 18 min between individuals using a water rinse (n=10) after tooth brushing compared to those using AmF/SnF_2 mouthrinse (n=19; Fig. 2.2). Within the test-group (n=19), the response of interproximal plaque to sucrose rinsing showed a different pattern than that of buccal plaque, as was also found at the baseline measurements. At 18 min, the lactic acid concentration in buccal plaque was still elevated compared to resting values (Fig. 2.1) while in interproximal plaque the values after 20 min had almost reached the resting level (Fig. 2.2).

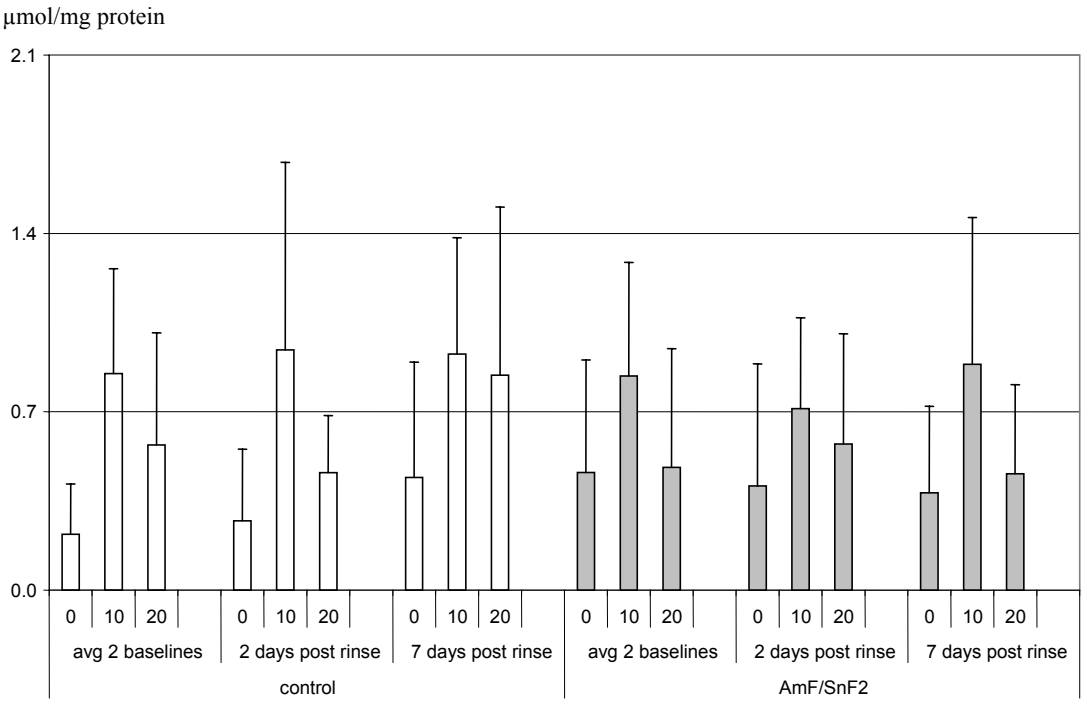
Figure 2.1: Average lactic acid concentrations upon sucrose challenge in buccal plaque before (average of 2 baseline measurements) and at the 2nd and 7th day after 3-wks daily use of AmF/SnF_2 mouthrinse (n=19) or a control water rinse (n=10); 0 min before and 8 and 18 min after sucrose



Discussion

This experiment studied plaque formation at the buccal and interproximal upper lateral tooth surfaces and the lactic acid concentrations in the contralateral buccal and interproximal upper plaque after 3-wk use of AmF/SnF_2 mouthrinse and NaF toothpaste. The Quigley and Hein plaque index (1962) was chosen for plaque assessments [Fischman, 1986]. Our results did not show statistically significant differences in average plaque scores after AmF/SnF_2 mouth rinsing compared to water rinsing. The first evaluation was on the 2nd day after the last rinse, which might have been too late to detect an effect in plaque amounts. However, significant reductions on plaque growth have been reported after the use of AmF/SnF_2 mouthrinse.

Figure 2.2: Average lactic acid concentrations upon sucrose challenge in interproximal plaque before (average of 2 baseline measurements) and at the 2nd and 7th day after 3-wks daily use of AmF/SnF_2 mouthrinse (n=19) or a control water rinse (n=10); 0 min before and 10 and 20 min after sucrose rinsing.



In these studies the rinse was combined with AmF/SnF_2 toothpaste [Zimmermann *et al*., 1993; Mengel *et al*., 1996; Paraskevas *et al*., 2004] enhancing the alleged effect on plaque formation [Banoczy *et al*., 1989] or the rinse was prescribed to be used twice a day [Brecx *et al*., 1993; Guarnelli *et al*., 2004] which is not in accordance with the manufacturer's instructions for use though it seems recommendable considering the reported results. Also the time period after the rinsing protocols in which the plaque assessments were made varied in the cited studies from 3-20 hrs. In contrast, our participants did not use a combination of AmF/SnF_2 products and the first assessments were done 2 days after discontinuing the 3-wks rinsing period.

At baseline and evaluation measurements, higher lactic acid concentrations were found in resting interproximal plaque than in resting buccal plaque. To our knowledge no such data on plaque acidogenicity at different sites have been published from *in vivo* studies. However, our finding is in agreement with Jensen and Schachtele (1983) who reported lower pH values in resting interproximal plaque compared to resting buccal plaque. The higher resting lactic acid concentrations might be related to reduced in- and outward diffusion in interproximal plaque. Dawes *et al*. (1989) postulated that the slow movement of the unstimulated salivary film leads to accumulation of diffusants from dental plaque, which reduces the concentration gradient for diffusion from plaque and prolongs the clearance time of metabolic products like acids. The clinical relevance of the higher resting values in interproximal plaque is not clear. It can be speculated that it leads to a lower pH [Jensen and Schachtele, 1983] and that it burdens the buffer potential of the plaque. Both conditions may promote the caries risk in the interproximal area.

After the sucrose challenges, the increase in lactic acid concentrations in the buccal plaque was 8-fold the resting value in contrast to a 2-fold increase in the interproximal plaque at all measurements. This difference might be due to incomplete penetration of sucrose in interproximal plaque as compared to buccal plaque since diffusion in the interproximal area is slow [Dawes *et al*., 1989]. This might be related to plaque thickness and to a smaller contact surface between plaque and rinsing

solution. But also differences in plaque composition and structure may contribute to different diffusion characteristics.

It is interesting that estimating from the amount of lactic acid produced within 20 min after a sucrose rinse, one might expect that buccal plaque would be more cariogenic than interproximal plaque. However, more caries develops in the approximal surfaces than in the buccal surfaces [Mejare *et al*., 2004]. This may be related to that people in general do not allow buccal plaque to develop undisturbed for 18 hrs, as in this experiment, while interproximal plaque may even be older. But also other characteristics than the immediate response to sucrose might determine the higher cariogenicity of the interproximal dental plaque such as the amount of acids in resting plaque, the pH or reduced diffusion of inorganic acids and therapeutics [Jensen and Schachtele, 1983; Dawes *et al*., 1989].

The use of AmF/SnF_2 mouthrinse did not have an inhibitory effect on acid production at either plaque collection site on the 2nd day after discontinuation of mouth rinsing. Previous work showed an inhibitory effect of AmF/SnF_2 on lactic acid production in buccal plaque when evaluated 3 and 6 hrs after use [Damen *et al*., 2002; Gerardu *et al*., 2006]. In those studies, the plaque was directly exposed to AmF/SnF_2 mouthrinse, while in the current study plaque was grown after discontinuation of using the AmF/SnF_2 mouthrinse. This would mean that an effect could only exist when AmF/SnF_2 was still present in the oral cavity or when there had been a change in plaque amount or in plaque composition. Zimmerman *et al*. (1993) studied the effect of a daily AmF/SnF_2 mouthrinse on plaque amounts and the composition of supragingival plaque in a 7-month clinical, double blind trial. They found significant changes in plaque composition in the AmF/SnF_2 group when the AmF/SnF_2 rinse was used in addition to AmF/SnF_2 toothpaste. Based on the current data that an effect of AmF/SnF_2 mouthrinse had already disappeared on the 2nd day after discontinuing its use additionally to F^- toothpaste, we conclude that none of these mechanisms were present after 3 wks. Whether a reduction in plaque amounts coincides with reduced plaque acidogenicity and vice versa remains to be studied in future research.

2.5 Conclusion

Higher lactic acid concentrations were found in resting interproximal plaque than in buccal plaque. The average response to sucrose challenge was stronger in buccal than in interproximal plaque, and the lactic acid concentrations were still elevated after 18 min in sucrose challenged buccal plaque.

No effect of AmF/SnF_2 on the acidogenicity of dental plaque after 3-wk use could be found on the 2^{nd} day after the last rinse. The 3-wk use of AmF/SnF_2 rinse once daily did not result in a prolonged reduction of acid formation in plaque nor in reduced site related plaque formation after discontinuation use of the rinse.

Chapter 3

The Effect of a Single Application of 40% Chlorhexidine Varnish on the Numbers of Salivary Mutans Streptococci and Acidogenicity of Dental Plaque

In *Caries Research* 2003; 37: 369-373
http://content.karger.com/produktedb/produkte.asp?typ=fulltext&file=CRE20030370053 69

V.A.M. Gerardu, M.J. Buijs, J.M. ten Cate, C. van Loveren

Abstract

The relationship between the numbers of salivary mutans streptococci and the acid production in dental plaque after a single application of the 40% chlorhexidine varnish EC40®, has been studied. Thirteen healthy subjects were treated with EC40-varnish. Saliva samples were taken before and up to 12 wks after treatment to count mutans streptococci and lactobacilli. At the same time points plaque samples were taken before and after sucrose challenge and these samples were analysed for protein and organic acid. Suppression of salivary mutans streptococci was observed together with a reduced production of lactic acid in sucrose challenged dental plaque in 9 subjects while inhibition of acid production without significant suppression of mutans streptococci was observed in the other 4 participants. The duration of the effects differed among the individuals but never exceeded 6 wks. We conclude that a prolonged suppression of mutans streptococci and acid production was not achieved by a single treatment with EC40 varnish in all subjects. Moreover, reduced acidogenicity of dental plaque after chlorhexidine treatment was not necessarily predicted by suppression of mutans streptococci in saliva.

3.1 Introduction

In spite of the overall decline of caries prevalence in developed countries [Marthaler *et al*., 1996] caries is still a concern among certain groups of individuals. Apparently, the available fluoride therapies do not give sufficient protection to these individuals. Therefore, additional antimicrobial therapy against mutans streptococci might be considered. For antimicrobial therapy against *Streptococcus mutans*, treatments for professional and for home use are available. For both types of treatments various antimicrobial agents with different application methods, concentrations and retentive properties have been developed: for chlorhexidine for instance, there are rinse-solutions, gels and varnishes. Chlorhexidine has a significant effect on the amount and

composition of dental plaque [Löe and Schiøtt, 1970; Rølla and Melsen, 1975]. Zickert *et al*. (1982), Lindquist *et al*. (1989) and Emilson (1981, 1994) have shown that frequent applications of 1% chlorhexidine gel reduced salivary levels of mutans streptococci concomitantly with a reduction of caries increment in children with high numbers of mutans streptococci. A meta-analysis of van Rijkom *et al*. (1996) reported a caries-inhibiting effect of 46% [95% CI: 35% - 57%] of chlorhexidine treatments by either a rinse or a gel, with 0.02% and 1% chlorhexidine, respectively. Chlorhexidine varnishes of 1%- to 40% showed effects on the numbers of mutans streptococci but the results were not conclusive on the efficacy of the treatments in caries prevention [Sandham *et al*., 1988; Ie and Schaeken, 1993; Twetman and Petersson, 1999; Forgie *et al*., 2000; van Lunsen *et al*., 2000]. However, the varnishes are of interest in caries prevention because they allow infrequent use and require little compliance. The caries preventive effect of the chlorhexidine varnishes is believed to consist of reduced cariogenicity of dental plaque after the suppression of mutans streptococci [Schaeken *et al*., 1989; Marsh, 1993]. Therefore, the question was raised whether a decrease in salivary mutans streptococci, after the application of a chlorhexidine varnish, is in time accompanied by a decrease in organic acid production in dental plaque.

In order to study their possible relationship, the suppression of salivary mutans streptococci and the acid production in dental plaque upon sucrose challenge after a single treatment of a 40% chlorhexidine varnish was monitored for 12 wks in a group of subjects.

3.2 Materials and Methods

Subjects

The study included 13 subjects who agreed to participate after informed consent (4 women, 9 men, mean age 25 years) and who all were dentally aware and in good general and dental health. Two weeks before the start of the experiment, and during the experiment, all participants brushed their teeth twice a day with sodium fluoride toothpaste (Prodent® Cool Mint 1450 ppm F^-, Sara Lee, Veenendaal, The

Netherlands). Neither dental hygiene instructions nor professional tooth cleaning were given.

Plaque and saliva sampling

Plaque and saliva samples were collected at five occasions to determine baseline values and at one day and one, three, six, nine and twelve wks after treatment. All subjects had been asked to refrain from oral hygiene eighteen hours before the collection of samples and were not allowed to take any food or drinks for two hours prior to sampling. Chew stimulated saliva samples to determine microbiological profiles were taken before the plaque samples were collected. Before a sucrose rinse, supragingival plaque was taken with a Teflon spoon from the buccal site of an upper molar in the first quadrant. To stop metabolic activity the plaque samples were immediately spun down into 50 µl MilliQ-water in a precooled vial (Eppendorf centrifuge 5415, Hamburg, Germany) and set on ice until further processing within one hour [Damen *et al*., 2002]. Immediately after sampling, the subjects rinsed for two min with 10 ml of a 10% sucrose solution and 8 min thereafter a contra-lateral sample was taken and processed in the same way as the first sample. The plaque sampling procedure itself never exceeded one minute.

Plaque processing

To release acids, all plaque samples were heated at 80ºC for 5 min and again cooled on ice [Damen *et al*., 2002]. Then the vials were centrifuged at 13,000 rpm for 15 min at 4ºC (Haereus centrifuge, Dijkstra BV, Lelystad, the Netherlands), their supernatants were transferred into vials with a micro spin filter (Ultrafree-MC 0.22 µm, Millipore, Bedford, Mass., USA) and centrifuged at 12,000 rpm for 5 min at 4ºC [Damen *et al*., 2002]. Finally, plaque pellets and filtered supernatants were stored at -80ºC. Later, the plaque pellets were thawed, resuspended in 200 µl MilliQ-water and sonicated on ice for 20 x 1 second (Kontes K-88140, Vineland, N.J., USA: maximum output) and analysed for protein content according to Bradford (1976) with the Bio-Rad protein analysis kit (Bio-Rad Laboratories GmbH, München, Germany) using bovine serum albumin as standard (Sigma Chemicals, St. Louis, MO, USA).

Organic acids analysis

Organic acids were determined as their anions by capillary electrophoresis on the Waters Capillary Ion Analyser (Milford, Mass., USA). Duplicate samples were run and Millennium Chromatography Manager software version 3.05 was used for data analysis. Peak identification and peak area integration were manually corrected if necessary. Sodium salts of formic, acetic, propionic, butyric, succinic and lactic acid (Sigma) were used to prepare single and mixture standard solutions in MilliQ-water. Calibration curves were made for each acid separately. As an internal standard, 0.12 mM $NaNO_3$ was included in all samples. Formic, butyric, succinic, propionic, acetic, and lactic acid were determined.

Treatment

The complete dentition was isolated with cotton rolls and air dried before EC40® tooth varnish (Explore, Nijmegen, The Netherlands) was applied according to the manufacturer's instructions. Once EC40 was applied, it was moistened to set and removed after 7.5 minutes.

Microbiology / saliva

Approximately 10 ml of paraffin chewing stimulated saliva was collected in a tube. These samples were kept on ice; sonicated 20 x for 1 second and serially diluted in Cystein Peptone Water [van Palenstein Helderman *et al*., 1975], (pH 7.3; g/L: 5 Yeast (Difco, Becton & Dickinson, Maryland, USA), 1 Peptone (Difco), 8.5 NaCl (Merck, Darmstadt, Germany), 0.5 Cysteine-HCl (Sigma). Appropriate dilutions were cultured on blood agar, TYC agar, TYCSB agar [van Palenstein Helderman *et al*., 1983] and on Rogosa agar (Difco). Total viable flora, all streptococci, mutans streptococci and lactobacilli were counted on the respective media. The plates were incubated anaerobically for 4 days at 37ºC using BBL GasPak (H_2 + CO_2; Becton & Dickinson, USA). Bacteria were counted using a microscope, using 60-250 times magnification (Wild Heerbrugg, Switzerland). Mutans streptococci and lactobacilli were identified on colony morphology and texture of the microorganisms. In case of doubt,

identification was made by gram staining, phase contrast microscopy and with the use of a rapid ID 32 strep test (bioMerieux, Lyon, France).

Statistics

Bacterial counts were transformed into log units before statistical analyses. All data, bacterial counts, acetate and lactate concentrations, from day 1, week 1, 3, 6, 9 and 12 were compared to the averaged values of the five baseline measurements with paired-samples t test using SPSS 10.0.

3.3 Results

Microbiology

The total viable flora as well as total streptococci counts did not show a significant reduction after application of EC40 (Fig. 3.1). A reduction was detected in counts of mutans streptococci and lactobacilli. The average log of the numbers of mutans streptococci decreased significantly from 5.6 ± 0.5 to 2.1 ± 2.1 CFU per ml saliva ($p<0.001$) at day one after treatment. After 1 week the values for the log numbers of mutans streptococci were increased to 4.6 ± 0.9 ($p=0.026$) (Fig. 3.1). At three wks the values for mutans streptococci were not significantly different ($p>0.05$) from the baseline value. For lactobacilli counts, the average log of the numbers of colony-forming units (CFU) decreased significantly, from 4.8 ± 0.9 to 3.8 ± 1.7 per ml saliva at day one ($p=0.024$). One week after treatment, values for lactobacilli had recovered to baseline levels again being and 4.7 ± 1.0 ($p>0.05$) (Fig. 3.1). Not all subjects showed a response to the chlorhexidine treatment; 4 out of 13 individuals did not show any significant reduction of salivary mutans streptococci or lactobacilli counts after the EC40 application.

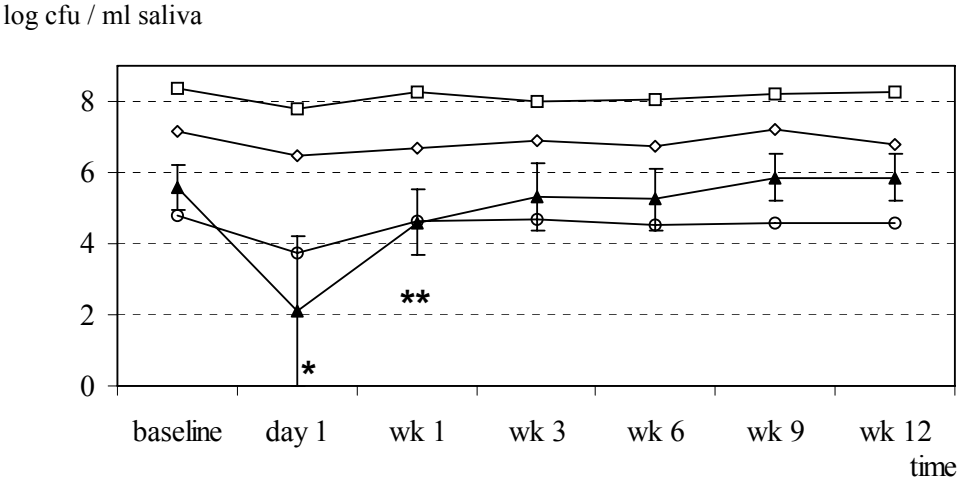


Figure 3.1: Average log (± SD) of salivary numbers of total viable flora (□), all streptococci (◊), mutans streptococci (▲) (* $p<0.001$; ** $p=0.026$) and lactobacilli (○) of all subjects before and after a single treatment with EC40.

Acid concentrations

Formic acid was often below detection limit; butyric and succinic acid concentrations contributed very little to the total acid concentration and therefore their values were not included in the results. Propionate, acetate and lactate concentrations in plaque before sucrose challenge did not show significant differences throughout the experiment. In sucrose challenged plaque, propionic as well as acetic acid concentrations were lower, although not statistically significant, at day one after treatment, and returned to baseline concentrations within one week.

The averaged post-sucrose lactate concentrations decreased significantly from the baseline value of 1.27 (± 0.1) μmol/mg protein to 0.47 (± 0.1) at day one ($p=0.002$) and rose back to baseline values of 1.24 (± 0.2) at week 3 ($p>0.05$) (Fig. 3.2). For the 4 subjects in whom the number of salivary mutans streptococci was not reduced, lactic acid production was, however, reduced comparably to the reduction in the total group.

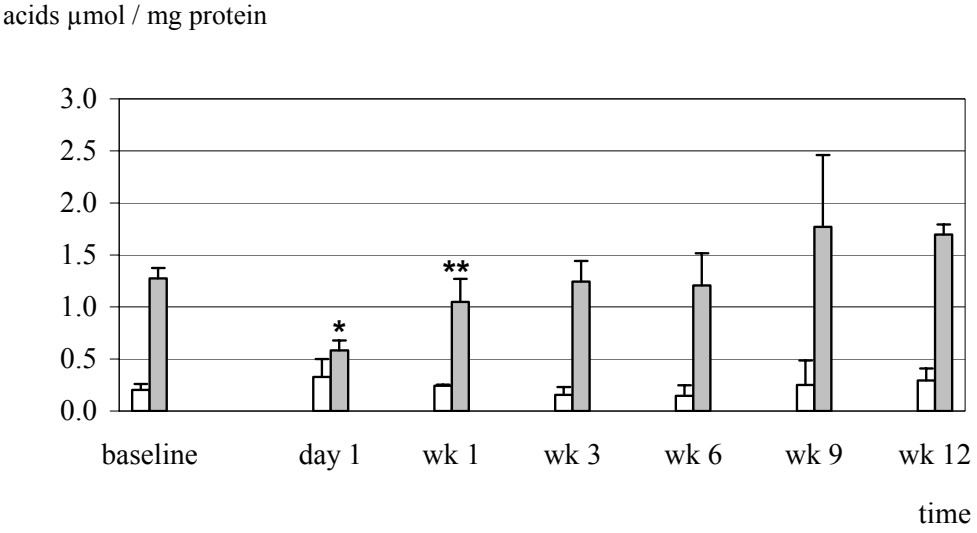


Figure 3.2: Average concentrations (± SD) of lactate in µmol/mg protein in dental plaque before (□) and after (■) sucrose challenge for all subjects (* p=0.002; ** p=0.045).

3.4 Discussion

Our findings showed no significant effect of a 40% chlorhexidine varnish on the total viable flora, total streptococci or lactobacilli counts in saliva. These results are in accordance with those of Schaeken *et al*. (1989), as is the suppression of mutans streptococci that we observed. In our study, however, the suppression of mutans streptococci lasted less than 3 wks, which is much shorter than the period reported by others [Zickert *et al*., 1987; Schaeken *et al*., 1989; Emilson 1994; Heintze and Twetman, 2002]. And there was a significant variation between participants. Failure of a single EC40 application on the numbers of mutans streptococci was also observed by van Lunsen *et al*. [2000]. One of the reasons for the short-lived suppression of salivary mutans streptococci might be the short application time of 7.5 min in our study (according to the manufacturer's instructions), while others left the varnishes on for 15 min or applied chlorhexidine for several times [Zickert *et al*., 1987; Schaeken *et al*., 1989; Emilson 1994; Heintze and Twetman, 2002]. The short application time could

have caused that relatively large numbers of mutans streptococci were only paralyzed and not killed. These paralyzed cells may have recovered relatively quickly. A short application time may also reduce the oral retention and substantivity of chlorhexidine after removal of the varnish, thereby hampering any sustained effect. And the short duration of the suppression may also be related to factors promoting the outgrowth of mutans streptococci, such as poor oral hygiene, high intake of sugars, and a remaining acidogenic flora. But the participants were dentally aware and were not expected to have poor oral hygiene habits or a sugar rich diet. An acidogenic flora was not detected immediately after treatment (see our lactic acid results) and it is not reasonable to assume that remaining bacteria have promoted the recovery of *S. mutans*. A reason for the variation among the participants is the variety in sensitivity of mutans streptococci to chlorhexidine. *In vitro* studies showed that minimum inhibitory concentrations of chlorhexidine may vary 8-fold between various *S. mutans* strains [Ashley, 1984].

During the suppression of salivary mutans streptococci, acid production in plaque was decreased. We found, however, subjects in whom a suppression of salivary mutans streptococci was not detected but a significant reduction of lactic acid production in plaque was. One explanation for this could be that bacterial counts in saliva samples do not reflect the bacterial composition in plaque, although the number of bacteria in saliva is generally considered to be a reasonable measurement of the entire dentition's microbial load [Schaeken *et al*. 1987; Lindquist *et al*., 1989a, b].

Another explanation might be that the non-mutans streptococci were responsible for the acid production [van Ruyven *et al*., 2000], and that these strains were inhibited by chlorhexidine. Indeed, in the four respective subjects, total streptococci counts showed a tendency to decrease, although not significantly after the chlorhexidine-treatment (data not shown). A third explanation is that cells were inhibited by chlorhexidine in dental plaque, but that detachment of chlorhexidine from these cells occurred when the cells were rinsed off the dental plaque by saliva. Also diluting the samples for the counting procedure might have washed away the chlorhexidine from the cells.

Our question was whether suppression of mutans streptococci is a good indicator for reduced acid production. From the results, we conclude that suppression of the number of mutans streptococci is accompanied by a decrease in acidogenicity of dental plaque and the regrowth of the number of mutans streptococci by a recovery of acid production. Sustained suppression of the number of mutans streptococci and reduced acidogenicity (over 3 wks) was not achieved in any of the subjects. We found 4 subjects in whom a suppression of mutans streptococci was not observed, but a significant reduction of lactic acid production was. This implies the non-suppression of mutans streptococci in saliva to be a poor indicator for reduced acid production after chlorhexidine treatment.

Acknowledgements

We wish to thank all subjects for their participation and compliance during the study and dr. I.H.A. Aartman for her statistical advice.

Chapter 4

Effect of an Intensified Treatment with 40% Chlorhexidine Varnish on Plaque Acidogenicity

In press in: *Clinical Oral Investigations* 2006

V.A.M. Gerardu, M.J. Buijs, J.M. ten Cate, C. van Loveren

Abstract

Previous work showed that a single application of 40% chlorhexidine varnish, EC40®, reduced plaque acidogenicity upon sucrose challenge only less than 3 wks. It was questioned whether lactic acid production could be reduced significantly longer when the treatment was intensified. Therefore, the effects of 3 consecutive EC40 applications on plaque acidogenicity were evaluated.

Nine subjects who participated in the previous study received 3 full mouth EC40 applications within 1 wk. Before the 1st application and up to 9 wks after the 3rd application, plaque samples were taken after a 10% sucrose rinse and analysed for organic acids with capillary electrophoresis.

At baseline, the mean provoked lactic acid concentration was 1.64 (± 0.69) μmol/mg protein. At the 1st and 7th day after the 3rd application there was too little plaque to measure acid concentrations. At 2 wks after the 3rd application, lactic acid concentrations were significantly reduced ($p<0.05$). The acid concentrations 3 wks after the 3rd application (1.61 (± 0.99) μmol/mg protein) did not differ from the values at baseline (paired t test, $p>0.05$). We conclude that a triple 40% chlorhexidine varnish treatment did not affect plaque acidogenicity for more than 3 wks. From comparison with a previous study, we conclude that the triple treatment with EC40 within 1 wk was not more effective in reducing plaque acidogenicity than the single one.

4.1 Introduction

Chlorhexidine can be used in addition to fluoride for patients with a high caries risk. As caries is a continuous process, the aid of chlorhexidine would require long-term use. Because of the side effects - such as bad taste and discoloration of the teeth - and the required compliance, long term daily use of mouthrinses is unrealistic. Therefore, dental varnishes with lasting antimicrobial effects developed to be applied every 3-6 months would be a desirable and suitable alternative [Emilson, 1994; Schaeken *et al*.,

1994]. Our previous study on the effect of a single 40% chlorhexidine varnish application showed a suppression of salivary mutans streptococci that was paralleled by a decrease in the acidogenicity of dental plaque. The subsequent regrowth of the salivary mutans streptococci within less than 3 wks was accompanied by a recovery of the acidogenicity [Gerardu *et al*., 2003].

It is doubtful whether a 3-wk period of reduced acidogenicity of dental plaque contributes substantially to the reduction of caries when the treatments are repeated with a 3- to 6-month interval. A more frequent application of chlorhexidine varnish e.g. once every 3 wks might be a solution but would require a lot of compliance of the participants and would be costly as a caries preventive measure.

For varnishes containing 1-10% chlorhexidine it has been demonstrated that multiple applications within short periods of time prolonged the suppression of salivary [Sandham *et al*., 1988; Sandham *et al*., 1991] and plaque mutans streptococci [Bratthall *et al*., 1995]. For 40% chlorhexidine varnish, a small advantage in suppression of the number of mutans streptococci was reported after 2 applications with a 1-wk interval compared to a single application [Schaeken and de Haan, 1989; Ie and Schaeken, 1993]. The clinical importance of the reported suppression of mutans streptococci in time or in numbers can be questioned. Furthermore the effect on the acidogenicity of dental plaque was not assessed in these studies.

The aim of this study was to evaluate the effects of a triple application of EC40® on acid production in sucrose challenged plaque.

4.2 Materials and Methods

Subjects

Six months after the single application study [Gerardu *et al*., 2003], all 13 dental students were invited for this intensified application program. Four students could not participate in this second trial because of scheduling conflicts to the strict design. The remaining 9 subjects (5 men, 4 women, mean age 25 (± 9) years) signed an informed

consent letter that was reviewed by the scientific board of the dental school. They were all in good general health, and showed a fairly good overall level of oral hygiene.

Set up

Two weeks before the chlorhexidine applications, and throughout the study period, the participants brushed their teeth twice a day with regular sodium fluoride toothpaste (Prodent® Cool Mint 1450 ppm F^-, Sara Lee, Veenendaal, The Netherlands). Neither dental hygiene instructions nor professional tooth cleaning were given before the start of the treatment. All participants received 3 applications of EC40® (Explore, Nijmegen, The Netherlands) every second day within 1 wk. At baseline and after the 3rd chlorhexidine application, sucrose challenged plaque was sampled as described below at various time intervals up to 9 wks.

Treatment

After the participants were asked to brush their teeth preceding the application, the complete dentition was isolated with cotton rolls and air dried before EC40 was applied from a syringe with a blunt end needle in the fissures, approximal areas and to all tooth surfaces that were close to the gingiva. On average approximately 1.2 ml varnish was applied containing 0.44 mg chlorhexidine each application. Once EC40 tooth varnish had been applied, it was moistened to set and removed after 7.5 min according to the manufacturer's instructions. The application was repeated twice within 1 wk. All varnishes were applied by the same operator.

Plaque sampling

Plaque samples were collected at baseline just before the 1st application, and at day1, week 1, 2, 3, 6 and 9 wks after the 3rd application. The participants refrained from oral hygiene 18 hrs before plaque sampling. They were not allowed to take any food or drinks for 2 hrs before sampling. At each visit, subjects rinsed with 10 ml of a 10% sucrose solution for 2 min to induce acid formation; 8 min thereafter a plaque sample was taken with a Teflon spoon from the buccal surfaces of the 1st and 2nd molar in the upper left jaw. Plaque was immediately spun down at 16.100 *g* for 30 s into 50 µl

MilliQ-water in a precooled vial and put on ice until further processing within 1 hr. The plaque sampling procedure itself never exceeded 1 min.

Plaque processing

The plaque samples were processed as described by Damen *et al*. (2002). In brief: the samples were heated to stop metabolic activity and cooled on ice to release the acids. Plaque samples were centrifuged in micro spin vials (Ultrafree-MC 0.22 μm, Millipore, Bedford, Mass., USA) and the plaque pellets stored at -80ºC separately from their supernatants. Later, plaque pellets were thawed, resuspended in 200 μl MQ and sonicated on ice for 20 x 1 s (Kontes K-88140, Vineland, N.J., USA: maximum output). The protein content was determined [Bradford, 1976] using the Bio-Rad protein analysis kit with bovine serum albumin as standard (Sigma Chemical Co. St. Louis, MO, USA).

Organic acids analyses

In the supernatants, the organic acids were determined as their anions by capillary electrophoresis on the Waters Capillary Ion Analyser (Milford, Mass., USA). Sodium salts of formic, acetic, propionic, butyric, succinic and lactic acid (Sigma Chemical Co. St. Louis, MO, USA) were used to prepare mixture standard solutions in MilliQ-water. As an internal standard, 0.12 mM $NaNO_3$ was included in all samples. Calibration curves were made for each acid separately. Samples were analysed in duplicate and Millennium Chromatography Manager Software version 3.05 was used for data analyses. Peak identification and peak area integration were manually corrected if necessary.

Statistics

The Statistical Package for the Social Sciences (SPSS version 10.0) was used to perform statistical analyses. Paired-samples t tests compared the acetic and lactic concentrations at the various evaluations to the baseline measurements. The minimally detectable difference between the response variables, using 9 participants, the level of

significance set at $p<0.05$ and a power of 0.8, is 0.30. This is based on the assumption that the within patient standard deviation of the response variable is 0.2.

4.3 Results

All plaque samples were analysed for formic, butyric, propionic, succinic, acetic, and lactic acid. The formic, butyric, propionic and succinic acid concentrations were not included in the results because their combined contribution to the total acid concentration was less than 15%. We present the results of the single and triple applications in the same 9 participants. The attrition of 4 students had not significantly affected the results of the single treatment as reported previously [Gerardu *et al*., 2003]. The results of the single chlorhexidine varnish application on the acetic and lactic acid concentrations in buccal plaque of these 9 subjects are shown in Fig. 4.1.

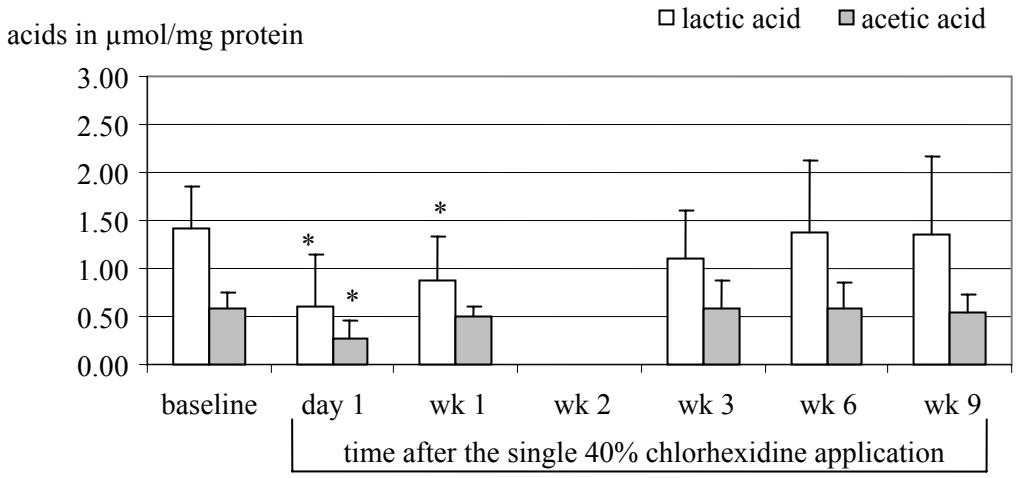


Figure 4.1: Average lactic (□) and acetic (■) acid concentrations (± SD) in µmol/mg protein in sucrose challenged dental plaque after the single 40% chlorhexidine varnish application; n=9 (*$p < 0.05$ significantly different from baseline)

After the single application, the concentration of acetic acid in sucrose challenged plaque was significantly reduced at day 1 (p=0.01). The average post-sucrose lactic acid concentration decreased significantly from the baseline value of 1.42 (± 0.44) µmol/mg protein to 0.60 (± 0.54) at day one (p=0.03) and rose back to values of 1.10 (± 0.51) at wk 3 (p>0.05).

After the 3rd application, plaque samples could not be taken on the first 7 days. Therefore, an additional examination was inserted after another week, at which one of the subjects still produced too little plaque for sampling. Two weeks after the 3rd application, the average post-sucrose acetic acid concentration did not differ from the baseline values (Fig. 4.2).

The average post-sucrose lactic acid concentration at wk 2 after the 3rd application (1.22 ± 0.53 µmol/mg protein) was lower (p=0.05) than the baseline value (1.64 ± 0.69). At wk 3 after the 3rd application the average lactic acid concentration (1.61 ± 0.99) did not differ from the average baseline value.

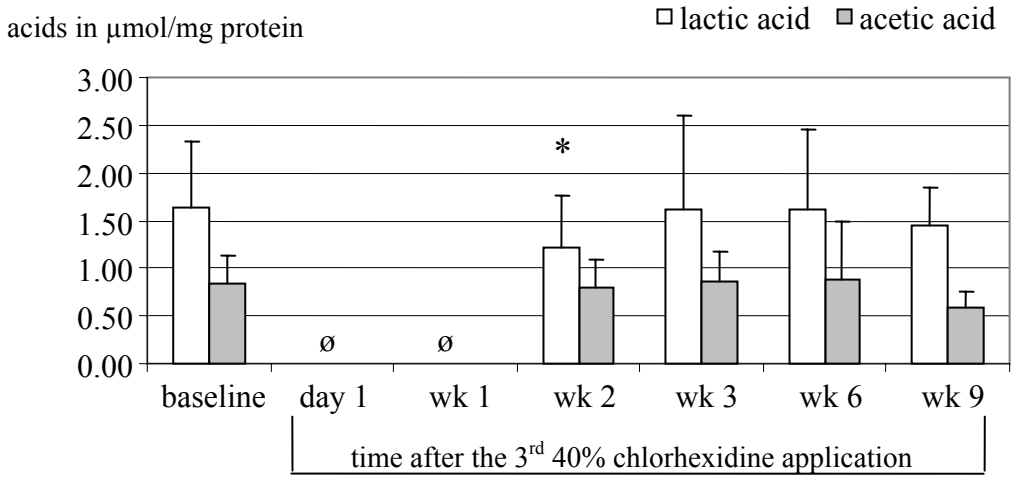


Figure 4.2: Average lactic (□) and acetic (■) acid concentrations (± SD) in µmol/mg protein in sucrose challenged dental plaque after 3 applications with 40% chlorhexidine varnish; n=9 (*p < 0.05 significantly different from baseline; ø =too little plaque to sample)

Despite the average reduction in lactic acid production 2 wks after the 3rd application, the effect had already disappeared in 3 subjects. In these 3 subjects the effect had disappeared also very rapidly in our 1st study [Gerardu *et al*., 2003] (data not shown).

4.4 Discussion

Most studies on antimicrobials have measured both salivary and plaque mutans streptococci as an output parameter for efficacy of antimicrobial treatments [Marsh, 1993; Emilson, 1994].
In our single EC40 application study, we evaluated not only the number of salivary mutans streptococci but also the acidogenicity of dental plaque. We found that the reduction of salivary mutans streptococci was closely paralleled by the reduction of lactic acid in dental plaque [Gerardu *et al*., 2003]. But, in some patients salivary mutans streptococci recovered immediately while acid production continued to be reduced. Since acid is the metabolite of mutans streptococci causing caries, we chose acid production as the output parameter in the current clinical trial. In our opinion the evaluation of the effect of our intensified antimicrobial treatment by acid concentrations in plaque is of sufficient clinical relevance.

Different modes of chlorhexidine application have been explored in patients with moderate or high caries incidence [Sandham *et al*., 1988; Schaeken *et al*., 1991; Fennis-le *et al*., 1998]. Because the killing efficacy of chlorhexidine is supposed to increase with repeated applications [Emilson *et al*., 1987], several authors have compared the effects of a single treatment on mutans streptococci in plaque to those of repeated treatments [Schaeken *et al*., 1989; Ie and Schaeken, 1993; Twetman and Petersson, 1997; Heintze and Twetman, 2002]. Ie and Schaeken (1993) reported a 2 months prolonged suppression of mutans streptococci in plaque in fissures of (pre-) molars after a double EC40 treatment compared to a single or a placebo treatment. In that study, however, the time point of evaluating the double treatment was counted from the day of the 1st application. By doing so, they compared 4-wks results after the single application with 3-wks results after the double application. So, in fact two

variables –the treatment modality and the time since the last application- were evaluated which could both explain the results. To avoid this complication, we chose to evaluate the intensified treatment on the described time points after the 3rd application. A comparison with the single application study evaluated purely the length of the effect of the treatment modality and not the benefit of the fact that the treatment itself took more time.

Others found that chlorhexidine had a continued suppressive effect on the numbers of salivary mutans streptococci after 4-7 applications of 1% chlorhexidine gel or 20% chlorhexidine varnish respectively [Maltz *et al*., 1981; Sandham *et al*., 1991]. Twetman (1999) and Petersson (1999, 2000) studied the effect of a triple 1% chlorhexidine/thymol-containing varnish treatment (Cervitec®, Vivacare, Schaan, Liechtenstein) on the incidence and progression of approximal caries in schoolchildren anticipated to be at caries risk. Children who exhibited a less marked suppression of levels of interdental mutans streptococci after a triple 1% application showed a significantly higher progression than the children with a high suppression of mutans streptococci ($p<0.01$). Based on this literature, we also chose for 3 applications within 1 wk for our intensified 40% chlorhexidine varnish treatment.

Delayed bacterial regrowth after chlorhexidine varnish treatment is based on the principle of colonisation resistance [Emilson *et al*., 1987; Schaeken and de Haan, 1989]. It has been shown that the effectiveness of this mechanism depends on the percentage of the mutans population initially killed [Emilson and Lindquist, 1988]. This may explain the benefit of the multiple treatments with lower concentrations (1-20%) of CHX [Maltz *et al*., 1981; Sandham *et al*., 1991; Twetman and Petersson, 1999; Petersson *et al*., 2000]: each treatment killed an additional fraction of the *S. mutans* population [Sandham *et al*., 1992]. Then, after each treatment, it would be more difficult for the mutans population to recover. We had expected that repeated applications of 40% chlorhexidine varnish would also result in a longer period of reduced numbers of mutans streptococci and therefore of acid production in dental plaque. Although the total absence of dental plaque in the first wk after the 3rd application suggests a higher killing efficacy of the intensified program, this did not result in a prolonged reduction of the acidogenicity of dental plaque beyond a period

of 3 wks. Prolonged colonisation resistance is not necessarily achieved in dental plaque by an intensified chlorhexidine application regime. Probably, the killing of the mutans streptococci by the first 40% chlorhexidine application may have been so effective that a second and third application shortly after the first one did not reduce the remaining mutans population significantly further.

We studied the effects of a triple chlorhexidine application on acid production in dental plaque. From comparison with our previous study [Gerardu *et al*., 2003], we conclude that an intensive treatment with EC40® is not superior to a single treatment in reducing acidogenic properties of dental plaque, although it is more effective in inhibiting plaque regrowth during the 1st week after intensified treatment.

Acknowledgements

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Chapter 5

Increased Salivary Fluoride Concentrations after Post-brush Fluoride Rinsing Not Reflected in Dental Plaque

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Abstract

The aim of the present study was to assess fluoride concentrations in unstimulated saliva and buccal dental plaque 6 hrs after an oral hygiene procedure that consisted of brushing with an AmF/SnF_2 dentifrice and different post-brush rinsing protocols: expectorating the excess of dentifrice foam and rinsing with tap water, expectorating only, or rinsing with 10 ml AmF/SnF_2 mouthwash. The fluoride concentrations in plaque and saliva were increased after all three experimental protocols compared to F^--free periods. The increase of the fluoride concentration in saliva was more pronounced after AmF/SnF_2 mouthrinse as compared to rinsing with water and expectorating the excess of dentifrice foam. Such an effect was not seen in the dental plaque. It is concluded that the potentially beneficial effect of not rinsing or fluoride rinsing after tooth brushing is not reflected in an increased fluoride concentration in newly formed dental plaque 6 hrs after brushing.

5.1 Introduction

The wide spread use of fluoride dentifrices has led to a marked decline in the prevalence of dental caries in many industrialized countries over the past three decades [Bratthall, 1996]. Through the daily application of fluoride dentifrices the teeth are continuously exposed to significantly elevated salivary fluoride concentrations [Duckworth *et al*., 1987, 1992]. Elevated salivary fluoride concentrations and elevated plaque fluoride concentrations are suggested to be predominant ways by which fluoride exerts anti-caries effects by reduction of the rate of dissolution of tooth mineral under acidic conditions, and by enhancement of remineralisation at the crystallite surface at pH levels above the critical value [Featherstone, 1999; ten Cate, 1999].

After tooth brushing, it is common practice to rinse the mouth with tap water. It has, however, been reported that abundant rinsing or rinsing after spitting out the

toothpaste affects the retention of fluoride, resulting in reduced approximal plaque and salivary fluoride concentrations [Sjögren and Melin, 2001; Duckworth *et al*, 1991]. In contrast, no spitting, rinsing with a dentifrice foam-water slurry or with a fluoride solution could elevate the salivary fluoride concentration up till 180 min after brushing [Sjögren and Birkhed, 1994; Sjögren and Melin, 2001]. It has been demonstrated that the retention of fluoride is dependent on the source of ionic fluoride [Duckworth *et al*., 1994]. Recently, Issa and Toumba (2004) demonstrated that amine fluoride (AmF) dentifrice displayed higher salivary fluoride concentrations compared to other types of fluoride containing toothpaste for at least 120 min after brushing, which was in agreement with a previous experiment of Attin and Hellwig (1996).

It is of interest to know how long increased concentrations of fluoride continue, since the duration could influence the efficacy of fluoride uptake in newly formed plaque during the day [Duckworth and Morgan, 1991]. Sidi and Wilson (1991) concluded that the potentially beneficial effect of a raised concentration of fluoride in approximal plaque observed shortly after brushing is lost during the following 24 hrs. In most studies the samples were collected between 30-180 min after the use of the products [Duckworth and Morgan, 1991; Sjögren and Birkhed, 1994; Campus *et al*., 2003; Issa and Toumba, 2004]. Since little is known about F^--levels between 3 and 24 hrs after brushing, it is interesting to study this period. A 6-hrs post-brush assessment could be relevant as an assessment approximately mid-way between two habitual brushing exercises.

The aim of the present experiment was to assess fluoride concentrations in saliva and dental plaque samples 6 hrs after an oral hygiene procedure that consisted of brushing with an AmF/SnF_2 dentifrice and different post-brush rinsing protocols.

5.2 Materials and Methods

Subjects

Thirty non-dental students (22 female, 8 male, mean age 26.8 (± 7.3) years) were recruited for this study. The inclusion criteria were: good general and dental health, no

antibiotic treatments during the last 4 wks, at least 24 natural teeth and an unstimulated salivary flow of 0.25-0.35 ml/min. Subjects were informed about the aims and objectives of the study and signed an informed consent.

Study design

After screening and before the start of the trial, the subjects were asked to use NaF dentifrice (Prodent® Cool Mint 1450 ppm F^-, Sara Lee, Veenendaal, The Netherlands) for 2 wks. During the next 2-week washout period the subjects used F-free dentifrice (Ultradent®, Kruidvat, Renswoude, The Netherlands). Subsequently, the participants followed three different rinsing protocols after tooth brushing with AmF/SnF_2 dentifrice (1400 ppm F^-; meridol®, GABA International AG, Münchenstein, Switzerland) in a crossover design. Participants' randomization numbers were set using a computer-generated allocation schedule. Subjects were asked to brush twice daily with the AmF/SnF_2 dentifrice for 1 week with one of the following post-brush rinsing protocols: (1) expectorating the excess of dentifrice foam followed by 30-second rinse with tap water (+H_20); (2) expectorating the excess of dentifrice foam only (-H_20), and (3) after expectoration of the toothpaste rinsing with 10 ml AmF/SnF_2 mouthwash (250 ppm F^-; meridol®) for 30 s (+Rinse). Each protocol was followed by a 2-week washout period with F-free dentifrice (Ultradent®) (Table 5.1). In total 8 appointments were scheduled. At the end of each visit, subjects were instructed about the procedure for the subsequent period. They were provided with a new toothbrush for every new fluoride period. In an attempt to optimize compliance, all participants received text messages on their mobile phones on the day of the appointment to remind them of their scheduled time of brushing and the time from which they had to refrain from using any food and drinks.

Sampling procedures

One examiner who was not aware of the protocol assignment collected all samples. Prior to sampling, subjects had to refrain from food and drinks for 1.5 h. At the start of each appointment (6 hrs after the last tooth brushing), the individuals were asked to drool for 5 min in a sterile plastic cup, to collect a non-stimulated whole saliva sample.

The saliva samples were stored at -80°C until analysis. Supra-gingival plaque was harvested from the buccal surfaces of the (pre-) molars with a sterile Teflon spatula in the 1st and 3rd quadrant separately. The collected plaque samples were immediately spun down, at 16,100 *g* (Centrifuge 5415D, Eppendorf, Hamburg, Germany), in pre-weighed (to the nearest 0.01 mg) Eppendorf tubes. The tubes were stored on ice until they were weighed to assess the wet weight of the plaque sample and the samples were stored at -80°C.

Fluoride analysis

Fluoride was determined with gas chromatography through methods modified for plaque and saliva [van Loveren *et al*., 2005]. Mean fluoride values at the start and end of the three rinsing protocols were calculated from triplicate measurements for saliva and for plaque samples.

Plaque

To extract the fluoride from the plaque, 75 µl 1 *M* $HClO_4$ was added to thawed plaque samples. When the plaque weight exceeded 3 mg, 25 µl $HClO_4$ was added for each extra mg of plaque. The samples were incubated for 60 min at room temperature and subsequently spun down for 15 min at 16,100 *g* at 4°C. For analysis, 75 µl of the supernatant was mixed with 75 µl 1 *M* $HClO_4$ and subsequently 80 µl toluene reagent (containing 0.025% trimethylchlorosilane and 0.005% isopentane) was added.

Saliva

Saliva samples were thawed and centrifuged for 5 min at 16,100 *g*, 1 ml of clear supernatant was mixed with 0.4 ml of 1 M $HCLO_4$ and 0.4 ml of toluene reagent. After overnight incubation the vials were shortly centrifuged to separate phases. Fluoride was analysed in samples taken from the toluene phase. Calibration curves were made from standard solutions containing known amounts of fluoride.

Table 5.1: Study design: the use of NaF (control) or F-free (washout) toothpaste for 2-week periods and the use of AmF/SnF_2 toothpaste with different rinsing protocols (experiment) in randomized order for 1-week periods. An asterisk indicates provision of a new toothbrush.

Control	Washout	Experiment	Washout	Experiment	Washout	Experiment	
NaF toothpaste + H2O rinse	F-free toothpaste + H2O rinse	AmF/SnF_2 toothpaste + H2O rinse or AmF/SnF_2 rinse or no rinse	F-free toothpaste + H2O rinse	AmF/SnF_2 toothpaste + H2O rinse or AmF/SnF_2 rinse or no rinse	F-free toothpaste + H2O rinse	AmF/SnF_2 toothpaste + H2O rinse or AmF/SnF_2 rinse or no rinse	
Week 0*	2	4*	5	7*	8	10*	11

Statistics

Wilcoxon-tests were used to analyse changes between pre- and post-experimental fluoride values. Friedman tests were used to compare the different protocols and to compare the fluoride values of all four quadrants at baseline. Values of $p \leq 0.05$ were accepted as statistically significant. The study design had a power of >80% to discern a difference of 6.4 ppm in plaque fluoride concentration between regimens with a standard deviation of 12 ppm for pooled, within-subject differences. For salivary fluoride concentration, a difference of 0.0064 ppm could be established with a pooled with-in-subject standard deviation of 0.012 ppm.

5.3 Results

All subjects completed the trial. Seven subjects failed to produce enough saliva at one of the appointments and in 4 subjects the plaque samples at one of the appointments were too small for further reliable analysis. These saliva and plaque samples were not included in the data analyses.

Saliva

The mean pre-experimental (F-free) values before the three procedures were not statistically significantly different and ranged between 0.009-0.011 ppm of fluoride. The fluoride concentration at the end of $+H_2O$ procedure was 0.013 ± 0.005 ppm, at the end of $-H_2O$ procedure 0.013 ± 0.005 ppm and at the end of +Rinse procedure 0.021 ± 0.015 ppm. These values were statistically significant different from the values of the respective F-free periods. The salivary fluoride concentration at the end of the three rinsing protocols appeared also to be significantly higher than the fluoride concentration after the 2-week use of NaF (0.011 ± 0.007 ppm). The fluoride concentration at the end of the +Rinse period was significantly higher as compared to at the end of the $+H_2O$ ($p=0.013$) and $-H_2O$ ($p=0.005$) periods.

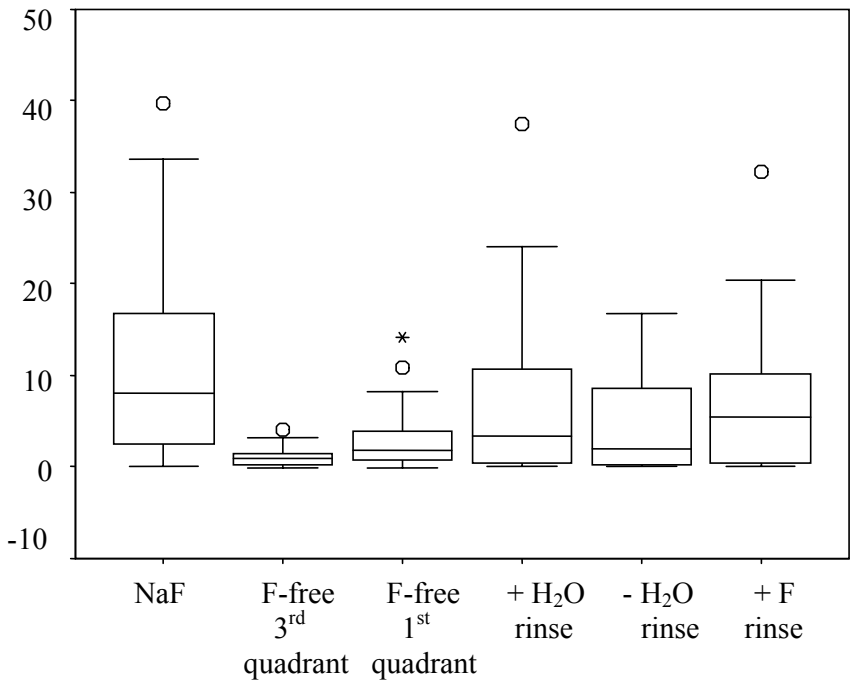

Figure 5.1: Individual fluoride concentrations (ppm) in plaque after the use of NaF toothpaste, F-free toothpaste (3^{rd} and 1^{st} quadrants separately) and after the use of AmF/SnF_2 toothpaste with three different rinsing protocols after tooth brushing. A circle indicates between 1.5 and 3 box lengths from the upper edge. An asterisk indicates >3 box lengths from the upper edge.

Plaque

In order to validate the choice to collect plaque samples only from the 1^{st} and 3^{rd} quadrant the fluoride concentrations were additionally assessed in the 2^{nd} and 4^{th} quadrant after the NaF period. The concentrations in the four quadrants varied between 11.9 ± 16.7 and 13.0 ± 16.8 and were not statistically significantly different (p=0.3). Also in the other F periods no significant differences were found between the fluoride concentrations in the 1^{st} and 3^{rd} quadrants. Therefore, the 1^{st} and 3^{rd} quadrant data were combined and averaged for further analyses. Interestingly, however, the mean pre-experimental fluoride values (after 2 weeks' brushing with F-free dentifrice) differed significantly being 2.4 ± 3.6 in the 1^{st} quadrant and 1.1 ± 1.3 in the 3^{rd} quadrant. The mean fluoride concentrations at the end of the three rinsing protocols were not significantly different being 9.1 ± 12.8 ppm for the $+H_2O$ regimen, 5.6 ± 6.6 ppm for

the -H_2O and 7.9 ± 9.1 ppm for the +Rinse. The fluoride concentration after the -H_2O regimen was significantly lower than after the NaF period (12.3 ± 12.3 ppm). Figure 5.1 shows the individual variation in the fluoride concentrations in dental plaque after brushing with Am/SnF_2 and the three post-brush protocols in box-plots. This illustrates clearly that no significant differences were found between the three rinsing protocols.

The correlations between the three rinsing protocols and the NaF-period for plaque and salivary fluoride concentrations were calculated. A significant correlation was found between AmF/SnF_2 and NaF both with post-brush water rinsing in 6hrs old plaque (r=0.455, p=0.011). The correlation coefficients between fluoride in plaque of NaF dentifrice with the -H_2O (r=0.211) and with the +Rinse (r=0.064) post-brush procedure were not statistically significant.

5.4 Discussion

In the present study the fluoride retention of AmF/SnF_2 dentifrice followed by three different rinsing protocols in saliva and dental plaque was evaluated. The values were compared to the fluoride concentrations after the use of a NaF dentifrice followed by rinsing with water according to subject's usual routine. The present study confirmed that using fluoride containing toothpaste increased the fluoride concentrations both in plaque and saliva compared to brushing with non-fluoride dentifrice irrespective of the rinsing protocol. Furthermore, it was found that the combination of AmF/SnF_2 dentifrice and AmF/SnF_2 mouthrinse significantly elevated the fluoride concentration in saliva when compared with brushing followed by rinsing with water or expectorating only. Such an effect was not found for the concentration of fluoride in plaque.

Omitting the usual water rinse after brushing with AmF/SnF_2 dentifrice did not result in significantly elevated salivary fluoride concentrations. This result was surprising, considering the results of other, previously performed fluoride experiments, in which post-brush water rinsing was not allowed. In these studies, an increase of salivary fluoride content was found. Fluoride concentrations were followed up to 3hrs

after brushing [Duckworth and Morgan, 1991; Sjögren and Birkhed, 1994; Sjögren and Melin, 2001; Issa and Toumba, 2004]. One may wonder whether such differences would last up to 6 hrs. If any differences would have been present shortly after brushing and rinsing in the present study, apparently they were not present after 6 hrs.

Our results did not confirm that omitting rinsing with H_2O after brushing or rinsing with a fluoride solution increased the fluoride concentration in dental plaque. In fact, there was a tendency to less fluoride in the dental plaque in the -H_2O period. Except for the above-mentioned difference in duration of the experiments, an explanation might be that in previous experiments plaque was evaluated that had survived the oral hygiene procedure and was topically treated by the fluoride application [Sjögren and Melin, 2001]. In the present experiments plaque formed after the oral hygiene procedures was evaluated. The fluoride concentration in newly formed plaque results from the uptake of fluoride from saliva. The efficiency of this process not only depends on the availability of fluoride, but also on the 'availability' of dental plaque. If plaque re-growth is less or delayed, or less binding sites are available in the dental plaque, less fluoride will be taken up. An inhibition of plaque accumulation has been observed after using AmF/SnF_2 dentifrice and/or mouthrinse in several studies [Zimmermann *et al*., 1993; Mengel *et al*., 1996; Paraskevas *et al*., 2004]. If the AmF/SnF_2 had a similar effect on the plaque growth in the present study, then this could have reduced fluoride uptake. The finding that the AmF/SnF_2 rinse resulted in higher salivary fluoride concentrations but not in higher plaque fluoride concentrations may thus be related to an antimicrobial effect on plaque re-growth.

An interesting observation in the present study was that the mean pre-experimental fluoride values, after 2 weeks' brushing with F-free dentifrice, were significantly higher in the plaque of the 1st quadrant than in the 3rd quadrant. The design of the present investigation does not permit an explanation for this finding. Yao and Grøn [1970] found similar concentrations of fluoride in parotid and submandibular saliva when collected separately but simultaneously, suggesting a homogenous fluoride concentration in the oral fluid throughout the mouth. Twetman *et al*. (1998) demonstrated, however, that after a 2-week F-free period the fluoride concentration was higher in stimulated submandibular and sublingual saliva than in parotid saliva,

suggesting that the fluoride concentration of the oral fluid may differ at various sites of the mouth. It is the question whether the buccal plaque in the lower and upper jaw is uploaded from the same saliva or whether the composition of the saliva differs at both sites. Also difference in fluoride clearance between the lower and upper buccal sulcus as demonstrated by Weatherell *et al*. (1986) may be involved.

A statistically significant correlation was observed between the fluoride concentrations in dental plaque after brushing with NaF plus water rinsing and brushing with AmF/SnF_2 dentifrice plus the water rinse. The fluoride concentrations after the other experimental protocols showed no significant correlations with the NaF dentifrice. This indicates that these two protocols which in essence were identical, except for the dentifrice formulation, provide individual fluoride retention patterns comparable within each subject. In the two remaining protocols a different individual pattern in fluoride retention was found, illustrated by the lack of correlation. Little is known about the variables responsible for individual fluoride retention. Oral reservoirs obviously are a factor in this.

In summary, all three post-brush rinsing protocols after the use of Am/SnF_2 dentifrice increased fluoride concentrations as compared to F-free dentifrice. The increase of fluoride concentration in saliva was more pronounced after brushing with AmF/SnF_2 dentifrice followed by rinsing with AmF/SnF_2 mouthrinse as compared to post-brush rinsing with water and expectorating excess dentifrice foam only. Such an effect was not found for the concentration of fluoride in plaque.

Acknowledgements

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Chapter 6

Effect of Various Rinsing Protocols after the Use of AmF/SnF_2 toothpaste on the Acid Production of Dental Plaque and Tongue Flora

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http://content.karger.com/produktedb/produkte.asp?typ=fulltext&file=CRE20060400032 45

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Abstract

The aim of this clinical study was to evaluate the effect of various rinsing protocols on oral acid production 6 hrs after tooth brushing with an AmF/SnF_2 toothpaste. After a 14 day period of using F-free toothpaste, thirty participants followed three experimental protocols each followed by F-free washout periods in a randomized crossover trial. They used AmF/SnF_2 toothpaste twice daily for one week and after brushing they rinsed with tap water, omitted the post-brush rinse, or rinsed with an AmF/SnF_2 mouthwash. In the F-free washout periods the participants brushed their teeth without further instructions. Six hours after the last brushing (± rinsing) of each period, subjects rinsed with 10 ml 10% sucrose solution for 2 min. A tongue film sample and a buccal plaque sample were taken, 4 and 8 minutes after the sucrose challenge, respectively. Metabolic acid ions were determined by capillary electrophoresis. The results show that 1) omitting the post-brush water rinse did not reduce the production of lactic, acetic or minor acids in plaque, or on the tongue and that 2) the additional use of AmF/SnF_2 mouthrinse after brushing reduced the acid production in plaque and tongue samples for at least 6 hrs. The distributions of acids produced in the plaque or tongue samples were not statistically different between experimental periods. It is concluded that an increase of the antimetabolic effect of AmF/SnF_2 toothpaste in between two daily brushing exercises is not achieved by omitting the post-brush water rinse. The additional use of AmF/SnF_2 mouthrinse after brushing is effective in reducing the acid metabolism in dental plaque and tongue flora.

6.1 Introduction

After tooth brushing, it is common practice to rinse the mouth with tap water. However, it has been reported that reducing the thoroughness of rinsing after brushing with fluoride containing toothpaste increased the substantivity of fluoride from

toothpaste, resulting in prolonged elevated salivary [Duckworth *et al*., 1991; Sjögren and Birkhed, 1993] and plaque [Sjögren and Melin, 1991] fluoride levels. The question should therefore be raised: should common post-brush rinsing practices be restricted?

Recently, Issa and Toumba (2004) studied salivary fluoride retention after single brushing exercises with or without subsequent water rinsing, using toothpastes that differed in fluoride concentration and in fluoride formulation. They found higher concentrations of fluoride in whole saliva up to 2hrs after brushing when not rinsing was compared with rinsing and when amine fluoride (AmF) toothpaste was compared with sodium fluoride (NaF) toothpaste (the toothpastes contained 1400 and 1450 ppm F-, respectively).

Toothpastes containing the AmF/SnF_2 complex have antimicrobial properties due to the amine and stannous parts and are well known for their inhibitory effects on growth of oral microorganisms and for the retardation of plaque growth [Banoczy *et al*., 1989; Brecx *et al*., 1993; Axelsson, 1993; Zimmermann *et al*., 1993; Madlena *et al*., 2004]. Antimicrobial properties of oral care products may in addition to fluoride contribute to caries prevention by lowering the acidogenic potential of dental plaque [Marsh and Bradshaw, 1993; Damen *et al*., 2002].

Previous work in our department on the acidogenicity of newly formed dental plaque, however, showed no differences between the regular use of either AmF/SnF_2 or NaF toothpaste, when the measurements were done 6hrs after the participants' normal brushing exercises [Damen *et al*., 2000]. For the effect on plaque that is formed after brushing, the oral reservoir may be crucial in delivering the active compounds. Loss of the active compounds of AmF/SnF_2 toothpaste from oral reservoirs by rinsing after brushing could be one explanation for Damen's findings. Therefore it is of interest to study whether the omission of the water rinse increases the substantivity of the AmF/SnF_2 compounds in the oral reservoirs, thereby enhancing the delivery of the active compounds to the plaque formed after brushing. Furthermore, it is of interest to study whether an additional antimicrobial mouthrinse after the use of AmF/SnF_2 toothpaste would have such an effect. The oral reservoir comprises plaque,

teeth, soft tissues and various stagnation sites. The tongue may be an important representative, because of its large surface area [Duckworth and Jones, 1993].

This paper describes a randomized crossover clinical trial on the effect of an AmF/SnF_2 toothpaste on acid concentrations in sucrose challenged dental plaque and tongue flora 6hrs after brushing, followed by one of three post-brush rinsing protocols: 1) a water rinse 2) no rinse 3) an AmF/SnF_2 mouthrinse. They are compared to each other and to F-free washout periods.

6.2 Materials and Methods

Subjects

Thirty non-dental students (22 women, 8 men, mean age 26.8 (± 7.3) years) were recruited through an advertisement. The inclusion criteria were: good general and dental health, no antibiotic use during the last 4 wks, at least 24 natural teeth and an unstimulated salivary flow of 0.25-0.35 ml/min. When meeting the inclusion criteria, judged by one operator, and after signing an informed consent, the subjects participated in this single centred *in-vivo* study that was approved by the ethical board of the dental faculty.

Design

The participants used F-free toothpaste twice daily for 2 wks without any instructions. Subsequently, they followed three 1-week experimental protocols in a randomized crossover design. After each protocol, a 2-week F-free washout period was incorporated. In total 7 visits were scheduled for sampling (Fig. 6.1). Participants' randomization numbers were set using a computer generated allocation schedule. To increase compliance, all participants received written instructions weekly and text messages on their mobile phones regularly.

Treatment

During the 1-week experimental protocols, participants used toothpaste containing amine fluoride and stannous fluoride (meridol®, GABA International AG, Münchenstein, Switzerland) twice daily. After brushing, they 1) rinsed once for 30 s with a pull of tap water or 2) spat out only and did not rinse their mouth or 3) rinsed for 30 s with 10 ml AmF/SnF_2 mouthwash (meridol®, GABA International AG, Münchenstein, Switzerland). In the 2-week washout periods (at the start of the experiment and after each week of AmF/SnF_2 toothpaste use), the participants used F-free toothpaste twice daily (Ultradent®, Kruidvat, Renswoude, The Netherlands) and after brushing rinsed with water without further instructions. A new toothbrush was provided at the start of every new experimental period, and interdental and tongue cleaning was not allowed throughout the study period.

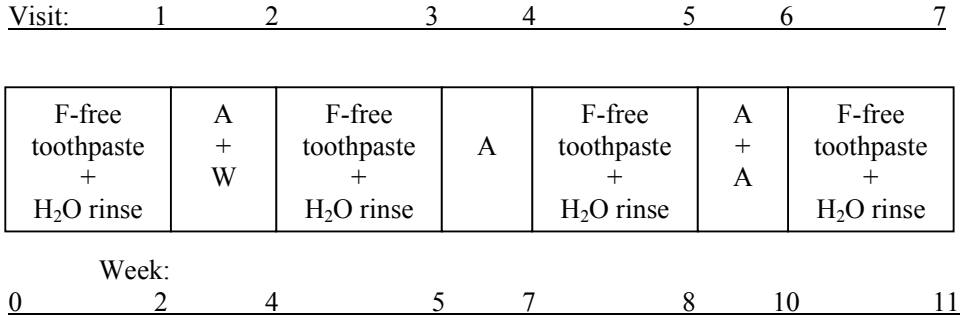

Figure 6.1: Experimental design: Three 1-week periods of brushing with AmF/SnF_2 toothpaste twice a day: with a water rinse (A+W), no after-brush rinse (A), or additional antimicrobial rinse (A+A) alternated with 2-week washout periods using F-free toothpaste and water rinse twice a day. The order was randomized among the participants. Visits 1-7: samples were collected 6 hrs after last brushing, 1.5 hrs after fasting (except H2O). At each visit a tongue film sample and a buccal plaque sample were collected at 4 min and 8 min, respectively, after a 2-min rinse with 10ml 10% sucrose solution.

Sampling procedures

All samples were collected treatment-blind by one examiner. Six hours after the last brushing, with or without rinse, the participants rinsed with 10 ml 10% sucrose for 2 min prior to sampling, they had refrained from food and drinks for 90 min. Four minutes after the sucrose challenge, a cotton swab was placed on the middle of the tongue and twisted around to become soaked with tongue fluid. Eight minutes after the sucrose challenge, a buccal plaque sample was collected with a Teflon spoon from 2 molars in the second quadrant. The bud of the cotton swab was cut, put up-side up within one minute in an Eppendorf vial and spun down for 10 s (Eppendorf centrifuge, 5415 Hamburg, Germany). Then, the bud itself was removed immediately from the vial to prevent the tongue sample fluid from being re-absorbed. The vial containing tongue sample fluid was put on ice for 5 min to stop metabolic activity. Next, the vial was heated for 5 min at 80°C in order to release internal acids from the bacterial cells and put on ice again [Damen *et al*., 2002]. Later the collected tongue samples were transferred into micro spin filter vials and centrifuged at 13,684 x *g* for 5 min at 4°C. The filtered tongue samples were stored at -80°C until further processing.

Plaque was spun down within 1 min into 50 µl MilliQ water in an iced vial (Eppendorf centrifuge, 5415 Hamburg, Germany) and thermocycled as described above. The vials containing plaque were centrifuged at 16,100 x *g*. The supernatants were transferred into micro spin filter vials (Ultrafree-MC 0.22 µm, Millipore, Bedford, Mass., USA) and centrifuged at 13,684 x *g* for 5 min at 4°C. Finally, plaque pellets and filtered plaque supernatants were stored at -80°C.

Organic acids analysis

Plaque pellets were thawed, resuspended in 200 µl MQ and sonicated on ice for 20 x 1 s (Kontes K-88140, Vineland, N.J., USA: maximum output) to analyse for protein content according to Bradford (1976) with the Bio-Rad protein analysis kit using bovine serum albumin (Sigma Chemicals, St. Louis, Mo., USA) as standard. The acids of the dental plaque were measured in the thawed filtered supernatants. For the analysis of the acids formed on the tongue, the filtered samples were thawed and 5 µl of the sample was diluted to 40 µl with MilliQ water. All organic acids were

determined as their anions by capillary electrophoresis on the Waters' Capillary Ion Analyser (Milford, Mass., USA) with UV detection at λ=185 nm. Separation was performed on capillary column of 60 cm x 75 μm (id) using an electrolyte containing 10 mM Na2HPO4 and 2 mM Osmotic flow modifier (OFM/OH, Waters Millford, Mass, USA) buffered at pH 6.0. Run conditions were: -15 kV, constant current 18 μA, 25°C and hydrostatic injection for 30 s. Sodium salts of lactic, acetic, formic, succinic, propionic and butyric acid (Sigma) were used to prepare mixed standard solutions in MilliQ water. As an internal standard, 5 μL 0.12 mM $NaNO_3$ was included in all samples. Calibration curves were made for each acid separately. Duplicate samples were run and Empower Pro Chromatography Manager software version 5.0 was used for data analysis. Peak identification and peak area integration were corrected manually if necessary.

Statistics

We enrolled 30 participants. Calculations of the number of participants for a 25% difference to be significant with a p-value of <0.05 and a power of 0.8 and assuming a standard deviation of 0.38, revealed the necessity to include 28 subjects. The proportional distributions of all acids after each experimental period were tested using χ^2-test. Differences in lactic and acetic acid concentrations were tested appropriately using General Linear Model (SPSS 10.0), repeated measures and paired t tests.

6.3 Results

Buccal plaque samples

All participants completed the trial. The proportional distributions of all acids after all 7 periods were not significantly different (χ^2-test). Lactic and acetic acids constituted 85 (± 2)% of the acids at all measurements. Of the minor acids, propionic acid was the most important one. The others - formic, succinic and butyric acid - were either below the detection limit, being 0.01 mmol/l, or contributed little. The lactic and acetic acid concentrations were not significantly different at the end of the F-free periods (data not

shown). The experimental conditions in the period preceding the F-free periods had no effect on the acid production at the end of these periods. This indicates that the washout periods were sufficiently long. The table shows the mean (± SD) acid concentrations for lactic and acetic acid and the total of the minor acids after the 4 F-free, the water rinse, the no rinse and the AmF/SnF_2 rinse periods. The lactic acid concentrations after the AmF/SnF_2 rinse period were significantly different from the other periods ($p \leq 0.01$). For acetate, a significant difference was only found between AmF/SnF_2 rinse and water rinse period ($p=0.018$).

Tongue samples

The proportional distribution of the acids in the tongue samples revealed no differences in the acid distributions after the various periods (χ^2-test). Lactic and acetic acid constituted 84 (± 2)% of the total acid amount at all measurements. The experimental conditions in the period preceding the F-free periods had an effect on the lactic acid concentration at the end of the F-free periods. This was due to two persons who showed a prolonged effect after the AmF/SnF_2 rinse period. Therefore we could not average all F-free measurements and we compared lactic acid concentrations after each AmF/SnF_2 protocol with the lactic acid concentrations at the end of its relevant F-free period. The mean lactic acid concentration (in mM) at the beginning of the AmF/SnF_2 toothpaste with post-brush water rinsing period (water rinse: 6.70 (± 2.74)) was significantly lower compared to the other AmF/SnF_2 periods (no rinse: 7.80 (± 3.32), AmF/SnF_2 rinse: 7.80 (± 2.28)). Acetic and minor acid concentrations at the end of the F-free periods were not related to the preceding protocol and could therefore be averaged; the mean acetic acid concentration was 4.18 (± 1.54) mM. Lactic, acetic and minor acid concentrations in tongue samples are shown in the table. After the water and AmF/SnF_2 rinse periods, the lactic acid concentrations were significantly lower compared to their relevant F-free period (paired t test, $p=0.013$ and $p<0.001$ respectively). Also, for acetic acid and the minor acids, the reductions were statistically significant when the use of AmF/SnF_2 toothpaste was followed by additional rinsing with AmF/SnF_2 mouthwash compared to all other periods ($p \leq 0.02$ and $p \leq 0.001$ respectively).

Table 6.1: Mean lactic, acetic and minor acid concentrations upon sucrose challenge in plaque and tongue samples after the 4 F-free and the AmF/SnF_2 brushing periods with the different post-brush rinsing protocols

	Four F-free toothpaste (tp) wash out periods + water rinse	AmF/SnF_2 tp + water rinse	AmF/SnF_2 tp + no rinse	AmF/SnF_2 tp + AmF/SnF_2 rinse
Plaque, µmol/mg protein				
lactic acid	1.20 (0.39)	1.32 (0.58)	1.19 (0.49)	0.90 (0.40)[a]
acetic acid	0.51 (0.26)[ab]	0.56 (0.26)[a]	0.50 (0.22)[ab]	0.42 (0.26)[b]
minor acids	0.10 (0.14)	0.12 (0.11)	0.10 (0.08)	0.08 (0.07)
Tongue, mM				
lactic acid	6.70 (2.74) - 7.80 (3.32)[d]	5.37 (3.09)[c]	5.72 (3.89)	2.23 (2.39)[c]
acetic acid	4.18 (1.54)	4.28 (1.98)	4.11 (1.92)	2.33 (1.82)[a]
minor acids	0.50 (0.59)	0.52 (0.63)	0.59 (0.85)	0.24 (0.38)[a]

[a,b] Statistical significance compared with all other groups not denoted a or b (General Linear Model, Bonferroni)

[c] The concentration range of the three F-free periods preceding each AmF/SnF2 period (see text)

[d] Significant different from its preceding F-free period (t test)

6.4 Discussion

The 6-hour post-brushing interval is substantially longer than the period of 30-180 min evaluated in other studies on the substantivity of fluoride [Duckworth and Morgan, 1991; Sjögren and Birkhed, 1994; Campus *et al*., 2003; Issa and Toumba, 2004]. Such short periods would, however, not have been feasible in our study because too little plaque would have developed for reliable sampling and measurements. Moreover, we think that a period of 6 hrs or longer is clinically more relevant, since oral care products are normally used twice a day and any effect should bridge as much as possible the intermediate periods. We did, however, not choose a period longer than 6 because in previous experiments in our lab we could not find an acid reducing effect of AmF/SnF_2 toothpaste 6 hrs after a single use with post-brush water rinsing [Damen *et al*., 2000].

To assess caries preventive effects, a lot of emphasis has been put on the microbial composition of plaque and saliva after preventive measures [Krasse, 1988; Houte van, 1993; Netuschil *et al*., 1995]. We chose to evaluate the acid reducing effect by measuring acid production in dental plaque and in tongue flora after these had been challenged with sucrose. Measurements of the acid concentration as a parameter of microbial activity in plaque and in saliva might serve well for evaluating putative caries risk, compliance with antimicrobial therapy and efficacy of oral care products [Oppermann, 1979; Gerardu *et al*., 2003; Zhang *et al*., 2004]. It should be realized that we did not measure the amount of dental plaque. Therefore, no effect in our experiments does not necessarily mean that the product is without any preventive value. It may be that the growth rate of dental plaque was reduced, but the acid concentration per plaque unit had remained unchanged.

Plaque results

Omitting the usual water rinse after brushing with AmF/SnF_2 toothpaste was not more effective in reducing acid formation than rinsing with water after brushing. This result was unexpected considering the fluoride experiments in which not rinsing after tooth brushing increased the fluoride content in saliva and plaque [Duckworth *et al*., 1991;

Sjögren and Birkhed, 1993; Sjögren, 2001; Sjögren and Melin, 2001; Issa and Toumba, 2004]. However, in those experiments fluoride was followed for no longer than 3 h. At those time points the observed differences were very small and it may be questioned whether such differences would last for another 3 h. If so, it has to be realized that this does not necessarily hold true for amine and stannous cations. A binding mechanism of amine and stannous ions to plaque is not known, but as divalent cations they may bind to plaque bacteria as Rose *et al*. (1996) have shown for Ca^{2+}, Mg^{2+} and Zn^{2+}.

No differences in acid reduction were found when the use of AmF/SnF_2 toothpaste with water rinsing was compared to the F-free control toothpaste with water rinsing. Damen *et al*. (2000) also found no differences when they compared the acid reducing effects of AmF/SnF_2 to NaF 6 hrs after a single tooth brushing. This would indicate that the AmF/SnF_2 paste and control toothpastes had either no effect or a similar acid reducing effect. All toothpastes have antimicrobial properties that are related to the preservatives they contain. The preservatives in the control toothpaste could have been as effective as the preservatives plus AmF/SnF_2 complex in the AmF/SnF_2 toothpaste. Altogether, it can be concluded that omitting the water rinse after brushing does not increase the effectiveness of the antimicrobial compounds of the AmF/SnF_2 toothpaste for over 6 hrs.

Tongue results

In all AmF/SnF_2 brushing periods there was a significant inhibitory effect on the acid concentration of salivary tongue film samples compared to the F-free washout period. However, the use of AmF/SnF_2 toothpaste with or without water rinsing showed comparable lactic acid concentrations, but these were significantly higher than after the period of using AmF/SnF_2 toothpaste followed by AmF/SnF_2 mouthrinse. In agreement with Duckworth and Jones (1993), our results suggest that the tongue is a significant oral reservoir probably due to the large papillate surface or the debris on the tongue, as it was not cleaned beforehand. However, also the release of active compounds from the oral mucosa to the tongue cannot be ruled as an explanation for the long duration of acid reduction in the tongue flora.

In our experiments, the additional rinse with AmF/SnF_2 mouthwash significantly decreased the acid concentrations in both plaque and salivary tongue samples. A subsequent study is needed to discriminate whether the AmF/SnF_2 mouthrinse prolongs the substantivity of the AmF/SnF_2 toothpaste or whether it is significantly effective itself.

In conclusion, the acid reduction in newly formed dental plaque following use of AmF/SnF_2 toothpaste is not prolonged over a 6 hrs period when the water rinse after tooth brushing is omitted. The use of AmF/SnF_2 mouthrinse after brushing with AmF/SnF_2 toothpaste does prolong the acid reducing effect in dental plaque for a period of 6 hrs. Therefore, the combined use of AmF/SnF_2 toothpaste and mouthrinse is preferred over single use of the toothpaste in reducing the acid concentrations in the oral reservoir including dental plaque.

Acknowledgements

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We are grateful to all volunteers who participated.

Chapter 7

Comparison of Clinpro Cario L-Pop Estimates to CIA Lactic Acid Estimates of the Oral Flora

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Abstract

Clinpro Cario L-Pop (CCLP, 3M ESPE, Seefeld, Germany) is a semi-quantitive test claimed to determine the general potential for caries development and to monitor the individual caries risk. This test translates the capacity of the tongue flora to produce lactic acid into a score between 1 and 9, indicating a low, medium or high risk for caries development.

Aim of this crossover randomized clinical trial was to evaluate the CCLP on its variation in time and its capacity to monitor the effect of 3 different oral hygiene procedures. The CCLP-readings were compared to measurements of lactic acid in tongue biofilm and plaque samples by capillary ion electrophoresis.

After 4 washout periods, the distribution of scores in the low, medium and high risk categories was 10%, 16% and 74%, respectively. Out of 30 subjects, 11 scored constantly in the same category. The coefficient of variance of lactic acid concentrations was 31% for tongue and 25% for plaque samples. After using antimicrobial toothpaste and mouthrinse, the number of high-risk scores was reduced to 33%; reduced acidogenicity was also found in tongue and plaque samples.

We conclude that CCLP could be used to monitor and stimulate compliance to an antimicrobial oral hygiene protocol.

7.1 Introduction

In modern dentistry the early detection of caries and the selection of high-risk patients are of significant importance. The level of oral hygiene, use of fluorides, recent caries experience and dietary habits are commonly used to assess caries risk [Reich *et al*., 1999]. However, these parameters do not give information on the current bacterial activity in the oral cavity. Therefore, a biochemical test, Clinpro™ Cario L-Pop™ (CCLP, 3M ESPE, Seefeld, Germany) was developed.

It is claimed by the manufacturer that this semi-quantitive test determines the general potential for caries development and monitors the individual caries risk. The test is performed on the dorsum of the tongue and is based on the determination of lactic acid that is an indicator of metabolic activity of caries-causing bacteria [Geddes, 1975]. Associations between the acidogenicity of tongue and plaque samples have been reported [Tanner *et al*., 2002]. The acidogenicity of the tongue flora might reflect the overall acidogenic potential of the oral flora.

To monitor the potential of caries development, a test has to meet certain criteria. Consistent test results under similar conditions and discriminating power between different conditions are required. Also, the outcome of a newly developed test has to be validated against the outcome of conventional or gold standard tests. When these criteria are met, the CCLP-test could be used to provide both the clinician and the patient objective information on the patient's oral metabolic activity and potential caries risk.

The aim of this randomized crossover clinical trial was to evaluate the CCLP regarding its variation in time under similar oral hygiene conditions, and on its capacity to monitor the effect of antimicrobials in the oral cavity. We compared the CCLP-test results to tongue biofilm and plaque samples, which were both analysed for lactic acid contents by capillary ion electrophoresis (CIA).

7.2 Materials and Methods

Subjects

Thirty volunteers (22 women, 8 men, mean age 26.8 (± 7.3) years) were recruited through an advertisement. The inclusion criteria were: good general and dental health, no antibiotic treatments during the preceding 4 wks, at least 24 natural teeth and an unstimulated salivary flow of 0.25-0.35 ml/min. When meeting the inclusion criteria and after signing an informed consent, the subjects participated in this single centre in-vivo study that was approved by the ethical board of the dental faculty. DMFS numbers (Decayed Missing Filled Surfaces) were recorded for all subjects.

Design

In the randomized, crossover design all subjects followed three 1-wk oral hygiene protocols during which an antimicrobial toothpaste and a specific protocol of post-brush rinsing was prescribed. A 2-wk washout period was scheduled before and after each experimental week. Randomization numbers were set using a computer generated allocation schedule. To increase compliance subjects received written instructions on every visit and text messages on their mobile phones on the day of the appointment to remind them of their scheduled time of brushing and the 1.5 hrs fasting period before sampling.

Treatment

In the washout periods all subjects used F-free toothpaste without any additional antimicrobial additives (Ultradent®, Kruidvat, Renswoude, The Netherlands) twice daily. During the 1-wk experimental protocols, they used an amine fluoride/stannous fluoride containing toothpaste twice daily (meridol® 1400 ppm, GABA International AG, Münchenstein, Switzerland). After tooth brushing, they either 1) rinsed once for 30 s with a pull of tap water (protocol A+W), 2) spat out only and did not rinse their mouth (protocol A) or 3) they rinsed with 10 ml of an antimicrobial mouthwash (meridol®, protocol A+A). New toothbrushes were provided at the start of every new experimental protocol. Interdental and tongue cleaning were not permitted.

Sample procedures

At the end of each of the experimental and washout periods all samples were collected by one operator 6 hrs after the last brushing exercise of the participants and after at least 1.5 hrs of fasting. The operator was not aware of the preceding protocol. First, a CCLP-test was performed according to the instructions for use (3M ESPE, Seefeld, Germany) except for the fact that the time span between brushing and CCLP-testing exceeded the prescribed 2h. Then, the subjects rinsed with 10 ml 10% sucrose solution for 2 min. Four minutes thereafter, the dorsum of the tongue was sampled with a cotton swab which was immediately spun down in an Eppendorf vial at 16.110 x *g* for 30 s. The cotton swab was removed directly from the vial to prevent

reabsorbtion of tongue biofilm into it. Subsequently, the vials were stored on ice until further processing. Eight minutes after the sucrose challenge, a buccal supragingival plaque sample was taken from an assigned first upper molar and immediately spun down in an ice-cooled vial containing 50 µl MilliQ-water. The plaque sample was stored on ice for 5 min to stop metabolic activity.

Samples were processed according to Damen *et al*. (2002). In brief: plaque and tongue biofilm samples were heated for 5 min at 80°C to release all acids and re-stored on ice. Then the plaque vials were centrifuged at 16,100 x g, their supernatants were transferred into micro spin filter vials (Ultrafree-MC 0.22 µm, Millipore, Bedford, Mass., USA) and the plaque pellets were stored at –80°C. The tongue samples were transferred into similar micro spin filter vials. All filter vials were then centrifuged at 13,148 x *g* for 5 min at 4°C and stored at –80°C until further processing.

CCLP-test

After twisting around the CCLP-stick 4 times on the tongue, the sampled microorganisms form lactic acid from sucrose impregnated in the cotton bud of the stick. The lactic acid is oxidised by lactate dehydrogenase to pyruvate. This enzymatic reaction is coupled with redox-indicators generating a blue signal, varying within a 9-scale colour range. The blue signal must be read after exactly 2 min. The 9 possible CCLP-scores were divided in 3 risk-categories indicating a low (CCLP scores 1-3), medium (CCLP scores 4-6) or high level (CCLP scores 7-9) of lactic acid metabolism.

Lactic acid analysis by CIA

Plaque pellets were thawed, resuspended in 200 µl MilliQ-water and sonicated on ice for 20 x 1 s (Sonics & Materials Inc, Vibracell 130, Newtown, CT., USA, output watts: 1). Sonicated plaque samples were analysed for protein [Bradford, 1976] with the Bio-Rad protein analysis kit (Bio-Rad Laboratories GmbH, München, Germany) using bovine serum albumin as standard (Sigma Chemicals, St. Louis, MO., USA). The lactic acid concentration was determined in duplicate in plaque supernatants and in diluted tongue biofilm samples by capillary electrophoresis on the Waters Capillary Ion Analyser (Milford, Mass., USA). As an internal standard, 5 µL 0.12 mM $NaNO_3$

was included. Calibration curves were made and data analysed (Empower Pro Chromatography Manager software version 5.0). Peak identification and peak area integration were corrected manually if necessary.

Statistics

Data were tested using Wilcoxon-test with SPSS 10.0. Distributions were tested with χ^2 and Spearman's or Pearson's correlations (r) were calculated.

7.3 Results

Dental examination of the participants showed DMFS numbers of 0 to 42 with an average DMFS number (± SD) of 6.6 (± 10.5) and a median of 2.5. No active caries was observed.

Table 7.1: The distributions of the CCLP-scores assessed after 4 washout periods over the categories low, medium and high risk (n=30)

No. of subjects	Low risk	Medium risk	High risk
11			4
6		1	3
3	1		3
1*			3
1		3	1
4	1	1	2
1	2		2
1	1	2	1
1		2	2
1	2	2	

*This person did not show up at one of the appointments

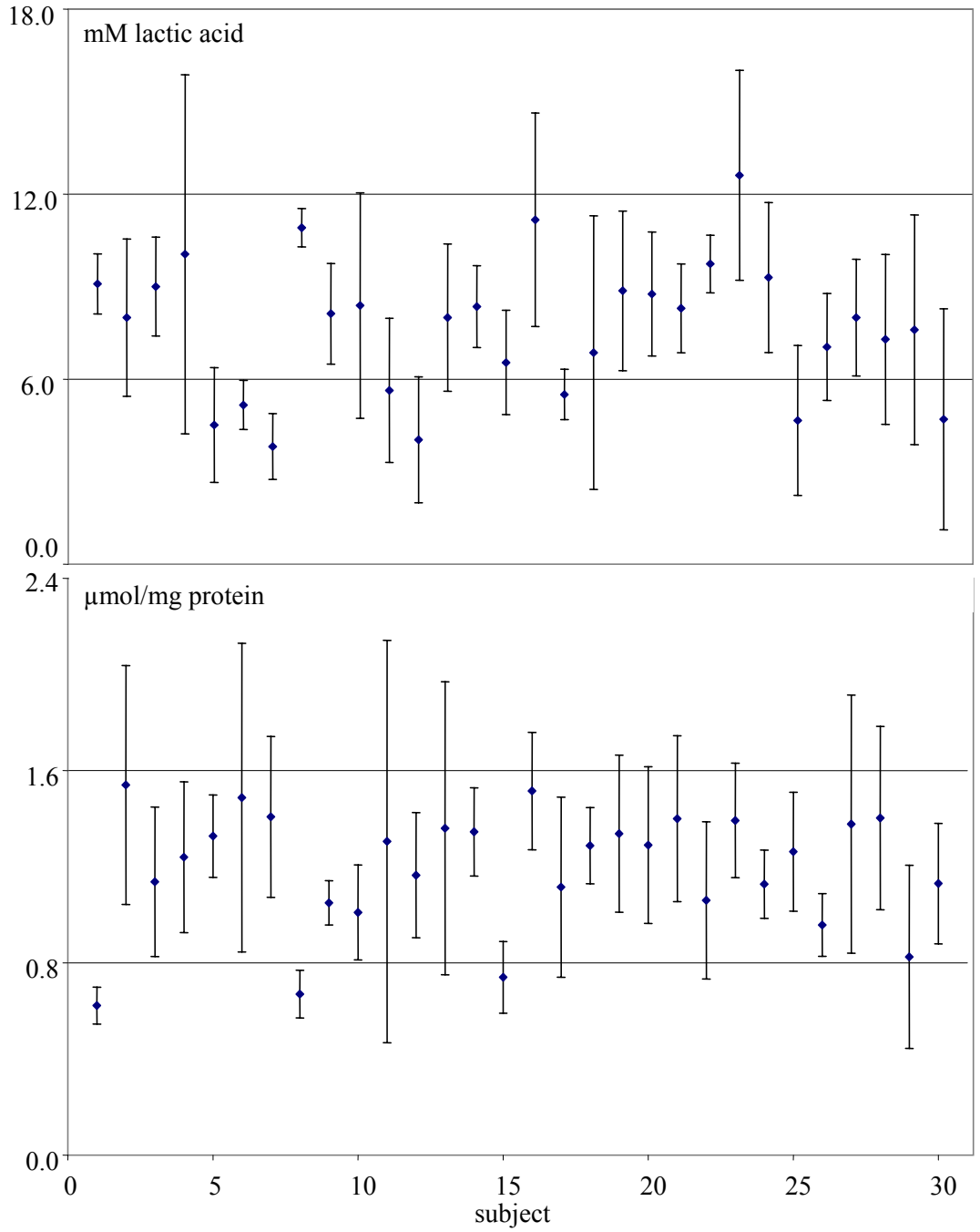

Figure 7.1: Average lactic acid concentrations (± standard deviation) after four washout periods in tongue biofilm (upper plot) and buccal plaque samples (lower plot) per subject. In both panels the subjects are in the same order.

Consistency after 4 washout periods

After the 4 washout periods, a total of 119 CCLP-tests was performed. Out of 30 subjects, 11 scored constantly in the same risk-category after the 4 washout periods, *i.e.* 37% reproducibility (Table 7.1). The distribution of the test scores in the low, medium and high risk category was 10%, 16% and 74%, respectively (Table 7.2).

The average (± SD) lactic acid concentrations in the tongue biofilm swabs and the buccal plaque samples after 4 washout periods measured by capillary ion analysis (CIA) are shown for each subject in Fig. 7.1. The coefficient of variance of the lactic acid concentrations after 4 washout periods was 31% for tongue biofilm and 25% for plaque samples. The individual variations in CCLP-scores and the lactic acid concentrations in tongue biofilm and plaque samples at the end of the washout periods were not related to the preceding experimental protocol.

Monitoring acidogenic potential after various oral hygiene protocols

After the use of antimicrobial toothpaste and AmF/SnF_2 mouthrinse (protocol A+A), the distribution of the scores in the low, medium and high category was 40, 27 and 33% respectively (Table 7.2). Statistical analyses (χ^2-test) showed that this distribution differed significantly from those in the other groups (washout, A+W and A). After the use of antimicrobial toothpaste with or without post-brush water rinse (protocols A+W and A), only 1 subject had changed from the high risk in the preceding washout period to the low risk category.

Table 7.2 Average lactic acid concentrations (± standard deviation) in the tongue biofilm and buccal plaque samples in relation to the Clinpro™ Cario L-Pop™ (CCLP) scores after the four washout periods and after use of antimicrobial toothpaste with an additional antimicrobial mouthrinse (A + A) or with a water rinse (A + W) or without post-brush water rinsing (A)

	CCLP-score			lactic acid concentration		correlation coefficients and p-values		
protocol	category	(n)	% distribution	tongue biofilm (mmol / L)	plaque (µmol/mg protein)	cclp tongue	cclp plaque	tongue plaque
Washout	Low	12	10	5.27 (2.46) [a]	1.17 (0.24) [a]			
(4 periods)	Medium	19	16	7.20 (3.26) [a]	1.13 (0.34) [a]	r = 0.28	r = 0.04	r = 0.03
	High	88	74	8.14 (3.11) [a]	1.19 (0.40) [a]	*P < 0.01*	*P* = 0.69	*P* =0.77
	All	119	100	7.71 (3.16)	1.18 (0.37)			
A + A	Low	12	40	1.30 (0.85) [a]	0.82 (0.34) [a]			
	Medium	8	27	2.17 (1.72) [b]	1.07 (0.44) [b]	r = 0.67	r = -0.01	r = 0.15
	High	10	33	5.63 (2.53) [c]	0.83 (0.41) [a]	*P < 0.01*	*P* = 0.98	*P* = 0.53
	All	30	100	2.60 (2.36)	0.90 (0.40)			
A + W	Low	2	7	1.44**	2.27 (0.61) [a]			
	Medium	5	17	4.76 (1.17) [a]	1.32 (0.62) [b]	r = 0.24	r = -0.19	r = 0.30
	High	22	76	5.93 (3.23) [a]	1.40 (0.69) [b]	*P* = 0.23	*P* = 0.35	*P* = 0.16
	All	29	100	5.56 (2.97)	1.43 (0.70)			
A	Low	2	7	4.53 (4.71) [a]	1.68 (1.19) [a]			
	Medium	4	14	7.18 (3.73) [b]	1.53 (0.35) [a]	r = 0.01	r = -0.36	r = 0.46
	High	23	79	6.14 (4.05) [b]	1.08 (0.41) [b]	*P* = 0.97	*P* = 0.07	*P = 0.02*
	All	29	100	6.40 (3.81)	1.19 (0.43)			

□Spearman correlation; ■ Pearson correlation; ** one sample was lost, the value is not included in the statistical evaluation.a, b, c: different letters denote statistically significant differences at a 95% probability level.

Correlations between CCLP and CIA-measurements

Table 7.2 also shows the average (± SD) lactic acid concentrations in the tongue biofilm and plaque samples per CCLP-score for each protocol. No significant correlations were found between the CCLP-scores and lactic acid concentrations measured in the dental plaque. Significant positive correlations were found between the CCLP-scores and lactic acid concentrations determined in the tongue biofilm samples after the washout period and after the A + A protocol (Fig. 7.2). We observed that subjects with high acid levels assessed by CIA scored more consistently in the CCLP assessment. These scores were predominantly in the highest CCLP-category. The CCLP-scores in every risk category corresponded with a wide range of average lactic acid concentrations in tongue biofilm and plaque samples. However, the ranges corresponding with the low and high risk did not overlap each other.

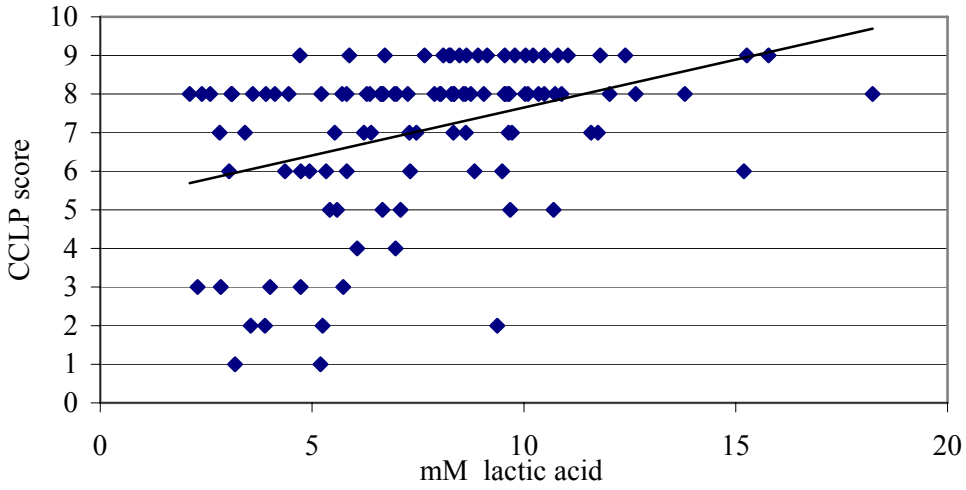

Figure 7.2: Correlation between CCLP-scores and lactic acid production in tongue biofilm samples (mM) (after A + A protocol)

7.4 Discussion

We compared three assessments of lactic acid production in the oral cavity: the CCLP-test and CIA measurements in tongue biofilm and plaque samples. Also the capacity of the CCLP to detect a change in acidogenic potential as the result of the use of various antimicrobial oral hygiene procedures was studied. After 4 washout periods, only 37% of the subjects showed reproducible CCLP-scores, being 4 scores in the same category. This is low compared to the results of others who reported over 80% reproducibility [Häberlein *et al*., 2003; Schiffner and Torres-Quintero, 2005]. However, our results showed also a wide range of lactic acid concentration in the tongue biofilm and plaque samples after the washout periods. A consistent result of one of the methods did not necessarily mean that the other measurements were comparably consistent for that same subject. The variation in the outcomes for one person does not mean that the measurements were not accurate. The acidogenicity of the tongue biofilm and plaque flora may fluctuate over time due to intra-individual differences such as plaque composition and salivary clearance [Oliveby *et al*., 1990].

The 74% of the CCLP-scores in the high category after the washout periods suggest that the group has a high potential for caries development. We presume, however, that the group did not present a potential risk at baseline considering the absence of carious lesions by visual examination and a median DMFS number of 2.5. We cannot give an explanation for the large number of CCLP-scores in the high-risk category range. In contrast to our results, Schiffner and Torres-Quintero (2005) reported 17 out of 20 individuals in the low risk in a group with stable oral conditions. In their study, the CCLP-test was performed 5 min after tooth brushing, while we performed the test 6 hrs after brushing. It could be that when the test is performed shortly after brushing the antimicrobial toothpaste substances retained on the tongue biofilm have resulted in lower CCLP-scores. This would not have been observed in our experiment. However, we decided to sample 6 hrs after tooth brushing to enable sufficient plaque growth for sampling. Alternatively, the test might be right in indicating a high risk for our subjects. A high risk, however, does not necessarily

result in the development of caries, since the risk might have been controlled by the use of fluoride toothpaste.

The correlations between CCLP-scores and acid production in tongue biofilm and in plaque samples were poor and only statistically significant for the correlation CCLP and tongue biofilm acids after the washout periods and after the use of antimicrobial toothpaste and mouthrinse together (Table 7.2). It is remarkable that the same amount of lactic acid in the tongue biofilm sample corresponds with low risk CCLP-scores after the washout protocol, but with high CCLP-scores after the A + A protocol. We have no explanation for this finding. The poor correlation does not necessarily devaluate the value of each method individually. In this respect, we emphasize that all three samples were collected at different time points after the sucrose challenge and at different sites. Therefore the data might reflect different stages of bacterial activity. Also the site-specific acid production in the oral cavity may have caused the correlations to be poor.

The CCLP-scores were recorded on an ordinal scale. To compare these to the average lactic acid concentration in the corresponding tongue biofilm samples (mM), all scores can be recalculated into mM according to the calibration curve in the CCLP's technical manual. The calibration curve is biphasic and the CCLP-scores 1 to 6 (low and medium risk) represent values between 0 and 3 mM lactic acid, while the high-risk category (scores 7, 8, 9) represents values between 3.0 and 16 mM lactic acid. Taking into account the CIA-estimations of the tongue biofilm samples (Fig.7.1) it is not that surprising that we found so many CCLP-scores in the high risk category, although there are marked differences between both methods regarding the sucrose challenge and the time point of the readings. If our study population represents the population in which the CCLP is meant to be used, it would be advisable to make the method more sensitive in the high-risk category.

We conclude that the CCLP-readings were reproducible in 37% of our subjects and that the readings did not correlate to lactic acid concentrations in plaque samples as measured by CIA. The readings correlated to lactic acid concentrations in tongue biofilm samples after the washout period and the A + A protocol. CCLP was able to detect a change in acidogenicity after the use of antimicrobial toothpaste and

mouthrinse. The CCLP could be used to monitor and stimulate compliance to an antimicrobial oral hygiene protocol.

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Chapter 8

General Discussion

&

Conclusions

The benefits of fluoride in the prevention of caries have been proven frequently, are widely acknowledged and fluoride use has been generally accepted by the public. Despite the daily use of fluoride toothpaste, caries is not eradicated in Western industrialized countries. Most caries (± 80%) occurs in a small group of individuals (± 20%) which number is of little value for health policy makers to consider caries as a dental health problem today. The attention for prevention of caries is decreasing and in my opinion this is not justified. More input in caries prevention is required from the professional dental field. The skewed distribution in caries prevalence and the identification of an individual susceptible to caries development should be of our concern. The individual who lacks compliance to caries preventive measures and dental education and who lacks access to dental health services may benefit from behavioural encouragements and dental consultations. Furthermore, the individual at risk for caries development might benefit from improved and additional preventive measures. For that reason, it is important to study caries preventive measures that could be carried out in addition to daily tooth brushing with fluoride toothpaste. The **aims** of this thesis were chosen as they could contribute to more effective caries prevention to individuals at risk. The effects of several antimicrobial interventions on microbial acid production in dental plaque and the substantivity of caries preventive agents were studied, as well as a method to select individuals at risk for caries development.

Well-designed randomized, controlled clinical trials with a high external validity are required to provide evidence for preventive programs and treatment protocols. The only 'true' output parameter or endpoint in such trials would be caries. However, the large number of participants, the extensive follow-up time, the costs and ethical approval required in clinical trials do not facilitate the evaluation of caries preventive measures in such trials today. Therefore, randomized clinical trials with other endpoints than caries and *in situ* trials are necessary to provide evidence for the efficacy of preventive measures.

Endpoints are defined as conditions or events that are associated with individual study subjects and that are used to assess treatment efficacy [Hujoel and DeRouen, 1995]. The endpoint is 'true' when the evidence reflects unequivocal benefit to the

subject and it has a 'surrogate' character when it represents a measure of the condition or the disease. Examples of surrogate outcomes are: reduction in plaque amounts and elimination of the caries-associated microorganisms from plaque or saliva. These endpoints do not necessarily correlate with caries [Caufield *et al*., 2001; Dasanayake *et al*., 2002; Anderson, 2003]. The question remains: what is a relevant surrogate endpoint?

Because acid is the metabolite of the mutans streptococci causing caries, acid production in dental plaque is expected to be a clinically relevant surrogate endpoint in controlled trials evaluating caries preventive measures, such as antimicrobial agents. Others [Oppermann, 1979; Giertsen and Scheie, 1995; Zhang *et al*., 2004] have studied metabolic effects in dental plaque as surrogate endpoints. In this thesis, acid production in dental plaque was chosen as endpoint to study the efficacy of caries preventive measures in randomized clinical trials.

Caries prevention with antimicrobial agents

In **Chapter 2**, the effects of 3-weeks' daily rinsing with an AmF/SnF_2 mouthrinse (meridol®, GABA International AG, Münchenstein, Switzerland) after tooth brushing with NaF toothpaste on plaque formation and on the acid production in buccal and interproximal plaque were studied in a randomized clinical trial (n=30). At baseline, lactic acid concentrations in resting plaque (without sucrose challenge) were higher in interproximal sites than in buccal sites. Buccal plaque responded, however, with a higher acid production to the sucrose challenge than interproximal plaque. It would seem from this response that buccal plaque is more cariogenic than interproximal plaque, while more caries develops in interproximal plaque than in buccal plaque [Mejare *et al*., 2004]. Buccal plaque is usually younger than interproximal plaque and therefore less dense. The diffusion of sucrose, acids and antimicrobials through less dense, buccal plaque might result in different concentration gradients compared to in plaque from retention sites that usually harbour thicker plaque. If sucrose is more likely to penetrate young buccal plaque than older interproximal plaque, it does not

necessarily mean that the outward diffusion of the acids produced after carbohydrate fermentation follows the same characteristics. Differences in these diffusion patterns remain to be studied. In addition, microorganisms growing in older plaque display an increased resistance to antimicrobial agents compared to those in younger plaque. To what extent these mechanisms affect the mode of action and efficacy of antimicrobials is not fully understood.

The home use, during 3 wks, of AmF/SnF_2 mouthrinse additionally to daily tooth brushing with fluoride toothpaste did not cause a reduction in average plaque amounts nor in sucrose metabolism in plaque at the 2nd day after discontinuation of the rinse. AmF/SnF_2 mouthrinse has an effect on plaque formation and on plaque acidogenicity up to 6 hrs after use (Chapter 6). However, it does not cause a prolonged effect after 3 wks daily use. Future studies are required to understand the retention and release of AmF/SnF_2 mouthrinse in the oral cavity before the duration of an alleged antimicrobial effect can be implemented in caries prevention.

Professional Chlorhexidine Applications

Chapter 3 showed that suppression of the number of mutans streptococci in saliva and reduction of acidogenicity in plaque did not extend a period beyond 3 wks after application of 40% chlorhexidine (CHX) varnish (EC40®, Explore, Nijmegen, The Netherlands). This period was much shorter than the period reported by others [Zickert *et al*., 1987; Schaeken *et al*., 1989; Emilson 1994; Heintze and Twetman, 2002]. A shorter application time compared to other clinical studies and a variety in sensitivity of mutans streptococci to CHX may explain this difference. The suggestion to count mutans streptococci in plaque instead of in saliva has been raised but the number of bacteria in saliva was generally considered to be a reasonable measurement of the entire dentition's microbial load [Schaeken *et al*. 1987; Lindquist *et al*., 1989a, b] and therefore a meaningful surrogate endpoint.

Although the killing efficacy of CHX is supposed to increase with repeated CHX applications [Emilson *et al*., 1987], the 3-wks' period of reduced acid production

in plaque after a single 40% CHX varnish application (Chapter 3) was not prolonged by 2 more applications within the same week in **Chapter 4**. The killing of the mutans streptococci by the first 40% CHX application may have been so effective that a second and third 40% application shortly after the first one did not significantly reduce the remaining mutans population further. The percentage of the mutans population initially killed determines the efficacy of colonisation resistance of dental plaque [Emilson and Lindquist, 1988]. This might explain the benefit of the multiple treatments with lower concentrations (1-20%) of CHX [Maltz *et al*., 1981; Sandham *et al*., 1991; Twetman and Petersson, 1999; Petersson *et al*., 2000]: each following treatment kills an additional fraction of the S. mutans population [Sandham *et al*., 1992]. This cannot be achieved shortly after a 40% CHX treatment because there is no or only a very minor remaining fraction to be killed. Prolonged colonisation resistance is not necessarily achieved in dental plaque by an intensified 40% CHX application regime. Would it, in this respect, be interesting to repeat the first 40% CHX application after 3 wks instead of after 2 days? Yes, as Chapter 3 and 4 showed an average reduction in plaque acidogenicity of 3-wks. The consequence, a 3-week treatment interval, should at least outweigh the drawbacks, being laborious for both professional and patient, and costly.

Substantivity of caries preventive agents

An increase in the substantivity of fluoride and antimicrobial agents is aimed at obtaining a larger beneficial effect of these agents. **Chapter 5** confirmed substantivity of fluoride both in saliva and plaque 6 hrs after tooth brushing with fluoride toothpaste compared to brushing with non-fluoride toothpaste.

The combined use of AmF/SnF_2 toothpaste and mouthrinse significantly elevated the fluoride substantivity in saliva when compared to tooth brushing followed by rinsing with water or expectorating toothpaste foam only. In dental plaque, however, the substantivity was neither increased after rinsing with AmF/SnF_2 solution nor after omitting the water rinse after brushing with AmF/SnF_2 toothpaste compared

to rinsing with water. Sjögren and Melin (2001) concluded from their data that a smaller water rinsing volume increased the retention of fluoride in saliva and plaque compared to a larger volume. It is possible that if the salivary fluoride content increases after omitting the water rinse, saliva could in turn upload the newly formed plaque. The substantivity of fluoride in newly formed plaque (Chapter 5) implies that fluoride retained in saliva does upload the newly formed plaque; however, this mechanism is not affected by the mode of rinsing after tooth brushing with fluoride toothpaste. It is doubtful whether the salivary fluoride increase exhibits an additional preventive effect. Besides, a large amount of fluoride may already have disappeared through the sink or by swallowing saliva.

The efficiency of the fluoride uptake in newly formed plaque from saliva not only depends on the availability of fluoride, but also on the availability of dental plaque. Reduced plaque accumulation has been reported after using AmF/SnF_2 toothpaste and/or mouthrinse in several studies [Zimmermann *et al*., 1993; Mengel *et al*., 1996; Paraskevas *et al*., 2004]. If the AmF/SnF_2 had a comparable effect on plaque regrowth in the study reported in Chapter 5, then this could have reduced the fluoride uptake in plaque. The result that the AmF/SnF_2 rinse caused higher fluoride concentrations in saliva but not in plaque may thus be related to an antimicrobial effect on plaque re-growth. The contribution of fluoride and plaque amounts, of salivary flow and of site dependent clearance to the total fluoride retained in the oral cavity is not elucidated yet.

In the tongue flora (**Chapter 6**), a significant inhibitory effect on the acid concentration was found after AmF/SnF_2 brushings compared to non-fluoride brushings. The use of AmF/SnF_2 toothpaste with or without water rinsing showed comparable lactic acid concentrations in the tongue samples, but these were significantly higher than after the period of using AmF/SnF_2 toothpaste followed by AmF/SnF_2 mouthrinse. These results suggest, in agreement with Duckworth and Jones (1993), that the tongue is an important oral reservoir.

Omitting the usual water rinse after brushing with AmF/SnF_2 toothpaste showed no difference in acid concentrations in plaque 6 hrs after brushing compared to

rinsing with water. Yet, no studies have reported on the capacity of saliva to deliver its retained antimicrobial components to the site of action: dental plaque. Omitting the post-brush water rinse after tooth brushing did not increase the antimetabolic effect of AmF/SnF_2 toothpaste in plaque. It may be that the growth rate of dental plaque was reduced, but the acid concentration per plaque unit had remained unchanged. An increased substantivity of caries preventive agents after omitting the post-brush water rinse could not be found 6 hrs after tooth brushing (Chapter 5 and 6). The hypothesis that the water rinse after tooth brushing should be omitted as an additional preventive measure to increase the efficacy of caries preventive agents is not confirmed in this thesis.

Caries Risk Assessment by CCLP

In clinical practice, the experienced naked eye or clinical expertise of the professional often assesses the caries risk of the individual patient. No test with sufficient power to predict whether patient will or will not develop caries is available. Most caries diagnostic tests that have been developed assess the caries risk by the numbers of mutans streptococci in saliva. It was shown (Chapter 3) that salivary mutans counts are not a good indicator for the acidogenic capacities of caries causing bacteria in plaque.

Today, a new tool for caries risk assessment is available: 'Clinpro Cario L-Pop' (CCLP, 3M ESPE AG, Seefeld, Germany). The CCLP test evaluates the production of lactic acid after sucrose consumption by bacteria living on the dorsum of the tongue. This diagnostic tool could be useful to the professional and underscore his clinical judgement.

A diagnostic test for potential caries development has to be consistent under similar conditions and discriminatory between different caries risk categories. In **Chapter 7**, the CCLP test results were validated and compared to lactic acid concentrations analysed by capillary ion electrophoreses (CIA) in tongue biofilm and plaque samples after different oral hygiene procedures. The reported reproducibility of the CCLP test

was low (37%) and coincided with a wide range of lactic acid concentrations in the tongue and plaque samples. It was also remarkable, considering the absence of caries and a low past caries experience (DMFS 2.5) in the group, that the test results of most participants displayed a high potential for caries development. The CCLP tests were performed 6 hrs instead of the prescribed 5-115 min after tooth brushing. When CCLP was evaluated sooner after brushing [Häberlein *et al*., 2003; Schiffner and Torres-Quintero, 2005], antimicrobial toothpaste substances could have been retained on the tongue surface and consequently have resulted in lower CCLP scores. However, CCLP was able to detect a change in the acidogenic potential of the tongue flora after combined use of an antimicrobial toothpaste and mouthrinse.

As long as a diagnostic risk test is not consistent under similar conditions and cannot discriminate between different risk categories in a population, it should not be used. In addition, the endpoint to be evaluated in a caries risk test should be strongly related to the true outcome that is aimed to be prevented.

The CCLP should not be used to assess the potential for caries development in the individual patient, but it could be used to detect a change in tongue acidogenicity after an antimicrobial oral hygiene protocol as well as to stimulate compliance to this protocol.

Conclusions

Both a single and a triple application of EC40 applied within one week reduce plaque acidogenicity only for a period of 3 wks.

The effect of 3-wks daily use of AmF/SnF_2 mouthrinse on plaque formation and on acid production in plaque does not persist two days after discontinuation of the rinse.

The combined use of AmF/SnF_2 toothpaste and mouthrinse reduces the metabolic activity in the oral reservoir including dental plaque better than toothpaste alone.

Omitting the habitual water rinse after tooth brushing does not result in more fluoride in newly formed dental plaque. The hypothesis that the water rinse after tooth brushing should be omitted as an additional preventive measure to increase the efficacy of caries preventive agents has not been confirmed in this thesis.

The validity of the diagnostic test, CCLP, to assess an individual's risk for caries development is not confirmed in this thesis. However, CCLP can detect a change in the oral metabolic activity after an antimicrobial oral hygiene regime. In clinical practice CCLP may be suitable to monitor and stimulate compliance to an antimicrobial oral hygiene protocol.

Summary

Chapter 1 describes the aetiology, prevalence and risk factors of dental caries. The important contribution of fluoride to the prevention of caries is well established and fluoride is well accepted by the public. Yet, the widespread daily use of fluoride in western industrialized countries has not been completely successful. The eradication of caries is still a distant goal. Therefore, there is a need to study caries preventive measures that could be employed in addition to daily tooth brushing with fluoride toothpaste. These measures could comprise the use of antibacterial agents at home as well as professional application of antimicrobial varnishes in the dental office. Besides, an increase in the substantivity of daily used fluoride and antimicrobial substances pursued by different rinsing procedures after tooth brushing could be beneficial.

In this thesis, the aims were to study 1) the effects of AmF/SnF_2 mouthrinse (meridol®) and of 40% chlorhexidine varnish (EC40®) on the acidogenicity of dental plaque; 2) the retention of fluoride and of antimicrobials in saliva and plaque after tooth brushing followed by the usual water rinse, compared to tooth brushing followed by rinsing with AmF/SnF_2 solution or by expectorating toothpaste foam only; and 3) the ability of a newly developed diagnostic test (Clinpro™ Cario L-Pop™) to assess a person's individual caries risk.

In **Chapter 2** a randomized clinical trial was done to study the effects of an AmF/SnF_2 mouthrinse (meridol®) after 3-wks daily rinsing additionally to tooth brushing with fluoride toothpaste on plaque formation and on the acid production in plaque at different sites in the mouth (n=30). Plaque samples were collected, before and after sucrose rinsing, from interproximal and buccal surfaces of upper (pre)molars at baseline and on the 2nd and 7th day after discontinuation of the 3-wks rinsing period. The results at baseline showed 1) higher lactic acid concentrations in resting interproximal plaque (considered to be a stagnation area with higher risk for caries) than in buccal plaque (smooth surface or open area with lower risk for caries) and 2)

that buccal plaque responded with a higher acid production to the sucrose challenge than interproximal plaque. After 3-wks use of AmF/SnF_2 mouthrinse, neither the amount of plaque nor the acidogenicity of dental plaque was reduced on the 2nd day after the last rinse compared to after 3-wks use of water.

In **Chapter 3** the relationship between the numbers of salivary mutans streptococci and the acid production in dental plaque after a single professional application of 40% chlorhexidine varnish (EC40®) was studied. Thirteen healthy subjects were treated with EC40. Saliva samples were taken before and up to 12 wks after treatment to assess mutans streptococci and lactobacilli. At the same time points plaque samples were taken before and after sucrose challenge and these samples were analysed for organic acid concentrations. Suppression of salivary mutans streptococci was observed together with a reduced production of lactic acid in sucrose challenged dental plaque in 9 subjects, while inhibition of acid production without significant suppression of mutans streptococci was observed in the other 4 participants. The duration of the two effects differed among the individuals but never exceeded 6 wks, with a mean duration of 3 wks

To conclude: a prolonged (> 6 wks) suppression of mutans streptococci and acid production was not achieved by a single treatment with EC40® varnish; the reduced acidogenicity of dental plaque shortly after the 40% chlorhexidine treatment was not necessarily predicted by a suppression of mutans streptococci in saliva.

The results from Chapter 3 showed that a single application of EC40® reduced (lactic) acid production in sucrose challenged plaque during a period of -on average- 3 wks. It was questioned (**Chapter 4**) whether lactic acid production could be reduced significantly longer when the treatment was intensified. Therefore, nine subjects ('good and bad responders') from the 'single EC40' study participated and received three full-mouth EC40 applications within 1 week. At baseline and up to 9 wks after the 3rd application, plaque samples were taken after a 10% sucrose rinse and analysed for organic acids. At the 1st and 7th day after the triple treatment there was too little

plaque to allow sampling. At 2 wks after treatment, lactic acid concentrations were significantly reduced. However, after 3 wks this effect had disappeared.

Conclusion: Three applications of 40% chlorhexidine varnish (EC40®) within 1 week did not increase the duration of the effect on plaque acidogenicity of a single application.

It is suggested that the substantivity of caries preventive agents such as AmF/SnF_2 toothpaste can be increased by omitting the usual water rinse after tooth brushing. By doing so, a higher concentration of the preventive agent remains adsorbed onto the oral surfaces that act as a reservoir from which the agents subsequently could reach the plaque. The objective in **Chapter 5** was to assess fluoride concentrations in unstimulated saliva and in buccal dental plaque in response to tooth brushing that was followed by 3 different post-brush rinsing protocols. In a randomized crossover trial, 30 participants used AmF/SnF_2 toothpaste twice daily for 1 week and after brushing they rinsed with tap water (protocol 1) or with an AmF/SnF_2 mouthwash (protocol 2) or omitted the post-brush rinse (protocol 3). Before and after each protocol, washout periods were scheduled during which the participants brushed their teeth with F^--free toothpaste without further instructions. The fluoride concentrations in saliva and plaque were increased after all 3 protocols compared to F^--free periods. Salivary fluoride concentrations were higher after the AmF/SnF_2 mouthrinse as compared to rinsing with water and to only expectorating the excess of toothpaste foam. In dental plaque, no significant differences in fluoride concentrations were found between the rinsing protocols.

It was concluded that the presumably beneficial effect of not rinsing, or of a fluoride rinse, immediately after tooth brushing, is not reflected in an increased fluoride concentration 6 hrs after tooth brushing in dental plaque.

In **Chapter 6** the effects of different rinsing protocols (as in Chapter 5) on the oral acidogenicity were determined. A tongue biofilm sample (collected 4 min after sucrose challenge) and a buccal plaque sample (collected 8 min after sucrose challenge) were taken 6 hrs after the oral hygiene protocols. Metabolic acid ions in these samples were

determined by capillary electrophoresis. The results showed that 1) omitting the post-brush water rinse did not reduce the production of lactic, acetic or minor acids in plaque, nor on the tongue; and 2) the post-brush use of AmF/SnF_2 mouthrinse did reduce the acid production in plaque and tongue samples for at least 6 hrs. The relative distribution of the various acids produced in the plaque or tongue samples were not statistically different between experimental groups.

It was concluded that the antimetabolic effect of AmF/SnF_2 toothpaste is not increased by omitting the post-brush water rinse. The post-brush use of AmF/SnF_2 mouthrinse did reduce the sugar metabolism of dental plaque and in the tongue flora.

Although various caries risk groups for the development of dental caries are identified in epidemiological surveys, it appears that no single test is available to identify a given individual with the risk for caries development. A new diagnostic test 'Clinpro™ Cario L-Pop™' (CCLP) was developed. The CCLP claims to assess the general potential for caries development and to monitor the individual caries risk.
The CCLP-test evaluates the production of lactic acid on the dorsum of the tongue. The more lactic acid, the higher the potential of the lactic acid producing bacteria could cause caries in the individual. In **Chapter 7**, the CCLP risk scores of 30 subjects were compared to continuous measurements of lactic acid concentrations in tongue saliva and in plaque samples measured by capillary electrophoresis (CIA). This was done in a crossover clinical trial to monitor the effects of three oral hygiene protocols (described in Chapter 5 and 6). The CCLP results showed that 11 subjects (total n=30) scored consistently in time *i.e.* within the same risk category after 4 comparable washout periods of the trial. After the use of antimicrobial toothpaste and post-brush mouthrinse (protocol 2 in Chapter 5) the number of high-risk CCLP scores was reduced with 44% compared to the washout period. The acidogenicity was significantly reduced in tongue saliva and in plaque samples. The correlation between the CCLP scores and lactic acid concentrations was significant after the washout period and after protocol 2 in the tongue samples, but not in plaque samples.

Conclusion: CCLP-results were consistent in 37% of the participants and did not correlate with the CIA lactic acid concentrations in plaque samples. After the

washout periods and after use of toothpaste followed by antimicrobial mouthrinse, the CCLP-scores did correlate to lactic acid concentrations in tongue samples. The CCLP could be used to monitor and to stimulate compliance to antimicrobial oral hygiene protocols.

Samenvatting

In **hoofdstuk 1** worden de etiologie, de prevalentie en de risicofactoren van cariës beschreven. De belangrijke rol van fluoride in cariëspreventie is onomstotelijk bewezen en algemeen bekend, maar ondanks het succesvolle, dagelijks gebruik van fluoridetandpasta in de westerse maatschappij, komt cariës nog steeds voor. Het is dan ook belangrijk om cariëspreventieve maatregelen te bestuderen die een aanvullende waarde kunnen hebben op het dagelijkse tandenpoetsen met fluoridetandpasta. Een dergelijke maatregel is bijvoorbeeld het gebruik van antimicrobiële middelen, thuis of in de tandheelkundige praktijk. Ook zou een hogere substantiviteit (=effectieve aanwezigheid) van fluoridetandpasta en antimicrobiële stoffen in de mond nagestreefd kunnen worden door na het tandenpoetsen op verschillende manieren de mond te spoelen.

Verder worden in hoofdstuk 1 de onderwerpen van dit proefschrift ingeleid. Dit zijn: 1) het effect van AmF/SnF_2 mondspoeling (meridol®) en van 40% chloorhexidine tandlak (EC40®) op het zuurvormend vermogen van tandplaque; 2) de aanwezigheid van fluoride en antimicrobiële stoffen in speeksel en plaque na het tandenpoetsen gevolgd door i) spoelen met water, ii) spoelen met meridol® mondwater, iii) alleen uitspugen van de tandpasta,dus niet spoelen; 3) de evaluatie van een nieuwe cariësdiagnostische test (Clinpro™ Cario L-Pop™) die beoogt het individuele cariësrisico te indiceren.

Hoofdstuk 2 beschrijft een onderzoek waarin het effect van het dagelijks gebruik van AmF/SnF_2 mondwater (meridol®) na het tandenpoetsen met fluoridetandpasta op tandplaque wordt bestudeerd. Dit werd geevalueerd in een gerandomiseerde klinische studie met 30 deelnemers. Het antimicrobiële effect van het mondwater op de vorming en het zuurproducerend vermogen van plaque werden onderzocht op verschillende plaatsen in de mond. Voorafgaand aan de spoelperiode van drie weken, en op de tweede en zevende dag na afloop van deze periode, werd plaque verzameld van buccale en approximale tandoppervlakken. Dit gebeurde telkens voor en na een suikerspoeling.

Resultaten: 1) zonder suikerspoeling was in approximale tandplaque (d.w.z. 'resting' plaque) een hogere concentratie lactaat (melkzuur) aanwezig dan in buccale plaque; 2) na een suikerspoeling werd in buccale plaque meer lactaat gevormd dan in approximale plaque; 3) er werden geen significante verschillen in hoeveelheden plaque gevonden na drie weken spoelen met AmF/SnF_2 mondwater in vergelijking met water; 4) er werden geen significante verschillen gevonden in het effect op het zuurvormend vermogen van de plaque tussen spoelen met AmF/SnF_2 mondwater en het spoelen met water op de tweede dag na drie weken spoelen

Conclusie: er was geen reductie in de hoeveelheid plaque en ook niet in het zuurvormend vermogen van plaque, kort na een drieweekse periode waarin dagelijks gespoeld werd met AmF/SnF_2 mondwater.

In **hoofdstuk 3** wordt het verband tussen het aantal mutans streptococcen in speeksel en de zuurproductie in plaque bekeken, nadat een 40% chloorhexidinetandlak (EC40®) professioneel was geappliceerd. Dertien gezonde deelnemers werden behandeld met EC40®. Voorafgaand aan, en tot 12 weken na de behandeling, werden speekselmonsters verzameld om het aantal mutans streptococcen en lactobacillen te bepalen. Op dezelfde tijdstippen werden, voor en na een suikerspoeling, plaquemonsters verzameld waarvan de zuurconcentraties werden berekend.
Resultaten: 1) bij negen deelnemers trad een vermindering van het aantal mutans streptococcen in het speeksel op en ook een verminderde lactaatproductie in de buccale tandplaque; 2) bij vier deelnemers liet de verminderde zuurproductie geen significante onderdrukking zien van de mutans streptococcen; 3) de periode van mutansonderdrukking en verminderde zuurproductie verschilde van persoon tot persoon, maar duurde gemiddeld niet langer dan drie weken en nooit langer dan zes weken.

Conclusie: een langdurige (> 6 wkn) onderdrukking van mutans streptococcen en verminderde zuurproductie kon niet worden verkregen middels een EC40® tandlak behandeling. De onderdrukking van mutans streptococcen in het speeksel betekende niet zonder meer een verminderd zuurvormend vermogen van de tandplaque na een 40% chloorhexidine-applicatie.

De resultaten in hoofdstuk 3 laten zien dat een EC40® applicatie de (melk-) zuurproductie na een suikerstoot in plaque reduceert gedurende een periode van gemiddeld drie weken. De vraag is of de lactaatproductie voor een langere periode significant kan worden verminderd wanneer de behandeling geïntensiveerd wordt. Hiervoor werd het volgende onderzoek gedaan dat in **hoofdstuk 4** wordt beschreven: Negen deelnemers uit de eerste EC40® studie werden behandeld met drie tandlak applicaties binnen één week. Er werd plaque verzameld voor en na een 10% suikerspoeling. Dit gebeurde voorafgaand aan de tandlakapplicaties en vervolgens tot negen weken na de derde behandeling. Van de plaquemonsters werden de zuurconcentraties bepaald. Op de eerste en zevende dag na de derde behandeling was er onvoldoende tandplaque aanwezig om een monster te nemen.
Resultaat: De lactaat concentraties waren twee weken na de derde behandeling nog steeds lager ten opzichte van de concentraties na een eenmalige EC40 behandeling. Dit effect was na drie weken verdwenen.

Conclusie: Drie 40% chloorhexidine applicaties (EC40®) in één week waren niet effectiever in het verminderen van het zuurvormend vermogen van plaque dan één applicatie.

De substantiviteit van cariëspreventieve middelen zoals AmF/SnF_2 tandpasta kan vergroot worden door na het tandenpoetsen niet na te spoelen met water. Op deze manier kan een hogere concentratie van het preventieve middel uit de tandpasta in de mond geadsorbeerd worden. Vanaf de adsorptieplaatsen in de mond kan het middel de nieuw gevormde tandplaque bereiken en zo beïnvloeden. In **hoofdstuk 5** wordt een onderzoek beschreven waarin de fluoride concentraties in ongestimuleerd speeksel en in buccale tandplaque werden bepaald bij verschillende manieren van mondspoelen na het tandenpoetsen. Dertig deelnemers hebben in een gerandomiseerde crossover studie drie experimentele spoelregimes (protocol 1-3) gevolgd in afwisseling met fluoride-vrije poetsperiodes. In het protocol poetsten de deelnemers 2x-dgs met AmF/SnF_2 tandpasta en spoelden vervolgens met kraanwater, of met AmF/SnF_2 mondwater, of ze spoelden niet. Dit alles gedurende één week In de 2-weken durende fluoride-vrije periode werden geen poets- en spoelinstructies gegeven.

Resultaten: 1) tijdens de fluoride-vrije periode waren de fluoride concentraties in speeksel en plaque lager dan tijdens de week dat een protocol werd toegepast; 2) de fluoride concentratie in speeksel was hoger na spoelen met AmF/SnF_2 mondwater dan na spoelen met kraanwater of na het enkel uitspugen van de tandpasta; 3) in tandplaque werd geen verhoogde fluoride concentratie gemeten na spoelen met AmF/SnF_2 mondwater.

Conclusie: Het potentiële positieve effect (namelijk een verhoogde fluoride concentratie) van niet spoelen, of van fluoride spoelen na het tandenpoetsen, werd zes uur na het tandenpoetsen niet gemeten in nieuw gevormde tandplaque.

In **hoofdstuk 6** wordt het onderzoek naar het effect van verschillende spoelregimes na tandenpoetsen (protocollen als in hoofdstuk 5) op het zuurvormende vermogen in speeksel en plaque beschreven. Zes uur na het uitgevoerde protocol werden een tongspeekselmonster van de tongrug (4 min. na suikerspoeling) en een buccaal plaque monster (8 min. na suikerspoeling) genomen. De metabolieten in deze monsters, organische zuren, werden als hun anion via capillaire ionanalyse bepaald.
Resultaten: 1) *niet* spoelen na het tandenpoetsen had geen invloed op de zuurproductie in speeksel op de tong, of op de zuurproductie in de plaque; 2) naspoelen met AmF/SnF_2 mondwater verminderde de zuurproductie in speeksel op de tong, en in plaquemonsters. Beiden werden gemeten na zes uur. 3) De verhouding tussen de concentraties van de verschillende geproduceerde zuren in het totale tongspeekselmonster was statistisch niet verschillend bij de drie experimentele protocollen.

Conclusie: het antimetabole effect van AmF/SnF_2 tandpasta werd niet vergroot door spoelen na het tandenpoetsen achterwege te laten. Spoelen met AmF/SnF_2 mondwater reduceerde het suikermetabolisme in plaque en in de flora op de tong.

Hoewel verschillende cariësrisicogroepen zijn beschreven in epidemiologisch onderzoek, bestond er nog geen test die het individu met een cariësrisico identificeert. Vandaar dat een nieuwe diagnostische test 'Clinpro™ Cario L-Pop™' (CCLP) werd ontwikkeld. De CCLP zou het risico op cariësontwikkeling kunnen vaststellen en op

die manier het individuele risico in kaart kunnen brengen. De CCLP test evalueert de lactaat-productie van bacteriën op de tongrug: hoe hoger deze productie, hoe groter de kans dat de lactaat producerende bacteriën cariës veroorzaken. In hoofdstuk 7 worden CCLP risico scores van 30 proefpersonen vergeleken met hun respectievelijke lactaat-concentraties in tongspeeksel- en plaquemonsters. In een klinische crossover studie werd het effect van drie verschillende mondhygiene regimes gevolgd.
Resultaat: 1) 11 deelnemers (totaal n=30) scoorden consistent in de tijd d.w.z.. in dezelfde risicocategorie na vier vergelijkbare poetsperiodes (=fluoride vrije / 'washout periods'); 2) na het gebruik van de antimicrobiële tandpasta en mondspoeling (protocol 1), werd het aantal hoge risicoscores gereduceerd met 44% vergeleken met de 'washout periods'; 3) het zuurvormend vermogen in tongspeeksel en tandplaque was significant verminderd na protocol 1.

Conclusie: De CCLP scores waren bij slechts 37% van de deelnemers reproduceerbaar en correleerden niet met de lactaatconcentraties in de plaquemonsters. De CCLP scores correleerden met lactaatconcentraties in de tongspeekselmonsters na de fluoride-vrije periodes en na protocol 1. De CCLP zou gebruikt kunnen worden om het uitvoeren van antimicrobiële poetsregimes te vervolgen en de patiënt hierin te stimuleren.

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Curriculum Vitae

Véronique Anne Marie Gerardu werd geboren op 8 december 1973 te Maastricht. In 1992 behaalde zij haar gymnasium B diploma aan het Sint Maartenscollege te Maastricht. In hetzelfde jaar begon zij haar studie tandheelkunde aan het Academisch Centrum Tandheelkunde Amsterdam (ACTA). In 1997 ontving ze haar tandheelkunde bul en in september van dat jaar werd zij als tandarts-docent verbonden aan de vakgroep Cariologie Endodontolgie Pedodontologie. Zij nam waar in diverse tandartspraktijken te Amsterdam en begon in 1999 haar eigen praktijk waarin ze sindsdien parttime werkt. In 2001 startte zij in part-time dienstverband haar onderzoek bij de vakgroep Cariologie Endodontolgie Pedodontologie.