In vivo evaluations of the neutralizing effect of a hydroxyl ion-releasing resin composite and a prophylactic gel on plaque acidogenicity - measured by the microtouch method

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”Utan tvivel är man inte klok”
Tage Danielsson
Abstract

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The prevalence of dental caries has decreased dramatically in most developed countries since the 1960s and the number of remaining teeth in adults has increased during the last decades. Elderly as a group will during aging become an increasing risk group for caries. Especially older individuals with hyposalivation are at increased risk for coronal and root caries and need increased oral care. A large part of the time spent by the dental team is occupied by prevention and treatment of secondary caries, which is the main reason of replacement or repair of restorations. Traditionally, prevention of caries is directed against the different risk factors of the individual such as oral hygiene, intake of fermentable carbohydrates, cariogenic microflora and oral dryness. In some restorative materials the release of fluoride can be used to decrease the risk of secondary caries alone or in combination with other preventive methods. New alternative preventive methods are necessary to complete traditional methods in order to decrease the caries risk in elderly and/or to prevent secondary caries. Addition of methods with buffering properties have been suggested and developed to supplement the biological buffering capacity of saliva.

The neutralizing effects of a hydroxyl ion-releasing resin composite and a prophylactic gel containing buffering properties on dental plaque acidogenicity was evaluated by the microtouch method. In this method, a skin reference electrode was validated in comparison with a glass capillary reference electrode and used in the subsequent studies. Change of plaque acidogenicity on proximal surfaces of aged restorations of the hydroxyl ion-releasing resin composite was compared intra-individually a conventional hybrid resin composite and a non-filled enamel proximal surface. Relative frequencies of cariogenic microorganisms in plaque on these surfaces were studied. The effect of a single application of the prophylactic gel with buffering substances was evaluated on plaque acidogenicity in healthy individuals with low and normal salivary secretion rate. It was compared intra-individually with the same gel without buffering substances. The effect of multiple applications of the prophylactic gel on plaque acidogenicity was studied in institutionalized elderly individuals with subjectively reported oral dryness and compared intra-individually with the same gel without buffering substances.

Major results and conclusions from the studies are:

• The use of the skin reference electrode, in combination with the microtouch electrode, showed high validity in comparison with the conventional glass capillary reference electrode
• The hydroxyl ion-releasing composite resin countered plaque acidogenicity fall at two time points of the aged restorations and maintained it at levels where lesser demineralization occurs. No influence of the ion-release on the amount of cariogenic plaque microorganisms on the resin composite surface was observed
• A single application of the prophylactic gel with buffering substances showed a neutralizing effect of plaque pH in healthy individuals with normal salivary secretion rate. No effect was observed in low secretion rate individuals.
• Multiple applications of the prophylactic gel did not neutralize dental plaque acidogenicity in elderly individuals with subjective oral dryness

Key words: caries, clinical, buffering, elderly, plaque pH, resin composite, restorations, microorganisms
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INTRODUCTION

**Dental caries**

Dental caries is caused by an interplay between tooth tissues, aciduric microorganisms and access of fermentable carbohydrates. Fermentation of accessible carbohydrates by cariogenic bacteria, usually mutans streptococcus and lactobacilli, in organics acids like lactic acid and formatic acid results in a plaque pH-fall (Neff 1967, Scheie *et al* 1996). Many oral factors, such as the type and number of cariogenic microorganisms, plaque composition, amount and composition of saliva, oral motorics and tooth anatomy influence the plaque pH value (Nyvad and Fejerskov 1994), Marsh and Martin 1999). If plaque-pH decreases under the critical level, 5.3-5.5 for enamel (Larsen and Bruun 1994) and 6.2-6.5 for dentin surfaces (Hoppenbrouwers *et al* 1987), an unsaturation of calcium and phosphate ions in the surrounding plaque fluid and liquid phase on the tooth surface will start the demineralization of the hard tissues. If this process continues without or to low possibilities to remineralize the tissues, the caries lesion will progress and will finally cavitate and become irreversible. Caries lesions are particularly likely to develop in undisturbed plaque niches, like fissures, below the contact point in proximal surfaces, cervical tooth areas, root surfaces as well as contiguous to gingival gaps of restorations. The etiology of both primary and secondary caries occurs due to disturbance of the demineralization-remineralization balance (Neff 1967, Kidd 1990, Fitzgerald 1994). Secondary caries has been the main reason of replacement or repair of restorative materials as reported in cross sectional studies and make today up an important part of restorative dental treatment (Rasmussen and Lundin 1995, Mjör 1997). After a fermentable carbohydrate challenge, changes in plaque pH can be followed over time and presented in a pH curve.

**Microtouch method**

Assessment of plaque pH with plaque acidity tests has become an important tool in determining the individuals risk of caries (Huang and Guo 2000), evaluating food cariogenicity (Simpson *et al* 2001, Neff 1967) and sugar-free substances (Imfeld *et al* 1995). Different methods for studying the process of bacterial fermentation in situ have been used since Stephan (1940) published his investigations to measure acid production in dental
plaque. There are today three main methods of assessing plaque-pH, namely the sampling, the microtouch and the telemetric method. In the microtouch method a plaque pH curve is the final result of the response of dental plaque, involving many host factors, to the intake of fermentable carbohydrates. The microtouch method includes an iridium microelectrode with a diameter of 0.1 mm. This metal electrode is applied directly in the dental plaque on tooth surfaces in different areas in the mouth and a direct reading of the dental plaque-pH is possible. Multiple sites can be measured at each test session. Intermittent readings can be obtained at different time intervals after ingestion of the test product. A reference system, often using a glass capillary electrode reference electrode, is included (Lingström et al 1993). Calibration of the electrode is performed before starting, during and after each test session. The strength of the microtouch method is that it is a rather simple technique without sophisticated instruments which can easily be transported. A disadvantage may be a possible disruption of plaque structure and fragile electrodes which may lack stability. Only intermittent readings can be obtained. To dip the reference electrode in a beaker during the test session with non-optimal cooperating individuals like elderly or children is also a disadvantage.

Dental materials

The replacements of lost hard tissue by caries, erosion, abrasion or trauma are performed by different types of dental materials. For cavities of different sizes, filling materials are used, while for larger replacements crown therapy is used. The filling material amalgam, which has been used for more than 200 years in dentistry, is replaced to-day increasingly in Sweden by resin composites. Their use has increased during the last years due to a growing demand for esthetics and concern about the biocompatibility of amalgam (van Dijken 1994, Geurtsen and Schoeler 1997). However, the main disadvantage associated with composite resins is still their shrinkage during conversion of the monomer molecules into a polymer network. This results in a high shrinkage stress which may cause debonding from the cavity walls and increase the risk for microleakage and occurrence of secondary caries (Davidsson and Feilzer 1997). Since the introduction of silicate cements, fluoride release from restorative materials has been advocated to prevent secondary caries and to enhance the rate of remineralization. A continuous release of fluoride ions from these materials result in precipitation of levels of CaF$_2$ crystals on the tooth surface and contiguous to the restoration especially at low pH (Larsen and Bruun 1994). Calcium ion saturation in the liquid phase reduces the degree of
demineralization and accelerates the process of remineralization (ten Cate and van Duinen 1995). Fluoride ions in high concentration may also inhibit the growth of oral bacteria or interfere with bacterial acid production and acidurance (Maltz and Emilsson 1982). Several investigations have demonstrated that fluoride impairs the growth of mutans streptococci by inhibiting acid production and electrolyte metabolism within these bacteria (Seppä et al 1992, Hamilton 1990). Conventional and resin-modified glass ionomers are the main ion-releasing materials used today. The amount of fluoride released is high during the first day after setting of the material, but declines fairly rapidly over the next weeks to finally stabilize at a lower level. The release increases under acidic conditions and by hydrolysis in saliva (Geurtsen et al 1998, Karantikis et al 2000). Glass ionomer cements have been found to inhibit growth of mutans streptococci in vitro, which have been explained by their high initial fluoride release (Palenik et al 1992, Scherer et al 1989). Two studies in vivo showed that glass ionomer cement reduced the levels of mutans streptococci in human plaque samples (Berg et al 1990, Svanberg et al 1990). However, the distribution of mutans streptococci and lactobacilli did not differ significantly among the surfaces of aged resin modified glass ionomer cement, compomer and composite resin restorations (van Dijken et al 1997). Recently a posterior resin composite was developed which, apart from conventional glass particles, also contains additional alkaline, calcium silicate glass fillers. As a consequence, both fluoride and calcium-ions are released. After contact with water, hydroxyl-ions are formed on the surface of the material which may neutralize part of the organic acids produced by the cariogenic bacteria.

**Saliva**

In the oral cavity both hard and soft oral tissues are protected by saliva, which play an essential role in the maintenance of oral health (Mandel 1987). The saliva consists of mixed secretions from major and minor salivary glands, with three major functions: digestion, protection and lubrication. The multiple functions of saliva are related both to the rate of fluid and to specific organic components (Lagerlöf and Oliveby 1994). In a 24-hours period the daily production of saliva ranges from 0.5 to 1.5 L. The normal rate of mixed stimulated secretion is 1 to 3 ml/min and for unstimulated 0.3 ml/min (Whelton 2004). Saliva is composed of more than 99% water and less than 1 % solids of proteins and electrolytes. It plays an important role in the demineralization process by delivering calcium and phosphate to the tooth surface. Both the salivary secretion rate and the buffering capacity of saliva
protect the tooth against acids produced by cariogenic microorganisms. The salivary secretion rate protects by its rinsing effect, solubility of food, taste-substances, bolus formation, food and bacterial clearance, dilution of detritus, lubrication of oral soft tissues and facilitation of mastication and swallowing (Nauntofte et al 2003). Saliva contains three different buffer systems of which the bicarbonate system is the most important (Bardow et al 2000).

Impaired saliva secretion is often observed in elderly patients because of a higher frequency of systemic diseases and/or high intake of medication in this population. Xerostomia is defined as a subjective complaint of oral dryness. Salivary gland hypofunction is an objective evidence of diminished saliva secretion. Xerostomia is not always associated with diminished saliva secretion. An individual may present xerostomia in absence of an objective evidence of reduced saliva flow rates (Nederfors 2000). Conversely, there are individuals, who have reduced salivary output, but are asymptomatic. It has been reported that xerostomia in healthy adults is experienced when their unstimulated salivary secretion is reduced by approximately 50% (Daves 1987). A higher frequency of diseases and medical treatments, as well psychogenic conditions may result in hyposalivation (Bergdahl and Bergdahl 2000). Almståhl and Wikström (1999) reported that patients with hyposalivation had a significantly increased number of lactobacilli and a tendency toward higher proportions of mutans streptococci. It has been reported that salivary flow rate and pH buffering capacity among the oldest and most frail individuals at an acute care geriatric ward were lower than in younger individuals with a better general health. (Pajukoski et al 1997). Patients with hyposalivation are considered to be at increased risk for coronal caries and root caries as well as periodontal disease (Fure and Zickert 1990, Fure and Zickert et al 1997, Morse et al 2002, Fure 2004).

**Buffer capacity**

The pH of a solution is a measure of its concentration of H\(^+\) ions. Buffer capacity is a measure of an ability to resist pH changes in dental plaque and/or saliva after a challenge. Saliva contains three buffer systems of which the bicarbonate system is the most important and has its principal buffer at pH 7 down to pH 5. Also the type and amount consumed carbonates may affect the pH response. After intake of fermentable carbohydrates and acid production within the dental plaque, the hydrogen ion concentration will drive the dissociation equation to the left. The carbonate ions take up hydrogen ions and produce more carbonic acid which
in turn produces more carbon dioxide and water. The concentration of the bicarbonate ion in saliva depends strongly on the flow rate. The phosphate system has its maximum buffer capacity close to neutral pH. Proteins and amino acids components perform their buffering roll from pH 5 down to pH 4 (Bardow et al 2000). In dental plaque, saliva helps to regulate pH in several ways. Bicarbonate, phosphate and histidin-rich peptides act directly as buffers once they have diffused into the plaque (Mandel 1987). Salivary urea is converted by bacterial urea’s to ammonia, which can neutralize acids. Normal levels of urea present in saliva are predicted to cause an elevation of the pH in fasted plaque (Dibdin and Hawes 1998, Imfeld 1995). Other pH-raising factors in dental plaque may be the presence of microorganisms like the lactate consuming Veillonella or alkali producing S salivarius and S sanguis (Marsh and Nyvad 2003).

**Prevention**

The individuals own human defence mechanism in saliva combined with basic preventive measures like information about the caries disease, good oral hygiene, dietary recommendations and a fluoride supplement will decrease the caries risk in the majority of our patients. A more intensive prophylactic programme, based on the individual’s risk, will be used in higher risk patients. An individual based more intensive fluoride treatment, diet information, intensive oral cleaning, and antibacterial therapies, separate or in combination are generally used. However, many of our preventive measures depend on the cooperation of the individual. Due to different circumstances like diseases, high age, handicaps, non-cooperating patients, many of the preventive measures will not function at all or less optimally. The occurrence of caries in these individuals has to be inhibited in other ways. Addition of buffering agents, like bicarbonate and phosphate in the oral cavity is one way. These supplement the buffering action of saliva and will maintain plaque pH at a high level during periods of caries activity. Imfeld (1983) found that rinsing with sodium bicarbonate increase pH of human plaque after previously been lowered by exposure to fermentable carbohydrates. Sucking on a sugar-free lozenge, containing bicarbonate and phosphate buffers, elevated the pH of human plaque and saliva after a previous sucrose rinse (Nilner et al 1991). A sorbitol-containing chewing gum supplemented with sodium bicarbonate has been found to enhance the ability of plaque-pH to maintain at elevated level following a cariogenic challenge (Igarashi et al 1988). Also the addition of sodium bicarbonate to a fluoridated
dentifrice was effective in reducing plaque acidity with neutralizing effects lasting up to 60 min after treatment (Blake-Haskins et al. 1997). However, the bicarbonate concentration of saliva increases as saliva is stimulated, which may partly explain the neutralizing effects of acids by certain bicarbonate-containing agents (Mandel 1987).

It is desirable to complete the present number of preventive methods with techniques which are easy to apply also in populations with poor compliance.
AIMS

I. To evaluate the clinical performance of a skin reference electrode in combination with the microtouch electrode for intraoral plaque pH measurements.

II. To evaluate the effect of a hydroxyl ion-releasing composite resin on plaque acidogenicity and to compare it intra-individually with a conventional resin composite and non-filled enamel surfaces.

III. To evaluate the effect of aged ion-releasing composite resin restorations on cariogenic microflora of dental plaque and to compare it intra-individually with a universal hybrid composite resin and non-filled enamel surfaces.

IV. To evaluate the effect of a single application of a prophylactic gel with buffering substances on plaque acidogenicity and plaque fluoride concentration in healthy individuals with normal and low stimulated whole salivary secretion rate.

V. To evaluate the effect of multiple daily applications of the gel on plaque acidogenicity in elderly institutionalized individuals.
MATERIALS AND METHODS

The subjects and methods involved in the included papers are shown in Table 1.

Table 1. Subjects involved and analysis used in the included papers.

<table>
<thead>
<tr>
<th>Age (mean/range)</th>
<th>N</th>
<th>Subjects</th>
<th>Analysis</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>47 (32-55)</td>
<td>10 Staff members</td>
<td>Plaque pH</td>
<td>Skin reference electrode/ glass capillary electrode</td>
</tr>
<tr>
<td>II</td>
<td>63 (43-85)</td>
<td>22 Patients attending university clinic</td>
<td>Plaque pH</td>
<td>Proximal surfaces of IRRC/RC/enamel</td>
</tr>
<tr>
<td>III</td>
<td>64 (49-86)</td>
<td>19 Patients attending university clinic</td>
<td>Levels of mutans streptococci and Lactobacilli in plaque samples</td>
<td>Proximal surfaces of IRRC/RC/enamel</td>
</tr>
<tr>
<td>IV (A)</td>
<td>62 (50-70)</td>
<td>12 Patients attending university clinic</td>
<td>Plaque pH</td>
<td>Prophylactic gel with/ and without buffering components/ nutrition solution rinse/ water rinse</td>
</tr>
<tr>
<td>V (B)</td>
<td>62 (52-74)</td>
<td>12</td>
<td>Fluoride</td>
<td>Prophylactic gel with buffering components/0.2% fluoride solution/water</td>
</tr>
<tr>
<td></td>
<td>77 (61-90)</td>
<td>11 Elderly subjects at a nursing home</td>
<td>Plaque pH</td>
<td>Prophylactic gel with/ and without buffering components/ water rinse</td>
</tr>
</tbody>
</table>

IRRC = ion releasing resin composite; RC = resin composite

All studies were approved by the ethics committee at the University of Umeå and written consent was obtained from all subjects prior to study.
Experimental design

To be able to perform plaque-pH measurements with the microtouch method and sample plaque for microbial and fluoride analysis the subjects in papers I, II, III and IV were instructed not to clean their teeth for 3 days prior the test sessions. On day four, the subjects participating in the microtouch measurements were not allowed to eat, drink or use tobacco for two hours prior to the measurement of plaque pH. In paper V all subjects had visible plaque in proximal areas. The assisting personnel were instructed to keep the regular oral hygiene habits of the participants during the study. After measuring baseline plaque pH (0 min), the subjects rinsed for 1 minute with 10 ml of a 10% sucrose solution (SIGMA, St. Louis, Miss, USA; papers I, II) or they rinsed with a nutrition solution (Semper vanilj, Arla Foods, Stockholm, Sweden; papers IV, V).

Microtouch method

To measure plaque-pH in dental plaque, an iridium microtouch electrode with a diameter of 0.1 mm (Beetrode®; NMPH-1, W.P. Instruments, Sarasota, Flor, USA) was used. The pH electrode was connected to a pH/ISE meter (Orion SA 290 A and Orion SA 720 respectively; Orion Research, Boston, Mass, USA). Before each test session, the pH electrodes were calibrated against standard buffers pH 7.00 and 4.01 for verification of the electrode slope and function (Thermo Orion, Beverly, Mass, USA) (Lingström et al. 1993, Scheie et al. 1992). The pH electrode was inserted into the proximal plaque, cervical of the contact point and a direct reading was obtained. After rinsing with the sucrose or nutrition solution, intermittent readings were obtained at different time intervals. The glass capillary reference electrode (MERE 1, W.P. Instruments) was connected to the pH meter. A salt bridge was created during measurements by having the test subject to dip one of his/her fingers into a beaker with a 3 M KCl solution into which the reference electrode also was placed (Scheie et al. 1992). Prior to the measurements, the pH electrode was calibrated against the standard buffer pH 7.00 and 4.01 (Thermo Orion). Plaque-pH measurements were performed up to 30 or 60 min after the mouthrinse. Individual pH-curves were obtained for each of the measured experimental surfaces after the measurements. These were analyzed regarding the following variables: baseline pH (0 min), minimum pH, maximum pH decrease and final pH (60 min). The area under each plaque-pH curve (AUC₅.₇ and AUC₆.₂) was calculated (Edgar 1982).
The materials and methods used in these studies are described in detail in respective papers. A brief summary of each of the studies follows.

**Paper I - Evaluation of a skin reference electrode used for intraoral pH measurements in combination with a microtouch electrode**

To use a skin reference electrode in combination with the microtouch method a validation between the skin reference electrode and a glass capillary reference electrode was performed (Fig. 1). For this system, a plate of silver-silver chloride (ECG: type Syntectics Medical, Stockholm, Sweden) was applied on either the left or right forearm (Millward et al 1997, Huang and Guo 2000, Simpson et al 2001). A conductive electrode gel was applied to the electrode plate (Lectro Derm, Viroderm, Stockholm, Sweden). Thereafter the plate was placed on the forearm and attached with an adhesive plaster. After attachment to the skin and before each measurement, a one-point calibration was performed with the subject’s finger and the pH electrode in a beaker containing a standard buffer pH 7.00 (Thermo Orion).

![Figure 1](image1.png)  
**Figure 1.** Capillary reference electrode (left) and skin reference electrode (right).

Two identical microtouch pH measuring systems were set up and measurements were carried out at two sites in the upper jaw, in the premolar/molar and front regions. One of the microtouch electrodes was combined with a skin reference electrode and the other with a glass capillary reference electrode. Plaque pH was then measured at 0, 2, 5, 10, 15, 20 and 30 minutes. At each time point, plaque pH was measured consecutively with the two systems with only a few seconds between the two measurements. After registering pH with the first system, measurements were directly repeated using the second system. The order of the two
systems was randomly alternated from one subject to another, but they were used in the same order for each individual. Two operators (AP and PL), who were experienced with the microtouch method, performed the measurements. The electrode was inserted into the proximal area by one person and the value, when stabilized, was read independently on the pH meter by the other (Fig. 2).

Figure 2. Microtouch electrode.

**Paper II - Effect of a hydroxyl ion-releasing composite resin on plaque acidogenicity**

In paper II all subjects had one aged proximal ion-releasing resin composite (IRRC) restoration and a none-restored proximal enamel surface located in the same jaw and if possible in similar location to make an intraindividual comparison possible. The mean age of the IRRC restorations was at the time point of the first pH measurements 15 month (referred to as 15 month old IRRC restorations). The pH-measurements were repeated about 1.5-2 yrs later with a mean age of 34 month (referred to as 34 month old IRRC restorations). In this part of the evaluation an aged universal hybrid resin composite (RC) surface was also included in each of the intraindividual comparisons. The RC was situated in similar location as the IRRC. The IRRC was applied according to the instructions of the manufacturer as previously described (van Dijken, 2002). The IRRC contained Bis-GMA, urethane dimethacrylate and dimethacrylate monomers. The filler types included were ytterbium trifluoride, Ba-Al- fluorosilicate glass, highly dispersed silica and silanized alkaline glass filler (48 wt%). All restorations had been made by one and the same operator (JvD). The participants refrained from oral hygiene for 3 days before start of the test session. Plaque-pH measurements were performed at 0, 2, 5, 10, 15, 20, 30, 40, 50 and up to 60 min after the mouthrinse as described earlier. The individual measurements of the two (IRRC and enamel) respective three experimental surfaces (IRRC, RC and enamel) were performed in a randomised order.
**Paper III - Levels of mutans streptococci and lactobacilli in plaque on aged restorations of an ion-releasing and a universal hybrid composite resin**

In paper III all subjects, had as in Paper II, one aged proximal IRRC restoration, one aged universal hybrid resin composite and a none-restored proximal enamel surface located in the same jaw and if possible in similar location to make intra-individual comparisons possible. At day three supragingival plaque was collected from each subject from cervical areas of the two proximal restorations and the non-filled enamel surface. Plaque was collected using the tip of a sterile applicator tip (Applicator Tips, Dentsply/De Trey, Konstanz, Germany). To determine the total number of bacteria (total bacteria) aliquots of the samples were cultured on blood agar plates. Mitis salivararius agar (Difco, Becton, Dickinson and Company, Sparks, MD, USA) supplemented with bacitracin (Gold et al 1973) and Rogosa selective lactobacilli agar (Merck, Darmstadt, Germany) were used to estimate the numbers of mutans streptococci, and lactobacilli, respectively. All plates were incubated in 5% CO₂ and 95% air at 37°C for 2 days. Subsequently the numbers of bacteria on the plates were counted as colony forming units (CFU) and the relative numbers (% of total bacteria) of mutans streptococci and lactobacilli were calculated.

**Paper IV- Buffering effect of a prophylactic gel on dental plaque**

In paper IV two different test series (A and B) were performed. In series A, plaque acidogenicity was evaluated and in series B plaque fluoride concentration. The subjects in series A were divided in two groups according to their stimulated salivary secretion rate: normal secretion rate (≥0.7; median 1.65, range 1.16-3.60 ml/min), low secretion rate (<0.7; median 0.59, range 0.40-0.70 ml/min). The median buffering capacity in the normal secretion rate group was 6.50 (range 3.90-8.40) and in the low secretion group 5.15 (range 2.80-8.10). None of the subjects used any medication. In series A, each subject made four visits to the dental clinic during which the following four treatments, in different test sessions, were carried out: 1) Profylin Fluoride gel with buffering components (Prophylactor AB, Stockholm, Sweden; active gel), 2) Profylin Fluoride gel without buffering components (placebo gel), 3) water rinse (water), and 4) no treatment. All four test sessions were followed by rinsing with a nutrition solution (Semper vanilj, Arla Foods, Stockholm, Sweden).

Each session started with registration of baseline pH. In test session 1 and 2, 0.2 ml of respective gel was applied on each test site. The gels were applied with a syringe (BD
Plastipak, Becton Dickinson, Madrid, Spain) at two proximal sites in the upper jaw, one in the premolar and one in the front region. The gels were kept in place for 5 min, during which the subjects were asked to move their cheeks. This was followed by a mouthrinse with 10 ml nutrition solution for 1 min. In session 3, rinsing with 10 ml water was performed for 1 min followed by rinsing with the nutrition solution as described above. In session 4, only a mouthrinse with the nutrition solution was performed. Plaque pH measurements were performed using a microtouch electrode at 0, 2, 5, 10, 15, 20, 30, 40, 50 and 60 min after the treatment (Lingström et al. 1993). The test sessions were distributed in a randomized order with at least one week interval between each visit.

In series B, professional dental cleaning was performed at baseline after which the subjects refrained from oral hygiene for 3 days before each test session. During this time period they were asked not to use any products containing fluoride. On day four, the subject came to the clinic without eating, drinking or using tobacco for the last two hours prior to visit.

Each subject visited the dental clinic at three test sessions at which one of the following three treatments were performed at random: 1) Profylin Fluoride gel with buffering components (active gel), 2) 0.2% sodium fluoride solution (Dentan Ipex, Medical AB, Danderyd, Sweden; 0.2% NaF), and 3) water rinse (water). In session 1, 1 ml gel was applied at all proximal and buccal surfaces. After 5 min the subjects gargled the slurry around the dentition with active movements of the tongue and cheeks. In session 2 and 3, a mouthrinse with 10 ml sodium fluoride or water was performed for 2 min. Each site was then shortly air dried to remove saliva before supragingival plaque was collected with a dental scaler which was placed in a 0.5 ml pre-weighed Eppendorf tube. Within 2 min after collection, the tube was weighed and then stored at -80°C until analysed at the laboratory. For fluoride analysis, the plaque samples were first centrifuged for 2 min in an Eppendorf centrifuge after which 200 µl of distilled water and 20 µl of TISAB III were added (Sjögren et al. 1996). After sonication for 7 s, the samples were left at room temperature for 24 hr after which the concentration of fluoride was determined with an ion-sensitive electrode (Orion 96-90 electrode, Orion Research, Cambridge, MA) connected to an Orion SA 720 pH/ISE Meter (Orion Research, Boston, MA). The F analyses were performed using standard solutions from 0.01 ppm to 10 ppm F. Fluoride concentration was expressed as ng F per mg plaque.

**Paper V- Buffering effect of a prophylactic gel on dental plaque in institutionalized elderly**

The study was performed as an intraindividual comparison of the new prophylactic fluoride gel with buffering components with two other treatments. The three sessions were carried out
in randomized order and evaluated double-blindly. Fourteen elderly having their own teeth and observed by the personnel to have a subjective oral dryness were asked to participate by the responsible nurse at the nursing home. The following three treatments were carried out: 1) Profylin Fluoride gel with buffering components (active gel; Prophylactor AB, Stockholm, Sweden). 2) Profylin Fluoride gel without buffering components (placebo gel) and 3) water rinse. Before the start of the study, the personnel at the nursing home were informed and motivated about the project and instructed how to perform the three treatments. The gels or water rinse were asked to be applied by the ordinary personnel four times a day during the whole treatment interval (15 days), by preference at the time points medicines were distributed. Approximately 0.3 ml of respective gel was applied under the tongue by a push from the pump flask. The subjects were encouraged to move the lips and cheeks to spread the gels on the teeth and mucosa. The personnel marked on a protocol every time the gel was applied.

All subjects had visible plaque in the proximal areas and the personnel were instructed to keep the regular oral hygiene habits of the participants during the whole study. Two hours before all test sessions the subjects were not allowed to eat, drink, or use tobacco. The subjects were also asked whether they felt oral dryness at any time during the day and/or night time. Paraffin-stimulated whole saliva was collected between 10 and 11 a.m at day 1 and day 15 in order to analyze secretion rate, buffer capacity, determination of mutans streptococci and lactobacilli. To study the presence of Candida albicans on the oral mucosa, samples were collected by swabbing the surface of the mucous membranes of the palate, tongue and cheeks. At day 2 and day 16, pH measurements were performed in dental plaque. The subjects were during the test sessions seated in a comfortable position in a chair or the bed. After rinsing with 10 ml nutrition) solution for 1 min (Semper vanilj, Arla Foods, Stockholm, Sweden), the plaque pH was measured at 0, 2, 5, 10, 15, 20, 30, 40, 50 and 60 min (Lingström et al 1993).

**Different parameters used when estimating the pH curve**

- **Resting plaque-pH** refers to plaque conditions at least 2 hours after the last intake of dietary carbohydrates.

- **Minimum plaque-pH** is the lowest value reached during the measurement and it is corresponding with the greatest concentration of lactate produced during the pH fall.
• **Maximum pH decrease** is the difference between resting and minimum plaque pH. It reflects presence of exogenous factors, as rapidly fermentable carbohydrates and low buffering capacity of saliva at unstimulated flow rate.

• **Final pH** is observed when the pH is rising back to resting level and the value observed at the last time point of each test series. It is influenced by the factors mentioned above, including diffusion of acids out of the plaque into saliva and by base production in plaque.

• **Area under the curve** (AUC). pH is estimated against time (pH x time). It describes the total time period during which plaque pH is below a certain critical pH level.

**Statistical analysis**

**Paper I.** The data were analyzed separately for the two sites, as well as for the mean of the two sites. The difference between the two pH systems was calculated for each time point, after which the mean pH difference for the seven sites was calculated. The total area of the pH response curve below pH 5.7 (AUC₅₇) and pH 6.2 (AUC₆₂) was calculated for each individual pH curve. To compare the two systems, the correlation coefficient \( r \) was calculated. Two-way analysis of variance (ANOVA) was used to test the significance of the different pH variables. When ANOVA rejected the multisample hypothesis of equal means, multiple-comparison testing was performed with Fisher’s PLSD.

**Paper II.** Analysis of variance and Fisher’s PLSD (Possible Least Significance Difference) was used to compare the pH of the IRRC, the RC and the enamel surface at each of the measured time points and for baseline pH, minimum pH, maximum pH decrease, final pH and AUC. For comparison of the results from the IRRC and enamel surfaces at 15 and 34 months, correlation coefficients \( r \) were calculated.

**Paper III.** Data were analyzed with Kolmogorov-Smirnov goodness of fit tests for normality. Wilcoxon’s signed rank test was used for the intra-individual comparisons.

**Paper IV.** Analysis of variance and Fisher’s PLSD were used to compare the pH of the experimental treatment groups at each of the measured time points and for minimum pH, maximum pH decrease, AUC₅₇ and AUC₆₂.

**Paper V.** Bacterial counts were transformed to log₁₀ in order to normalize their distribution. Descriptive statistics was used to describe the frequencies. Differences before and after treatment and between the treatments were tested with paired t-test. For plaque-pH,
individual pH curves were obtained for each of the treatment methods. These were analyzed using the following variables: baseline pH (0 min), minimum pH, maximum pH decrease, final pH (60 min), AUC$_{5.7}$ and AUC$_{6.2}$. Differences between mean values of the studied pH-variables were analyzed using a one-or two-factor analysis of variance (ANOVA) and Fisher’s PLSD.

P-values < 0.05 were considered statistically significant.
RESULTS

Paper I

*Evaluation of a skin reference electrode used for intraoral pH measurements in combination with a microtouch electrode*

The two reference systems resulted in almost identical mean plaque pH for the ten individuals during the whole test period, with the lowest pH values registered at 10 minutes (Fig. 3). When all the time points were taken together, the mean plaque pH was 5.53 ± 0.72 for the system using the skin reference electrode and 5.58 ± 0.64 when using the glass capillary reference electrode. Thus, only a small variation in pH units between the two systems at the different time points was found. The mean difference, when all the time points were taken together, was 0.03±0.08 pH units. High agreement was found between the two methods, with a correlation coefficient (r) of 0.97 (Fig. 4). When the total area of the pH response curve was calculated, no statistically-significant differences were found when comparing the two reference systems. For the skin reference electrode, AUC₅.₇ was 13.1±12.1 and AUC₆.₂ 24.9±15.4 (pH x pH). The corresponding values for the glass capillary reference electrode were 11.5±7.4 for AUC₅.₇ and 24.2±9.6 (pH x pH) for AUC₆.₂. For both AUC values, a high correlation was found (r=0.81 and r=0.86 respectively).

![Figure 3](image-url)  
*Figure 3.* Changes in pH of human dental plaque after a mouthrinse with 10% sucrose when using the skin and the glass capillary reference electrodes in combination with a microtouch electrode.
Figure 4. Correlation between the skin and the glass capillary reference electrodes. Each circle indicates the mean pH value at one time point.

**Paper II**

- Effect of a hydroxyl ion-releasing composite resin on plaque acidogenicity

**15-month old restorations**

The mean pH values for the 15-months aged restorations after the sucrose mouthrinse are shown in Fig 5. The most pronounced pH fall during the whole 60 min time period was found for the enamel surfaces. The differences in plaque pH between the two sites varied between 0.2 and 0.6 pH units at the different time points with a mean of 0.4 pH units when all sites were taken together. Statistically significant differences between the IRRC and enamel surfaces were found at 10 min. None of the surfaces had recovered back to baseline values at 60 min. The baseline pH, minimum pH and final pH were all higher for the IRRC surfaces compared to the enamel surfaces. Only the minimum pH differed significantly (Table 2). The maximum pH decrease was less for IRRC surfaces but the difference was not significant. Larger AUC$_{5.7}$ and AUC$_{6.2}$ values for all three time intervals were found for the enamel surfaces. Significant differences between the two surfaces were found for both AUC$_{5.7}$ and AUC$_{6.2}$ (pH x min) for the 0-5 min and 5-15 min time intervals. The total AUC-values (0-60 min) for IRRC and enamel were AUC$_{5.7}$ 8.4 ± 15.7 and 19.5 ± 28.7 (pH x min)
respectively and for AUC$_{6.2}$ 19.5 ± 28.7 and 34.6 ± 38.0 (pH x min), respectively (ns).

![Graph of plaque-pH changes](image)

**Figure 5.** Changes in plaque-pH at 15 months for the ion-releasing resin composite (IRRC) and enamel surfaces after the mouthrinse with 10% sucrose. pH 6.2 and 5.7 are indicated as dotted lines.

**Table 2.** Baseline pH, maximum pH decrease, minimum pH and final pH at 15 months for the ion-releasing resin composite (IRRC) and enamel surfaces. Statistically significant differences between the two surfaces are shown.

<table>
<thead>
<tr>
<th>Variable</th>
<th>IRRC</th>
<th>Enamel</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline pH</td>
<td>6.99 ± 0.47</td>
<td>6.82 ± 0.56</td>
<td>ns</td>
</tr>
<tr>
<td>Maximum pH decrease</td>
<td>1.50 ± 0.77</td>
<td>2.47 ± 1.76</td>
<td>ns</td>
</tr>
<tr>
<td>Minimum pH</td>
<td>5.49 ± 0.74</td>
<td>5.02 ± 0.84</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Final pH</td>
<td>6.44 ± 0.72</td>
<td>6.21 ± 0.72</td>
<td>ns</td>
</tr>
</tbody>
</table>

**34-month old restorations**

In the second part of the study 16 of the subjects participated. The drop-out was related to four fractures of IRRC restorations and two subjects who did not show up. Sixteen intraindividual comparisons were performed on altogether 48 surfaces (16 x 3). The mean pH values for the three experimental surfaces after a mouthrinse with sucrose are shown in Fig. 6. The most pronounced pH fall during the whole 60 min time period was found for the CR and the least for the IRRC. Statistically significant differences were found at 5, 20 and 60 min. Baseline, minimum and final pHs were equal or higher on the IRRC surfaces compared to enamel and RC. The difference was significant for final pH (Table 3). Maximum pH
decrease was less for IRRC (ns). The lowest AUC$_{5.7}$ and AUC$_{6.2}$ values for all three time intervals were found for the IRRC surface. Comparison of the AUC, calculated at 15 and 34 months, showed a high correlation for AUC$_{6.2}$ both for IRRC (r = 0.75, p < 0.001) and enamel (r = 0.60, p = 0.01), but not for AUC$_{5.7}$, either for IRRC (r = 0.14, p > 0.05) or enamel (r = 0.39, p > 0.05).

**Figure 6.** Changes in plaque-pH at 34 months for the ion-releasing resin composite (IRRC), universal hybrid resin composite (RC) and enamel surfaces after a mouthrinse with 10% sucrose. pH 6.2 and 5.7 are indicated as dotted lines.

**Table 3.** Baseline pH, maximum pH decrease, minimum pH and final pH at 34 months for the ion-releasing resin composite (IRRC), enamel and universal hybrid resin composite (RC) surfaces. Statistically significant differences between the three surfaces are also shown.

<table>
<thead>
<tr>
<th>Variable</th>
<th>IRRC</th>
<th>Enamel</th>
<th>RC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline pH</td>
<td>6.82 ± 0.42</td>
<td>6.82 ± 0.46</td>
<td>6.61 ± 0.36</td>
<td>ns</td>
</tr>
<tr>
<td>Maximum decrease</td>
<td>1.52 ± 0.74</td>
<td>1.76 ± 0.62</td>
<td>1.77 ± 0.64</td>
<td>ns</td>
</tr>
<tr>
<td>Minimum pH</td>
<td>5.33 ± 0.76</td>
<td>5.06 ± 0.84</td>
<td>4.85 ± 0.72</td>
<td>ns</td>
</tr>
<tr>
<td>Final pH</td>
<td>6.90 ± 0.54</td>
<td>6.49 ± 0.93</td>
<td>6.24 ± 0.96</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>
Nineteen subjects participated in this study. Plaque samples were collected from 57 surfaces (3x19). The amount of total bacteria recovered from the three surfaces and the relative numbers of mutans streptococci and lactobacilli, respectively, are given in Table 4. The total numbers of bacteria in the plaque samples varied between $4 \times 10^5$ and $5.7 \times 10^8$. No significant differences were observed between the three surfaces. Mutans streptococci were detected on all surfaces. The relative numbers of mutans streptococci bacteria were for, IRRC 0.59%, CR 0.40%, and enamel 0.22%. No significant differences were found in the relative numbers of mutans streptococci among the different surfaces. IRRC showed two outlier surfaces with high numbers of mutans streptococci (relative numbers 17% and 24%). Excluding the outliers resulted in a relative number of 0.33%. Lactobacilli were detected in the plaque from only 9 surfaces and at very low proportions. The relative numbers were <0.01% for all three surfaces. The total numbers of bacteria from the restored surfaces were not different from those from enamel.

**Table 4.** The number of total bacteria and relative numbers of mutans streptococci and lactobacilli (% of total bacteria) shown as median (range) for the three surfaces. Enamel, IRRC (ion-releasing resin composite), RC (resin composite).

<table>
<thead>
<tr>
<th></th>
<th>Enamel</th>
<th>IRRC</th>
<th>RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>total bacteria x10^8</td>
<td>0.92</td>
<td>1.12</td>
<td>1.33</td>
</tr>
<tr>
<td>(0.09-5.7)</td>
<td>(0.19-4.9)</td>
<td>(0.004-3.8)</td>
<td></td>
</tr>
<tr>
<td>mutans streptococci</td>
<td>0.22</td>
<td>0.59</td>
<td>0.40</td>
</tr>
<tr>
<td>(% of total bacteria)</td>
<td>(0.00-2.10)</td>
<td>(0.00-23.6)</td>
<td>(0.00-4.95)</td>
</tr>
<tr>
<td>lactobacilli</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(% of total bacteria)</td>
<td>(0.00-0.055)</td>
<td>(0.00-0.11)</td>
<td>(0.00-3.11)</td>
</tr>
</tbody>
</table>
Paper IV
- Buffering effect of a prophylactic gel on dental plaque

Plaque pH measurements

All subjects: Changes in plaque pH after the four treatments in series A are given as mean values in Fig. 7. The active gel containing buffering components resulted in the highest pH values during the whole test period. Statistically significant differences between the treatment groups were observed within the first 20 min; active gel vs no treatment at 2 and 5 min (p<0.001) and at 10, 15 and 20 min (p<0.05); active gel vs water at 2 and 5 min (p<0.001) and at 15 min (p<0.05); placebo gel vs no treatment at 5 and 10 min (p<0.01) and at 2 and 15 min (p<0.05); placebo gel vs water at 2 and 5 min (p<0.05). No statistically significant differences were observed between the two gels. The AUC5.7 and AUC6.2 variables for the four groups are shown in Fig 8. The highest values for both AUC-values were found for no treatment. Statistically significant differences were observed for AUC5.7 between no treatment and the active gel (p<0.05) and for AUC6.2 between no treatment and the active respective placebo gels (p<0.05).

Subjects with normal salivary secretion rate: A similar pattern of plaque-pH response as for all patients was observed for the participants with normal salivary secretion rate with the least pronounced pH fall after use of the active gel. Statistically significant differences for this group were found within the first 15 min of measurements, which were almost similar to those observed for “all subjects”. In addition, a significant difference was also found between active and placebo gel at 2, 5, 15 and 50 min (p<0.05).

Subjects with low salivary secretion rate: For the participants with low salivary secretion rate, the active gel resulted in a generally lower pH compared to those with normal salivary secretion rate with a mean difference in plaque-pH of 0.7 pH-units. Significant differences were found for the active gel vs no treatment at 5 min (p<0.05) and the placebo gel vs no treatment at 15 min (p<0.05).

Significant differences between low and normal salivary secretion rate subjects were found in the active gel group at the time points 2, 5 and 15 min (p<0.001, p<0.05, p<0.01) as well as for minimum-pH (p<0.05) and AUC6.2 (p<0.05). The corresponding AUC5.7 values for the low and normal salivary secretion groups were 13.2 ± 11.2 respective 1.8 ± 3.6 (pH x min) for the active gel and 8.5 ± 5.1 respective 7.4 ± 4.9 (pH x min) for the placebo gel.
Figure 7. Changes in plaque pH for the four treatments (active gel, placebo gel, water and no treatment) given as mean values at each of the time points for all individuals. (n=12).

Figure 8. The AUC_{5.7} and AUC_{6.2} (pH x min) for the four treatments (active gel, placebo gel, water and no treatment) for all participants.

**Fluoride analyses**

The plaque fluoride concentrations expressed as ng F/mg plaque are shown in Table 5. The 0.2% NaF rinsing resulted in significantly higher plaque F concentrations compared to both the active gel and the water rinse (p<0.001).
**Table 5.** Plaque fluoride concentrations (ng F/mg plaque) for all subjects after: application of active gel, rinsing with 0.2% NaF and rinsing with water.

<table>
<thead>
<tr>
<th></th>
<th>Fluoride concentrations ng/mg</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Significance level</td>
</tr>
<tr>
<td>Active gel</td>
<td>0.24 ± 0.12</td>
<td>0.07 – 0.44</td>
<td>a</td>
</tr>
<tr>
<td>0.2% NaF</td>
<td>1.22 ± 0.70</td>
<td>0.30 – 3.06</td>
<td>b</td>
</tr>
<tr>
<td>Water</td>
<td>0.09 ± 0.06</td>
<td>0.01 – 0.18</td>
<td>a</td>
</tr>
</tbody>
</table>

a vs b: p<0.001

---

**Paper V**

- *Buffering effect of a prophylactic gel on dental plaque in institutionalized elderly*

Three drop outs were observed during the study, one participant died during the study period and 2 wanted to discontinue the treatments. Thus, eleven participants (8 women and 3 men), with a mean age 76.6 years (range 61-90) fulfilled the study. The participants in this study had a mean number of 16 remaining teeth (range 6 – 28). All participants had a diagnosed disease and they used 3-10 different medications. Salivary secretion rates and buffer pH before and after the treatments are shown in Table 6. Six participants showed stimulated secretion rates <0.7 ml/min and 9 showed a low buffer capacity (<5.2). No statistically significant differences regarding salivary secretion rate and buffering pH were found when comparing data before and after the treatments, between or within each of the three groups.

*Oral microflora*

For number of CFU of mutans streptococci, no statistically significant differences were found between the groups comparing before and after the treatments. CFU of lactobacilli were significantly increased after the active gel treatment (p=0.02), but not for the other treatments (Table 7). Low levels of *Candida albicans* were found in 8 and no detectable colonies in 3 participants before and after the 3 treatments (n.s.).
Table 6. Salivary stimulated secretion rate (ml/min) and buffering capacity (final pH) before (day 1) and after (day 15) the treatments. No statistically significant differences were found between the three test periods. Median (Q25-Q75%).

<table>
<thead>
<tr>
<th></th>
<th>Active Before</th>
<th>Active After</th>
<th>Placebo Before</th>
<th>Placebo After</th>
<th>Water Before</th>
<th>Water After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary secretion rate ml/min</td>
<td>0.80</td>
<td>0.70</td>
<td>0.70</td>
<td>0.50</td>
<td>0.90</td>
<td>0.61</td>
</tr>
<tr>
<td>Q25%</td>
<td>0.29</td>
<td>0.29</td>
<td>0.40</td>
<td>0.40</td>
<td>0.24</td>
<td>0.40</td>
</tr>
<tr>
<td>Q75%</td>
<td>1.50</td>
<td>1.30</td>
<td>2.10</td>
<td>2.00</td>
<td>1.60</td>
<td>1.90</td>
</tr>
<tr>
<td>Buffering final pH</td>
<td>5.20</td>
<td>5.10</td>
<td>5.00</td>
<td>4.80</td>
<td>4.80</td>
<td>5.10</td>
</tr>
<tr>
<td>Q25%</td>
<td>4.70</td>
<td>4.4</td>
<td>4.50</td>
<td>4.50</td>
<td>4.20</td>
<td>4.60</td>
</tr>
<tr>
<td>Q75%</td>
<td>7.80</td>
<td>6.40</td>
<td>5.80</td>
<td>5.60</td>
<td>6.90</td>
<td>5.80</td>
</tr>
</tbody>
</table>

Table 7. The number of mutans streptococci and lactobacilli in stimulated whole saliva, expressed as mean (±SD) and range of Candida albicans on oral mucosa tissues and tongue given as % of total microorganisms (min-max) * p<0.05.

<table>
<thead>
<tr>
<th></th>
<th>Active Before</th>
<th>Active After</th>
<th>Placebo Before</th>
<th>Placebo After</th>
<th>Water Before</th>
<th>Water After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutans streptococci</td>
<td>6.25±1.05</td>
<td>6.01±1.43</td>
<td>5.64±1.72</td>
<td>5.91±1.68</td>
<td>6.00±1.43</td>
<td>6.38±0.86</td>
</tr>
<tr>
<td>(Log10 CFU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>5.44±1.41</td>
<td>*</td>
<td>5.22±1.34</td>
<td>5.56±1.34</td>
<td>5.72±0.89</td>
<td>5.54±1.29</td>
</tr>
<tr>
<td>(Log10 CFU/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans (%)</td>
<td>0.00-0.1</td>
<td>0.00-0.1</td>
<td>0.00-0.4</td>
<td>0.00-0.9</td>
<td>0.00-0.06</td>
<td>0.00-0.03</td>
</tr>
</tbody>
</table>

Plaque-pH measurements
Changes in plaque pH before and after each of the three treatments are shown as mean values in Fig. 9 a-c and Fig. 10. Baseline pH, minimum pH, maximum pH decrease, final pH, AUC5.7 and AUC6.2 are shown in Table 8. No significant differences were observed before and after each treatment group, between or within the three treatments groups.
**Figure 9a.** Changes in plaque pH day 16, before and after multiple treatments with the active prophylactic gel. pH 6.2 and 5.7 are indicated as dotted line.

**Figure 9b.** Changes in plaque pH day 16, before and after multiple treatments with the placebo prophylactic gel. pH 6.2 and 5.7 are indicated as dotted line.

**Figure 9c.** Changes in plaque pH day 16, before and after multiple treatments with water rinsing. pH 6.2 and 5.7 are indicated as dotted line.
After multiple treatments

**Figure 10.** Changes in plaque pH, day 16 after multiple treatments with the active and placebo gel and water rinsing. pH 6.2 and 5.7 are indicated as dotted line.

**Table 8.** Baseline pH, minimum pH, maximum pH fall, final pH and AUC5.7 and AUC6.2 values (pH x min) before and after the three treatments. Mean±SD

<table>
<thead>
<tr>
<th></th>
<th>Active</th>
<th>Placebo</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Baseline pH</td>
<td>6.58±0.63</td>
<td>6.39±0.79</td>
<td>6.63±0.84</td>
</tr>
<tr>
<td>Minimum pH</td>
<td>5.23±0.67</td>
<td>5.19±0.65</td>
<td>4.99±0.64</td>
</tr>
<tr>
<td>Maximum pH fall</td>
<td>1.35±0.36</td>
<td>1.20±0.58</td>
<td>1.64±0.51</td>
</tr>
<tr>
<td>Final pH</td>
<td>5.99±1.01</td>
<td>5.85±0.69</td>
<td>5.92±0.91</td>
</tr>
<tr>
<td>AUC5.7</td>
<td>15.4±22.4</td>
<td>18.8±22.5</td>
<td>19.2±18.7</td>
</tr>
<tr>
<td>AUC6.2</td>
<td>30.3±33.2</td>
<td>36.0±33.7</td>
<td>38.5±27.8</td>
</tr>
</tbody>
</table>
Dental caries is one of the two main oral diseases in the mouth. The prevalence of caries has decreased dramatically in most developed countries since the second half of the twentieth century. As a consequence, most young individuals have very low DFS values and the number of remaining teeth in adults and elderly has increased during the last decades. The improved oral health is a result of the extended use of fluorides, improved oral hygiene and a greater access to dental service with favourable dental insurance systems (Palmqvist et al 2001, Twetman et al 2003). Elderly people experience today a longer lifetime and a better quality of life (Steen 1998). Major efforts in dental care have to be performed to maintain their oral health (Fure and Zickert 1990, Fure and Zickert 1997, Fure 2004). A good oral health is necessary for proper chewing, to start digestion, swallowing and for verbal communication which influence their quality of the life (Peltola et al 2005). A Finish study showed that elderly with a poor oral health had more functional, psychosocial and pain/discomfort-related limitations (Pajukoski et al 1997). Elderly will on group level be at increased risk for caries. Several studies show that many elderly suffer from poor oral hygiene and increased levels of cariogenic bacteria (Almståhl et al 1999, Fure 2004). Oral dryness (xerostomia) is a common clinical complaint in the elderly which in most cases is caused by their medication (Österberg et al 1994, Bergdahl et al 2000). Saliva is one of the most important defending factors for dental caries. Apart from its water content, it contains several buffer- and antimicrobial systems which influence the individual’s caries activity. Older individuals with hyposalivation are considered to have an increased risk for coronal and root caries (Fure et al 1990, Fure 2004).

A good buffer capacity has the ability to resist pH changes in dental plaque and/or saliva after a challenge. The bicarbonate buffering system is the most important action of saliva in order to influence caries challenge (Bardow et al 2000, Edgar and Higham 2004). Other buffer systems like the phosphate system and proteins and amino acids components in saliva have minor impact (Imfeld et al 1995, Dibdin and Dawes 1998). Bicarbonates will diffuse into plaque and act as a buffer by neutralizing acids present in plaque and increase the time for remineralization of early caries (Mandel 1987).
Dental caries is caused by an interplay between tooth tissues, aciduric microorganisms and access of fermentable carbohydrates. Fermentation of accessible carbohydrates by cariogenic bacteria, in the first place by mutans streptococcus and lactobacilli, result in formation of organics acids like lactic acid and formatic acid and a plaque pH-fall (Neff 1967, Scheie et al 1996). Many oral factors, such as the type and number of cariogenic microorganisms, plaque composition, amount and composition of saliva, oral motorics and tooth anatomy influence the plaque pH value (Nyvad and Fejerskov 1994, Marsh and Martin 1999). Caries lesions are particularly likely to develop in undisturbed plaque niches. These include fissures, the area below the contact point in proximal surfaces, cervical tooth areas, root surfaces as well as contiguous to gingival gaps of restorations. Secondary caries, like primary caries, occurs due to disturbance of the demineralization-remineralization balance (Neff 1967, Kidd 1990, Fitzgerald et al 1994). Basic caries prophylactic treatments of individuals in Sweden consist of disease information, oral hygiene instruction and the use of fluoride toothpaste. Individuals with increased caries activity or risk should have an individually adapted prophylactic programme which starts with a risk evaluation followed by extensive disease information, expended individual adapted fluoride treatment and completing treatments of different risk factors such as diet information or use of antimicrobial components. Fluoride tablets and rinsing, together with a reduction of sugar and optimal oral hygiene, are the most frequently used preventive tools against caries in elderly (Fure et al 1998, Fure 2004). Despite all efforts caries is still a main problem in elderly and found to be the main reason for tooth extraction (Fure and Zickert 1997). Other ways to prevent disease and especially dentin caries in elderly are therefore necessary.

Besides different methods to stimulate or replace saliva in individuals with oral dryness, several attempts have been made during the last years to decrease caries activity by addition of substances with buffering and/or remineralizing effects. One way is to add buffering agents, like bicarbonate and phosphate, to the oral cavity, which may supplement the buffering action of saliva and maintain pH at a high level during periods of caries activity. Rinsing with sodium bicarbonate, sucking on a sugar-free lozenge containing bicarbonate and phosphate buffers, sorbitol-containing chewing gum and fluoridated dentifrice supplemented with sodium bicarbonate, have been found to enhance the ability of plaque-pH to maintain at an elevated level following a cariogenic challenge (Imfeld 1983, Igarishi et al 1988, Nilner et al 1991, Blake-Haskins et al 1997). However, the bicarbonate concentration of saliva increases as saliva is stimulated, which may partly explain the neutralizing effects
of acids by certain bicarbonate-containing agents (Mandel 1987). To prevent secondary
caries, fluoride-releasing materials have been used during the last decades in operative
dentistry starting during the 1960s with silicate cements followed in the late seventies by
glass ionomer cements. Unfortunately, the physical and mechanical properties of these
materials were found to be insufficient in the posterior areas of the mouth (van Dijken 2002).
This thesis includes the evaluation of two alternative methods assumed to decrease caries
activity by their buffering properties. The overall aim in this thesis was to evaluate the
neutralizing effect of a hydroxyl ion-releasing resin composite and a prophylactic gel on
plaque acidogenicity measured by the microtouch method. The resin composite was
suggested as an improved version of the available “smart” fluoride releasing restorative
materials in order to prevent secondary caries, while the gel was proposed to be a suitable
prophylactic method especially in elderly at risk.

Different plaque acidity tests to estimate for example the cariogenic potential of foods have
been frequently used since the introduction of the Stephan curve in 1940. Changes in plaque
pH after a fermentable carbohydrate challenge can be followed over time and is usually
presented as a pH curve. Registration of plaque pH with the microtouch method has become
an important tool in assessing the cariogenic potential of food products (Lingström et al
1993, Imfeld et al 1995). This technique has never been applied to evaluate prophylactic
agents or the effect of aged dental materials on plaque pH. Pilot studies performed with the
microtouch method in combination with the traditional reference electrode (usually glass
capillary electrode) showed that the reference system is complicated for patients who are
unable to fully cooperate. The test subject needs to keep both his/her head and finger in
stable position during the measurements, which can be difficult or even impossible for
certain individuals. Therefore we developed a modified, easier to apply, reference electrode
method in combination with the microtouch method. Within the medical field, a silver/silver
chloride plate (ECG; type Syntectics Medical, Stockholm, Sweden) attached to the skin has
been used to record the oesophageal pH in vivo (Kahrilas and Quigley 1996). A similar
approach and technique was developed and tested at our department in order to be used for
intraoral pH measurements.

The validation performed between the skin reference electrode and the glass capillary
reference electrode resulted in almost identical mean plaque pH values during the whole test
period. A high agreement was found between the two methods, with a correlation coefficient
of 0.97. From a technical point of view the skin reference electrode was regarded as being much easier to handle as it requires less cooperation from the test subject. The very small differences seen in pH registration between the two techniques is believed to be related to the difference in placement of the electrode at time for measurement and that the environment, dental plaque, changes over time. Due to the positive experience with the skin attached electrode, this was used in the subsequent studies.

To reduce the development of secondary caries with different kind of preventive measures is important for a majority of our patients. Traditionally, prevention is directed against the different risk factors of each individual by treating factors like oral hygiene, intake of fermentable carbohydrates, cariogenic microflora or oral dryness. To recommend the individual an increased use of fluoride is a common method to decrease the caries risk. In some restorative materials the release of fluoride can be used to prevent decrease the risk of secondary caries - alone or in combination with other preventive methods. We evaluated a so called “smart restorative material”. The term “smart material” has earlier been used for fluoride releasing materials which release higher fluoride concentrations during periods of low pH. The posterior composite material has a suggested neutralizing effect of plaque pH not only by the release of fluoride ions, but also by setting calcium and especially hydroxyl-ions free. Its neutralizing effect was demonstrated in the present studies and shown to be similar at 15 and 34 months age of the restoration. This means that the neutralizing capacity of the material was stable during aging. It also showed that the mechanism behind the release of ions was different from that of the glass ionomer cements. These cements show a high initial fluoride release peak during the first weeks and depend later on a continuous uptake of fluoride from other sources like tooth paste to be able to release fluorides but on a lower release level. The ion-releasing resin composite behaves different. Due to a continuous hydrolytic degeneration of the alkaline filler by water, the filler is able to release hydroxyl, calcium and fluoride ions during aging in the mouth. However, the neutralizing effect of the ion-releasing resin composite could also be due to a lower number of acid producing bacteria due to its ion release. In paper III, mutans streptococci were observed on all test surfaces, but no significant differences were found and it was therefore concluded that the neutralizing effect of the material was not related to changes in microflora. The data shows that the neutralizing effect of the material contributes during long term to lower the recurrent caries risk of the adjacent tooth surface. In a 3-year follow-up study of the composite material used
for class II cavities no secondary caries was observed. (van Dijken 2002). The increased water uptake of the composite resulted in an increased fracture rate of the restorations and teeth involved.

In the second part of the thesis we evaluated another alternative way to neutralize plaque pH after intake of fermentable carbohydrates. In several earlier studies incorporation of buffering agents, especially bicarbonates and phosphates, have been suggested to be used in order to inhibit caries (Blake-Haskins et al 1997). In our university clinic we have earlier, with positive experiences, used a tablet with the same buffering substances included as in the tested prophylactic gel (paper IV and V) (Nilner et al 1991). As earlier mentioned, the growing problem with an increasing amount of elderly patients with high caries risk needs new prophylactic approaches (Gabre et al 2005). To cover energy and nutrition needs in elderly with physical or psychological handicaps, nutrition solutions are frequently used. We observed that the intake of nutrition solutions in these individuals caused severe pH falls. Many of these individuals belong to an exposed group with poor oral hygiene, malnutrition, oral dryness and fragile oral mucosa. Dental hygienists in Sweden often takes a responsibility for the oral health prevention of these patients and to find suitable preventive methods for this group is urgent. The idea to use a prophylactic gel combining buffering and lubricating properties in caries active patients especially elderly was therefore of great interest. To investigate the effect of the prophylactic gel, a study with a group of healthy individuals was started. Individuals with normal and low stimulated secretion rate were selected to simulate the situation in the elderly population. The short time effect of a single application of the gel on plaque pH was measured after 5 minutes. A significant better neutralizing effect was observed for the active gel in individuals with normal secretory rate while no improved effect was shown in the low secretory rate group. Surprisingly, a low fluoride release was observed from the gel compared to a traditional fluoride rinse. In paper V the gel was then tested in elderly with subjective oral dryness. It was expected that the subjects with low salivary secretion would show an inferior basic defence. Multiple applications were therefore applied to investigate if an accumulation of the gel substances occurred in the plaque of these high caries risk individuals. Accumulated gel components would in this way be able to function as a depot which could be available during fermentable carbohydrate intakes. The long term effect of the multiple applications was studied by measuring pH changes after frequent use of the gel and two hours after the last gel
application instead of 5 minutes after a single application as in paper IV. No differences in plaque pH changes were found between the active gel and the other test groups indicating that probably no accumulation of the buffering substances had occurred. The results from both studies pointed out that the gel had no buffering effect in individuals with low secretory rates. There might be several possible explanations for these findings. One may speculate that the non-effective buffering action of the active gel is caused by too low concentrations of the buffering agent accumulated in the plaque as suggested above. Another possibility is an impaired solubility of the gel resulting in a too low release of the active substances out of the gel. However, we observed in paper IV that individuals with normal secretion rate showed an improved buffering effect 5 minutes after application of the active gel, indicating a good solubility of the gel substances in these individuals shortly after application. But in individuals with oral dryness no buffering effect was observed. It may therefore be assumed that a certain amount of saliva is needed to release the buffering agents. A reduced uptake of the active gel components may therefore be expected in these subjects.

In paper IV it was shown that a 0.20% sodium fluoride rinsing solution showed almost a 5 times higher uptake in plaque compared to the release of fluoride in plaque from the 0.15% fluoride containing prophylactic gel. An improvement of the solubility of the gel may increase the transport of active substances into the plaque also in individuals with low salivary secretion rate. It was observed that all elderly participants in study V had a thick aged plaque, which also may affect the uptake of substances into the plaque. This most likely also holds for the release of fluoride. Paper IV clearly showed that the buffering gel has the capacity to reduce plaque acidogenicity when applied directly prior to a sugar challenge. Paper V showed that an insufficient accumulation of the substances did occur in the plaque. The impaired uptake and availability of the gel substances also suggest that it may be favourable to apply the gel more closely in time prior to the carbohydrate intakes.

A not expected finding in study V was a tendency to higher amounts of cariogenic microorganisms in both gel groups. Frost et al (2006) showed also increased levels of streptococcus mutans and lactobacilli in patients with oral dryness after use of an intraoral lubricating gel containing lactoperoxidase, glucose oxidase and xylitol. It can be assumed that the gel, when applied in the oral cavity, may act as a substrate which increase the retention of bacteria and promote towards increased numbers of microorganisms. An increase in cariogenic plaque microorganisms probably results in an increased plaque acidogenicity,
which was confirmed by the lower baseline pH-levels for both the active and placebo gels after the treatments.

The non-neutralizing effect of the gel may be caused by a combination of several factors like the oral dryness of the individuals, low solubility, low release and no accumulation of gel substances in plaque, and by increased plaque acidogenicity. The present data corresponds well with a recent study which have shown an increased plaque lowering potential of dental plaque in dry mouth subjects (Eliasson et al 2006), Higher number of cariogenic microorganisms were also found in these subjects compared to matched healthy controls. Our findings points to the need for further evaluation of methods in order to increase the buffering effect of dental plaque particularly aimed for elderly and dry mouth subjects. An enhanced understanding of the interaction between plaque, saliva and intake of fermentable carbohydrates during dry mouth conditions is needed. It is also suggested to further study the possibility to use dental materials as a vehicle for different buffering agents. It is in this respect important not only to focus on release, but also on a possible uptake of such active buffering components.
The use of the skin reference electrode, in combination with the microtouch electrode, showed high validity in comparison with the conventional glass capillary reference electrode.

The hydroxyl ion-releasing composite resin countered plaque acidogenicity fall at two time points of the aged restorations and maintained it at levels where lesser demineralization occurs. No influence of the ion-release on the amount of cariogenic plaque microorganisms on the resin composite surface was observed.

A single application of the prophylactic gel with buffering substances showed a neutralizing effect of plaque pH in healthy individuals with normal salivary secretion rate. No effect was observed in low secretion rate individuals.

Multiple applications of the prophylactic gel did not neutralize dental plaque acidogenicity in elderly individuals with subjective oral dryness.
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REFERENCES


Huang GF, Guo MK Resting dental plaque values after repeated measurements at different sites in the oral cavity. Proc Natl Sci Counce ROC (B) 2000; 24:187-192.


Karantakis P, Helvatjoglou–Antoniades M, Theodoridou-Pahini S, Papadogiannis Y.


Stephan RM. Changes in hydrogen-ion concentration on tooth surfaces and in caries lesions. JADA 1940; 27:718-723.

