On Approximal Caries Prevention using Fluoridated Toothpicks, Dental Floss and Interdental Brushes

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Abstract

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Although dental health has improved during the last 40-50 years, approximal caries still constitutes a problem in many age groups. It is important that fluoride (F) toothpaste is used when brushing the teeth. In some subjects, there may be a need for supplementary F products, especially in the caries-prone approximal area. **Aim:** The aims of this thesis were: i) to study the F release of F-containing approximal oral hygiene aids both *in vitro* and *in vivo*, ii) to evaluate different methods for the administration of F *in vivo*, iii) to study the effect of the frequent use of F-containing toothpicks and floss on demineralised enamel and dentine *in situ* and iv) to evaluate recommendations and the use of oral hygiene products for approximal cleaning in a Swedish adult population. **Material and methods and Results**: The F release of 26 brands of toothpicks and floss was followed for 24 hrs *in vitro*. A large variation in the release between these products was found; in general, toothpicks resulted in larger amounts of F compared with floss. The release *in vivo* was studied using single and multiple fluoridated toothpicks and dental floss, as well as in combination with toothbrushing or a mouthrinse with 0.2% NaF. Moreover, the administration of F by an interdental brush dipped in 0.2% NaF gel (here called the "Inter Dental Brush Gel Method") was evaluated. Approximal saliva was collected, using paper points, before and up to 60 min after treatment. Both toothpicks and floss resulted in enhanced F concentrations *in vivo*. An interdental brush dipped in 0.2% NaF gel and a mouthrinse with 0.2% NaF resulted in the same F concentration as after using multiple toothpicks. All combinations of toothpicks and dental floss with F rinsing resulted in higher concentrations than after only toothbrushing or in combination with brushing. The most optimal order was to use toothpicks and dental floss after toothbrushing and before rinsing. Fifteen adults with full dentures, in which demineralised enamel and dentine specimens had been mounted, were included in an *in situ* experimental caries model. Toothpicks or floss, impregnated with NaF and amine fluoride (AmF), were used regularly for four weeks. All the products inhibited continuous demineralisation - dental floss somewhat more than toothpicks. A reduction in plaque micro-organisms was also found. Recommendations made by dental staff in relation to approximal cleaning aids were evaluated by a questionnaire sent to 500 dentists, 500 dental hygienists and 1000 patients in Sweden. The ability to remove approximal plaque was also evaluated in 60 regular users of approximal aids. Recommendations by dental staff are mostly given to children and adolescents in order to prevent dental caries and to older individuals to prevent gingivitis and periodontal disease. Approximal plaque appears to be more easily removed by regular users of interdental brushes compared with the use of toothpicks and dental floss. **Conclusions:** The use of fluoridated interproximal aids appears to be important in order to reduce approximal caries. An interdental brush dipped in a NaF gel is an interesting method for increasing approximal F concentration.

Key words: Approximal caries • Dental flosses • De- and re-mineralization • Fluoride • Interproximal aids • Interproximal area • Oral hygiene • Questionnaire • Toothpicks

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Contents

Original papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-IV).

I. Särner B, Lingström P, Birkhed D. **Fluoride release from NaF- and AmF-impregnated toothpicks and dental flosses in vitro and in vivo.** Acta Odontol Scand 2003;61:289-296.

II. Särner B, Birkhed D, Lingström P. **Approximal fluoride concentration using different fluoridated products alone or in combination.** Caries Res 2008;42:73-78.

III. Särner B, Birkhed D, Huysmans MCDNJM , Ruben JL, Fidler V, Lingström P. **Effect of fluoridated toothpicks and dental flosses on enamel and dentine and on plaque composition in situ.** Caries Res 2005;39:52-59.

IV. Särner B, Birkhed D, Andersson P, Lingström P. **Recommendations by dental staff and use of toothpicks, dental floss and interdental brushes for approximal cleaning in an adult Swedish population.** 2008, submitted.

Introduction

Dental caries

Dental caries is the most common oral disease worldwide and it affects the majority of individuals in all age groups during their lifetime (Petersen et al., 2005). It is regarded as an infectious disease in which changes in the oral environment lead to a pathological shift in the oral biofilm, which then results in the localised destruction of the hard tooth tissues. A triad of indispensable factors, the host, bacteria and fermentable carbohydrates, are therefore necessary in order for disease to occur (Keyes and Jordan, 1963; Selwitz et al., 2007). The inter-relationship between these three key elements may be influenced by a large number of biological and socioeconomic factors and dental caries is therefore regarded as a disease of multifactorial origin.

Since the middle of the last century, a decline in dental caries has been seen among children, adolescents and adults, but there are still a large number of individuals and populations in whom the caries prevalence remains at a high level (Sundberg, 1996; Fure, 1997; Marthaler, 2004; Hugoson et al., 2005; Hugoson et al., 2008). The mean numbers of decayed and filled surfaces (dfs/DFS) have decreased in the age groups of 15-50 years, while a slight increase in caries has been found for the oldest age groups (70-80 years) in a Swedish population over a 30-year period (Hugoson et al., 2005). Although a reduction in caries prevalence among pre-school children has been found, a tendency towards stagnation in this decline since the end of the 1980s has been reported (Stecksén-Blicks et al., 2004). Dental caries affects 46% of children aged 4 (Stecksén-Blicks et al., 2004) and 80% of 15-year-olds (Hugoson et al., 2008). It has been calculated that, every year, around 20-30% of the adult population develop new carious lesions which require treatment (Zickert et al., 2000, Bader, et al., 2005).

The most caries-prone areas of the teeth are the approximal surfaces, the fissures and the gingival third of the smooth surfaces (Seppä et al., 1991). In addition, the exposed root surface can be regarded as an area at high risk. Lesions at this site are frequently found in the elderly in particular (Fure, 1997). A large number of lesions are found in the approximal area. The mean DFS of approximal surfaces in 19-year-olds in 2005 was 1.3. Of the adolescents affected, the corresponding mean DFS for the approximal area was 3.2 (Swedish Board of Health and Welfare, 2008). These data only included manifest lesions. The number of DF on approximal surfaces, including initial lesions, was 3.0 for 15-year-olds in the Jönköping study (Hugoson et al., 2005). This indicates that the problem is underestimated by figures presenting only manifest lesions (Moberg Sköld et al., 1995). Approximal caries has also been found to be unevenly distributed between surfaces (Mejàre et al., 1998; 1999). In an examination of

premolars and molars in children from 11 to 22 years of age, the majority of the lesions were found on the distal surface of the first molar (6d) in the lower jaw and on the distal surface of the second premolar (5d) in the upper jaw (Mejàre et al., 1999). The lowest caries prevalence was observed for the mesial surface of the second premolar (5m) and the distal surface of the first premolar (4d) in the lower jaw.

Like the total caries prevalence, approximal caries is also unevenly distributed in the population. An increased prevalence of approximal caries has been found in relation to high caries risk (Mejàre et al., 1999) and approximal surfaces are regarded as especially high-risk sites for caries in individuals with high sugar consumption (Sundin et al., 1992; Arnadottir et al., 1998). In our modern society, there are a large number of children with no caries whatsoever or a low level of caries. However, a larger number of new lesions actually occur in the large low-risk group than in the small high-risk group (Moberg Sköld, 2005; Baelum et al., 2008). This is even more pronounced when enamel lesions are included in caries reports (Moberg Sköld, 2005). A relationship between caries in early childhood and manifest approximal caries prevalence in the posterior teeth at 15 years of age has recently been shown (Alm et al., 2007).

So, even if the prevalence of approximal caries has decreased in recent decades, it still constitutes a major problem for many individuals and it is important to identify preventive methods for use in different age groups. Studies have demonstrated that approximal caries still poses a problem in adolescents and that new methods for the prevention of these lesions are important (Bjarnason et al., 1992; Hugoson et al., 1995; Crossner and Unell, 1996). As caries may occur on both enamel and exposed root surfaces, it is also thought to constitute a significant problem in the future in adults, not least as the number of elderly people is expected to increase in the future.

The approximal area

The high caries prevalence on the approximal surfaces can be explained by the unique characteristics of this area. There are a number of caries-promoting factors, which are specific to the interproximal area. Due to the size and shape of the approximal region, it constitutes an ecological niche in which an undisturbed biofilm can form. Oral micro-organisms and food products easily become attached approximally and access by saliva to this site is limited. A wide variation in oral biofilm formation between different areas is known to exist. Plaque at interproximal sites has been reported to be more acidogenic than in other areas of the mouth (Igarashi et al., 1989). Furthermore, a reduced clearance rate from this area has been demonstrated compared with more readily accessible tooth surfaces. The higher plaque acidogenicity and prolonged clearance rate are factors that are both known to be of importance in terms of the development of caries.

A difference in interproximal size is found between the primary and permanent dentition, with larger interproximal areas in the latter. Smaller contact points are seen in the front region, while there are larger areas in the premolar/molar region. Further anatomical changes may also occur during aging. With increasing age, the retraction of the gingiva may result in larger interproximal areas. Due to the rougher surface structure of the root, plaque formation may even be higher on these surfaces. Thus, there are several factors that may influence the retention of plaque and bacteria in the area, which may in turn explain the higher risk of disease occurring.

Caries prevention

Caries prevention is regarded as a measure designed to prevent the disease as such, but also the clinical symptoms of caries disease appearing. It also includes treatment for the early signs of illness (initial caries) to prevent further progress and subsequent cavities formation. This action is designed to work primarily against the tooth, the bacteria and the fermentable carbohydrates (Lingström et al., 2003; Selwitz et al., 2007). Consequently, frequent exposure to fluoride, optimal oral hygiene and a reduction in the substrate for bacterial fermentation, i.e. the intake of fermentable carbohydrates or replacing the sugar with different sweeteners, are all important (Selwitz et al., 2007). The basic methods for preventing dental caries are the same, regardless of tooth site, but different methods may be more suitable for certain areas. In this context, the application of fissure sealants is a method that is specifically recommended for caries prevention on the occlusal surface (Mejàre et al., 2003).

Different preventive strategies, such as population-based and high-risk strategies, have been used in economically developed countries (Seppä, 2001). Regardless of preventive action, it is important that the most suitable technique and/or product is used in relation to anatomic and biological variations and in relation to the level of risk of disease.

Fluoride in caries prevention

The decline in dental caries that has been seen in most western countries since the middle of last century can be primarily attributed to the introduction of fluoridated toothpaste (Arnold et al., 1962; DePaola, 1983; Mellberg, 1990; Bratthall et al., 1996; Marinho et al., 2003; Twetman et al., 2003). Daily toothbrushing with fluoridated toothpaste is currently regarded all over the world as the most important action for the prevention of dental caries. A number of reviews have recently been published in support of this consept (Clarkson et al., 1993; Lewis et al., 1995; Marinho et al., 2003). The recommendation given today is that toothbrushing with fluoridated toothpaste should be performed twice a day and should start at the time point when the

first primary tooth erupts. For caries prevention in individuals at increased risk, additional fluoride for home-care use or professional fluoride application may be used (Zimmer, 2001; SBU, 2002; Ellwood et al., 2008).

In recent years, our understanding of the cariostatic effect of fluoride (F) has increased and the use of fluoride has become the most important factor for caries prevention (Rölla and Ekstrand, 1996; Featherstone, 1999). The mechanism of fluoride results primarily in the inhibition of demineralisation of both enamel and dentine and the stimulation of remineralisation (ten Cate & Duijsters, 1983; ten Cate, 1990; ten Cate et al., 1998). Although NaF also has some antimicrobial effect, the anticaries effect is exerted by its topical action on tooth surfaces in the oral cavity (Ellwood et al., 2008). In addition to toothpaste, a wide range of products, such as mouthrinse solutions, gels, tablets, chewing gums, fluoridated toothpicks and dental floss, are currently suitable.

The most commonly used fluoride sources are sodium fluoride (NaF) and sodium monofluorophosphate (MPF). Both these compounds mainly act by reducing demineralisation and increasing remineralisation. Moreover, fluoride compounds to which an antimicrobial component, such as amine (Am) or stannous (Sn), has been added are also frequently used. Both amine fluoride (AmF) and stannous fluoride (SnF_2) have been found to possess antibacterial activity, in addition to the effect by the fluoride ion, when used in dental oral hygiene products (Shani et al., 1996). Amine fluoride compound has been used as an active ingredient in toothpaste for more than 30 years (Marthaler, 1968; Cahen et al., 1982). In an animal study, its caries-preventive effect has been considered to be comparable to that of NaF (Warrick et al., 1999). A fluoride uptake of clinically intact dental enamel have also been found *in vitro* by various fluoride solutions (Kirkegaard, 1977). A recent study has shown that the daily application of an AmF-containing dentifrice slurry had a remineralising effect on primary tooth carious lesions *in vitro* (Petersson & Kambara, 2004). Amine fluoride has also been found to be able to inhibit acid production by plaque bacteria (Capozzi et al., 1967), reduce enamel solubility (Mühlemann et al., 1957), prevent bacterial adhesion and affect the vitality of bacteria (Shern et al., 1970; Shani et al., 1996). However, the extent to which the organic amine and the fluoride contribute to the total antibacterial activity of the amine-fluoride molecule is not completely clear (Shani et al., 1996).

Both the uptake and retention of fluoride have been shown to be greater after treatment with amine fluoride than with various inorganic fluorides such as NaF (Mühlemann et al., 1968; Barbakow et al., 1983; Schmid et al., 1984). Mok et al. (1990) have shown that AmF produced significantly higher F uptake than NaF, especially in approximally located enamel. In explaining the differences found between treatment sites, it was postulated that AmF had a greater affinity for porous enamel than NaF and that some of the proximal sites may have had early preclinical carious lesions.

Administration of fluoride into the approximal area

A large number of techniques and products are used in order to deliver fluoride into the oral cavity. Even if the approximal area benefits with other tooth surfaces from general fluoride exposure, it has previously been found that the effect of fluoride-containing products, such as dentifrices and tablets, is less pronounced on the approximal surfaces (Granath et al., 1978; Li et al., 1994; Øgaard et al., 1994). The frequent application of fluoride varnish in this area has been found to reduce the prevalence of approximal caries (Moberg-Sköld et al., 2005). Some products specially designed to distribute fluoride to the interproximal site, such as toothpicks and dental floss, can currently be found on the market.

The effect of fluoridated toothpicks has previously been studied by Kashani (1998). Wooden toothpicks, made of both birch and lime, were found quickly to release fluoride into the approximal area. A maximum *in vivo* fluoride concentration of 9.2 mM was found after using a commercial toothpick impregnated with 4% NaF (Kashani et al., 1998b). In addition, an antimicrobial effect resulting in lower numbers of mutans streptococci after the frequent use of toothpicks impregnated with NaF, SnF_2 and chlorhexidine was found (Kashani et al., 1998c). Furthermore, an *in situ* reduction in the enamel and dentine demineralisation of approximal sites was found after four weeks' frequent use of fluoride-impregnated toothpicks (Kashani, 1998). The effect of other commercial toothpicks, such as products made of plastic, is unknown. Furthermore, no study has evaluated the fluoride release into the approximal area or the caries-prevention effect of a fluoridated dental floss.

A limited number of other techniques used for delivering fluoride into the interproximal area and the effect on different clinical parameters are described in the literature (Keene et al., 1977; Gisselsson et al., 1999). The method that is most frequently described is the application of a gel with a syringe at the entrance to an approximal space, after which the gel is moved into the interproximal space with dental floss. After three years of frequent professional flossing with 1% NaF or SnF_2 gel, a reduction in caries of around 30% and 39% respectively was found (Gisselsson et al., 1999). A reduction in the number of mutans streptococci when delivering 10% SnF_2 into the approximal area with dental floss has been observed (Keene et al., 1977).

Caries-preventive school programmes with fluoride varnish applied to the approximal surfaces with a 1.2-ml syringe have been used (Moberg Sköld et al., 2005). Another way to administer fluoride to the approximal area is to use a mouthrinse (Twetman et al., 2004).

Importance of oral hygiene

The daily oral hygiene procedure that is performed at home constitutes an important part of caries prevention. Optimal plaque control is one of the key elements in the prevention of disease and it is recommended that it should be carried out twice daily. It is also important to pay special attention to the cleaning of the interproximal area. Plaque accumulation is promoted by poor self-cleaning in this dental region. The interproximal area is a narrow zone that is difficult to reach with a toothbrush in order to obtain optimal plaque control. It has been shown that dental cleaning is less effective in the approximal areas (Axelsson, 1993). No toothbrushing technique has been found to be able to provide interdental cleanness (Kinane, 1998; Löe, 2000). Apart from brushing, it is therefore necessary to add other cleaning aids, such as toothpicks, dental floss or interdental brushes, in order to disrupt and remove interproximal plaque. A large number of commercial products for cleaning the interproximal area can currently be found on the market. Both toothpicks and dental floss have been found to be effective for mechanical plaque control (Bergenholtz et al., 1980; Waerhaug, 1981). The relative plaque-reducing effect of both toothbrushes and aids specifically designed for the approximal area has been compared and show varying results.

Of the products specifically designed for approximal cleaning, toothpicks have been used for the longest time (Kashani, 1998). A wide range of toothpick products, including both wooden and plastic toothpicks, is currently available on the market. The most commonly used wooden toothpicks are made of birch and lime.

Dental floss started to be recommended for interproximal tooth cleaning at the end of the 1960s (Drum, 1968). Dental floss is clearly recognised as an effective method for removing approximal plaque (Gjermo and Flörta, 1970; Spolsky et al., 1993; Anderson et al., 1995; Löe 2000). An automated flossing is now also available on the market (Hague et al., 2007). Flossing also has been found to reduce gingivitis (Hill et al., 1973). The daily use of dental floss once a day for six weeks resulted in a reduction in both plaque scores and gingivitis (Cronin and Dembling, 1996). No difference in plaque removal has been found between waxed and non-waxed floss (Lamberts et al., 1982; Wunderlich et al., 1982). The effect on the use of dental floss on caries is unclear. A recent systematic review evaluating the effect of professional flossing on interproximal caries risk was unable to demonstrate any reduction in caries risk (Hujoel et al., 2006). While no effect on the caries rate was found after supervised daily flossing for three years (Horowitz et al., 1980), a caries-prevention effect was found in a group of 10- to 11-year-old children using fluoride-free dental floss more than every second day for two years (Gisselsson et al., 1983). In a clinical trial, professional tooth cleaning over a 20-month period has demonstrated a 50% reduction in the incidence of primary proximal caries (Wright et al., 1979).

It was also found that the beneficial effect of flossing increased the longer flossing was continued.

Interdental brushes constitute a third group of aids for approximal cleaning. Little is known about their caries-prevention effect, although they are believed to have a similar effect to toothpicks and dental floss.

Oral hygiene behaviour

Nowadays, toothbrushing is performed once or twice a day by the majority of individuals in the industrialised countries (Saxer and Yankell, 1997). A cross-sectional study performed at 10-year intervals found that the frequency of toothbrushing had increased in Sweden over a 30-year period (Hugoson et al., 2005). Between 80-93% of Swedish individuals between 3-80 years of age brush their teeth twice a day or more. However, a number of individuals report that they only brush their teeth now and then or never; the highest figures were found for subjects aged < 30 years.

Fewer data are available regarding the use of aids for approximal cleaning in different age groups. The use of toothpicks varies according to different studies. In 2003, it was found that toothpicks were regularly used by < 15% of subjects aged 20-40 years in a Swedish population (Hugoson et al., 2005). This was a decrease compared with previous years. On the other hand, the number of users in the older age groups increased between 1973 and 2003 (Hugoson et al., 2005). The corresponding data for dental floss vary between 12-30% for individuals aged between 20-80 years, with the highest figure in the youngest age group (Hugoson et al., 2005). This study did not include any questions related to the use of other cleaning aids for the proximal region. Although oral hygiene is reported to be performed on a regular basis by many individuals, it is important to remember that a large variation in toothbrushing behaviour, including brushing technique and brushing time, has been reported (Bradnock et al., 2001; Christensen et al., 2003).

In oral health promotion, it is important both to increase the patients' knowledge of factors behind the disease and to develop individual oral health skills. In this respect, the promotion of self-care is important (Sheiham, 1992). Little is still known about the factors influencing this process in order to reduce the prevalence of approximal caries. This also includes knowledge about the factors that influence the choice of products and the exact use of the different cleaning aids. Information about products and recommendations for use are primarily given to patients by oral health professionals and both dental hygienists and dentists play an important role in this respect. Nowadays, information is also passed on by individuals outside the traditional dental arena, such as the mass media (Mårtensson, 2004). A large number of both clinical and behavioural factors are also closely linked to the self-care promotion. This applies not least to approximal cleaning and approximal fluoride administration.

Limited information is available on the impact of the different factors that influence the individual performance of daily oral hygiene.

Aims

The overall aim of the work in this thesis was to examine ways of optimising fluoride application in the approximal area using different fluoridated approximal cleaning aids in order to prevent approximal caries. In more detail, the aims were:

- to determine the fluoride release from toothpicks and dental floss *in vitro* (Paper I)
- to determine the fluoride release from toothpicks and dental floss and to evaluate various approximal administration methods *in vivo* (Papers I and II)
- to measure the approximal fluoride concentration using different fluoride-containing products either alone or in combination *in vivo* (Paper II)
- to evaluate the effect of different toothpicks and dental floss on demineralised enamel and dentine using an *in situ* model (Paper III)
- to evaluate the effect of different toothpicks and dental floss on plaque microflora (Paper III)
- to evaluate the recommendations given by dental hygienists and dentists as well as to evaluate the self-care practices of using approximal cleaning aids (Paper IV)
- to study the ability to remove dental plaque when using toothpicks, dental floss and an interdental brush (Paper IV)

Material and Methods

Study design

Paper I
The release of fluoride from toothpicks and dental floss was evaluated *in vitro* and *in vivo* in two test series (Series I and II) in an experimental cross-sectional study. The fluoride release when using an interdental brush dipped in fluoride was also evaluated.

Paper II
The release of fluoride when using different fluoride-containing products for approximal fluoride distribution was evaluated *in vivo* in two different test series (Series I and II) in an experimental cross-sectional study. The products were evaluated alone or when used in combination with either brushing or rinsing.

Paper III
The effect of different toothpicks and dental floss on demineralised enamel and dentine and on plaque microbial composition was evaluated using an *in situ* model with a double-blind, randomised, cross-over design. It consisted of four test periods, the first two comparing a birch toothpick with AmF and a birch toothpick with NaF and the other two comparing a dental floss with AmF + NaF and a dental floss without fluoride. Between these four periods, each lasting for four weeks, there was a control period without any approximal cleaning. The study was performed double blind with a cross- over design with respect to the two toothpicks and the two dental floss periods.

Paper IV
Factors determining recommendations by dentists and dental hygienists (Series I) and the use of approximal cleaning aids by subjects in different age groups (Series II) were evaluated in this cross-sectional study. Both categories answered a randomised questionnaire. A clinical evaluation of the use of toothpicks, dental floss and interdental brushes, together with a questionnaire, was also performed in a smaller sample of subjects.

Subjects

Paper I
A total of 12 adult volunteers, recruited from patients and personnel at the Institute of Odontology, participated. In Series I, six healthy subjects (5 women and 1 man) aged 42 ± 11 years (mean ± SD) were included. They had a DMFT of 10 ± 8. For Series II, six other healthy subjects (4 women and 2 men) aged 45 ± 9 years with a DMFT of 14 ± 5 were recruited. They all had a normal

stimulated salivary secretion rate (2.1 ± 0.8 mL/min in Series I and 2.1 ± 1.0 mL/min in Series II). No glass ionomer fillings were found in the approximal region where the treatments and samplings were carried out. All the subjects were instructed not to use any F-containing oral hygiene products and to reduce their intake of F-containing drinks and food products to a minimum 48 h prior to each test occasion. Fluoride-free toothpaste was given to all participants to be used during the 48-hour period. They were not allowed to eat/drink, use tobacco or snuff or brush their teeth one hour before each test session. For each individual, the tests were carried out at the same time of the day at the Department of Cariology. They made a total of 10 visits to the laboratory.

Paper II

Ten healthy volunteers (7 women and 3 men) aged 47 ± 13, recruited from patients and personnel at the Institute of Odontology, participated in the two series (I and II). The following inclusion criteria were used: 1) fully dentate region 16-26 and region 46-36, 2) stimulated salivary secretion rate > 1.0 ml/min and 3) no glass ionomer fillings in the approximal region where the treatment was carried out. The salivary secretion rate for the included subjects was 1.5 ± 0.4 mL/min (mean ± SD). Home-care procedures were performed as described for Study I.

Paper III

Fifteen adult subjects with full dentures, nine men and six women, with a mean age of 64 ± 11 years, were included in the study. They were all patients at the Public Dental Clinic in Mölnlycke, Sweden. The subjects visited the clinic six times during the total experimental period of 20 weeks.

Paper IV

In Series I, a questionnaire was sent to a total of 500 dentists and 500 dental hygienists. The dentists were randomly selected from a register belonging to the Swedish Association of Public Dental Officers and the Swedish Association of Private Dental Practitioners and the dental hygienists from lists belonging to the Swedish Dental Hygienist Society. In Series II, a total of 1,000 individuals in the following age intervals were included: 15-20 years, 21-40 years, 41-60 years and >60 years. For each age group, 250 persons were identified, of whom half were men and half were women. All the individuals were randomly selected from a population register belonging to the local area of Västra Götaland in the south west of Sweden.

In Series III, 60 individuals with a mean age of 54.8 yrs (range 20-81 yrs), divided into three different groups of 20 subjects, participated. They were all recruited from Göteborg, Sweden, and from the Institute of Odontology according to their regular use of dental floss, toothpicks or interdental brushes. The inclusion criteria were that, during the past five years, they should have

received information and instructions regarding the use of the individual approximal aids. They each made one visit to the laboratory.

Test products and treatments

Paper I

Twelve different types of toothpick and 14 different types of dental floss were evaluated *in vitro* (Table 1). At the time of the study, they were all available on the Swedish market or in the form of prototypes of new products. From 10 packages of each brand, one toothpick or 20 cm of dental floss was used.

For *in vivo* Series I, two toothpicks and four types of dental floss were tested (Table 1). In Series II, the following seven products and application methods were compared for the administration of fluoride to the approximal area: 1) a single toothpick (TePe Björk), 2) multiple toothpicks (TePe Björk), 3) a single dental floss (J&J Dentotape), 4) multiple types of dental floss (J&J Dentotape), 5) an inderdental brush + 0.2% NaF solution, 6) an interdental brush + 0.2% NaF gel, 7) a mouthrinse with 0.2% NaF (control) (Table 1).

In Series I, the treatment was performed at two approximal sites, i.e. 45/46 and 25/26. The toothpick was used for one minute at each site and in a similar manner, while a 20-cm-long piece of dental floss was used. In Series II, the treatment for methods 1-6 was performed in all the approximal areas between teeth 16-26 (a total of 11 sites). For methods 1 and 3, one toothpick and one piece of dental floss respectively were used for all sites. For methods 2 and 4, a new toothpick and piece of dental floss respectively were used for each site. For methods 5 and 6, the inderdental brush was dipped into the F solution or F gel prior to the treatment of each site (Fig. 1). For method 7, a mouthrinse was carried out with 10 ml solution for one minute. In Series II, each site was treated for 10 sec.

Paper II

In Series I, the following products and combinations of products were tested: 1) "brushing"; i.e. toothbrushing with fluoridated toothpaste (Pepsodent Xylitol, 0.15% NaF; Lever Fabergé, Stockholm, Sweden), 2) "rinsing"; i.e. a mouthrinse with 0.2% NaF solution (Dentan; Ipex Medical AB, Sweden), 3) "toothpick"; i.e. a NaF-containing toothpick (TePe Björk; TePe munhygienprodukter AB, Sweden), 4) "dental floss"; i.e. a NaF-containing dental floss (Johnson & Johnson Dentotape; Johnson & Johnson AB, Sweden), 5) "brushing + toothpick", 6) "brushing + dental floss", 7) "toothpick + brushing", 8) "dental floss + brushing", 9) "rinsing + toothpick", 10) "rinsing + dental floss", 11) "toothpick + rinsing" and 12) "dental floss + rinsing". The toothpick and dental floss were therefore used for tests 5-12 either before or after brushing and rinsing.

In Series II, the following products or combinations of products were tested:

Fig. 1. The use of an interdental brush first dipped in NaF gel and then inserted into region 15/14.

1) "0.2% gel"; i.e. interdental brush (IDB) and 0.2% NaF gel (Apoteksbolaget, Sweden; IDB/0.2% gel), 2) "0.32% gel"; i.e. IDB and 0.32% NaF gel (Apoteksbolaget, Sweden; IDB/0.32% gel), 3) "brushing"; toothbrushing with fluoridated toothpaste (same as No 1 in Series I), 4) "brushing + 0.2% gel" and 5) "brushing + 0.32% gel". Within each trial, the treatments were distributed in randomised order with at least one week between each test.

All treatments, except for brushing and rinsing, were carried out in a standardised manner by one of the authors (BS). The toothpick and dental floss were used for 10 sec in two approximal areas in the upper jaw (16/15 and 12/11). A new toothpick and piece of dental floss (20 cm long) was used for each site. For the IDB (interdental brush), a 0.5-mm brush was dipped in 0.2% NaF gel or 0.32% NaF gel and used for the treatment of each site (16/15 and 12/11) for 10 sec ("Inter Dental Brush Gel Method"; Fig. 1). Toothbrushing was performed with 1 g of toothpaste for two minutes, followed by post-brushing rinsing with 2 x 10 ml of water. The mouthrinse was performed with

10 ml of solution for one minute. When the combination of products was tested, the two techniques were used directly after each other.

Paper III
The following four products were tested: 1) a birch toothpick with AmF (called "AmF toothpick"), 2) a birch toothpick with NaF (called "NaF toothpick"), 3) a dental floss with a mixture of NaF and AmF (called "NaF/AmF floss") and 4) a dental floss without fluoride (called "F-free floss"). Products 1, 3 and 4 were manufactured by GABA, Münchenstein, Switzerland, and product 2 was manufactured by Te-Pe, Munhygienprodukter, Malmö, Sweden.

The two toothpick periods were carried out first (called periods 1 and 2), followed by a four-week period without any approximal cleaning (called control period or period 3). Finally, the two dental floss periods were performed (called periods 4 and 5). The participants were carefully instructed and trained in how to use the toothpicks and dental floss by one of the investigators (BS) before the study started. The cleaning procedure was always carried out with the dentures in place in the mouth. A fresh toothpick or a 20-cm-long piece of dental floss was used for half a minute for each sample holder. The patients were told to pay attention to both the mesially and distally oriented specimen in each sample holder. The approximal cleaning was carried out three times a day (after breakfast, after lunch and before bed time), except during the four-week control period. An F-free toothpaste (BlåVitt, Konsum, Stockholm, Sweden), a soft toothbrush (Te-Pe Mjuk) and tap water were used for cleaning the dentures (except the area where the sample holders were located) twice a day in the hand-basin; this was carried out in the morning and evening shortly before using the toothpicks and dental floss. Otherwise, the dentures were worn day and night, including at meals.

Fluoride analysis

For the *in vitro* fluoride analyses in Paper I, each toothpick and piece of dental floss was immersed in a Petri dish containing 10 mL of water and 1 mL of TISAB. The dish was gently stirred at intervals. Samples were collected after 30 min and 24 h and the fluoride concentration in the solutions was determined.

For the *in vivo* evaluation of the amount of fluoride in approximal saliva in Papers I-II, the method originally described by Kashani et al. (1998a) was used. Approximal saliva was sampled before treatment (0 min) and at different time points up to 60 min. For the collection of approximal fluid, a standardized triangular-shaped paper point (1.5 x 5 mm), cut from Munktell filter paper no. 1600 (Grycksbo Pappersbruk, Sweden), was inserted into each approximal area with a pair of forceps. The paper point, which sucks up approximately 4 μL, was kept in place for 20 sec until it was soaked in saliva from the approximal area

Table 1. Test products used for the studies *in vitro* (Paper I), *in vivo* (Papers I and II) and *in situ* (Paper III).

Product/ Brand name	Manufacturer, country	Fluoride compound	*In vitro*	*In vivo*	*In situ*
Toothpicks					
Flossbrush	Dentac AB, Sweden	NaF	I		
Jordan Dubbel	Jordan, Norway	NaF	I		
Sanodent	Cederroth, Sweden	NaF	I		
Jordan Enkel	Jordan, Norway	NaF	I		
Proxident Plaststicka	Athena Nordic AB, Sweden	NaF	I		
Blåvitt Fluor	Konsum, Sweden	NaF	I		
TePe Björk	TePe munhygienprodukter AB, Sweden	NaF	I	I, II	III
TePe Lind	TePe munhygienprodukter AB, Sweden	NaF	I		
Butler	Butler, USA	NaF	I		
Prototype A	GABA, Switzerland	AmF	I	I	III
TePe Björk Smal	TePe munhygienprodukter AB, Sweden	NaF	I		
Proxident Trätandsticka	Athena Nordic, Sweden	NaF	I		
Dental floss					
Blåvitt Fluor	Konsum, Sweden	NaF	I		
Jordan Fresh	Jordan, Norway	NaF	I		
Jordan Easy Slide	Jordan, Norway	NaF	I		
Colgate Total	Colgate, USA	NaF	I		
Jordan Active Care	Jordan, Norway	NaF	I		
elmex dental floss (old version)	GABA International AG, Switzerland	AmF	I	I	
Johnson & Johnson Dentotape	Johnson & Johnson AB, Sweden	NaF	I	I, II	
Dentosal	Dentosal, Sweden	NaF	I		
Sanodent	Cederroth, Sweden	NaF	I		
elmex dental floss unwaxed	GABA International AG, Switzerland	AmF + NaF	I		
Proxident Elastisk Tandtråd	Athena Nordic AB, Sweden	NaF	I		
elmex dental floss waxed	GABA International AG, Switzerland	AmF + NaF	I		
Prototype B	GABA International AG, Switzerland	AmF + NaF	I	I	
Prototype C	GABA International AG, Switzerland	AmF + NaF	I	I	III
Fluoride-free dental floss	GABA International AG, Switzerland	-			III
Fluoride gel					
0.2%	Apoteksbolaget, Sweden	NaF		I, II	
0.32%	Apoteksbolaget, Sweden	NaF		II	
Other products					
Dentan 0.2%	Ipex Medical AB, Sweden	NaF		I, II	
Pepsodent Xylitol, 0.15%	Lever Fabergé, Stockholm, Sweden	NaF		II	
Interdental brush 0.5 mm	TePe munhygienprodukter AB, Sweden	-		I, II	

(the capacity of 20 paper points was tested and resulted in a mean ± SD of 4.0 ± 0.5 μL). It was transferred to a 0.5-mL Eppendorf tube (covered with a lid) containing 200 μL of de-ionised water + 20 μL of TISAB III (Orion Research, Boston, Mass., USA). All the samples were kept frozen until analysed.

All the *in vitro* samples (Paper I) were analysed fresh. For the *in vivo* samples (Papers I-II), the test tubes were first thawed, after which they were kept in a refrigerator for 24 h in order to allow the absorbed fluoride to diffuse from the paper point into the solution. The samples were then mixed by vibration for 10 s. One hundred μL was transferred to a Petri dish, after which the fluoride concentration was determined. For all *in vitro* and *in vivo* samples, an ion-specific electrode (Orion 96-09, Boston, Mass., USA) was used. The detection for all samples was 0.01 ppm (≈ 0.00053 mM F).

Transversal microradiography

The enamel and dentine specimens mounted into the prosthesis in Paper III were prepared from freshly extracted, sound human premolars and molars and mounted in sample holders. Both the enamel and dentine samples were demineralised for 10 days in a 6% CMC gel containing 0.1 M lactic acid (titrated to pH 5 with 10 M KOH), resulting in subsurface lesions of around 100 μm. A total of 216 demineralised specimens were used to provide starting values for the initial lesion depth and mineral loss. In a small, rectangular sample holder (Evergreen scale models, Kirkland, Wash., USA), measuring 9.5 x 6.3 x 5.0 mm (length x height x depth), one demineralised enamel specimen and one demineralised dentine specimen were embedded in acrylic (Candulor Autoplast, Wangen, Switzerland). They were inserted in the holder to form a triangular, approximal-like space (Fig. 2). Two sample holders were mounted in the premolar-molar region of the denture in either the upper or lower jaw (depending on available space in the denture), one on the left and one on the right side. They were mounted in such a way that a toothpick or a piece of dental floss could be used for approximal cleaning. Due to lack of space, only one holder could be mounted in six of the 15 patients.

After each four-week-period, the sample holders were taken out and replaced with new holders for the next four-week period. The specimens were kept in a plastic jar, containing wet cotton rolls, and sent to Department of Dentistry and Oral Hygiene, University of Groningen, the Netherlands, where they were analysed by transverse microradiography (TMR).

At the laboratory in Groningen, the enamel and dentine specimens were first cleaned with a multitufted toothbrush under running tap water for half a

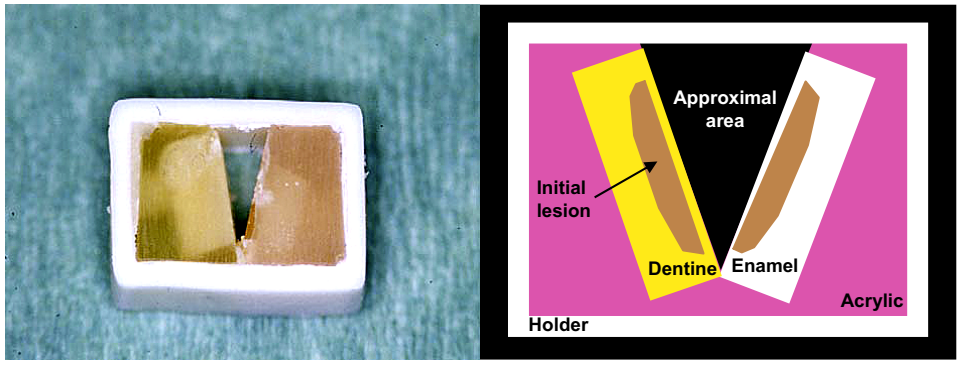

Fig. 2. Clinical and schematic figure showing the sample holder, with the artificially formed approximal area in which the treatment took place, inserted in the prosthesis.

minute to remove plaque. Subsequently, two sections were cut from the central part of each specimen (~150 µm thick for enamel and ~350 µm for dentine). These sections were ground down together with the control samples to around 80 µm for enamel and 140 µm for dentine and then microradiographed (Dijkman et al., 1986; Øgaard et al., 1986; Arends and ten Bosch, 1992). Densitometric scanning of the microradiographs was carried out using computer-assisted video densitometry (CAV) (Inaba et al., 1997). Three scans (400 x 300 µm) were made of each enamel and dentine microradiograph. The average of these three measurements was used for further analysis. For each sample, two parameters were assessed: 1) lesion depth (Ld, in µm) and 2) mineral loss (ΔZ, in vol% x µm).

Microbiological analyses

After each test period in Paper III, plaque samples from the experimental approximal sites were collected using a sterile, triangular-shaped, fluoride-free birch toothpick. The tip of the toothpick with plaque was cut off and transferred to a bottle containing pre-reduced transport medium (Syed and Loeche, 1972) and glass beads. The plaque samples were sonically dispersed for 10 sec and serially diluted in 0.05 M phosphate buffer with 0.4% KCl (pH 7.1). They were then plated on four different solid media for the growth of the following bacteria: 1) blood agar for total count, 2) CFAT agar (Zylber and Jordan, 1982) for actinomyces, 3) mitis salivarius bacitracin agar (MSB) (Gold et al., 1973) for mutans streptococci and 4) Rogosa SL agar (Difco Laboratories, Detroit, MI, USA) for lactobacilli. The blood and CFAT agar plates were incubated in a gas

mixture of 95% N_2 and 5% H_2 at 37ºC for two days. The MSB agar plates were incubated in a candle jar at 37ºC for two days. The SL agar plates were grown in air at 37ºC for three days. Colonies with morphology characteristic of mutans streptococci and actinomyces were counted on MSB agar and CFAT agar respectively. On blood agar and SL agar, all colonies were counted.

Questionnaires

An anonymous self-administered questionnaire was sent to the dentists and dental hygienists, together with a coded envelope to be used to return the document (Paper IV - Series I). If no answer was received within two weeks after the first questionnaire was distributed, a reminder was sent. The structured questionnaire consisted of 23 semi-closed questions focusing on recommendations relating to approximal cleaning aids and the criteria for recommendations in relation to different age groups. The dental personnel were also asked to use a 10 cm line (VAS scale) to mark the effect from "no effect" to "large effect" of dental floss and toothpicks in order to prevent dental caries and gingivitis/periodontitis. A number of socio-demographic variables and information about the clinic were also included in the questionnaire.

In an identical manner to that described above, a self-administered anonymous questionnaire was mailed to the subjects (Paper IV - Series II). The questionnaire comprised a total of 21 semi-closed questions focusing on general oral health care and approximal oral hygiene habits and the recommendations that had been given in relation to oral hygiene. A number of socio-demographic variables were also included, as well as information about visits to dentists and dental hygienists.

Plaque reduction

The subjects in Paper IV - Series III were asked to refrain from approximal cleaning for 24 hours before their visit to the clinic. Firstly, the visible plaque index (VPI) was registered for all approximal surfaces in the upper and lower jaw (teeth 17-27, 47-37). The plaque index was assessed according to a dichotomous system with plaque/no plaque after staining the plaque with Diaplack® (Wallco AB, Kista, Sweden). Assessments were carried out before and after using the individual approximal cleaning aid, after which the mean plaque reduction for each individual was calculated (%). The patients were observed and their skills were assessed using the following three variables: 1) motor function, 2) technique and 3) overall capability. The skill for each variable was graded in one of the following five categories: i) "very good", ii) "good", iii) "acceptable", iv) "poor" and v) "very poor". After the clinical evaluation, the

subjects were asked to complete a self-administered questionnaire with 11 closed questions related to their use of the individual approximal cleaning aids.

Ethical considerations

The four studies were approved by the Ethics Committee at the University of Gothenburg (S 415-00, S 633-01, Dnr 444-05 and Dnr 482-06). Both verbal and written information about the individual study was given to the subjects. Written informed consent was obtained from all subjects prior to the start of each study. All the studies were conducted in accordance with the Helsinki Declaration. All the subjects were coded when entering the individual studies and the statistical analyses were performed with unidentifiable data.

Statistical methods

All the analyses were performed using Statview (Paper I-III) or SPSS 14.0 (Statistical Package of Social Sciences) (Paper IV). The mean ± SD for each product analysed *in vitro* was expressed both as ppm and as mM fluoride (Paper I). Only mM fluoride was used for the *in vivo* results (Papers I and II). The mean values for the two treated and untreated sites for each individual were calculated, after which the mean ± SD for all test subjects was calculated (Paper I). For the four products tested in duplicate, the mean of the two test series was calculated. For *in vivo* Series II, the mean ± SD of the fluoride concentration of each site and method was calculated. The *in vivo* values were also transformed to logarithmic values. The area under the curve ($AUC_{0\text{-}60\ min}$) was calculated for each individual curve (on a non-logarithmic scale), after which the corresponding mean ± SD was calculated for the treated and untreated sites for the six methods (Series I) and for the four individual sites and the mean of the four sites for the seven methods (Series II). Two-way analysis of variance (ANOVA) was used to test the significance of differences between the treatments. When the ANOVA rejected the multisample hypothesis of equal means, multiple comparison testing was performed with Fisher's PLSD. When comparing the duplicate tests (Series I), correlation coefficients (r) were calculated. In Paper II, no significant differences were found between the two test sites and the mean values for the two sites were therefore used for all further calculations. The outcome variables were F concentrations at two and 60 min and the area under the curve ($AUC_{0\text{-}60\ min}$). The AUC was calculated for each individual and each treatment. Statistical comparisons were made using two-way analysis of variance (ANOVA). When the ANOVA rejected the multisample hypothesis of equal means, multiple comparison testing was performed with Fisher's PLSD.

For Paper III, the mean values for the specimens on the left and right side of the mouth (for the subjects in whom two samples were inserted) were calculated for each individual and each treatment. For the microbiological data, all the values were transformed to logarithmic values. Student's t-test was used to compare the group results for the TMR data with the initial (starting) values. A linear mixed statistical model was used to compare the effects of the two toothpicks and the two types of floss, taking account of possible differences between periods. The model included a random patient effect and fixed effects due to treatment and period. This analysis was carried out separately for each of eight variables (Ld and ΔZ for dentine and enamel and CFU for the four microbiological counts). The presented p-values were not adjusted for multiple testing. To account for multiple tests (separately for the four TMR variables and the four microbiological variables), the results were considered significant at the 5% level when the adjusted p-values did not exceed 1%. The effect of the two toothpicks compared with the control period on the one hand and the two types of floss compared with the control period on the other hand was also analysed using the same statistical method.

For Paper IV, both a descriptive and an analytical approach were used for the data analysis. Bivariate analyses were performed using the chi-square test for the statistical evaluation of proportions. The correlation of toothbrushing and the use of approximal aids was analysed using Pearson's coefficient. For all data, $p < 0.05$ was considered statistically significant.

Results

Fluoride release *in vitro*

A wide range of fluoride concentrations was found *in vitro* among the twelve toothpicks and types of dental floss (Paper I). For all products, the majority of the fluoride was released at 30 min. The fluoride concentration for toothpicks at 30 min ranged between 3.1-37.8 ppm; corresponding to 0.7-2.0 mM (Fig. 3). The highest fluoride values were found for the Proxident trätandsticka and TePe Björk smal, while the Flossbrush and Jordan Dubbel produced the lowest.

For most products, dental floss resulted in lower fluoride values compared with toothpicks (Fig. 3). For three of the dental floss types, little or no release was found after 24 h, with values below ≤0.1 ppm fluoride (corresponding to ≤0.003 mM fluoride). Of the five products producing the highest values, four contained a mixture of AmF and NaF. The two prototypes, B and C, resulted in the highest overall release, which was higher than any of the toothpicks. However, a large standard deviation was found for Prototype C.

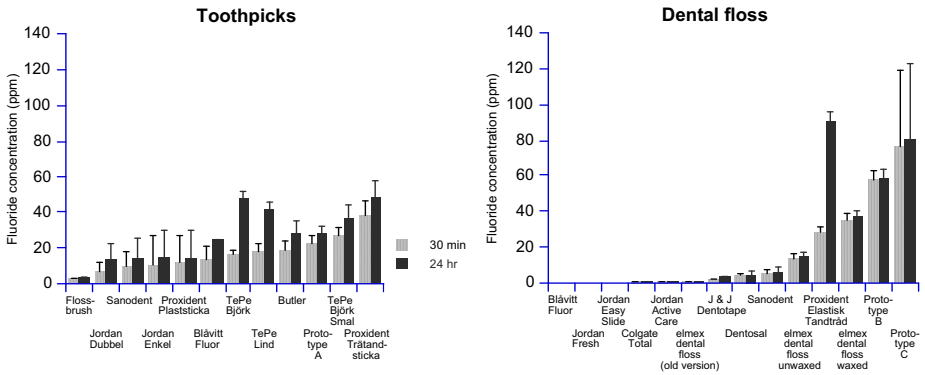

Fig. 3. Fluoride concentration (ppm F) *in vitro* after release in water at 30 min and 24 hr for the altogether 26 toothpicks and dental flosses in Paper I. The data represent the mean ± SD for 10 packages for each product.

Fluoride release *in vivo*

Toothpicks and dental floss

No difference was found when comparing the values from the upper and lower jaw (Paper I – Series I). The peak fluoride concentration was found at two minutes and the values were still above baseline after 60 min, especially at the treated sites. For the toothpicks, the highest values at the treates sites were found after treatment with the TePe Björk (Fig. 4a). Dental floss also resulted in peak values at two minutes and Prototype C produced the highest values (Fig. 4b). In both Series I and Series II, the fluoride concentration at 2 min and AUC was several times higher at the treated sites than at the untreated ones (Table 2). The two toothpicks resulted in higher values when compared with commercial floss. A high correlation for the $AUC_{0\text{-}60\ min}$ was found for the duplicate samples. Similar findings were made for the toothpick and dental floss in Paper II (Fig. 7).

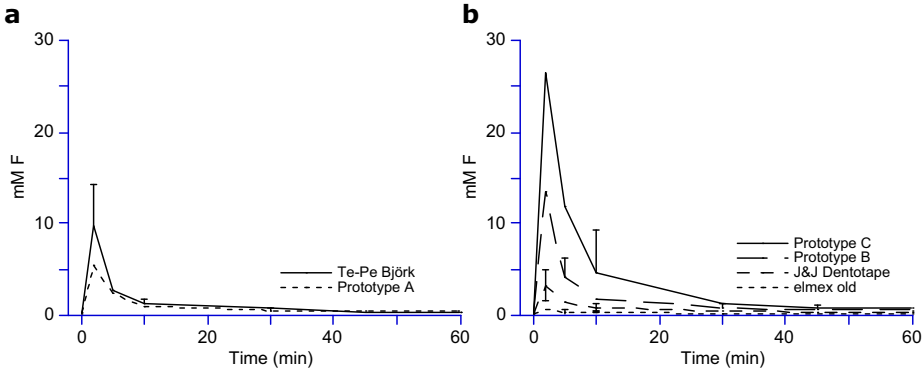

Fig. 4. Fluoride concentration (mM) at the approximal sites after using toothpicks (a) and dental floss (b) in Paper I. Mean values for six and 10 individuals respectively.

Table 2. Fluoride concentration in the approximal area *in vivo* expressed as $AUC_{0\text{-}60\ min}$ (mM F x min) at the treated and non-treated sites after using toothpicks and dental floss in Paper I. Mean values for six individuals.

Product	$AUC_{0\text{-}60\ min}$ (mM F x min)	
	Treated sites	Non-treated sites
Toothpicks		
Te-Pe Björck	66.3 ± 27.8	35.7 ± 17.0
Prototype A	51.8 ± 22.5	46.8 ± 39.0
Dental floss		
elmex old	13.4 ± 2.7	10.4 ± 2.3
J & J Dentotape	39.3 ± 10.8	23.3 ± 2.3
Prototype B	91.3 ± 68.4	46.9 ± 25.7
Prototype C	104.1 ± 50.4	48.1 ± 13.0

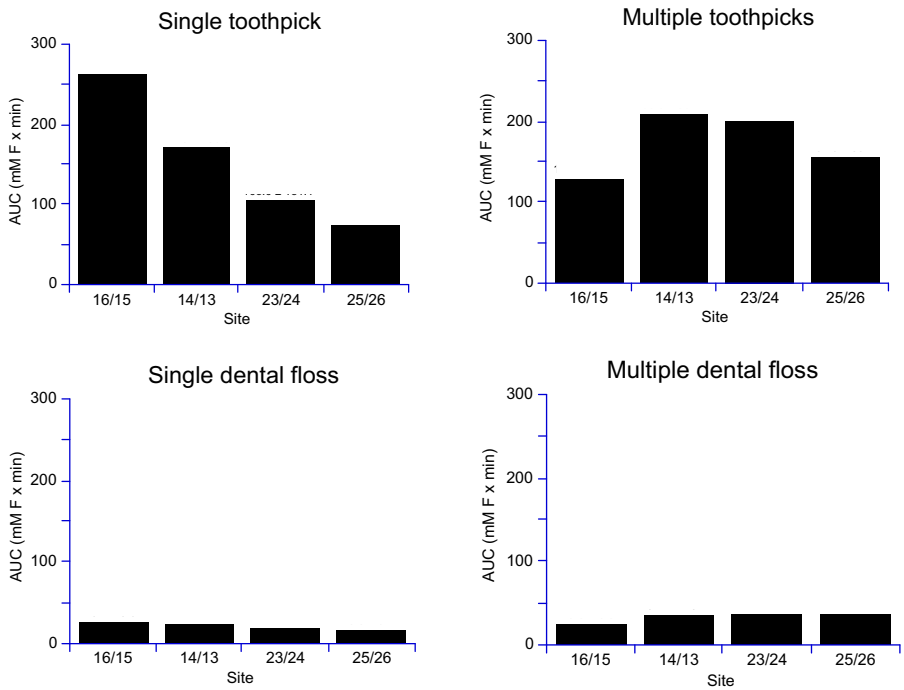

Fig. 5. Fluoride concentration at four approximal sites *in vivo* expressed as $AUC_{0\text{-}60\ min}$ (mM F x min) after using a single and multiple toothpicks and a single piece or multiple pieces of dental floss in Paper I. Mean values for six individuals.

The use of a single toothpick and a single piece of dental floss resulted in a stepwise reduction in fluoride concentration when used from site 16/15 to 25/26 (Fig. 5). The values were more consistent when multiple products were used (Fig. 5). The same thing applied to the use of an interdental brush dipped in 0.2% NaF, an interdental brush dipped in 0.2% NaF gel and mouthrinse with 0.2% NaF solution (data not shown).

Inter dental gel brush method

As shown in Figure 6, the interdental brush dipped in 0.2% NaF gel resulted in the highest approximal fluoride concentration, followed by the 0.2% NaF mouthrinse and both ways of using toothpicks (Paper I – Series II). For site 16/15, treatment with the interdental brush dipped in 0.2% NaF gel, the mouthrinse with 0.2% NaF and toothpick resulted in significantly higher values when compared with dental floss ($p<0.05$ or $p<0.01$).

The highest overall F concentration, both when evaluated as actual F values and AUC, was found after using an interdental brush dipped in 0.32% NaF gel (Fig. 7a). This value was significantly increased when compared with 0.2% NaF gel ($p<0.05$) (Paper II – Series II). Both gels also differed significantly resulting in higher fluoride values when compared with brushing (Fig. 7a and Fig. 8).

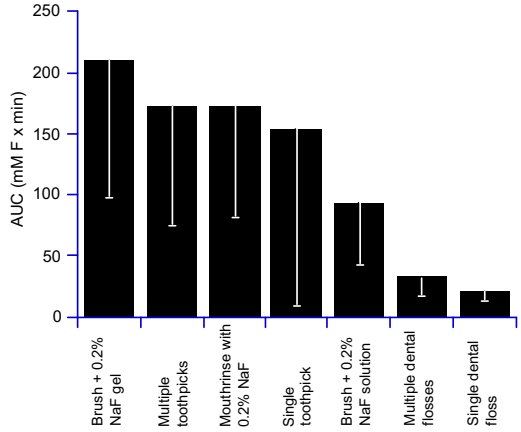

Fig. 6. Fluoride concentration *in vivo* expressed as $AUC_{0\text{-}60\ min}$ (mM F x min) for the different methods tested in Paper I. Mean values ± SD for six individuals and four sites per individual.

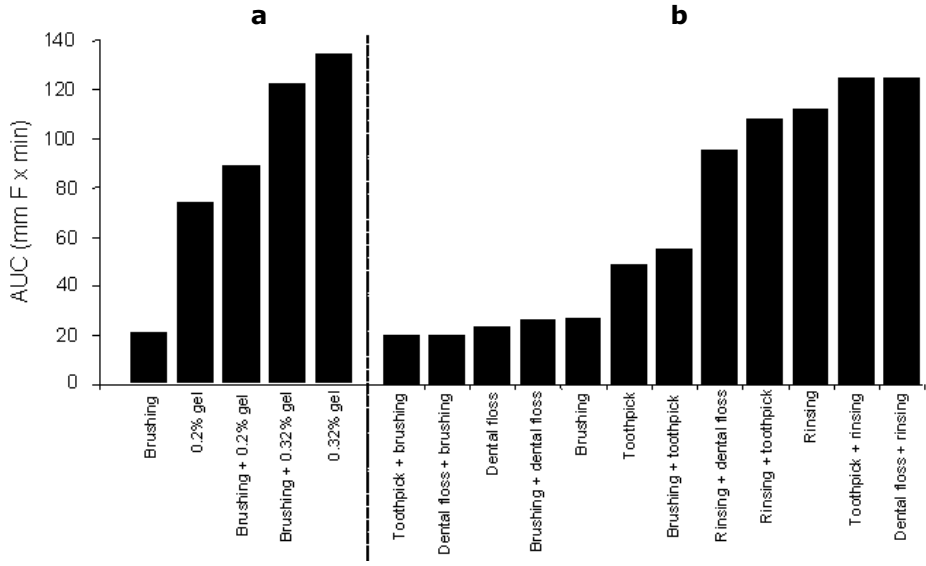

Fig. 7. Fluoride concentration *in vivo* expressed as $AUC_{0\text{-}60\ min}$ (mM F x min) after using the five (a) and 12 methods (b) respectively in Paper II. Mean values for 10 individuals and two sites per individual.

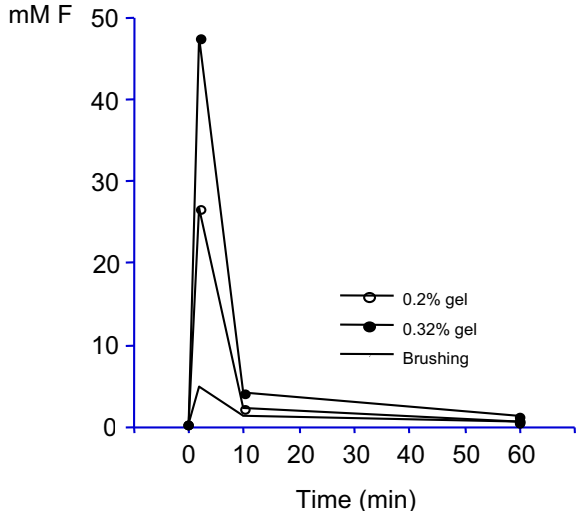

Fig. 8. Fluoride concentration in the approximal area after using 0.2% NaF gel, 0.32% NaF gel and brushing in Paper II. Mean values for 10 individuals and two sites per individual.

Combination of products

The highest F concentration at two minutes was found for the combination of toothpick + rinsing, followed by dental floss + rinsing and rinsing alone in Paper II (Fig. 9). When presented as the AUC, rinsing alone and all four combinations with rinsing resulted in higher values than brushing or any combination with brushing ($p<0.05$, $p<0.01$ or $p<0.001$) (Fig. 7b). Using a toothpick or dental floss before the mouthrinse resulted in the highest AUC values (Paper II). When brushing was combined with either a toothpick or flossing, the most pronounced fluoride values were seen when the approximal aids were used after toothbrushing (Fig. 7b).

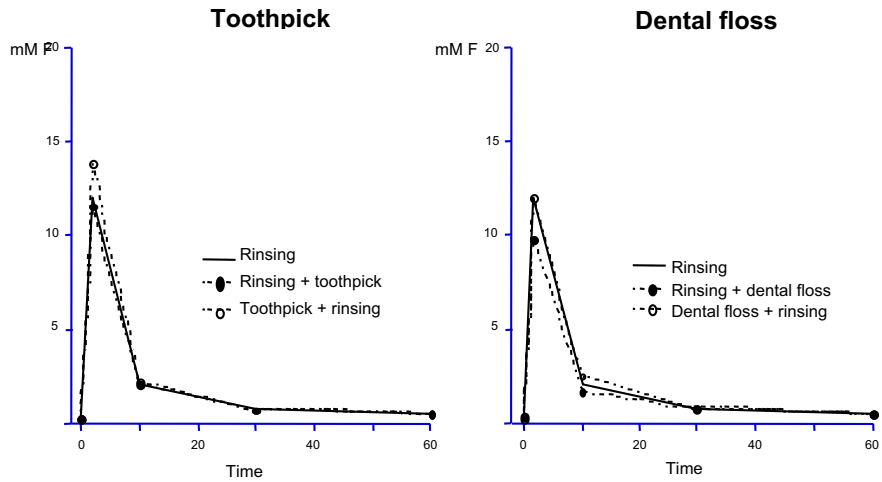

Fig. 9. Fluoride concentration in the approximal area after using toothpicks and dental floss before and after rinsing in Paper II. Mean values for 10 individuals and two sites per individual.

Mineralisation *in situ*

The mean (± SD) initial demineralisation values in enamel were 105.5 ± 10.7 µm for Ld and 5,588 ± 938 vol% x µm for ΔZ (Paper III). In dentine, the corresponding values were 106.8 ± 17.5 µm and 3,558 ± 657 vol% x µm respectively. Both the NaF and AmF toothpick showed mineral gain when compared with the initial demineralisation values (Table 3). There was a lesion

depth reduction for enamel, but a lesion depth increase for dentine ($p<0.01$). The control period resulted in an increase in both mineral loss and lesion depth for dentine ($p<0.01$) but no significant changes for the enamel samples. The two types of dental floss also resulted in remineralisation compared with the initial demineralised samples in terms of both lesion depth and mineral loss ($p<0.01$).

When comparing the two toothpicks, the NaF toothpick reduced the lesion depth in enamel more than the AmF toothpick ($p=0.004$; Table 3). The mineral loss difference in enamel was of borderline significance ($p=0.011$). The results for dentine showed the same trend, but there were no significant differences. In the toothpick analysis, a significant effect ($p=0.001$) for the period was found; period 2 resulted in higher lesion depth (enamel and dentine) and mineral loss (dentine) values. When comparing the two types of dental floss, the floss containing NaF + AmF displayed a trend towards less lesion depth and mineral loss for enamel than the fluoride-free floss. The difference was statistically significant for lesion depth in dentine (mean difference 31 μm; $p=0.007$). No "period effect" was found for the floss (Paper III).

Table 3. Lesion depth (μm), mineral loss (vol% x μm) for the enamel and dentine for the five test runs and the initial demineralisation in Paper III. The data represent mean values ± SD for 15 individuals and two specimens per individual.

Variable / Test run	Lesion depth (μm)	Mineral loss (vol% x μm)
Enamel		
NaF toothpick	50.4 ± 5.6	1955 ± 382
AmF toothpick	81.3 ± 5.7	3362 ± 451
F-free floss	74.7 ± 21.3	3296 ± 1003
NaF/AmF floss	74.0 ± 26.5	2745 ± 1313
Control	109 ± 13.7	5320 ± 781
Initial demineralisation	105.5 ± 10.7	5588 ± 938
Dentine		
NaF toothpick	125.6 ± 38.8	2537 ± 1119
AmF toothpick	120.5 ± 34.0	2718 ± 805
F-free floss	76.5 ± 41.6	1599 ± 1034
NaF/AmF floss	45.8 ± 29.9	1128 ± 672
Control	192.8 ± 15.2	5785 ± 490
Initial demineralisation	106.8 ± 17.5	3558 ± 657

Plaque microflora *in situ*

The analysis of oral microflora samples from the experimental approximal sites revealed a general trend for all micro-organisms (total count, actinomyces, mutans streptococci and lactobacilli), indicating that the control period resulted in the highest counts for all types of micro-organism, while the AmF toothpick period resulted in the lowest counts (Table 4). Lower CFU values were found after the test period using the AmF toothpick compared with the NaF toothpick. For most bacterial variables, only minor differences were found between the two types of dental floss. However, the smallest number of bacteria were found for the NaF/AmF floss, with a significant difference for mutans streptococci ($p=0.007$) (Paper III).

Table 4. Plaque microflora (total count, actinomyces, mutans streptococci and lactobacilli) for the five test runs in Paper III. The data represent mean values ± SD for 15 individuals and two specimens per individual.

Variable / Test run	Total count (Log CFU)	Actinomyces (Log CFU)	Mutans streptococci (Log CFU)	Lactobacilli (Log CFU)
NaF toothpick	7.7 ± 0.3	6.9 ± 0.5	3.7 ± 2.1	4.6 ± 1.2
AmF toothpick	7.0 ± 1.0	6.0 ± 1.4	3.0 ± 1.8	3.7 ± 1.2
F-free floss	7.7 ± 0.4	6.8 ± 0.8	4.4 ± 2.0	4.2 ± 1.5
NaF/AmF floss	7.5 ± 0.7	6.6 ± 1.1	3.7 ± 1.6	4.6 ± 1.3
Control	8.0 ± 0.4	6.9 ± 0.6	4.9 ± 1.4	5.0 ± 1.8

Questionnaire data dental personnel – patients

Questionnaire to dental personnel

Of the questionnaires sent to dental hygienists and dentists, 412 and 398 respectively were returned. Of these, four and three respectively had to be excluded and the final number that was analysed was 408 (82%) and 395 (79%) respectively. The mean age was 43.4 ± 11.0 for dental hygienists and 48.4 ± 10.5 for dentists. About two thirds of the dental hygienists worked in the public dental health service, while about half the dentists worked in the public dental health service and the private sector respectively. Of the dental hygienists, 55%

had >10 years' work experience, while the corresponding figure for the dentists was 80%.

Dental hygienists more frequently gave specific recommendations about the use of approximal cleaning aids compared with dentists. This difference between the two professions was statistically significant for the choice of product, how often it should be used, how long it should be used and when, in relation to toothbrushing, approximal cleaning should be performed ($p<0.01$ or $p<0.001$). The recommendation that was most frequently given by dental hygienists related to the choice of product (100%), while dentists most frequently gave recommendation relating to the way the product should be used (98%). The least frequent recommendation related to how long the product should be used (35% and 25% respectively). Approximal oral hygiene is discussed more frequently by dental hygienists in private clinics compared with those working in the public dental health service ($p<0.01$).

A large variation for both professions could be seen when it came to the recommendations given in relation to patient age. Dental floss was the most commonly recommended approximal cleaning aid for pre-school children and children/adolescents (14-52%), while a combination of two or more products was most frequently recommended for all other age groups (78-95%). Only minor differences could be seen when comparing dental hygienists and dentists. The main reasons for recommending interproximal cleaning aids varied in relation to age groups (Table 5). Recommendations were given in relation to caries for pre-school children and children/adolescents and in order to prevent gingivitis and periodontitis for the three oldest age groups.

Questionnaire to patients

The response rate for the questionnaires sent to the four patient groups varied between 150-182 and the final number of questionnaires that were analysed varied between 60-72%. The number of women was higher in the three lowest age groups (55-59%), while men dominated in the oldest age group (55%).

The majority of the individuals (82-92%) stated that they brushed their teeth twice a day or more (Fig. 10). A small percentage of individuals (0.6-3.2%) reported that they only brushed their teeth "once or a few times a week"; the highest figure was reported by 15- to 20-year-olds. The combination of a toothbrush and other cleaning aids was used on a regular basis by between 57-81% of the individuals in the four age groups. A manual toothbrush dominated in all four age groups compared with an electric toothbrush or a combination of the two.

The use of approximal cleaning aids showed a large variation in relation to age, with dental floss dominating in the two youngest age groups and an interdental brush in subjects aged >60 years. The mean use of approximal aids once a day or more frequently, when all the individuals were taken together, was

Table 5. Recommendations by dental hygienists and dentists in relation to interproximal cleaning aids given in relation to different oral conditions for the five age groups (%).

Dental profession/ Oral condition	Age group (yrs) 0-6	7-19	20-40	41-65	>65
Dental hygienists					
Dental caries	29.6	33.8	10.3	1.0	1.2
High plaque amount	17.3	18.4	18.6	10.5	12.0
Deep periodontal pockets	0.4	1.7	11.5	25.2	18.9
Gingivitis	2.0	15.4	27.0	11.0	7.6
Periodontitis	0.2	0.2	4.2	24.3	33.1
Other oral conditions	3.2	0.4	1.7	1.7	12.3
Combination of above	7.1	20.3	21.1	21.1	20.8
No recommendation	4.7	2.2	0	0	0
No answer	35.0	7.1	5.1	5.2	5.1
Dentists					
Dental caries	24.9	32.2	16.2	4.3	4.8
High plaque amount	8.6	14.7	14.2	10.6	11.4
Deep periodontal pockets	0.5	0.8	11.2	22.8	14.2
Gingivitis	3.0	16.0	20.3	8.9	5.3
Periodontitis	0.2	0.5	4.1	20.3	31.2
Other oral conditions	4.6	12.7	17.8	1.5	1.5
Combination of above	6.6	22.1	28.7	27.7	27.4
No recommendation	6.8	3.3	0.5	0.5	0.5
No answer	44.7	9.1	3.0	3.3	3.6

21% for toothpicks, 13% for dental floss and 9% for an interdental brush. Both the time point throughout the day, use in relation to brushing and place showed large variations, with evening, after brushing and bathroom as the most frequent answers. Recommendations relating to use had been given to 69-81% and instructions to 53-70% of the individuals in the four age groups. When all the age groups were taken together, the subjects reported that most recommendations about products that should be used for approximal cleaning had been given by dentists (39%), followed by different dental personnel (32%) and dental hygienists (21%). When it came to instructions relating to a model or in the mouth, the two youngest age groups reported that they had primarily been given instructions by dentists (37%), while the two older groups had received most instructions from dental hygienists (35%). Between 29-34% (mean for all four groups) reported that instructions had been given by different dental personnel.

The clinical evaluation of approximal oral hygiene showed a significantly higher plaque-reducing capacity for the users of interdental brushes than for the

individuals using toothpicks and dental floss ($p<0.05$). All 60 subjects had "very good" or "good" skills in handling the three interproximal aids.

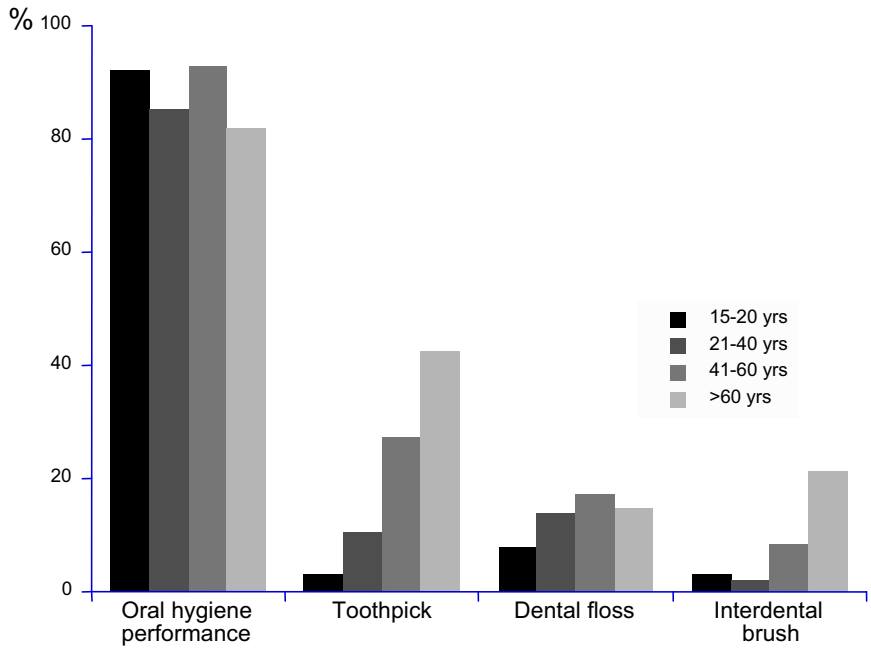

Fig. 10. Distribution (%) of oral hygiene performance twice a day or more often and use of approximal cleaning aids (toothpick, dental floss and interdental brush) once a day or more often for the four age groups; n = varying between 148-177.

Discussion

This study is based on the concept that, even in the 21st century, the approximal area is an area at risk of dental caries. In order to prevent the initiation and further progression of caries in this region, different caries-preventive measures should be recommended to the patient. They include a reduction in total sugar intake and sugar frequency, frequent fluoride administration and optimal oral hygiene techniques. The current data focus on the frequent application of fluoride in the interproximal region using fluoridated interproximal cleaning aids. However, it is important to remember that, at the same time, this has a mechanical effect, which will reduce the plaque in this area. This thesis emphasize some clinical and behavioural factors related to the recommendations for and use of approximal fluoridated cleaning products and their caries-preventive effect.

Variation in fluoride release *in vitro*

One interesting finding was the large variation in fluoride released *in vitro* from the total of 26 toothpicks and dental floss types that were evaluated. Among the toothpicks, the product resulting in the highest fluoride concentration at 24 h (Proxident Trätandsticka) released almost 14 times as much fluoride compared with the product with the lowest fluoride-releasing capacity (Flossbrush). It has previously been found that both a high uptake and release of fluoride from toothpicks *in vitro* may take place (Mörch & Bjørvatn, 1981; Petersson et al., 1994). The variation is believed to be primarily related to the difference in material and texture of the various products. One explanation for this is that chemical reactions between fluoride and different organic and inorganic substances present in the wood may take place and protract or inhibit the release of the fluoride that is retrieved (Mörch & Bjørvatn, 1981). In comparison to other fluoride products, it is not possible for technical reasons to assess the maximum fluoride content incorporated in a specific product.

The first time point for measurements of fluoride released *in vitro* in the present thesis was 30 minutes, which is not a realistic time point from a clinical point of view. However, previous *in vitro* studies have found an almost instant release of fluoride when the products were soaked in water. Petersson et al. (1994) found that 16% of the incorporated fluoride was released after just one minute and 69% after 24 h. Of the total amount released after 24 h, the mean amount released from the 26 products after 30 min in the present study was 77%. This is slightly higher compared with another study in which 60% was found to be released after one hour (Kashani et al., 1995). It can be expected that a high fluoride concentration will also be released at a very early point

during use *in vivo*. Previous studies have suggested that the rapid release of fluoride is probably explained by the fact that there are fluoride crystals on the surface, in porosities beneath the surface and within the bulk of the wooden toothpick (Petersson et al., 1994; Kashani et al., 1995). Although not studied in detail in the present investigation, previous investigations have shown that different wood textures may differ in their capacity to take up fluoride. Only a small numerical difference in fluoride-releasing capacity was found between the birch and lime toothpicks (Kashani et al., 1995).

The previous discussion is also believed to apply to different kinds of dental floss. They displayed a wide variation in their fluoride-releasing capacity. For some of the products, only a very small amount of fluoride (≤0.06 ppm fluoride) was released. The two floss types that produced the highest F concentrations at 30 min were the two prototypes. The products were ranked in almost identical order when comparing the 30-minute and 24-hour release. The Proxident Elastisk Tandtråd floss was an exception in this case, as about three times as much fluoride was released after 24 h compared with 30 min. This product, which is fairly new on the market, is made of an elastic material in which NaF is incorporated at an early stage of the manufacturing process. It has long been considered difficult to impregnate dental floss with a large amount of fluoride, but, in the last few years, new technology has increased this potential and there are currently several products on the market which display good fluoride-release properties.

Another aspect to be considered is the fact that the products have been soaked in distilled water and 10% TISAB and not in saliva, which is the case when used intraorally. Mörch and Bjørvatn (1981) have previously demonstrated that the fluoride release may differ depending on the media that are used. A higher fluoride release has been found for toothpicks impregnated with NaF when soaked in water compared with saliva, while the opposite has been observed for AmF-impregnated toothpicks. However, when used clinically, a wide range of factors may eventually determine the amount of fluoride that is released and the present data should primarily be regarded as the relative capability of the different products to release fluoride.

Fluoride release *in vivo* of toothpicks and dental floss

Methodological aspects. All the toothpicks and dental floss evaluated *in vivo* in Papers I and II produced elevated fluoride levels in the oral fluid collected in the approximal area throughout the entire test period (60 min). The peak for all the test series was found after two minutes and was followed by the classical elimination pattern after fluoride exposure, with a fast initial phase followed by a slower elimination period (Weatherell et al., 1986). However, the fluoride concentration was lower *in vivo* than that found *in vitro*. One reason is the fact

that only the outer tip (approximately 1/3) of the toothpick and a small part of the dental floss is used *in vivo*. Although only a small volume of saliva was collected at each time point (~ 4 μL) and saliva was collected at a limited number of time points (6 and 4 time points respectively in Papers I and II after using the individual products), the possibility that the sampling technique itself may also influence the fluoride concentration cannot be disregarded. Higher approximal fluoride values have been found when the first sampling did not take place until 10 or 20 min after the treatment (Kashani et al., 1998b). A higher, not lower fluoride concentration may therefore be expected during realistic use. The fact that no statistically significant differences were found between the two sampling sites in either Paper I or Paper II indicates the high reproducibility of the method. This is also indicated by the duplicate samples in Paper I revealing high correlation coefficients when evaluating the two test series. The slightly higher baseline fluoride concentrations found in Paper II compared with Paper I can most probably be explained by anatomic differences, as well as variations in dietary intake prior to testing.

The fluoride release *in vivo* is closely related to and influenced by the saliva. Both the salivary flow rate and the volumes of saliva in the mouth before and after swallowing are of great importance for the oral fluoride concentration (Lagerlöf et al., 1987). This means that the product that is used may be influenced differently both intra- and inter-individually. The volunteers were told first to moisten the toothpick before use, as this is known to speed up the fluoride release into the interproximal site. All subjects participating in Papers I and II had normal salivary secretion rates, but in future studies it would be interesting to evaluate the fluoride release in relation to different secretion rates.

Variations between products. A variation in fluoride-releasing capacity among the tested products was found *in vivo*. For all the tested products (Papers I and II), elevated values were still seen at 60 min after treatment compared with baseline. For all series, there was a smaller range among the products at 60 min when compared with two minutes, which was the first sampling occasion. The highest concentration of fluoride in Paper I was found for the two prototype floss types impregnated with NaF+AmF when evaluating both the concentration at two minutes and the total area under the curve during the entire 60-min period (Paper I). However, the two commercial dental floss types resulted in less fluoride compared with the two toothpicks, which corresponds well with the *in vitro* data. Furthermore, the data from the toothpicks correspond well with previous data relating to fluoridated toothpicks (Kashani et al., 1998b).

Treated vs. non-treated sites. One interesting finding in Paper I was the fact that fluoride was able to migrate from the treated site to the adjacent untreated site. The amount of fluoride found at this site was lower compared with the treated site (5-45%), but the variation between the different products was also smaller. It is not thought that any fluoride was carried from the opposite side, as the

opportunity for fluoride to migrate from one side of the mouth to another is known to be limited (Weatherell et al., 1984; Sjögren et al., 1997). After a fluoride tablet (Weatherell et al., 1984) or chewing gum (Sjögren et al., 1997) was placed at one asymmetrically located position in the mouth, the fluoride that was released failed to produce an overall elevation in fluoride in the oral cavity.

Single vs. multiple use. As expected, a large difference in fluoride concentration was found when using one and the same toothpick or dental floss for the whole cavity or when a fresh product was used for each of the eleven sites (Paper I). The gradual reduction was more pronounced when using a single toothpick in comparison to a single dental floss. This may be explained by the high initial release of fluoride from the product. However, a certain variation was also found when using a new product for each of the four sites. As previously mentioned, this may be due to both product- and individually related factors, where product quality and variations in the site in relation to salivary glands are believed to play a major role. However, the area under the curve (AUC; mM F x min) summarises the fluoride concentration during the whole 60-min period and other normal activities such as swallowing and tongue movement may influence the elimination process. The subjects were otherwise told to remain quiet during the whole sampling period. It can be questioned how realistic a recommendation to use a new product for each interproximal site may be. It may instead be advisable to start to use the toothpick or dental floss at the sites where the need for fluoride is the greatest, often in the molar-premolar region or at sites where initial carious lesions have been identified.

Combination of products. The results differed when evaluating the optimal order when using a toothpick or dental floss in combination with a mouthrinse or toothbrushing in Paper II. In combination with rinsing, the highest fluoride values were obtained when using a toothpick and dental floss prior to rinsing. When combined with toothbrushing, the order was the opposite, i.e. the toothpick and dental floss should be used after brushing. This was particularly obvious in the case of the toothpick. The data from the combination with rinsing indicate that the relatively high fluoride concentration in the solution easily penetrates the interproximal area. The subsequent use of a toothpick or dental floss will remove previously retained fluoride from the area. The opposite is supposed to apply to the combination of the two approximal aids with brushing. The fact that the toothpick compared with dental floss for both series appeared to add more fluoride to the area, when used as the second product, corresponds well with the data on the single product. For the present series, a new toothpick or dental floss was used for each of the two sites (16/15 and 12/11). In the clinical situation, the majority of individuals most probably use one and the same item for all sites. No statistically significant difference was found between the two test sites in the present study. However, it can be

expected that the final fluoride concentration at the individual sites may differ if a combination of products is used for a larger number of interproximal sites.

Fluoride compound. No clear trend could be seen between the products in relation to the fluoride compound used (NaF, AmF or NaF + AmF) in Paper I. The fact that the prototype toothpick impregnated with AmF resulted in lower fluoride values compared with the commercially available NaF-impregnated toothpick, while the opposite was found for the four dental floss types, is believed to be more closely related to the concentration of fluoride incorporated into the product and the opportunity for the material to release fluoride than the fluoride compound itself. When used in toothpaste and mouthwash, AmF has been found to elevate fluoride values in saliva (Campus et al., 2003).

Other administrative methods. A single mouthrinse with 0.2% NaF solution for one minute was also one of the methods resulting in the highest fluoride concentrations as evaluated in Papers I and II, exceeding those seen for the single use of toothpick and dental floss. However, no statistically significant difference was found in Paper I when compared with the use of either single or multiple toothpicks, the latter even resulting in almost identical values when compared with the mouthrinse. The present positive findings for the mouthrinse in comparison to brushing (Paper II) correspond well with clinical data where it has been found to have a favourable effect on preventing caries in both adolescents, when used to supplement the daily use of fluoridated toothpaste (Moberg Sköld et al., 2005), and in the elderly, when compared with other home-care fluoride programmes (Fure et al., 1998). Apart from the slightly higher fluoride concentration, a solution more easily penetrates into different areas when swished around the mouth. When it came to this method, a numerical variation was also found between the four sampled sites, in spite of the fact that instructions had been given to the subjects to perform active movements of the tongue and cheeks during rinsing. The variation in fluoride concentration indicates once again that both anatomic and behavioural factors may influence the final fluoride concentration at a specific intraoral site and that, for the optimal use of this technique, it is also important to give instructions on use.

Surprisingly, brushing with toothpaste containing 0.15% NaF alone in Paper II produced lower fluoride concentrations than several of the other methods that were evaluated. It resulted in about a quarter of the AUC value (mM F x min) obtained for rinsing with 0.2% NaF. Rinsing with 2 x 10 ml of water post-brushing is believed to remove some of the fluoride from the oral cavity, but the more complex composition most probably also influences the results. However, it is important to remember that regular toothbrushing is believed to be the most important measure in overall terms when it comes to the prevention of dental caries (Bratthall et al., 1996). The present study has only

focused on techniques designed to obtain optimal fluoride concentrations on the approximal surfaces.

Inter Dental Brush Gel Method

The highest fluoride concentrations were found for the interdental brush dipped in 0.2% or 0.32% NaF gel when used alone (Papers I and II) or in combination with brushing (Paper II). As expected, the 0.32% NaF gel resulted in the highest overall fluoride levels, when evaluated both as mM F and area under the curve (AUC; mM F x min). With its higher substantivity, the gel is more easily retained interproximally. Used in combination with professional flossing, a NaF gel has previously been found to be able to reduce approximal caries in 13- to 16-year-old schoolchildren (Gisselson et al., 1999). It has recently also been found to be beneficial in the remineralisation of demineralised enamel both *in vitro* and *in situ* (Sawyer & Donly, 2004).

Based on the previous data relating to the combination of methods, only the order of interdental brush + gel used after brushing was tested (Paper II). In the same way as when brushing was combined with a toothpick or dental floss, higher fluoride values were obtained when combining two products compared with brushing alone (8.8 and 4.5 times higher respectively). The amounts that were obtained revealed only minor differences when compared with gel treatment alone. The gel with the higher fluoride concentration (0.32%) was most favourable from this respect as well.

The technique evaluated in the present study is thought to be used primarily for home care. It may be of special interest for individuals with parodontal problems and the elderly, with their larger approximal areas, where there is a higher risk of root caries in particular. Fluoride has been found to be particularly beneficial when it comes to remineralising the dentine. The two patient categories mentioned above are also the individuals for whom an interdental brush is most frequently the first choice of approximal cleaning aid. It has also been found to be more effective in the removal of plaque compared with dental floss (Christou et al., 1998). Consequently, the objective when dipping an interdental brush in fluoride gel, known as the "Inter Dental Brush Gel Method", is to obtain optimal cleaning and optimal fluoride concentrations. In this respect, it could be interesting to discuss in greater detail the order in which the brush + gel treatment should be carried out when used in relation to toothbrushing. Only use after brushing was evaluated in this study. As toothbrushing including rinsing with water followed by gel application resulted in lower fluoride concentrations compared with the single use of an interdental brush + gel, it is thought that use before brushing will produce even lower values. Even if it is a slightly complicated procedure, the most efficient way, from both a plaque and fluoride perspective, may be first to use the interdental

brush for cleaning, then perform regular toothbrushing and rinse with water, after which the brush is dipped in the gel and fluoride is finally applied.

Lower fluoride values were found when dipping the brush in 0.2% NaF solution (Paper I). Although the two fluoride products had the same fluoride concentration, the gel with its higher retention is more easily retained both on the brush and approximally.

Effect on enamel and dentine as well as plaque microflora

The finding that the frequent use of fluoridated toothpicks and dental floss reduced the demineralisation of approximally situated enamel and dentine when analysed with Transverse Microradiography (TMR) is in agreement with previous studies in which fluoride has been found to have a beneficial effect on both enamel and dentine (Bjarnason & Finnbogason, 1991; ten Cate, 1997; Oliveby, 1991; Zimmer, 2001; Petersson & Kambara, 2004). It was found that all four toothpicks and dental floss types inhibited further demineralisation compared with the control period with no treatment. In this respect, the two dental floss types were somewhat more effective than the toothpicks. A comparison of the data for the toothpicks with a similar *in situ* experimental model reveals very similar results, with the reduced demineralisation of both enamel and dentine (Kashani et al., 1998a). The fact that no remineralisation was found may, according to the authors, be explained by tighter approximal surfaces.

The effect on dentine was slightly greater in comparison to enamel. Although the remineralisation process is similar to that for enamel, it is known that a significantly higher fluoride level is required in order to enhance remineralisation.

The design of the study was chosen in order to compare the two toothpicks and the two dental floss types with a cross-over design for each individual group. A certain period effect was found for the toothpicks, with more attenuated values for lesion depth and mineral loss for both tooth tissues for period 2, while no such effect was found for the two floss types. No effective explanation for this can be given. The compliance of the subjects could be discussed, but all the participating volunteers assured us that they had followed the instructions they had been given regarding the use of the prosthesis, the approximal cleaning and the general oral hygiene regimen.

It is possible to discuss how realistic the use of an approximal aid for half a minute per site and three times per day is. As shown by the *in vivo* data, both the fluoridated toothpick and dental floss result in elevated approximal fluoride concentrations. Even if recommendations are given to use cleaning aids in relation to toothbrushing in a bathroom, the questionnaire data from Paper IV revealed that approximal cleaning aids are used "twice a day or more" by the

oldest age groups. This applied particularly to toothpicks, which are easier to use alone throughout the day in different environments at time points when brushing is not performed. An extra fluoride application of this kind has a favourable effect on the risk area and should be recommended to individuals at risk of caries. This may be particularly suitable after food and drink intake. The level of fluoride applied may seem low, but delivery of relatively low fluoride concentrations for longer time has been suggested favourable for inhibiting demineralisation and enhancing remineralisation (Featherstone et al., 1990; 1999; Lynch et al., 2004). The experimentally constructed interproximal spaces were wider than those usually seen in the approximal area in the natural dentition. The more rapid clearance of fluoride from this area may therefore be expected to have occurred. The fact that the subjects wore complete dentures is not thought to have influenced the results, as comparisons of data from previous studies have shown similar salivary fluoride levels after toothbrushing when comparing dentate individuals with those wearing complete dentures (Sjögren & Birkhed, 1994; Sjögren et al., 1995).

The effect of the frequent use of toothpicks and dental floss on plaque microflora resulted in a reduction in micro-organisms compared with the control period. These data correspond well with the TMR data. The AmF-impregnated toothpick and the dental floss impregnated with AmF/NaF resulted in the smallest number of micro-organisms. This corresponds well with previous studies where it has been found that amine fluorides have an antimicrobial effect on cariogenic bacteria (Shani et al., 1996; Shani et al., 2000), as well as an anticaries effect (Rosin-Grget & Lincir, 1995). In the present study, a slight difference was found between the two approximal vehicles, as only a significant reduction was found for mutans streptococci when comparing the two dental floss types, while all four bacterial parameters (total count, actinomyces, mutans streptococci and lactobacilli) differed significantly when comparing the two toothpicks. The fact that the toothpick seem to be more favourable than the floss cannot be fully explained. However, although the floss *in vitro* and *in vivo* (Paper I) was found to result in higher total fluoride concentrations, it is suggested that a higher concentration of AmF was released from the toothpick.

The effect of fluoride on plaque adogenicity has not been evaluated in the present studies. However, it has previously been shown that slightly elevated levels of fluoride in saliva during daytime are sufficient to change the acidogenicity of dental plaque *in vivo* (Ekstrand et al., 1985).

Clinical recommendations for use of approximal aids

From the results of the questionnaire answered by dental hygienists and dentists, it can be seen that both dental professions give recommendations about oral hygiene and the use of approximal cleaning aids. Moreover, the respondents in

the four age groups reported that they had largely been given recommendations related to approximal cleaning by dental hygienists and dentists. Although these findings are positive, there is no evidence from the present data to indicate that the recommendations have resulted in changes in behaviour. However, a systematic review focusing on oral health promotion has concluded that chairside oral health promotion is more effective than other methods of health promotion (Kay & Locker, 1998).

Dental hygienists were found to give more specific recommendations related to the use of approximal cleaning aids compared with dentists when it came to the choice of product, how often the product should be used, how long it should be used each time and when, in relation to toothbrushing, approximal cleaning should be performed. Furthermore, approximal oral hygiene was discussed more frequently by dental hygienists working privately compared with those working in the public dental health service. Even if no statistically significant difference was found in relation to years in practice, dental hygienists at group level had fewer years in practice compared with dentists. It is therefore likely that they completed their education more recently. Both modern methods and procedures for prevention are currently available. Furthermore, the importance of individual action is currently being stressed and oral hygiene practices at home are regarded as an important part of the prevention of both dental caries and periodontal disease.

One interesting finding was the fact that recommendations relating to interproximal cleaning were primarily given for dental caries for the two youngest age groups and in order to prevent gingivitis and periodontitis for the three oldest age groups. Even if dental caries is the dominant problem for the younger generations, it also constitutes – together with gingivitis and periodontitis – a significant problem for adults and the elderly, not least in relation to the root surface (Bader et al., 2005; Hugoson et al., 2008). As expected, the recommendations associated with products also varied in relation to patient age, with dental floss dominating among pre-school children and children/adolescents and a combination of two or more products for all other age groups (78-95%).

The recommendation to use an interdental brush increased with increasing age, while only a small variation was seen in relation to age when it came to the use of toothpicks. Recommendations for products that should be used for approximal cleaning, as well as instructions for use, had been given to the majority of the individuals. These figures are higher than those previously reported for the UK and Lithuania (Kelly et al., 2000; Vysniauskaitè & Vehkalahti, 2007). The subjects in the four age groups reported that most recommendations regarding products for approximal cleaning were given by dentists and this is most probably related to the fact that dentists are visited by individuals more frequently than dental hygienists. For all the age groups,

between 65-88% of the individuals reported that they visited the dentist once a year or more often, while the same figure for visits to dental hygienists for the four groups varied between 20-40%. Between 45-53% of the subjects reported that they had never visited a dental hygienist. It is interesting to note that the individuals aged ≥ 41 years were mainly the ones that reported that they had received instructions on use from a dental hygienist. Older individuals are thought to visit dental hygienists more frequently than younger persons due to periodontally related problems.

The daily oral hygiene procedure, using fluoridated toothpaste, that is performed at home constitutes an important part of overall caries prevention. The figures found for toothbrushing twice a day or more often in the present study are in accordance with data previously reported by a Swedish adult population (Hugoson et al., 2005) but higher than the figure that has recently been reported for a Danish population, where a mean of 68% was found among 3,435 individuals aged 16 years or more. These figures can be compared with data from a study of adult populations worldwide which found that the level of toothbrushing more than once a day varied between 57 and 85% for individuals aged 35-44 years (Chen et al., 1997). The use of toothpicks every day or more frequently reported by the individuals in the present study is slightly lower (28%), while the figure for dental floss was almost identical (11%) compared with what has been reported from almost identically aged individuals from Denmark (Christensen et al., 2003). The data related to the use of dental floss also correspond well with the data presented by Petersen and Nortov (1994). No previous data regarding the use of interproximal brushes have been reported. Although the response rate varied between the four age groups, with the lowest figure for the two youngest groups, the data correspond well with data from previous studies.

Patient use of approximal aids

It was found to be easier for regular users of interdental brushes to clean the approximal area from plaque in contrast to the use of toothpicks and dental floss. Within the past five years, according to their oral health status and anatomy, all 60 participants had been informed and instructed about the use of the individual cleaning aids. This means that the individuals varied from an oral perspective but also due to age and other medical and behavioural factors. Taken together with the small number of subjects in each group, this could limit the generalisation of the present findings. However, the present data indicate that it is easier for regular users of an interdental brush to clean the approximal area in comparison to using toothpicks and dental floss. Only three products recommended for cleaning the interproximal area were studied. At the present time, a wide range of products, including plastic toothpicks and single-tufted

brushes, is available on the market. The variation in plaque-removal efficacy of four types of dental floss has been presented in a recent study by Terézhalmy et al. (2008). It would therefore be of interest to study the performance of a larger number of products in a realistic home-care situation in greater detail. The skills were found to be satisfactory when assessed against their specific criteria. However, as this part was performed at the clinic, the possibility that the subjects performed more careful cleaning than usual. It has previously been found that patients are not always aware of their exact performance and that the time spent on oral hygiene is overestimated (Zeedyk et al., 2005). Cleaning efficacy has also been found to vary in different parts of the mouth (Macgregor and Rugg-Gunn, 1979; Söder et al. 2003). So more detailed studies would also be of interest from this perspective.

Clinical implications

The use of fluoridated toothpaste is the key factor for caries reduction. These studies indicate that fluoridated toothpicks, dental floss and interdental brushes for approximal use may be of special importance when it comes to reducing caries in this target area. It is important to make the individual the starting point and to look at both oral and behavioural factors and the importance of giving instructions on the use of tools for approximal cleaning and approximal fluoride administration. Apart from their fluoride delivering effect, their plaque-reducing properties are also important, which is beneficial for gingival health. In order to obtain high fluoride concentrations, it is important to recommend the order when using the combination of toothpicks and dental floss with brushing and rinsing.

Dental hygienists, together with dentists and other oral health professionals, have an important role to play in this work. They have gradually become an important part of the dental team and as health educators, particularly in the developed countries (Öhrn, 2004). However, the subjects also mentioned "other sources of information" in the questionnaire regarding the choice of products. .The possibility that, apart from dental professionals, knowledge and information in the future can be transferred via other media should therefore not be ruled out (Bradnock et al., 2001; Mårtensson, 2004).

Both the clinical data and information collected from the questionnaires clearly indicate the importance of advising subjects running a high risk of caries to optimise the use of approximal cleaning aids impregnated with fluoride in order to obtain a good caries-preventive effect. However, when giving advice regarding the use of approximal aids, it is important to consider both the cariological and parodontal aspects. All individuals are recommended to perform approximal cleaning in addition to regular toothbrushing. Depending on age and anatomic features, as well as the risk of disease, different vehicles should be

recommended. From a caries perspective, the use of dental floss is recommended to start when the last permanent tooth has erupted and the approximal area is closed. It is important throughout life that dental personnel continuously modify recommendations according to these biological and age changes. They should emanate from the individual need.

Recent work at our department has focused on approximal caries prevalence and its related factors (Alm, 2008). This work has concluded that the prevalence of approximal caries is underestimated in official caries data, as initial lesions are not included in the calculations. Furthermore, good oral hygiene habits, established in early childhood, were found to provide a foundation for a low experience of approximal caries in adolescence (Alm et al., 2008). These data, together with the findings from the present thesis, stress the importance of establishing optimal preventive measures at an early stage in order to prevent new lesions developing, but also in order to prevent initial lesions progressing to manifest lesions. The use of both approximal cleaning aids and fluoride is a natural step in this respect. This thesis has demonstrated the benefits when combining the use of these two preventive measures.

Conclusions

The main conclusions from this thesis are that:

- there were large variations in the fluoride release from various brands of toothpicks and dental floss *in vitro* (Paper I)

- both fluoridated toothpicks and dental floss produce elevated fluoride concentrations up to 30 min after use *in vivo* (Papers I and II)

- order of use when combining products may be of importance when it comes to obtaining high fluoride levels at the approximal sites (Papers II)

- an interdental brush dipped in a NaF gel, the "Inter Dental Gel Brush Method", was found to be favourable when it comes to obtaining a high approximal fluoride concentration *in vivo* (Papers I and II)

- the frequent use of fluoridated toothpicks and dental floss may inhibit further demineralisation and promote remineralisation of enamel and dentine (Paper III)

- the frequent use of fluoridated toothpicks and dental floss results in lower counts of micro-organisms in plaque compared with a control period; the AmF toothpick results in a higher reduction compared with the NaF toothpick (Paper III)

- the recommendations given by dental hygienists and dentists regarding approximal cleaning vary between the two professions but also particularly in relation to oral health and the age of the patient (Paper IV)

- the individual self-care practices when using approximal cleaning aids are influenced by a large number of factors (Paper IV)

- the ability to remove plaque is high for regular users of toothpicks, dental floss and interdental brushes (Paper IV)

Svensk sammanfattning (Swedish summary)

Trots en generellt förbättrad tandhälsa hos barn, ungdomar och vuxna under de senaste årtiondena ses fortfarande i dessa samtliga åldersgrupper en hög förekomst av approximala kariesangrepp i premolar- och molarregionen. Det är viktigt att fluor (F) tillförs dessa tandytor för att motverka utvecklingen av karies. **Frågeställning:** Syftet med avhandlingen var att: i) studera fluoravgivningen av fluorinnehållande approximala munhygienhjälpmedel *in vitro* och *in vivo*, ii) utvärdera olika administrationssätt *in vivo*, iii) studera effekten av frekvent användning av fluoravgivande tandstickor och tandtrådar på demineraliserad emalj och dentin *in situ*, samt iv) kartlägga rekommendationer och användning av munhygienprodukter för approximal rengöring. **Metodik och resultat:** Fluoravgivning *in vitro* av 26 olika fluoriderade tandstickor och tandtrådar utvärderades upp till 24 tim. En stor variation i fluoravgivning sågs mellan produkterna; generellt resulterade tandsticka i högre fluorhalter jämfört med tandtråd. Fluoravgivningen *in vivo* studerades hos friska vuxna efter varierande användningssätt av fluoriderade tandstickor/tandtrådar enskilt eller i kombination med tandborstning alternativt munsköljning med 0,2% natriumfluorid(NaF)-lösning. Även administration av fluor via interdentalborste doppad i 0,2% eller 0,32% NaF-gel (”Inter Dental Brush Gel Method”) utvärderades enskilt eller i kombination med tandborstning. Approximal saliv insamlades, med små papperspoints, före och upp till 60 min efter behandling. Fluorhalten analyserades med hjälp av en jon-specifik elektrod. Både tandstickor och tandtrådar gav förhöjda fluorhalter *in vivo* under hela testperioden. Interdentalborste doppad i 0,2% NaF-gel och munsköljning med 0,2% NaF gav samma fluorhalt som efter användning av flera tandstickor i rad. Samtliga kombinationer av tandstickor/tandtråd med F-sköljning och F-gel gav högre fluorkoncentrationer än vad som sågs efter enbart tandborstning eller i kombination med tandborstning. Vid kombination av tandsticka/tandtråd med munsköljning och tandborstning erhölls högst fluorhalt ifall dessa användes efter sköljning och före borstning. Även om uppmätt fluorhalt var lägre i obehandlade jämfört med behandlade områden sågs möjlighet för fluor att spridas från ett approximalrum till ett annat. *In situ* inkluderades 15 vuxna med helprotes i vilka demineraliserade emalj- och dentinbitar monterats. Regelbunden användning av tandstickor eller tandtrådar, impregnerade med NaF och aminfluorid, skedde under 4 veckor. Effekten på tandbitar, med hjälp av transversell mikroradiografi (TMR), och oral mikroflora studerades. Samtliga produkter hämmade fortsatt demineralisation jämfört med en kontrollperiod; tandtråd var något mer effektiv jämfört med tandsticka. Den största reduktionen av plackfloran uppmättes efter användning av tandsticka impregnerad med aminfluorid. Kartläggning av de rekommendationer som idag ges beträffande

approximala munhygienhjälpmedel genomfördes genom att en enkät utsändes till 500 tandläkare, 500 tandhygienister och 1000 patienter. Dessutom deltog 60 patienter i en klinisk utvärdering avseende deras förmåga att avlägsna approximalt plack vid användning av olika approximala munhygienhjälpmedel. Resultaten visade att tandhygienister ger något mer specifika rekommendationer avseende approximal munhygien jämfört med tandläkare. I första hand sker rekommendationer i syfte att förbättra den parodontala hälsan. Regelbundna användare av interdentalborste har lättare att få rent från approximalt plack jämfört med regelbundna användare av tandsticka och tandtråd. **Slutsatser:** Användningen av fluorinnehållande munhygien-hjälpmedel interproximalt bedöms vara en viktig åtgärd för att reducera karies på approximalytan. En interdentalborste doppad i fluorgel kan vara ett intressant administrationssätt för att höja den approximala fluorhalten.

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