On Fluoridation of Chewing Sticks (Miswaks) with Respect to Dental Caries

Hosam Baeshen

Department of Cariology
Institute of Odontology at Sahlgrenska Academy
University of Gothenburg, Sweden

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A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarises the accompanying papers. They have already been published or accepted for publication. The photos in this thesis were taken by the author *Hosam Baeshen.*

Abstract

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Hosam Baeshen, Department of Cariology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Box 450, SE-405 30 Gothenburg, Sweden

The chewing stick known as a "Miswak" is a natural toothbrush that is widely used for cleaning the teeth. It has been used for thousands of years in Asia, Africa and the Middle East. The aim of this thesis was to evaluate the Miswak as a vehicle for fluoride delivery in the oral cavity. *In vitro* studies showed that both fresh and old Miswaks take up fluoride, which can even reach the pulp of the stick. *In vivo*, the fluoride release from Miswaks impregnated in 0.5% NaF was rapid and was estimated to be around 0.4 mg. A large variation in fluoride release was observed between Miswaks purchased from different stores. This variation may depend on differences in wood properties or the fact that some stores sell fresh Miswaks, while others sell older types. Based on *in vivo* data, it is recommended to use fresh Miswaks impregnated in 0.1% NaF or a maximum of 0.5% NaF on daily basis. The mean salivary fluoride concentration for Miswaks impregnated in 0.1-0.3% NaF produced about the same fluoride level in saliva as toothpaste containing 0.32% NaF.

The fluoride concentrations at the various sites in the oral cavity were higher before than after debonding in orthodontic patients. Moreover, products with a high fluoride content (toothpaste, solution and Miswaks) resulted in higher fluoride retention than the corresponding products with a lower fluoride content. In whole saliva, the highest area under the curve (AUC) values were found in patients using 0.2% NaF rinsing solution, followed by 1.1% NaF toothpaste ($p < 0.05$). The mean fluoride concentration in approximal saliva was higher for Miswaks impregnated in 0.5% NaF compared with other fluoridated products ($p < 0.001$). Consequently, presence of fixed orthodontic appliances appears to increase the oral fluoride retention for all the tested home-care fluoride products.

The treatment effect of fluoridated Miswaks was evaluated on white spot lesions (WSL) in healthy adolescents with a minimum of 4 WSL after completing the orthodontic treatment. They participated in a double-blind, randomised, longitudinal trial, lasting for 6 weeks, and were divided into two groups using: 1) fluoridated Miswaks impregnated in 0.5% NaF (test group, n=19) and 2) non-fluoridated Miswaks (control group, n=18). A custom-made mouth tray, covering half the dentition in the upper jaw, was used while brushing with the Miswaks 5 times/day. The lesions were scored at baseline and 2, 4 and 6 weeks after debonding. The DIAGNOdent readings and the International Caries Detection and Assessment System (ICDAS II) index of the WSL decreased in the test group on the uncovered side of the dentition but not on the covered side, during the 6-week trial ($p < 0.0001$). This indicates that the frequent use of fluoridated Miswaks had a remineralising effect on WSL.

In conclusion, NaF-impregnated Miswaks produced a rapid release of fluoride *in vitro*, as well as *in vivo*, and may be an interesting vehicle for home-care use for caries prevention in countries where they are frequently used.

Key words: Approximal area, caries lesions, chewing stick (Miswak), fluoride, fluoride retention, fluoride solution, fluoride toothpaste, impregnation, orthodontic patients, saliva, Salvadora Persica

ISBN 978-91-628-8039-2
Corresponding email: drbaeshen@me.com

Contents

Original papers

This thesis is based on the following four papers, which will be referred to in the text by their Roman numerals:

I. Baeshen H, Kjellberg H, Lingström P, Birkhed D. Uptake and release of fluoride from fluoride-impregnated chewing sticks (Miswaks) in vitro and in vivo. Caries Res 2008;42:363–368.

II. Baeshen H, Birkhed D. Release of fluoride from fresh and old fluoride-impregnated chewing sticks (Miswaks) in vitro and oral retention in vivo. Oral Health Prev Dent;2010;1:93-99.

III. Baeshen H, Kjellberg H, Birkhed D. Oral fluoride retention in orthodontic patients with and without fixed appliances after using different fluoridated home-care products. Acta Odontol Scand 2010, in press.

IV. Baeshen H, Lingström P, Birkhed D. Effect of fluoridated Miswaks (chewing sticks) on white spot lesions in post-orthodontic patients evaluated by DIAGNOdent pen and ICDAS II. Am J Orthod Dentofacial Orthop 2010, accepted.

Introduction

Dental caries is the localised destruction of susceptible dental hard tissues by acidic by-products from the bacterial fermentation of dietary carbohydrates [Marsh, 1999; Marsh et al., 2003; Fejerskov et al., 2003]. The disease process is initiated within the bacterial biofilm (dental plaque) that covers a tooth surface. The very early changes of caries in the enamel cannot be detected with traditional clinical and radiographic methods [Bader et al., 2001; Ismail, 2004; Ko et al., 2008].

Caries prevention and fluoride

Frequent exposure to fluoride, optimal oral hygiene and a reduction in the intake of fermentable carbohydrates are important factors to be considered in caries prevention [Lingström et al., 2003; Selwitz et al., 2007]. The basic methods for preventing dental caries are generally the same, regardless of the tooth site and surface. However, some methods may be more suitable for certain areas. For example, the application of fissure sealants is a method recommended for caries prevention on occlusal surfaces [Mejàre et al., 2003], while fluoridated toothpicks, flosses and an interdental brush combined with fluoride gel are methods recommended for the approximal area [Kashani et al., 1998a; Särner et al., 2003; Särner et al., 2008].

The observed decline in dental caries in most industrialised countries over the last four decades can be attributed primarily to the daily use of fluoridated toothpaste [Bratthall et al., 1996; Marinho et al., 2003c; Twetman et al., 2003]. The bulk of modern research has confirmed the anticariogenic properties of fluoride and its key role in caries prevention [Featherstone, 1999; Marinho et al., 2003b]. The anticariogenic action of fluoride depends mainly on its ability to inhibit the demineralisation of enamel and dentine, to stimulate remineralisation [ten Cate, 1997; ten Cate et al., 1998; ten Cate, 1999; ten Cate et al., 2008] and to affect the metabolism of cariogenic bacteria [Selwitz et al., 2007; Wiegand et al., 2007; Stoodley et al., 2008]. Nowadays, brushing with fluoridated toothpaste twice a day as soon as the eruption of the first primary tooth takes place is highly recommended [Twetman et al., 2003; Alm, 2008]. In addition to toothpaste, a wide range of

fluoridated products, such as mouthrinse solutions, gels, tablets, chewing gums, toothpicks and dental floss, is currently available.

Research has shown that the effect of fluoride-containing products, such as toothpaste and tablets, is less pronounced on approximal surfaces [Granath et al., 1978; Li et al., 1994; Øgaard et al., 1994]. The prevalence of approximal caries can be reduced by the frequent application of fluoride varnish [Moberg Sköld et al., 2005].

Miswaks and oral hygiene

Good oral hygiene habits can prevent or retard the development of caries and periodontal diseases [Axelsson et al., 2004]. Toothbrushes and toothpastes are the most widely used teeth cleaning tools. The toothbrush, which is relatively modern, was introduced in Europe around 300 years ago [Elvin-Lewis, 1980; Penick, 2004]. Historically, the first known oral hygiene device is the chewing stick. “Miswak” is an Arabic word meaning tooth cleaning stick and it has various local names in different Arabic dialects and countries. It is known as Miswak and Siwak in the Middle East, Mswaki in Tanzania, Mefaka in Ethiopia and Datun in India and Pakistan [Khoory, 1983; Hattab, 1997; Petersen and Mzee, 1998]. In English, it is known as the “toothbrush tree”, “mustard plant”, the “toothbrush tree of the Orient” and the “Persian toothbrush tree”. The use of the chewing stick began more than 7,000 years ago. The Babylonians were the first to use it, followed by the Greeks and Romans and then the Jews, Egyptians and Muslims [Asadi and Asadi, 1997; Wu et al., 2001; Al-Otaibi, 2004]. The Japanese used Koyoji, while the Romans used Mastic to rub their teeth and the Jews used a kind of wooden stick called Qesam in Hebrew [Gerrit 1993].

Chewing sticks are used by 90% of Nigerian people and the inhabitants of rural regions of Tanzania and Zanzibar [Elvin-Lewis, 1980; Norton and Addy, 1989; Petersen and Mzee, 1998]. In Saudi Arabia, a large study of 1,155 patients, 65% were found to use chewing stick every day [Al-Otaibi et al., 2003b]. Another study by Guile et al., [1998] based on a sample of 3,117 people, reported chewing stick use by 50% of 15-year-old children. In India, more than 65% of the rural population and 43% of the urban population used chewing stick regularly [Boghani, 1978]. In Pakistan, more than 50% of the rural population used a chewing stick [Asadi and Asadi, 1997].

The Miswak material is harvested from a plant called Salvadora Persica or Are tree (synonymous with Araak). It is a small, upright, evergreen shrub with white branches and aromatic roots [Wu et al., 2001; Al-Otaibi, 2004], The roots, twigs and stems have been used for centuries for oral hygiene and are commonly used nowadays in the Middle East, as well as in Asia, Africa and South America [Elvin-Lewis, 1980; Eid and Selim, 1994; Wu et al., 2001].

Most chewing sticks are pencil-sized sticks, 15-20 cm long, with a diameter of 1-1.5 cm [Al-Sadhan et al., 1999; Wu et al., 2001; Al-Otaibi, 2004]. There are about 200 plant species that used for preparing brush sticks, of which more than 150 are found in Africa [Elvin-Lewis, 1982; Al-Otaibi, 2004] and the most widely used is *Salvadora Persica*, which is distributed geographically in Asia, Africa and the Middle East [Khoory, 1983; Wu et al., 2001]. They are prepared from the root, stem, twigs or bark of the trees. However, the plant is spongy and can easily be crushed between the teeth (Fig. 1A). Fig. 1B shows how one end of the stick is chewed or tapered until it becomes frayed into a brush. These sticks are sold in kiosks or small shops (Fig. 1C). In the Kingdom of Saudi Arabia, Miswaks are also available in most pharmacies in a ready-made hygienic air vacuum plastic pack (Fig. 3D). A Miswak and a toothbrush are similar in that they have bristles, which are used to remove plaque from tooth surfaces mechanically. Unlike a conventional toothbrush, the bristles of the Miswak lie in the same long axis as its handle. The angulation in the toothbrush enables it to adapt more easily to the buccal and occlusal surfaces, particularly in the posterior teeth [Danielsen et al., 1989].

The stick is usually chewed only briefly to fray the fibres and it is then used as a brush applied to the teeth, gums and tongue (Fig. 1B). It may be soaked in water or left in the mouth for a couple of minutes to stimulate salivation and enhance cleaning. It should be kept moist when not in use [Al-Sadhan et al., 1999; Almas and Al-Lafi, 1995]. With each use, worn bristles are snipped away and new bristles are prepared by further chewing or tapering [Mohammad and Turner, 1983; Wu et al., 2001].

For Muslims, it is customary to use the chewing stick prior to each prayer (five times a day). The Miswak should be freshly cut so that it is flexible, easily chewed and still rich in active constituents [Almas and Al-Lafi, 1995; Al-Otaibi, 2004]. The root should be whitish-brown in colour; a dark brown colour indicates that the Miswak is no longer fresh [Grant, 1990]. A very dry, hard Miswak can be expected to

damage the gums and other oral tissues. If dry, the end should be soaked in fresh water for 24 hours. However, soaking for unduly long periods causes the loss of active constituents and diminishes the therapeutic properties but without any loss of the mechanical effects on the teeth [Almas and Al-Lafi, 1995; Al-Sadhan et al., 1999]. Before use, the end of the chewing stick should be washed with water. It is then chewed repeatedly until the fibres stand out like the bristles of a toothbrush. These fibres should be clipped off every 24 hours.

Fig. 1. A) A Saudi man showing how easily Miswaks can be crushed between the teeth. **B)** Miswaks become frayed like a brush when used orally. **C**) Kiosk or small shop sells Miswaks. **D)** A ready-made hygienic plastic vacuum-packed Miswak.

The value of chewing sticks is believed to lie in their mechanical cleansing action. Miswaks were also reported to inhibit the formation of dental plaque chemically and exert an antimicrobial effect on many oral bacteria [Al-Otaibi et al., 2003a; Al-Otaibi et al., 2004; Sofrata et al., 2007; Sofrata et al., 2008]. Several studies have reported that the prevalence and incidence of both dental caries and periodontal disease were low among chewing stick users compared with toothbrush and toothpaste users [Carl and Zambon, 1993; Schier and Cleaton-Jones, 1995; Sathananthan et al., 1996; Darout et al., 2000; Batwa et al., 2006]. A recent study in Saudi Arabia by Al-Otaibi et al., [2003a] found that the Miswak was more effective than toothbrushing in reducing plaque and gingivitis, when preceded by professional instructions on its correct application. Batwa et al., [2006] concluded that the Miswak was as effective as a toothbrush in reducing plaque on buccal teeth surfaces. Current studies frequently lack specific details relating to the time, duration and frequency of Miswak use, which prevents meaningful assessments of the mechanical cleansing role of Miswaks in oral health [Hardie and Ahmed, 1995a, b].

Extracts from the roots and stems of *Salvadora Persica* have shown antimicrobial activity, 5% sulphur as a major antimicrobial constituent [Ezmirly et al., 1979]. Al lafi and Ababneh, [1995] reported that trimethylamine, benzylisothiocyanate, B-sitosterol, m-anisic acid, salvadourea, chlorides, silica, large amounts of NaCl, KCl, sulphur, vitamin C, tannins, saponins, flavenoids and sterols, all of which are present in Miswaks, contributed to the Miswak's antibacterial effect on *Staphylococcus aureus* and other aerobic and anaerobic bacteria. Benzylisothiocyanate, a component exhibiting antiviral and antimycotic activity, has been shown to inhibit *in vitro* growth and acid production by mutans streptococci, but its mode of action has not been clearly delineated [Al-Bagieh et al., 1994]. The substantial amount of silica detected in *Salvadora Persica* ashes has been thought to contribute to the Miswak's mechanical action in plaque removal [Almas and Al-Lafi, 1995]. The potential contribution by fluoride was considered unlikely, as it is soluble and its total content in the Miswak, especially that released when soaked in water, is negligible (< 0.07 μg/ml) [Hattab, 1997; Wu et al., 2001; Al-Otaibi, 2004].

Despite increasing numbers of clinical surveys and epidemiological studies of chewing sticks and their oral health benefits, relatively few studies have looked into the antimicrobial effect of chewing stick extracts on oral pathogens associated with

caries and periodontal disease. In 2004, Almas and Al-Zeid, [2004] conducted a clinical study to assess the antimicrobial activity of the Miswak and they revealed that mutans streptococci were more susceptible to Miswak antimicrobial activity than lactobacilli. Mouth rinsing with an aqueous extract of Miswaks significantly reduced the bacterial counts in salivary samples obtained up to 3 hours after rinsing [Gazi et al., 1992]. The extracts also inhibited glycolytic reactions by the salivary bacteria for up to 90 min post-rinsing. In a recent study by Almas et al., [2005], comparing the antimicrobial effect of a Miswak-based mouthwash with other commercially available non-alcohol mouth rinses, a mild reduction in mutans streptococci was noted.

The World Health Organisation (WHO) has recommended and encouraged the use of chewing sticks as an effective tool for oral hygiene [World Health Organisation, 1987]. With a better understanding of the properties, clinical effectiveness and the development of effective techniques with the emphasis on the frequency and thoroughness of the cleaning, chewing sticks may represent an equivalent or alternative instrument to the toothbrush for the prevention and control of dental diseases in developing countries [Al-Otaibi, 2004].

Fluoridation of toothpicks and other products

At the beginning of the 1980s, the idea of impregnating toothpicks in fluoride solutions stimulated two Norwegian researchers, [Mørch and Bjørvatn, 1981], who studied fluoride-impregnated toothpicks in the laboratory. The effect of fluoridated toothpicks was studied in a thesis by Kashani, [1998]. Toothpicks made of both birch and lime were found quickly to take up and release fluoride into the approximal area. Kashani and co-workers [Kashani et al., 1995; Kashani, 1998; Kashani et al., 1998b] carried out extensive studies on toothpicks impregnated in 1%, 2%, 3% and 4% NaF for 30 sec, 30 min or 3 days, which were used *in vivo* for 2 min. The results revealed that there was a clear dose-response effect and that the concentration of fluoride was highest during the first 5 min. Significantly higher values were found in saliva for toothpicks impregnated in 4% NaF compared with 1% and 2% NaF. *In vivo*, when impregnated toothpicks were compared with some other fluoride products like mouthrinse solution, tablets and dentifrice, the result found that the fluoride concentration in whole saliva increased up to 60 min.

A recent thesis at our department Särner, [2008] showed that there are large variations in the fluoride release from various fluoridated toothpicks and dental flosses. Treatment with a fluoridated toothpick or dental floss can be expected to produce elevated fluoride concentrations in the approximal area for up to 60 min. Another interesting method for administering fluoride into the approximal area is to use an interdental brush dipped in a fluoride gel [Särner et al., 2008].

Use of chewing sticks in the remineralisation of caries lesions

In individuals with a high caries risk, such as orthodontic patients, extra means of fluoride home care and professional fluoride application are recommended [Zimmer, 2001; SBU, 2002; Ellwood et al., 2008]. The demineralisation of enamel adjacent to the brackets is a clinical problem during treatment and even after debonding, as shown in Fig. 2. They develop as a result of dietary carbohydrate and acid production in plaque, resulting in an imbalance between the demineralisation and remineralisation of the enamel [Benson et al., 2004]. This is an interrupted process, with periods of remineralisation and demineralisation, depending on the state of the oral environment in terms of the prolonged accumulation and retention of bacterial plaque on the enamel surface, the standard of individual oral hygiene and the fluoride exposure [Featherstone, 2000; Aoba, 2004]. A white spot lesion (WSL) is the precursor of frank enamel caries. The white appearance of early enamel caries is due to an optical phenomenon, which is caused by mineral loss in the surface or subsurface enamel. Enamel crystal dissolution begins with subsurface demineralisation, creating pores between the enamel rods. The affected area is a consequence of both surface roughness and the loss of surface shine and alterations in internal reflection. This results in greater visual enamel opacity, since porous enamel scatters more light than sound enamel [Gorelick et al., 1982; Øgaard, 1989b]. The demineralisation process may encompass the full thickness of the enamel and some of the dentine before the relatively hyper-mineralised surface layer is actually lost.

It is generally accepted that the insertion of fixed orthodontic appliances creates stagnation areas for plaque and makes tooth cleaning more difficult. The irregular surfaces of brackets, bands, wires and other attachments also limit naturally occurring self-cleaning mechanisms, such as the movement of the oral musculature and saliva [Ng'ang'a and Øgaard, 1993]. This in turn encourages a lower plaque-pH in the

presence of carbohydrates and accelerates the rate of plaque accumulation. These changes in the local environment appear to favour colonisation by aciduric bacteria, such as mutans streptococcus and lactobacilli. It has been reported that these levels can increase up to fivefold during orthodontic treatment [Sudjalim et al., 2006]. Since orthodontic treatment may result in temporary elevations in the number of cariogenic micro-organisms, the use of antimicrobials, such as chlorhexidine, may be justified in some patients [Derks et al., 2004].

It has been reported that there is a significant increase in the prevalence and severity of enamel demineralisation after orthodontic treatment when compared with untreated control subjects [Lovrov et al., 2007]. The overall prevalence of WSL among orthodontic patients has been reported to be anywhere between 50% and 96% [Gorelick et al., 1982; Øgaard et al., 1988; Gorton and Featherstone, 2003; Boersma et al., 2005]. Once active orthodontic treatment has been completed and the patient has been debonded, the demineralisation process is normally expected to decline due to positive changes in local environmental factors. Some WSL may remineralise and return to either normal or at least a visually acceptable appearance. On the other hand, some lesions may persist, leading to an aesthetically unacceptable result. In severe cases, restorative treatment may be required [Øgaard, 1989a; Sudjalim et al., 2006; Sudjalim et al., 2007].

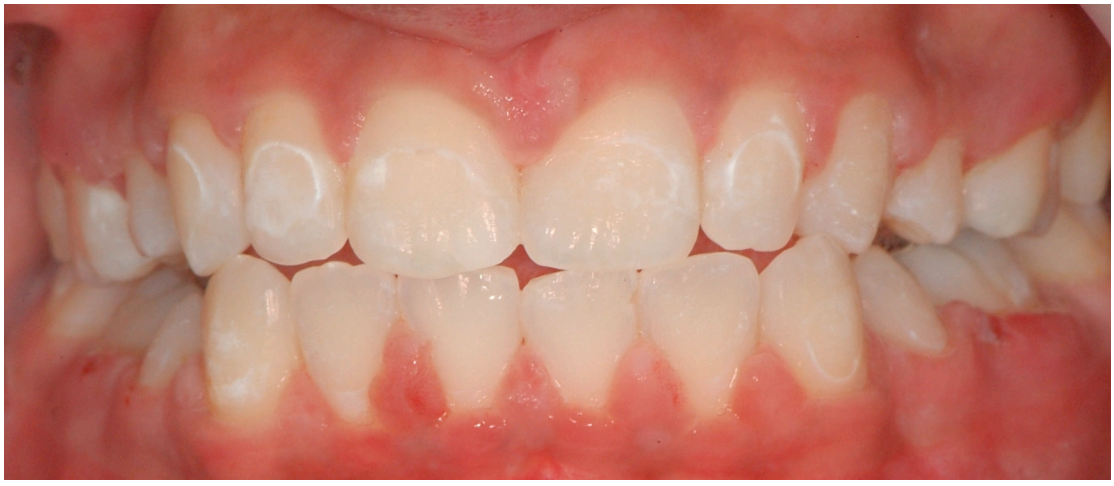

Fig. 2. White spot lesions after orthodontic treatment at debonding visit.

The overall management of WSL takes methods to prevent demineralisation and encourage remineralisation into consideration. Preventive measures play a pivotal role

and are challenging, especially when treating patients with a high caries activity. In addition to regular professional oral hygiene visits, dietary control and the application of appropriate fluoride products, successful preventive strategies include oral health promotion, patient education and patient motivation.

From this point of view, the impregnation of chewing sticks with fluoride appears to be worth studying. This thesis was conducted to evaluate possible ways of fluoridating Miswaks, to study the fluoride release both *in vitro* and *in vivo* and to examine the effect of fluoridated Miswaks on remineralisation of white spot lesions (WSL).

Aims

General aim

The present thesis aimed at evaluating the uptake and release of the fluoride from fluoridated Miswaks *in vitro* and the oral fluoride retention and its effect *in vivo*.

Specific aims

1. To study the uptake and release of fluoride from fluoridated Miswaks both *in vitro* and *in vivo* (Papers I & II)
2. To evaluate the optimal and standard level of fluoride concentration in the fluoridated Miswak *in vivo* to be used on a daily basis (Paper II)
3. To evaluate the oral fluoride retention and concentration in whole saliva after using different concentrations of fluoridated Miswaks compared with brushing with fluoride toothpaste *in vivo* (Papers I, II & III)
4. To compare the oral fluoride retention and concentration after using fluoridated Miswaks in orthodontic patients before and after removal of their fixed appliances *in vivo* (Paper III)
5. To investigate the effect of fluoridated Miswaks on the remineralisation of white spot lesions (WSL), which have developed around orthodontic bands and brackets during orthodontic treatment *in vivo* (Paper IV)

Material and Methods

Study design

Paper I

The uptake and release of fluoride from fluoridated Miswaks in four different NaF concentrations and three different impregnation times were evaluated *in vitro* and *in vivo* in a clinical experimental study.

Paper II

The release of the fluoride from different NaF concentrations of fluoridated Miswaks was evaluated *in vitro* in two different test series (Series I & II) in an experimental study. The same products were tested to evaluate the oral fluoride retention *in vivo* compared with fluoride toothpaste as a control in a double-blind, randomised, cross-over experimental study.

Paper III

The effects of fluoride release and oral retention of six different home-care fluoride products were tested in orthodontic patients before and after the removal of their fixed appliances in a randomised, cross-over clinical study *in vivo*.

Paper IV

The remineralisation effect of fluoride released from fluoridated Miswaks on WSL in post-orthodontic patients was evaluated in a 6-week longitudinal, randomised, double-blind clinical study.

Subjects, test products and treatments

Paper I

In vitro. Three experimental series were conducted using 200 prepared Miswaks. In the first series, 120 pieces (10 pieces x 4 concentrations x 3 impregnation times) were impregnated in 1%, 2%, 3% and 4% NaF for 3 hours, 1 day and 3 days. A total of 12 different types of fluoridated Miswak were evaluated for both the uptake and release of fluoride. In the second series, 20 Miswaks were impregnated in 3% NaF for 1 day. The third series was identical to the second, except that the impregnation time was

prolonged to 3 days. The bark was separated from the pulp and the two parts placed in separate vials.

In vivo. Nine healthy adult volunteers, mean age 60 years, were recruited from the staff at the Institute of Odontology in Gothenburg. All the subjects were instructed not to use any fluoride-containing oral hygiene products and to reduce their intake of fluoride-containing food products to a minimum of 48 hours prior to each test occasion. They were not allowed to eat/drink, use tobacco or snuff or brush their teeth one hour before each test session. Three different fluoridated products were used: 1) Miswaks impregnated in 3% NaF for 1 day, 2) Miswaks impregnated in 3% NaF for 3 days and 3) 1 gram of fluoride toothpaste (containing 1450 ppm fluoride as NaF) applied to a toothbrush. The cover of the Miswak was first removed. It was then chewed for a short time and moved around the dentition to clean all the buccal and buccally oriented approximal surfaces for 2 min (Fig. 1A). Toothbrushing was carried in a normal manner for 2 min. For each individual, the tests were carried out at the same time of the day. There were a total of 27 visits for all patients, testing 3 products (two different fluoridated Miswaks and one regular fluoridated toothpaste) during an experimental period of 4 weeks.

Paper II

For both the *in vitro* and the *in vivo* experimental studies, a total of around 400 pieces of Miswak were used.

In vitro. Two series were performed using 200 prepared Miswaks. In the first, 100 pieces (50 old and 50 fresh) were impregnated in 0.01%, 0.1%, 0.5%, 1% and 3% NaF for one day and were used to evaluate the difference in fluoride release between old and fresh Miswaks. In the second series, a total of 100 pieces, selected randomly from 10 different stores (10/store), were impregnated in 0.5% NaF for one day and used to evaluate whether there were any differences in the fluoride release between the Miswaks that were purchased from 10 different stores.

In vivo. A total of 20 healthy adult volunteers were recruited from patients and the staff at the Institute of Odontology in Gothenburg. They participated in four series (called I-IV); in Series I and II, there were 10 healthy adults (6 men and 4 women) and, in Series III and IV, there were 10 other healthy adults (6 women and 4 men). All the subjects were instructed not to brush their teeth with fluoride-containing

toothpaste on the day of the examination and not to eat or drink for at least 2 hours before the test. Five Miswaks, fluoridated in 0.01%, 0.1%, 0.5%, 1% and 3% NaF for 1 day (Series I), were tested to evaluate the "fluoride intake" when using a Miswak. The subject was instructed not to swallow any saliva but instead to spit into a beaker during this 2-min period and for the next 10 min. After this 2+10=12-min period, the patient finally rinsed his/her mouth with 20 ml of distilled water for 30 sec and spat it out into the same beaker. In the second (Series II), another five Miswaks impregnated in 0.01%, 0.1%, 0.5%, 1% and 3% NaF for 1 day were used to study the fluoride concentration in saliva after using a fluoridated Miswak for 2 min. Series III & IV were identical to Series I & II, except that a more narrow range of NaF impregnation solutions (0.05%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% NaF for 1 day) was used. Moreover, the subjects brushed with 1 g of fluoride toothpaste (containing 0.32% NaF) applied to a toothbrush.

Paper III

Nine healthy orthodontic patients (7 females and 2 males), with a mean age of 16 years (range 14-19), almost at the stage of debonding, were recruited from the specialist orthodontic clinics at the Institute of Odontology in Gothenburg. They used six different home-care fluoridated products before and after the removal of their fixed appliances. Each patient completed the 12 visits within 3 months. The products were: 1) toothpaste with 0.32% NaF (Pepsodent, Lever Fabergé, Stockholm, Sweden), 2) toothpaste with 1.1% NaF (Duraphat, Colgate-Palmolive, Glostrup, Denmark), 3) mouthrinse solution with 0.05% NaF (Dentan, Meda, Stockholm, Sweden), 4) mouthrinse solution with 0.2% NaF (Dentan, Meda), 5) Miswaks impregnated in 0.05% NaF for one day and 6) Miswaks impregnated for one day in 0.5% NaF. Prior to each test, the subjects chewed on paraffin wax for 5 min and rinsed with distilled water for 30 sec.

Toothpaste. 1 g of paste was applied to a wet toothbrush and the subject brushed his/her teeth for 2 min. After brushing, the remaining toothpaste was spat out and the mouth was rinsed with 5 ml of water for 5 sec.

Mouthrinse solution. 10 ml of the solution were swished around in the mouth for 2 min with active movements of the cheeks and lips.

Miswak. The cover was first removed and the participants then chewed on the stick for a short time (3-5 sec), before it was moved around the dentition to clean all the buccal tooth surfaces and all the buccally oriented approximal surfaces for 2 min.

Paper IV

Thirty-seven orthodontic patients (11 males and 26 females), with a mean age of 17.2 years, from three different dental hospitals in Jeddah, Saudi Arabia: 1) the King Fahad Armed Forces Hospital, 2) the King Faisal Specialist Hospital and Research Centre and 3) the King Fahad General Hospital were recruited after completion of their orthodontic treatment. The inclusion criterion was at least 2 WSL on both the left and right side of the dentition in the upper jaw, i.e. a minimum of 4 WSL/subject (total of 152 WSL in the test group and 140 WSL in the control group). Two different products were used: 1) fluoridated Miswaks impregnated in 0.5% NaF for one day and 2) non-fluoridated Miswaks (natural fresh Miswaks). The participants were randomly divided into two groups: 1) a test group with 19 patients using fluoridated Miswaks and 2) a control group with 18 patients using non-fluoridated Miswaks. The two types of Miswak were identical except for their fluoride content. An individual plastic mouth tray (Essix, Dentsply, Parkland, USA) covering half the dentition was used (Fig. 3). The tray was applied to the teeth immediately after toothbrushing and was kept in place, while using the Miswak and for a minimum of 30 min afterwards. The Miswaks were used 5 times/day. The WSL were scored using a DIAGNOdent pen, as well as the International Caries Detection and Assessment System (ICDAS II) index, at baseline and 2, 4 and 6 weeks after debonding.

The DIAGNOdent device has been studied extensively both *in vivo* and *in vitro* for detection of caries lesions on occlusal and smooth surfaces [Lussi et al., 1999; Shi et al., 2000; Attrill and Ashley, 2001; Lussi et al., 2001; Alwas-Danowska et al., 2002; Costa et al., 2002; Ouellet et al., 2002; Baseren and Gokalp, 2003; Cortes et al., 2003; Francescut and Lussi, 2003; Kordic et al., 2003; Bader and Shugars, 2004; Fung et al., 2004; Mendes et al., 2005]. Recent studies by Anttonen et al., [2003, 2004] and Sköld-Larsson et al., [2004] support the application of the method for detection and monitoring of caries. The DIAGNOdent has been tested *in vitro* for quantification of lesions adjacent to fixed orthodontic appliances [Staudt et al., 2004].

The International Caries Detection and Assessment System (ICDAS), based on

visual inspection, was developed for use in clinical research, clinical practice and for epidemiological purposes [Pitts, 2004]. The system was intended to be feasible for use in epidemiological surveys and to detect cavitated and non-cavitated caries lesions with acceptable reliability [Pitts, 2004; Ismail et al., 2007].

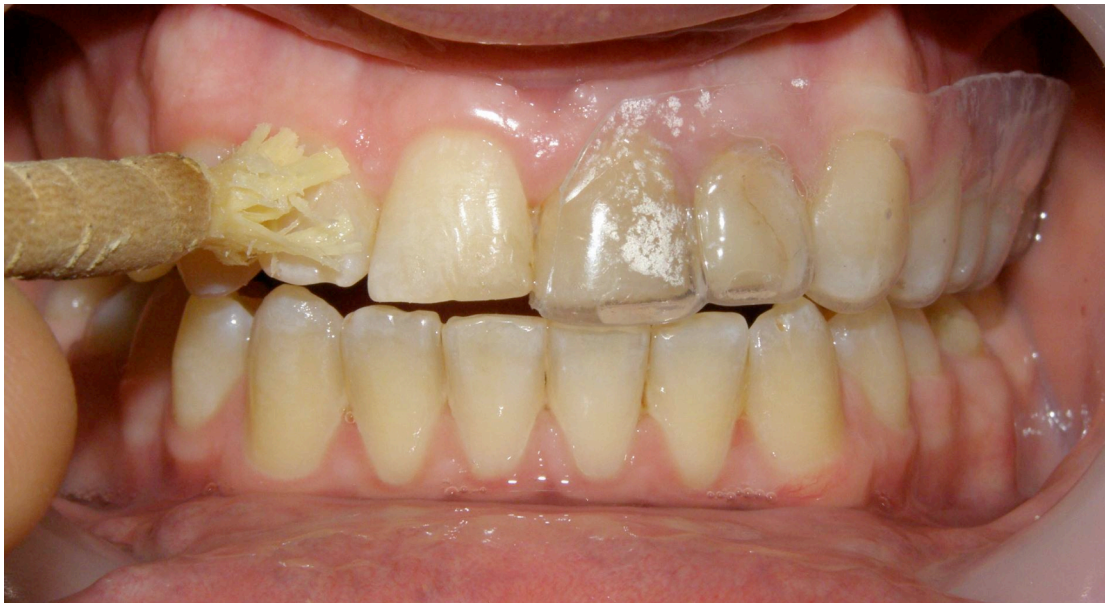

Fig. 3. Use of fluoridated Miswaks for cleaning the teeth with a custom-made mouth tray covering half the dentition in the upper jaw in Study IV. The cleaning was only performed in the non-covered area.

Miswaks preparation and fluoridating procedure

Fresh Miswaks bundles were purchased and transported from the Kingdom of Saudi Arabia to Sweden and kept in a refrigerator for 2 weeks prior to the start of each study. Each bundle contained 15-cm long sticks from the *Salvadora Persica* tree. From these bundles, five different sticks were selected randomly and cut with a scalpel into different lengths (3-4-cm long pieces). Fluoridation was carried out at Department of Cariology in Gothenburg for all four studies (I-IV). Fluoridated Miswaks were packed and transported back to Saudi Arabia one week prior to the start of Study IV.

Miswaks are natural products and there are great variations in size, diameter and colour (Fig. 4A). We tried to control for these factors, by visual inspection, in order to have all the specimens as similar as possible, especially with regard to size and weight (Fig. 4B). After cutting and coding, they were kept in distilled water for 6 hours (in order to clean them from dust). They were then dried in an oven at 40°C overnight

(Fig. 4C). For each concentration, Miswaks were placed in a vial with 500 ml of NaF solution for 3 hours, 1 day and 3 days in Paper I and for one day in Papers II-IV, i.e. with an excess of solution (Fig. 4D). After impregnation, the Miswaks were removed from the bottle (with forceps) and placed on paper at room temperature overnight in order to dry (Fig. 4E). They were packed in a clear plastic sealed nylon bag (Fig. 4F) and stored in a refrigerator to keep them clean.

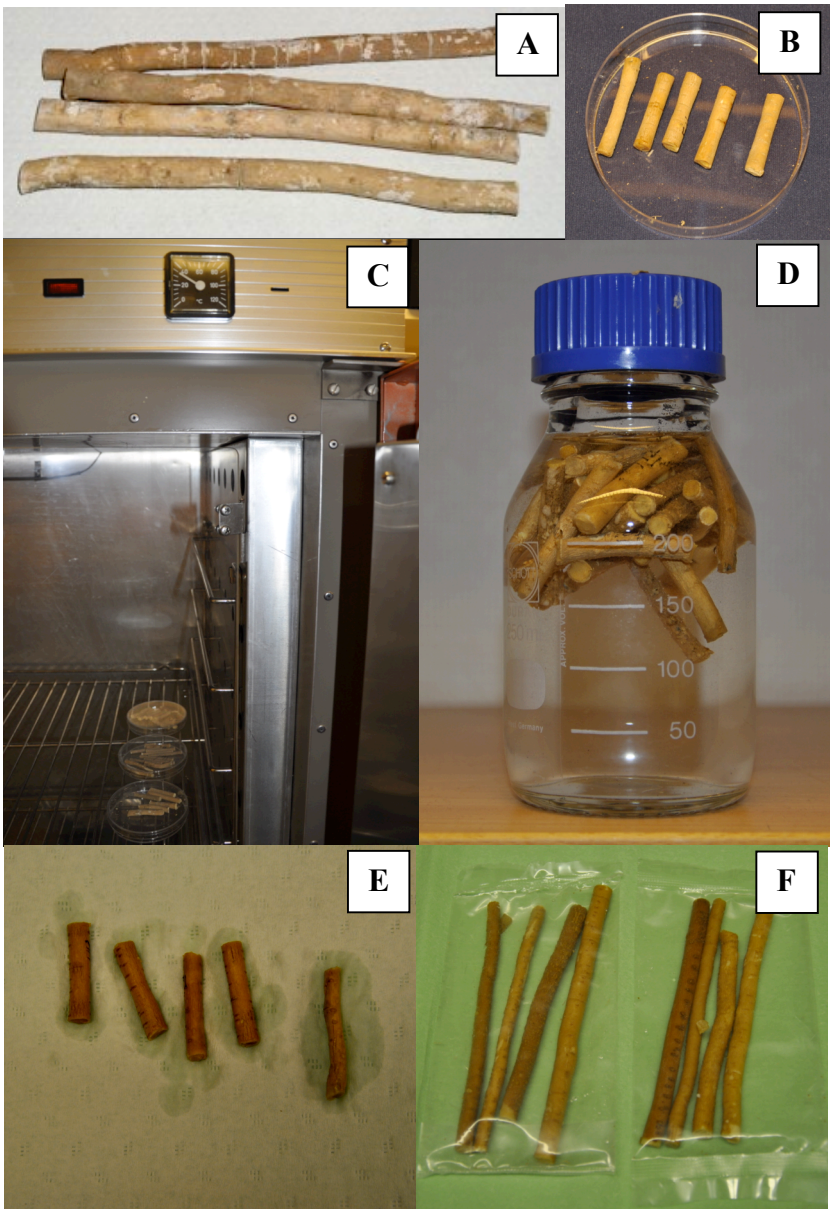

Fig. 4. A) Miswaks showing variations in size, colour and diameter. **B)** 3-cm-long pieces of Miswak cut to be as similar as possible. **C)** Drying of Miswaks in the oven at 40°C. **D)** Miswaks impregnated in NaF solution. **E)** Drying Miswaks after impregnation on paper at room temperature overnight. **F)** Packing Miswaks in a sealed clear plastic bag.

Scanning electron microscope (SEM)

One non-impregnated Miswak and one Miswak impregnated with 3% NaF were air-dried, spattered with gold and analysed in a Scanning Electron Microscope (SEM) (Leica S420; Leica Microsystems, Heidelberg, Germany, equipped with LEO Software 15XX) in order to examine the NaF crystal on the surface of the Miswak. Figures 5A and C show a magnified cross-section of the fluoridated Miswaks, showing clear multiple shiny dots representing NaF crystal deposition inside the pulp and on the bark of the Miswaks. Figures 5B and D show non-fluoridated Miswaks with no NaF crystals.

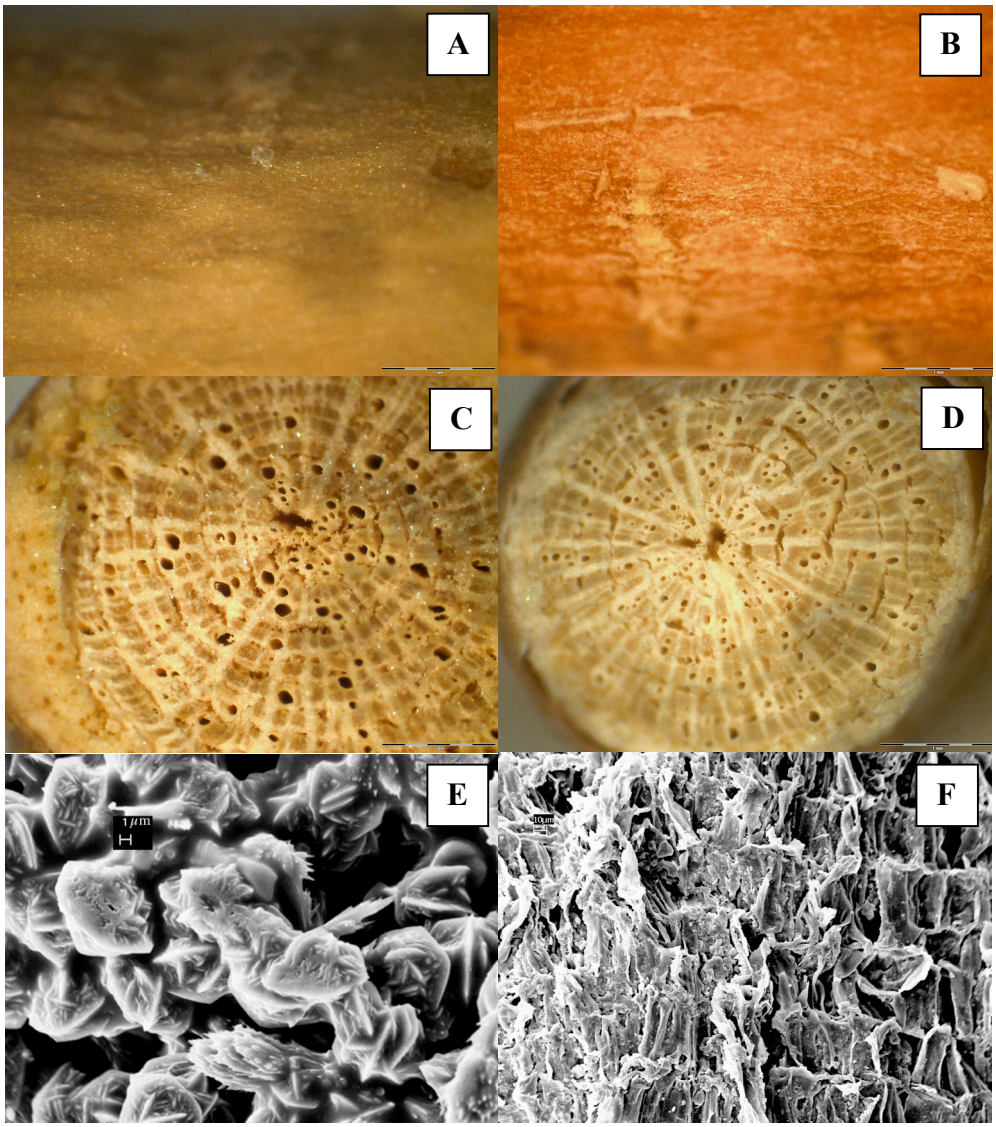

Fig. 5. A) White NaF crystal on the bark of a fluoridated Miswak. **B)** The bark of a non-fluoridated Miswak. **C)** Multiple shiny dots in a cross-section of a fluoridated Miswak showing a deposit of NaF crystals. **D)** Cross-section of a non-fluoridated Miswak. **E)** SEM of the outer surface (bark) of a fluoridated Miswak. **F)** SEM of the outer surface (bark) of a non-fluoridated Miswak.

SEM analysis images of a Miswak impregnated in 3% NaF is shown in Fig. 5E. There were "snowflake"-like crystals on the outer surface (bark) of the Miswak. However, the shape of these crystals looked different compared with NaF crystals found on a glass surface. Figure 5F shows SEM analysis images of a non-fluoridated Miswak. There were mesh-shaped fibres on the outer surface (bark) of the Miswak with no signs of any crystal deposits.

Sampling procedure

In vitro. After drying, each piece was transferred to a 20-ml bottle with 15 ml of distilled water and 1.5 ml of TISAB III solution (Orion Research, Boston, MA, USA). 0.3-ml aliquots were sucked up with a pipette on nine occasions (0, 1, 2, 5, 10, 15, 30, 40 and 60 min) and transferred to a 2-ml beaker covered with a lid. The samples were then kept in a refrigerator for up to one week before the fluoride analysis.

In vivo. Sampling of two approximal sites in Paper I (14/15 and 24/25) and in Paper III (15/16 and 24/25) was carried out before (0 min; baseline) and after 1, 3, 5, 7, 9, 15, 30 and 60 min after finishing the 2-min cleaning procedure. The sampling method according to Kashani et al. [1998] was used. Small triangle-shaped paper points (base: 1.5 mm; length: 5 mm) were inserted in the approximal area for 30 sec in order to suck up around 4 µl of saliva (Fig. 6). The paper points were then transferred to Eppendorf tubes, containing 200 µl of de-ionised water and 20 µl of TISAB III buffer solution (dilution 10:1; Thermo Electron, Waltham, Mass., USA).

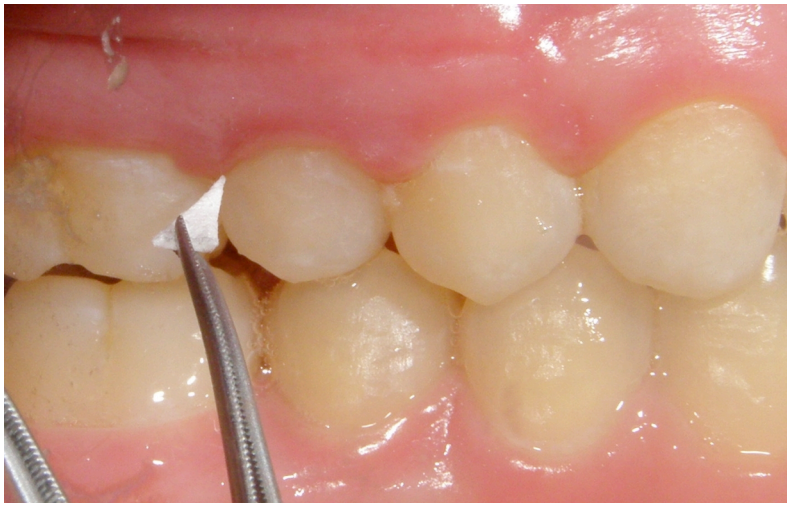

Fig. 6. Sampling of approximal fluid with triangular paper points.

In Papers II & III, resting whole saliva was collected just before (0-min sample; baseline) and after 1, 3, 5, 10, 15, 30 and 60 min, making 8 samples/test. For Paper II, a total of 800 saliva samples were collected for the *in vivo* study (9 subjects x 5 products x 1 site x 8 time points x 2 series). For Paper III, a total of 864 saliva samples were collected (9 subjects x 6 products x 1 site x 8 time points x 2 occasions).

Fluoride analysis

All the samples were analysed blind in terms of the subjects and methods. The same technician analysed all the samples.

In vitro. The fluoride concentration was determined by means of a fluoride-sensitive electrode connected to an expandable ion analyser (Orion Research, Boston, MA, USA). Ionic strength was stabilised by adding a buffer solution (TISAB III) according to the manufacturer's instructions. All the analyses were performed using standard solutions from 0.526 μM (0.01 ppm) to 5.26 mM (100 ppm) fluoride. The detection level of fluoride was approximately 0.5 μM.

In vivo study. Each paper point was transferred to a 0.5-ml Eppendorf tube, containing 200 μl of distilled water and 20 μl of TISAB III (Orion Research). The absorbed fluoride was allowed to diffuse from the paper point into the solution for 24 hours in a refrigerator. Prior to analysis, the samples were thoroughly mixed by vibration for 10 sec. The fluoride concentration was then analysed using the ion-specific electrode.

Ethical considerations

The first three studies (I-III) were approved by the Ethics Committee at the University of Gothenburg. Study IV was approved by the Ethics Committee at 1) the King Fahad Armed Forces Hospital, 2) the King Faisal Specialist Hospital and Research Centre and 3) the King Fahad General Hospital. Both verbal and written information about the individual studies was given to the subjects. Written informed consent was obtained from all the subjects prior to the start of each study. All the subjects were coded when entering the individual studies and the statistical analyses were performed with unidentifiable data.

Statistical methods

The sample size was based on a simple power analysis that was performed before the start of each study (Papers I-III) and from earlier studies by Kashani et al., [1998] and Särner et al., [2003]. Even if statistically significant differences were obtained with six individuals, it was nonetheless decided to include 9-10 subjects in all the *in vivo* series.

In Papers I & II, the area under the curve (AUC) was calculated using the KaleidaGraph software program version 4.0 (Synergy Software, Reading, Pa., USA) for each individual and each treatment. Statistical comparisons were made using two-way analysis of variance (ANOVA), $p < 0.05$ was considered statistically significant. In Paper II, an unpaired t-test was applied to the 60-min fluoride values *in vitro* in order to compare old and fresh Miswaks, as well as Miswaks from different stores.

In Paper III, means and standard deviations were calculated for each F product with and without the fixed orthodontic appliances. AUC was measured then the mean differences between the AUC values with and without orthodontic appliances and between the various products were compared using a paired t-test ($p < 0.05$ was considered to be statistically significant). Statistical comparisons using three-way analysis of variance (ANOVA) were also performed, which confirmed the result of the paired t-test.

In Paper IV, a power analysis was performed before the start of the study and a sample size of 17 patients/group was suggested. Means and standard deviations were calculated for each visit and patient for both ICDAS II and DIAGNOdent pen measurements. The mean values were calculated for the teeth in one and the same quadrant. The mean changes (Δ) for baseline vs. 6 weeks for the covered and a non-covered side were analysed using a paired t-test. For comparisons between the test and control group, a non-paired t-test was used. As a multiple t-test was used, the correlation between the ICDAS II index score and the DIAGNOdent readings at baseline and at 6 weeks was analysed using Pearson's correlation coefficient.

Results

Studies I & II

In vitro. A clear dose-response effect with respect to fluoride concentration and impregnation time was found; the higher the NaF solution used and the longer the impregnation time, the greater the release of fluoride (Paper I). Moreover, fresh Miswaks produced a slightly higher fluoride release than old Miswaks, especially at high NaF impregnation concentrations (i.e. at 1% and 3% NaF). However, there were no statistically significant differences between fresh and old Miswaks at 60 min for any of the five NaF concentrations (Paper II).

The release of fluoride was linear with time, but a more rapid release during the first 10 min, especially when impregnated in 3% and 4% NaF, was found in Studies I & II (Fig. 7).

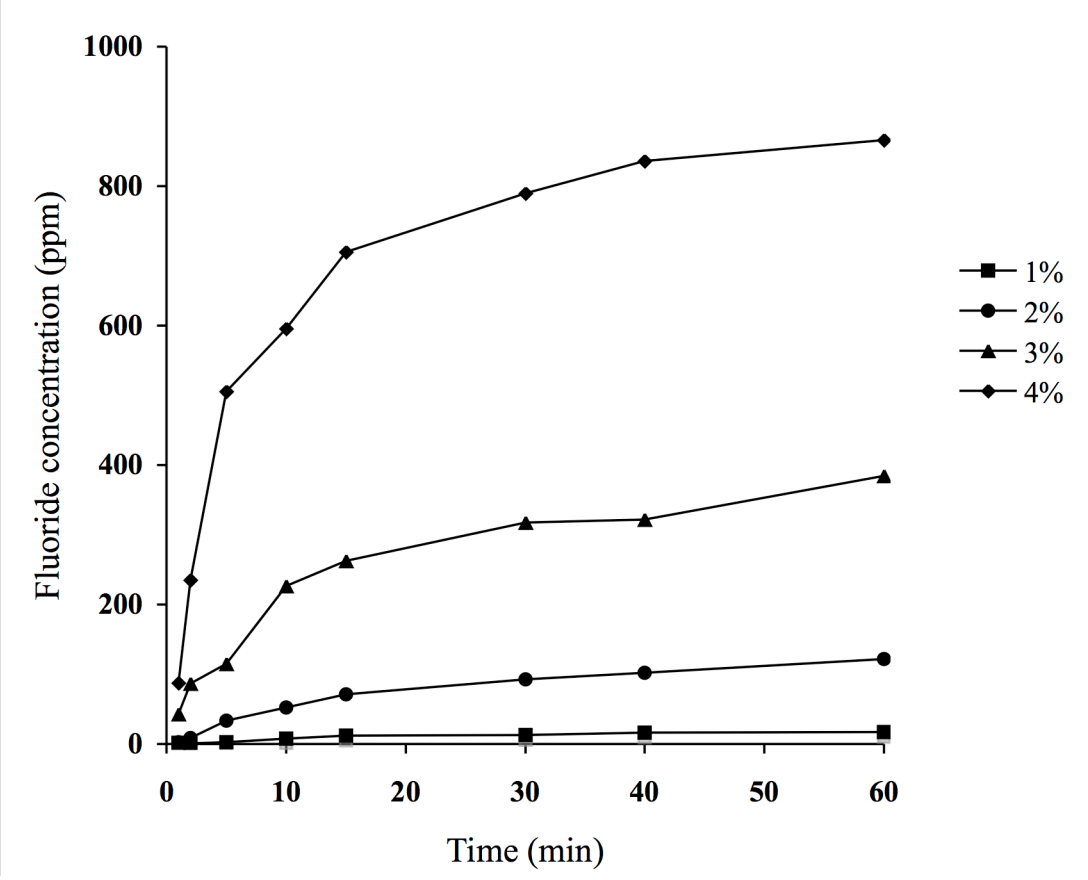

Fig. 7. The release of fluoride *in vitro* from Miswaks during 60 min. Each Miswak was impregnated in 1%, 2%, 3% or 4% NaF for 3 days and dried and placed in 5 ml of distilled water (mean values of 10 samples/test).

The bark released more fluoride than the pulp, but both parts produced a fast, high release, especially during the first 10 min. The results for impregnation in 3% NaF for 3 days showed parallel curves for the bark and the pulp, as shown in Fig. 8.

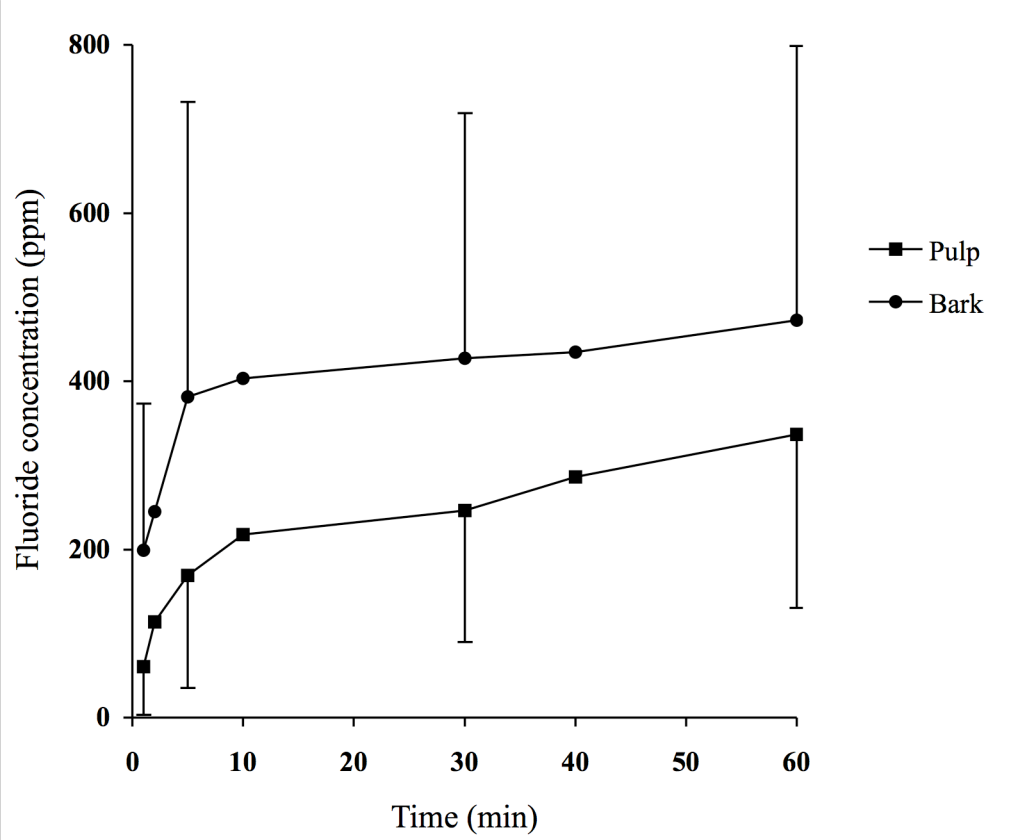

Fig. 8. The release of fluoride from the bark and the pulp of Miswaks impregnated in 3% NaF for 3 days (mean values of 20 samples/test). The standard deviation in one direction is given for every second value.

The differences in the weight of dried Miswaks before and after 3 hours' impregnation with NaF solution are shown in Table 1. The higher the NaF concentration used, the greater the uptake of fluoride by the Miswaks (Papers I & II).

Table 1. The weight (in grams) of 3-cm Miswaks after drying before and after impregnation in NaF solution for 3 hours and the difference, which indicates the uptake of NaF (mean values ± SD of 10 pieces/test).

NaF concentration	Difference (uptake) Between before and after impregnation
1%	0.20±0.04
2%	0.24±0.03
3%	0.42±0.11
4%	0.47±0.06

In vivo. For Study I, the fluoride concentration in the approximal area was elevated during the entire 30-min period and the AUC was twice as high for the Miswaks impregnated for 3 days compared with 1 day and four times higher compared with fluoride toothpaste ($p < 0.05$ and $p < 0.01$ respectively). The mean salivary fluoride concentration and the AUC values of Miswaks impregnated in 0.1-0.3% NaF produced about the same fluoride level as brushing with fluoride toothpaste (Fig. 9). The mean AUC in Series II was twice as high for the Miswaks impregnated in 3% NaF compared with 0.5% NaF ($p < 0.0001$) and four times higher compared with 0.1% NaF ($p < 0.0001$). In Series IV, the mean AUC was three times as high for Miswaks impregnated in 0.5% NaF compared with 0.05% NaF and twice as high compared with 0.1% NaF.

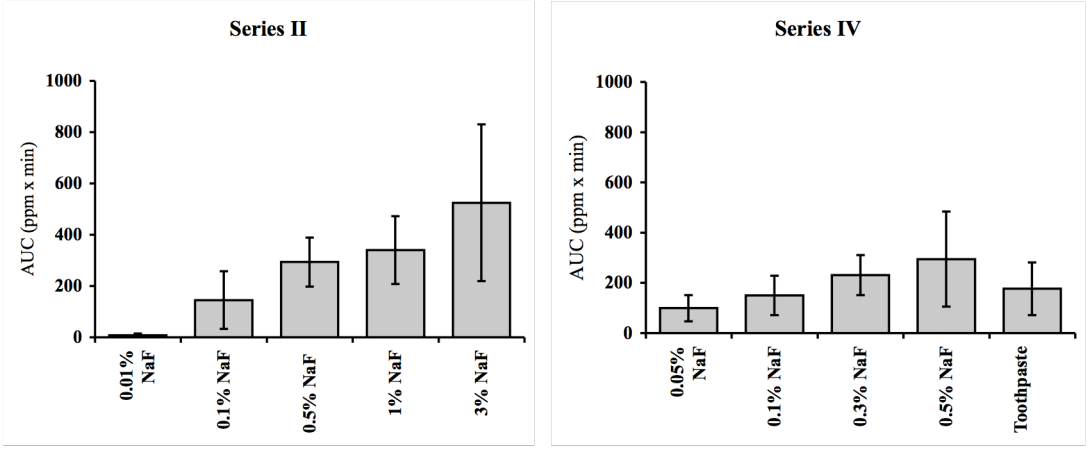

Fig. 9. The AUC values (mean and SD; n=10) in saliva *in vivo* from Series II (0.01%, 0.1%, 0.5%, 1% and 3% NaF) and Series IV (0.05%, 0.1%, 0.3%, 0.5% and toothpaste; the data from 0.2% and 0.4% are not shown).

The mean ± SD for the oral "fluoride intake", which is expressed as mg of fluoride for 6 of the 11 different NaF impregnation solutions, are shown in Table 2. Approximately 27-29 ml of saliva were collected during the 12 min, including the final mouthrinse with 20 ml of water. The calculated "fluoride intake" increased at higher NaF impregnation concentrations. At 0.1% NaF, it was a mean of 0.21 mg of fluoride (range 0.06-0.44) and, at 0.5% NaF, about twice as much, i.e. 0.39 mg of fluoride (range 0.21-0.61).

Table 2. The results from Series I and III *in vivo*. Both the total volume of saliva (ml), collected during 10+2=12 min, and the fluoride concentration (ppm) in saliva, including a mouth rinse with 20 ml of water, are shown. The "fluoride intake" was calculated as ml saliva x ppm fluoride and expressed as mg fluoride (mean ± SD and range of 10 individuals).

Impregnation solution (NaF)	Saliva + water rinse		"Fluoride intake" (mg F)*	
	Volume (ml)	F (ppm)	Mean ± SD	Range
0.1%	27.7±4.8	7.7±3.6	0.21±0.11	0.06-0.44
0.5%	28.1±5.7	14.0±5.6	0.39±0.13	0.21-0.61
3%	29.1±5.0	31.1±17.1	0.91±0.50	0.22-1.80

* Calculated as volume (ml) x ppm F / 1000 = mg F

Study III

Figure 10 shows the means and standard deviations of AUC in approximal saliva from the patients with orthodontic appliances; the six fluoride products are given in ranking order. 0.5% NaF-impregnated Miswaks produced the highest fluoride values, which was significantly higher compared with the other products ($p < 0.05$).

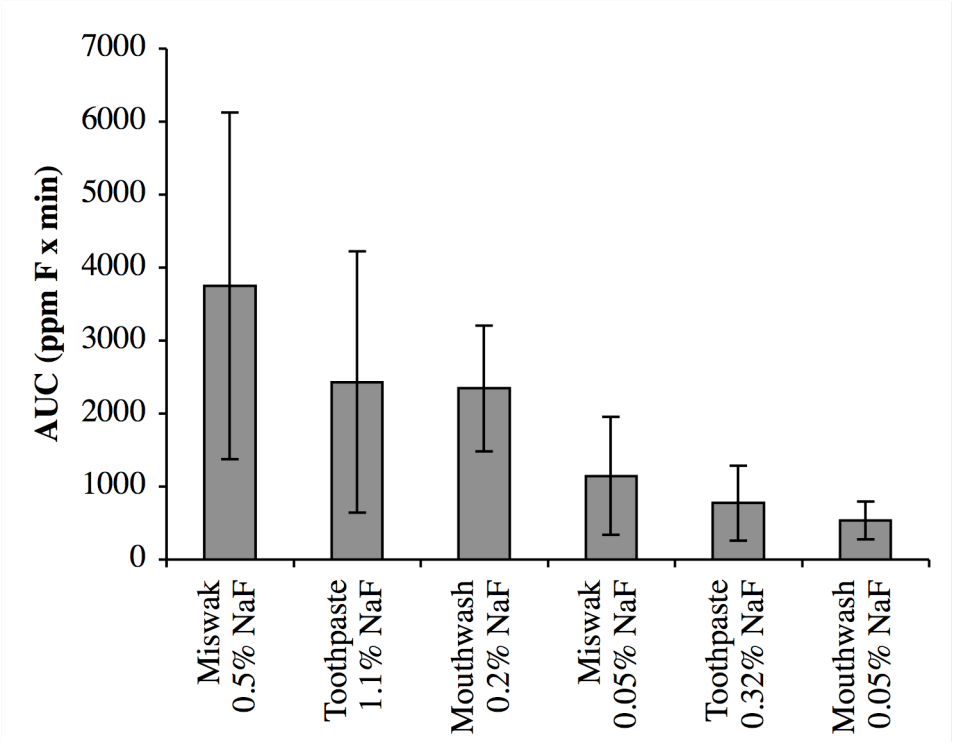

Fig. 10. The AUC values (mean ± SD) of the fluoride concentration in approximal fluid (mesial 25) from six different fluoride products, arranged in order from the highest to the lowest, in patients (n=9) wearing fixed orthodontic appliances.

Generally, in all the tests, the numerically highest fluoride release was mesial 25, followed by mesial 16. Using 0.5% NaF-impregnated Miswaks, both with and without orthodontic appliances, resulted in the highest fluoride retention in approximal saliva, especially at mesial 25, with statistically significant differences compared with all the other F products ($p < 0.001$).

The mean the AUC values with orthodontic appliances for 0.2% NaF mouthrinse solution, 1.1% NaF toothpaste and 0.5% NaF Miswaks are shown in Fig. 11. The fluoride concentration was high, especially during the first 10 min; there were no significant differences between the three sampling sites, except for Miswaks, which obtained higher values at the two approximal sites than in whole saliva ($p < 0.05$). Both the fluoride concentration and the AUC were about one and a half times higher for 0.5% NaF-impregnated Miswaks compared with 0.2% NaF mouthrinse solution and 0.5% NaF toothpaste ($p < 0.05$).

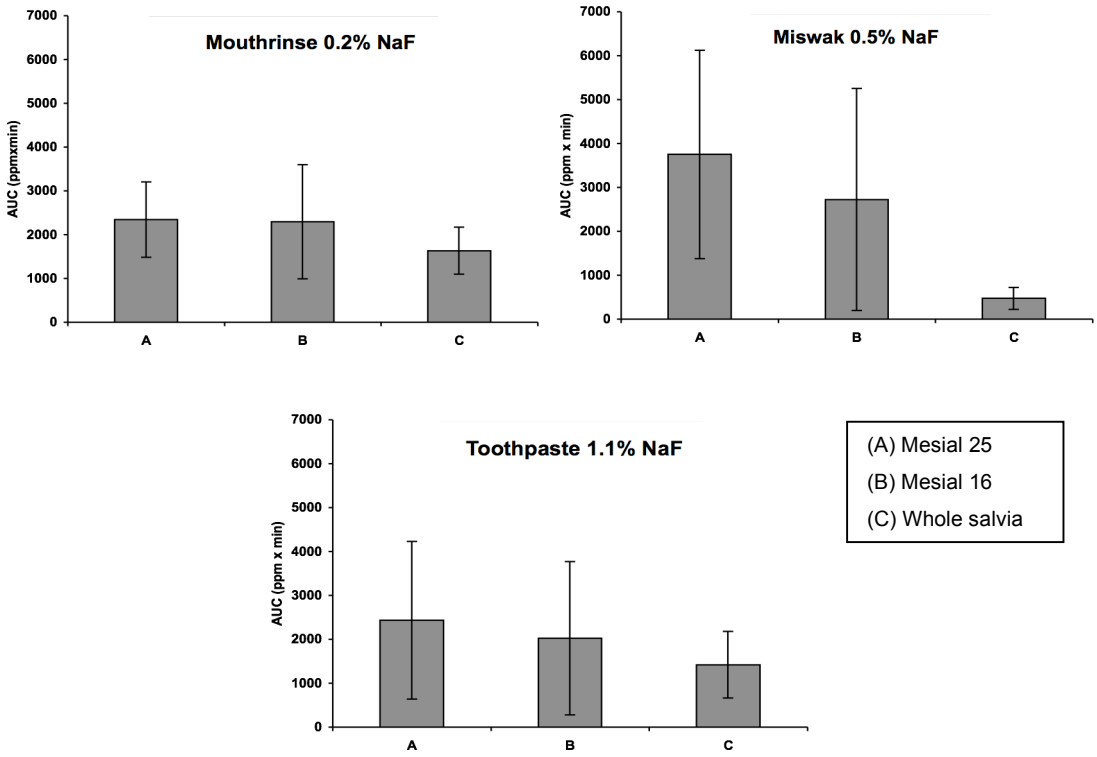

Fig. 11. Mean values (n=9) of the AUC values (0–60 minutes) both in saliva and at two approximal sites after using three fluoride products (0.2% NaF mouthrinse, 1.1% NaF toothpaste and 0.5% NaF-impregnated Miswaks) in orthodontic patients with fixed appliances.

Study IV

Figures 12 show the means and standard deviations of the readings from the DIAGNOdent pen at baseline and after 6 weeks for the non-covered quadrants. There was a gradual decrease for non-covered surfaces treated with fluoridated Miswaks in the test group ($p < 0.0001$). In the control group, the values stayed more or less the same. The result was the same with the International Caries Detection and Assessment System (ICDAS II) index values. It was found that the DIAGNOdent values correlated well with the ICDAS II clinical index ($r=0.76$). Thus, the progression/regression of WSL can be registered by both methods, even during such a short period as 6 weeks.

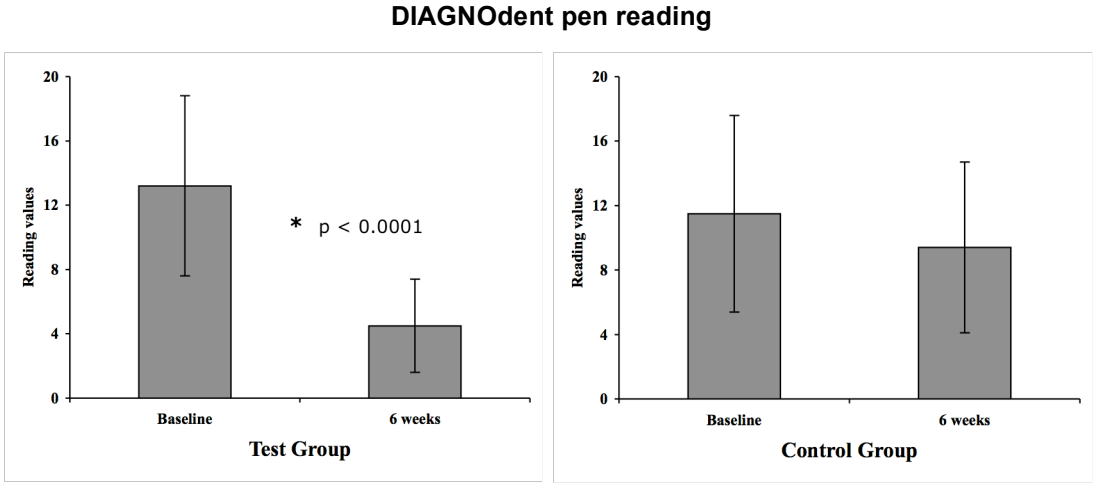

Fig. 12. Means and standard deviations of the DIAGNOdent pen readings for non-covered quadrants in the test (n=19) and control (n=18) groups at baseline and at 6 weeks.

Fig. 13A & B show an example of intra-oral clinical photographs taken for one patient in the control and one in the test groups both at baseline and at 6 weeks, illustrating the remineralising effect after using fluoridated Miswaks on WSL (test group) compared to non-fluoridated Miswaks (control group).

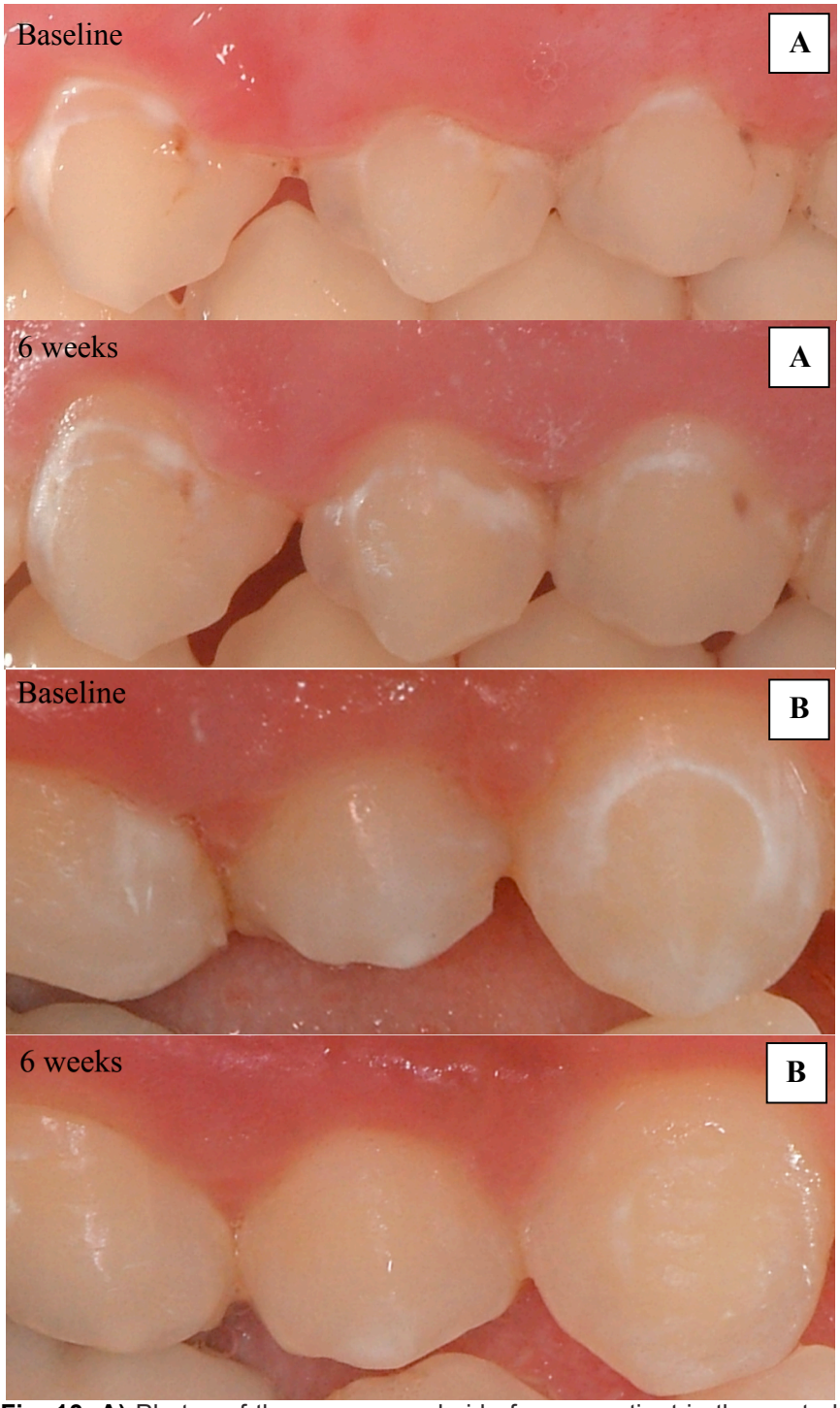

Fig. 13. A) Photos of the non-covered side for one patient in the control group showing no improvement of the WSL from baseline to 6 weeks after using non-fluoridated Miswaks. **B)** Photos of the non-covered side for one patient in the test group showing the WSL improvement from baseline to 6 weeks after using fluoridated Miswaks.

Discussion

This thesis was based on the concept that chewing sticks are frequently utilised in many countries around the world for cleaning purposes and are often used up to 5 times/day in Arabic countries. The prevalence of dental caries is high in different countries like Saudi Arabia [Alamoudi et al., 1996; Al-Shammery, 1999; Gandeh and Milaat, 2000; Wyne et al., 2001; Amin and Al-Abad, 2008]. In order to reduce and prevent dental caries in these countries, it may be interesting to study the idea of fluoridating Miswaks in order to have a dual effect, combining the mechanical cleaning effect and the anticariogenic effect achieved through fluoride release into the oral cavity.

In the present series of studies, the effect of fluoridated Miswaks was investigated in terms of the uptake and release of fluoride (Papers I & II), the fluoride clearance in the saliva and oral fluoride retention (Paper II), oral fluoride retention with and without orthodontic appliances (Paper III) and the remineralisation effect on white spot lesions "WSL" (Paper IV). Data from Papers I-IV revealed that Miswaks were generally suitable for both the uptake and release of NaF. This is in agreement with previous studies of fluoridated toothpicks [Mørch and Bjørvatn, 1981; Petersson et al., 1994; Kashani et al., 1995; Särner et al., 2003; Särner et al., 2008]. The fluoride uptake of Miswaks impregnated in 3% NaF for 3 hours in the present study was twice as high as the Miswaks impregnated in 1% NaF. As fluoride is released rapidly, this indicates that the fairly simple diffusion of NaF occurs through the surface (bark) into the Miswak pulp. This finding was confirmed in Study I, when fluoride release from the bark and the pulp was analysed separately.

The variation in the uptake and release of fluoride depends on several factors, such as the size, nature and degree of porosity within the Miswak. Mørch and Bjørvatn, [1981] suggested that a chemical reaction between fluoride and various organic or inorganic substances present in wood might take place and protect or inhibit the release of fluoride. In comparison to other products, it is not possible for technical reasons to assess the maximum fluoride content incorporated in a specific product. Fluoride incorporation probably occurs in both the bark and pulp of the Miswak. It appears that NaF crystals are absorbed to some extent within the Miswaks and fluoride is released intra-orally from the pulp after using the Miswak for 2 min

and diminishes after 1 hour (Papers I-III). The release of fluoride from the bark of the Miswak was found to be higher than that from the pulp (bristles), but both parts produced a high, rapid fluoride release, especially during the first 10 min. The reason for this could be that the bark is thin and more porous, which may be due to the fact that a Miswak is originally the root of a plant, which is characterised by water absorption (Fig. 3).

There was a clear dose-response effect with respect to both the fluoride concentration and the impregnation time (Papers I&II). The higher the NaF concentration, the more fluoride that was absorbed, leading to more fluoride release from the Miswak, as found in studies of fluoridated toothpicks [Petersson et al., 1994; Kashani et al., 1995; Kashani, 1998; Särner et al., 2003; Särner et al., 2008]. High concentrations, such as 3% or 4% NaF solutions, were used for the impregnation of Miswaks in Papers I & II and produced a fluoride release that was 4-5 times higher compared with the toothpaste (0.32% NaF) when calculated as AUC. On the other hand, lower concentrations, such as 0.05% and 0.5% NaF solutions, were used in Papers II & III and produced a lower fluoride release. Miswaks impregnated in 4% NaF solution for 3 days displayed a very high level of fluoride release compared with any other fluoridated product, in addition to demonstrating a whitish layer consisting of NaF crystals (unpublished observations). To avoid this whitish discoloration of Miswaks, it is suggested to use Miswaks impregnated in 0.1% or 0.5% NaF for one day (Paper II).

The total uptake of fluoride in Miswaks impregnated in 4% NaF solution for 3 hours is about 0.47 mg (Paper I). About 60-70% of this fluoride was released after 1 hour of storage in water. The *in vivo* release is, however, much lower because the Miswak is used in the mouth for a much shorter time than one hour. In spite of the large uptake, a fair amount of fluoride is still released and swallowed during the use of Miswaks in the mouth. For this reason, only the bristle part of the Miswak should be in contact with the tooth surface and only part of the bark.

In Study II, fresh Miswaks released slightly more fluoride than old ones, even if the difference was not statistically significant. Both types are sold on the open market, but the fresh Miswaks are softer, taste better, are more flexible and somewhat more expensive. Moreover, there was a large difference in fluoride release from Miswaks purchased from different stores, with a factor of around 2. The reason for this

variation could be that some of the stores sell fresh Miswaks, while others sell older types. The difference may also depend on the properties of the Miswak itself, even if we tried to control for both the size and weight of the pieces.

Large surface areas of the bristles of the Miswaks are in contact with the tooth surface during brushing, which means that a great deal of the released fluoride can then be subsequently swallowed. In Study II, Miswaks impregnated for one day in 0.5% NaF produced a mean "fluoride retention" of 0.39 mg of fluoride. If the Miswak is to be used five times a day, around 2 mg of fluoride will be swallowed every day. The daily fluoride intake of an adult has been estimated to be around 1-2 mg from food and fluoride toothpaste [Villa et al.]. This amount is considered safe for an adult from a toxicological point of view. However, for safety reasons and due to individual variations, the recommended impregnation is with 0.1% NaF, with a net intake of $0.21x5 \approx 1$ mg fluoride/day.

Previous studies of various fluoride products have usually involved measurements of the fluoride content of the whole saliva and plaque, but very few data on fluoride in the approximal area are available. Generally, the fluoride concentration values for all fluoridated products and for all patients with fixed orthodontic appliances showed higher fluoride retention values with than without the appliances. In Papers I & III, the fluoride concentration at various approximal sites measured after using fluoridated Miswaks, dentifrices and mouthrinse solutions. Fluoridated Miswaks was equivalent or superior to the other products. The result was that 0.5% fluoridated Miswaks were the best at delivering fluoride directly into the approximal area. In Paper III, 0.5% NaF Miswaks retained 1.5 times more fluoride than the 0.2% NaF rinsing solution and 0.5% NaF toothpaste in the approximal saliva. The advantage of Miswaks is that there is no need for post-brushing water rinsing, as there is after using toothpaste, and this could explain why fluoride retention in the approximal area is higher than with other fluoridated products. Several studies have reported the caries preventive effect of rinsing with fluoride mouthwash [Fure et al., 1998; Marinho et al., 2003a; Marinho et al., 2004b,a; Twetman et al., 2004]. A higher salivary fluoride concentration interproximally when using fluoride mouthrinses is attributed to better accessibility and spread, especially between brackets and wires, when compared with other fluoridated products. This is in agreement with the study by Särner et al., [2003], which showed that rinsing with 0.2% NaF resulted in higher approximal fluoride

concentrations compared with fluoridated toothpicks and dental flosses.

The combined mechanical cleaning effect (achieved by the Miswak bristles) and the chemical effect of the fluoride impregnation regimens in the treatment and prevention of WSL were investigated in Paper IV. When it came to the remineralisation effect on WSL, fluoridated Miswaks were superior to non-fluoridated Miswaks. This difference may be due to fluoride release from the fluoridated Miswaks (chemical effect), the mechanical cleaning effect or both. The DIAGNOdent and clinical index methods were included in the study in order to compare them with one another. The study indicated that the DIAGNOdent provided a consistent performance and reliability as found previously by several studies [Lussi et al., 1999; Shi et al., 2000; Costa et al., 2002; Baseren and Gokalp, 2003; Cortes et al., 2003]. It could therefore be suitable for the longitudinal monitoring of caries lesions and subsequently aid in clinical decision-making with respect to the management of orthodontically induced smooth surface lesions. All the WSL in Study IV were also examined visually during the de-bonding visit and 6 weeks later. The differences between the clinical index readings at baseline and at the final examination were statistically significant. The clinical index correlated well with the DIAGNOdent values (r=0.76). The DIAGNOdent pen thus offered a possible means of detecting and quantifying minor changes that had occurred during the 6-week study period. These findings confirm the results of previous studies applying the DIAGNOdent method [Al-Khateeb et al., 1998; Aljehani et al., 2004; Bamzahim et al., 2004]. The advantage of the pen is that it is easy to carry, accurate, reproducible and the readings can be shown to the patient and may thus have a pedagogic value. On the other hand, it is expensive and time consuming. The advantages of using the ICDAS II are that it is easy to use, inexpensive and less time consuming. However, it is more subjective and less informative for the patient.

WSL that form around brackets during orthodontic treatment may possibly be remineralised over a relatively short period of time, as shown in Study IV. Other studies have reported longer periods of time that may extend to some years [Øgaard et al., 1988]. In our study, the test group patients were provided with fluoride toothpaste for use twice daily, combined with fluoridated Miswaks 5 times/day. The remineralisation is thus a combination of the cleaning effect of the Miswaks and their fluoride release. Since the control group was also given fluoride toothpaste and was

using non-fluoridated Miswaks, the remineralisation of WSL can be mainly attributed to the fluoride release from the Miswaks. The slight regression of WSL in the covered sites in the subjects using the fluoridated Miswaks indicated that the mouth tray did not totally prevent fluoride from reaching the buccal surfaces. However, the effect was still stronger on the treated sites.

Several studies have assessed the presence of WSL by using photographic techniques. However, there are difficulties in achieving consistency in lightning, reflection and angulation. In Study IV, the light was standardised by using a ring flash with cross-polarisation filters and by fixing the focal length distance of the lens at 50 cm. In spite of this, it was not possible to obtain identical photos in order to score the WSL in an optimal way.

The successful fluoride impregnation of Miswaks can be efficiently incorporated in future community-targeted preventive programmes, especially in countries where Miswak use is common or a tradition. The advantages of fluoridated Miswaks as an alternative to the toothbrush are that it is inexpensive, readily available in both urban and rural areas, easy to carry, has medicinal properties, is hygienic and its use is not limited to the bathroom. On the other hand, accessibility to the lingual surfaces of the posterior teeth may be problematic when using a Miswak, as its bristles lie in the same long axis as its handle, as different from the toothbrush. In addition, specific written instructions should be given to the patient to avoid potential damage to the gingival tissues (gingival recession).

More extensive research on fluoridated Miswaks as a preventive regimen, should be applied in large populations such as schoolchildren, especially in countries where its use is very common, such as Saudi Arabia. In addition, it should be compared with fluoride toothpaste and mouthrinse. Taking account of the availability of plastic-packed chewing sticks in various pharmacies around Saudi Arabia, a clever way to commercialise the fluoridated Miswak in a more modern and hygienic fashion would be to introduce it in the market with special protective carriers, thereby facilitating its transport and keeping it fresh and clean.

Conclusions

The main conclusion from this series of studies is that fluoridated Miswaks are suitable vehicles for the uptake and release of fluoride both *in vitro* and *in vivo*. More specifically, the conclusions are as follows:

- The uptake and release of fluoride from fluoridated Miswaks both *in vitro* and *in vivo* are rapid processes (Papers I-II and III).
- Miswaks impregnated in 0.1% to 0.5% NaF for one day could be considered the "optimum level" with respect to both the fluoride concentration in saliva and the "fluoride retention" (Papers II-IV).
- The oral "fluoride retention" increased at higher NaF impregnation concentrations. At 0.1% NaF, it was a mean of 0.21 mg F and, at 0.5% NaF, it was 0.39 mg fluoride. Miswaks impregnated in 0.1% to 0.3% NaF produced about the same salivary fluoride level in saliva as brushing with fluoride toothpaste containing 0.32% NaF (Paper II).
- The oral fluoride retention was higher for all fluoride products when the patients were wearing fixed orthodontic appliances (Paper III).
- Fluoridated Miswaks impregnated with 0.5% NaF have a remineralisation effect on WSL compared with non-fluoridated Miswaks (Paper IV).

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