CHAPTER 1

INTRODUCTION

Prevalence of dental caries among 3-12 years old children

Dental caries is a major problem of oral disease in both adults and children for many decades. The fifth Thai national oral health surveys was conducted in 2000-2001. This survey found that the prevalence of dental caries among children aged 3-12 years old are still high (Table 1). The number of decayed, missing and filled teeth (dmft and DMFT) varied between 1-6 teeth/person, which increased by age. The highest mean of decayed teeth/person was found in 5-6 years old children, dmft were 5.53 teeth/child (The fifth national oral health surveys report, 2000-2001).

Table 1. Percentage of decayed, missing and filled teeth in all age groups (The fifth Thai national oral health survey in 2000-2001).

Age group (years)	Decayed teeth	Missing teeth	Filled teeth
3	65.7	4.8	2.7
5-6	87.5	16.8	7.7
12	57.3	5.4	22.8

Mechanism of fluoride in caries prevention

Fluoride was introduced to reduce dental caries. The efficacy of fluoride in caries prevention has been well documented (Seppa *et al.*, 1982; Chow, 1990: White and Nancollas, 1990; ten Cate, 1999). Both systemic and topical fluoride has been used to prevent caries development. In the past two decades, topical fluoride effects had dominated in preventing caries development and enhancing caries remineralisation.

Professionally applied topical fluoride such as fluoride gel and varnish has been cleared to be an effective way to deliver fluoride onto enamel surface. (Beltran-Aguilar *et al.*, 2000; Rozier, 2001; Stronhmenger and Brambilla, 2001). The contact time between the tooth surface and topical fluoride agent has been found to affect the preventive efficacy (Retief *et al*., 1980; ten Cate, 1997).

The enamel mineral is calcium phosphate, namely hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. The two main theories on the cariostatic mechanism of fluoride are: 1) when high fluoride concentration is present in enamel apatite the enamel is supposed to be resist to caries, and 2) when the concentration of fluoride in the surrounding medium is high the enamel will not be dissolved according to the law of mass action. The two theories do not seem to exclude the other (Larsen, 1990; White and Nancollas, 1990; ten Cate, 1997).

When enamel is exposed to high concentrations of fluoride a calcium fluoride like material will form on the surface of enamel. This calcium fluoride acts as a fluoride ion reservoir on enamel and in plaque. As saliva is undersaturated with respect to calcium fluoride, saliva eventually dissolves the salt. The release of fluoride from the reservoir depends to some extent on pH, as a pH drop increases the release. At low pH, when enamel apatite was dissolved under development of a caries lesion, a high fluoride concentration in the surrounding medium could induce a fluorapatite formation in the enamel surface layer (Figure 1). If there is no fluoride present, a well-mineralized surface layer will not formed, the resulting lesion being an erosion. The higher the fluoride concentration in the aqueous phase, the more supersaturated with respect to fluorapatite it is and the more well-mineralised becomes the surface layer. The calcium fluoride formation at the tooth surface is strongly depended on the duration of contact between the fluoride agent and the mineral (Retief *et al*., 1980; ten Cate, 1997; Strohmenger and Brambilla, 2001). The high level and firmly bound fluoride on tooth surface are beneficial for caries inhibition because of the low solubility of fluorapatite (White and Nancallas, 1990).

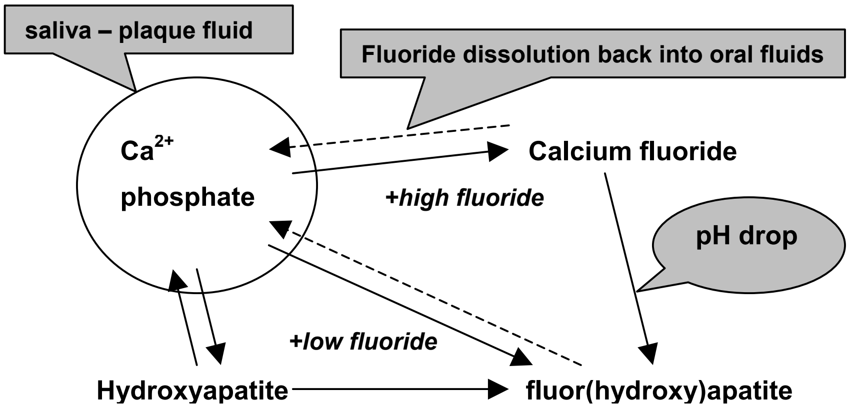

Figure 1. Mechanism of fluoride in caries prevention (ten Cate, 1999).

Effect of fluoride in demineralising solution

Several studies (ten Cate and Duijsters, 1983a, 1983b; Borsboom *et al.*, 1985; Larsen, 1986; Margolis *et al.*, 1986; Featherstone, 1999) have suggested that fluoride in solution surrounding tooth is effective in inhibiting enamel demineralisation. ten Cate and Duijsters (1983a, 1983b) and Featherstone *et al*. (1990) reported that the calcium loss from enamel is shown as a function of pH and fluoride concentration in demineralisation solution. Enamel dissolution proceeds faster at low pH due to effect of protons on the thickness of the diffusion layer (ten Cate and Duijsters, 1983a). Arends *et al*. (1983) reported that 19 mg/L fluoride in demineralising solution could inhibit enamel dissolution. This finding is in the same trend with the study of ten Cate and Duijsters (1983b) who found that 2 mg/L fluoride in 0.05 mol/L acetic acid at pH 5 was sufficient to inhibit enamel demineralisation. Featherstone *et al.* (1990) suggested that inhibiting of enamel demineralisation was shown to be logarithmically function of fluoride concentration in demineralising solution. Fluoride 2 mg/L reduced the initial calcium dissolved approximately 40% compared with the non fluoride buffer. Moreover, fluoride in solution was influenced in caries like lesion formation in enamel (Borsboom *et al.*, 1985; Margolis *et al.*, 1986; Larsen, 1991). Borsboom *et al.* (1985) reported that subsurface lesion formed seem to be cause by the presence of 0.12 mg/L fluoride ion in liquid whereas erosion formed when no fluoride ion present in liquid. This finding consistent with the study of Larsen (1973, 1991) who reported that at high fluoride concentration in the aqueous phase, the more saturated with respect to fluorapatite it became which increased the uptake of fluoride in the enamel resulting in a well-mineral surface layer. Margolis *et al.* (1986) reported that a demineralising solution containing as little as 0.024 and 0.054 mg/L fluoride had changed the enamel destruction pattern. The enamel exposed to demineralising solutions containing 0.024, 0.054 and 0.154 mg/L fluoride appeared whitish and, when examined in the polarized light microscope, was seen with a subsurface demineralisation instead of surface erosion. A demineralising solution containing 1 mg/L fluoride had slightly dissolved enamel surface which could hardly be observed by the scanning electron microscope (SEM) when compared to normal surface enamel.

Development of fluoride varnish

Fluoride varnish was invented during the late 1960s in an effort to improve effects of topical fluoride, such as fluoride gel or mouthrinse, by prolonging contact time between tooth enamel and fluoride. By 1980s, fluoride varnish was widely used in European countries (Clark *et al.,* 1987; Beltran-Aguilar *et al.*, 2000; Rozier, 2001; Strohmenger and Brambilla, 2001). In 1994, the fluoride varnish was approved by U.S. Food and Drug Administration (FDA) to be used as cavity liner and dentine desensitising agent. Since then the studies in the efficacy of fluoride varnish on dental caries prevention in the United States have been started (Beltran-Aguilar *et al.*, 2000; Vaikuntam, 2000).

Fluoride varnish has some advantages over fluoride gel. It is often used among handicapped and unco-operative children. The thickness and rapid setting of fluoride varnish reduces the amount of fluoride ingestion, eases application, higher fluoride concentration can be used and the fluoride adherence to tooth structures (Beltran-Aguilar *et al.*, 2000; Strohmenger and Brambilla, 2001; Hicks *et al.*, 2001).

Type and application technique of fluoride varnish

Several types of fluoride varnish are available commercially. Table 2 shows product name, presentation, fluoride concentration and manufacturer of several types of fluoride varnish (Beltran-Aguilar *et al.*, 2000; Vaikuntam, 2000). Duraphat® is a 5% sodium fluoride formulation in a viscous colophonium base (Beltran-Aguilar *et al.*, 2000; Vaikuntam, 2000). It was the first commercial fluoride varnish and the product most commonly used in Thailand. This is the reason of our used of Duraphat® in this study.

The application technique of fluoride varnish is easier than fluoride gel. Tooth brushing is considered to clean the teeth sufficiently before application. The teeth are dried with gauze or cotton roll and 0.3-0.5 ml of fluoride varnish is applied with a brush directly. Dental floss can be used to ensure that the varnish reaches interproximal areas. The varnish will be set within a few seconds. To maximize contact between the varnish and the teeth, patients are instructed to avoid eating for two to four hours and to avoid brushing their teeth for 24 hours (Beltran-Aguilar *et al.*, 2000; Vaikuntam, 2000).

Table 2. Product name, presentation, fluoride concentration and manufacturer of fluoride varnish.

Product name	**Presentation**	**Fluoride concentration**	**Manufacturer**
Duraphat	Tube (10 ml)	5% sodium fluoride (2.26% F, 22.6 mg/ml F or 22600 mg/L F)	Colgate Oral Pharmaceuticals
Duraflor	Tube (10 ml)	5% sodium fluoride (2.26% F, 22.6 mg/ml F or 22600 mg/L F)	Pharmascience Inc.
Fluor Protector	Single dose of 0.4 ml/vial (20 vials/pack)	1% difluorsilane (0.1 % F, 1.0 mg/ml F or 1000 mg/L F)	Ivoclar-Vivadent
Cavity Shield	A unit dose of 0.25 ml/pakage or 0.4 ml/package	5% sodium fluoride (2.26% F, 22.6 mg/ml F or 22600 mg/L F)	Omnii Products

Effect of fluoride varnish on reducing enamel dissolution

In several *in vitro* studies (Retief *et al.*, 1980, 1983; Acuna *et al.*, 1990; Eronat *et al.*, 1993) the uptake of fluoride in enamel after fluoride varnish application has been examined. Retief *et al.* (1980) determined the in vitro fluoride uptake and retention by human enamel after a single application of APF, Duraphat® and Fluor Protector®. They found that Fluor Protector® gave a greater uptake of fluoride in the outermost 7.5 µm of enamel than did the Duraphat® and APF gel. Furthermore, the fluoride uptake increased by extending the contact time to 24 hours.

Retief *et al.* (1983) showed that the highest fluoride uptake was seen among teeth treated with Duraphat® and Fluor protector® in comparison with APF gel. Moreover, fluoride varnish lead to better crystal formation, that is fluorapatite, whereas an APF application led to alkali-soluble fluorides.

Acuna *et al.* (1990) reported that fluoride uptake from Duraphat® in dentine was greater than in enamel. Eronate *et al.* (1993) reported that a topical fluoride application of APF, 2% neutral sodium fluoride, Duraphat® and Fluor protector® caused more fluoride uptake in enamel when compared to a non fluoride varnish.

Furthermore, the uptake in sound enamel was less than it was in carious enamel (Chan *et al.*, 1991). The amount of calcium fluoride precipitated on sound enamel by topical fluoride is easily lost when compared to carious lesion because calcium fluoride may harbour within micropores of caries lesion and serve as a slow releasing reservoir of fluoride (Bruun and Givskov, 1991; Larsen and Richards, 2001).

Several *in vitro* studies (Seppa, 1988; Hicks *et al.*, 2001;Tazel *et al.*, 2002) have shown a caries preventive effect of fluoride varnish. Seppa *et al.* (1988) found that Duraphat® prevented enamel softening after immersion in acid. Fluoride varnish appeared more effective in reducing enamel dissolution than did sodium fluoride solution. Moreover, he found that the higher fluoride concentration, the greater was the fluoride uptake by enamel. This finding agreed with the observations of Hicks *et al.* (2001) who reported that fluoride varnish (Duraphat®, Duraflor® and Cavity-Shield®) improved the caries resistance of primary teeth to a continuous cariogenic challenges. Moreover, they found that fluoride varnish significantly decreased the carious lesion depth. Later study of Tazel *et al.* (2002) also reported that titanium fluoride varnish (TiF_4) was more effective than sodium fluoride and amine fluoride varnish in preventing artificial caries.

In numerous clinical trials the efficacy of fluoride varnish in preventing dental caries have been examined. An overall decline of caries increment has been shown following fluoride varnish application, ranged from 18 to 77% (Seppa *et al.*, 1982, 1983, 1995; Helfenstein and Steiner 1994; Bawden, 1998; Vaikuntam, 2000; Autio-Gold and Courts, 2001).

Application frequencies of fluoride varnish

In the past decades, fluoride varnish has been applied after three different schedules: 1) single application every six months, 2) single application every three months, and 3) three applications within one week period (intensive application). The frequency of application depends on individual caries risk (Beltran-Aguilar *et al.*, 2000; Vaikuntam, 2000). Among low to moderate caries risk patients, a single application at every six months should be enough while among high caries risk patients, either application every three months or three times a week (intensive application) is recommended. Seppa and Tolonen (1990) studied 254 low caries risk children, aged 9-13 years old for over 2 years and found that the caries prevention of a single application at every six months and a single application at every three months did not differ significantly. Petersson *et al.* (1991) showed that intensive applications within one week inhibited proximal caries progression more than did single application every six months. This result revealed that intensive applications enhanced caries reversal and increased remineralisation of white carious lesion. No clinical study has compared the caries preventive effect of a single application every three months with intensive applications in high caries risk patients.

In a couple of *in vitro* studies the fluoride uptake and the inhibition of enamel dissolution after fluoride varnish reapplication have been examined (Retief *et al.*, 1983; Seppa, 1988). Retief *et al.* (1983) found that reapplication of topical fluoride (Duraphat® and Fluor Protector®) 1 month after the initial application did not increase enamel fluoride uptake. This finding consistent with the study of Seppa (1988), who observed that the enamel solubility of a single application did not statistically differ from that of enamel given intensive applications.

SEM studies of enamel surface

SEM observation of surface enamel is the one of several methods to estimate enamel dissolution (Silverstone *et al*., 1975; Shellis and Hallsworth, 1987; Haikel *et al*., 1983; Margolis *et al*., 1986; Moller and Schroder, 1986). Silverstone *et al.* (1975) reported 3 type SEM pattern of enamel after phosphoric acid etching. Type I or “honey comb” pattern, the prism junction regions are elevated above the prism cores. Type II pattern is characterized by a widening of the prism junction that are seen as cleft surrounding the prism cores while type III appears with an irregular surface without features relating to the prism structure. Haikel *et al.* (1983) reported a prismatic pattern of destruction and an irregular type of destruction from SEM observation of non-cavitated incipient caries. This finding was consistent with Holmen *et al.*’s studies (1985). At higher magnification SEM shows that an opening up of the intercrystalline space after artificial caries formation by acid. Moller and Schroder (1986) found that the surfaces of remineralised initial enamel caries after reapplied Duraphat® every 10th day for 8-10 weeks had more regular, densely packed and larger crystals compare to untreated lesion.

Although the effect of fluoride in demineralising solutions has been examined in many studies, no study has parallely investigated the effect of fluoride varnish application and fluoride concentration in the demineralising solution. Moreover, few studies have compared the effect of a single application to intensive application (Seppa, 1988; Petersson *et al*., 1991). Both studies aiming at its effect in demineralisation enamel. Therefore, in present study aimed to examine the effect of fluoride level in demineralisating solution in addition to fluoride uptake from fluoride varnish application and the effect of intensive applications of fluoride varnish on enamel dissolution and thereby its possible impact on caries prevention.

Objective : The objectives of the present study were to:

1. Assess the effect of fluoride concentration in demineralising solution on enamel dissolution.
2. Examine enamel dissolution after a single and intensive applications of fluoride varnish.
3. Examine by scanning electron microscope the demineralised enamel surface after treatment with single and intensive applications of fluoride varnish.