CHAPTER 4

DISCUSSION

The present study has shown that high fluoride concentration in solution reduces enamel dissolution which is confirm the results of a number of previous studies (ten Cate and Duijsters, 1983a, 1983b; Borsboom *et al.*, 1985; Margolis *et al.*, 1986; Larsen, 1986; Featherstone, 1999). Further, intensive fluoride varnish application appeared to enhance the dissolution preventive effect.

Several previous studies have reported that fluoride is taken up into enamel after a fluoride varnish application (Retief *et al.*, 1980, 1983; Acuna *et al.*, 1990; Eronat *et al.*, 1993). Retief *et al.* (1980, 1983) and Seppa (1988) suggested that the uptake of fluoride depends on fluoride concentration and the contact time between fluoride and the tooth surface. In this study, we used Duraphat® that has been reported to be the most consistent in regard to fluoride concentration uniformity when compared to Duraflor® and Cavity Shield® (Shen and Autio-Gold, 2002; Hazelrigg *et al.*, 2003). The order in which Duraphat® varnish was dispensed from the same tube does not affect the fluoride concentration (Hazel *et al.*, 2003). The composition of enamel has been reported to vary among teeth and even somewhat within the same tooth (Weatherell *et al.*, 1974). In order to reduce the effect of varying enamel composition in this study, the specimens of all groups were from the same teeth. Thus, a large variations in mineral content and trace elements between teeth were avoided. However the minor surface to surface variation within the tooth still remained. In group A and B, all factors that could influence the fluoride uptake in enamel were similar, except for the fluoride concentration in the demineralising solution was 1 and 10 ppm, respectively. It indicated that the higher fluoride level in demineralising solution resulted in less dissolution of

enamel (p<0.005). Fluoride concentrations in demineralising solutions in the previous studies have varied between 0.154-19 mg/L (Table 3). Increasing fluoride concentration in acid decreases the solubility of enamel logarithmically (Larsen, 1986; Featherstone *et al.*, 1990). The fluoride concentration in demineralising solution that could reduced enamel dissolution depends on a number of factors. First, and most important, the concentration of calcium, phosphate and pH. Also other factors as concentration of acid, volume of aqueous solution, ion strength and type of enamel are of significance (Larsen, 1986; Miura *et al.*, 1993; Amaechi *et al.*, 1999; Hughes *et al.*, 2000).

Table 3. Studies of the fluoride level in demineralising solution on reduction enamel dissolution.

Study	year	F level in acid (mg/L)	Ca, PO$_4$ level in acid (mM)	pH of acid	Conc. of acid (mol/L)	Volume of acid (ml/mm^2)	Enamel type
ten Cate and Duijster	1983	2	2.2 mM Ca 2.2 mM PO$_4$	4.5	0.05 mol/L acetic acid	0.4	bovine enamel
Arends *et al.*	1983	19	-	4.5	0.1 mol/L acetic acid	1.11	Bovine enamel
Borsboom and Arends	1985	0.12	2.2 mM Ca 2.2 mM PO$_4$	5	0.05 mol/L acetic acid	0.8	Human enamel
Margolis, Moreno and Murphy	1986	0.154	11.7 mM Ca 6.0 mM PO$_4$	4.3	0.1 mol/L lactic acid	0.6	Human enamel
Featherstone *et al.*	1990	2	-	4.5	0.01 mol/L acetic acid	200 ml/pellet	Synthetic enamel
This study		10	-	4	0.05 mol/L acetic acid	1.5	Human enamel

At a high concentration of calcium and phosphate in demineralising solution, it becomes saturated with respect to the calcium phosphate, and the dissolution of enamel was reduced (ten Cate and Duijsters, 1983; Larsen, 1986). The extensive

dissolution of enamel could occur at low pH and high acid concentration more than at high pH and low acid concentration (ten Cate and Duijsters, 1983; Larsen, 1986; Miura *et al.*, 1993; Amaechi *et al.*, 1999). To prevent enamel dissolution in this study, as we used the higher concentration of acid, lower pH and did not add calcium and phosphate into solutions used, therefore it requires higher fluoride concentrations than other studies. In addition, the relatively large volume of acid demands large amount of mineral to saturate the solution (Larsen, 1990). Bovine enamel is a more porous material than is human enamel (Arends *et al.*, 1989) and is therefore more rapidly demineralised (Featherstone and Mellberg, 1981) than is human enamel. Featherstone *et al.* (1990) reported that dissolved calcium was logarithmically reduced about 40% from 4.0 mg/L/h to 2.4 mg/L/h when the fluoride concentration was increased from 1 to 10 mg/L. In the present study, the amount of dissolved calcium was similarly reduced to that of Featherstone *et al.*'s study. When the fluoride concentration was increased from 1 to 10 mg/L, the amount of dissolved calcium decreased approximately 27% from 46.85 mg/L to 33.67 mg/L. In this study, the capacity of fluoride to reduce the amount of dissolved calcium was less than that of Featherstone *et al.*'s study may be because of the lower pH and higher concentration. Moreover, Featherstone *et al.* used synthetic enamel whereas this study used human enamel. This study used non parametric statistic, Kruskal-Wallis and Wilcoxon, to compare the dissolved calcium data between groups because the dissolved calcium data did not meet the assumption of parametric test. The data were not in normal distribution and the number of samples in each group were less than 30.

Enamel dissolution occurrs during dental caries formation. When pH drop, the hydroxyapatite will be dissolved into calcium, phosphate and hydroxyl ion. Therefore, dissolved calcium measurement could be used to determine the dissolution of enamel. To the mechanism of fluoride on caries prevention, during demineralisation if there is

no fluoride in the solution, a vast demineralisation will occur. The present of fluoride in demineralising solution, reduces the dissolution rate. The role of fluoride is to accelerate supersaturate fluoraptite in solution which will lead to deposition of fluoride on the outermost surface layer. This surface layer is the barrier preventing the mineral (Larsen, 1973, 1990, 1991). Increasing of fluoride in demineralising solution to 10 mg/L will increase the thickness of surface layer at the same time it reduces the lesion depth. (ten Cate and Duijster, 1983b; Fejerskov *et al.* 1996).

This study assessed the effect of fluoride in solution and intensive fluoride varnish application by artificial caries formation of enamel. They are several methods that have been used to stimulate artificial caries lesion development. They may be divided into three groups: agitated aqueous systems, non-agitate aqueous systems and gelled systems. This experiment used agitated aqueous system to stimulate artificial caries development which has been used in several in vitro studies (Larsen, 1973, Seppa, 1988; Hicks *et al.*, 2001; Tazel *et al.*, 2002). This system has some advantages over the gelled system. The solution enable us to determine the exact amount of calcium and phosphate lost both during demineralisation and during remineralisation experiments. The mineral uptake is determined by measuring concentration changes in solution (ten Cate and Duijsters, 1983a). In this experiment, 1 mg/L fluoride was added to the demineralising solution as a purpose of making artificial caries lesion. If fluoride is not present, the erosion like lesion will occur (Larsen, 1973, 1990, 1991; ten Cate and Duijster, 1983a; Borsboom and Arends, 1985).

To imitate the pH change during ingestion of carbohydrate in our daily life and enhance fluoride uptake of enamel during pH drop (Ogaard *et al.*, 1982), the pH cycling was cycled from 4.5 to 7 in this experiment. There are several ways to cycle pH (ten Cate *et al.*, 1982, 1988; Seppa, 1988; Damato *et al.*, 1990; ten Cate *et al.*, 1995; Robinson *et al.*, 1992; Maia *et al.*, 2003), depending on type of acid used and period of

tooth sample exposed to acid. In principle, a demineralisation for a short period up to 6 hours daily is followed by a remineralisation during the rest of 24 hours (ten Cate, 1988). This study included three acid challenges to copy three meal periods of the day that is breakfast, lunch and dinner.

This experiment showed that intensive fluoride varnish application had significantly reduced enamel dissolution compared with single fluoride varnish application. Retief *et al*., (1980, 1983), Acuna *et al.* (1990) and Eronat *et al.* (1993) found that fluoride from fluoride varnish was taken up in the outermost layer of enamel. The SEM studies show that the fluoride richest enamel surface layers were dissolved. It is likely that our series of three applications had increased the fluoride content of enamel more than did a single application. This finding inconsistent with the couple of in vitro studies that investigated the reapplication effect of fluoride varnish (Retief *et al.*, 1983; Seppa, 1988). Retief *et al.* (1983) observed that reapplication 1 month after the initial topical treatment did not increase enamel fluoride concentration. It might be that Retief *et al.* did not use pH cycling during their experiment, as Ogaard *et al.* (1983) claimed that pH cycling could enhance the enamel fluoride uptake. The pH cycling established a dynamic series of demineralisation and remineralisation during which fluoride is gradually transferred from "loosely" to a "firmly bound" state (Ogaard *et al.,* 1983). Seppa (1988) showed that the solubility of enamel after a single application was not significantly differ from that after multiple applications. Our results are inconsistent with those of Seppa's study. It may be caused by several reasons. First, the enamel slabs in Seppa's study were softened by immersion in acid before the experiment. Because the enamel slabs had pores in the surface after the immersion in acid, fluoride varnish could have been trapped within the pores and were not easily removed by scalpel. Therefore, residual fluoride varnish could act as a fluoride reservoir. Further, because reapplication would not add more fluoride varnish to these samples,

reapplication showed no effect. Second, Seppa used enamel slabs, presumably from different tooth. Thus, the mineral content of samples might have varied between groups. Third, Seppa used acid without fluoride whereas we used acid with 10 mg/L fluoride. Without fluoride in the demineralising solution, a vast demineralisation is necessary for the saturation of the aqueous phase. In this situation, the fluoride taken up as fluorapatite after intensive fluoride application might not be enough to resist the dissolution, or otherwise the enamel may be dissolved deeper though the layer in which fluoride was taken up.

Moller and Schroder (1986) found that the reapplication of varnish caused a remineralisation and improved surface texture of carious lesions. The SEM figure revealed densely packed regular, homogeneous enamel crystals. It indicated that reapplication of fluoride varnish every 10th day for 8-10 weeks enhanced remineralisation of initial carious lesion. The remineralisation effect of intensive fluoride varnish application has also been investigated. A 3-year study of *Peterson et al.* (1991) showed that intensive application of fluoride varnish inhibited progression of proximal caries more than did single application every six months. It enhanced remineralisation of initial caries rather than it reduced demineralisation.

When enamel is exposed to fluoride varnish, the calcium fluoride will form on enamel surface. When pH drop, enamel apatite and calcium fluoride are dissolved. Consequently, fluoride ion will replace the hydroxyl group of hydroxyapatite to be fluorapatite in the enamel surface layer (figure 1). This is a preventive effect of fluoride as the solubility of fluorapatite is lower than that of hydroxyapatite (White and Nancallas, 1990). The high frequency of fluoride varnish application might increase the uptake of fluoride in the apatite and it reduces enamel dissolution.

Although intensive fluoride varnish application had significantly reduced calcium dissolution from enamel in our study, the effect was minor compared to that of the

fluoride concentration in solution. Indicating that fluoride in liquid is more efficient in reducing enamel dissolution than fluoride in the solid phase (fluorapatite). This finding is consistent with a study of Nelson *et al*. (1983) who reported that fluoride in solution surrounding carbonate synthetic apatite crystals is much more effective in inhibiting enamel demineralisation than fluoride incorporated into the crystal at the level found in the enamel.

In SEM figures of this study, when increasing fluoride concentration in the demineralising solution to 10 mg/L, we observed a reduction of enamel destruction. In the single fluoride varnish application groups (group A and B), the lesion depth and holes in enamel of group B seem to be less than Group A. These holes were the ends of the rods, which demineralising solution has dissolved. The larger size of holes in group A indicated that the rod wilder and deeper dissolved than group B. This finding inconsistent with the finding of Margolis *et al.* (1986) who investigated the effect of fluoride in demineralising solution on inhibiting enamel dissolution by SEM. They found that even 1 mg/L fluoride in demineralising solution had a remarkable protection of enamel surface whereas it was not seen in this study. The reason of this difference could be that this study used higher concentration of acid, lower pH and did not add calcium and phosphate into the solution.

Regarding repeated fluoride application, dissolved calcium in the group of intensive fluoride varnish application were significantly less than the single application group. In SEM figure (Figure 13B and C), the size of some erode rods in group C slightly smaller than group B. This finding had confirmed the dissolved calcium measurement by ICP-OES.

With respect to clinical used, if fluoride is present in the plaque fluid at the time when bacteria generate acid it will reduce the dissolution of enamel. The maintenance of sufficient fluoride concentrations in plaque and saliva during acid attack is an

effective method of caries prevention (Ekstrand and Oliveby, 1999; Larsen and Richards, 2001). Although application of fluoride varnish creates elevated concentrations of fluoride in saliva, it is only lasts for 6-12 hours (Twetman *et al.*, 1999). Regularly used fluoride products such as fluoride dentifrice or mouthrinse can maintain a useful high concentration of fluoride in saliva (Larsen and Richard, 2001; Campas *et al.*, 2003) and to precipitation of calcium fluoride on the enamel surface (Larsen and Richards, 2001). Bruun *et al.* (1984) reported that total fluoride in saliva increased from 0.016 mg/L to 135 mg/L after brushing with a 1000 mg/L sodium fluoride containing dentifrice. Consequently, after expectoration and subsequent rinsing, the mean total fluoride in saliva was reduced to 3-11 mg/L in 3 minutes and the fluoride level declined almost exponentially to 0.1-0.3 mg/L after 30 minutes. However it still remained higher than baseline for more than 60 minutes (Bruun *et al.*, 1984). Duckworth and Morgan (1991) reported that salivary fluoride after tooth brushing decreased in two distinct phases: an initial rapid phase which lasted for 40-80 minutes, and a second slower phase lasting for 6 hours.

In this study, fluoride uptake after intensive fluoride varnish application was more than single application. Fluoride uptake as fluorapatite might remain in enamel for a long time and could resist acid attack, whereas fluoride level in saliva depends upon the regular use of fluoride dentifrice (Larsen and Richards, 2001). Among high caries risk patients, the cariogenic challenge may occur several times daily. Intensive fluoride varnish application may be the useful way to increase enamel resistance to cariogenic challenges that could add up the fluoride uptake on top of the regularly used of fluoride dentifrice in such high caries risk patients.

The limitation of this study is that it has been done *in vitro.* The results may not be directly applied for clinical use. Moreover, we neither investigated the amount of fluoride uptake after intensive fluoride varnish application nor measured the depth of

lesion in the SEM. In the SEM procedure, the specimens were not cleaned with ultrasonic cleanser before surface texture examination. The remnant of bile product of demineralisation might deposited on enamel surface, thus the sign of dissolution in the rod areas could not be clearly seen.

Furthermore, this study used ICP-OES to determine the concentration of dissolved calcium. This method has very high sensitivity. It can determine the calcium concentration as low as $\mu g/L$ (part per billion) while dissolved calcium concentration in this study were in mg/L (part per million). To reduce the cost of the study, an atomic absorption spectrometer (AAS) would be recommended.