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Effect of three fluoride agents on remineralization and fluoride uptake on enamel lesion

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Abstract This study is to compare the fluoride uptake of three fluoride agents and the effect of remineralization on artificial enamel lesions. Eight human third molars were all divided into four slabs. The slabs were randomly assigned to the following four groups: group A (9,040 ppm F, APF gel), group B (968 ppm F, SnF_2 home gel), group C (22,600 ppm F, fluoride varnish, FV) and a control group. After 72-hour demineralizing and 10-day pH-cycling period with the application of corresponding fluorides. The distribution of fluoride and mineral change was analyzed with EPMA and CMR respectively. There was a significant increase in the amount of F uptake after the application of the three fluoride agents compared to the control group. Group A showed an extremely greater F uptake compared to group C and B. The amount of F uptake from group C was greater than that of group B. The three tested groups showed signs of significantly greater amounts of remineralization as compared to the control group. Remineralization of group C was detected to be the greatest, followed by group A and group B, but there was no significant difference in them under statistical analysis. It can be concluded that all of the tested agents were effective in regaining mineral loss. FV may be recommended as a professional topical fluoride. It is as effective, if not more so, as traditionally used APF gel. Also 0.4% SnF_2 home gel is an efficient self-applied topical fluoride for daily use.

Key words
Demineralization,
Fluoride uptake,
Human molar enamel,
Remineralization

Introduction

Fluoride therapy has been proven to be the most important scientific discovery in caries prevention. A steady decline of caries incidence has been obtained due to the systemic and topical use of fluoride over the past 50 years. It is now accepted that the primary mode of action of fluoride is post-eruptive. The post-eruptive action of fluoride has resulted in new methods of delivering fluoride[1]. One trend that stands out in applying fluoride agents is the development of increasingly more user-friendly and safer products.

The most commonly used professional topical fluoride is acidulated phosphate fluoride (APF) gel and its effectiveness in preventing caries is widely accepted. However, the use of APF products may cause some side effects. For example, frequent use of APF products may cause etching of porcelain restorations. Excessive ingestion may cause gastro-intestinal upset, including abdominal pain, nausea, and vomiting. Additionally, some patients cannot tolerate the use of tray for fluoridation[2].

Fluoride varnish was first introduced in 1964[3]. It is a nonaqueous solution with a resin or synthetic base. It easily adheres to the tooth surface. In 1994 the U.S. Food and Drug Administration approved its use as a cavity liner and for treating hypersensitive teeth[4], but fluoride varnish as a topical fluoride therapy is not yet allowed in the U.S.[5], nor in Japan. Two clinical studies showed that fluoride varnish was even more effective in caries reduction than APF gel[6,7]. And it was also considered to be ingested

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less and to be a more user-friendly product than APF gel[8].

Zero point four percent SnF_2 gel is a self-applied fluoride agent used on a daily basis, and not recommended for children under the ages 4–6 years. These studies were disclosed that it was effective in inhibiting demineralization in orthodontic patients[9] and preventing root caries in patients with radiation-induced xerostomia[10].

Although some studies have been carried out concerning the reduction of mineral loss and caries incidence with the use of these mentioned fluoride agents. A few easily controlled laboratory comparisons of remineralization and intensity of F uptake of the three products have not yet been made. This study was designed to provide such data in this field, and to provide some information for the clinical selection of topical fluoride agents.

Materials and Methods

Tooth sample preparation

Eight extracted young human third molars fixed in 10% buffered formalin were utilized in the study. All were free from defects and showed no signs of caries or hypoplastic lesions. The smooth surfaces of the crown all underwent sonic scaling and brushing with a non-fluoride dentifrice. Each tooth crown were made a cross section from occlusal surface with a band saw (KT-100, Meiwa Co., Ltd., Japan), and then four gained slabs were randomly divided into four groups. A $4\times4\,mm^2$ size window was exposed on the smooth surface with sticky dental wax covering the remaining area.

Demineralizing treatment

All specimens were then subjected to 3-day acid-challenge to acquire artificial caries-like enamel lesions in 37°C conditions according to the regimen of Oshino *et al.*[11] (Table 1). After thoroughly washing with distilled water and drying at room temperature, half of the window was again covered with sticky dental wax, leaving a $2\times4\,mm^2$ area uncovered.

pH-cycling treatment

After 10 successive days of 4-hour demineralizing and 20-hour remineralizing process in 37°C circumstance was carried out with the regimen as shown[11] (Table 1). Each group was immersed in 200 m*l* of de- or remineralizing solution. The solution was renewed every 24 hours. In addition, the fluoride agents were all applied to the tested window of the three fluoride groups with a cotton swab for 4 minutes before both demineralizing and remineralizing treatment twice every 24 hours. The tested groups were group A (9,040 ppm F, 2% NaF acidulated phosphate fluoride, APF gel, Stone Pharmaceuticals Co., Ltd., U.S.A.), group B (968 ppm F, 0.4% SnF_2 home gel, Oral Care Co., Ltd., Japan), group C (22,600 ppm F, 5% NaF varnish, Duraphat FV, Colgate Oral Pharmaceuticals Inc., Germany). The control group was left untreated. After application, the agents on the slabs were then removed with a cotton swab and the slabs were rinsed with distilled water for 1 minute each. All specimens were dried with tissue paper. The above procedures were repeated over 10 successive days.

Table 1 The de- and remineralizing regimen

Ingredients	Demineralizing solution	Remineralizing solution
$CaCl_2$	3.0 mM	3.0 mM
KH_2PO_4	1.8 mM	1.8 mM
$CH_3CH(OH)COOH$	20 mM	
$CH_3CH(OH)COONa$	80 mM	
$NaHCO_3$		8.3 mM
NaCl		4.8 mM
KCl		137 mM
NH_2CONH_2		2.5 mM
pH	4.5	7.0

Preparation for EPMA and CMR analysis

In the end of the pH-cycling period, all the slabs were dewaxed with xylene and then dehydrated in graded ethanol and embedded in polyester resin (Rigolac; Nisshin EM Co., Ltd., Japan) as described previously[12]. Each slab was cross-sectioned in a way to include the untreated, pH-cycling treated and demineralized regions. Then the sectioned surfaces of each group were ground and polished with a refine polisher (61A103, Refine Tec Co., Ltd., Japan) using different sand paper successively (C#320, C#800, C#1500). After a thin layer of carbon was applied with a carbon coater (TB500, Emscope Co., Ltd., England), the Kα intensity of F in untreated and pH-cycling treated regions of each slab was analyzed with an Electron Probe Micro Analyzer (EPMA) (JXA-8200, WD/ED combined microanalyzer, JEOL, Japan) under the condition

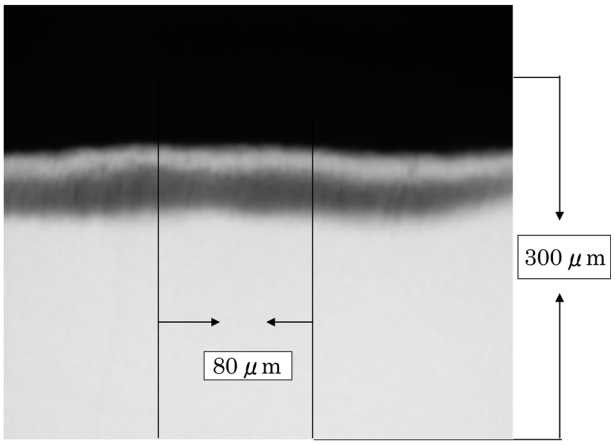

Fig. 1 The measurement of mineral loss (by CMR)

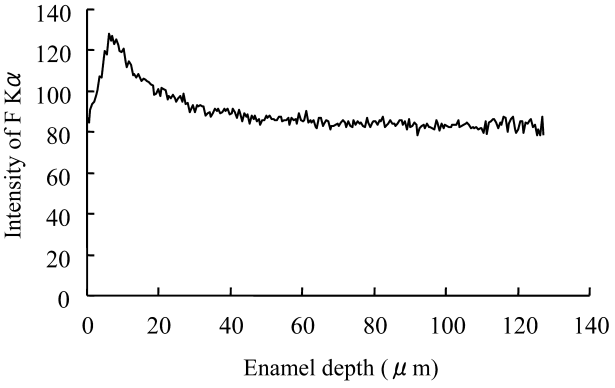

Fig. 2 The base line intensity of F Kα in all groups

Table 2 The amount of F intake in inner layer (80μm–120μm)

Group	N	Amount of F intake in inner layer (Kα•μm) Means ± SD
A	8	1002.15 ± 538.97
C	8	1257.60 ± 1102.46
P		>0.05

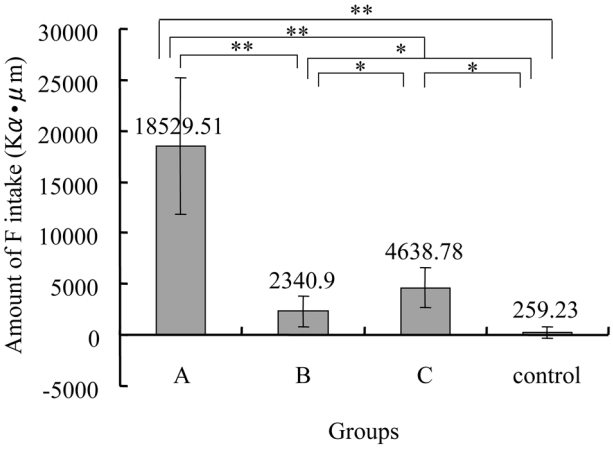

Fig. 3 The amount of F intake in enamel after pH-cycling
Asterisk shows significant differences by one-way Anova, *$P<0.05$, **$P<0.001$.

of an accelerating voltage of 10 kv, a current of 5×10-8A and beam diameter of 5μm.

Among all the specimens, five slabs from each group were further sectioned to 200μm with a microtome (SP1600, Leica Co., Ltd., Germany) and then about 100μm-thick slices were prepared with two pieces of refined grindstone. Contact micrographs were then taken with a soft X-ray generator (Softex CMR-3, Softex Co., Ltd., Japan) under the operating condition of an accelerating voltage of 10 kv and a specimen current of 3 mA for 25 minutes. An aluminum stepwedge used as a contrasting standard was placed beside the slices on the film (HRP-SN-2, Konica Minolta Opto, Inc., Japan) during the operation. The film was developed at the same way according to the method by Miake *et al.*[12]

Then Contact Microradiography (CMR) images were analyzed by an Image Analyze System (HC-2500/OL, Olympus Co., Ltd., Japan), and the data from the demineralized region and pH-cycling region were obtained as the average value of the three corresponding areas (each with 80μm width and 300μm depth). The amount of mineral loss in the demineralized (vol%•μm) (ΔZ1) and pH-cycling region (vol%•μm) (ΔZ2) was calculated by setting the totally black area of the film as 0% and the sound enamel area as 100% (Figure 1). Amount of remineralization (vol%•μm) $\Delta\ \Delta$Z was calculated as $\Delta\ \Delta Z = \Delta Z2 - \Delta Z1$.

Statistical analysis

SPSS (version 11.0) was used to analyze the data with one-way Anova method at a standard of 0.05.

Results

EPMA

No significant difference was found in the base-line intensity of F (F Kα) distributions in each group. Figure 2 shows the change of F concentrations from

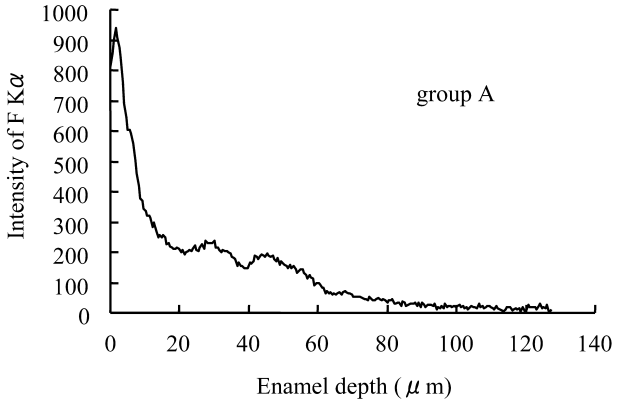

Fig. 4 The increasing of F Kα in enamel lesion of group A

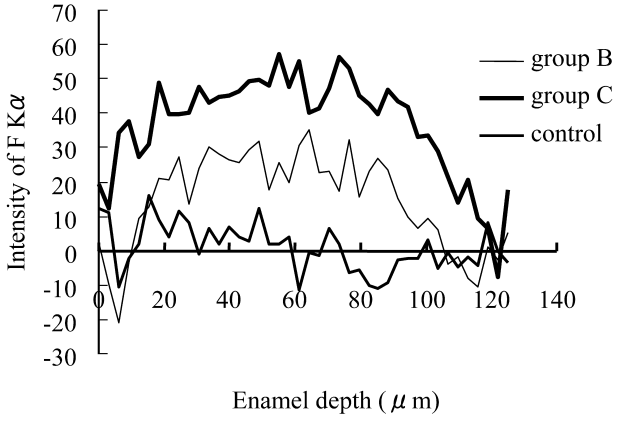

Fig. 5 The increasing of F Kα in enamel lesion of group B, C and control

Table 3 The base line value of the amount and depth of mineral loss

Group	N	Amount of mineral loss (vol%•μm) Means ± SD	Depth of mineral loss (μm) Means ± SD
A	5	5391.80 ± 1057.59	109.40 ± 6.54
B	5	5000.40 ± 949.14	113.20 ± 6.69
C	5	5564.40 ± 1910.00	118.80 ± 25.33
control	5	3364.60 ± 1487.24	100.60 ± 8.38
P		>0.05	>0.05

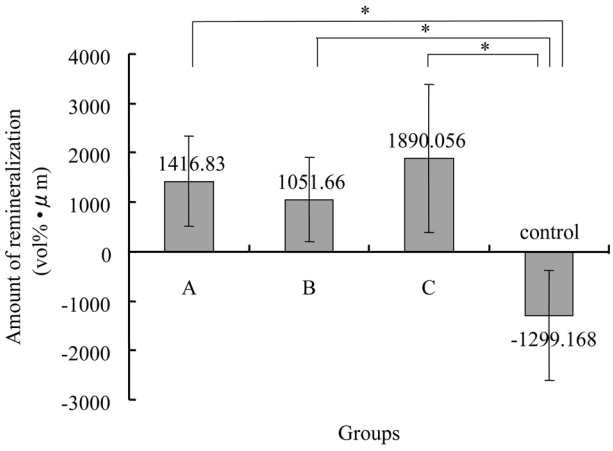

Fig. 6 The amount of remineralization (vol%•μm)
Asterisk shows significant differences by one-way Anova, *P<0.05.

the enamel surface to the inner layer. F Kα reached a maximum at about 5–10μm depth and remained approximately unchanged after about 35μm depth.

There was a significant increase in the amount of F intake. After application of the three fluoride agents were compared with the untreated group (P<0.001). There was a significant difference in the amount of F intake among the three tested groups. Group A showed an extremely greater F intake compared to group C and B (P<0.001). The value in the inner layer of group C was found to be greater than group A. However there were no statistical differences in groups C and A (Table 2). The amount of F intake of group C was greater than that of group B (P<0.05) (Figure 3).

The extremely rapid increase of F distribution in group A was roughly within the outer 20μm enamel, while group B and C approximately reached their greatest F uptake in the depth of 20μm to 90μm; The increase of F distribution in both groups A and C were found to reach the depth of about 120μm, and that in group B to the depth of 100μm (Figures 4, 5).

CMR

The amount and depth of mineral loss at the base line showed no statistical difference (P>0.05) (Table 3).

The tested groups showed a significantly greater amount of remineralization compared to the control group (P<0.05). Among the tested groups, remineralization of group C was detected to be the greatest, following that was group A and group B was the lowest, but no significant difference was shown under statistical analysis (Figure 6).

As to the lesion depth reduction after pH-cycling regimen, both group A and group C

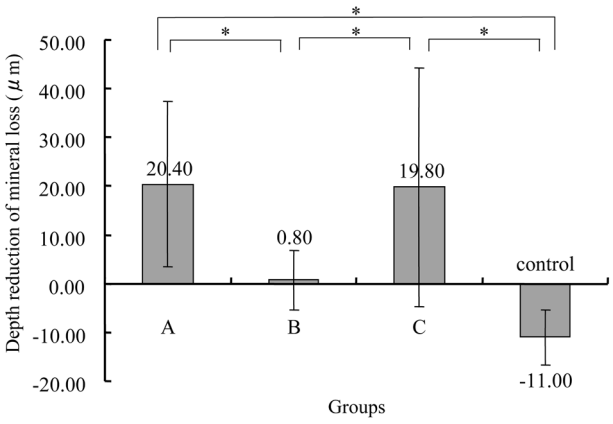

Fig. 7 The depth reduction of mineral loss (μm)
Asterisk shows significant differences by one-way Anova, *$P<0.05$.

showed statistically significant depth reduction in demineralized lesions in contrast to the control group ($P<0.05$), and no difference was found between the two groups. Group B did not show any significance in depth reduction (Figure 7).

Discussion and Conclusion

The study showed that each of fluoride containing agents had a significant remineralizing effect on enamel lesion, regardless of their different F concentrations, forms and type of compound. It provided *in vitro* support for the clinical use of these three agents.

Based on the EPMA analysis, the amount of F uptake of the home gel group was lower, and the depth reached was shallower, compared with the other two fluoride agents. This result agreed well with expectations. Though some studies reported no significant difference between the amount of fluoride uptake in enamel and the fluoride concentration in the agents[13–15]. The tested home gel showed lower fluoride uptake due to its large difference of fluoride concentration, in contrast to FV and APF gel.

The analysis of CMR results disclosed that the FV was as effective as APF in the effect of remineralization, and even showed an inclination to be more effective. This result was in consensus with the previous clinical studies which reported fluoride varnish to have a similar or greater caries reduction ability than APF gel[6,7]. The amount of F intake of FV was less than that of the APF group, especially in the outer layer. But the value in the inner layer of FV group was found to be greater, though without statistical significance. This may suggest that the degree of remineralization was not only related to the integral amount of F intake but also to the distribution of fluoride in enamel layers and its preservation time.

The FV is not officially permitted to be used as a topical fluoride in some countries yet, such as U.S.A. and Japan, because of its high concentration of fluoride. A previous safety study of FV reported it produced much lower plasma F concentration other than APF gel and a 0.2% NaF mouthrinse[16]. Also a clinical trials review found little evidence of side effects of FV[17]. Furthermore, an investigation showed that the operators preferred FV to APF in terms of easier application, short time consumption, no need for moisture control and ability to control patient ingestion. While patients also preferred to it in the aspects of comfort and taste[8].

The tested 0.4% SnF_2 gel in this study also proved to be effective in improving mineral deposition. A tendency for a lower remineralizing ability compared with FV and APF was found, but the difference in the amount of remineralization showed no statistical significance. In this study, the three tested agents were applied to the artificial enamel lesions under the same circumstance and using the same method. Usually APF and FV are recommended to be used in a biannual model, and the home gel used on a daily base. So the amount of mineral regain from the home gel can be expected to be as much, if not more, as the other two tested agents when they are applied on 6-month interval. However, the data of depth reduction in demineralized lesions suggests that the home gel may be not very efficient in any cases of deep demineralized lesions.

A correspondingly higher remineralizing effect at a lower fluoride concentration in the home gel group was found in this study; the combined use of xylitol with 0.4% SnF_2 in this gel may well explain the phenomenon, since remineralizing effect of xylitol on demineralized enamel was recognized previously[12].

In conclusion, the remineralization effects of the tested fluoride agents were all proved in this vitro study. FV and home gel proved to be as efficient topical fluoride agents in retrieving mineral loss as traditional APF gel, in some degrees. Practitioners should also consider patient acceptance, age, and economic condition, etc. when making a clinical selection of a topical fluoride.

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References

1) Clarkson, J.J. and McLoughlin, J.: Role of fluoride in oral health promotion. *Int Dent J* **50**: 119–128, 2000.
2) Newbrun, E.: Evolution of professionally applied topical fluoride therapies. *Compend Contin Educ Dent* **20**(1 Suppl): 5–9, 1999.
3) Schmidt, H.F.: Ein neues touchierungsmittel mit besonders lang anhaltendem intensivem fluoridierungseffekt. *Stoma* **17**: 14–20, 1964.
4) Beltran-Aguilar, E.D., Goldstein, J.W. and Lockwood, S.A.: Fluoride varnishes. A review of their clinical use, cariostatic mechanism, efficacy and safety. *J Am Dent Assoc* **131**: 589–596, 2000.
5) Duncan, T.B., Duncan, W.K. and De Ball, S.: Fluoride: a review—Part II: Topical fluorides. *Miss Dent Assoc J* **55**: 34–36, 1999.
6) Tewari, A., Chawla, H.S. and Utreja, A.: Comparative evaluation of the role of NaF, APF & Duraphat topical fluoride applications in the prevention of dental caries—a 2 1/2 years study. *J Indian Soc Pedod Prev Dent* **8**: 28–35, 1991.
7) Seppa, L. and Tolonen, T.: Caries preventive effect of fluoride varnish applications performed two or four times a year. *Scand J Dent Res* **98**: 102–105, 1990.
8) Warren, D.P., Henson, H.A. and Chan, J.T.: Dental hygienist and patient comparisons of fluoride varnishes to fluoride gels. *J Dent Hyg* **74**: 94–101, 2000.
9) Boyd, R.L.: Long-term evaluation of a SnF_2 gel for control of gingivitis and decalcification in adolescent orthodontic patients. *Int Dent J* **44**(1 Suppl 1): 119–130, 1994.
10) Al-Joburi, W., Clark, C. and Fisher, R.: A comparison of the effectiveness of two systems for the prevention of radiation caries. *Clin Prev Den* **13**: 15–19, 1991.
11) Oshino, K., shintani, T. and Yamagishi, A.: Effect of calcium fluoride on remineralization of initial caries-like enamel lesions. *Jpn J Ped Dent* **41**: 580–587, 2003. (in Japanese)
12) Miake, Y., Saeki, Y., Takahashi, M. and Yanagisawa, T.: Remineralization effects of xylitol on demineralized enamel. *J Electron Microsc (Tokyo)* **52**: 471–476, 2003.
13) Dijkman, A.G., Tak, J. and Arends, J.: Fluoride deposited topical applications in enamel. KOH-soluble and acquired fluoride. *Caries Res* **16**: 147–155, 1982.
14) Saxegaard, E. and Rolla, G.: Fluoride acquisition on and in human enamel during topical application *in vitro*. *Scand J Dent Res* **96**: 523–535, 1988.
15) Rosin-Grget, K., Lincir, I. and Andrijanic, A.: *In vitro* fluoride uptake by enamel from different amine fluoride concentrations. *Caries Res* **36**: 266–269, 2002.
16) Ekstrand, J., Koch, G. and Petersson, L.G.: Plasma fluoride concentration and urinary fluoride excretion in children following application of the fluoride-containing varnish Duraphat. *Caries Res* **14**: 185–189, 1980.
17) Marinho, V.C., Higgins, J.P., Logan, S. and Sheiham, A.: Fluoride varnishes for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2002. CD002279.