In vitro fluoride uptake by bovine enamel in using cyanoacrylate adhesives containing fluoride compounds

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To study the *in vitro* uptake of fluoride from 3-year-old bovine enamel, enamel biopsies for fluoride analysis were performed using five successive etchings. At 10 μm etch depth the uptake of fluoride was maximum for all methyl-α-cyanoacrylates containing six kinds of fluoride compounds. The fluoride concentration was pronounced by 1 month of immersion, rather than by 1 week of immersion in distilled water. In particular, fluoride compounds such as BiF_3, NaF, SnF_2 and ZnF_2 had a significant difference ($p < 0.01$) from KF and Na_2FPO_4 at 1 month of immersion.

1. Introduction

It is known that there is an obvious necessity to clarify the effective method of fluoride utilization in children and adults who remain caries-active. The methods of NaF dentifrice and NaF rinse delivered fluoride effectively to human enamel [1]. The *in vitro* fluoride uptake was controlled solely by the area of bovine enamel surface of the apatitic mineral when equilibrated in buffered solutions containing 10^{-5} to 10^{-3} M fluoride [2]. By enamel biopsies for fluoride analysis [3–5], successive etching was done to determine the fluoride concentration at individual and cumulative etch depths. The enamel to be treated was healthy enamel, and thus a sufficient basic knowledge of the solubility and adhesion of enamel surface was needed to avoid causing harmful effects [6]. The method of bonding various materials to hard tissues has often been attempted [7–9], and the cyanoacrylate adhesives were effective for caries-preventive occlusal sealing. The sealing of the pits and fissures of the molar needs an extremely strong adhesion to the walls of the pits and fissures [6–10].

The present studies were undertaken to clarify the uptake of fluoride in bovine enamel by a successive etching method, and to examine which fluoride compounds in alkyl-α-cyanoacrylate adhesives were effective to the uptake of fluoride for the application of pits and fissure sealant in a dental field.

2. Materials and methods

The alkyl-α-cyanoacrylate of methyl-α-cyanoacrylate (Taoka Chemical Co., Osaka, Japan) was used by including fluoride compounds (BiF_3; Mituwa Chemicals, Osaka, Japan; KF and SnF_2, Kishida Chemicals, Osaka, Japan; Na_2FPO_4 and ZnF_4, Hanawa Chemicals, Osaka, Japan; MaF, Wako Chemicals, Osaka, Japan). Safety considerations were checked for the commercial dental product containing NaF and SnF_2 [11]. In the first series of experiments 3-year-old bovine enamels were given in distilled water for 1 week or 1 month at 37° C (Figs 1a and b). Before sampling, the bovine enamel surface was cleaned with a cotton pellet soaked in alcohol. The adhesive disc of diameter 8 mm was placed on the enamel surface before coating with nail varnish. The thin films on bovine enamels were removed manually. At each stage (I to V) the bovine enamel was immersed in 0.5 M $HClO_4$ (1 ml) for 1 min to calculate the fluoride concentration at each enamel layer [3–5]. An acid buffer such as 0.5 M $HClO_4$/1 M $Na_3C_6H_7O_5 \cdot 2H_2O$ was selected at the

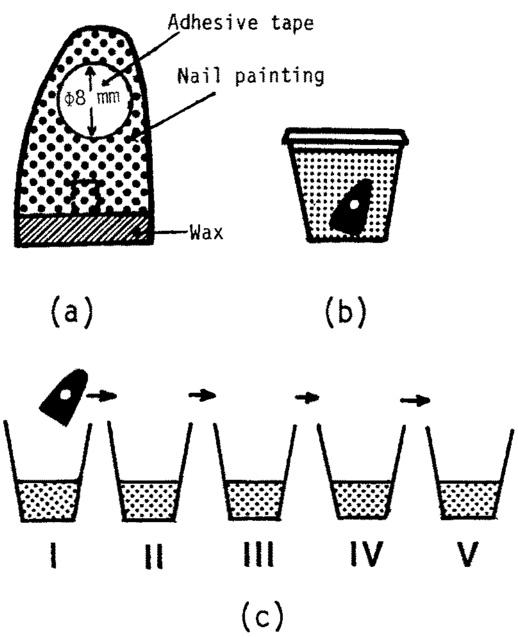

Figure 1 Experimental designs. (a) The treated bovine enamel having diameter 8 mm circle without nail varnish. (b) Solubility of fluoride of (a) in distilled water immersed at 37° C (50 ml). (c) Procedures at five stages (I to V) removing the bovine enamel with 1 ml $HClO_4$ (0.5 M).

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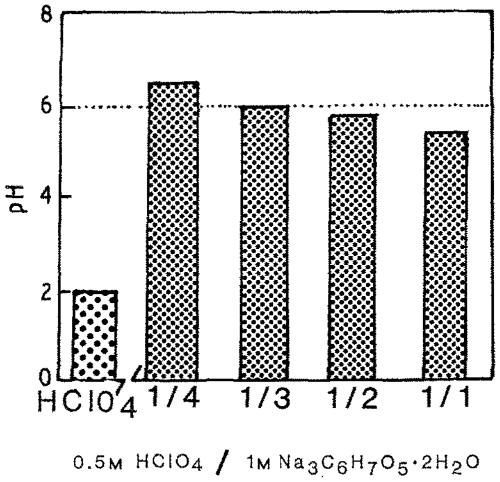

Figure 2 pH of 0.5 M $HClO_4$ and 0.5 M $HClO_4$/1 M $Na_3C_6H_7O_5 \cdot 2H_2O$ (1/4, 1/3. 1/2 and 1/1).

ratios of 1/4, 1/3, 1/2 and 1/1 to obtain a pH above 6.0, as shown in Fig. 2 (pH electrode 91-03; Orion Research, Chicago, USA). In Fig. 3 the pH is shown for different ratios of the solution (0.5 M $HClO_4$ and 1 M $Na_3C_6H_7O_5 \cdot 2H_2O$). The ratio of 1/4 was appropriate for measuring the calcium and fluoride concentrations [5].

In the second series of experiments the bovine enamels obtained in Fig. 1c were given according to the calibration curves of calcium and fluoride concentrations (Figs 4 and 5). The calcium concentration in the solution to which the acid buffer was added was measured by means of an AA-670 spectrophotometer (Shimadzu Co., Kyoto, Japan), and the fluoride concentration was assessed by digital ionanalyser 810A (Orion Research, Chicago, USA), using a 96-09 fluoride electrode. The fluoride concentration was then calculated at 10 μm intervals of the enamel layer between 0 and 70 μm [5, 12]. Care was taken to avoid contamination when the procedure was done, as shown in Fig. 1c. The measurement was repeated three times for each solution obtained in Fig. 1c.

3. Results

After immersion of bovine tooth in distilled water (Figs 1a and b), the fluoride concentration was

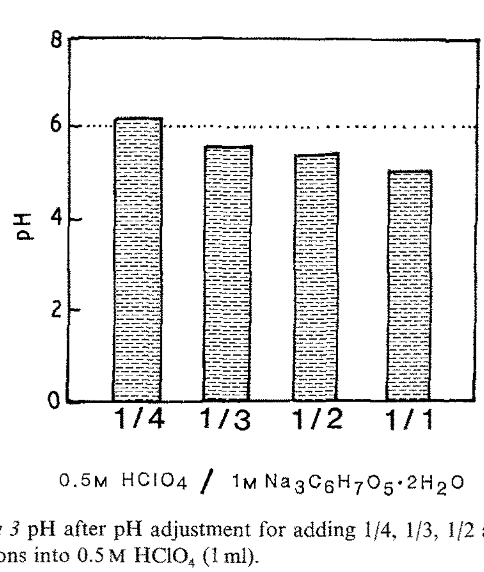

Figure 3 pH after pH adjustment for adding 1/4, 1/3, 1/2 and 1/1 solutions into 0.5 M $HClO_4$ (1 ml).

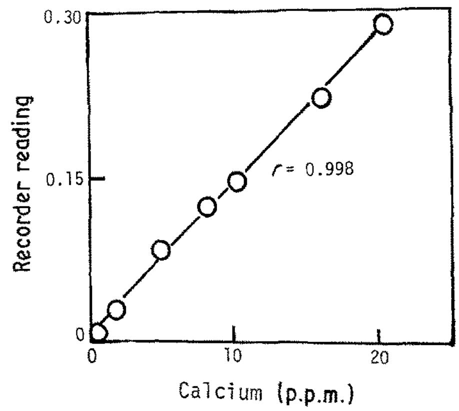

Figure 4 Calibration curve of calcium ion (absolute value had been read from an atomic absorption flame emission spectrophotometer).

checked at different depths of the bovine enamel. Fluoride must be measured in the solution indicating a pH above 6.0 because the fluoride measured in the solution agreed with the total fluoride soluble in the solution [5, 12]. In order to examine the fluoride concentration in the solution of 0.5 M $HClO_4$ (1 ml), five successive etchings were done, the pH adjustment was carried out as shown in Figs 2 and 3. As a result, the solution had a pH above 6.0 when the solution (1/4 = 0.5 M $HClO_4$/1 M $Na_3C_6H_7O_5 \cdot 2H_2O$) was added to 0.5 M $HClO_4$ solution.

For the measurement of fluoride carried out according to Figs 1 to 5, Fig. 6 shows the mean value of the fluoride concentration in bovine enamel when it was etched at each enamel layer of 0 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50 and 50 to 60 μm in the etching solution. The value ranged from 25 to 37 p.p.m. for the bovine enamel. In Figs 7 and 8 the fluoride concentration in bovine enamel is shown at each depth of 10 to 70 μm in using each agent containing fluoride compounds in methyl-α-cyanoacrylate adhesive (the depth of 10 μm in the figure corresponded to 0 to 10 μm interval in this study). A steep fluoride gradient in the outermost enamel was found [13], and the amount of fluoride in a given biopsy obtained from a standarized delineated area (diameter 8 mm) depended on the depth of the biopsy which was equivalent to [mass of enamel/(density of enamel $\times$ biopsy surface area)] [5, 12]. The density of bovine enamel was measured to be 2.91 $g\,cm^{-3}$ as shown in [12] (its value was 2.95 $g\,cm^{-3}$ in [14]). The mass of enamel in the etching solution of 0.5 M $HClO_4$ was calculated by measuring that bovine enamel contained 34% [12].

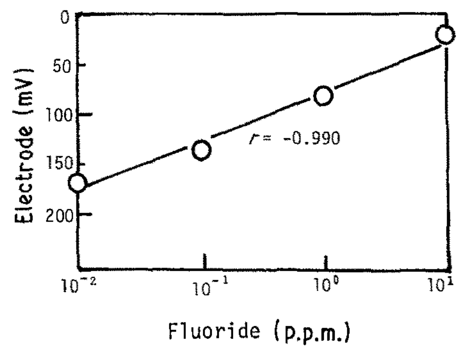

Figure 5 Calibration curve of fluoride ion (millivolt reading was done by electrode potential).

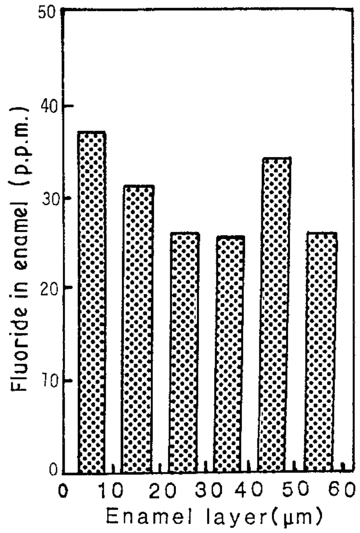

Figure 6 Fluoride concentration in bovine enamel without cyanoacrylate adhesives (control) for each enamel layer, 0 to 10, 10 to 20, 20 to 30, 30 to 40, 40 to 50 and 50 to 60 μm from the enamel surface.

The standardized depth of bovine enamel for each etching was thus calculated in this study.

Data in Fig. 7 were rewritten for every depth of the bovine enamel, as shown in Figs 8a to c. The difference of the cumulative uptake of fluoride obtained from the solution (pH 6.0) was found for the bovine enamel coated with methyl-α-cyanoacrylate containing BiF_3, KF, NaF, Na_2FPO_4, SnF_2 and ZnF_2 compounds. Also, the effect of the immersion time on the mean

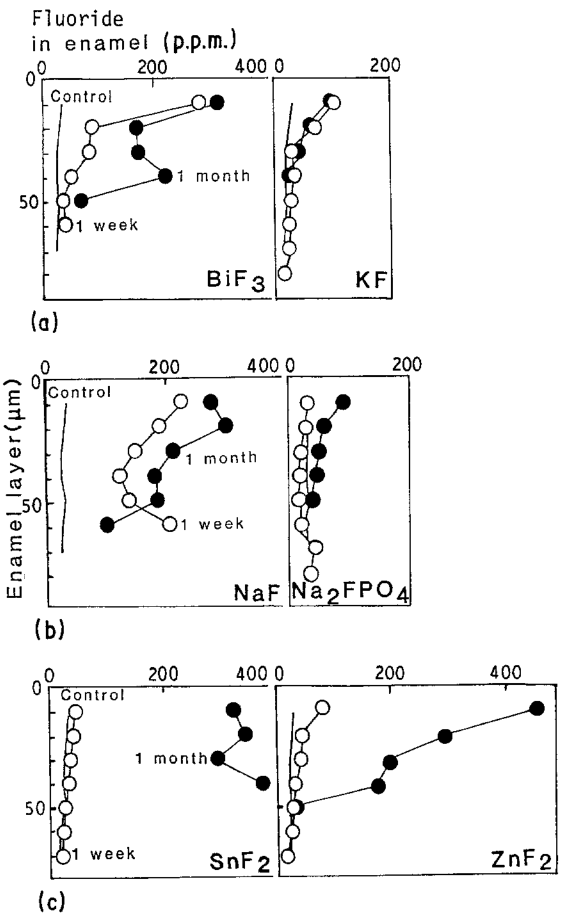

Figure 7 Fluoride concentration after immersion for (○) 1 week and (●) 1 month in bovine enamel coated by the following methyl-α-cyanoacrylate adhesives, containing fluoride compounds: (a) BiF_3 and KF, (b) NaF and Na_2FPO_4 and (c) SnF_2 and ZnF_2.

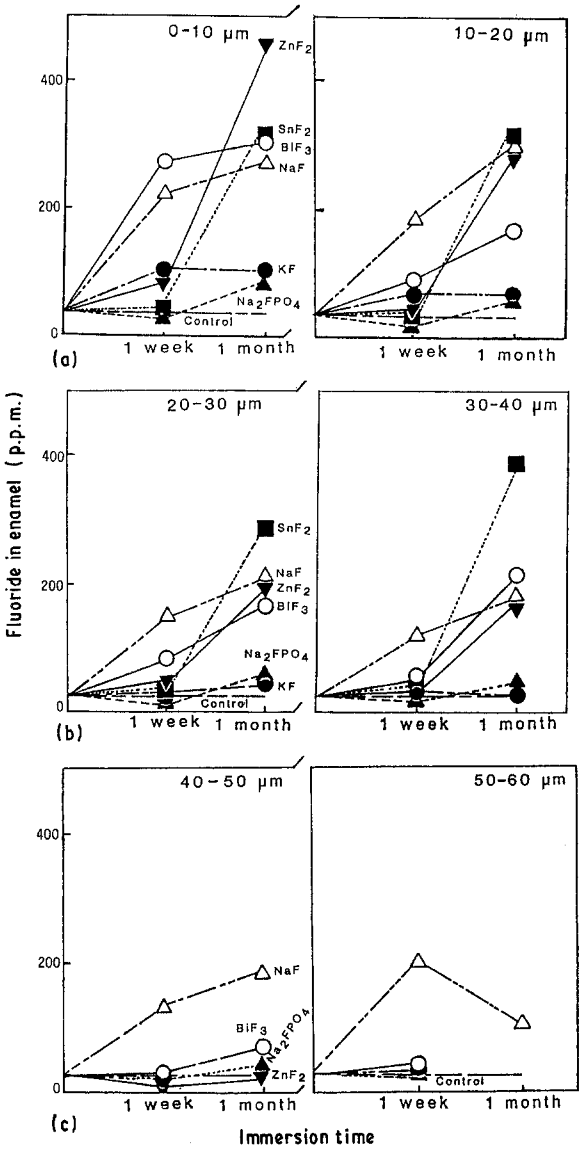

Figure 8 Fluoride concentration at the following enamel layer (the application of each methyl-α-cyanoacrylate adhesive containing fluoride compounds; for key, see Fig. 7): (a) 0 to 10 and 10 to 20 μm, (b) 20 to 30 and 30 to 40 μm and (c) 40 to 50 and 50 to 60 μm.

fluoride concentration was detected. The value at 1 month was larger than that of the bovine enamel immersed for 1 week except for the one including KF fluoride compound, but the fluoride concentration (about 100 to 450 p.p.m.) at etch depths of 10 to 30 μm was larger than that (about 30 p.p.m.) of the bovine enamel without fluoride compounds (control). At successive etched layers of bovine enamel (Figs 8 and 9), the value at the 10 μm, layer 0 to 10 μm was larger than that at the other layers in the immersion times, such as 1 week and 1 month. The maximum value (mean) at 10 μm depth was about 280 p.p.m. for a cyanoacrylate adhesive containing fluoride compound (1 week of immersion) and about 430 p.p.m. for ZnF_2-containing methyl-α-cyanoacrylate adhesive (1 month of immersion). The data for the enamel fluoride concentration of the bovine enamel at each of the five etch depths (Fig. 9b) showed that statistical significance was obtained between KF or Na_2FPO_4 and the other fluoride compounds ($p < 0.01$) for the 10 to 40 μm etch depth.

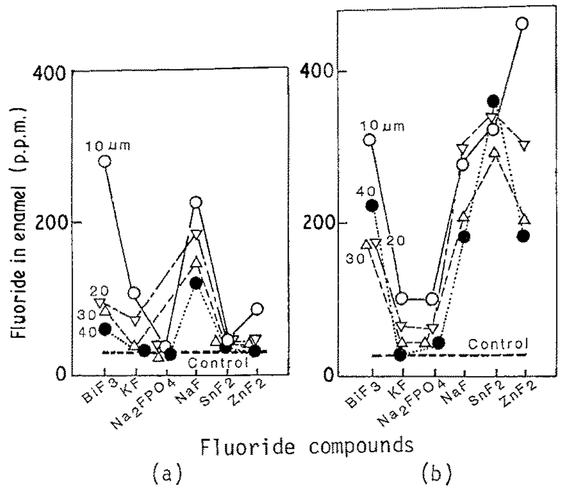

Figure 9 Fluoride concentration in bovine enamel coated by methyl-α-cyanoacrylate containing fluoride compounds (for key, see Fig. 7). Immersion for (a) 1 week and (b) 1 month, both at 37° C.

4. Discussion

Attempts were made to examine whether the fluoride in a methyl-α-cyanoacrylate adhesive was taken up in the bovine enamel. A significant difference due to various types of fluoride compounds was found for the uptake of fluoride concentration (Figs 7 to 9). The treatment by immersion in distilled water was pronounced at 1 month, rather than 1 week. The enamel fluoride concentration at 10 to 40 μm etch depths for methyl-α-cyanoacrylate adhesive with BiF_3, NaF, SnF_2 and ZnF_2 compounds was above 180 p.p.m. as a mean value, but the fluoride concentration for each methyl-α-cyanoacrylate adhesive containing KF and Na_2FPO_4 compounds ranged from 30 to 100 p.p.m. In these fluoride compounds (KF and Na_2FPO_4) the solubility in distilled water was 100%, and thus the fluoride in the adhesive may be out from it and soluble. The other fluoride compounds, such as BiF_3, NaF, SnF_2 and ZnF_2, showed no solubility in the water for BiF_3 and a partial solubility for the last three, but the solubility in the water was not dependent on the uptake of fluoride.

In comparison with the effect of immersion time in distilled water on the uptake of the fluoride concentration, the fluoride in the SnF_2- and ZnF_2-containing methyl-α-cyanoacrylate adhesives was taken up slowly after 1 week of immersion, but the two adhesives had a larger value that the others (Fig. 9b). However, the cyanoacrylate adhesives containing BiF_3 and NaF had an approximately saturated value of uptake of fluoride concentration at 1 week of immersion, showing about 280 p.p.m. for BiF_3 and about 220 p.p.m. for NaF.

Three mechanisms by which fluoride can be taken up in bovine enamel are proposed: (1) exchange for hydroxyl and other ions on the crystal surfaces and in the hydration shells [15]; (2) incorporation into the lattice structure of the growing apatite crystals [16]; and (3) binding by the matrix proteins [17]. In the present study the fluoride uptake is not indicated as being at least partially reversible, but it was suggested that a diffusion-limiting membrane for fluoride from the extracellular fluids into the mineralizing matrix could be present [4]. The hypotheses described above would hold for the fluoride uptake in this study.

We are concerned which methyl-α-cyanoacrylate adhesive containing various types of fluoride compounds is effective to the uptake of fluoride in bovine enamel. Examining the surface of premolars extracted from 10- to 14-year-old children, the relationship between the enamel fluoride concentration at 0 to 10 μm etch depth and dental caries experience was observed [5]. It was thus deduced that fluoride exerted cariostatic activity. In the present study the uptake of fluoride in bovine enamel was clarified when the coated bovine enamel was immersed in distilled water for 1 month at 37° C. Therefore, each fluoride compound which was mixed with methyl-α-cyanoacrylate adhesive had a good effect on the uptake of fluoride, compared with the bovine enamel without fluoride compounds (control). This result shows the probability of application to the human enamel as a fissure sealant at a first stage.

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