alizing buffer solution. During remineralization, water, calcium, and phosphate diffuse through the cellulose film and through a region immediately adjacent to the tooth surface, which contains the micronized powders. Therefore, calcium and phosphate ions were supplied from both the buffer and the dissolution of these powders, while fluoride was supplied directly from the dissolution of I. The higher diffusivity of ions through the cellulose film and the lack of a polymer network on the tooth surface would be expected to lead to much more remineralization by Device B than was accomplished using Device A. Figure 5 shows the fluoride concentration profiles measured in the teeth remineralized with Device B. The results were considerably higher (1000–2000 ppm at depths as great as 40–50 μm) than the results obtained with Device A (<300 ppm). Furthermore, Device B containing only I was more effective in remineralizing the teeth than was Device B containing the I–II mixture, implying that calcium and phosphate readily diffused through the film from the solution.

The fluoride concentration profile of teeth remineralized in saliva instead of the buffer solution are shown in Fig. 5. The results were almost the same as those obtained from remineralization in the remineralizing buffer solution. As was the case when remineralizing with buffer solutions, the system containing I was much better than the I–II mixture. Even in saliva, an appreciable fluoride concentration was achieved using Device B.

DISCUSSION

The results obtained with Device B show that satisfactory remineralization occurred when I was held in proximity to the tooth surface for 24 hr. These initial experiments with Device A, a prototype of a practical means for holding I near the tooth surface, show that the main problem to overcome is to find a polymer film that adheres to the tooth and from which I is released rapidly.

Since these studies showed that remineralization can be achieved in the model systems using bovine teeth, it then was verified that human teeth respond in similar manner (4, 5). These studies provide justification for proceeding in the development of the appropriate polymer system for use with Device A.

SUMMARY

Delivery Device A, a possible prototype of a clinically feasible remineralization system, was not successful with the polymer systems studied. In each case, the film permeability was not high enough for ions to penetrate at a sufficient rate.

Delivery Device B, while not practical for clinical use, had a much higher permeability to ions than did Device A and was used to simulate behavior that might be expected from Device A if a more permeable film could be developed. Device B was quite effective in remineralizing teeth, yielding fluoride levels of ~1000 ppm at depths up to 50 μm, *even in saliva.* Calcium fluoride alone gave better results than the I–II mixtures studied.

These results imply that a practical clinical remineralization procedure based on Device A is feasible, with the primary problem being development of a suitable polymer film.

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ACKNOWLEDGMENTS

Supported by National Institute of Dental Research Grants DE04600 and DE02731.

Novel Topical Fluoride Delivery System II: Remineralization of Human Teeth

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Received June 30, 1980, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.* Accepted for publication January 26, 1981.

Abstract ☐ A recently conceived calcium fluoride-containing remineralization system was tested using human teeth *in vitro.* The influence of several variables (surface pretreatment, demineralization time, and remineralization time) was studied. Appreciable levels of fluoride taken up by pumiced human teeth were found at depths up to 50 μm when remineralization was carried out in either the remineralizing solution or saliva. The successful performance of the delivery device in these laboratory studies is encouraging and indicates that the logical evolution of the crude devices studied thus far could lead to clinically practical fluoride delivery devices.

Keyphrases ☐ Delivery devices—fluoride, remineralization of human teeth *in vitro*, effect of surface treatment on remineralization ☐ Fluoride—delivery devices for remineralization of human teeth *in vitro*, effect of surface treatment on remineralization ☐ Remineralization—human teeth *in vitro*, fluoride delivery devices, effect of surface treatment on remineralization ☐ Teeth, human—remineralization *in vitro* using fluoride delivery devices, effect of surface treatment on remineralization

As a result of *in vitro* studies demonstrating the effectiveness of a controlled demineralization pretreatment in enhancing subsequent fluoride remineralization (1), investigations recently were initiated to develop techniques for a practical clinical remineralization procedure. In the previous study, Yonese *et al.* (2) worked with a delivery device that was capable of physically holding particles of calcium fluoride (I) adjacent to the tooth surface while remaining permeable to calcium, phosphate, and fluoride ions as well as water. This device was capable of depositing high levels of fluoride (1000–2000 ppm) at depths of up to 50 μm, using bovine enamel as a test substrate.

This paper demonstrates the effectiveness of demineralization–remineralization for human teeth and examines additional variables that are clinically relevant but that were not studied with the bovine teeth.

EXPERIMENTAL

Human teeth removed for orthodontic reasons were obtained from dentists in the Chicago area.

The buffer solutions used for demineralization and remineralization were prepared as described previously (2). The demineralizing solution

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consisted of an acetate buffer solution 16% saturated with respect to the thermodynamic solubility of hydroxyapatite (0.1 *M* acetate buffer containing 3.5 m*M* calcium and phosphate, adjusted to pH 4.5 by the addition of sodium hydroxide and to an ionic strength of 0.5 by the addition of sodium chloride). The remineralizing solution also was 0.1 *M* acetate buffer adjusted to pH 4.5 with an ionic strength of 0.5 *M*, but it contained 12 m*M* calcium and phosphate and 10 ppm of fluoride. All chemicals used in the buffers were reagent grade, and all water was distilled twice.

Dialysis bags[1] were used for the cellulose film in fabricating the fluoride delivery device.

The experimental procedures were similar to those reported previously (2). In these experiments, the fluoride delivery device was a cellulose film that physically held calcium fluoride (I) or a mixture of I and dicalcium phosphate dihydrate (II) against the tooth surface (Device B).

Teeth surfaces were conditioned in one of two ways. They were ground with rotating sandpaper (No. 400 first, followed by No. 600) to remove pellicle, or they were pumiced with a non-fluoride-containing pumice.

Prior to remineralization, the tooth received the following pretreatment. The tooth surface was covered by a dental inlay wax[2] except for a 0.25-cm^2 area on the front surface. Then the tooth was demineralized in 10 ml of the 16% partially saturated buffer solution for 0–48 hr while being shaken gently with a wrist-action shaker. The solution was kept at 30 ± 0.1°. The extent of demineralization was assessed by measurement of the amount of phosphate dissolved.

The fluoride delivery device was attached to the tooth surface prior to remineralization as follows. Calcium fluoride, sieved by a 270-mesh sieve (52-μm openings), was spread evenly on the tooth surface and covered by a cellulose film (0.2 cm^2 in area), the periphery of which was fixed in place by adhesive[3]. The teeth with the device attached were remineralized for 24–72 hr in the remineralizing buffer or in saliva. Details of the remineralization procedure were elaborated in the previous paper (2).

All experiments were done in duplicate except where noted, and the two trials generally were within 10–15% of each other. Results of one of the two are presented for clarity in the figures.

The fluoride delivery device was peeled off carefully from the adhesive to avoid damaging the tooth surface. Then the tooth surface was washed with water to remove the particles of I. The teeth were etched in 1 ml of 0.5 *M* $HClO_4$ in a small glass tube without stirring for successive intervals of 30, 60, 120, 200, and 200 sec. The etched surface was washed with 1 ml of water after each etching step. Concentrations of phosphate, fluoride, and calcium 45 in the etching solution were analyzed in the same manner as reported previously (2).

RESULTS

Effect of Pellicle on Remineralization—In the previous experiments, bovine teeth were ground by rotating a fine sandpaper so as to remove the pellicle completely. However, during *in vivo* application of a clinically practical anticaries treatment, the pellicle might not be as readily removable. Therefore, the effects of the pellicle on demineralization and remineralization were investigated.

To test for a possible pellicle effect, one of three types of surface conditioning (grinding, pumicing, or simply washing) was done prior to demineralization. The conditioned tooth was demineralized in 16% partially saturated acetate buffer solution for 0–24 hr and then remineralized in the remineralizing solution. The fluoride delivery device was not used in this experiment.

Figure 1 shows the fluoride concentration profiles obtained for both ground and pumiced teeth. After grinding, the tooth demineralized for 2 hr had a high fluoride uptake while that of the nondemineralized tooth was much lower. The results were similar to those obtained previously with ground bovine teeth. The molar ratio of calcium 45 to fluoride uptake was near the value of 5.0 expected for completely fluoridated hydroxyapatite. For the pumiced teeth, however, the fluoride concentration profiles attained after demineralization–remineralization were lower and at about the same level as for the nondemineralized ground tooth. Thus, the type of surface conditioning greatly affected the degree of remineralization.

Figure 2 shows the cumulative fluoride uptake from the surface to a depth of ~80 μm as a function of the duration of demineralization. The fluoride uptake of both ground and pumiced teeth increased with the duration of demineralization, and the levels attained in the ground teeth were much greater than in the pumiced teeth.

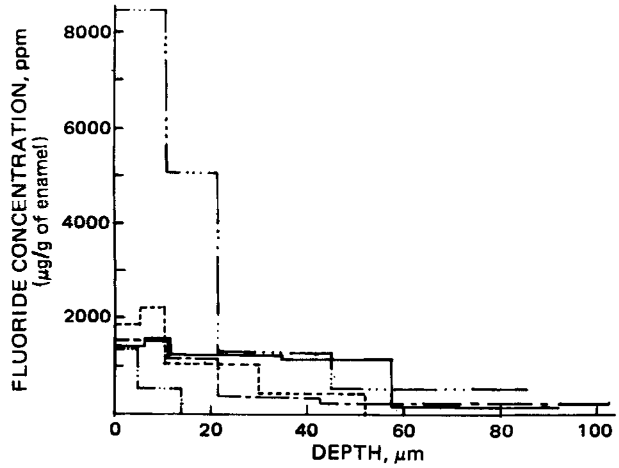

Figure 1—*Fluoride concentration profiles of ground and pumiced human teeth remineralized in buffer solution. Key:* -····-, D_2–R_{24}, *ground teeth;* — — —, D_0–R_{24}, *ground teeth;* ---, D_{24}–R_{24}, *pumiced teeth;* —, $D_{16.5}$–R_{24}, *pumiced teeth; and* -·····, D_0–R_{24}, *pumiced teeth. D and R represent demineralization and remineralization, respectively, and the lower subscripts represent the time in hours.*

Remineralization with Device B in Buffer Solution—Extrapolation of the data for the pumiced teeth in Fig. 2 suggests that prior demineralization for ~48 hr might be necessary to attain fluoride levels in pumiced teeth comparable to the levels attained in ground teeth demineralized for only 2 hr prior to remineralization. Therefore, all pumiced human teeth in these experiments were demineralized for 48 hr to achieve appreciable fluoride uptake. Figure 3 shows the fluoride concentration profiles of these pumiced human teeth after application of Device B containing only I. These teeth were remineralized for 24–72 hr in the acetate buffer. The fluoride concentrations in these teeth were 1000–2000 ppm at depths as great as 40 μm.

Figure 4 shows the cumulative fluoride uptake from the surface to about an 80-μm depth as a function of the remineralization time after application of Device B containing I or I and II (1:6). The device containing I alone appeared to be marginally more effective than the device containing I–II. Furthermore, fluoride uptake was almost independent of the duration of remineralization; a plateau was achieved after ~24 hr of remineralization, as in the previous study using bovine teeth (2).

As the next step in the approach to the *in vivo* situation, remineralization was carried out in saliva. The fluoride uptake concentration profiles of teeth remineralized in saliva are shown in Fig. 5. The fluoride uptake by the tooth remineralized for only 4 hr was very low, as expected. However, the teeth remineralized for 48 hr had fairly high fluoride levels up to a depth of ~50 μm.

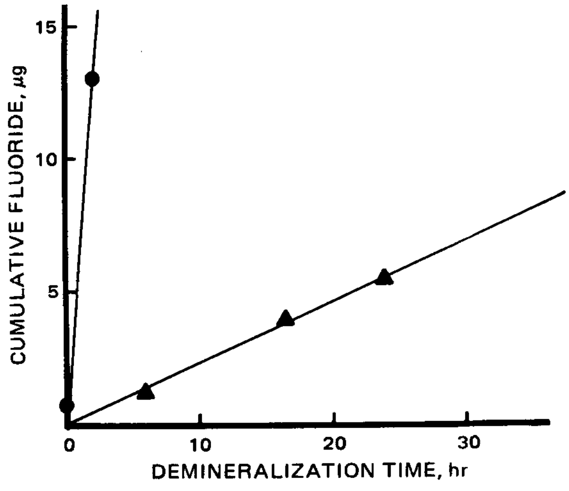

Figure 2—*Influence of surface treatment on cumulative fluoride uptake of human teeth demineralized for different time intervals. Key:* ●, *ground; and* ▲, *pumiced.*

[1] Spectrum Medical Industries, Los Angeles, Calif.
[2] Ivory inlay casting wax, Kerr Sybron Co.
[3] Endur M, Ormco Co.

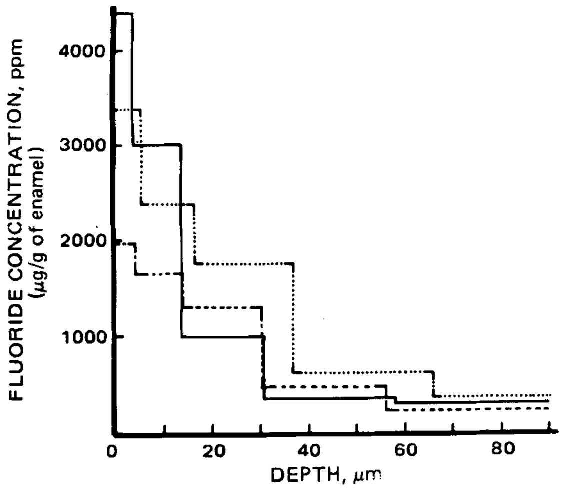

Figure 3—*Fluoride concentration profiles of pumiced human teeth treated with Device B containing calcium fluoride. Key:* —, D_{48}–R_{72}; – · · · –, D_{48}–R_{48}; *and* - - - - -, D_{48}–R_{24}.

To determine whether loosely bound material at the tooth surface affects the interpretation of results, a tooth was brushed with a soft toothbrush[4] after remineralization (Fig. 5). The concentration peak seen at a depth of 5–20 μm in nonbrushed teeth was not present after brushing, implying that some relatively loose material may have been included in previous measurements of fluoride levels near the nonbrushed tooth surface. However, even in the brushed teeth, ~1000 ppm of fluoride was found at a depth of ~50 μm.

DISCUSSION

Teeth used in this study were ground or pumiced prior to demineralization. A few trials also were conducted using teeth with no surface treatment and, therefore, with an intact pellicle. These teeth generally had poor fluoride uptake and considerably larger sample-to-sample variation and, therefore, were not studied further. Although ground teeth had much better fluoride uptake than pumiced teeth, the impracticality of grinding in a clinical setting also limited the number of experiments done with ground teeth. Furthermore, the length of prior demineralization of ground teeth was limited to only 2 hr because of the excessive loss of mineral occurring with longer demineralization times.

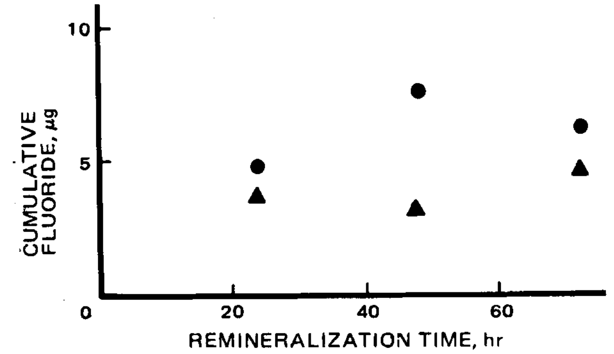

Figure 4—*Cumulative fluoride uptake of pumiced human teeth remineralized for different time intervals with Device B. Each point represents a single experiment. Key:* ●, D_{48}–R_T, *I alone; and* ▲, D_{48}–R_T, *I–II.*

[4] Colgate.

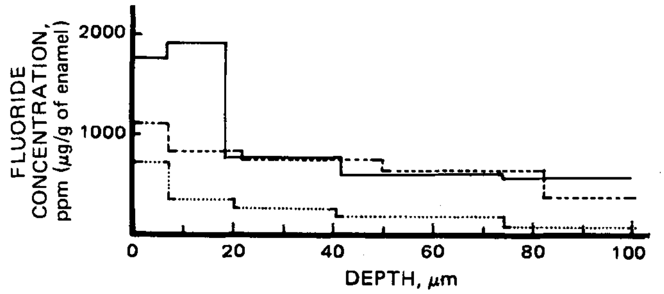

Figure 5—*Effect of brushing after remineralization on fluoride uptake of pumiced human teeth with Device B in saliva. Key:* —, D_{48}–R_{48}, *nonbrushed;* - - -, D_{48}–R_{48}, *brushed; and* – · · · · –, D_{48}–R_4, *nonbrushed.*

The studies conducted with Device B show that the remineralization approach used has promise. In particular, the results show that remineralization is essentially complete after 24 hr of treatment and that appreciable fluoride levels are achieved at depths up to ~50 μm. Furthermore, the results are not greatly dependent on whether I alone or I–II is used. This finding may mean that there is considerable room to manipulate the formulation so that maximum efficiency of fluoride delivery is achieved. Although the mixture appeared to be slightly less effective in delivering fluoride than I alone, the efficiency was quite good (~3–5% of the total fluoride applied was deposited in the tooth).

The experiments conducted with saliva were encouraging, showing that saliva can be exploited as a source of calcium and phosphate even though a physical barrier exists between saliva and the tooth surface. Furthermore, this barrier prevents salivary proteins and other macromolecules from contacting the surface directly and possibly interfering with remineralization.

SUMMARY

Although the surface conditioning of a tooth affects both remineralization and demineralization, human and bovine teeth have the same demineralization–remineralization behavior when the pellicle is removed by grinding. The degree of remineralization attainable increases in proportion to the amount of mineral removed during demineralization.

Appreciable fluoride levels by pumiced human teeth with Device B attached were found at depths of up to 50 μm when remineralization was carried out in either the remineralizing buffer solution or saliva.

The salient features of the fluoride delivery system used in this study were the use of a relatively insoluble fluoride salt (I), which is inherently long acting and also provides a source for calcium as well as fluoride, and the provision for a relatively long contact time (24–48 hr) so that the low fluoride levels provided by I could be sustained.

The successive performance of the delivery device in these laboratory studies is encouraging and indicates that the logical evolution of the crude devices studied thus far could likely lead to clinically practical fluoride delivery devices. Pilot *in vivo* studies (3) with Device B have already yielded results comparable to those obtained in these *in vitro* experiments.

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Supported by National Institute of Dental Research Grants DE04600 and DE02731.