alization for various lengths of time. In both the enamel and pellet systems, the degree of remineralization attainable was directly related to the extent of prior demineralization, although in no instance was the demineralized material 100% recovered in remineralization. Fluoride levels up to several thousand parts per million were found at depths as great as 50 μm from the surface in some cases. The stoichiometry of the remineralized material and electron microprobe examination were consistent with the formation of fluoridated hydroxyapatite rather than I. The detailed characterization of the behavior of the remineralized material is now being addressed in studies in these laboratories.

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Novel Topical Fluoride Delivery System I: Remineralization of Ground Bovine Teeth

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Received June 30, 1980, from the **College of Pharmacy, University of Michigan, Ann Arbor, MI 48109*, and the ‡*American Dental Association, Chicago, IL 60611.* Accepted for publication January 26, 1981.

Abstract □ Laboratory studies were carried out on a newly conceived fluoride-containing remineralizing system with bovine teeth. The prototype fluoride delivery device involved micronized calcium fluoride maintained at the tooth surface with a cellulose film. Together with salivary calcium and phosphate (or simulated saliva), this system was able to generate and maintain the appropriate thermodynamic activity driving force for significant fluorapatite deposition in a reasonably short time (~48 hr).

Keyphrases □ Delivery devices—fluoride, remineralization of bovine teeth, effect of film thickness and particle size □ Fluoride—delivery devices for remineralization of bovine teeth, effect of film thickness and particle size on remineralization □ Remineralization—bovine teeth, fluoride delivery devices, effect of film thickness and particle size on remineralization □ Teeth, bovine—remineralization using fluoride delivery devices, effect of film thickness and particle size on remineralization

Recent *in vitro* studies in these laboratories (1) showed that the amount of fluoride incorporated in a remineralization treatment can be increased substantially if the tooth is demineralized carefully prior to the remineralization. This finding suggests that successful remineralization might be attained *in vivo* if the teeth could be demineralized to the same extent as in the *in vitro* experiments. This paper describes the initial studies in the development of a fluoride topical delivery system designed to achieve *in vivo* results similar to the results obtained *in vitro*.

In vitro remineralization can be very successful when the ionic activity product, K_{FAP} ($a^{10}_{\mathrm{Ca}^{2+}} \cdot a^{6}_{\mathrm{PO}_4^{3-}} \cdot a^{2}_{\mathrm{F}^-}$), is $\sim 10^{-108}$ (1). Less concentrated solutions provide less driving force for remineralization; more concentrated solutions may result in the rapid precipitation of calcium fluoride (I) or dicalcium phosphate dihydrate (II) in the prepared solutions themselves or in enamel pores, thereby blocking or retarding remineralization. It was decided to control the solution conditions at the enamel surface by supplying fluoride in the form of I suspended in a film adhering to the enamel surface.

Calculations have shown that mixtures of I and II or I alone in the film should result in solution compositions appropriate for remineralization at the enamel surface. Furthermore, the relatively low solubility of I limits the rate at which the suspended particles dissolve, so that fluoride applied in this way is inherently long acting, even with no moderation from the film. This fact makes the film design problem much simpler than when a more soluble fluoride source such as sodium fluoride is used and the film must then control the release rate. Therefore, the problem of film design is not to find a film with a narrowly prescribed set of properties but rather to find a film that can hold the particles of I in place while being porous to saliva and interfering minimally with particle dissolution.

EXPERIMENTAL

Materials and Methods—Bovine teeth from 8-week-old, crate-fed, strictly kosher calves were obtained from packing houses in the Chicago area. These animals were chosen because each is subjected to rigidly controlled and uniform environmental conditions (including diet) and

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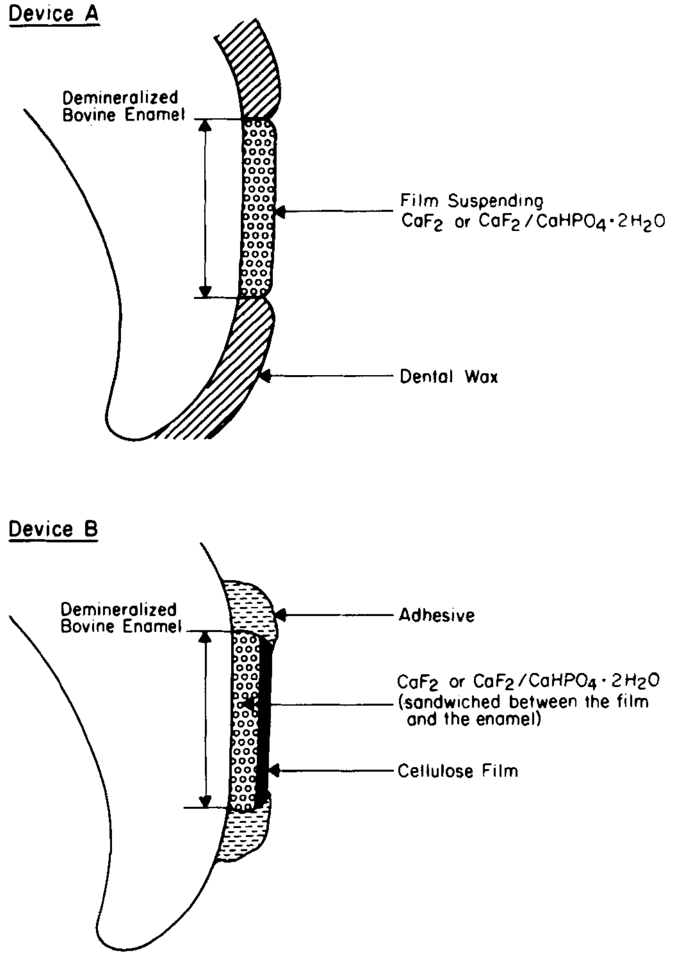

Figure 1—*Fluoride delivery devices.*

each is slaughtered at precisely the same age. Thus, each tooth has very nearly the same past history so there is little experimental variation from one tooth to another. In addition, these teeth provide large flat surfaces for kinetic studies and have no detectable fluoride ($\ll$1 ppm in the enamel). All bovine teeth were ground with a rotating sandpaper (No. 600) to remove any pellicle and further reduce any sample to sample variation.

Chemicals—Ethylcellulose[1] and povidone[2] (average mol. wt. 10,000) were used as supplied from the manufacturer. The cellulose film was cut from dialysis tubing[3]. All other chemicals were analytical grade, and all water was distilled twice.

Demineralization Experiments—In the demineralization experiments, a tooth was covered by inlay wax except for ~0.25 cm^2 of the labial surface. The tooth then was demineralized in 10 ml of a 16% saturated buffer solution (0.1 *M* acetate buffer at pH 4.5, containing 3.5 m*M* total phosphate and total calcium, with the ionic strength adjusted to 0.5 by the addition of sodium chloride) with gentle shaking for 1–48 hr. The extent of demineralization was assessed by measuring the amount of phosphate dissolved. The solution was always kept at 30 ± 0.1°.

The attachment of fluoride delivery devices followed demineralization. Two kinds of devices were used (Fig. 1).

Mixed Polymer Film with Suspended I or I–II (Fluoride Delivery Device A)—Powders of I or II passed through either a 100-mesh sieve (150-μm openings) or a 270-mesh sieve (52-μm openings) were suspended in a 5% (w/v) ethanolic solution of the selected polymer mixture. The tooth was dipped for various times in the polymer solution. Before each dipping, the polymer solution on the tooth surface was dried so as not to drop off. Therefore, the film thickness could be controlled by the dipping times and was varied from 10 to 60 μm. After 2 min of drying, the tooth with the film was immersed in water for 30 min to permit ethanol to leach from the film.

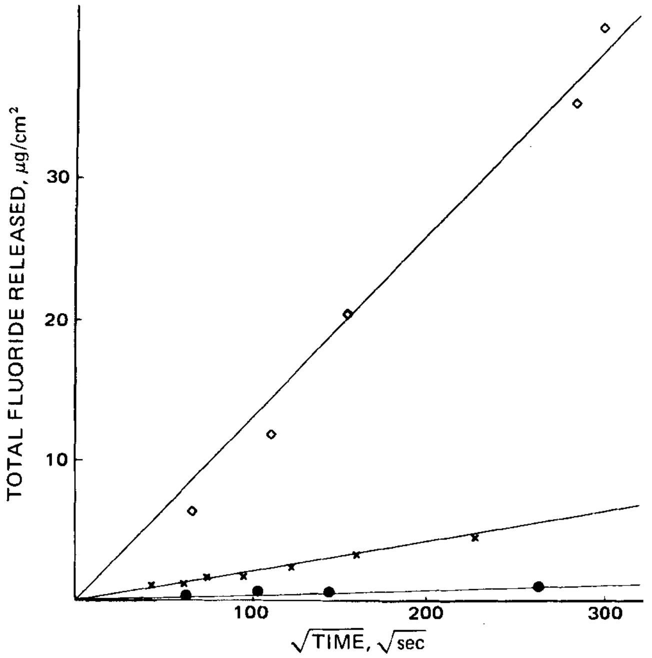

Figure 2—*Plots of Q* versus *the square root of time for determining the diffusivity of calcium fluoride in the polymeric films. Key:* ◇, *calcium fluoride–ethylcellulose–povidone (1:4:1);* ×, *calcium fluoride–ethylcellulose (1:5); and* ●, *calcium fluoride–ethylcellulose–glycerin (1:5:0.22).*

Cellulose Film Containing Micronized I or I–II (Fluoride Delivery Device B)—Powders of I and II passed through a 270-mesh sieve (52-μm openings) were mixed in various ratios. The mixture was scattered evenly on the surface (0.2 cm^2) and was covered by a cellulose film with its periphery fixed by adhesive[4]. The quantities used were 0.5 mg of I or 2 mg of a 1:6 mixture of I and II/0.2 cm^2.

Remineralization Experiments—In the remineralization experiments, the tooth with the attached fluoride delivery device was remineralized for 24–72 hr in buffer for remineralization (0.1 *M* acetate buffer at pH 4.5 containing 12 m*M* of total calcium and total phosphate with its ionic strength adjusted to 0.5 by the addition of sodium chloride). The buffer solution was shaken gently and kept at 30 ± 0.1° as in demineralization. A buffer solution volume of 100 ml was used so that the buffer composition during remineralization would not change significantly.

The remineralization in saliva was done in a test tube with enough saliva for dipping the tooth. The tooth was fixed at the tip of a small glass rod ~1–2 cm shorter in length than the test tube. The test tube was fixed on a rotating wheel (2 rpm) in an electric oven kept at 37°. This was accomplished in such a way that the tooth was prevented by the glass rod from moving down with the saliva as the tube rotated end-over-end and instead was immersed intermittently in the saliva. All remineralization experiments were done in duplicate.

The fluoride delivery system was removed after remineralization. For Device A, the film was dissolved by ethanol, which was renewed at least five times and shaken vigorously. For Device B, the film was peeled off carefully so as not to damage the tooth surface, and the tooth was washed with water to remove any I or II particles adhering to the surface. Then the surface was rewaxed, except for the remineralized area, and etched in 1 ml of 0.5 *M* $HClO_4$ 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.

Assay of Phosphate, Fluoride, and Calcium 45—Phosphate, fluoride, and calcium 45 concentrations in each etching solution were analyzed when the tooth was remineralized in the buffer containing calcium 45. Phosphate and fluoride were analyzed when the tooth with the fluoride delivery device was remineralized in saliva.

Phosphate concentrations were determined by the method of Gee *et al.* (2) in which the phosphoammonium–molybdate complex formed was reduced by stannous chloride. The absorbance of the resulting blue color was determined after 15 min at 720 nm[5].

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[1] Ethocel, Dow Chemical Co.

[2] Matheson, Coleman and Bell.

[3] Spectrum Medical Industries.

[4] Endur M, Ormco Co.

[5] Hitachi 690 spectrophotometer.

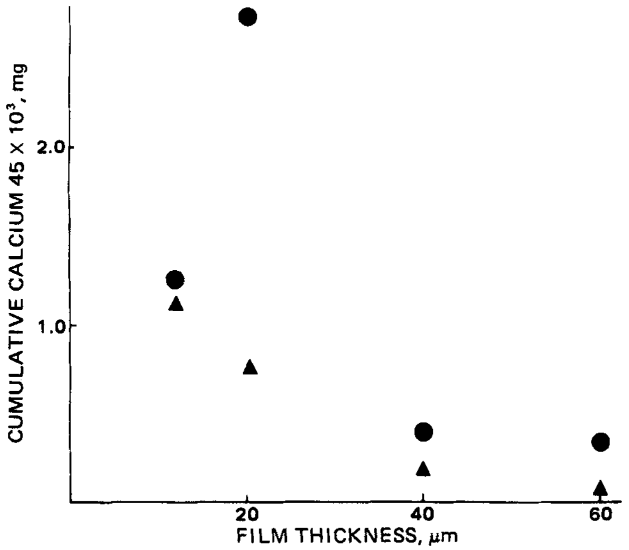

Figure 3—*Effect of film thickness and particle size on the cumulative uptake of calcium 45 with Device A. Teeth were demineralized for 6 hr and remineralized for 24 hr. Calcium fluoride particles passed through a 270- (●) or a 100- (▲) mesh sieve.*

Fluoride concentrations were determined by a combination fluoride electrode[6] using a low-level total ionic strength-adjusting buffer.

Calcium 45 was determined by a scintillation counter[7]. Nonradioactive calcium was measured by atomic absorption[8]. Because of possible phosphate interference, strontium chloride was added to each sample in the latter procedure.

Apparent Diffusivity of I through Ethylcellulose, Ethylcellulose–Glycerin, and Ethylcellulose–Povidone Films—When a solute is suspended in a film, its diffusivity, D_e, through the film can be obtained by measuring the amount of solute released from the film as a function of time according to (3):

$$Q = (2D_e tAC_s)^{1/2} \qquad \text{(Eq. 1)}$$

where Q is the amount of solute released per unit area after time t, A is the total amount of solute in the film per unit volume, and C_s is the solubility of the solute in the film.

The diffusivities of I through ethylcellulose, ethylcellulose–glycerin, and ethylcellulose–povidone films were measured as follows. Calcium fluoride (I) was suspended in 0.3 ml of an ethanolic solution of the polymer or polymer mixture. The suspension was poured into a shallow (1-mm depth) cylindrical depression (0.88-cm radius) on a polytef disk and dried for 13 min in an electric oven at 50°. The film on the disk was then immersed into water that was stirred constantly and kept at 30°. The fluoride released from the film was determined at several sampling times with a fluoride electrode.

RESULTS

Fluoride Delivery Device A—*Diffusivity of I in Polymer Films*—The plots for amounts of I released *versus* the square root of time for the three films are shown in Fig. 2. Effective diffusivities of I then were calculated from the slopes using Eq. 1. The values of D_e for I in ethylcellulose, ethylcellulose–glycerin, and ethylcellulose–povidone films were $1.44 \pm 0.18 \times 10^{-10}$, $3.20 \pm 0.58 \times 10^{-12}$, and $2.20 \pm 0.25 \times 10^{-8}$ cm²/sec, respectively. Since the last film had a higher effective diffusivity and adhered to the tooth better, it was selected for Device A.

Effects of Film Thickness and Particle Size of Suspended I on Remineralization—The teeth demineralized for 6 hr (D_6) were covered by varying thicknesses (10–60 μm) of an ethylcellulose–povidone film containing I, which was sieved by a 100- or 270-mesh sieve (150- or 52-μm openings, respectively). Then these teeth were remineralized for 24 hr in buffer (R_{24}). Figure 3 shows the cumulative uptake of calcium 45 at depths up to 80 μm as a function of the film thickness. Fluoride uptake decreased with increasing film thickness for both particle sizes of I studied and were very small, with levels of only ~1/50 or less of those attained previously in solution remineralization experiments (1). Even in the system with smaller particles, appreciable uptake was not found. These results indicate that the diffusion of ions out of or through the film is the rate-limiting step for remineralization in this system and that the ionic activity product, K_{FAP}, simply does not reach a high enough value ($>10^{-108}$) in the tooth.

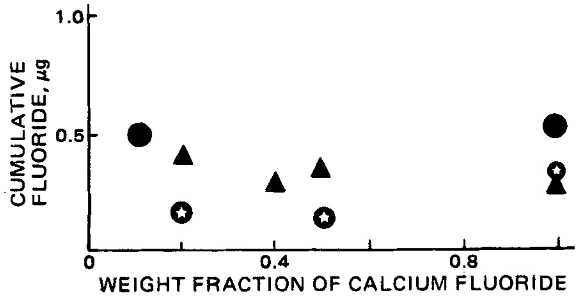

Figure 4—*Effect of ratio of calcium fluoride (CaF_2) to calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) on cumulative fluoride uptake with Device A. Key: ●, D_6–R_{24}, 40-μm film thickness; ✪, D_6–R_{72}, 40-μm film thickness; and ▲, D_{24}–R_{72}, 40-μm film thickness.*

Effects of Longer Remineralization Times and All Three Ion Sources—Assuming that the solubility of I in the aqueous pores of the film phase is equal to its aqueous solubility implies that the fluoride concentration in the pores of the film is ~7 ppm. This concentration is high enough for the formation of fluoridated hydroxyapatite in the remineralization step, suggesting that higher permeabilities of the film to ions and longer remineralization times could increase the degree of remineralization. Furthermore, higher calcium and phosphate concentrations in the film might improve remineralization even if the permeability remains low. Therefore, the following experimental systems were examined.

Teeth demineralized for 6 or 24 hr were covered by a 40-μm film (ethylcellulose–povidone) containing mixtures of I and II and remineralized for 24 or 72 hr. Although a thinner film would present less of a barrier for ions to diffuse in from the external solution, the 40-μm film was selected to accommodate the particles that ranged in size up to ~50 μm. Figure 4 shows the cumulative fluoride uptake (in micrograms) to a depth of 89–90 μm as a function of the weight fraction of I in the I–II mixture. These results ranged from 0.2 to 0.5 μg and did not depend significantly on either the weight fraction of I or the demineralization and remineralization times. Fluoride concentrations in these teeth were much less than expected, a few hundred parts per million at a depth of ~40 μm.

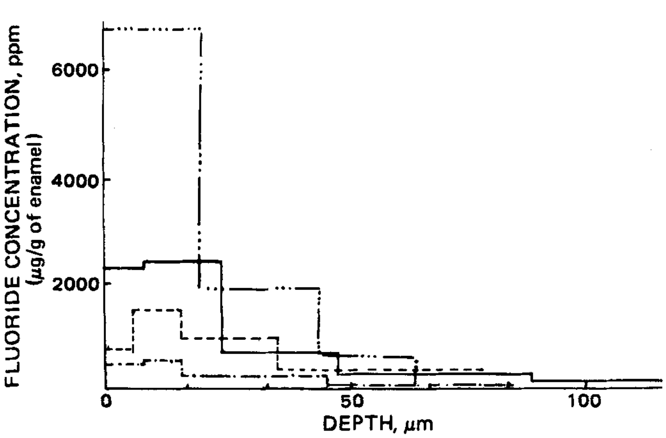

Figure 5—*Remineralization of ground bovine tooth using Device B in saliva and in remineralizing solution. Key: -····-, calcium fluoride with remineralization in saliva; —, calcium fluoride with remineralization in buffer solution; - - -, calcium fluoride and calcium phosphate dihydrate (1:6) with remineralization in saliva; and -·-, calcium fluoride and calcium phosphate dihydrate (1:6) with remineralization in buffer solution.*

Fluoride Delivery Device B—Calcium fluoride or a I–II mixture was dispersed on the surface of a demineralized tooth and covered by a cellulose film in which the ions were estimated to have an effective diffusivity of ~10^{-6} cm²/sec. The tooth was remineralized for 24 hr in the reminer-

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[6] Model 96-09, Orion Co.
[7] Liquid scintillation system, Beckman Instruments.
[8] Perkin-Elmer atomic absorption model 303.

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 $\sim$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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Novel Topical Fluoride Delivery System II: Remineralization of Human Teeth

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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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