

In vivo Remineralization Using a Sustained Topical Fluoride Delivery System

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The efficacy of a new remineralization system was determined in vivo by maintaining a low concentration of approximately 1 ppm fluoride for 48 hrs against a demineralized human tooth. Human subjects were selected who wore removable partial dentures containing two or more of the demineralized teeth with film system. The findings indicate levels of fluoride uptake to 500 ppm at 50 micron depths in experimental sites.

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Introduction.

Many researchers have demonstrated that, in both laboratory and clinical investigations, substantial amounts of fluoride are deposited in human enamel from topical treatments. Unfortunately, a considerable portion of the fluoride deposited from a topical treatment leaches away in a short period of time, and only a small amount is permanently retained.¹⁻³ To increase the amount of retained fluoride in enamel, multiple treatments, as well as sealants and etching, have been studied.⁴⁻¹³ The use of a single application which will provide a prolonged and unhindered treatment appears to be essential to meet the needs of dental health programs.

The purpose of this study was to deter-

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mine, in a pilot clinical program, the efficacy of a new remineralization system employing calcium fluoride. We also tested the hypothesis that a film capable of sustaining a local aqueous fluoride concentration of approximately 1 ppm and permeable to water, calcium, and phosphate ions can remineralize demineralized human tooth enamel with prolonged contact.

Materials and methods.

The material consisted of 11 pairs of homologous contralateral non-carious teeth (extracted for orthodontic expediency). One tooth from each pair was used in the clinical study. The other tooth was used for baseline evaluation of fluoride content for each pair. Residual tissue was cleaned from the tooth with Bunting scalers 5 and 6, and the tooth was brushed under warm tap water with a soft toothbrush. Each pair was individually sterilized by exposure to ethylene oxide gas[§] for five hrs at 63°C, 40% humidity and at eight pounds psi pressure. To verify the sterilization procedure, two additional non-carious bicuspid teeth were sterilized and then incubated at 37°C for 48 hrs in triplicate soya culture media. The results were negative, since no bacterial growth was observed. The verification procedure was repeated after the 11 pairs of teeth were sterilized, and the same negative results were obtained. The enamel surface had a prophylaxis treatment with a non-fluoride pumice.

The roots of the teeth were sectioned with a carborundum disk approximately 2mm from the cemento-enamel junction. The teeth were then covered with a blue inlay wax, except for a region of approximately 0.20 cm² in the center of the buccal and lingual surfaces.

[§] Penngas, Pennsylvania Engineering Co., Philadelphia, PA

The exposed portion of each tooth was then demineralized in a 16% partially saturated buffer solution (pH = 4.5, 0.1M acetate, 3.5mM Ca and PO₄, adjusted to ionic strength 0.5 with NaCl) for 48 hrs. One tooth from each pair was maintained in the laboratory in a high humidity environment, which was obtained by placing a folded 2x2 gauze wet with distilled water at the bottom of a closed glass bottle. The tooth was kept out of contact with the gauze. In this manner, the tooth was prevented from becoming dehydrated. The other tooth from each pair had the wax removed and was used in the clinical study.

Four subjects, ranging from 42 to 74 years of age, were selected. Each subject had at least two posterior edentulous sites on either arch. There were no significant contributing findings in the health histories. Alginate impressions and stone casts were made of the edentulous and opposing arches. Two male subjects each wore a maxillary appliance, while two female subjects each wore a mandibular appliance. Two, three, or four demineralized teeth were adapted to the ridge area of each subject.

The teeth were placed on one or both sides of the arch. The remineralization film was applied to either the facial or lingual surface of the teeth as shown in Figs. 1a and b. The remineralizing system consisted of a water-permeable cellulose dialysis film[†] with 1 mg of CaF₂ powder placed on its surface. The powder and film were sealed against the tooth with a dimethacrylate resin[‡] as shown in Figs. 2a and b. The CaF₂ powder had been ground to a particle size of 50 microns and filtered with a #270 micromesh. The corresponding buccal or lingual surface of the adjacent tooth served as the control (See Figs. 1a and b). Wrought wire clasps, using 0.022-inch-round orthodontic wire, were adapted for retention. Cold-cure acrylic resin** was used to form the denture base for the removable partial dentures.

Each subject was instructed not to remove the denture except for cleaning after meals. The appliance was worn during the

night. The denture and teeth were cleaned lightly under warm water only. The subject's own dentition was cleaned with a soft toothbrush and baking soda. No toothpaste was used. The denture was worn for 48 hrs.

After that time period, the teeth were removed and sectioned with a carborundum disk into facial and lingual halves. The film was removed from the experimental sites. The segments were again covered with a blue inlay wax, except for the region which had originally been exposed for demineralization. All sites were rinsed for a short period of time with double distilled water. The experimental sites with the remineralization system were brushed lightly for five secs to remove residual CaF₂ powder.

Five enamel layers, approximately 5, 10, 20, 30, and 30 microns thick were etched off each tooth by exposing the enamel to 1 ml 0.5M perchloric acid for about 30, 60, 120, 200, and 200 secs, respectively. The enamel was then washed with a 1 ml volume of double distilled water which was added to the original sample. The fluoride concentration was determined with a fluoride electrode.^{††}

The amounts of fluoride uptake in the experimental and control teeth were compared using the nonparametric Mann-Whitney U Test to determine if any significant differences existed.

Results.

The results are summarized in Table 1 and Fig. 3. When the experimental surfaces were analyzed 48 hrs after a single application of the experimental fluoride delivery system, the mean fluoride uptake in the first enamel layer was 600 ppm. The uptake was significant when compared to the control surfaces ($p < 0.01$). Also, the fluoride uptake became highly significant ($p < 0.001$) when the second through fifth enamel layers were analyzed with values of 800 ppm, 500 ppm, 250 ppm, 150 ppm, respectively. The depths reported in Fig. 3 represent the midpoints for each successive enamel layer. The differences between etched enamel layer thickness in the base-

[†]Dialysis Bag, Spectrum Medical Industries, Los Angeles, CA

[‡]Endur-M by ORMCO, Glendora, CA

**Perm repair and rebase cold cure acrylic resin, Hygienic Manufacturing Co., Akron, OH

^{††}Model 96-09 Combination Fluoride Electrode used in conjunction with a Model 701A Digital pH/mv Meter, Orion Research, Inc., Cambridge, MA

TABLE I
 FLUORIDE CONCENTRATIONS (PPM) IN FIVE SUCCESSIVE SURFACE LAYERS (MICRONS)
 OF UNTREATED DEMINERALIZED TEETH (BASELINE); DEMINERALIZED TEETH AFTER
 TREATMENT FOR 48 HRS WITH SALIVA AS THE ONLY REMINERALIZATION SYSTEM
 (CONTROL); AND DEMINERALIZED TEETH AFTER TREATMENT FOR 48 HRS WITH
 EXPERIMENTAL REMINERALIZATION SYSTEM (EXPERIMENTAL).

Layer	Number of tooth surfaces	Base (n = 11)		Control (n = 11)		Experimental (n = 11)	
		Mean (microns) Layer Thickness	Mean F. Conc. ppm	Mean (microns) Layer Thickness	Mean F. Conc. ppm	Mean (microns) Layer Thickness	Mean F. Conc. ppm
1	33	5	474	5	713	5	1100
2	33	11	381	9	540	10	1216
3	33	20	299	19	266	19	789
4	33	31	282	28	242	29	549
5	33	33	144	33	170	32	303

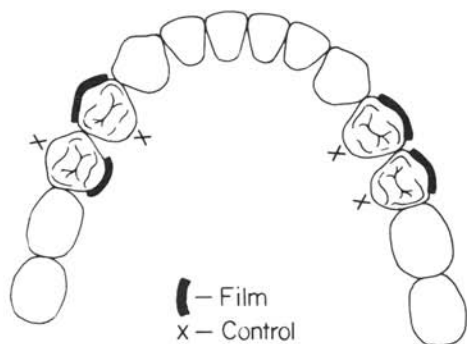


Fig. 1a - Schematic placement of remineralization system for appliance worn by Patient #3.

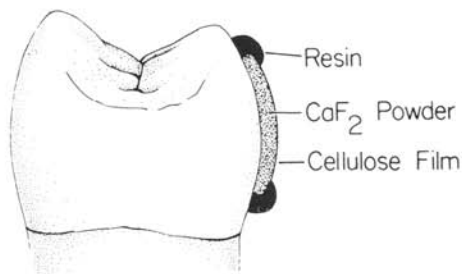


Fig. 2a - Schematic side view of remineralization system on the lingual surface of a bicuspid.

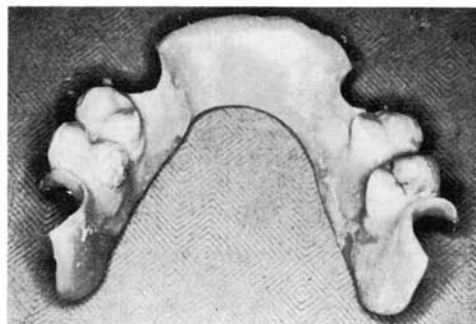


Fig. 1b - Photograph of appliance worn by Patient #3.

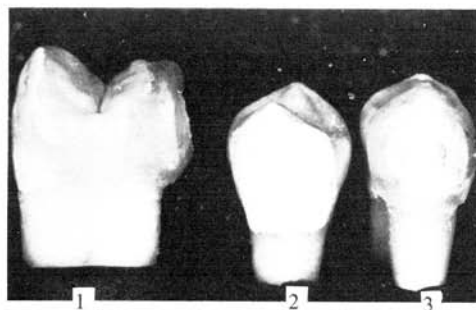


Fig. 2b - (1) Side view with film on lingual surface and demineralized facial surface; (2) front view of a demineralized surface; and (3) front view of a demineralized surface with film system.

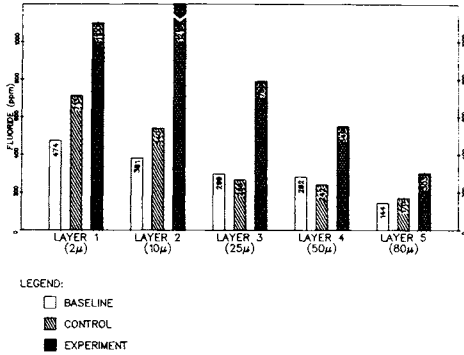


Fig. 3 - Fluoride concentrations (ppm) in five successive layers of baseline, control, and experimental surfaces. The number in parentheses represents the mid-point depth (in microns) for each layer. $N = 11$ for each column.

line, control, and experimental groups were not statistically significant ($p > 0.05$).

Both female subjects who wore the mandibular appliances had non-fluoridated water sources. However, using the Mann-Whitney U Test, no significant difference ($p > 0.05$) was found in fluoride uptake for both control and experimental surfaces between patients living in fluoridated and non-fluoridated areas. Therefore, the results of maxillary and mandibular appliances could be summarized together. Furthermore, no significant difference ($p > 0.05$) was found in fluoride uptake when comparing facial with lingual surfaces for either control or experimental sites. Thus, the data for maxillary, mandibular, facial, and lingual sites could be tabulated together to determine if there was statistical significance in fluoride uptake in enamel layers one through five, as shown in Table 1.

Discussion.

These results support the hypothesis that an artificially demineralized tooth surface or an incipient carious lesion may be relatively rapidly remineralized *in vivo* with an appropriate delivery system. In previously reported studies,^{14,15} it was shown that both demineralized bovine teeth and human teeth are rapidly remineralized (~24 hr or less) when the solution conditions correspond to a fluorapatite activity product ($K_{FAP} = a_{Ca}^{10} a_{PO_4}^6 a_{F}^{-2}$) of around 1×10^{-108} . The deposited mineral phase in

these experiments was shown to possess the fluorapatite stoichiometry both by the perchloric acid etch method and by the electron microprobe assay.

Thermodynamic calculations show that, when CaF_2 is added to saliva, the K_{FAP} value for the system would be in the neighborhood of 1×10^{-108} , and it is relatively independent of pH in the 5 to 7 range. This, then, has been the basis for the rationale that CaF_2 , together with saliva, may be an effective remineralization system. *In vitro* experiments¹⁵ showed that this is so, and the present data support the *in vivo* applicability of the concept.

Before this approach may be considered for human therapeutic applications, answers to two questions should be found. One of these is whether the remineralization has significantly enhanced the tooth resistance to caries. The other is the retention of the remineralized phase and, therefore, the question of the frequency of application.

Conclusions.

A concept for rapid remineralization using a fluoride/saliva system for sustaining the microenvironment K_{FAP} levels has been examined *in vivo*. Micronized CaF_2 applied with a cellulose film was the fluoride source which, together with salivary phosphate and calcium, provided the necessary thermodynamic driving force for the remineralization process. The results of the *in vivo* experiments together with earlier *in vitro* data show that relatively high levels of fluoride (in the form fluorapatite or fluorhydroxyapatite) at substantial depths (~50 microns) may be deposited in a relatively short period of time (~24-48 hrs).

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