# Reduction in Dentin Permeability Using a Slurry Containing Dicalcium Phosphate and Calcium Hydroxide

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Abstract: Treatments that obdurate dentin tubules have been used for reducing dentin hypersensitivity. The purpose of this study was to determine the effect of a treatment with a slurry of micron sized calcium phosphate on the hydraulic conductance  $(L_p)$  of etched dentin discs *in vitro*. The treatment slurry was prepared by mixing a powder mixture of dicalcium phosphate anhydrous and calcium hydroxide with a solution that contained sodium fluoride and carboxymethyl cellulose. The mean baseline  $L_p$  (in mL cm<sup>-2</sup> s<sup>-1</sup> H<sub>2</sub>O cm<sup>-1</sup>) was 2.07 ± 1.45 (mean ± SD; n = 13)). After one treatment and 2, 4, and 7 days of incubation in a protein-free saliva-like solution (SLS), the mean relative  $L_p$ , presented as % of baseline, were  $65 \pm 16$ ,  $42 \pm 27$ ,  $36 \pm 26$ , and  $33 \pm 27$  (n = 13), respectively. The  $L_p$  values of the baseline and treatment after incubation in the SLS were significantly (p < 0.05) different. Scanning electron microscopic examination showed partial obturation of dentin tubules in the treated dentin. X-ray diffraction and chemical analyses indicated the major product formed from the slurry was a fluoride-containing hydroxyapatite. Treatment appeared effective in further reducing  $L_p$  of dentin discs after incubation in the SLS. © 2006 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 78B: 291–295, 2006

Keywords: calcium phosphate; slurry; obturation; dentin permeability; hydraulic conductance; dentin hypersensitivity

## INTRODUCTION

Dentin hypersensitivity is an intermittent chronic experience caused by exposed dentin and demonstrated by an exaggerated pain response to tactile, chemical, thermal, or osmotic stimulus. It has been reported to affect 10% of adults.<sup>1</sup> The mechanism of dentin hypersensitivity has been associated with Brännström's hydrodynamics theory.<sup>2</sup> Reactions that can produce sufficient amounts of mineral precipitated within dentin tubules for obturation are potentially useful for desensitization treatments. If treatment products are insoluble under both neutral and acidic oral conditions, the desensitizing effects are likely to last longer. Different calcium phosphates,<sup>3–7</sup> oxalates,<sup>8</sup> and fluorides<sup>9</sup> have been studied in the occlusion of dental tubules. At present, there have not been

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sufficient data to establish the long-term clinical efficacies of these professionally administered treatments. Calcium phosphate containing solutions have rather limited capacity as a reservoir of mineral ions because these solutions are relatively dilute and not all the ions are available for precipitation. After a fraction of the ions are precipitated, the degree of mineral saturation diminishes. In contrast, a slurry containing solid components that can form an apatite or other calcium phosphate precipitate continuously during oral application has the potential of precipitating larger amounts of mineral. We describe here such a slurry that contained micrometer sized dicalcium phosphate anhydrous (DCPA), Ca(OH)<sub>2</sub>, and sodium fluoride. This slurry formed fluorapatite (FAp) in a saliva-like environment (saliva-like solution), a salt less soluble than hydroxyapatite in acidic medium, within minutes and was evaluated for its ability to reduce the hydraulic conductance of etched dentin discs in vitro.

### MATERIALS AND METHODS

## Sample (Dentin Discs) Preparation

Dentin discs with a thickness of  $0.5 \pm 0.05$  mm (in the text and tables,  $\pm$  refers to standard deviation, which is used as a

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<sup>\*</sup> Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the NIST or the ADAF, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

measure of standard uncertainty) and a typical diameter of about 8 mm were prepared from the middle third of noncarious human third molars (extracted for treatment reasons unrelated to this study and were collected from local oral surgeons) stored in distilled water containing a mass fraction of 0.1% thymol (a disinfectant). Both sides of the dentin discs were first etched by immersion in a solution with mass fraction of 6% citric acid for 3 min and rinsed in distilled water to produce totally opened tubules. Each dentin disc was mounted on a Plexiglas ring (26.0 mm in diameter and 6.0 mm in thickness) that had a 2.0-mm diameter hole located in the center by cementing (Dow Corning 3145RTV Silicone Adhesive, Midland, MI\*) the enamel portion of the disc to the Plexiglas.<sup>10</sup> The hydraulic conductance ( $L_p$ ) (in mL cm<sup>-2</sup> min<sup>-1</sup> H<sub>2</sub>O cm<sup>-1</sup> or 10.20  $\mu$ L cm<sup>-2</sup> min<sup>-1</sup> kPa<sup>-1</sup>) of each sample was measured as described below.

#### **Treatment Slurry**

The treatment slurry was prepared by combining a powder, consisting of mass fraction of 73% CaHPO<sub>4</sub> (DCPA) and mass fraction of 27% Ca(OH)<sub>2</sub>, and an aqueous liquid containing mass fractions of 2% carboxymethyl cellulose (CMC) and 4% NaF. Commercially obtained DCPA (Baker analyzed, Baker, Phillipsburg, NJ) and Ca(OH)<sub>2</sub> (Fisher Scientific, NJ) were individually ground in 95% ethanol and cyclohexane, respectively, in a 250-mL agate jar with 120 agate balls (10 mm diameter) for 24 h in a ball mill (Retsch PM4, Brinkman, NY). After ethanol or cyclohexane was completely evaporated, the powder was sifted through a fabric sieve (Spectrum/mesh, Houston, TX) with 5 mm openings to obtain powder with a median particle size of 1.0 mm, measured by a centrifugal particle analyzer (model SA-CP3, Shimadzu, MD). Slurries with three different powder/liquid mass ratios (2/1, 1/1, 1/2) were prepared. These flow properties of the pastes were evaluated by placing the paste into a plastic column (6 mm ID) that was filled with glass beads with an average diameter of 0.1 mm (Thomas Scientific, NJ). A regulated N<sub>2</sub> gas of 0.14–0.21 kg/cm<sup>2</sup> pressure was applied on the top of column for 20 s to force the paste into the spaces between glass beads. The slurry with a powder/ liquid mass ratio of 1/1 was selected because this slurry had the consistency of a homogeneous soft paste and was able to flow through the glass beads filled column without being separated into particles and liquid phases.

The treatment slurry was freshly prepared by mixing all the ingredients with the aid of ultrasonication (Branson Ultrasonics, Danbury, CT) for 3 min, during which the slurry container was placed in ice water to prevent the mixture from overheating.

### **Characterization of Products From the Treatment Slurry**

To understand the reaction that will occur in the treatment slurry, samples of the slurry were removed at 3 min, 30 min, and 2 h after mixing and analyzed by powder X-ray diffraction (XRD) (model DMAX 2200, Rigaku/USA, Danvers, MA) with graphite-monochromated copper  $K_a$  radiation ( $\lambda = 0.154$  nm) generated at 40 kV and 40 mA.



**Figure 1.** Schematic drawing of a modified Pashley's flow cell for measuring hydraulic conductance of dentin samples.

Some of the F originally present in the treatment slurry would be incorporated into the treatment product. The following chemical analyses were conducted to determine quantitatively the amounts of F incorporated in the forms of CaF<sub>2</sub> and FAp. Three samples of the treatment slurry were placed in 100% humidity, 37°C box for 2 h. Each sample, with mass in the range of 0.516–0.534 g, was filtered, dried, and weighed before being equilibrated with 5 mL of 1 mol/L KOH in an end-to-end shaker for 24 h to dissolve CaF<sub>2</sub>.<sup>10</sup> The suspension was filtered and the supernatant was analyzed by F ion specific electrode to determine the amount of F present in the form of CaF<sub>2</sub>. The precipitate was then dissolved in 0.5 mol/L HClO<sub>4</sub> for analysis of F in the form of FAp.<sup>11</sup>

## Incubation Solution

All samples were incubated in a protein-free SLS<sup>12</sup> before and after treatment to simulate the effects saliva may have *in vivo* on the treatment products and their effects on dentin permeability. The SLS contained 1.2 mmol/L CaCl<sub>2</sub>, 0.72 mmol/L KH<sub>2</sub>PO<sub>4</sub>, 30 mmol/L KCl, 50 mmol/L HEPES, and 1% thymol. The pH was adjusted to 7.0 with KOH.

# Permeability Cell and Hydraulic Conductance Measurements

A modified Pashley flow system<sup>13</sup> (Figure 1) that consisted of two parts was used to measure  $L_p$ . The top part was connected to a flow rate measuring device and the phosphate buffered saline (PBS) (Biofluid, Bethesda, MD) reservoir that was situated 1.44 m above the cell. The lower part was the Plexiglas ring with the mounted dentin sample as described above. The flow rate was determined by measuring the length of time for a small bubble in the PBS to travel a 20-cm distance in the capillary glass tube. PBS contained 0.15 mol/L NaCl, 1.7 mmol/L KH<sub>2</sub>PO<sub>4</sub>, and 4.95 mmol/L Na<sub>2</sub>HPO<sub>4</sub> and had a pH of 7.2  $\pm$  0.01.

#### **Treatment Procedures**

Thirteen mounted dentin discs were first brushed with an electric toothbrush (Braun type 4728, Oral-B, Belmont, CA) on both sides for 1 min prior to baseline incubation. The soft



**Figure 2.** XRD patterns ( $\lambda = 0.154$  nm) of the reactions of dicalcium phosphate anhydrous (DCPA) and Ca(OH)<sub>2</sub> in 2% CMC containing 4% NaF solution after 3 min, 30 min, and 2 h.

bristle electric toothbrush can generate 2,800 rpm and 60g of vertical force during brushing. The same brushing procedure was also applied before all  $L_p$  measurements. To establish the baseline  $L_p$  value for each sample,  $L_p$  measurements were conducted before SLS incubation, and during various time points, that is, 2, 4, and 7 days after the sample was immersed in 20 mL of SLS (replaced daily) at 37°C. Following the baseline  $L_{\rm p}$  measurements, the dentin specimen received the treatment regimen as follows. The disc was first blotted dry with tissue wipes. The treatment slurry was then applied to the upper side of the disc with a small disposable brush. The treated surface was gently dried by blowing a water-presaturated N<sub>2</sub> gas of 0.14-0.21 kg/cm<sup>2</sup> pressure for 20 s. The specimen was then placed in 100% humidity, 37°C box for 15 min, and then immersed in 20 mL of SLS for 2 h. At the end of incubation, the surface of the treated side was brushed again for 1 min before  $L_p$  measurements. The treated specimen was subsequently incubated in a SLS (replaced daily) for a total of 7 days, with  $L_{\rm p}$  measurements taken at the same day points as in the procedure for determining the baseline  $L_{\rm p}$ .

Because the dentin discs exhibited a wide range of baseline  $L_p$  values, relative hydraulic conductance  $(L_p(\%))$ , which is the hydraulic conductance of a sample at any time point expressed as a fraction of the baseline  $L_p$  of the same sample, was used in the data analysis.

#### Scanning Electron Microscopic Analysis

Scanning electron microscopy (JEOL JSM-5300, JEOL USA, Inc., Peabody, MA) was used to observe the surface morphology of the samples after they were treated and incubated in the SLS. Two samples from each time point, with and without fractured longitudinal cross section, were coated with gold (DESK II Cold Sputter/Etch unit, Denton Vacuum, LLC, Stephene City, VA) and examined under the condition of 15 kV and 45 mA.

#### **Statistical Analysis**

The baseline  $L_p$  for dentin specimen was established by averaging the four  $L_p$  values measured during the "incubation" regimen. The relative  $L_p$  of the treated sample was expressed as  $L_p$  (%), which is the fraction relative to the baseline  $L_p$  of the same sample. Analysis of variance (ANOVA) and Student Newman-Keuls Multiple Comparisons Tests were used to analyze the treatment differences at 0.05 level of significance. Pearson's coefficient of correlation was used to examine correlations between baseline and treatment  $L_p$  values.

#### RESULTS

XRD analyses (Figure 2) at 3 min, 30 min, and 2 h after preparation of the treatment slurry showed that the product formed was a low crystallinity apatitic material, and formation of the apatitic material increased with time. Chemical analysis results showed that 2 h after the preparation of the treatment slurry, F, which was originally present in the liquid phase of the slurry, was incorporated into the slurry solid. The mass fractions of F in the forms of CaF<sub>2</sub> and FAp accounted for 89.9%  $\pm$  10.1% and 10.5%  $\pm$  0.52% (n = 3) of the F in the solid phase of the slurry, respectively.

Table I shows the baseline  $L_p$ , that is, the  $L_p$  (in mL cm<sup>-2</sup> min<sup>-1</sup> H<sub>2</sub>O cm<sup>-1</sup>) values of the etched dentin samples measured during the "incubation" regimen (see treatment procedure above). The  $L_p$  values for a given sample showed random fluctuations rather than a significant change with time. Thus, the baseline  $L_p$  for each sample was obtained by averaging the four  $L_p$  values measured during the incubation

TABLE I. Mean Hydraulic Conductance Values ( $L_p$ ) and  $L_p$ % of Dentin Samples Before Treatment With Dicalcium Phosphate Anhydrous and Ca(OH)<sub>2</sub> and After Incubation in a Saliva-Like Solution at Four Time Points

	Hydraulic Conductance $(L_{\rm p}, \mu {\rm L} {\rm cm}^{-2}{\rm min}^{-1}{\rm H_2O}{\rm cm}^{-1})$	Relative Hydraulic conductance (As % of Initial Reading)
Day 0 before incubation	1.96 ± 1.37   *	100 *
Day 2 after incubation	$2.00 \pm 1.44$	$101 \pm 12$
Day 4 after incubation	$2.07 \pm 1.47$	$104 \pm 21$
Day 7 after incubation	$2.15 \pm 1.50$	$108 \pm 19$
Baseline incubation average	$2.07 \pm 1.45$	

\*Values within each column are not significantly different (p > 0.05).

	$L_{\rm p} \ (n = 13; \ \mu L \ {\rm cm}^{-2} \ {\rm min}^{-1} \ {\rm H}_2 {\rm O} \ {\rm cm}^{-1})$	$L_{\rm p}$ (As % of Mean Baseline Incubation)
Baseline incubation average	$2.07 \pm 1.45$  *	100  *
Day 0 after treatment	$1.35 \pm 0.98$	$65 \pm 16$
Day 2 after incubation	$1.09 \pm 1.09$	$42 \pm 27$
Day 4 after incubation	$0.94 \pm 0.98$	$36 \pm 26$
Day 7 after incubation	$0.88 \pm 0.98$	33 ± 27

TABLE II. Mean Hydraulic Conductance Values ( $L_p$ ) and  $L_p$ % of Dentin Samples After Incubation in a Saliva-Like Solution and Treatment With Dicalcium Phosphate Anhydrous and Ca(OH)<sub>2</sub> at Four Time Points

\*Values within each column are not significantly different (p > 0.05).

period. The baseline  $L_{\rm p}$  values of the 13 dentin samples ranged from 0.50 to 4.47, leading to an averaged baseline  $L_{\rm p}$  value of 2.07  $\pm$  1.45 (n = 13).

Table II shows the individual and the mean relative  $L_{\rm p}$  (%) values of the thirteen samples at each time point in the treatment regimen. Multiple comparison tests indicated that the relative  $L_{\rm p}$  (%) values fell into three populations (p < 0.05). The mean relative  $L_{\rm p}$  (%) measured after treatment prior to SLS incubation was significantly lower than the mean baseline relative  $L_{\rm p}$  (%). The 2-, 4-, and 7day mean relative  $L_{\rm p}$  (%) values were not different from each other but were lower than the 0-day and baseline  $L_{\rm p}$  (%) values. Correlation analysis showed that changes in  $L_{\rm p}$  by the treatments did not correlate to the baseline  $L_{\rm p}$  value of that sample.

Scanning Electron Microscopic pictures of the fractured surfaces of the treated and untreated sides (Figures 3 and 4, respectively) of a sample 7 days after treatment show that dentin tubules were partially obturated by crystalline deposits formed inside the dentin tubules after incubation in an SLS.

# DISCUSSION

Before treatment, the baseline incubation was established to investigate the effects of SLS on the permeability of dentin discs during the experiment. The results from multiple comparison analysis indicated that incubation in SLS without a treatment produced no effects on  $L_p$ .

Micrometer sized (median particle of 1 mm) DCPA and  $Ca(OH)_2$  were used in this study to increase the rates of dissolution of these mineral reservoirs and precipitation of hydroxylapatite (HA), with the possibility of some penetration of the particles into dentin tubules. Inclusion of 2 mass % CMC and 4 mass % of NaF in the liquid phase of the slurry was to increase the coherence of the slurry and to promote the formation of FAp, respectively.

For each treatment, the treatment slurry needed to be freshly prepared and applied to the dentin surface immediately, because apatite began to form within 3 min after mixing. The regulated air pressure from  $N_2$  might have facilitated the entry of the treatment slurry onto dentin discs, when air equilibrated with water, the air may have prevented the slurry from further dehydration. To mimic oral treatment, specimens were placed in 100% humidity for 15 min, after being treated with the slurry. The SLS incubation time was 2 h because the formation of apatite was nearly completed as shown by XRD where the peaks of the DCPA were greatly reduced.

The present *in vitro* experiment (Table II) showed that there was a significant reduction (35%) in relative  $L_p$  after a single treatment and a 67% reduction after 7 days of incuba-



Figure 3. The treated side of a sample after 7 days of SLS incubation, showing mineral deposits in tubules (arrows).



Figure 4. The other side of the same sample (untreated) after 7 days of SLS incubation, showing no mineral deposits in tubules (arrows).

tion in an SLS. There was no significant difference in  $L_p$  among samples incubated for 2, 4, and 7 days. The obturated material was not washed off during the incubation in SLS, which contains calcium and phosphate ions, is supersaturated with respect to HA and was reported to promote the formation of HA.<sup>5</sup> Thus, SLS may have precipitated additional amounts of HA on treated dentin, and further reduced the dentin permeability. However, this is in contrast to the finding that incubation in SLS before treatment did not change the baseline  $L_p$ , suggesting that HA precipitation on dentin from SLS did not occur without the treatment.

SEM analysis on the fractured surfaces on the treated side of dentin showed precipitated apatite crystals within the tubules after incubation in SLS for 2 days, but not in samples immediately after treatment. It may explain the observation that the reduction in dentin permeability occurred not only by the treatment, but continued with the apatite formed during the incubation in SLS.

The results from chemical analysis suggested that F in the treatment slurry was nearly totally converted to both  $CaF_2$  and FAp at a ratio of about 9:1 at the end of 2 h. However  $CaF_2$  could eventually dissolve and release more F for possible reincorporation into HA as apatically bound F. Since both HA and FAp are insoluble in resting oral fluids and FAp is less soluble than HA under acidic conditions, it is possible that under both resting and cariogenic oral conditions the reduced dentin permeability produced by such a treatment would not be lost significantly with time. Further studies are warranted to promote the formation of FAp with higher percentage of more soluble F compounds in the liquid phase, such as  $NH_4F$  or KF, as well as to evaluate the long-term effectiveness of treatments that form FAp as the main tubule occluding deposits.

In conclusion, it is feasible to partially obdurate dentin tubules with this micrometer sized mixture of DCPA and  $Ca(OH)_2$  because of the formation of acid resistant FAp within the dentin tubules. Although a single treatment did not lead to total obturation, multiple treatments may increase the possibility of total obturation.

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