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Reduction in dentin permeability using mildly supersaturated calcium phosphate solutions

A.M. Cherng*, L.C. Chow, S. Takagi

American Dental Association Health Foundation, Paffenbarger Research Center, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

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Calcium phosphate; Smear layer; Multiple treatments; Tubule obturation; Dentin permeability

Summary Treatments that obturate dentin tubules have been used for reducing dentin hypersensitivity. The objective of this study was to determine the effects of multiple treatments with a mildly supersaturated calcium phosphate solution on the hydraulic conductance (*Lp*) of partially occluded dentin discs in vitro. The treatment solution contained 6.5 mmol l^{-1} each of calcium and phosphate, 0.25 mmol l^{-1} fluoride, 30 mmol l^{-1} KCl, and 50 mmol l^{-1} HEPES buffer (pH adjusted to 7.0). The mean baseline *Lp* (in $\mu l\ cm^{-2}\ min^{-1}\ H_2O\ cm^{-1}$) was 0.108 ± 0.041 (mean $\pm$ S.D.; $n = 9$, $\mu l\ cm^{-2}\ min^{-1}\ H_2O\ cm^{-1} = 10.20\ \mu l\ cm^{-2}\ min^{-1}\ KPa^{-1}$) and after five consecutive treatments, the mean relative *Lp*, presented as percentage of baseline, were 71 ± 11, 58 ± 10, 46 ± 18, 40 ± 14, and 25 ± 10, respectively. The *Lp* values of the baseline and treatment groups were significantly ($P < 0.05$) different. Consecutive treatments appeared effective in further reducing *Lp* of dentin discs.
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Introduction

Dentin hypersensitivity has been associated with permeable dentin based on the hydrodynamics theory.[1] Therefore, one of the treatments for dentin desensitisation is to obturate dentin tubules with various tubule-occluding agents. Among mineral agents, the clinical efficacy of oxalates[4] and fluorides[11] on the treatment of dentin hypersensitivity had been studied. Some of these treatments were highly effective, but the effects were of limited duration.[10] The effects of calcium phosphates[5,6,8,16,17] on dentin permeability had been reported. At present, there have not been sufficient data to establish the clinical efficacies of these professionally administered treatments. Previous studies showed that calcium and phosphate levels in saliva[3] and plaque[18] were elevated for a period of 15 min with the use of calcium and phosphate-releasing chewing gums. The possibility exists that this may lead to precipitation of calcium phosphate salts on tooth surfaces that may partially obturate dentin tubules and, consequently, enhance the reduction of dentin permeability even if the recovery of complete occlusion may occur with time by nature. The objective of this study was to determine the short-term effects of multiple treatments with a calcium phosphate solution on the hydraulic conductance of partially occluded dentin discs in vitro. The calcium and phosphate concentrations of the treatment solution were similar to those of the saliva samples collected during the use of calcium phosphate releasing gums.[3]

Materials and methods

Sample (dentin discs) preparation

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

*Corresponding author.

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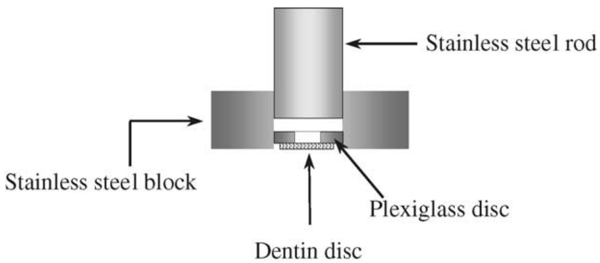


Figure 1 Schematic drawing of the device used to perform sanding of dentin discs.

standard uncertainty) were prepared from the middle third of non-carious human third molars stored in distilled water containing mass fraction of 0.1% thymol. 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 cemented (Dow Corning 3145RTV Adhesive, Midland, MI)[1] to a Plexiglas disc (26.0 mm in diameter and 6.0 mm in thickness) that had a 2.0 mm diameter hole located in the centre. The mounted dentin specimen was then sanded to produce a smear layer on one side with partially occluded dentine tubules as follows. The mounted dentin specimen, with the dentin facing down, was placed in the hole (Fig. 1) of a 10 mm thick stainless steel block. A stainless steel rod, approximately, 26.0 mm in diameter, 50.0 mm in length, and 135.0 g in mass, was placed through the hole and rested on top of the Plexiglas disc. The upper side of the dentin disc, held in this assembly, was sanded on wet 320-grit sand papers for 30 s, which consisted of 15 and 20 cm long reciprocal strokes. The specimen was then thoroughly rinsed with distilled water. The hydraulic conductance (*Lp*) (in $\mu l\ cm^{-2}\ min^{-1}$ $H_2O\ cm^{-1}$ or $10.20\ \mu l\ cm^{-2}\ min^{-1}\ KPa^{-1}$) of each sample before and after sanding were measured as described below.

Treatment solution

The composition of the treatment solution was determined based on the results of a preliminary test in which solutions that contained 30 mmol l^{-1} KCl, 50 mmol l^{-1} HEPES buffer (pH adjusted to 7.0 ± 0.01) and various levels of calcium, phosphate, and fluoride concentrations were prepared. The absorbance due to turbidity of the solutions at a wavelength of 500 nm was measured as a function of time with a spectrophotometer (Perkin-Elmer Lambda 3A UV/VIS, Norwalk, CT). A solution that contained 6.5 mmol l^{-1} each of calcium and phosphate and 0.25 mmol l^{-1} of fluoride developed measurable turbidity at about 1 min after preparation and the turbidity continued to increase in the subsequent 15 min period. This solution was selected as the treatment solution in this study. A calcium ion specific electrode (Orion 9300, Boston, MA) was also used to verify the depletion of calcium ion during the mineral precipitation process (Fig. 2). This solution was initially supersaturated with respect to hydroxyapatite, fluorapatite, octacalcium phosphate, and dicalcium phosphate dihydrate. It was undersaturated with respect to α-tricalcium phosphate, amorphous calcium phosphate, and calcium fluoride. The precipitated minerals formed in a test tube at the end of the reaction were analysed by powder X-ray diffraction (XRD) using a Rigaku DMAX 2200 (Rigaku/USA, Danvers, MA). In the experimental procedure, the treatment solution was prepared each time before use by combining 10 ml of a 13.0 mmol l^{-1} calcium-containing solution with 10 ml of a 13.0 mmol l^{-1} phosphate and 0.5 mmol l^{-1} F-containing solution, with both solutions containing 30.0 mmol l^{-1} of KCl and 50.0 mmol l^{-1} of HEPES background as described above.

Incubation solution

All samples were incubated in a phosphate buffered saline (PBS) before and between treatments. PBS contains 0.15 mol l^{-1} NaCl, 1.7 mmol l^{-1} KH_2PO_4 and 4.95 mmol l^{-1} Na_2HPO_4. The pH of PBS was 7.2 ± 0.01.

Permeability cell and flow rate-measuring system

A modified Pashley flow system[12] that consisted of two parts (Fig. 3) was used to measure *Lp*. The upper part was connected to a capillary glass tube (1 mm in diameter) flow rate measuring device and then the PBS reservoir that was situated approximately 1.74 m above the cell (17 KPa). The lower part was a dentin disc mounted on a Plexiglas disc that a smear layer was produced as described above (Fig. 1). The flow rate was determined by measuring the length of time for a small air bubble in the PBS to travel a 20 cm distance in the capillary glass tube.

Treatment procedures

Nine mounted dentin discs that had been sanded to produce a smear layer that partially occluded the

[1]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 ADAHF, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

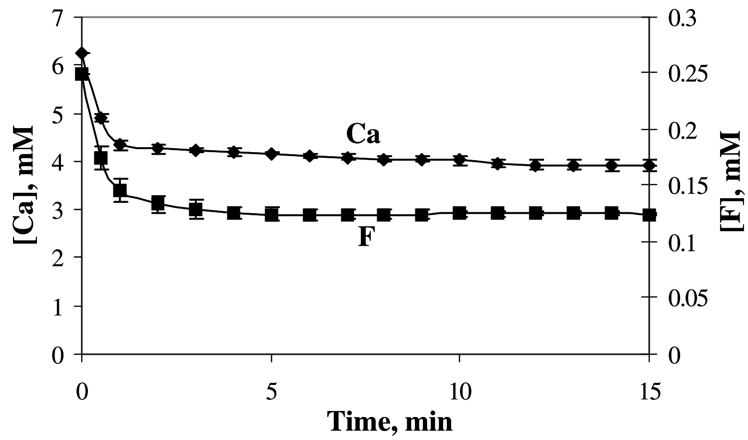


Figure 2 The electrode measurements of Ca^{2+} and F^- after mixing 12.5 mM Ca^{2+} with 12.5 mM $HPO_4^{2-}/H_2PO_4^-$ containing 0.5 mM F^-. Error bar indicates standard deviation ($n = 3$).

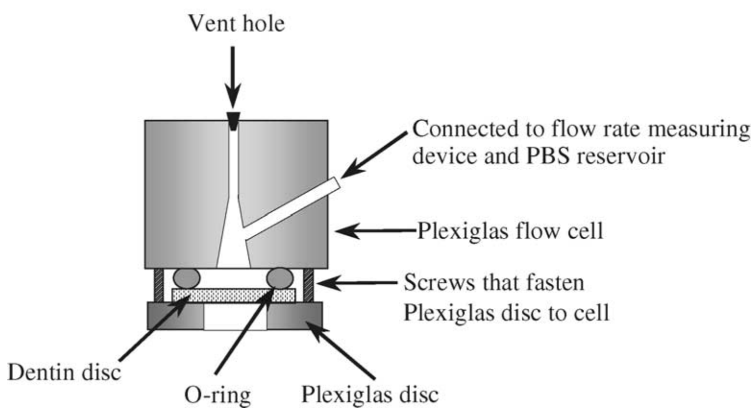


Figure 3 Schematic drawing of a modified Pashley's flow cell for measuring hydraulic conductance of dentin samples.

tubules were first brushed with an electric rotary toothbrush (Braun type 4728, Oral-B, Belmont, CA) for 1 min prior to baseline incubation. This electric toothbrush had soft nylon bristles and approximately 60 g of vertical force was generated during brushing. The 1 min brushing of the dentin sample surface was also conducted before each *Lp* measurement throughout the experiment. To establish a baseline *Lp* value, *Lp* measurements were conducted at various time points in the ''incubation'' regimen (Fig. 4). The regimen consisted of four cycles of 15 and 60 min incubation steps in which each samples was incubated in 10 ml of fresh PBS at 37 °C, plus a final 15 min incubation step (Fig. 4). Thus, nine *Lp* repeated measurements were conducted on each sample that was immersed in PBS for a total of 315 min. Once the baseline *Lp* values were obtained, the dentin specimens went through the treatment regimen (Fig. 4) in which the specimens received four treatment cycles. In each cycle, the upper side of the sample was exposed to the treatment solution for 15 min and then the sample was immersed in 10 ml of PBS for 60 min. Again, nine *Lp* repeated measurements were conducted on each sample during at various time points during the treatment regimen.

Computation of dentin hydraulic conductance (*Lp*) and relative hydraulic conductance (relative *Lp*)

Dentin hydraulic conductance, *Lp*, was calculated from the flow rate measurement data using the equation

$$Lp = \frac{Jv}{A(\Delta P)} \quad (1)$$

where Jv is fluid flow rate ($\mu l\ min^{-1}$), A is dentin surface area (cm^2) defined by the O-ring thorough which the fluid passes, ΔP is hydrostatic pressure gradient (cm of H_2O, 1 cm $H_2O = 0.098$ KPa) across dentin disc. *Lp* has the unit of $\mu l\ cm^{-2}\ min^{-1}\ H_2O\ cm^{-1}$.[12] As will be seen in the 'Results' section, for each dentin sample, the measured *Lp* values during ''incubation'' regimen fluctuated but did not show a trend of either increase or decrease with treatment.

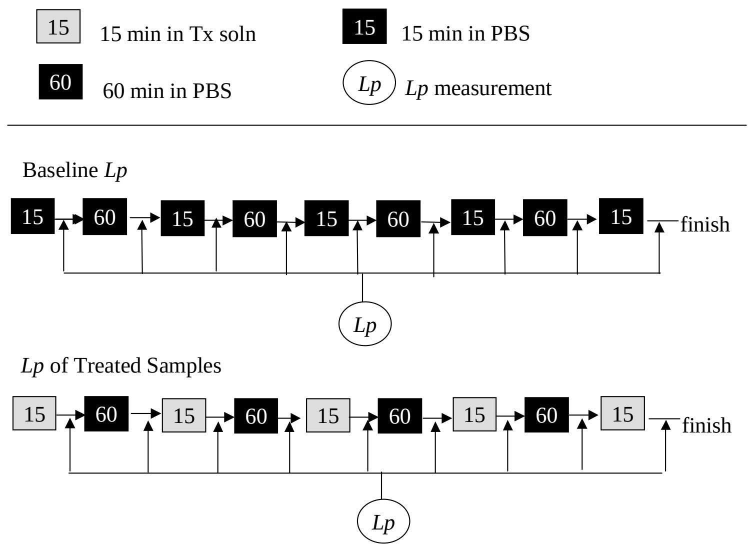


Figure 4 Schematic representation of the baseline incubation and treatment procedures.

Thus, the mean of the nine *Lp* values measured during the ''incubation'' regimen was taken as the *baseline Lp* for that sample. Relative hydraulic conductance, *relative Lp*, defined by Eq. (2), corresponds to the hydraulic conductance of a sample at any time point expressed as the faction relative to the *baseline Lp* of the same sample.

$$\text{Relative}\,Lp = \left(\frac{Lp}{\text{baseline}\,Lp}\right) \times 100 \tag{2}$$

The standard uncertainty of the *Lp* values from the combined standard uncertainties of the measured values is less than 1% of the mean and much less than the observed variation between treatments.

Other experimental methods

Scanning electron microscopy (SEM) (JEOL JSM-5300, JEOL USA, Inc., Peabody, MA) was used to observe the surface morphology of samples. The samples were coated with gold (DESK II COLDSPUTTER/ETCH UNIT, Denton Vacuum, LLC, Stephene City, VA) and examined under condition of 5 kV and 45 μA.

Powder XRD analysis was used to determine the phase composition of the product formed in the treatment solution. The estimated standard uncertainty in 2θ measurements is 0.01° and the minimum mass fraction of a calcium phosphate phase that can be detected by XRD is about 3%.

Statistical analysis

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 Lp* values.

Results

Although the treatment solution was initially supersaturated with respect to dicalcium phosphate dihydrate and octacalcium phosphate in addition to hydroxyapatite and fluorapatite, XRD analyses (Fig. 5) showed that the product formed was a low crystallinity apatitic material.

The average *Lp* value (in μl cm^{-2} min^{-1} H_2O cm^{-1}) of the nine etched dentin samples was (mean $\pm$ standard uncertainty) 1.7 ± 1.1 (range = 0.44—4.03). For each of the sanded samples, *Lp* measurements were conducted at nine times points during the ''incubation'' regimen (see 'Treatment procedures' section). Table 1 shows the mean *Lp*

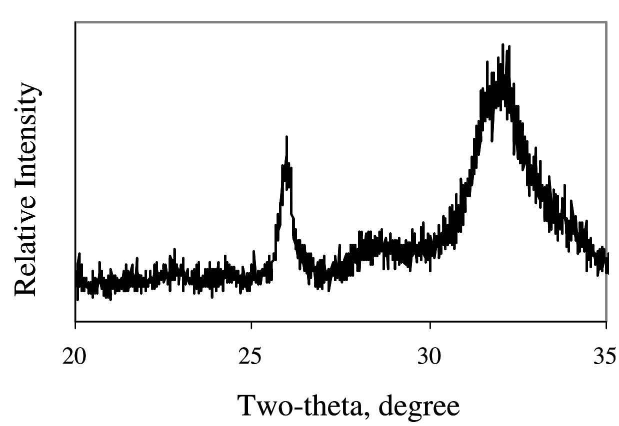


Figure 5 XRD of products of treatment solution (apatitic material).

Table 1 Mean hydraulic conductance values (*Lp*) of dentin samples at various time points in the ''incubation'' regimen.

Treatment	*Lp* ($\mu l\ cm^{-2}\ min^{-1}\ H_2O\ cm^{-1}$)	*Lp* expressed as percentage of initial reading
First		
15 min PBS	0.111 ± 0.046[a] [b]	100
60 min PBS	0.105 ± 0.046	95 ± 14
Second		
15 min PBS	0.102 ± 0.044	96 ± 19
60 min PBS	0.092 ± 0.035	89 ± 16
Third		
15 min PBS	0.093 ± 0.034	91 ± 14
60 min PBS	0.118 ± 0.065	111 ± 12
Fourth		
15 min PBS	0.118 ± 0.060	112 ± 13
60 min PBS	0.113 ± 0.053	107 ± 16
Fifth		
15 min PBS	0.114 ± 0.054	100 ± 14
Baseline (average)	0.108 ± 0.041	

[a] Mean ± standard deviation of nine dentin samples.
[b] Values connected by the same line are not significantly different ($P > 0.05$).

Table 2 Mean hydraulic conductance values of dentin samples after treatment with supersaturated calcium phosphate solution.

Treatment	*Lp* ($\mu l\ cm^{-2}\ min^{-1}\ H_2O\ cm^{-1}$)	Relative *Lp* (as percentage of baseline *Lp*)
Baseline	0.108 ± 0.041[a]	100
First		
15 min treatment	0.075 ± 0.028 [b]	71 ± 11
60 min PBS	0.074 ± 0.030	69 ± 10
Second		
15 min treatment	0.061 ± 0.022	58 ± 10
60 min PBS	0.058 ± 0.021	55 ± 9
Third		
15 min treatment	0.048 ± 0.019	46 ± 18
60 min PBS	0.044 ± 0.017	43 ± 20
Fourth		
15 min treatment	0.042 ± 0.017	40 ± 14
60 min PBS	0.035 ± 0.016	33 ± 10
Fifth		
15 min treatment	0.027 ± 0.013	25 ± 10

[a] Mean ± standard deviation of nine dentin samples.
[b] Values connected by the same line are not significantly different ($P > 0.05$).

values of the nine samples at each time point. Also shown are the mean of the *Lp* values expressed as percentage of the first *Lp* value of the same sample measured during the ''incubation'' cycle. ANOVA results showed that there were no significant differences ($P > 0.05$) among the mean *Lp* or mean percentage *Lp* values at different time points. Since the *Lp* values (Table 1) showed random fluctuations rather than a significant change with time, the *baseline Lp* value for each sample was obtained by averaging the *Lp* values measured at the nine time points. The *baseline Lp* values of the nine sanded dentin samples ranged from 0.063 to 0.165, and the mean *baseline Lp* value ($n = 9$) was 0.108 ± 0.041. The mean *baseline Lp* of the sanded sample was approximately 6% of the mean *Lp* of etched samples that had completely open tubules.

Table 2 shows the mean *Lp* values of the nine samples at each time point in the treatment regimen. Also shown are the mean *relative Lp* values of the samples at each time point. ANOVA tests showed that there were significant differences ($P < 0.05$) in *Lp* at different time points in the treatment regimen. The mean *Lp* values fell into four populations, whereas the mean *relative Lp* values fell into six populations. This was because the variance about the mean *relative Lp* was smaller as effects due to sample differences were not present. In either case, all treated dentin samples showed lower *Lp* values than the *baseline Lp*. Correlation analysis showed that changes in *Lp* by the treatments were not correlated to the *baseline Lp* value of that sample.

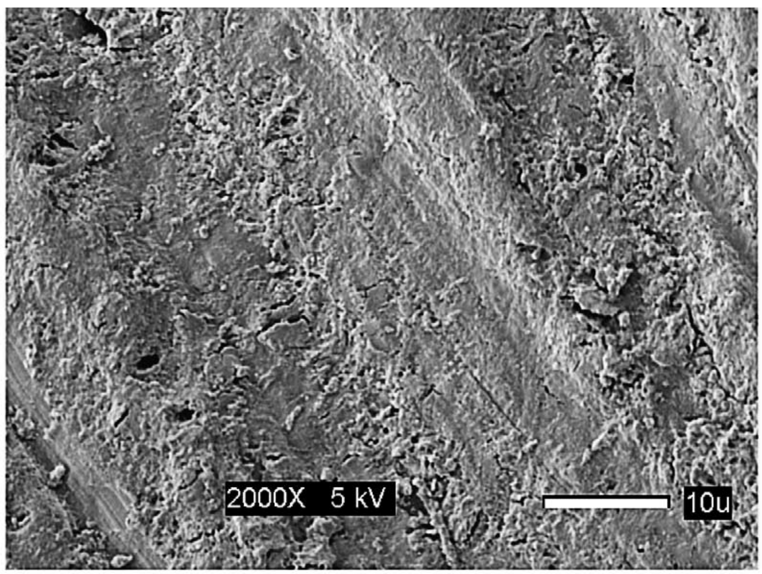
Discussion

Ca- and F-electrode measurements (Fig. 2) showed that after the preparation of the treatment solution the calcium concentration in the solution continued to decrease over the 15 min treatment time and fluoride was removed from the solution in proportion to the calcium consumption. These observations together with XRD of the product (Fig. 5) suggest that the product formed in the treatment solution was a fluoride-containing apatitic material and this mineral precipitate was formed continuously during this period.

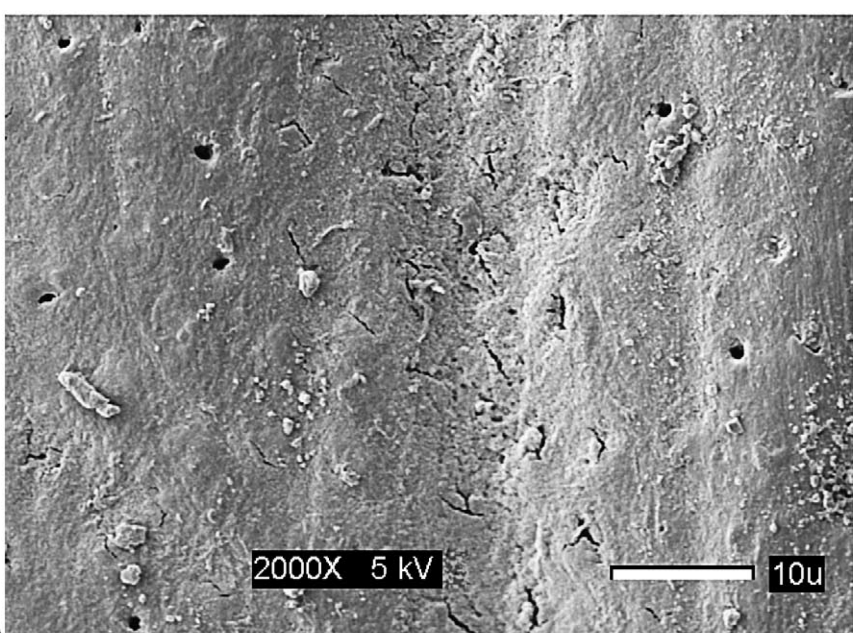
SEM micrographs of representative dentin samples after sanding, at the end of the ''incubation'' regimen, and at the end of the treatment regimen are shown in Figs. 6a–c, respectively. Fig. 6a shows the presence of debris, typical smear layer[2] that covered most of the tubule openings. Scratch marks from sanding were also apparent. Fig. 6b shows a cleaner looking surface with some tubule partially open. Fig. 6c shows a surface similar to that in Fig. 6b. It is noted that although the surface appearances of samples in Figs. 6a and b are quite different, the *Lp* values were similar. On the other hand, the surface appearances of samples in Figs. 6b and c were similar but their *Lp* values were significantly different. This suggests that for the type of treatments used in the present study, SEM was not an effective tool for discerning the treatment effects on dentin hydraulic conductance.

The modified Pashley flow cell design allows the dentin sample to be removed from the flow cell assembly to receive treatments, incubation, etc. between *Lp* measurements. The dentin surface subjected to fluid flow, defined by the O-ring, was held relatively constant in the repetitive measurements. This design was useful for the studies of the multiple effects on dentin permeability by treatments such as obturation, temperature cycling, re-mineralisation and demineralisation.

It is noted that in this study the direction of the PBS flow through the dentin disc was from the treated occlusal side to the pulpal side. To determine whether the direction of fluid flow would make a difference in dentin permeability, *Lp* measurements were conducted on five additional sanded dentin discs that had not received treatments with the calcium phosphate solution. *Lp* was measured



(a)



(b)

Figure 6 (a) SEM of the smear layer created with 320-frit sand paper; (b) SEM of dentin disc after baseline incubation; (c) SEM of dentin disc after five times treatments.

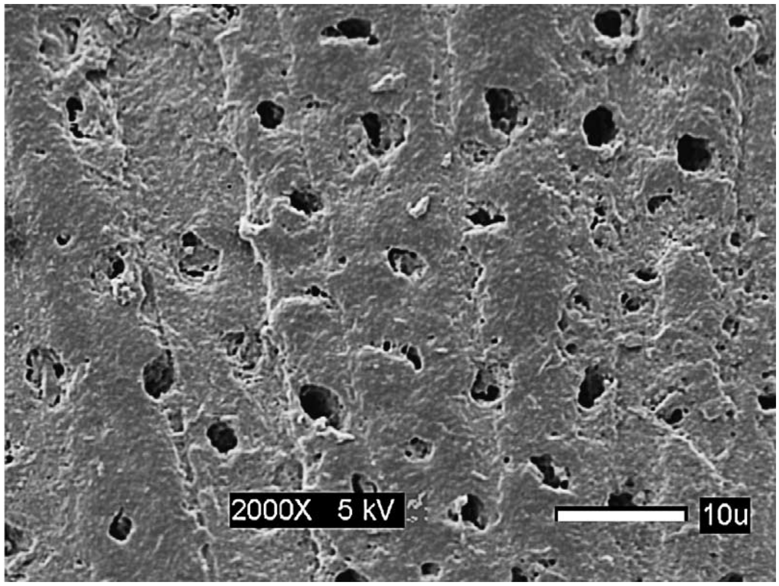
(c) 

Figure 6. (*Continued*).

under the conditions where the fluid flow was either occlusal-to-pulpal or pulpal-to-occlusal. The results showed no significant differences in measured *Lp* values. Further studies may be warranted to determine whether the *Lp* of treated dentin is affected by the direction of fluid flow, and if it is, in future studies *Lp* measurements should be performed under the condition that the flow is pulpal-to-occlusal as this is the direction of flow in vivo.

Before treatment, the baseline incubation was established to investigate the effects of PBS and the stability of smear layers during the experiment. Comparison analysis results indicated that there were no significant changes in *Lp*. In terms of sample selection, Pearson correlation test showed that changes in *Lp* after treatments were independent of baseline *Lp*, which varied in a wide range among nine samples.

In this study, the averaged *Lp* of etched samples was 1.71, which was higher than that reported in another study.[13] This may be due to differences in sample selection, the thickness of dentin discs, and hydraulic pressure.[7] However, the reduction in *Lp* produced by the sanding observed in this study (94%) was comparable to that (95%) reported in a previous study.[14] It was different from that (60%) reported in the other study.[2] The difference in the reduction of *Lp* may be as a result of various methods used to produce a smear layer.

The present in vitro experiment showed that there was a significant reduction in relative *Lp* after a single treatment and a 75% reduction after five treatments (Table 2) with a mildly supersaturated calcium phosphate solution. There is a significant linear relationship between relative *Lp* and number of treatments (correlation coefficient = 0.99). On the other hand, limited preliminary study results indicated that the same treatment regimen produced no significant reductions in *Lp* on acid etched dentin discs that had totally open dentinal tubules. Presumably, the treatment solution formed microscopic apatitic precipitates that became trapped within the tortuous fluid-filled channels between the grinding particles that make up the smear layer.[15] In the absence of a smear layer, the amount of mineral precipitation may be insufficient to occlude the tubules and the crystallites may have been forced through the tubules during incubation or the *Lp* measurement procedure.

The present results would suggest that it is feasible to use chewing gum[9] as a vehicle for the delivery of calcium and phosphate for the purposes of enhancing the process of the reduction of dentin permeability. This is because salivary calcium and phosphate concentrations, similar to those of the treatment solution used in this study, could be produced with the use of a calcium phosphate-releasing gum.[3] In that in vivo study, calcium and phosphate ions were released into saliva continuously by the chewing gum, and elevated levels of salivary calcium and phosphate were maintained for the 15 min time period studied. This in vivo condition is more favourable for precipitating calcium phosphate mineral than that produced by the treatment solution used in the present study in which the calcium and phosphate concentrations were the highest initially and they diminished with time.

However, the presence of mucins and other salivary constituents adsorbed to dentin may modify the interaction of this mineralised solution with dentin. Further clinical investigation is warranted to determine the actual effects of elevated salivary calcium phosphate levels produced by chewing gums or other vehicles on dentin permeability.

Acknowledgements

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