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Dentin Permeability Reduction by a Sequential Application of Calcium and Fluoride-Phosphate Solutions

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Abstract

Objective—A sequential topical application of calcium and fluoride-phosphate solutions was reported to occlude open dentin tubules, mainly with fluoroapatite precipitates by a rapid ionic reaction, and to be effective at treating dentin hypersensitivity. However, its ability to reduce dentin permeability (Lp) is unknown. The aim of this *in vitro* study was to evaluate the effect of this treatment on Lp.

Methods—Nine extracted human third molars were sectioned transversely to obtain 0.5mm-thick discs, which were then etched and rinsed. Aqueous solutions of 5% (w/w) disodium phosphate containing 0.3% (w/w) sodium fluoride (A) and 10% (w/w) calcium chloride (B) were prepared. The sequential application of the A&B solutions was repeated three times on each disc, which was then rinsed with distilled water. The Lp of the discs was measured before and after the application using a modified Pashley's fluid flow measuring system. The differences in the Lp values between the conditions before and after the solution applications were analyzed using a generalized estimating equation method and paired t-test. Scanning Electron Microscopy (SEM) was used to observe the dentin surfaces.

Results—All nine discs consistently indicated reduced Lp following the application of the A&B solutions. There was a significant decrease in the mean Lp [$\mu L/(cm^2 \cdot sec \cdot cmH_2O)$] from baseline (−0.27±0.25, p=0.011). Overall, an average decrease of 34% Lp occurred after the application of the A&B solutions. SEM observation indicated that the reaction products covered the entire dentin disc surface.

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Conclusion—The application of the A&B solutions was effective at reducing the Lp of the dentin discs.

Keywords

Dentin hypersensitivity; Desensitizing agents; Dentin permeability; Sequential application; Disodium phosphate; Sodium fluoride; Calcium chloride; Dentin tubules

Introduction

Dentin hypersensitivity (DH) is very prevalent, but its etiology is poorly understood (1). The current evidence (2–4) favors the "hydrodynamic theory" originally postulated by Gysi (5) and later developed by Brännström (6). According to this theory, an increased number of exposed dentin tubules can cause increased dentinal fluid movement and patient discomfort (6). The clinical strategies for ensuring patient comfort include blocking neural transmission and fluid transportation by occluding the tubules, a strategy that reportedly reduces DH (7–12).

Using minerals to alter dentin tubule contents or to create insoluble calcium complexes is one strategy for forming plugs mechanically or chemically. A stable solution containing fluoride, calcium, and phosphate was reported to precipitate calcium phosphate (13–14).

The use of mineral particles precipitated from saturated or non-saturated solutions was first introduced by Imai and Akimoto (15) in 1990 as an innovative method of treatment for DH. They proposed a method of precipitating insoluble calcium phosphate *in vitro* using a serial application of a 5% disodium phosphate solution and a 10% calcium chloride solution that immediately occluded the dentin tubules with calcium phosphate crystal precipitates as a result of an ionic reaction. In 1993, Ikemura (13) and Imai and Ikemura (16) reported that the addition of fluoride ions to the phosphate solution improved the acid resistance of the precipitate. In short, aqueous solutions of 5% disodium phosphate with 0.3% sodium fluoride (solution A) and 10% calcium chloride (solution B) were prepared. Solution A was applied to the patient's cervical dentin for 5 seconds using a cotton swab, immediately followed by the application of solution B for 5 additional seconds using another cotton swab; this application sequence was repeated three times. Preliminary clinical trials indicated that approximately 80% of the treated patients reported immediate relief from DH (15).

The outcomes of calcium phosphate on dentin permeability (Lp) have been reported (17–19). However, the effectiveness of using this innovative method of treating dentin hypersensitivity plus the A&B solutions to reduce Lp is unknown. The aim of this study was to use the A&B solutions to precipitate fluoroapatite onto human dentin discs *in vitro* and to evaluate the effect of the solutions on Lp.

Materials and Methods

Collection and selection of extracted human teeth

Fully erupted, non-carious, defect-free human third molars extracted for treatment reasons unrelated to this study were collected from local oral surgeons. The teeth were left under running water for one hour before being cleaned and stored in 10% formalin solution (a disinfectant) in polyethylene screw-cap jars until ready for use. Bucco-lingual and mesio-distal digital radiographs (Schick CDR, Schick Technologies, Inc., Long Island City, NY) of the teeth were taken in order to exclude teeth that had restorations, defects, or open apexes. Nine extracted permanent molar teeth were selected for this study.

Sample (dentin disc) preparation

The teeth were mounted in a precision sectioning saw (Isomet 1000, Buehler, Ltd. Lake Bluff, IL) and cut perpendicular to the long axis just cervical to the occlusal DEJ at 300 rpm to obtain a nominal thickness of 500 ± 50 μm and a typical diameter of about 8 mm. The flat surfaces of the dentin discs were first etched by immersion in 6% citric acid solution for 3 minutes to remove the smear layer and produce fully opened tubules and then rinsed in distilled water. The enamel side of each dentin disc was cemented (Dow Corning 3145RTV Silicone Adhesive, Midland, MI) on a Plexiglas disc (26.0 mm diam., 6.0 mm thick) with a 2.0 mm diameter hole in the center (20). As described below, this constituted the lower part of the modified Pashley's permeability measurement cell.

Treatment solutions (A&B solutions)

Aqueous solutions of 5 % (w/w) disodium phosphate with 0.3% (w/w) sodium fluoride (A) and 10% (w/w) calcium chloride (B) were prepared (Fisher Scientific, NJ). These two solutions are referred to as the "A&B solutions", respectively, in this paper.

Permeability cell and dentin permeability (Lp) measurements

A modified, two-part Pashley flow system (Figure 1) (14,20–23) was used to measure Lp. The top part was connected to a flow rate measuring device and to the phosphate-buffered saline (PBS) (Biofluid, Bethesda, MD) reservoir situated 174 cm above the cell (17 KPa). The PBS contained 0.15 mol/L NaCl, 1.7 mmol/L KH_2PO_4, and 4.95 mmol/L Na_2HPO_4 and had a pH of 7.2 ± 0.01. The lower part consisted of the Plexiglas disc with the cemented dentin sample as described above. The occlusal side of the dentin disc was placed upward on the high pressure side. The flow rate was determined by measuring the length of time it took for a small bubble in the PBS to move 10 cm in a horizontally positioned glass capillary tube (1 mm in diameter). The cell was disassembled between Lp measurements, allowing the dentin disc mounted on the Plexiglas disc to receive additional treatments as needed. With the use of two positioning pins, the upper and lower parts of the cell can be held together with a clamp in only one possible way. This assured that the area of the dentin disc subjected to the fluid flow measurement, which was defined by the o-ring (Figure 1), remained the same despite repeated disassembling and re-assembling of the cell.

Dentin permeability (Lp) measurement before the application of A&B solutions

The dentin permeability of the smear-free conditioned dentin discs was quantitatively measured before treatment with the A&B solution. This condition was defined as the baseline Lp value. The disc's permeability was measured three times with 5-minute equilibration intervals between measurements. The Lp [$\mu L/(cm^2 \cdot sec \cdot cmH_2O)$] was calculated from the fluid flow data using a standard equation (22–23).

Application of A&B solutions and dentin permeability (Lp) measurements

After the baseline Lp measurement, the permeability cell was disassembled and the top surface of the dentin disc received treatment as follows: solution A was applied to exposed lower part of the discs for 5 seconds using a cotton swab, immediately followed by an application of solution B for 5 additional seconds using another cotton swab. This application was repeated three times on each disc, which was then rinsed with distilled water. Immediately after the final application, three Lp measurements were conducted.

Statistical analysis

The generalized estimating equation (GEE) method (24) was used to investigate if there were significant differences among the discs' repeatedly measured Lp values for the nine samples before and after treatment with the A&B solutions. Changes in the Lp values before and after treatment, defined as relative dentin permeability calculated as (post-treatment Lp/pre-treatment Lp) × 100, were examined using a paired t-test. In the text, ± refers to the standard deviation, which is used in this paper as a measure of the standard uncertainty. All computations were carried out with the SAS v.9.1.3. statistical package (SAS Institute, Cary, NC).

SEM observation of dentin discs

Scanning electron microscopy (SEM) was used to observe the dentin surfaces of the samples before and after treatment with the A&B solutions and after the first and third Lp measurements of the treated samples. The discs were sputter-coated (DESK II Cold Sputter/Etch Unit, Denton Vacuum, LLC, Stephen City, VA) with gold and examined using SEM (JEOL JSM-5300, JEOL USA, Inc., Peabody, MA) using conditions of 15 kV and 58 mA.

Results

The GEE analysis showed that there were significant differences in the discs' repeatedly measured Lp values for the nine samples before and after treatment with the A&B solutions ($p=0.001$). The repeatedly measured Lp values for the treated samples consistently increased ($p=0.028$), as in the typical example shown in Figure 2. Therefore, only the Lp value obtained from the first measurement for each sample was used for the data analysis because it most closely represented the Lp of the sample after the treatment. When we compared the Lp values before treatment with the first post-treatment value, we also found significant differences in the values before and after treatment with the A&B solutions ($p=0.0008$). Since there were no significant differences in the discs' Lp measurements before treatment with the A&B solutions ($p=0.770$), relative dentin permeability [Lp (%)] was calculated for each treated sample as (first measurement Lp/average baseline Lp) × 100. The paired t-test showed that there were significant differences between the pre- and post-treatment values ($p=0.011$), which was consistent with the GEE analysis showing that there were significant changes in the Lp values after treatment with the solutions ($p=0.0008$).

The dentin permeability (Lp) values of the nine samples are summarized in Table 1. All nine discs consistently indicated a reduction in Lp. Following the application of the A&B solutions, there was a significant change in the mean Lp [$\mu L/(cm^2 \cdot sec \cdot cmH_2O)$] from the baseline ($-0.27 \pm 0.25$, $p=0.011$). The corresponding 95% confidence interval for the change ranged from −0.459 to −0.079. Overall, an average decrease of 34% Lp was observed after the application of the A&B solutions.

Figure 2 shows a typical example of the change in the relative Lp values with repeated Lp measurements after the application of the A&B solutions. In sample 9, the Lp value changed from the baseline value (100%) to 36%, 64%, and 80% in the first, second, and third repeated Lp measurements, respectively.

The SEM image of the dentin surface treated with 6% citric acid solution for 3 minutes showed that all dentin tubules were open (Figure 3A), which indicated that the smear layer was removed and the tubules were cleansed as a result of the citric acid treatment. The dentin tubules were completely free from debris. The peritubular dentin was compact and homogeneous.

The dentin surface treated with the A&B solutions can be seen in Figure 3B. The sequential application of the solutions readily yielded deposits consisting of reaction products. The dentin surface was completely covered with a dense layer of deposits, and all the dentin tubules were closed.

Figure 3C shows a SEM image of the dentin surface after the treatment with the A&B solutions and one Lp measurement. For this sample (sample 8), the Lp value decreased from 0.49 of the baseline value to 0.19 after the treatment. Almost all of the dentin surface was covered by the deposits; some of the dentin tubules were open.

An SEM image of the dentin surface treated with the A&B solutions after three Lp measurements is shown in Figure 3D. More open dentin tubules are visible compared to the number seen in Figure 3C.

Discussion

The sequential application of the A&B solutions on dentin discs readily yielded deposits, thus occluding the dentin tubules and effectively reducing the Lp. The difference in Lp between the samples before and after the A&B solution application was statistically significant. Overall, an average decrease in the Lp of 34% was observed after the application of the solutions.

While there was no significant change in the baseline Lp with repeated measurements, the values for all the treated samples did change. This finding may have been due to the presence of incompletely occluded tubules, and deposits may have been forced out of the tubules during the Lp measurement procedure (14). It is likely that the repeated Lp measurements caused the partial removal of the deposits, resulting in an increase in Lp. The deposits might have been dislodged due to the high hydraulic pressure and fast fluid flow rate used for the Lp measurements.

In this and previous studies (14,20), a hydraulic pressure of about 140 cm or 170 cm of H_2O was used, which was extremely high compared with the reported intrapulpal pressure of about 20 cm H_2O (23). While the high pressure and the resulting fast fluid flow rate facilitated the rapid Lp measurements, it appears that this situation inevitably produced artifacts by removing some of the deposits from the partially occluded tubules. Presumably under clinical conditions, the deposits will be preserved, and the Lp values after treatment should be similar to or lower than the Lp values recorded at the first measurement.

Our two previous studies reported a reduction in the relative Lp after treatment using mildly supersaturated calcium phosphate solution with fluoride (29%)(14) or a slurry mainly consisting of dicalcium phosphate, calcium hydroxide, and fluoride (35%)(20). These studies used the same *in vitro* model, including the relatively high hydraulic pressure, as in the present study, making the Lp values directly comparable. The reduction (34%) in relative Lp observed in our study is within the range of the reductions reported in the previous studies. In those studies, immersion in PBS or a saliva-like solution (SLS) after treatment and multiple treatments further decreased the Lp (10,16). It would be useful to determine the Lp values of the A&B solution-treated specimens after incubation in PBS or SLS. Gandolfi *et al.* (25) reported a reduction of 53% in the relative Lp produced by a treatment using a “dentin silicate coating” (DSC) in which a homogeneous amorphous layer occluded almost all tubule orifices. Because a lower hydraulic pressure (70 cm H_2O) was used for the Lp measurements in their study, it is not entirely clear whether the DSC treatment would be more effective than the calcium phosphate-based treatments. While dentinal tubules after the application of toothpaste like Dentosan S appeared largely open, a 33% Lp reduction was reported in their study, which was almost the same as the value in the present study. Kolker *et al.* (9) reported a reduction of 46% in the relative Lp produced by a treatment using a D/Sense 2, which is similar to the A&B solutions with respect to material components. While a lower hydraulic pressure (70 cm H_2O) was used, a crystal precipitate was unattached to the tubules and covered a part of them. On the contrary, in the present study, Lp reduction was 34% using a high hydraulic pressure. Nevertheless, almost all of the dentin surface was covered by the deposits and the precipitate was attached to the tubules.

Preliminary clinical trials of the A&B solutions indicated that approximately 80% of the treated patients reported immediate relief from DH (15). It seems that this level of Lp decrease (average of 34%) might be sufficient for producing a measurable positive clinical outcome. Future studies of the A&B solutions may include controlling the amount and rate of the deposit formation on the dentin discs under varying conditions, followed by a well defined clinical trial.

Conclusions

Within the constraints of this experimental design, the following conclusions can be drawn:

1. The sequential application of the A&B solutions on dentin discs immediately yielded deposits on the entire surfaces that occluded the dentin tubules, thereby effectively reducing the Lp of the dentin discs.
2. Overall, a statistically significant average of 34% Lp decrease was observed after the application of the A&B solutions *in vitro*.
3. The repeated Lp measurements caused the removal of the deposits, resulting in an increase in Lp as the number of measurements increased. This phenomenon may be due to the high hydraulic pressure of the fluid used for the Lp measurement in this study.

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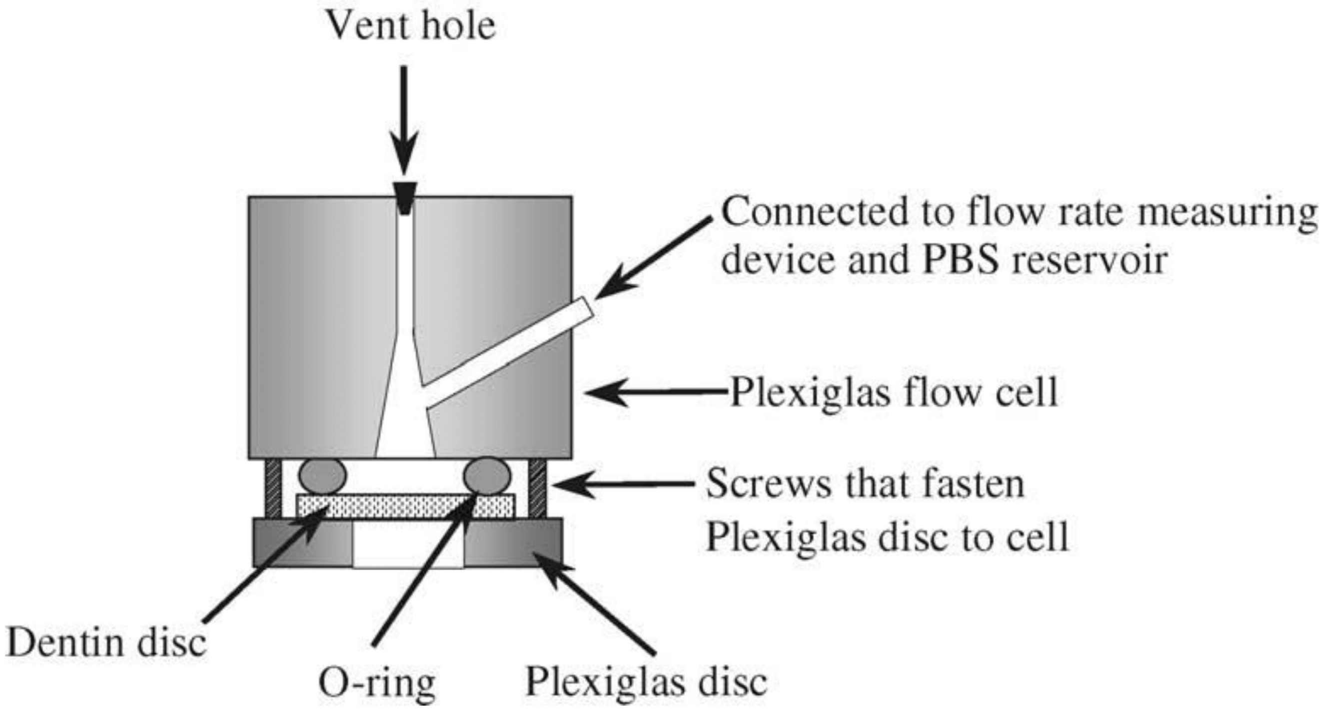

Figure 1.

Schematic drawing of a modified Pashley's flow cell for measuring dentin permeability (Lp) of samples

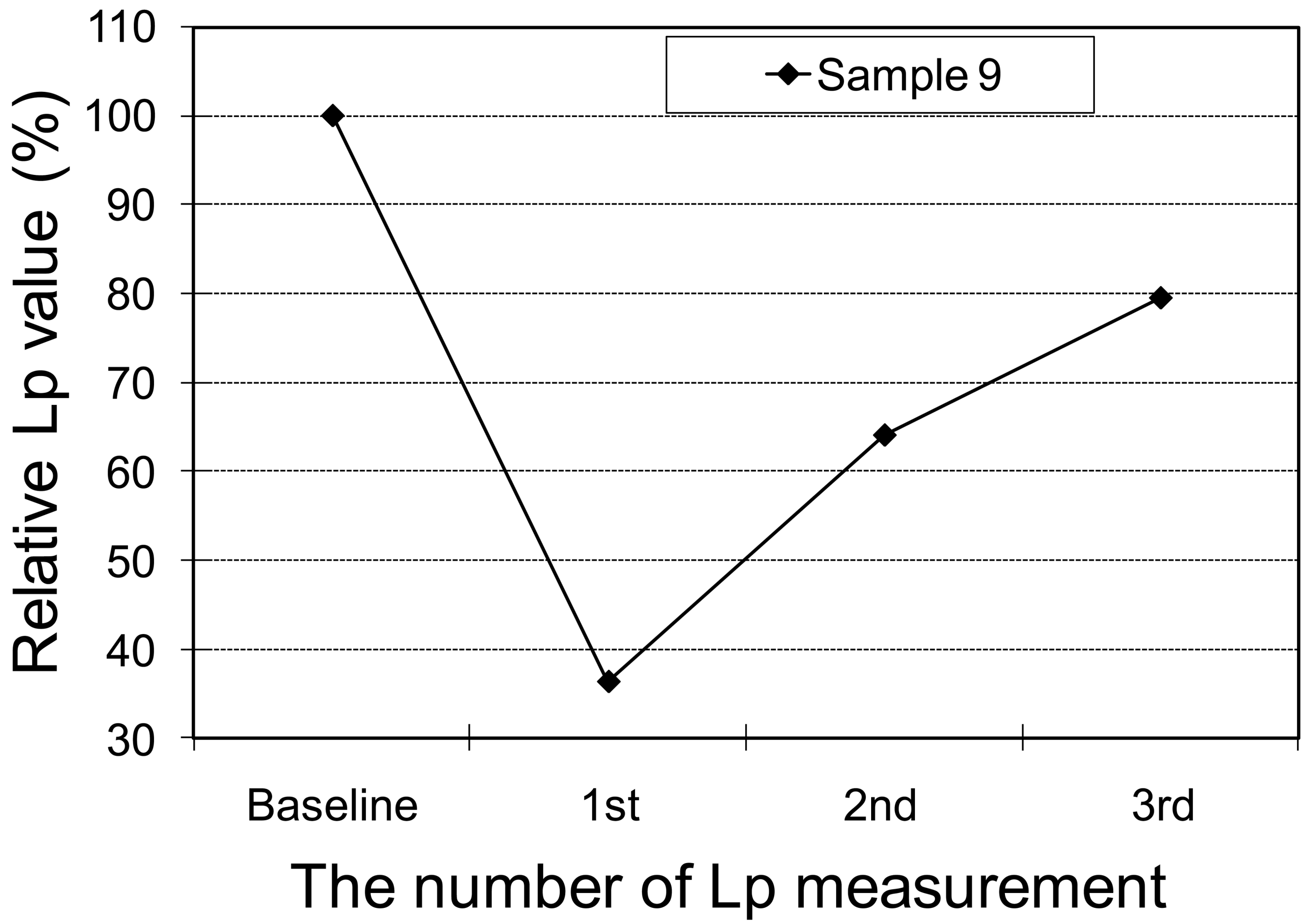

Figure 2.
Typical example of change of relative Lp value with repeated Lp measurement after the application of the A & B solutions (Sample 9)

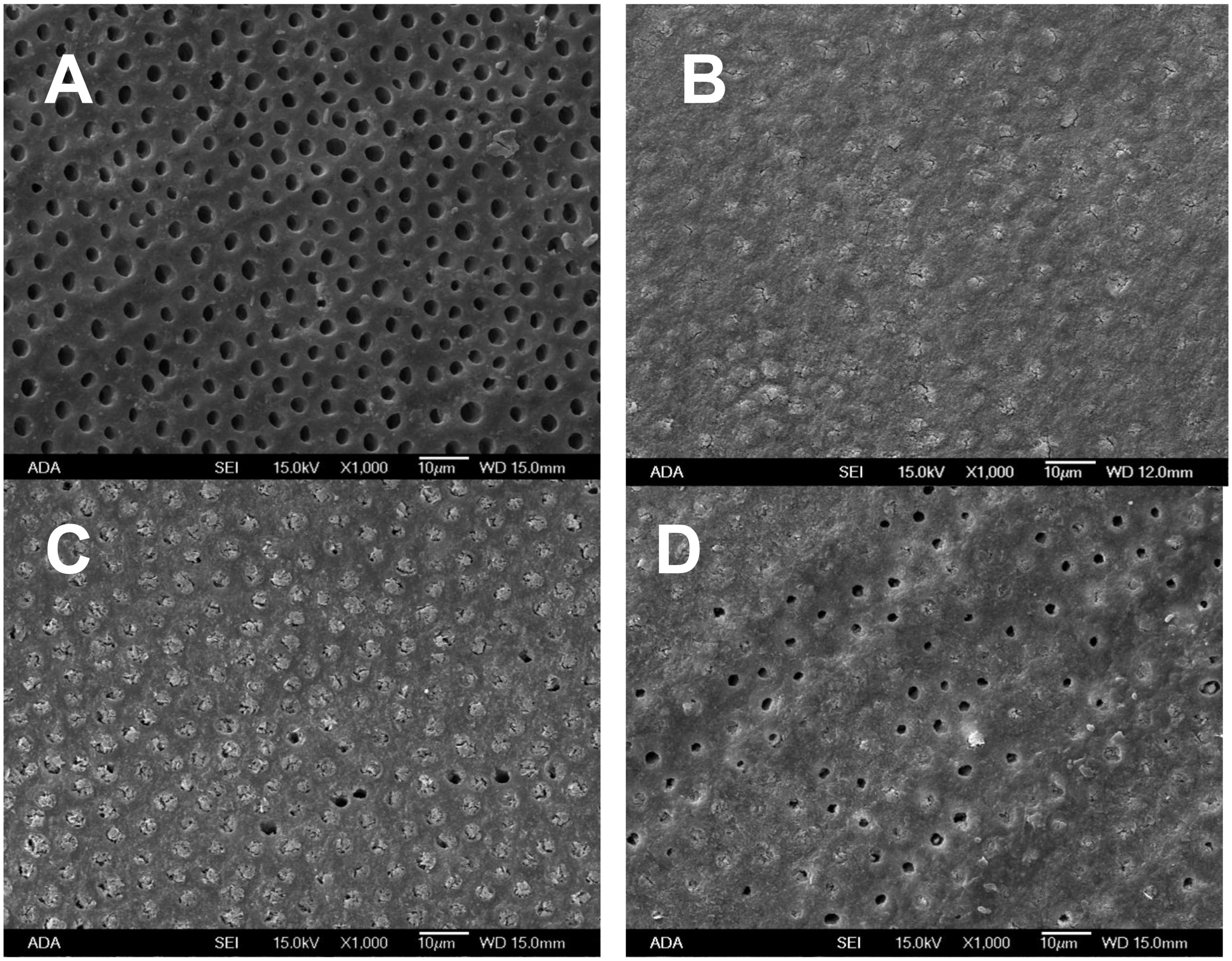

Figure 3.
SEM of dentin surfaces (Magnification, 1000 ×; bar, 10 μm)
(A) After treatment with 6% citric acid solution for 3 minutes
(B) After treatment with A & B solutions
(C) After treatment with A & B solutions and Lp measurement once
(D) After treatment with A & B solutions and Lp measurements three times

Table 1

Summary of Lp measurements for nine samples

Sample Number	Permeability (Lp, μL/(cm^2·sec·cmH_2O))		Relative Lp after treatment of A & B solutions[c]
	Baseline[a]	After treatment of A & B solutions[b]	
1	0.53	0.48	90
2	1.48	0.93	63
3	0.56	0.39	69
4	0.68	0.54	79
5	1.44	1.17	81
6	0.33	0.24	74
7	0.19	0.12	66
8	0.49	0.19	38
9	1.22	0.44	36
Mean	0.77	0.50	66
SD	0.48	0.35	18

[a]Mean of more than three measurements

[b]The first measurement data

[c]As percentage of baseline Lp