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Original Article

The effect of stannous fluoride desensitizing toothpaste on immediate dentin sealing ability of universal adhesive *in vitro*

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Abstract *Background/purpose:* The stannous fluoride pretreatment of dentin may compromise the bond formation of the dental adhesive system. This study aimed to determine the effect of stannous fluoride (SnF₂) desensitizing toothpaste on immediate dentin sealing ability (IDS) of universal adhesive in human intact dentin using dentin permeability test and scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS).

Materials and methods: Sixty sound dentin specimens were prepared from 60 extracted intact third molars, then treated with 17 % EDTA for 60 s to simulate hypersensitive dentin, and randomly divided into 2 series. Series 1 (dentin permeability test; n = 40) had 4 different treatment groups: (1) universal adhesive (self-etch mode), (2) universal adhesive (total-etch mode), (3) SnF₂ pretreatment + universal adhesive (self-etch), (4) SnF₂ pretreatment + universal adhesive (total-etch). The IDS of each group was the percentage decrease of hydraulic conductance of dentin after bonding. Series 2 (n = 20) was SEM-EDS analysis of different treated dentin before bonding. The data were analyzed using 2-way ANOVA and Tukey test.

Results: In groups of no SnF₂ pretreatment, the IDS of universal adhesive were 79.53 ± 20.71 and 77.34 ± 18.14 % for self-etch and total-etch mode, respectively. In groups of SnF₂ pretreatment, the corresponding values were 71.54 ± 15.56 and 69.56 ± 9.92 %, respectively. Although SnF₂ pretreated-dentin had the remarkable signals of silicon and tin, there were no significant reductions in the IDS of universal adhesive (*P* < 0.05).

Conclusion: SnF₂ desensitizing toothpaste has no adverse effect on the immediate dentin sealing ability of universal adhesive.

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Introduction

Dentin hypersensitivity is an important clinical problem that affects the patients' quality of life and has the wide range of prevalence varied from 1.3 % to 92.1 %.^{1,2} Dentin hypersensitivity is associated with dentin exposure, resulting in opened dentinal tubules and increased dental pulp nerve responsiveness to external environmental stimuli such as chemical, thermal, tactile or osmotic stimuli.³ Generally, dentin exposure can be caused by physical, chemical, pathological, biological challenges and developmental abnormalities that result in dental or periodontal damages.⁴ At present, the hydrodynamic theory is widely accepted for describing dentin hypersensitivity, as the theory has been proposed based on the fluid movement inside the open dentinal tubules which act as a hydraulic link between the environment of the dentin surface and dental pulp.⁵ The noxious stimuli, such as thermal, mechanical, evaporative and chemical near the exposed dentin increase the rate of dentinal fluid flow in tubules, further generate action potentials in intradental nerves, and the action potentials pass to the brain to cause dental pain sensation.⁶ Additionally, the exposed dentin becomes more hypersensitive to the pain stimuli when the smear layer and smear plugs are removed from dentin.⁷

Currently, there are two main therapeutic approaches for dentin hypersensitivity treatment. The first approach is to suppress the nerve impulse or nerve desensitization by desensitizing agents containing potassium salts (potassium nitrate, potassium chloride or potassium citrate), and another approach is to occlude the dentinal tubules by obliterating agents such as sodium fluoride, strontium chloride, stannous fluoride and dental adhesives.^{3,6,8,9} Stannous fluoride toothpaste is an effective at-home desensitizing agent, as a result of the reaction between the stannous ion and the dental hard tissue, leading to the formation of a stable layer of insoluble stannous compounds on the dentinal surface and precipitates within the dentinal tubules.^{8,10} The previous studies found that stannous fluoride provided statistically significant improvements in tactile dentinal hypersensitivity scores of 27.8 % and 42.0 % at the 4- and 8-week examinations.¹¹

However, some cases need further in-office treatment by dentists, and the application of dental adhesive may be an immediate solution to treat dentin hypersensitivity if the patients cannot be cured by using desensitizing toothpaste alone. Generally, the use of dental adhesive helps to provide an immediate dentin seal to protect pulpo–dentin complex, and prevent post-operative dentin sensitivity and pulpal inflammation.¹² Nowadays, the universal adhesive is the newest type of dental adhesive systems and can be applied with self-etch and total-etch mode on dentin.¹³ A recent *in vitro* study reported that the previous use of desensitizing dentifrices could not positively or negatively affect bond strength of eroded/abraded dentin when a universal adhesive was used.¹⁴ However, for the combined treatment of SnF₂ and dental adhesive, another previous study reported that 1-min pretreatment of 0.717 % stannous fluoride reduced the bond strength of the dentin bonding agent (Scotchbond 2 which employed a dentinal primer and a light curing dentin bonding agent), as the hydrophilic nature of the

stannous fluoride caused an unfavorable site for penetration by the dentinal adhesive resin.¹⁵ Nevertheless, it is still unclear whether the pretreatment of stannous fluoride desensitizing toothpaste affects the seal ability of universal adhesive which can be used in both self-etch and total-etch modes in hypersensitive dentin.

Therefore, the objective of this study was to determine the effect of stannous fluoride (0.454 % w/w SnF₂) desensitizing toothpaste on immediate dentin sealing ability (IDS) of universal adhesive in human dentin with simulated dentin hypersensitivity condition by using dentin permeability test, and to observe and analyze the characteristics of different treated dentin before bonding under a scanning electron microscopy (SEM) and an energy dispersive x-ray spectroscopy (EDS). The null hypothesis tested was that (1) stannous fluoride (SnF₂) desensitizing toothpaste had not the effect on the immediate dentin sealing ability (IDS) of universal adhesive, and (2) there were no effects of the application modes on the IDS of universal adhesive.

Materials and methods

Tooth selection and sample size determination

The experimental protocol was approved by the Institutional Review Board of the Faculty of Dentistry and Faculty of Pharmacy at Mahidol University (COE.No.MU-DT/PY-IRB 2022/031.0206), and complied with the principles of the Declaration of Helsinki. The ANOVA sample size was calculated to compare the percentage dentin sealing ability of adhesive using SigmaStat 3.5 (Systat Software Inc.; San Jose, CA, USA). Based on the data of previous study,¹⁶ the minimum required number of samples in each group was 8 ($n = 8$) at alpha set to 0.05 and desired power of 0.80.

Sixty extracted intact human third molars were gained from Oral and Maxillofacial Surgery Clinic, Dental Hospital, Faculty of Dentistry, Mahidol University, and used for the study in such a manner that there were no identifiable data of the donors. Therefore, the need for informed consent was waived by the Institutional Review Board of the Faculty of Dentistry and Faculty of Pharmacy at Mahidol University (COE.No.MU-DT/PY-IRB 2022/031.0206). The teeth were intact and without evidence of crack, craze line, dental caries or restoration, and extracted due to eruption problems or orthodontic purposes. The teeth were stored in 0.1 % thymol solution (M Dent, Nakhon Pathom, Thailand) and used within 1 month after extraction.

Preparation of tooth specimens

Sixty intact third molars were sectioned transversely 3 mm below and above the cemento–enamel junction using a diamond disc (NTI, Kahla, Germany) under water-cooling spray. Thereafter, the exposed occlusal dentin surface of each tooth specimen was abraded using a 600 grit silicon carbide abrasive paper for 15 s with water coolant to obtain an occlusal dentin surface with a standardized smear layer.¹⁷ At the pulpal side of each tooth specimen, coronal pulpal tissue was gently removed with tissue forceps, and

the pulp cavity was irrigated with distilled water to remove any remnant tissue.

Experimental design

After tooth preparation, the tooth specimens were randomly divided into 2 experimental series (Fig. 1); series 1: dentin permeability test was performed on 40 specimens and series 2: SEM-EDS analysis was performed on 20 specimens. In each series, the specimens were randomly categorized into 4 groups as follow:

Series 1: Seal ability test/dentin permeability test (n = 40)

Group 1: universal adhesive (self-etch mode; n = 10)

Group 2: universal adhesive (total-etch mode; n = 10)

Group 3: SnF₂ toothpaste + universal adhesive (self-etch mode; n = 10)

Group 4: SnF₂ toothpaste + universal adhesive (total-etch mode; n = 10)

Series 2: SEM-EDS analysis (n = 20)

Group 1: dentin treated with EDTA (n = 5)

Group 2: dentin treated with EDTA + 35 % phosphoric acid (n = 5)

Group 3: dentin treated with EDTA + SnF₂ toothpaste (n = 5)

Group 4: dentin treated with EDTA + SnF₂ toothpaste + 35 % phosphoric acid (n = 5)

Dentin permeability test

The remaining dentin thickness (RDT) of each specimen in each group was determined using Iwanson caliper, item no. 4733 (Kohler, Stockach, Germany). The mean RDT of different treatment groups were compared using 1-way ANOVA test to ensure that there was no significant difference among the groups (Table 1) because variation in dentin depths of specimens might affect the dentin permeability and dentin seal ability test. After that, the dentin permeability test was set up based on the study protocol of Kijisanmith et al.,^{9,17} where crown specimens were connected to the fluid filtration system of the dentin permeability measurement device to observe the fluid flow through dentin under simulated hydrostatic pressure of 100 mmHg.

In brief, each crown specimen was glued with cyanoacrylate (Koatsu Gas Kogyo Co. Ltd., Osaka, Japan) to an acrylic block containing a metal tube (gauge 18) that was

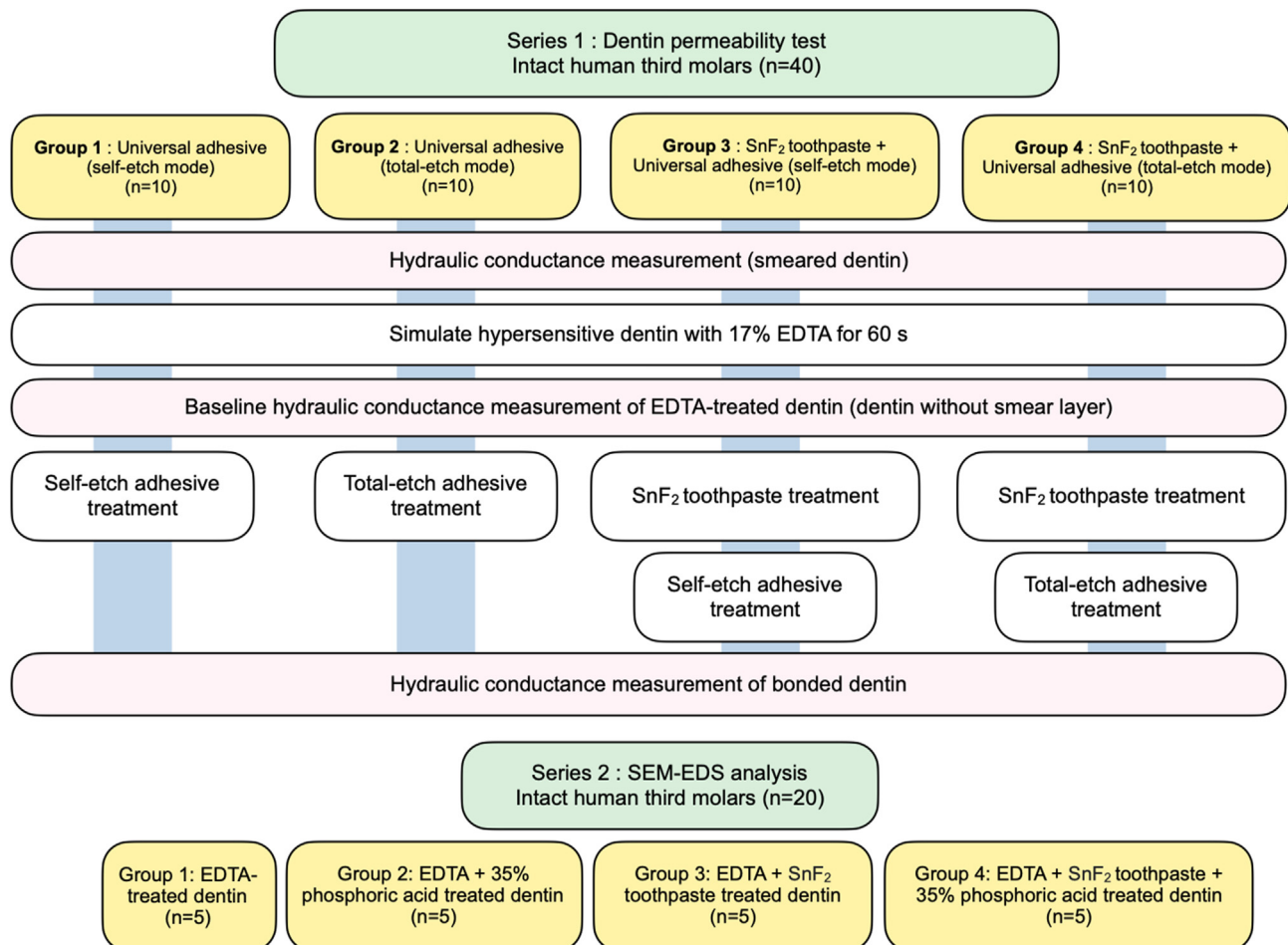


Figure 1 Flowchart of the experimental design.

Table 1 The mean and standard deviation (SD) of remaining dentin thickness (RDT) above the highest pulpal horn (RDT1) and above the middle of the roof of the pulp chamber (RDT2) of tooth specimens of 4 different treatment groups.

RDT (mm)	Group 1	Group 2	Group 3	Group 4	1-way ANOVA
RDT1	2.19 (0.40)	2.13 (0.47)	1.92 (0.45)	2.11 (0.54)	P = 0.606
RDT2	3.38 (0.38)	3.56 (0.31)	3.22 (0.39)	3.36 (0.70)	P = 0.458

There were no significant differences among the treatment groups. Abbreviations: Group 1: universal adhesive (self-etch mode); Group 2: universal adhesive (total-etch mode); Group 3: stannous fluoride toothpaste + universal adhesive (self-etch mode); Group 4: stannous fluoride toothpaste + universal adhesive (total-etch mode).

connected to a capillary tube (internal diameter of 300 μ m; DADE, Miami, FL, USA), and the tubing was attached from the capillary to a mercury manometer for controlling the pressure in the system.^{9,17} The pulp chamber of the specimen, capillary, and tubes in the fluid filtration system of the device were filled with ringer's solution, and the occlusal dentin surface of the specimen was covered with cotton soaked with ringer's solution to maintain dentin moisture condition.^{9,17}

The hydraulic conductance (HD) of smeared dentin was measured by observing the movement of a small air bubble which introduced into a capillary using visualizer and the pressure in the system was set at 100 mmHg above atmospheric.^{9,17} Thereafter, to remove smear layer and simulate hypersensitive dentin condition, the occlusal dentin surface was treated with 17 % EDTA (M Dent, Nakhon Pathom, Thailand) for 60 s using a microbrush applicator (Cotisen, Hebei, China), then the dentin surface was rinsed with distilled water for 30 s.⁸ The HD of EDTA-treated dentin was measured as described above, and served as the baseline value (assigned as 100 % dentin permeability). EDTA-treated dentin showed an increase in HD value compared to smeared dentin, this confirmed a mimic of hypersensitive dentin condition.

After that, each of 4 groups received different treatments (Fig. 1) as follows:

Group 1: universal adhesive (self-etch mode; n = 10). The dentin surface was applied with 2–3 consecutive coats of Single bond universal adhesive (3M Deutschland GmbH, Neuss, Germany), then rubbed for 20 s using a fully saturated microbrush (Cotisen), and gently air-dried for 5 s to evaporate the solvent.

Group 2: universal adhesive (total-etch mode; n = 10). The dentin surface was treated with 35 % phosphoric acid (Scotchbond™ Etchant; 3M ESPE, St. Paul, MN, USA) for 15 s, then rinsed with distilled water for 10 s, and blotted excess water using a cotton pellet until the surface appears glistening without pooling of water. After that, the dentin surface was applied with Single bond universal adhesive (3M Deutschland GmbH) as described in group 1.

Group 3: SnF₂ toothpaste + universal adhesive (self-etch mode; n = 10). The 1450 ppm fluoride toothpaste containing 0.454 % w/w stannous fluoride and 0.0721 % w/w sodium fluoride (Sensodyne rapid action; Glaxosmithkline, Pathumthani, Thailand) was applied on a moist dentin surface by agitating with a microbrush (Cotisen) for 1 min, and left undisturbed for 4 min, then the dentin surface was cleaned thoroughly with a moist

cotton swab until all visible toothpaste was removed.⁸ Thereafter, the dentin surface was treated with Single bond universal adhesive (3M Deutschland GmbH) in self-etch mode as described in group 1.

Group 4: SnF₂ toothpaste + universal adhesive (total-etch mode; n = 10). After the dentin surface was treated with SnF₂ toothpaste (Glaxosmithkline) as described in group 3, the dentin was applied with Single bond universal adhesive (3M Deutschland GmbH) in total-etch mode as described in group 2.

In each group, the dentin treatment was applied under simulated normal intrapulpal pressure of 11 mmHg.^{9,17} The chemical compositions and manufacturer's instructions of the universal adhesive system used in the study is presented in Table 2. After application of universal adhesive, the adhesive-dentin surface was light-cured with a halogen lamp-curing unit (3M ESPE Elipar™ 2500; 3M ESPE, St. Paul, MN, USA) according to the manufacturer's instructions. The HD of bonded dentin was immediately measured under the pressure of 100 mmHg as mentioned earlier, and the percentage of immediate dentin sealing ability was calculated for each group.

As the HD value of bonded dentin was repeatedly measured on the same dentin specimen and the percentage immediate dentin sealing ability of bonded dentin was expressed as a percentage decrease of HD related to the baseline HD value of EDTA treated dentin. Thus, each specimen served as its own control. For each specimen, the percentage immediate dentin sealing ability of bonded dentin (%IDS) was calculated by the following formula: the %IDS = [(HD value of EDTA treated dentin – HD value of bonded dentin)/(HD value of EDTA treated dentin)] $\times$ 100 %.

Scanning electron microscopic and energy dispersive spectroscopic analysis (SEM-EDS analysis)

The additional 20 intact dentin specimens were prepared and randomly divided into 4 treatment groups: group 1: dentin treated with 17 % EDTA; group 2: dentin treated with EDTA + 35 % phosphoric acid; group 3: dentin treated with EDTA + SnF₂ toothpaste and group 4: dentin treated with EDTA + SnF₂ toothpaste + 35 % phosphoric acid for SEM-EDS study, using a scanning electron microscope (JSM-6610 LV; JEOL, Tokyo, Japan) and energy dispersive x-ray spectrometer with an X-Max 20 mm² detector (Oxford Instruments Inc., High Wycombe, UK). Each specimen was

Table 2 Chemical compositions and manufacturer's instructions of the universal adhesive system used in the study.

Material used (lot number)	Manufacturer	Composition	Manufacturer's instruction	
Scotchbond etchant gel (NE67240)	3M ESPE, St. Paul, MN, USA	35 % phosphoric acid by weight (pH 0.6), fumed silica, water soluble surfactant	Total-etch mode: Apply etchant to the prepared dentin and allow it to react for 15 s. Rinse thoroughly with water and dry with water-free and oil-free air, or with cotton pellets; do not overdry.	Self-etch mode: Do not use etchant. Rinse thoroughly with water and dry with water-free and oil- free air, or with cotton pellets; do not overdry.
Single bond universal adhesive (20325A)	3M Deutschland GmbH, Neuss, Germany	10-MDP, Bis-GMA, phosphate monomer, HEMA, dimethacrylate resins, ethanol, water, methacrylate-modified polyalkenoic acids copolymer, filler, initiators, silane.	Application of universal adhesive: Use the disposable applicator to apply the adhesive to the entire dentin surface and rub it in for 20 s. If necessary rewet the disposable applicator during treatment. Subsequently direct a gentle stream of air over the liquid for 5 s until it no longer moves and the solvent has evaporated completely. Harden the adhesive with a commonly used curing light for 10 s.	

Abbreviations: MDP: methacryloyloxy-decyl-dihydrogen-phosphate; Bis-GMA: bisphenol-A- glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate.

observed to assess the characteristics of each treated dentin and SnF₂ precipitation on dentinal surface. In addition, the dispersive spectroscopic analysis of each specimen was performed at 2 separate locations on each dentin surface to determine elemental composition. As dentin is calcified tissue composed of mineralized hydroxyapatites and collagen fibrils, elements like calcium, oxygen and phosphorous represent the components of hydroxyapatite, meanwhile carbon, nitrogen and oxygen represent the organic components of collagen fibrils in dentin.^{10,17} Additionally, fluoride, tin, zinc and silicon represent the components of SnF₂ toothpaste (Glaxosmithkline) which deposit on dentin.^{10,11} Thus, the elemental analysis of 4 different treated dentin groups in terms of atomic% of elements (C—carbon, N—nitrogen, O—oxygen, F—fluoride, Si—silicon, Ca—calcium, P—phosphorous, Zn—zinc, Sn—Tin) were taken from 10 different areas of the 5 specimens per each treated group.

Statistical analysis

The percentage of immediate dentin sealing ability of bonded dentin and the atomic% of each element of treated dentin in each group are represented as mean and standard deviation (SD). The data were analyzed using SigmaStat 3.5 (Systat Software Inc.; San Jose, CA, USA). The Kolmogorov–Smirnov test was performed to test the normal distribution of the data. Regarding the different stannous fluoride pretreatment and bonding application modes, the mean percentage of immediate dentin sealing ability of bonded dentin were compared using two-way analysis of variance (2-way ANOVA). Where this

showed a significant effect, Tukey test was used for pairwise multiple comparisons.

For analysis of the atomic% of each element of treated dentin, the means of different treatment groups were compared using one-way analysis of variance (1-way ANOVA). Where this showed a significant effect, Tukey test was used for pairwise multiple comparisons.

For all statistical tests, *P* values less than 0.05 were considered significant.

Results

Percentage of immediate dentin sealing ability of bonded dentin

The mean and SD of percentage of immediate dentin sealing ability of bonded dentin using universal adhesive with self-etch or total-etch mode, and without or with SnF₂ toothpaste pretreatment are shown in Fig. 2. The percentage of immediate dentin sealing ability for self-etch mode without and with SnF₂ toothpaste pretreatment were 79.531 ± 20.713 % and 71.545 ± 15.558 %, respectively. The corresponding values for total-etch mode without and with SnF₂ toothpaste pretreatment were 77.345 ± 18.145 % and 69.565 ± 9.925 %, respectively.

The results of 2-way ANOVA showed that the effect of both factors (Factor 1: without or with SnF₂ toothpaste pretreatment and Factor 2: self-etch or total-etch modes) and their interaction on the determined percentage of immediate dentin sealing ability of bonded dentin was not

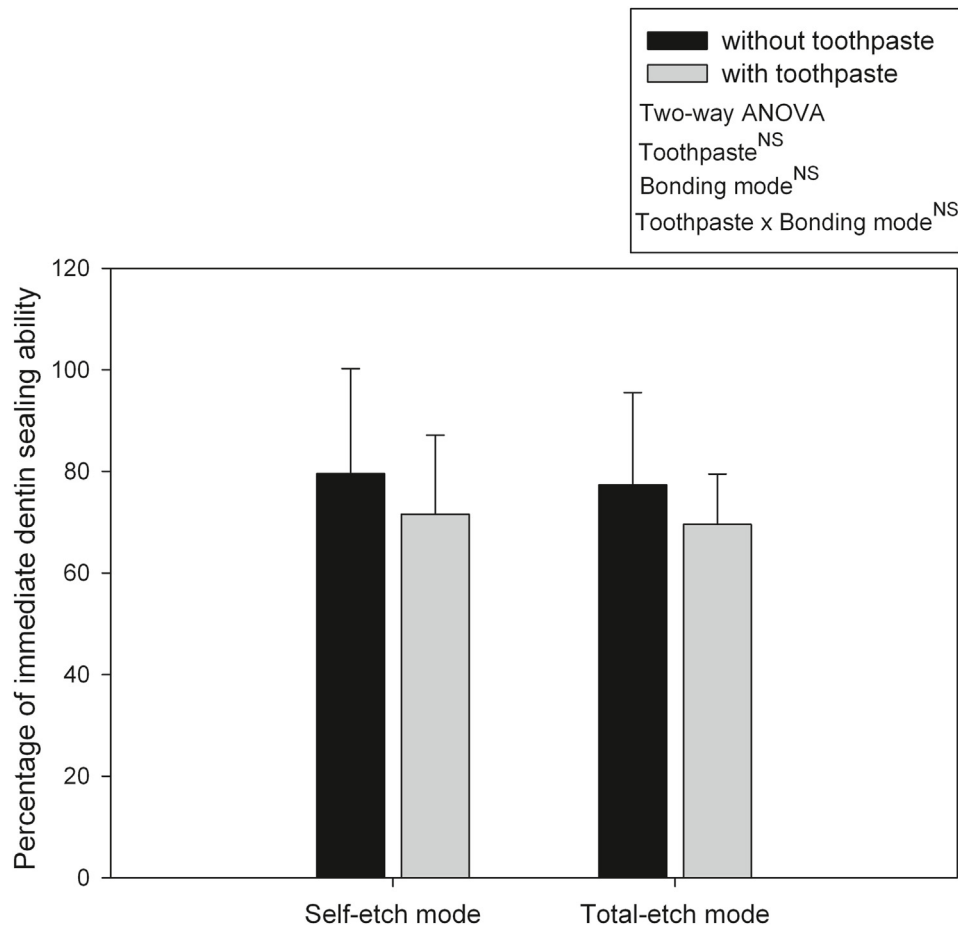


Figure 2 Mean (± 1 SD) percentage of immediate dentin sealing ability of bonded dentin using universal adhesive with self-etch or total-etch mode and without (black column) or with (gray column) stannous fluoride toothpaste pretreatment. NS represents no significant difference among the groups ($P > 0.05$).

significantly different (Factor 1: $P = 0.141$, Factor 2: $P = 0.694$, Interaction: $P = 0.984$).

Scanning electron microscopic and energy dispersive x-ray spectroscopic results

Dentin specimens treated with 17 % EDTA showed open dentinal tubules without smear layer (Fig. 3A), and dentinal tubules became more open with well-defined border in dentin specimens treated with 17 % EDTA +35 % phosphoric acid (Fig. 3B). Meanwhile, dentin specimens treated with EDTA followed by SnF₂ toothpaste, most dentinal tubules were occluded (Fig. 3C). However, dentin treated with EDTA + SnF₂ toothpaste+35 % phosphoric acid had some fine particles on the dentin surface but it was difficult to observe any occlusion of dentinal tubules (Fig. 3D).

For EDS analysis, the mean and SD of the chemical composition of different treated dentin are presented in Table 3. The examples of EDS spectra taken from different treated dentin surfaces are presented in Fig. 4. In group 1, dentin treated with 17 % EDTA exhibited C, N, O which represent the organic components of dentin, and Ca, O, P which represent the components of hydroxyapatite in dentin. In group 2, dentin treated with 17 % EDTA followed

by 35 % phosphoric acid showed higher atomic% of C and N, but lower atomic% of Ca, O and P, when compared to group 1 ($P < 0.05$). Meanwhile, in group 3, dentin treated with 17 % EDTA followed by SnF₂ toothpaste revealed significantly higher atomic% of F, Sn and Si, but lower atomic% of N when compared to the other groups ($P < 0.05$), this indicated that the components of SnF₂ toothpaste could deposit in dentin. In group 4, dentin treated with 17 % EDTA + SnF₂ toothpaste +35 % phosphoric acid showed the remarkable decrease in atomic% of F, Si, Sn, Ca, O, P but significant increase in atomic% of C and N, when compared to group 3 ($P < 0.05$). However, there was not significant difference in atomic% of elements between group 2 and 4 ($P > 0.05$).

Discussion

The present study demonstrated that stannous fluoride desensitizing toothpaste had no adverse effect on immediate dentin sealing ability of universal adhesive in both total-etch and self-etch mode. Although the results of SEM-EDS analysis showed that there were significant differences in atomic% of elements of treated dentin among the different treatment groups, both application modes of universal adhesive had the same ability of sealing dentin

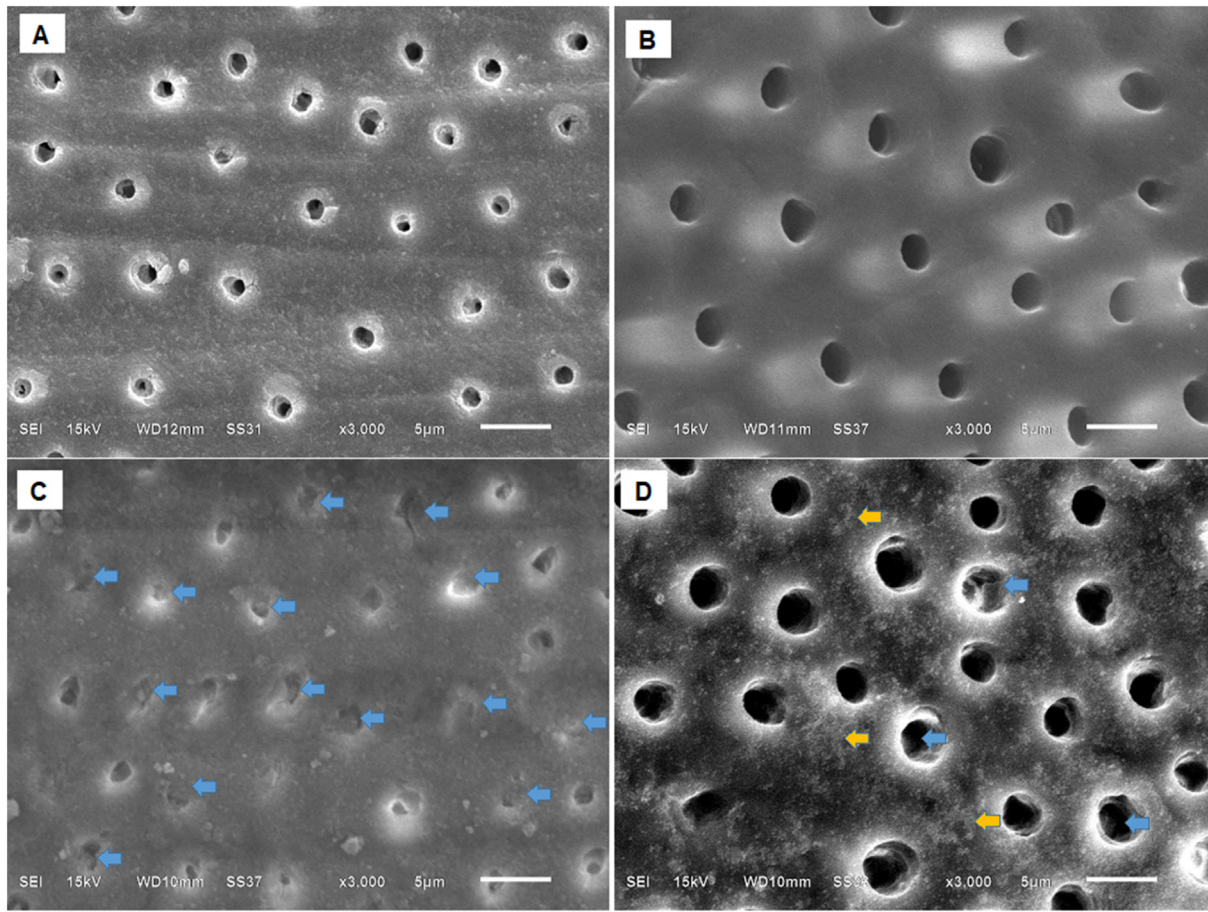


Figure 3 (A–D) Scanning electron micrographs of human dentin (original magnification X3000). (A) EDTA treated dentin; (B) EDTA+35 % phosphoric acid treated dentin; (C) EDTA + SnF₂ toothpaste treated dentin, blue arrows showing occlusions of dentinal tubules; (D) EDTA + SnF₂ toothpaste+35 % phosphoric acid treated dentin, yellow arrows showing fine particles still remained on the dentin surface.

with and without stannous fluoride pretreatment. Thus, the null hypothesis was accepted.

In this study, all specimens were treated with 17 % EDTA for 60 s to remove smear layer and simulate hypersensitive dentin before bonding; thus, the SEM results of EDTA-treated dentin found clean dentinal surface with open dentinal tubules.⁸ In agreement to the previous studies, after EDTA-treated dentin received SnF₂ toothpaste application, most dentinal tubules were occluded, and SEM-EDS analysis found higher atomic% of F, Si and Sn in group 3 (dentin treated with EDTA + SnF₂ toothpaste), when compared to group 1 (dentin treated with EDTA).^{8,10,11} This is due to the formation of insoluble stannous compounds from rapid oxidation of Sn and hydrolysis of stannous fluoride in the presence of water, contributing to the formation of a layer over the dentin and the reduction of its permeability by creating precipitates within the dentinal tubules.^{8,10,11} However, the difference in characteristic and atomic% of elements of dentin surface caused by the occlusive property of SnF₂ toothpaste did not influence the immediate dentin sealing ability of universal adhesive in self-etch mode, as confirmed by the results of dentin permeability test in the present study, which showed that SnF₂ pretreatment group yielded a similar percentage of

dentin sealing ability of adhesive, when compared to the control group (without SnF₂ pretreatment). According to the manufacturer's recommendation, 20 s scrubbing time of single bond universal adhesive into the dentin allows for proper self-etching effects to take place and for the penetration of adhesive into the dentinal tubules, resulting in the immediate dentin sealing of adhesive in both groups.¹⁴ Thus, it would be acceptable on the basis of the advantage of the presence of SnF₂ in the dentin, that it not only helps in remineralization of dentin but also reduces dentin hypersensitivity before operative treatment by dentists.^{8,10,11}

For universal adhesive treatment in total-etch mode, etching dentin with phosphoric acid is needed prior to bonding. Therefore, the SEM results of group 2 (dentin treated with EDTA + 35 % phosphoric acid) found wider open dentinal tubules, when compared to group 1. Additionally, the SEM image of group 4 (dentin treated with EDTA + SnF₂ toothpaste + 35 % phosphoric acid) showed larger open dentinal tubules with some silica particles precipitated on the dentin surface, when compared to group 3. Consistent with the prior studies,¹⁸ the chemical composition of group 2 and group 4 showed lower percentage of Ca, P and O as a result of the stronger mineral

Table 3 The elemental analysis of 4 different treated dentin groups in terms of atomic% of elements (C—carbon, N—nitrogen, O—oxygen, F—fluoride, Si—silicon, Ca—calcium, P—phosphorous, Zn—zinc, Sn—Tin). The mean value and standard deviation (SD) are shown and taken from 10 different areas of the 5 specimens per each treated group.

Element	Atomic%			
	Group 1	Group 2	Group 3	Group 4
C	31.895 (1.557) ^a	53.176 (3.981) ^b	28.405 (1.244) ^a	51.723 (4.411) ^b
N	4.302 (1.753) ^a	14.875 (2.393) ^b	0.094 (0.164) ^c	13.924 (1.385) ^b
O	44.251 (1.381) ^a	26.350 (2.486) ^b	49.209 (2.777) ^c	27.478 (1.521) ^b
F	0.026 (0.061) ^a	0.021 (0.066) ^a	0.253 (0.136) ^b	0.019 (0.043) ^a
Si	0.000 (0.000) ^a	0.000 (0.000) ^a	0.241 (0.164) ^b	0.089 (0.096) ^a
Ca	11.583 (1.246) ^a	3.454 (2.006) ^b	12.790 (2.030) ^a	4.150 (2.129) ^b
P	7.683 (0.755) ^a	1.946 (1.183) ^b	8.693 (0.995) ^a	2.460 (1.257) ^b
Zn	0.239 (0.052) ^{a,b}	0.169 (0.161) ^{a,b}	0.270 (0.079) ^a	0.145 (0.082) ^b
Sn	0.019 (0.016) ^a	0.010 (0.013) ^a	0.043 (0.027) ^b	0.014 (0.006) ^a

For each element, the same lowercase letter represents no significant difference among the treatment groups ($P > 0.05$, 1-way ANOVA and Tukey test). Group 1: dentin treated with 17 % EDTA; Group 2: dentin treated with 17 % EDTA followed by 35 % phosphoric acid; Group 3: dentin treated with 17 % EDTA followed by stannous fluoride toothpaste; Group 4: dentin treated with 17 % EDTA followed by stannous fluoride toothpaste and 35 % phosphoric acid.

dissolution of phosphoric acid, but higher percentage of C and N as a result of higher presence of exposed collagen fibers, when compared to group 1 and group 3, respectively. However, the morphological and chemical features of dentin had no effect on the immediate dentin bonding ability of 2 application modes of universal adhesive, as confirmed by the 2-way ANOVA results of dentin permeability test in the present study, which showed that in simulated hypersensitive dentin with 17 % EDTA, both factors (factor 1: SnF₂ toothpaste and factor 2: bonding mode) had not significant effects on immediate dentin sealing ability of universal adhesive. Thus, stannous fluoride desensitizing toothpaste could be recommended as home pretreatment in patients with dentin hypersensitivity before employing operative dental procedure as it had no adverse effect on the immediate dentin sealing ability of universal adhesive system.

This universal adhesive used in this study contains 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), which is a phosphate and bifunctional monomer, its pH is 2.7 for self-etching performance, and is able to bind chemically to hydroxyapatite via nano-layering of 10-MDP-calcium salts. Great bonding performance of 10-MDP based universal adhesive systems had been reported in many *in vitro* and *in vivo* studies.^{13,19} The 10-MDP monomer has a long and hydrophobic spacer chain to allow the formation of a rich MDP-Ca salt adhesive interface, improving the bond strength of adhesive.¹³ Moreover, self-assembled nano-layered structures are typical of the 10-MDP monomer, and thought to produce a better water-stable interface which is favorable to adhesion, leading to the higher adhesive stability of 10-MDP containing adhesive systems.¹³

In addition, the present study found that there were no effects of the application modes on the immediate dentin sealing ability of universal adhesive in dentin with and without SnF₂ pretreatment. In agreement with the previous investigations, universal adhesives could seal dentin, and the percentage reduction in dentin permeability between the self-etch and total-etch modes of universal adhesives were not statistically different.^{20,21} Additionally, in micro-

tensile bond strength (μ TBS) test, several previous studies reported that there were no effects of both application modes on the immediate μ TBS of universal adhesives, when applied the adhesive to dentin in either self-etch mode or total-etch mode.^{22–25}

Although, the total-etch mode, involving prior etching dentin with phosphoric acid, could increase the risk of exposed collagen collapse and lead to incompletely infiltration of bonding resin to the demineralized dentin depth, resulting in a decrease in the effectiveness of the adhesive system.^{14,26} In fact, the key prevention of collagen collapse is the preservation of adequate moisture of demineralized dentin, not overdrying. Additionally, the vitrebond copolymer in universal adhesive, a methacrylate-modified polyalkenoic acid copolymer, allows more consistent bond performance to dentin under varying moist dentin levels.²⁷ Furthermore, the hydrophilic nature before polymerization of universal adhesive might relatively help the penetration of the resin bonding into dentinal tubules during application prior to curing for optimum wetting of the tooth structure.²⁷ Therefore, these explanations support why both application modes of universal adhesive had the same ability of sealing dentin with and without stannous fluoride pretreatment. Moreover, the immediate dentin sealing ability of universal adhesive creates reocclusion of dentinal tubules via polymerized resin impregnation of dentinal tubules, resulting in blocking dentinal fluid movement in tubules and decreasing dentin permeability.^{3,6} Thus, the use of universal adhesive is effective in dentin desensitization and management of dentin hypersensitivity.

In conclusion, in simulated-hypersensitive dentin with 17 % EDTA, SnF₂ desensitizing toothpaste has no adverse effect on the immediate dentin sealing ability of universal adhesive, and both application modes of universal adhesive have the same ability of sealing dentin with and without stannous fluoride pretreatment. Hence, at-home SnF₂ treatment should be recommended to the patients for reducing dentin hypersensitivity prior to receiving the operative treatment with universal dental adhesive by dentists.

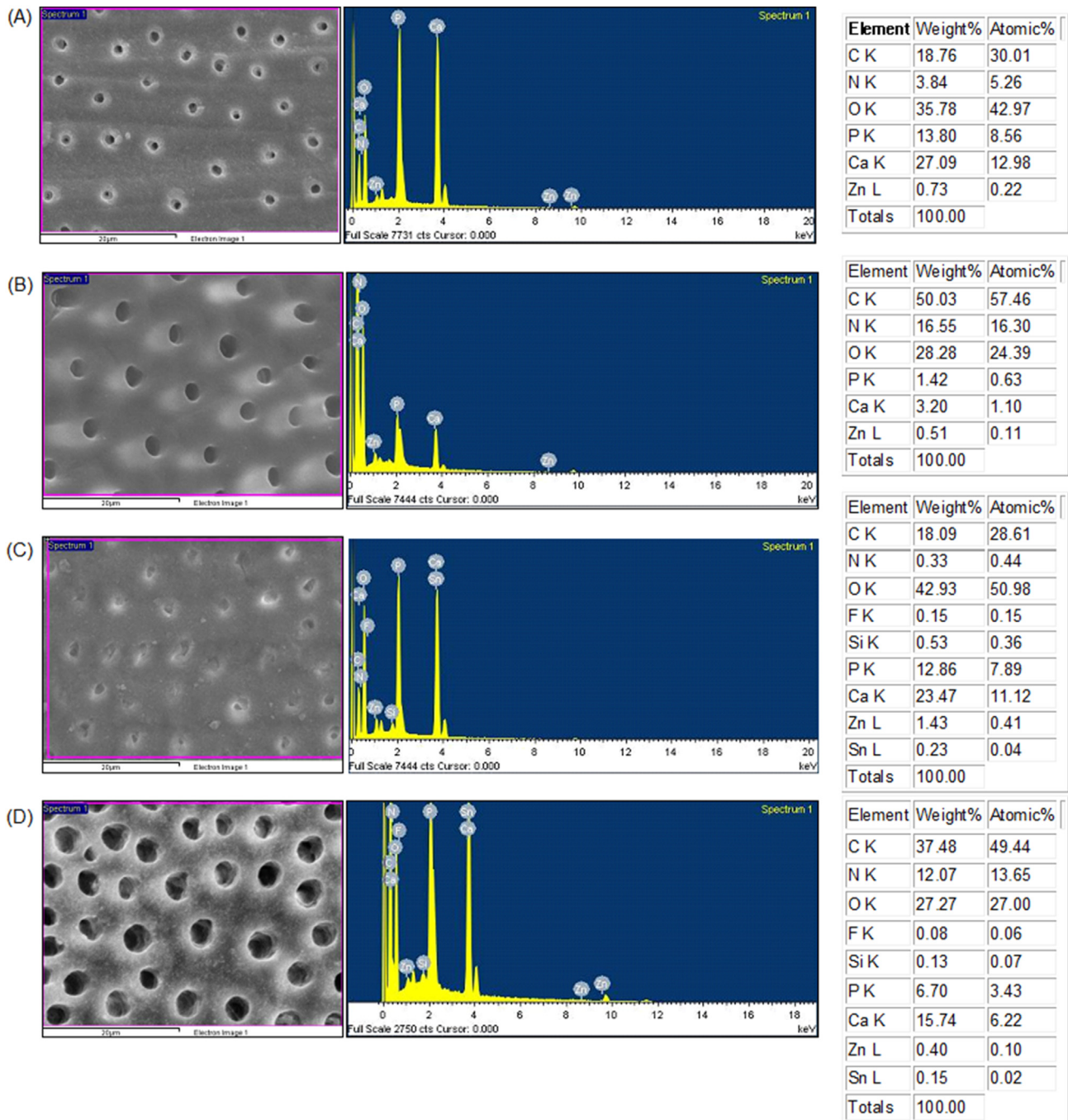


Figure 4 Energy-dispersive X-ray spectroscopy (EDS) spectra taken from the treated dentin surface of (A) 17 % EDTA treatment, (B) 17 % EDTA+35 % phosphoric acid treatment, (C) 17 % EDTA + SnF₂ toothpaste treatment, (D) 17 % EDTA + SnF₂ toothpaste+35 % phosphoric acid treatment with the right table for the weight and atomic percentage of the following elements: C—carbon, N—nitrogen, O—oxygen, F—fluoride, Si—silicon, Ca—calcium, P—phosphorous, Zn—zinc, Sn—Tin.

Declaration of competing interest

The authors have no conflicts of interest relevant to this study.

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