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Iontophoresis effects of two-step self-etch and total-etch systems on dentin permeability and sealing of composite restoration under simulated pulpal pressure

Orapin Ajcharanukul^{1*}, Peeraya Santikulluk¹, Palat Sasingha¹, Sirithorn Sabpawat¹ and Kanokporn Sukyanan²

Abstract

Background: Studies demonstrated the bond strength enhancement and the decrease in degradation of the adhesive interface after applying either self-etch adhesives or two-step, etch-and-rinse adhesives under an electric field. However, the presence of dentinal fluid driven by the pulpal pressure in vivo is a profound factor affecting both the sealing ability and bond strength of adhesives. This study aimed to evaluate the effect of three-step etch-and-rinse and two-step self-etch adhesives when applied with iontophoresis under simulated pulpal pressure on the permeability of dentin, resin infiltration, and the sealing ability of resin composite.

Methods: The experiments were done on 32 recently extracted premolars, randomly assigned into four groups ($n=8$) according to two adhesive systems (SBMP and SE), applied following the manufacturer's instructions (control) for two groups or with iontophoresis for the others (SBMPi and SEi). For the iontophoresis, the anodal current was applied at 75 μ A for 20 s through the cavity electrode during the bond. The fluid flow rate of dentin was recorded after cavity preparation (smear-layer-covered dentin; T1), bonding (T2), and composite restoration (T3) during the maintained pulpal pressure of 20 mm Hg. The flow rates were expressed as a percentage relative to the initial smear-layer-covered value for each specimen. Results were analyzed using repeated measures ANOVA. Scanning electron microscopy (SEM) was performed to observe the resin/dentin interface.

Results: There were no significant increases in the mean flow rates from T1 to T3 in the SBMP ($P=0.355$), while these changes in the SE were significant between T1 (100%) and T2 (166.77%) and T1 and T3 (221.16%) ($P=0.002$; one-way RM ANOVA; Holm-Sidak test). For the iontophoresis groups, the mean flow rates decreased significantly from T1 to T2 and T1 to T3 of both SBMPi ($T2=86.43$, and $T3=79.53$; $P<0.001$) and SEi groups ($T2=87.96$, and $T3=81.48$; $P=0.004$). The iontophoresis of both adhesives produced the optimal resin infiltration with improved quality of the hybrid layer and resin tags.

Conclusions: SBMP bonded with or without iontophoresis performed better sealing ability than SE under the same condition. Both adhesives applied with anodal iontophoresis significantly decreased the dentin permeability, contributing to the improved resin infiltration.

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Keywords: Dental bonding system, Dentin permeability, Iontophoresis, Resin composite, Hybrid layer

Background

Under physiologic pulpal pressure causing a continuous outward fluid flow through the dentin, studies demonstrated that restorative procedures, including dentin bonding and composite restoration, could not perfectly seal the tooth structure [1, 2]. When the hydraulic conductance technique was used to determine the adhesive permeability, various systems of dental adhesives allowed fluid transudation differently across the bonded dentin [3]. The results revealed that a three-step, etch-and-rinse adhesive such as Scotchbond Multi-Purpose (SBMP) sealed dentin better than other adhesives, whereas, for the self-etch adhesive, the appropriate dentin sealing could be achieved using Clearfil SE Bond (SE). However, most adhesives do not seal dentin as well as that smear layers [3, 4]. They permeate the demineralized dentin structure mainly via a diffusion mechanism which is highly variable depending on the adhesive system used, the dentin substrate, and the intervention of the operator [5, 6]. These factors result in different degrees of the poor seal within the hybrid layer along the adhesive interface, increasing bond degradation, and subsequent loss of retention of the composite restoration [7]. Some procedures are suggested to improve the impregnation of the resin monomers into dentin, such as using an additional layer of hydrophobic resin [8] and applying multiple layers of adhesives [9]. Although these were proven beneficial for some bonding agents [10], the iontophoretic effects on the bonding application could provide another option for further studies of the selected bonding agents to be used under certain clinical situations.

The use of iontophoresis to enhance drug delivery through the tooth structure was widely investigated, with promising results indicating the possibilities to apply to the field of restorative dentistry [11–13]. Studies demonstrated the bond strength enhancement and the decrease in degradation of the adhesive interface after applying either self-etch adhesives [14–16] or two-step, etch-and-rinse adhesives [17–20] under an electric field [21]. The intensity of the applied current contributing to the significantly higher bond strength was from 0 to 110 μ A. Since the three-step adhesive system was considered effective in reducing dentin permeability [3], the use of iontophoresis to enhance its penetration into dentin may increase the sealing ability and be a reference condition. Furthermore, the presence of dentinal fluid driven by the pulpal pressure in vivo is a substantial factor affecting both the sealing ability [1, 3] and bond strength of adhesives [22, 23]. Recent evidence indicated that the rate and direction

of continuous outward fluid flow through dentin could be changed when a direct current (DC) was passed from the dentin surface into the pulp using different polarities and intensities of the current [24]. Such phenomenon, so-called electroosmosis, is produced during the iontophoresis through a tissue causing an enhanced delivery of agents through dentin. Also, a DC device is necessary to guarantee the constant current applied during the drug delivery of the iontophoresis because the compositions of the tooth structure are highly variable among individuals, resulting in differences in the electrical resistances of the tooth areas [14, 21, 25]. Therefore, the bonding agent delivered with a DC iontophoresis can ensure its effect on the dentinal fluid movement with increasing infiltration of the adhesive interface.

By determining the dye penetration qualitatively through the resin-dentin interface under simulated pulpal pressure, N Gharizadeh, A Kaviani and S Nik [26] demonstrated that the microleakage scores reduced significantly after applying etch-and-rinse adhesive (Single Bond) with the electric current of 15 μ A. However, details of such effects on the other adhesive types, instrument set, application time, electrode polarity, and the quantitative evaluation of the dentin fluid flow under the experimental condition were absent, prompting the challenge in the potential application. Therefore, this study aimed to quantitatively evaluate the effect of three-step etch-and-rinse and two-step self-etch adhesives when applied with iontophoresis under simulated pulpal pressure on the permeability of dentin, resin infiltration, and the sealing ability of resin composite. The null hypothesis was that the application of iontophoresis with bonding agents created comparable effects to that of the conventional methods on the sealing ability of dentin under simulated pulpal pressure.

Methods

The experimental protocol was exempted from permission by the ethical committee of Srinakharinwirot University (SWUEC-321/2562X). The required sample size for ANOVA at the different conditions was determined based on previous experiments [27] by using the Sigma-Plot 11.0 (Systat Software Inc., San Jose, CA, USA). The minimum size was calculated as 5–7 for each treatment group with a desired power of 0.8 and a significance level of 0.05. The experiments were done on 32 extracted human premolars recently extracted for orthodontic purposes. Teeth were free of caries or restorations and

stored in 0.1% thymol solution at 4 °C for up to 4 weeks before use.

Sample preparation and fluid flow measurement

The sample preparation and testing were carried out by two operators while the other recorded fluid flow rates. The tooth was cut transversely 2 mm below the cemento-enamel junction with a diamond disc under streaming water. The coronal pulpal tissue was removed with fine tweezers, then irrigated with water using a triple syringe to remove any remaining tissue. Dentine was exposed at the tip of the buccal cusp by cutting a cavity (diameter 4 mm, depth 3 mm) with an air-rotor and straight cylinder diamond burs (Nos. 201 and 204, Intensive1, Viganello-Lugano, Switzerland) under cooling water. A

fluid flow measurement was set up in each specimen as shown in Fig. 1. The crown was sealed with cyanoacrylate cement (Alteco Inc., Osaka, Japan) to an acrylic block into which had been sealed a stainless-steel tube (18 G, o.d. 1.27 mm, i.d. 0.84 mm). Each tooth crown and acrylic block were held together in a vertical direction until the glue was set to prevent excess in the pulp chamber. The pulp chamber, stainless steel tube, and capillary were filled with Ringer’s solution and kept in the same horizontal plane during each flow rate measurement. The set-up was connected to a manometer and maintained at a pressure of 20 mm Hg above atmospheric to represent the normal tissue fluid pressure of the pulp in vivo [28]. Prior to measurements of fluid flow, the cavity floor was manually polished using a small piece of 600-grit abrasive

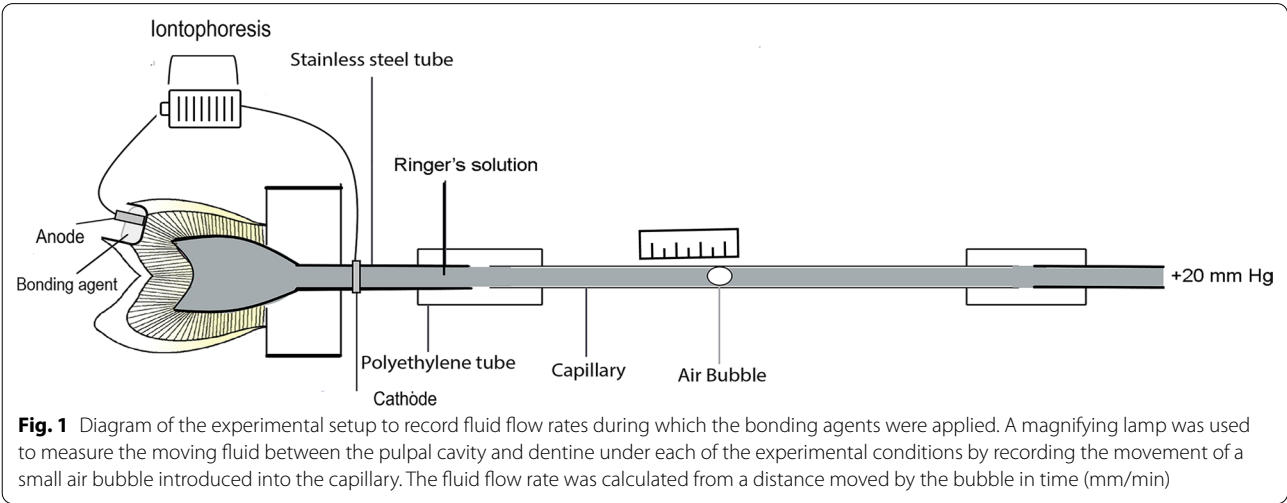


Table 1 Materials used in the study

Material	Classification	Composition	Application
Adper Scotchbond Multi-purpose (SBMP; 3 M ESPE, St. Paul, MN, USA)	Three-step total-etch adhesive	Etchant 35% phosphoric acid Primer HEMA, polyalkenoic acid polymer, water Bond HEMA, Bis-GMA, tertiary amines, photoinitiator	Acid etch for 15 s, water rinse for 10 s, gently air dry for 5 s Apply primer for 10 s, gently air dry for 5 s Apply bond. Gently air dry Light cure 10 s
Clearfil SE Bond (SE; Kuraray, Osaka, Japan)	Two-step self-etch adhesive	Primer 10-Methacryloyloxydecyl Dihydrogen phosphate (MDP), HEMA, hydrophilic dimethacrylates, photoinitiator, amine, water Bond MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, colloidal silica, photoinitiator	Apply primer for 20 s, gently air dry for 5 s Apply bond, Gentle air dry Light cure 10 s
Estelite quick (Tokuyama, Tokyo, Japan)	Nano-filled composite resin	Bis-GMA, TEGDMA, silica zirconia fillers, silica-titania fillers, photoinitiators	Resin composite placement through incremental technique with each 2.0 mm increment light-cured

HEMA (hydroxyethyl)methacrylate, BisGMA bisphenol A glycidylmethacrylate, MDP 10-methacryloyloxydecyl dihydrogen phosphate, TEGDMA triethylene glycol dimethacrylate

paper and water for 30 s to obtain a standardized smear layer [19, 20].

Samples were randomly assigned into four groups ($n=8$) according to the adhesive systems, (1) SBMP (3 M ESPE, St. Paul, MN, USA) and (2) SE (Kuraray, Osaka, Japan) (Table 1) applied following the manufacturers instruction for the control groups, and the use of iontophoresis during the bond application, (3) SBMPi, and (4) SEi groups. Each adhesive was light-cured for 10 s with a LED light-curing unit at 1200 mW/cm^2 (D-2000, APOZA; New Taipei City, Taiwan). Estelite Quick (Tokuyama, Tokyo, Japan) was used as a composite restoration for the prepared cavity (Table 1). Each increment of 1.5–2 mm was light-cured for 20 s using the same light-curing unit. For the SBMPi and SEi groups, an iontophoresis machine was used to pass a D.C. current of $75 \mu\text{A}$ for 20 s between the cavity electrode and the stainless-steel needle (return electrode) inserted into the cannula beyond the glass capillary (Fig. 1). The machine measured the electrical resistance along with the passing current at the end of the current application.

The fluid flow, the movement of a small air bubble introduced into the capillary, was recorded by using a microscope and graticule during the continuously maintained pulpal pressure of 20 mm Hg (Fig. 1). The flow rates were obtained from each sample after the cavity preparation (smear layer-covered dentin; T1), after adhesive (T2), and after composite restoration (T3). The flow direction that occurred during anodal iontophoresis with each bonding was observed to determine if the electroosmosis could affect the motion of the air bubble in the glass capillary. To compensate for the high variations in adhesive permeability, dentin structure, and composition among the samples, the flow measurements for each sample were also expressed as a percentage increase or decrease relative to the initial smear-layer-covered value. Thus, each sample served as its control.

Scanning electron microscopy (SEM) preparation

After finishing the fluid flow measurements, each sample was longitudinally fractured to examine the nature of the resin/tooth interface under the SEM. Representative samples of each group ($n=2$) were sectioned into two halves using a low-speed diamond disc under saline irrigation to clarify the distribution of the resin tags. The sectioned surfaces were polished with 1500 and 3000-grit silicon carbide sandpaper for 30 s before submitting to ultrasound water cleaning for 1 min. Subsequently, they were etched with 35% phosphoric acid gel for 1 min, followed by deproteinization in 5.25% NaOCl for 30 min as modified from N Nakabayashi and DH Pashley [29]. All SEM samples were dehydrated in ascending grades of

ethanol (50%, 75%, 95%, and 100% for 10 min each) and subjected to a critical-point dryer for 30 min. Then, they were coated with Au/Pt in a sputter-coater and examined under the SEM (JEOL, Model 5400, Tokyo, Japan).

The representative SEM images of all samples were also used to measure the remaining dentine thickness (RDT) between the floor of the cavity and the closest point of the pulp chamber along the dentinal tubules using measurement features of the SEM imaging software.

Statistical analysis

The data of the flow rates and the percentages increase or decrease relative to the initial smear layer covered dentin values (T1) were summarized as mean \pm 1 S.D. The data obtained from each group were analyzed statistically using one-way, repeated measures and analysis of variance (one-way RM ANOVA). Where this showed a significant effect, the Holm-Sidak test was used for pairwise comparisons between the individual means. Pearson correlations between percentage fluid conductance changes after adhesive and composite restoration of each group were determined by using linear regression analyses. P values < 0.05 were considered significant.

Results

The mean flow rates recorded at T1, T2, and T3 of each group are shown in Table 2. The fluid conductance expressed as percentage changes of the baseline smear-layer-covered values for each group were summarized in Fig. 2a. In the treatment groups without iontophoresis, there were no significant increases from T1 to T3 in the SBMP ($P=0.355$), while these changes of the SE group were significant between T1 (100%) and T2 (166.77%) and T1 and T3 (221.16%) ($P=0.002$; one-way RM ANOVA; Holm-Sidak test). This revealed that the conventional application of SBMP could seal the cavity's

Table 2 Effect of iontophoresis applied with bonding agents on fluid flow rate across smear layer-covered dentin (T1), bonded dentin (T2) and after composite restoration (T3)

Dentin condition	Conventional application (mm/min)		Iontophoresis application (mm/min)	
	SE	SBMP	SEi	SBMPi
T1	1.67 (1.3) ^A	3.58 (2.58) ^a	1.27 (0.43) ^A	1.28 (0.63) ^a
T2	2.54 (1.85) ^B	4.15 (2.68) ^a	1.11 (0.39) ^{AB}	1.0 (0.65) ^{ab}
T3	3.06 (1.8) ^B	3.68 (2.68) ^a	1.03 (0.43) ^B	0.94 (0.65) ^b

SE Clearfil SE Bond; SBMP Scotchbond Multi-Purpose; SEi Clearfil SE Bond applied with iontophoresis; SBMPi Scotchbond Multi-Purpose applied with iontophoresis
In the same column, subgroups with different letter superscripts indicate a significant difference ($p < 0.05$)

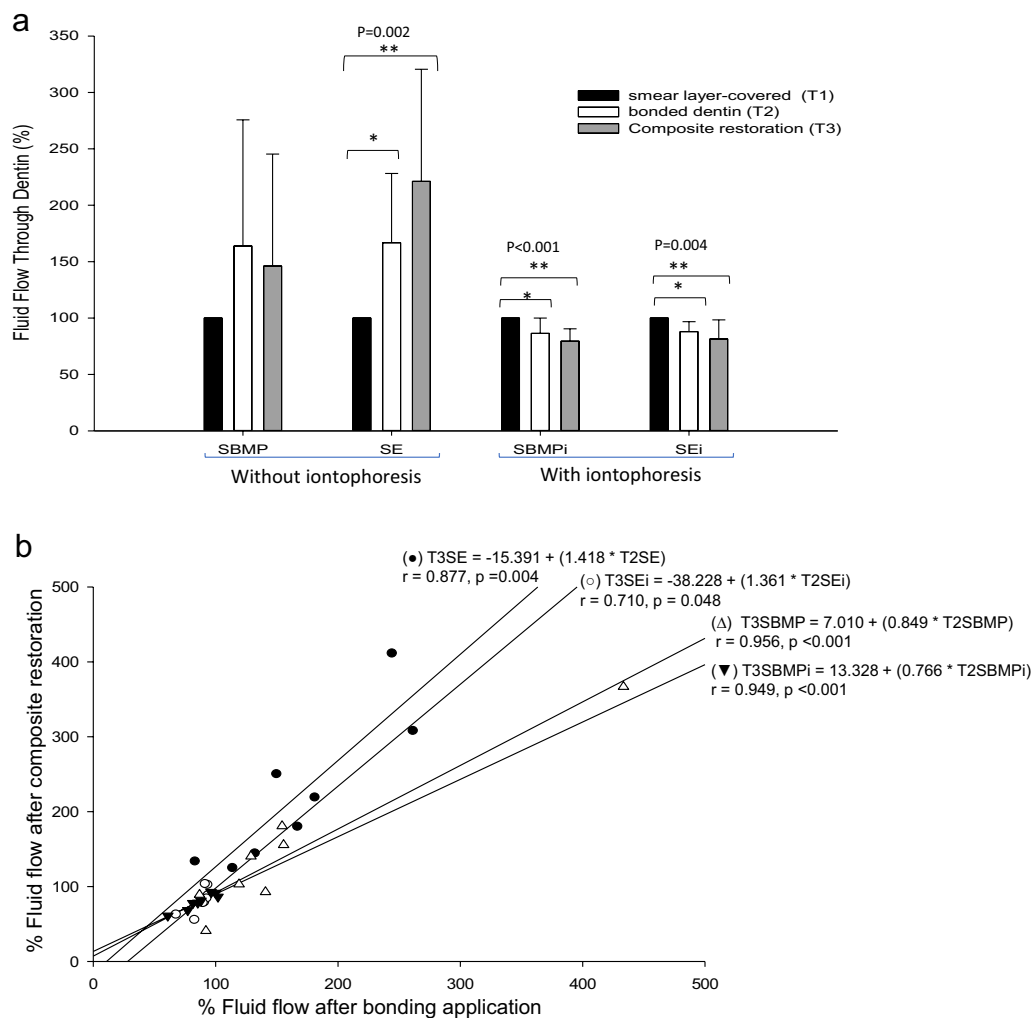
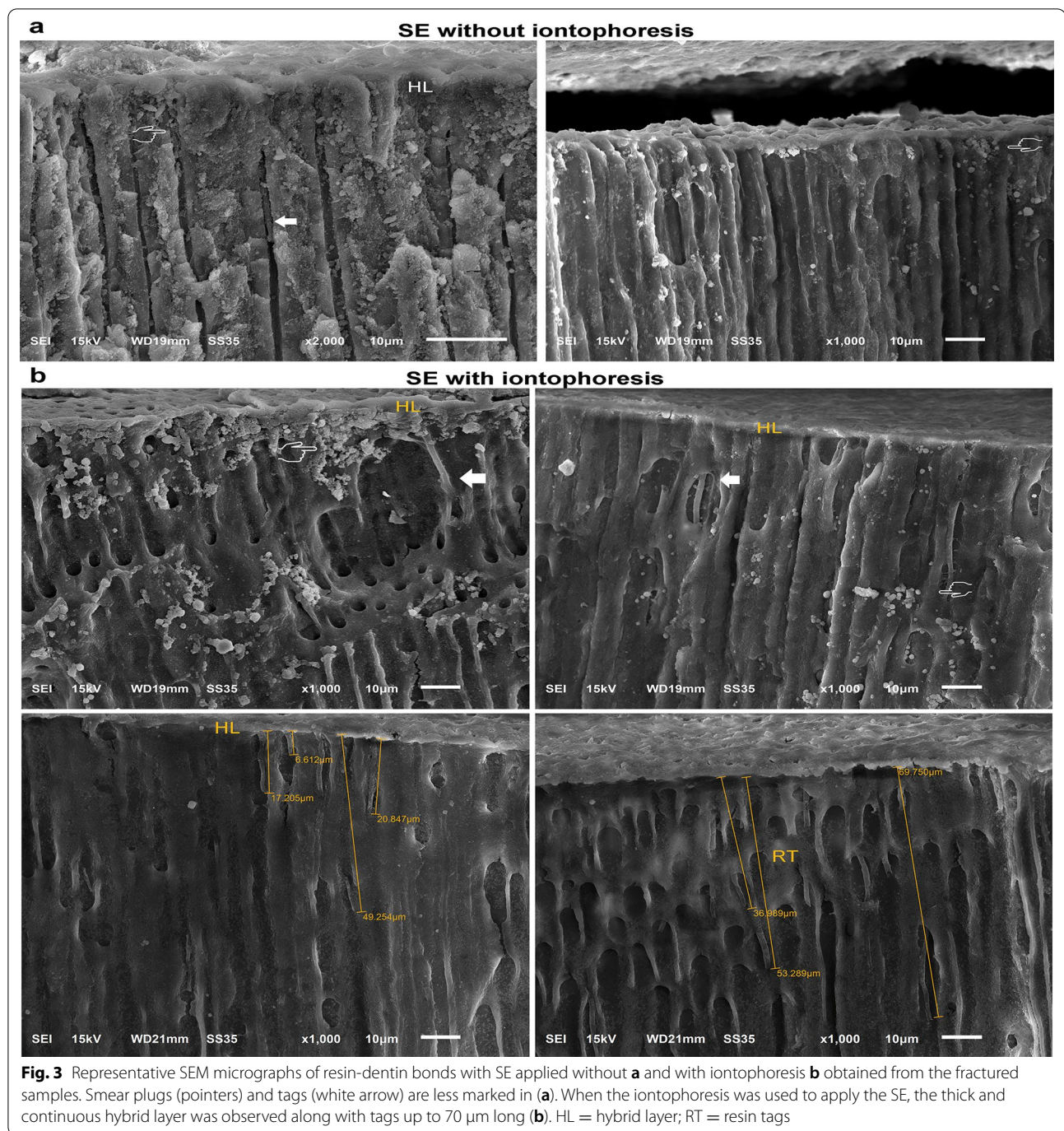


Fig. 2 **a** Bar charts showing the means and standard deviations of the percentage change of fluid flow rate from baseline obtained from that in the smear layer-covered dentin recorded with different adhesives and application either with or without iontophoresis. **b** Linear regression analyses comparing the percentage changes of fluid flow after bond associated with that obtained after composite restoration. SE = Clearfil SE Bond; SBMP = Scotchbond Multi-Purpose; SEi = Clearfil SE Bond applied with iontophoresis; SBMPi = Scotchbond Multi-Purpose applied with iontophoresis. $P < 0.05$ indicates statistical significance

wall as effectively as that smear layer-covered condition, whereas the sample bonded with the SE resulted in a significant increase in permeability even after composite restoration. For the iontophoresis, the mean percentage changes of the flow rates decreased significantly from T1 to T2 and T1 to T3 of both SBMPi ($T2 = 86.43$, and $T3 = 79.53$; $P < 0.001$) and SEi groups ($T2 = 87.96$, and $T3 = 81.48$; $P = 0.004$) (Fig. 2a), indicating that the application of anodal iontophoresis during the bonding step of both adhesives improved dentin sealing after adhesive and after composite restoration significantly better than that in the baseline smear layer.

When the percentage fluid conductance at T2 was plotted against T3 of each group, significant positive correlations were found and expressed as linear regression analyses in Fig. 2b. Furthermore, observations of the bubble movement in the capillary during the application of adhesives demonstrated that it was paused when the current was applied at the bonding in all samples of the iontophoresis groups, while this was outwardly continued during the adhesive application without iontophoresis.

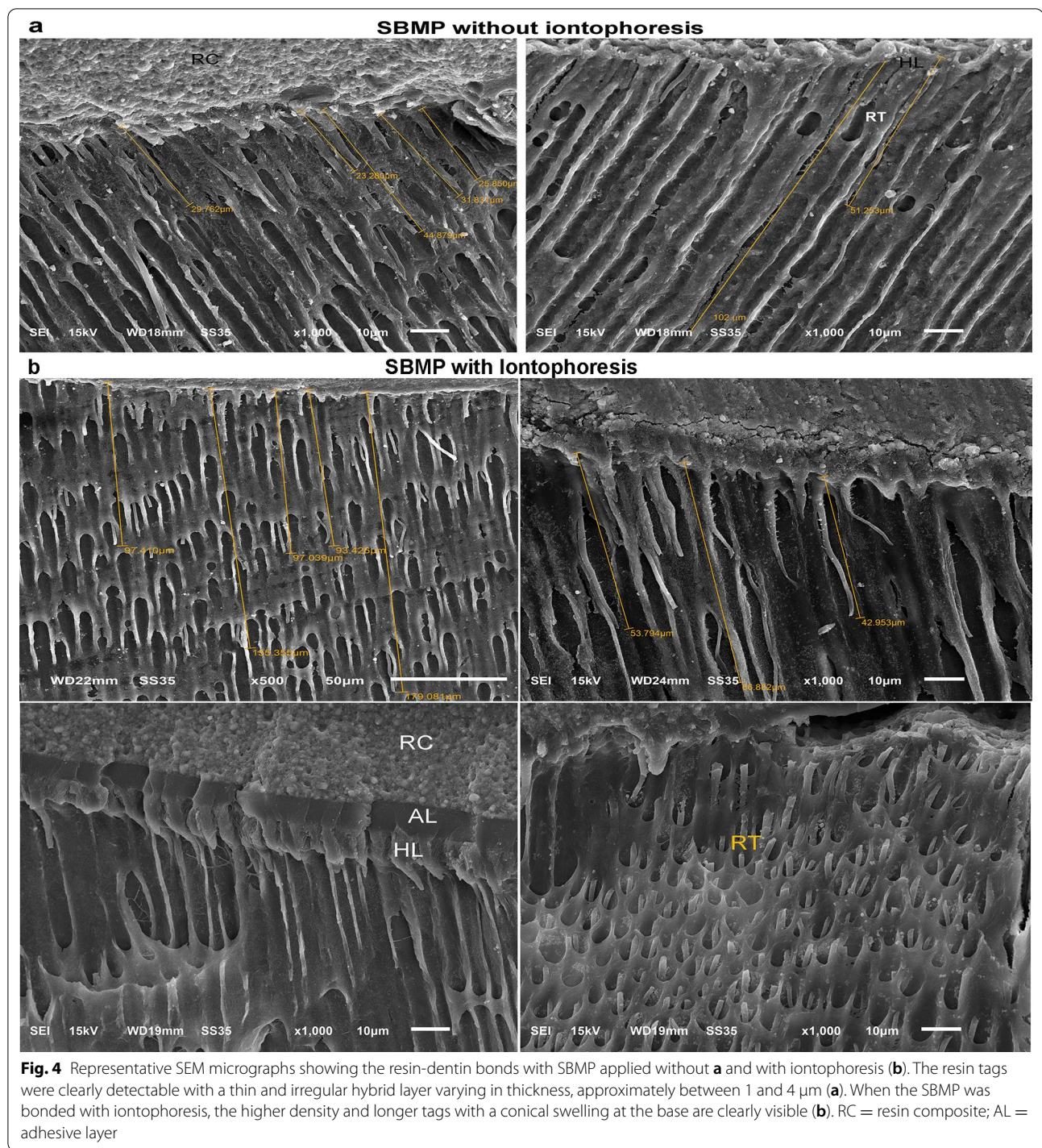
The means \pm SDs of the remaining dentine thicknesses obtained from all groups were not statistically significant differences (SE, 1.27 ± 0.37 mm; SEi, 1.2 ± 0.3 mm; SBMP,



1.1 ± 0.38 mm; SBMPi, 1.87 ± 0.34 mm; $P > 0.05$; one-way RM ANOVA).

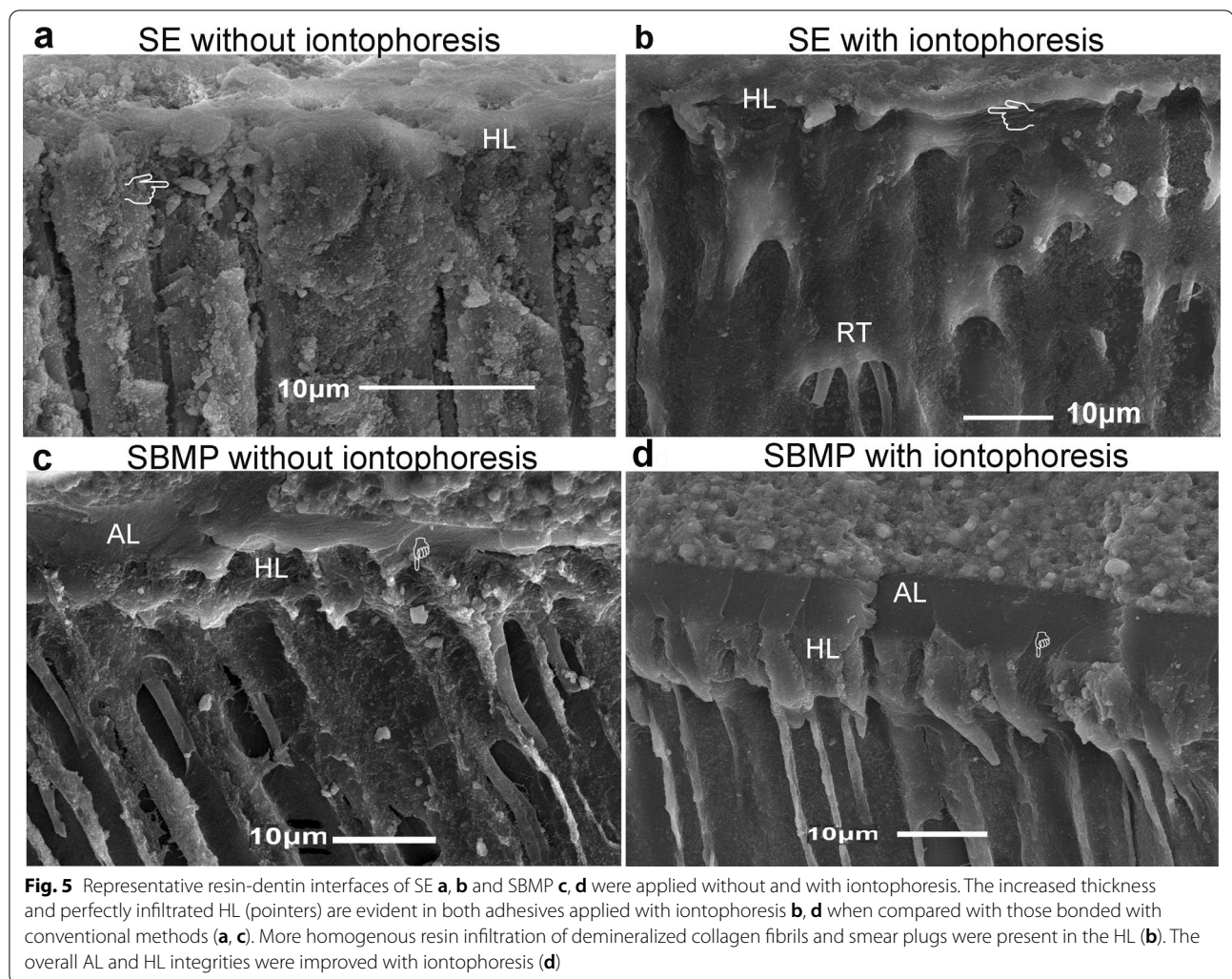
Representative SEM photomicrographs of bonded interfaces are shown in Fig. 3, 4, 5, and 6. Figure 3 is representative of resin-dentin bonds with SE applied without (Fig. 3a) and with iontophoresis (Fig. 3b). SE adhesives partially infiltrate no further than the smear layer, revealing a regional difference in morphology of the hybrid

layer (HL) and the rare evidence of resin tags (Figs. 3a and 5a). The bonded interface obtained with SE applied with iontophoresis has more condensed HL and tags up to 70 μm long with an increased accumulation of smear plugs inside the dentinal tubules (Figs. 3b and 5b). Resin tags are clearly detectable in the SBMP sample with partially infiltrated HL (Figs. 4a and 5c). For the SBMPi samples, the well-infiltrated HL (approximately 8 μm in



thickness), including the longer and greater numbers of tags up to 179 µm, have been observed. These tags typically show distinct conical enlargement at their bases (Figs. 4b and 5d), suggesting that SBMP applied with iontophoresis could fully envelop the deepest portion of the demineralized collagen fibrils within the HL.

The resin-enamel interfaces of SBMP samples are more detectable than those in SE. The representative SEM images of the SBMP applied without and with iontophoresis were demonstrated in Fig. 6. There are regional differences in the presence of the HL regardless of the SBMP's application mode. However, the



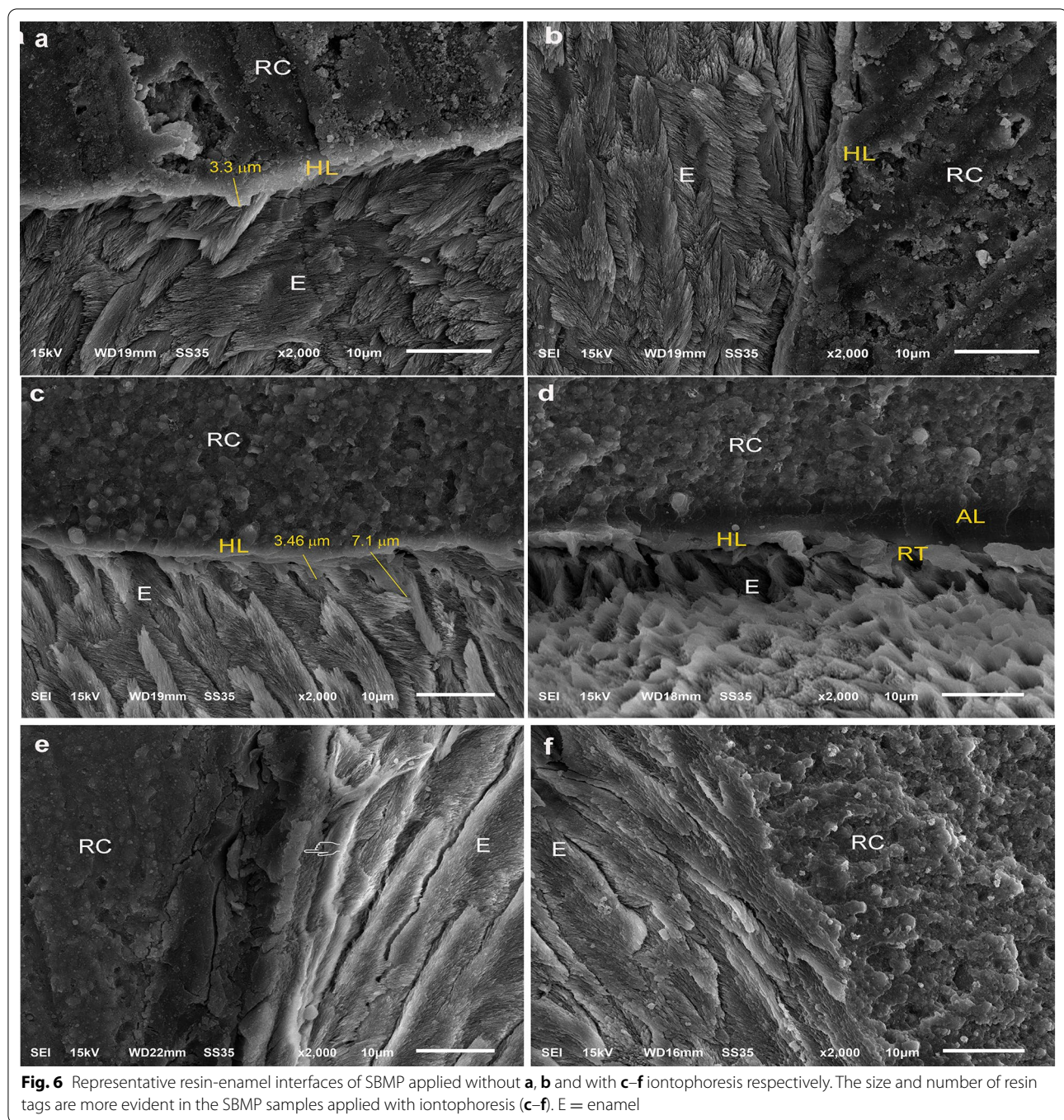
increases in size and number of resin tags, including the HL thickness, are more obvious in the SBMPi (Fig. 6c–f) than those in the SBMP samples (Fig. 6a, b).

Discussion

Under simulated pulpal pressure, the application of either three-step etch-and-rinse (SBMP) or two-step self-etch (SE) adhesives using the anodal iontophoresis significantly decreased the fluid flow after bond and composite restoration when compared with those applied according to the manufacturer's instructions without the electric current. The results well corresponded with the SEM analyses showing the optimal resin infiltration with a longer and greater number of resin tags obtained after applying electric current during the bond application of both adhesives. Considering the significant linear relationship between the permeabilities attained after bond and composite restoration, the slopes of the regression equations obtained after SBMP and SBMPi were lower

than those in SE and SEi samples (Fig. 2b), demonstrating a better sealing ability after composite restoration when using SBMP as adhesive. Furthermore, a slight decrease of the slopes found in both types of adhesives applied with iontophoresis indicates that the improved seal obtained after using the anodal current in the bonding step of both agents also facilitated the immediate reduction of the tooth permeability after composite restoration (Fig. 2b).

Although study designs, electric currents, and types of adhesives were different, this study was consistent with the previous results showing significant improvements in the bond strength and the bonding quality after applying self-etch [14–16] or two-step, etch-and-rinse adhesives [17–20] with the electric currents varying from 0 to 110 μ A [21]. The electric-current-assisted application of etch-and-rinse adhesive (Single Bond) also decreased microleakage scores of the class V cavities under simulated pulpal pressure [26], which corresponded to the



results of this study. However, the present data could evidently demonstrate the degree of flow rate changes following the crucial steps of restorative procedures, the applications of two different adhesive systems, and composite restoration under pulpal pressure. Such findings were well associated with the possible differences between the demineralization front and the adhesive interface, as revealed by the SEM images. Studies show

that the pulpal pressure in teeth in vivo caused increased permeability and decreased bond strength of the tooth-restoration bond [22, 23]. When the bonding of both adhesives with the anodal current of 75 μ A for 20 s was performed in the current study, the outward flow created during the applied pressure was paused, indicating the decrease in outward dentinal fluid movement into the dentinal tubules and therefore reducing its penetration

into polymerized hydrophilic monomer. Therefore, the electroosmotic flow resulting from the applied current could enhance the resin infiltration by decelerating the outward flow driven by the simulating pulpal pressure of 20 mm Hg across the dentin. This finding entirely agrees with the recent study showing that the direction of the electroosmotic flow with both polarities of applied current was from anode to cathode and that electroosmosis can deliver both uncharged and charged molecules through the dentin to the pulp [13, 24]. Furthermore, in a pilot study, it was found that the anodal current at the minimum of 250 μ A caused the inward flow from dentin into the pulp during the bond application, which this also consistent with the previous finding that the fluid flow rates through dentin produced by electroosmosis increased as the current intensity was increased regardless of the test solution's composition [24]. Since the higher current intensity (≥ 250 μ A) causing inward fluid flow could raise the possibility of the cytotoxicity of adhesives in vivo [30], the applied current in this study was set at approximately 75 μ A.

The outward flow rates obtained after bonding and restoration in the SE group were significantly increased, while these changes were not different in the SBMP samples, indicating the less ability of SE to prevent dentin fluid from permeating across the polymerized adhesive. The finding was in accord with the previous results also measuring the dentinal fluid flow during composite restoration [2, 31] and that by Carrilho et al. [32], showing the better sealing ability of the smear layer than adhesive resins. The possible explanations involved the presence of porosities with the low integrity of the bonded smear layer-cover dentin (Fig. 3a), allowing the fluid permeation, also reported in the literature [1, 23]. The increased fluid conductance across the tooth-restoration interface in the SE group could be explained by the fact that the initially weak bond strength of the SE to the cavity wall probably caused the higher degree of polymerization shrinkage stress of resin composite [33]. Moreover, it could be the result of complex interactions of several stimuli generated during the restorative procedure, including the structure and composition of the tooth, such as thermal expansion from the light sources [31].

Using smear layer-covered dentin as a reference condition in this study demonstrated the increased sealing efficacy of the tooth-restoration bond using both SBMP and SE applied with anodal iontophoresis under pulpal pressure. Even though the conventional application of SBMP generally performed better laboratory results than SE [3, 34], the increase in immediate bonding effectiveness produced by two different adhesive systems employed with anodal iontophoresis in this study suggested the comparable efficacy between SBMP and SE. Thus, the use of

simplified adhesive, SE, applied with iontophoresis is recommended to decrease the number of clinical steps with additional bonding effectiveness.

It appears that the prolonged application time was found to increase the immediate microtensile bond strength of two-step etch-and-rinse adhesive systems [35]. The limitation of this study was partly from the less application time of the bonding agents applied with the conventional methods compared to those with iontophoresis, requiring a duration of 20 s for conducting the electric current. In addition, the ultrastructure of the resin infiltration pattern should also be characterized using the transmission electron microscope [36, 37], as this would help confirm the bonding quality improved with iontophoresis. The fact that dentin is more complex, with high variables in the viscosities of dentinal fluid in vivo resulting in different degrees of wetter dentin than in vitro studies [38], could also affect the bonding efficacy for the clinical application. However, due to the simplicity of the clinical procedure and the safety of the minimum intensity of the electric current, in vivo use of anodal iontophoresis during bond application should be further investigated for possible use in dental practice.

Conclusions

Under simulated pulpal pressure, the anodal iontophoresis could cause a brief pause of the continuous outward fluid flow through the dentin, enhancing the resin infiltration at the adhesive/dentin interface of both three-step (SBMP) and two-step (SE) adhesives applied to the dentin as revealed by the SEM images. The process then contributed to the substantial reduction of the dentin permeability compared with the conventional methods. SBMP and SE, when applied with iontophoresis, produced a similar increase in the degree of sealing ability compared to that in conventional procedure.

Abbreviations

SBMP: Scotchbond Multi-Purpose; SE: Clearfil SE Bond; DC: Direct current; SBMPi: Scotchbond Multi-Purpose applied with iontophoresis; SEi: Clearfil SE Bond applied with iontophoresis; HEMA: (Hydroxyethyl)methacrylate; BisGMA: Bisphenol A glycidylmethacrylate; MDP: 10-Methacryloyloxydecyl dihydrogen phosphate; TEGDMA: Triethylene glycol dimethacrylate; SEM: Scanning electron microscopy; RDT: Remaining dentine thickness; RMANOVA: Repeated measures, analysis of variance; T1: Smear layer-covered dentin; T2: After adhesive; T3: After composite restoration; HL: Hybrid layer; RT: Resin tags; RC: Resin composite; AL: Adhesive layer; E: Enamel.

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Author contributions

OA contributed to design of the project, performed the experiments, analyzed the results, and prepared the manuscript. PS, PS, and SS conducted

the experiments and analyzed the results. KS assisted in the SEM studies. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All procedures performed and all treatments in these studies involving extracted human teeth were conducted strictly in full accordance with Declaration of Helsinki or comparable ethical standards. Informed consent was obtained from all subjects and/or their legal guardian(s). The experimental protocol was exempted from permission by the ethical committee of Strategic Wisdom and Research Institute, Srinakharinwirot University (SWUEC-321/2562X).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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