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ORIGINAL ARTICLE

Dentin infiltration ability of different classes of adhesive systems

Alina Langer · Nicoleta Ilie

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Abstract
Objectives This study evaluates the dentin infiltration ability of various types of adhesives and compares four classes of adhesive systems with regard to this property. The infiltration is determined quantitatively, characterized as tag length and ratio of infiltration, and qualitatively, characterized as homogeneity, regularity, and continuity of the resin tags.
Materials and methods Flat dentin surfaces from 140 halves of caries-free molars were bonded with four classes of adhesive systems. The adhesives (n=20) were labeled with rhodamine B isothiocyanate and applied on the occlusal dentin following the manufacturer's recommendations and were subsequently light cured, 20 s. Then a 2-mm thick composite layer was applied and light cured, 20 s. The specimens were stored in distilled water at 37°C, 24 h. Two slices were sectioned mesio–distally from each sample and were investigated with a confocal laser scanning microscope. The measurements were done at 0.5, 1.5, and 2.5 mm from the enamel–dentin junction. The data were analyzed by using analysis of variance and the general linear model.
Results The class of adhesive, the composition, and the dentin position were significant factors affecting the investigated parameters. The use of etch and rinse adhesives in comparison to self-etch adhesives provided the formation of longer, more homogeneous, very regularly distributed but mostly fractured tags.
Conclusions A comparison of adhesives confirmed that etch and rinse systems remain better in bond infiltration.
Clinical relevance While the importance of tags formation on bonding is still controversially discussed, adhesive systems with a high ratio of infiltration might better protect the tooth against microorganism contamination.

Keywords Adhesive systems · CLSM · Infiltration · Dentin penetration · Etch and rinse adhesives · Self-etch adhesives

Introduction

Nowadays available adhesives are classified according to the bonding strategies, as etch and rinse systems and self-etching systems [1]. The etch and rinse systems necessitate phosphoric acid etching and rinsing of enamel/dentin prior to applying multi-bottle or one bottle adhesives, whereas the self-etching systems contain acid monomers which can condition both enamel and dentin simultaneously, with no rinsing. [1]. The mechanism of modern adhesion is currently believed to be based on micromechanical interlocking rather than on primary chemical adhesion [2, 3]. Bonding to enamel has been demonstrated to be easy and durable [1] while bonding to dentin is far more challenging [1], due to its great morphological and physical variation (variable tubular structure, high organic content, and fluid flow) [4, 5]. The adhesion to tooth substrate implies an exchange process in which inorganic tooth material is replaced by synthetic resins [6–8]. This process consists of two phases: in the first phase, calcium phosphates are removed, exposing microporosities at both the enamel and dentin surfaces; the second phase is characterized by infiltration and subsequent in situ polymerization of resin within the created surface microporosities [7].

The resin infiltration into demineralized dentin permits formation of hybrid layers and resin tags thus producing micromechanical retention of the resin to the demineralized substrate [3, 9]. The bonding mechanism depends on the

A. Langer · N. Ilie (✉)
Department of Restorative Dentistry,
Dental School of the Ludwig-Maximilians-University,
Goethestr. 70,
80336 Munich, Germany
e-mail: nilie@dent.med.uni-muenchen.de

penetration of the primer and adhesive resin into the conditioned dentin surface in order to create micromechanical interlocking with the dentin collagen [4]. Special attention is given to the potential roles of both micromechanical and chemical bonding mechanisms through correlating morphologic and chemical interfacial characteristics of tooth–biomaterial interactions using diverse kinds of adhesives [7].

The contribution of the resin tags to the bond strength, relative to the role of the intertubular dentin, depends on the tested materials, on the orientation of the dentinal tubules, and on the dentin depth [9]. While the penetration of resin tags into dentinal tubules is believed to contribute little to the final bond strength, the adaptation to the inner tubule walls probably contributes significantly much more to bonding efficacy [3, 9, 10].

Hybridization by resin interdiffusion into the exposed dentinal collagen layer, combined with attachment of resin tags into the opened dentin tubules, appeared to be essential for a reliable dentin bonding [11]. The additional formation of an elastic bonding area as a polymerization shrinkage stress absorber and the use of a restorative composite apparently guaranteed an efficient clinical result [11]. However, the importance of the infiltration of adhesive resins into the acid-treated dentinal tubules remains uncertain. Current literature showed several contradictory interpretations regarding the formation of tags: some researchers found no correlation between bond strength and formation of resin tags [12, 13], while others appreciated that the resin tags may contribute about 30% to the total strength of the adhesive–dentin bond [3, 12, 14] or at least that the resin tags are a major factor influencing bond strength [15].

In accordance with the resin tags as an important factor for bond effectiveness, the morphology, length, and density of resin tags are used by some researchers for the qualitative and/or semiquantitative evaluation of the efficiency of adhesive systems [12, 16].

A twofold bonding mechanism (micromechanical and chemical bonding) is believed to be advantageous in terms of restoration durability. This mechanism has a micromechanical bonding component that may in particular provide resistance to abrupt debonding stress. The chemical interaction may result in bonds that better resist to hydrolytic breakdown and thus keep the restoration margins sealed for a longer period [17].

Regarding the clinical effectiveness in terms of durability of the dentin–adhesive interface compared with laboratory testing, functional laboratory tests such as bond strength measurements, microleakage evaluation, and marginal gap measurements are necessary to predict clinical behavior [11]. Clinically, three-step or two-step etch and rinse adhesives were shown to generate a durable dentin bonding if all cavity margins are located in the enamel. For cavities with margins ending in the dentin, the three-step etch and rinse adhesives are preferred [18].

The purpose of this study was to compare quantitatively (tag length and ratio of infiltration) and qualitatively (homogeneity, regularity, and continuity of resin tags) the resin infiltration of adhesives using a confocal laser scanning microscope (CLSM). Four classes of adhesives were compared: etch and rinse with three or two steps and self-etch with two or one step, comprising a total of 20 adhesives. Also investigated was the relationship between dentin infiltration parameters (tag length and ratio of infiltration) and adhesive types, solvent, and dentin position (where the measurements were done). The null hypothesis of the present study was that the class of adhesives system and the position of the dentinal tubules do not affect the resin infiltration quality.

Materials and methods

Seventy sound extracted human molars were selected by visual inspection. The teeth were stored in sodium azide to avoid microbial contamination and used within a maximum of 1 month after extraction. The position of the teeth in the mouth was determined, and the tooth side (vestibular, lingual or buccal), the mesio–distal line, and the occlusal coronary third were marked. The occlusal coronary third was cut with a low-speed diamond saw (Isomet, Buehler GmbH, Düsseldorf, Germany) under constant water cooling. The molars without the first coronary third were halved with the same machine on the mesio–distal line. The coronary part of each half was wet grinded (Wet-sharpen and Polishing system Leco VP 100, Leco of Instruments GmbH, Moenchengladbach, Germany) with sandpaper (LECO corporation, Grit 220) [19] to simulate the smear layer created by hand or rotary instruments during cavity preparation [20]. After grinding the surface, the primer of the three- and two-step etch and rinse adhesives was mixed with the fluorescence dye Nile blue chloride (NB) (Fluka ,USA) in a concentration of approximately 0.1%. The bond of the one-, two-, and three-step classes was mixed with the fluorescent dye rhodamine B isothiocyanate (RITC) (Polysciences Inc., Warrington, PA, USA) in a concentration of approximately 0.1%. The selected adhesives (bond and primer) were applied as described in Tables 1 and 2.

For the etch and rinse adhesives (Table 1), the phosphoric acid was applied on the dentin for 15 s, followed by water spraying and air drying. For the two-step self-etch adhesives (Table 2), the liquid A was mixed with liquid B prior to be applied.

For Syntac, the Heliobond was also mixed with the dye NB and applied as described in Table 1. The primer was colored only to visualize the primer's infiltration. There was no clear difference between the infiltration of the primer (NB) and the infiltration of the adhesive (RITC), thus for qualitative and quantitative analyses of the tags only the labeled adhesive was used.

Table 1 Etch and rinse adhesive systems

Group	Adhesive, manufacturer and batch number	Composition	Application
3 steps	Syntac (Ivoclar Vivadent); Total Etch K14609; Primer K08247, Bond K02656; Heliobond K01560	Total Etch (37% phosphoric acid)	Dentin was etched for 15 s, rinsed with water, and air burst
		Glutaraldehyde 5%, maleic acid, PEGDMA 20–40%, water	Primer was applied, rubbed for 15 s and gently air burst to disperse the excess
		TEGDMA 25%, maleic acid 4%, acetone, water	Adhesive was applied and rubbed for 10 s, then air burst.
		Bis-GMA, dimethacrylate, initiators and stabilizers	Heliobond was applied, gently air burst and light cured for 20 s
	Solobond Plus (Voco); Total Etch 791595; Primer 0803002; Bond 0803002	34.5% phosphoric acid	Dentin was etched for 15 s, rinsed with water, and air burst
		Maleic acid, water, acetone, natrium fluoride	Primer was applied on dentin, rubbed for 30 s, and gently air burst to remove the excess
		Methacrylic carbon acid ester, 2-HEMA, Bis-GMA, camphorquinone, BHT, acetone	Adhesive was applied and rubbed for 15 s then, light cured for 20 s
2 steps	Solobond M (Voco); Total Etch 791595; Bond 792382	34.5% phosphoric acid	Dentin was etched for 15 s, rinsed with water, and air burst
		Methacrylate phosphoric acid esters, HEMA, Bis-GMA, natrium fluoride, camphorquinone, butylhydroxytoluol, acetone	Adhesive was applied and rubbed for 15 s, air burst for 15 s, and light cured for 20 s
	Excite (Ivoclar Vivadent); Total Etch K14609; Bond K01754	Total Etch (37% phosphoric acid)	Dentin was etched for 15 s, rinsed with water, and air burst
		Phosphoric acid acrylate, HEMA, Bis-GMA, dimethacrylate nanofillers SiO2 (0.5%), ethanol, initiators, stabilizers	Adhesive was applied and rubbed for 10 s, air burst, and light cured for 20 s
	XP Bond (Dentsply); Total Etch (De Trey Conditioner); 0609001329; Bond 0609001329	36% phosphoric acid	Dentin was etched for 15 s, rinsed with water, and air burst
		PENTA, TCB, UDMA, TEGDMA, HEMA, initiators, stabilizers, tertiary butanol, nanofillers	Adhesive was applied and rubbed for 20 s, then gently air burst, and light cured for 20 s
	Prime & Bond NT (Dentsply); Total Etch (De Trey Conditioner); 0609001329; Bond 0705002648	36% phosphoric acid	Dentin was etched for 15 s, rinsed with water, and air burst
		Di- and trimethacrylate, amorphous functionalized silica, PENTA, photoinitiators, stabilizers, cetylaminehydrofluoride, acetone	Adhesive was applied and rubbed for 20 s, then air burst for 5 s, and light cured for 20 s

PENTA dipentaerytrithol-penta-acrylate-monophosphate, *UDMA* urethane dimethacrylate, *TEGDMA* triethylene glycol dimethacrylate, *HEMA* hydroxyethyl methacrylate, *Bis-GMA* bisphenol-a-glycidyldimethacrylate, *BHT* butylhydroxytoluol

At the end of each application process, all adhesives were cured for 20 s with the LED unit Freelight 2 (3 M ESPE, Seefeld, Germany, 1,100 mW/cm^2). Information on the 20 materials used in this study is presented in Tables 1 and 2.

At the end of the preparation, a 2-mm thick composite layer (Saremco Dental, B2, St. Gallen, Switzerland) was applied and light cured for 20 s. The specimens were stored in distilled water at 37°C for 24 h. Two slices of 300 μm were sectioned mesio–distally from each sample with a microtome saw (Leica SP 1600, Leica Mikrosystems Vertrieb GmbH, Bensheim, Germany) with a constant water coolant from the first cutting edge to the edge of the teeth. The slices were then wet polished with a series of sandpaper (1,200 and 2,500 grit) on an automatic polishing device (Wet-sharpen and Polishing system Leco VP 100, Leco of Instruments GmbH, Moenchengladbach, Germany). The slices were kept humid during the study minimizing the effects of drying and/or shrinkage. The sample preparation of molars is described in Fig. 1.

CLSM examination

The measurements of dentin infiltration were done at distances of 0.5 (in the superficial dentin), 1.5 (in the middle dentin), and 2.5 (in the deep dentin) mm from the enamel–

Table 2 Self-etch adhesive systems

Group	Adhesive, manufacturer and LOT	Composition	Application
2 steps	Silorane System (3 M ESPE); Primer 292319; Bond 292274	Phosphorylated methacrylates, Vitrebond™ copolymer, Bis-GMA, HEMA, water, ethanol, silane-treated silica filler, initiators, and stabilizers	Primer was applied and rubbed for 15 s, then gently air dried, and light cured for 10 s
		hydrophobic dimethacrylate, phosphorylated methacrylates, TEGDMA, silane-treated silica filler (6%), initiators, and stabilizers	Adhesive was applied, gently air burst, and light cured for 20 s
	Clearfil SE Bond (Kuraray); Primer 41586; Bond 41586	MDP, HEMA, hydrophilic DMA, water, camphorquinone, N, N-diethanol-p-toluidine, initiators	Primer was applied and rubbed for 20 s and then air burst
		Bis-GMA, HEMA, MDP, hydrophobic DMA, camphorquinone, N, N-diethanol-p-toluidine, silanized colloidal silica, initiators	Adhesive was applied, gently air burst, and light cured for 20 s
	Adper™ Scotchbond™ SE (3 M ESPE); Liquid A 7AH; Liquid B 7AG	2-HEMA, rose bengal colorant, water	Liquid A was applied
		Diurethandimethacrylate, D,L-camphorquinone, TEGDMA, dimethylaminoethyl benzoate, mono-HEMA phosphate, di-HEMA phosphate, tri-HEMA phosphate, hexandiol dimethacrylate, phosphoric acid methacryloxy-hexyl esters, propylidine trimethyl trimethacrylate, pyrophosphate methacrylate, nanofillers (15-25%)	Liquid B was applied and rubbed 20 s, air burst for 10 s, and light cured for 20 s
	Adhe SE (Ivoclar Vivdent); Primer JO6075; Bond K03345	Phosphoric acid acrylate, bis-acrylamide, water, initiators and stabilizers	Primer was applied for 15 s and rubbed for 15 s, then air burst to remove the excess
		Dimethacrylate hydroxylethylmethacrylate, highly dispersed silicon dioxide, initiators and stabilizers	Adhesive was applied, gently air burst, and light cured for 20 s
1 step	Futurabond NR (Voco); Liquid A 761101; Liquid B 761102	HEMA, water, ethanol, highly dispersed silicon dioxide, butylhydroxytoluol (BHT) ethanol, water, fluoride, hydrophilic adhesive monomers	1 drop liquid A was mixed with 1 drop liquid B and applied, rubbed for 20 s, then air burst for 5 s and light cured for 20 s
	XenoIII (Dentsply); Liquid A 0608002341; Liquid B 0608002669	HEMA, water, ethanol, BHT, highly dispersed SiO2 Pyro-EMA, PEM-F, UDMA, BHT, camphorquinone, EPD	1 drop liquid A was mixed with 1 drop liquid B for 5 s, applied and rubbed for 20 s, then gently air burst, and light cured for 20 s
	iBond (Heraeus Kulzer); Bond 010043	UDMA, MMA, 4-META, glutaraldehyde, acetone, water	Adhesive was applied and rubbed 20 s, air burst, and light cured for 20 s
	XenoV (Dentsply); Bond 0704000721	Bifunctional acrylate, acid acrylate, phosphoric acid ester, water, tertiary butanol, initiators (camphorchinone), stabilizers	Adhesive was applied and rubbed for 20 s, air burst for 5 s, and light cured for 20 s
	Hybrid Bond (Sun Medical Co., Japan); Bond LF2	UDMA, MMA,4-META, polymerizations initiators, acetone, water	Adhesive was applied and rubbed for 20 s, air burst, and light cured for 20 s
	Futurabond DC (Voco); Liquid 1 791595; Liquid 2 792382	Methacryl phosphoric acid esters, HEMA, Bis-GMA, natrium fluoride, camphorquinone, butylhydroxytoluol, acetone Bis-GMA, HEMA,TMPTMA, camphorquinone, BHT, catalysts, fluoride, ethanol	1 drop liquid 1 was mixed with 1 drop liquid 2, applied on dentin and rubbed for 20 s, then air burst for 5 s, and light cured for 20 s
	Bond Force (Tokuyama); Bond YT 20177	C2-4 alkyl, Methacryloyloxyalkyl acid phosphate, HEMA, Bis-GMA, TEGDMA, camphorquinone, water	Adhesive was applied and rubbed for 20 s, air burst for 5 s, and light cured for 20 s
	AQ Bond (Sun Medical Co., Japan); Bond SE 2	UDMA, MMA,4-META, HEMA, polymerizations initiator, acetone, water AQ sponge: polyurethane foam, initiators (pTSNa)	AQ sponge was soaked on adhesive, then the adhesive was applied, gently air burst for 5 s, and light cured for 20 s

Table 2 (continued)

Group	Adhesive, manufacturer and LOT	Composition	Application
	Adper™Easy Bond (3 M ESPE); Bond 302051	HEMA, BIS-GMA, methacrylated phosphoresters, hexandiol-1.6 dimethacrylate, Vitrebond™ Copolymer, silicon fillers (7 nm), ethanol, water, initiators	Pack was activated, the adhesive was applied and rubbed for 20 s, gently air burst for 5 s, and light cured for 20 s
	Adper Prompt L-Pop (3 M ESPE); Bond 2554565	Water, 10% HEMA, Bis-GMA, methacrylated phosphoric acid esters, photoinitiators, stabilizer	Pack was activated, the adhesive was applied and rubbed for 15 s, gently air burst for 5 s, and cured for 20 s

UDMA urethane dimethacrylate, *TEGDMA* triethylene glycol dimethacrylate, *HEMA* hydroxyethyl methacrylate, *Bis-GMA* bisphenol-a-glycidyldimethacrylate, *MMA* methyl methacrylate, *4-META* 4-methacryloxyethyl trimellitate anhydride, *TMPTMA* trimethylolpropane triacrylate, *BHT* butylhydroxytoluol, pyro-EMA phosphoric acid modified methacrylate, *PEM-F* monofluorophosphazene-modified methacrylate

dentin junction (DEJ) using CLSM (Confocal Laser Scanning Microscope LSM 510, Zeiss, Jena, Germany) equipped with a water immersion objective (Achroplan ×63/0.95 W). The layer was observed at 10.2 µm under the polished sample surface. The image size was 150×150 µm with a resolution of 2,656×2,924 pixels. The image analysis (that is, the quantitative and the qualitative analyses) and 3D reconstruction were carried out using the CLSM Image Browser 4.6 (Carl Zeiss). The slices were scanned with "multitracking", allowing the simultaneous evaluation of labeled adhesive and the density of the dentinal tubules. For that purpose, the CLSM provides separate colored channels that can be configured for simultaneous detection of light with different wavelength ranges. To visualize the RITC-labeled adhesive, a laser excitation wavelength of 488 nm was used. The emitted fluorescent light was detected on the first channel, using a HFT 405/488 beam splitter and a band pass filter (BP 530–600 nm) that blocked all wavelengths outside the fluorescence wavelength range. To count all dentinal tubules that are not infiltrated by the labeled adhesive, a second channel with a neutral beam splitter (NT 80/20) and a long pass filter (LP 420 nm) was added, and the dentinal tubules were then visualized in the reflection mode (excitation wavelength, 488 nm) of the CLSM. The counting of dentinal tubules and filled dentinal tubules was performed on images taken from an area of 10.2×100 µm.

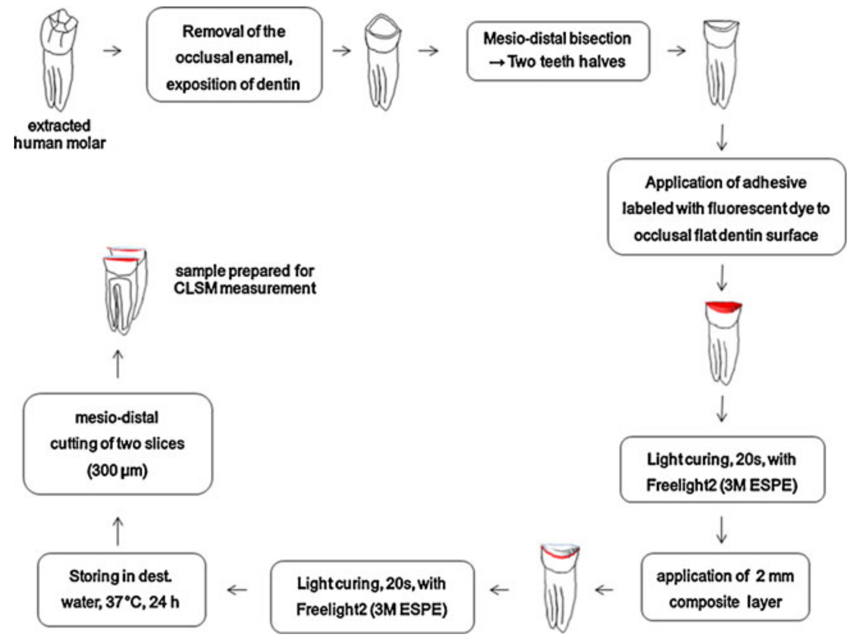
The quantitative penetration was estimated with two parameters:

1. Ratio of infiltration, calculated as the proportion of filled tags (colored) reported to the total amount of dentinal tubule (white) (Figs. 2 and 3).
2. Tag length, measured as the distance between the end of filled dentin tubule and the adhesive surface.

The qualitative penetration was estimated with three criteria:

1. Homogeneity describes the difference in the length of the tags. Following scores were used: 0 = all tags have the same length, 1 = more than 50% have the same length, 2 = less than 50% have the same length, and 3 = all tags have different lengths.
2. Regularity describes the uniformity of tag distribution. Following scores were used: 0 = all tags are uniformly distributed over the image area, 1 = more than 50% of the image area has tags, 2 = less than 50 % of the image area has tags, and 3 = no tags found.
3. Continuity describes whether the tags are interrupted or not. Following scores are used: 0 = 100% of the tags are uninterrupted, 1 = more than 50% of the tags are uninterrupted, 2 = less than 50% of the tags are uninterrupted, and 3 = all tags are interrupted.

Fig. 1 Sample preparation



Statistical analysis

The one-way analysis of variance ($p<0.05$) was applied to verify the statistical significance of the differences in the quantitative and qualitative infiltration parameters among the tested adhesives and also among the adhesives category. The Tukey's test was then used for post hoc comparison. The influence of the parameter adhesive type, dentin position, and solvent was assessed in a general linear model ($p=0.05$). A Pearson correlation analysis was used to assess the relationship between tag lengths and ratio of infiltration.

Results

All adhesives tested showed resin tags formation. The results regarding the quantitative and qualitative parameters of every tested adhesive and of the four classes of adhesive systems are summarized in Tables 3, 4, 5, 6, and 7.

Results regarding quantitative infiltration

The ratio of infiltration measured for AdheSE (91.8%), Solobond Plus (91.7%), XP Bond (90.6%), Prime&Bond NT (90.5%), and Solobond M (90.2%) was found to be the significantly highest of all adhesives. In contrast, the resin tags for Bond Force (7.3 µm), AdheSE (9.0 µm), Silorane System (9.1 µm), and AQ Bond (9.2 µm) were found to be significantly shorter compared to the other materials (Table 3). The Silorane System recorded the lowest ratio of infiltration (59.8%) with short (9.1 µm) resin tags (Table 3).

The materials were divided into four groups according to the adhesive system they belong to: three-step etch and rinse, two-step etch and rinse, two-step self-etch, and one-

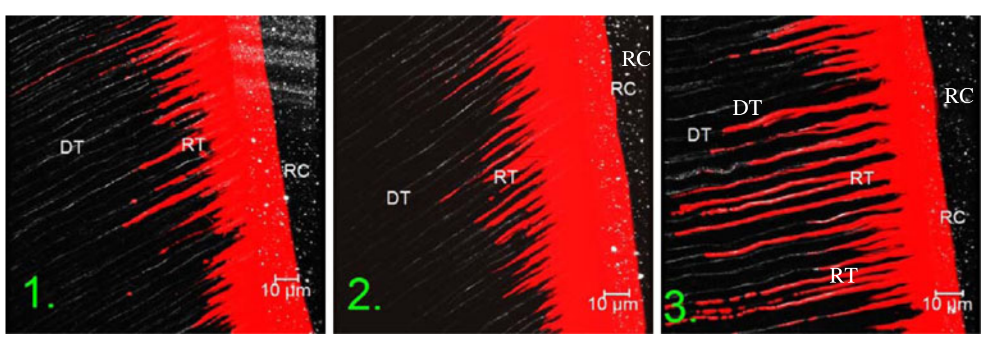


Fig. 2 CLSM images of the resin tags formed by XP Bond (two-step etch and rinse adhesive) into dentinal tubules in fluorescence mode: *DT* dentinal tubules, *RT* resin tags, *RC* resin composite. *1.* Penetration of the adhesive on first position at 0.5 mm from enamel–dentin junction (*1*); the tags are not homogeneous, very regularly distributed, and with many breaks. *2.* Penetration of the adhesive on second position at 1.5 mm from enamel–dentin junction (*2*); the tags are not homogeneous, less frequently interrupted, and very regularly distributed. *3.* Penetration of the adhesive on third position at 2.5 mm from enamel–dentin junction (*3*); the tags are longer and more homogeneous, less frequently interrupted, and very regularly distributed

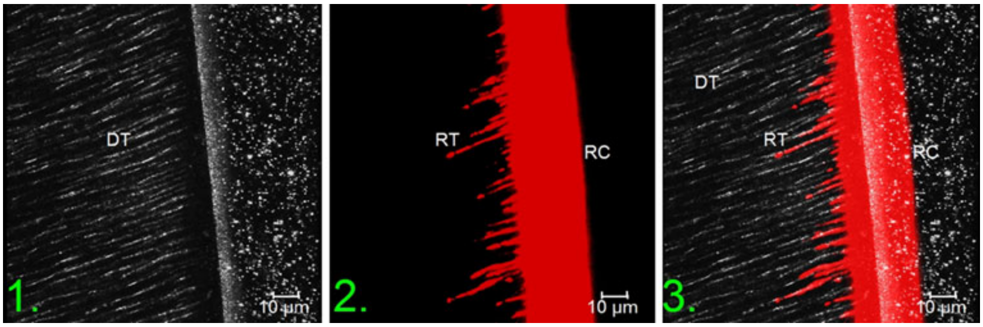


Fig. 3 CLSM images of the resin tags formed by Bond Force (one-step self-etch adhesive) into dentinal tubules in fluorescence mode: *DT* dentinal tubules, *RT* resin tags, *RC* resin composite. The tags are short, regularly distributed, and with breaks. *1.* Dentinal tubules appear *white* in reflection mode. *2.* Dentinal tags in fluorescence mode. *3.* Dentin tags and dentinal tubules in fluorescence and reflection mode

step self-etch. The highest ratio of infiltration was observed for the two-step etch and rinse adhesives (group 1) followed by the three-step etch and rinse (group 2), then the two-step self-etch (group 3), and finally the one-step self-etch (group 4). The three-step etch and rinse materials (group 2) showed the longest tags followed by the two-step etch and rinse (group 1) and the self-etch adhesives (groups 3 and 4). No significant difference in tag length was found between groups 3 and 4 (Table 4).

The type of adhesive was a statistically significant factor for both infiltration parameters—tag length ($p<0.0001$) and ratio of infiltration ($p<0.0001$). Similar was valid also for the solvent ($p<0.0001$ for both tag length and ratio of infiltration) and the dentin position where the measurements were done ($p=0.005$, for tag length and $p<0.0001$, for ratio of infiltration). The dentin position have a significant influence on the tag length and ratio of infiltration for almost all tested adhesive (Table 6) and adhesive classes (Table 7).

Results regarding qualitative infiltration

The resin tags formed by the three and two-step etch and rinse adhesives (Table 5) are more homogeneous (31.3% and 30.2% score 0) than the resin tags formed by the two-step self-etch adhesives (mostly score 1). While the difference between three- and two-step etch and rinse adhesives in tag homogeneity was not significant, the number of steps needed for the self-etch materials was an important factor

Table 3 Ratio of infiltration and resin tags length

Adhesive	Adhesive type	Ratio of infiltration [%]	Tags length [µm]
AdheSE	2-step self-etch	91.8K (8.9)	9.0A, B (6.2)
Solobond Plus	3-step etch and rinse	91.7K (8.8)	19.5I (11.5)
XP Bond	2-step etch and rinse	90.6J, K (8.4)	20.3I (12.0)
Prime&Bond NT	2-step etch and rinse	90.5J, K (10.7)	14.5G, H (10.2)
Solobond M	2-step etch and rinse	90.2J, K (10.4)	12.8E, F G (10.5)
Excite	2-step etch and rinse	87.9I, J (9.9)	10.5B, C, D (6.39)
Clearfil SE Bond	2-step self-etch	86.9I (12.4)	13.2F, G, H (7.8)
AQ Bond	One-step self-etch	86.6I, J (11.4)	9.2A, B, C (9.6)
AdperPrompt L-Pop	One-step self-etch	86.5I (13.3)	12.3D, E, F (12.7)
Futurabond NR	One-step self-etch	80.4H (17.7)	10.0B, C (8.3)
Syntac	3-step etch and rinse	78.5G, H (16.4)	20.8I (15.6)
Futurabond DC	One-step self-etch	78.5G, H (11.5)	14.9H (14.1)
Adper Easy Bond	One-step self-etch	78.1F, G, H (21.8)	11.0C, D, E, F (7.5)
Hybrid Bond	One-step self-etch	77.1F, G (15.8)	10.4B, C, D (6.9)
Adper Scotchbond SE	2-step self-etch	75.5E, F (17.5)	14.4G, H (11.6)
Bond Force	One-step self-etch	73.9D, E (14.0)	7.3A (5.0)
iBond	One-step self-etch	71.3C, D (19.8)	12.6E, F, G (8.2)
Xeno V	One-step self-etch	69.6B, C (20.1)	11.1C, D, E (10.1)
Xeno III	One-step self-etch	67.6B (18.9)	14.6G, H (15.6)
Silorane System	2-step self-etch	59.8A (15.9)	9.1A, B, C (6.1)

Same letters indicate statistically homogeneous subgroups (Tukey's HSD test, $\alpha=0.05$). Adhesives are presented in descending order of the infiltration rate

Table 4 Ratio of infiltration and resin tags length as a function of adhesive type are presented in mean values and standard deviation

No	Groups	Ratio of infiltration [%]	Tag length [μm]
1	2-step etch and rinse	89.9D (9.9)	14.8b (12.4)
2	3-step etch and rinse	85.7C (9.3)	20.1c (13.5)
3	2-step self-etch	81.0B (17.7)	11.6a (8.6)
4	One-step self-etch	78.1A (17.7)	11.2a (10.4)

Same letters indicate statistically homogeneous subgroup (Tukey's HSD test, α=0.05)

for this criterion. The number of steps within the etch and rinse materials (three or two steps) was not a significant factor for tag homogeneity since the percentile differences between scores was only about 1%, but was a significant factor within the self-etch materials (19.1% score 0 in the two-step self-etch group compared with 37.2% score 0 for one-step self-etch adhesives). The best homogeneity of tags showed the one-step self-etch materials with 37.2% score 0. Regarding regularity, the resin tags of the three-step and two-step etch and rinse adhesives were very regularly distributed on the tested dentin surfaces (84.8% and 91.7% score 0) followed by two-step (74.5% score 0) and one-step self-etch adhesives (62.5% score 0).

Regarding the criterion continuity, the three-step and two-step etch and rinse adhesives showed the most interrupted resin tags (12.4% score 3 and 3.8% score 3), followed by the two-step and the one-step self-etch adhesives (2.6% score 3 and 17.1% score 3). Significant differences between the two versions of etch and rinse (groups 1 and 2) and the two versions of self-etch (groups 3 and 4) materials could be detected.

The adhesive type (etch and rinse or self-etch) was a significant factor for homogeneity (p=0.006), regularity (p=0.007), and continuity of resin tags (p=0.005). The dentin position was a statistically significant factor for two parameter of the qualitative penetration (homogeneity, p= 0.003 and continuity, p=0.004), whereas the solvent showed a statistical influence only on tags regularity (p=0.001).

There was a significant but very poor correlation between tag length and ratio of infiltration (Pearson correlation coefficient = 0.04) when all measured adhesives were considered. Similar was valid also within one adhesive class, with the three-step etch and rinse adhesives reaching the highest correlation value (0.14).

Discussion

The CLSM method permits more detailed information regarding the infiltration and the distribution of adhesive than for instance the scanning electronic microscopy (SEM) did [21]. The advantage of CLSM involves a non-destructive examination [22]. Layers up to 100 μm below the surface can be visualized, depending however on substrate, while the SEM method shows only details of the surface. Additionally, an important source of artifacts is eliminated by using the CLSM, due to the fact that the samples do not need to be dried [22].

The incorporation of dyes in the adhesive components (primer or bonding) is based on a simple mixing process but the risk of nonhomogeneous dye distribution cannot be excluded [21, 23, 24]. On the other hand, the used dye RITC is extremely soluble in organic solutions [22] and is also stable under different pH conditions [25]. The fluorescence of the recorded CLSM images was uniform, thus allowing to assume a homogeneous distribution of the dye.

In our study, the adhesive with ideal resin penetration was considered as an adhesive that achieves high values in both quantitative parameters—ratio of infiltration and tag length. According to this definition, Solobond Plus (etch and rinse) which had almost the highest values in both parameters shows nearly ideal resin penetration.

Several studies [4, 17, 21, 26–29] showed that, regarding bonding effectiveness, etch and rinse adhesives performed better than self-etch adhesives. In general, in the same adhesive class, the version three-step etch and rinse performed better than two-step etch and rinse [26, 27], and the version two-step self-etch performed better than the one-step self-etch [17, 26, 28]. The superior bonding

Table 5 Qualitative evaluation of dentin penetration characterized by homogeneity, regularity, and continuity

Adhesive Type	Homogeneity				Regularity				Continuity			
	Score %				Score %				Score %			
	0	1	2	3	0	1	2	3	0	1	2	3
2 step self-etch	19.0	79.1	1.9	0	68.2	25.0	6.8	0	37.2	35.0	25.2	2.6
2-step etch and rinse	31.4	64.4	4.2	0	91.7	8.2	0.1	0	46.6	31.9	17.7	3.8
3-step etch and rinse	31.3	65.2	3.5	0	84.8	14.7	0.5	0	38.9	27.6	21.1	12.4
One step self-etch	37.2	57.9	4.9	0	62.5	31.1	6.3	0	33.4	31.6	17.9	17.1

Table 6 Influence of dentin position (distance from the dentin–enamel junction) on tag length and ratio of infiltration for every tested adhesive

Adhesive	Tag length [μm]			Ratio of infiltration [%]		
	0.5 mm	1.5 mm	2.5 mm	0.5 mm	1.5 mm	2.5 mm
Syntac	18.3A (11.7)	22.1A, B (19.4)	21.1A (14.0)	85.6a (16.9)	80.2b (15.01)	69.5c (12.8)
Adper Prompt L-Pop	12.1A (14.4)	12.8A (13.3)	11.9A (10.1)	89.4a (10.1)	88.9a (11.3)	81.3b (15.9)
Xeno III	17.6A (20.2)	15.0A (16.2)	12.0A, B (9.3)	67.9a (21.7)	68.2a (21.5)	68.2a (21.5)
Xeno V	13.6A (12.8)	9.9B (7.9)	10.6B (9.6)	60.3a (15.6)	75.8b (17.5)	70.1c (22.7)
Futurabond NR	10.8A (9.1)	9.9A (7.7)	9.2A (7.9)	85.4a (14.3)	78.2b (16.7)	77.4b (20.3)
Silorane System	8.3A (6.4)	10.8B (6.9)	8.3A (4.8)	62.8a (14.8)	55.9b (14.8)	60.6a (8.3)
iBond	12.2A (6.6)	13.56A (10.1)	11.9A (6.9)	75.4a (21.4)	76.5a (19.5)	61.0b (13.0)
Hybrid Bond	9.8A (6.7)	9.7A (5.5)	11.6B (8.0)	75.3a (17.7)	78.6a (14.2)	77.2a (15.6)
Excite	11.3A (7.2)	9.7A (6.0)	10.5B (5.7)	88.1a (10.0)	88.0a (11.1)	87.7a (8.72)
Solobond Plus	19.9A (10.6)	21.5A (12.6)	17.3B (10.7)	94.3a (7.0)	92.5a (9.5)	88.5b (8.71)
Solobond M	14.0A (10.9)	13.8A (12.7)	10.8B (6.7)	85.5a (11.6)	93.0b (8.9)	91.0b (9.8)
Futurabond DC	17.9A (16.9)	13.3B (11.8)	14.0B (13.3)	80.8a (13.6)	78.9a (10.2)	76.1b (10.2)
Prime&Bond NT	12.9A (7.3)	15.4A (11.5)	14.7A (10.5)	90.2a (13.8)	93.6b (10.6)	88.0c (8.04)
Clearfil SE Bond	13.2A (7.3)	12.6A (7.7)	13.7B (8.2)	94.7a (9.9)	86.9b (11.4)	80.5c (11.6)
XP Bond	21.5A (6.7)	16.4B (11.1)	23.1A (19.6)	95.6a (4.9)	91.7b (6.6)	85.4c (9.3)
AQ Bond	8.3A (8.7)	8.2A (6.6)	10.9B (12.2)	85.5a (8.7)	92.9b (10.9)	86.8a (7.9)
Scotchbond SE	12.7A (12.3)	16.5B (12.5)	13.9A (9.8)	81.7a (12.7)	74.6b (15.6)	71.0b (15.6)
Adhe SE	7.8A (5.1)	9.3B (5.6)	9.6B (7.1)	95.2a (6.7)	89.4b (10.6)	91.4c (8.0)
Bond Force	7.6A (4.9)	7.4A (5.0)	6.8A (4.9)	74.9a (14.8)	72.7a (13.2)	74.2a (13.9)
Adper Easy Bond	11.2A (7.4)	11.5B (7.8)	10.2B (7.1)	76.5a (14.8)	80.1b (20.1)	77.4b (24.5)

Same letters indicate statistically homogeneous subgroups within one adhesive (Tukey's HSD test, α=0.05)

effectiveness of etch and rinse adhesives is attributed to the acid etching step [30]. The removal of smear layer and smear plugs favored the development of a thicker hybrid layer, increased the dentin permeability, thus allowing for deeper resin infiltration into dentin tubules [3, 4, 30]. The formed tags will be slimmer, more distinct, and longer than the tags formed by using self-etch adhesives. Several studies [1, 4, 28] showed that self-etch adhesives demineralize dentin only partially and create a hybrid layer still containing hydroxyapatite crystals. However, when the dentin was etched, the hybrid layer was completely devoid of hydroxyapatite and very good distinct shape was formed [28]. In accordance with another study [21], our study confirmed that the type of adhesive system significantly affected the resin tag length: the tags formed with the tested etch and rinse adhesives were longer than those bonded with self-etch adhesives (Table 4).

Tags can also indirectly indicate how deeply a substrate was etched. Creating a deeper etching pattern assures a better resin penetration [31]. Accordingly, the etch and rinse adhesives etch deeper in the substrate than the self-etch adhesives, resulting in longer tags, as confirmed by our results.

The removal of the smear layer and smear plugs with acid solutions results in an increase of the fluid flow on the exposed dentin surface [5]. The outflow of tubular fluid in

Table 7 Influence of dentin position on tag length and ratio of infiltration for every class of adhesive

Type of adhesive	Tag length [μm]			Ratio of infiltration [%]		
	0.5 mm	1.5 mm	2.5 mm	0.5 mm	1.5 mm	2.5 mm
3 step etch and rinse	19.2A (11.2)	22.1B (16.1)	18.9B (12.3)	90.2a (13.3)	86.9a (13.8)	80.3a (14.2)
2 step etch and rinse	15.1A (12.7)	14.1B (11.0)	15.8A (13.2)	90.2a (10.9)	91.8b (9.6)	87.9c (9.1)
2 step self-etch	10.7A (8.62)	12.3A (8.9)	11.6A (8.2)	85.9a (18.0)	79.6b (17.5)	78.4c (16.8)
One step self-etch	11.8A (11.8)	10.9B (9.8)	10.8B (9.5)	78.6a (18.1)	79.9b (16.5)	76.0b (18.3)

Same letters indicate statistically homogeneous subgroups within one adhesive class (Tukey's HSD test, α=0.05) vital teeth may prevent penetration of the tubules by the bonding agent since hydrophobic resins do not adhere to hydrophilic substrates, even if resin tags are formed in the dentinal tubules. The presence of the smear layer is considered to be the major factor controlling the dentin permeability [4, 5].

The current study showed that both quantitative parameters measured for the Silorane System Adhesive (two-step self-etch) were lower compared to those of one-step self-etch adhesives. As concluded in the previous study [32], the reason for this behavior is the Silorane primer, containing etching monomers which have to be cured prior to the application of the Silorane bond.

It was previously shown that the removal of the smear layer when self-etch adhesives are used is dependent on the pH of the acidic primer [26, 33]. The self-etch systems investigated in the present study have different degrees of acidity: Bond Force (one-step self-etch) with a pH of 2.3 [30] can be considered as a mild self-etch adhesive and demineralizes dentin only superficially, whereas AdheSE Primer (self-etch), with a pH value of 1.5 [7] is regarded as a more aggressive solution, which completely solubilizes the smear layer and the smear plugs, building a hybrid layer with almost the same thickness as etch and rinse adhesives as well as a deeper resin extension into the dentin tubules [30, 34]. With regard to the acidity of adhesives, Bond Force showed a lower infiltration rate and shorter tags (Fig. 3) whereas for AdheSE, a higher ratio of infiltration but short tags was measured.

Considering the results of this study, the most effective solvent system on both infiltration parameters comprises water as one of its components. This was claimed to be able to promote reexpansion of collapsed fibrils [35]. The water present in the adhesive may be capable of simultaneously expanding collagen fibrils during the infiltration of solvated comonomers, thus allowing for a better resin infiltration [36]. Therefore, water as a solvent explained the positive results of infiltration for following adhesives: AdheSE (only ratio of infiltration), Solobond Plus (both parameters), and Syntac (very good ratio of infiltration and medium tag length). The excellent resin penetration of XP Bond (Fig. 2) can also be attributed to its components: a volatile solvent (tertiary butanol), which is totally miscible with water and the polymerizable resins, and phosphate esters that may chemically interact with the mineral apatite component of dentin [30, 37].

In this study, the tag length as well as the homogeneity of the tags was statistically influenced by the dentin position (where the measurements were done). The dentinal tubules in the first measurements (0.5 mm from DEJ) were oblique to the prepared dentin surface and spaced apart from the enamel–dentin junction; at the third measurement (2.5 mm DEJ) they are perpendicular to the prepared dentin surface. It was shown that for etch and rinse adhesives, the perpendicular orientation of the dentinal tubules is associated with longer resin tags [4]. Our results, according to another study [12], also pointed out the importance of considering the dentin area (superficial, middle, or deep dentin) where measurements are done, for instance in view of bond strength, since the deep dentin is characterized by larger and numerous tubules than superficial dentin [38]. It was also demonstrated that the position of the dentinal tubules relative to the bonded area, being perpendicular, oblique, or parallel to the investigated dentin surface, also affects the bond strength [4, 12, 39, 40].

Small and hydrophilic monomers as hydroxyethyl methacrylate (HEMA) can easily penetrate the dentin [29]. The adhesives which do not contain HEMA (in our study, AdheSE, and Xeno V) may account for short and thick resin tags [32]. The study of Santini [32] showed that the acrylamide contained in AdheSE is significantly less viscous than urethane dimethacrylate and bisphenol-A-glycidyldimethacrylate. There may be however other reasons for the shorter tags measured for AdheSE in this study.

Individually, every adhesive has its own (unique) interaction with dentin. The bonding performance depends not only on the type of adhesive but certainly also on their chemical composition [28]. For this reason, some previous investigations showed that some self-etch materials perform better or almost equal to etch and rinse adhesives [30, 41]. For example, the findings of Margvelashvili et al. [30] demonstrated that the bond strength achieved by one-step self-etch adhesive Bond Force and Xeno III were similar to that of the etch and rinse adhesives [30]. The findings of Knobloch et al. [41] indicated that the bond strength of the two-step self-etch adhesives Clearfil SE Bond, Optibond Solo Plus, iBond, and G-Bond was not significantly different from the two-step etch and rinse adhesive Prime Bond&NT [41]. These findings can be confirmed by our measurements, since some self-etch adhesives performed better than etch and rinse adhesives (Table 3).

The better homogeneity of three-step and two-step etch and rinse adhesives might be explained by the fact that the application of phosphoric acid before applying the adhesive demineralized the dentin surface layer to a determinate depth, facilitating penetration of the adhesive system. The etching phase removes totally or partially the smear layer and smear plugs. Remaining globular particles of both structures have the ability to obliterate some dentinal tubules, thus the tags will be wider (largely) with a constant length and better homogeneity than those of two-step self-etch adhesives [38, 39]. Interrupted tags could have occurred as a result of the extensive demineralization produced by the etch phase, the infiltration of resin inside the dentinal tubules, and polymerization shrinkage stresses [21].

The reason for a better regularity of the three-step and two-step etch and rinse adhesives is the etching phase that dissolved the smear layer, allowing for easier access of monomers to fill the dentinal tubules. Some adhesives have a very good clinical effectiveness, while laboratory tests show rather moderate results. In particular, though barely tags formation, adhesives can perform clinical efficient [42]. Moreover, in contrast with their laboratory performance, it was also shown that self-etch adhesives can perform clinically better as etch and rinse adhesives [43]. An example therefore is the self-etch adhesive Clearfil Bond SE showing a very good clinical behavior [42] though insufficient resin infiltration, as measured in laboratory tests, when compared with three-step etch and rinse adhesives. A reason for this behavior must be searched in the particular composition of the respective adhesive [44]. In this way, the two-step self-etch adhesives appeared to be related to the hydrolytic stability of the functional monomer itself and its interaction with dentin. The adhesive containing 10-MDP as a functional monomer, which effectively interacts chemically with hydroxylapatite within a clinically reasonable time, showed no signs of degradation in bond strength and interfacial ultrastructure. Intimate monomer–dentinal tissue interaction is therefore expected to extend bond longevity [44]. The two-step self-etch Clearfil Bond SE containing 10-MDP shows excellent clinical effectiveness also after 8 years of clinical functioning [42]. Nevertheless, the ultimate effectiveness of dentin adhesives has to be validated in controlled long-term clinical trials [11].

Conclusions

This study showed that not only the class to which an adhesive can be classified is an important factor for the adhesive infiltration into dentin, but also the composition of the adhesive and the dentin position where the measurement was done; thus, the null hypothesis tested in this study was rejected. Our findings regarding dentin infiltration confirmed that two-step etch and rinse adhesives performed better in terms of ratio of infiltration, while three-step etch and rinse adhesives generated significant longer tags. The clinical role of the length and quality of tags as well as the ratio of infiltration must be further investigated.

Conflict of interest The authors declare that they have no conflict of interest.

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