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Original Article

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Sodium hypochlorite effects on dentin bond strength and acid-base resistant zone formation by adhesive systems

Marina Di Francescantonio[1], Hamid Nurrohman[2], Tomohiro Takagaki[2], Toru Nikaido[2], Junji Tagami[2], Marcelo Giannini[1]

[1] Universidade Estadual de Campinas - UNICAMP, Piracicaba Dental School, Department of Restorative Dentistry, Piracicaba, SP, Brazil

[2] Tokyo Medical and Dental University, Department of Cariology and Operative Dentistry, Tokyo, Japan

Abstract

Aim: To evaluate the effects of 10% NaOCl gel application on the dentin bond strengths and morphology of resin-dentin interfaces formed by three adhesives. **Methods:** Two etch-and-rinse adhesives (One-Step Plus, Bisco Inc. and Clearfil Photo Bond, Kuraray Noritake Dental) and one self-etch adhesive (Clearfil SE Bond, Kuraray Noritake Dental) were applied on dentin according to the manufacturers' instructions or after the treatment with 10% NaOCl (ED-Gel, Kuraray Noritake Dental) for 60 s. For interfacial analysis, specimens were subjected to acid-base challenge and observed by SEM to identify the formation of the acid-base resistant zone (ABRZ). For microtensile bond strength, the same groups were investigated and the restored teeth were thermocycled (5,000 cycles) or not before testing. Bond strength data were subjected to two-way ANOVA and Tukey's test ($p<0.05$). **Results:** NaOCl application affected the bond strengths for One-Step Plus and Clearfil Photo Bond. Thermocycling reduced the bond strengths for Clearfil Photo Bond and Clearfil SE Bond when used after NaOCl application and One-Step Plus when used as recommended by manufacturer. ABRZ was observed adjacent to the hybrid layer for self-etch primer. The etch-and-rinse systems showed external lesions after acid-base challenge and no ABRZ formation when applied according to manufacturer's instructions. **Conclusions:**10% NaOCl changed the morphology of the bonding interfaces and its use with etch-&-rinse adhesives reduced the dentin bond strength. Formation of ABRZ was material-dependent and the interface morphologies were different among the tested materials.

Keywords: dental bonding; dentin; dental caries susceptibility; dentin-bonding agents; sodium hypochlorite; scanning electron microscopy.

Introduction

The success and durability of restorations depends on the type of adhesive technique and materials used. The advancement of Adhesive Dentistry produces restorations with excellent aesthetic and mechanical properties; even so, the restorative procedures still present some clinical problems related to microleakage and degradation. Such problems could occur from gap formations between tooth and adhesive restoration, which can lead to secondary caries and fractures in adjacent dental structures, affecting the longevity of restorations[1].

Many studies have evaluated the prevention and control of secondary caries around composite restorations, mainly in dentin. The focus of these investigations has been on e resin-adhesive interfaces after cariogenic challenge. Different methods

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Correspondence to:
Marcelo Giannini
Departamento de Odontologia Restauradora
Faculdade de Odontologia de Piracicaba – UNICAMP
Av. Limeira, 901, Piracicaba, SP, Brasil
CEP: 13414-903
Phone: + 55 19 21065340
Fax: + 55 19 21065218
E-mail: giannini@fop.unicamp.br

can be used to analyze the effects of cariogenic challenge on dentin-resin interfaces, such as scanning electron microscopy (SEM), polarized light microscopy, microhardness analysis, microradiography, confocal laser scanning microscopy and X-ray analytical microscopy[2-5]. Tsuchiya et al.[6] (2004) using SEM identified the formation of an acid-base resistant zone (ABRZ) beneath the hybrid layer, which was characterized by argon-ion etching technique. However, they reported that ABRZ was observed only for some categories of adhesive systems[7].

Sodium hypochlorite (NaOCl) is a well-known nonspecific proteolytic agent able to dissolve organic material and has been used in Restorative Dentistry as a deproteinizing agent for dentin[8-10]. The rationale for using NaOCl is that it facilitates the infiltration of adhesive resins into an etched dentin substrate, creating the bond without collagen fibrils and hybrid layer[11-13]. Depending on each test methodology and/or specific composition of dentin bonding agents, the application of NaOCl on acid etching may increase or decrease bond strengths[14-19].

This *in vitro* study was conducted to determine by SEM the effects of thermocycling and 10% NaOCl gel application after acid etching on the dentin bond strengths and resin-dentin interface morphology. The first tested hypothesis was that 10% NaOCl and thermocycling would not reduce the dentin bond strength, regardless the type of adhesive system used. The second was that 10% NaOCl application would not provide the ABRZ formation.

Material and methods

Eighty-four caries-free human third molars stored in an aqueous solution containing 0.2% thymol were used after approval by the Ethics Research Committee (process # 90/2009). This study tested three adhesive systems : two etch-and-rinse adhesive systems: One-Step Plus (Bisco Inc., Schaumburg, IL, USA) and Clearfil Photo Bond (Kuraray Noritake Dental, Tokyo, Japan); and one self-etch adhesive system: Clearfil SE Bond (Kuraray Noritake Dental). Table 1 displays the composition and lot number of these adhesive systems.

Bond strength test - Sixty teeth were used in this part of study (n=5). The occlusal enamel of teeth was removed using a low speed diamond saw (Buehler, Lake Bluff, IL, USA) to expose a flat middle-depth coronal dentin surface. The exposed dentin surface was ground using 600-grit silicon carbide paper (Fuji Star, Sankyo Rikagaku, Saitama, Japan) under water-cooling. Adhesive systems were applied to the dentin surfaces according to the manufacturers' instructions or following 10% NaOCl application (AD-Gel, Kuraray Noritake Dental). The experimental groups are described in Table 2.

When 10% NaOCl was used with two acid-etch adhesives (One-Step Plus and Clearfil Photo Bond), it was applied for 1 min after phosphoric acid etching, rinsed for 20 s and gently air-dried for 10 s, followed by adhesive application. For Clearfil SE Bond, 10% NaOCl was used after SE-Primer. Afterwards, acetone (for 30 s) and water rinsing (for 30 s) were used to remove functional monomers that did not react with calcium ions from the dentin surface. Next, the Clearfil SE Bond bonding resin was applied to dentin and light-activated for 10 s (Optilux 501, Demetron-Kerr Corp., Danbury, CT, USA).

A 6 mm-high composite block (Clearfil Majesty, Kuraray Noritake Dental, Tokyo, Japan and Aelite LS, Bisco Inc) was built-up incrementally over the bonded dentin. Three increments of 2.0 mm were placed and each layer was light activated for 20 s. After 24 h in distilled water at 37 °C, half of the stored specimens and the other halves were thermocycled 5,000 times in a water bath between 5 °C and 55 °C with a 30 s dwell time in each bath and a transfer time of 10 s. Afterwards, the teeth were serially sectioned perpendicular to the adhesive-dentin interface in mesiodistal and buccal-lingual directions to obtain bonded beams of approximately 1.0 mm^2 cross-sectional area using a slow-speed diamond saw. The specimens were tested individually by attaching them to a microtensile jig with cyanoacrylate glue (Model Repair II, Dentsply-Sankin, Tochiji, Japan). The bonded beams were tested in tension using a universal testing machine (EZ Test, Shimadzu Corp., Kyoto, Japan) at a 1.0 mm/min crosshead speed.

The load in kgf and the bonding surface area of the specimen were recorded and microtensile bond strength data

Table 1. Composition and batch number of the adhesive systems used in this study (information supplied by the MSDS of each manufacturers).

Material	Composition	Batch number
One-Step Plus	Biphenyldimethacrylate, HEMA, acetone, dental glass	0800005538
Clearfil Photo Bond	Catalyst liquid: Bis-GMA, MDP, HEMA, hydrophobic dimethacrylate, benzoyl peroxide, camphorquinoneUniversal liquid: N,N'-diethanol p-toluidine, sodium benzene sulûnate, ethyl alcohol	Catalyst: 00453A Universal:00549A
Clearfil SE Bond	Primer: HEMA, MDP, hydrophilic aliphatic dimethacrylate, dl-camphorquinone, water, accelerators, dyes and othersBond: HEMA, MDP, Bis-GMA, hydrophobic dimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica	Primer:01633A Adhesive: 01088B

Abbreviations: Bis-GMA- bis-phenol A diglycidyl, HEMA- 2-hydroxyethyl methacrylate, MDP- 10-methacryloyloxydecyl dihydrogen phosphate.

Table 2. Experimental groups and application technique for adhesive systems.

Restorative System Treatment (adhesive + composite)	Treatment
One-Step Plus + Aelite LS	Manufacturer's Instructions
One-Step Plus + Aelite LS	10% NaOCl application
Clearfil Photo Bond + Majesty	Manufacturer's Instructions
Clearfil Photo Bond + Majesty	10% NaOCl application
Clearfil SE Bond + Majesty	Manufacturer's Instructions
Clearfil SE Bond + Majesty	Primer removal with acetone and water+ 10% NaOCl application

were expressed in MPa. Statistical analysis was performed with statistical software (Minitab 15, Minitab, State College, PA, USA). Two-way analysis of variance (ANOVA) for the "restorative system" and "thermocycling" factors was performed, followed by a Tukey's post hoc test ($p<0.05$).

The fractured surfaces of the tested specimens were sputter-coated with gold (MED 010, Balzers Union, Balzers, Liechtenstein) and examined using a scanning electron microscope (VP 435, Leo Electron Microscopy Ltd., Cambridge, UK). Failure patterns were classified as: (1) failure within adhesive resin layer; (2) failure within hybrid layer; (3) cohesive failure in dentin; and (4) mixed failure involving the adhesive resin and hybrid layer.

Acid-base resistant zone evaluation - The methodology for specimen preparation (Figure 1) followed a previous report[20]. Twenty-four caries-free human third molars were used in this part of study (n=4). Teeth were prepared according to the bond strength methodology and the same application technique for adhesives and composites was used.

After bonding and composite placement, teeth were stored in distilled water at 37 °C for 24 h and then were vertically sectioned through the dentin-adhesive interface and embedded in an epoxy resin (Epoxicure Resin, Buehler). The embedded teeth were placed in 100 mL of a buffered demineralizing solution, containing 2.2 mmoL/L $CaCl_2$, 2.2 mmoL/L NaH_2PO_4 and 50 mmoL/L acetic acid (pH 4.5) for 90 min to create artificial recurrent caries[20]. After this, the teeth were immersed in 5% NaClO for 20 min to remove any demineralized dentin collagen fibrils, followed by rinsing

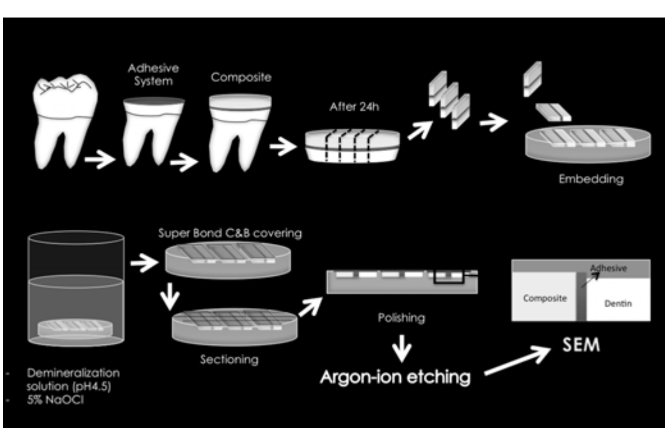

Fig. 1. Schematic illustration of the specimen preparation for SEM.

with water for 30 s. The 4-META/MMA-TBB resin (Super Bond C&B, Sun Medical, Moriyama, Japan) was used as infiltrant agent to protect the demineralized surface during polishing procedure. After curing of infiltrant resin, the samples were vertically sectioned through the dentin-adhesive interface to obtain 1-mm thick specimens and then polished with diamond pastes (Struers A/S, Copenhagen, Denmark). The polished surfaces were conditioned with a beam of argon-ions (EIS-IE, Elionix Inc., Tokyo, Japan) for 6 min. The operating conditions for argon ion beam etching were 1 kV accelerating voltage and 0.2 mA/cm^2 current density with the ion beam positioned directly perpendicular to the polished surface. The specimens were gold-sputter coated and the morphological changes at the dentin-adhesive interfaces produced by acid-base challenge were observed by SEM (JSM-5310LV, Jeol, Tokyo, Japan).

Results

Bond strength - The dentin bond strength means of the experimental groups are shown in Table 3. Two-way ANOVA indicated that the "restorative system" and "thermocycling" factors influenced the bond strength results ($p<0.0001$ and $p<0.0001$, respectively), and the interaction factor was also significant ($p=0.0004$).

Dentin bond strengths of One-Step Plus and Clearfil Photo Bond decreased following 10% NaOCl application ($p<0.05$), while Clearfil SE Bond was not affected ($p>0.05$). Thermocycling decreased the bond strength of Clearfil SE Bond after NaOCl application. Thermocycling affected also the bond strength of Clearfil Photo Bond (with or without NaOCl application) and One-Step Plus when following the manufacturer's instructions ($p<0.05$).

Table 4 shows de percentage distribution of failure modes. Failure within adhesive resin layer, within hybrid layer and mixed were observed for all experimental groups. In general, higher incidence of cohesive failure in dentin occurred when teeth bonded with Clearfil Photo Bond and Clearfil SE Bond were thermocycled.

Acid-base resistant zone - The SEM images of the dentin-resin interfaces after acid-base challenge are shown in Figure 2. The outer lesion created by the acid challenge was observed in all groups; it was approximately 10 to 20 ìm thick. One-Step Plus and Clearfil Photo Bond used following the manufacturer's instructions formed thick hybrid layers of approximately 3 to 4 µm (Figures 2A and 2C). However, the hybrid layer formed by One-Step Plus was destroyed by acid-base challenge (Figure 2C) and wall lesions were observed for it and Clearfil Photo Bond adhesives. The etch-and-rinse adhesives applied after 10% NaOCl did not form hybrid layer. The adhesive resins were bonded to irregular dentin wall created by the acid etching and no wall lesion was found along the adhesive interface (Figures 2B and 2D).

For Clearfil SE Bond, 2-ìm-thick ABRZ formation was observed (Figure 2E). A very thin ABRZ, approximately 0.5 ìm thick, was found with 10% NaOCl treatment after

Table 3. Mean dentin bond strengths (standard deviation) of restorative systems (composite and adhesive) after thermocycling (TC) or not (in MPa).

Restorative system	NaOCl Treatment	Without TC	With TC
One-Step Plus and	no	50.5 (3.3) A a	35.2 (4.5) BCD b
Aelite LS	yes	38.0 (3.4) C a	32.4 (2.7) D a
Clearfil Photo	no	50.4 (2.6) Aa	41.1 (4.1) ABC b
Bondand Majesty	yes	42.4 (5.1) BC a	34.5 (3.2) CD b
Clearfil SE Bond and	no	49.9 (6.5) AB a	44.2 (6.0) A a
Majesty	yes	44.1 (6.0) ABC a	32.5 (3.5) D b

Capital letters compare "Restorative System/NaOCl Treatment" within the same column. Lower case letters compare "Thermocycling or not" (row) for the same "Restorative System/NaOCl Treatment".

Table 4. Failure modes distribution (%) according to the experimental groups.

Adhesive	/ Treatment/ Thermocycling	1	2	3	4
One-Step Plus	/ No treatment / None	36	30	18	16
	/ No treatment / Thermocycled	26	35	10	20
	/ 10% sodium hypochlorite / None	13	51	10	39
	/ 10% sodium hypochlorite / Thermocycled	12	40	10	38
Clearfil Photo Bond	/ No treatment / None	23	25	0	52
	/ No treatment / Thermocycled	12	24	12	51
	/ 10% sodium hypochlorite / None	14	37	12	37
	/ 10% sodium hypochlorite / Thermocycled	11	36	17	36
Clearfil SE Bond	/ No treatment / None	34	24	0	62
	/ No treatment / Thermocycled	24	28	6	62
	/ 10% sodium hypochlorite / None	19	36	10	35
	/ 10% sodium hypochlorite / Thermocycled	13	30	18	39

1- failure within adhesive resin layer; 2- failure within hybrid layer; 3- cohesive failure in dentin; 4- mixed failure involving the adhesive resin and hybrid layer.

demineralization with the self-etch primer for Clearfil SE Bond (Figure 2F).

Discussion

The first tested hypothesis, that 10% NaOCl and thermocycling would not reduce dentin bond strengths regardless the type of adhesive system used, was rejected because the dentin bond strengths of adhesives were reduced with 10% NaOCl application and thermocycling. The second hypothesis stating that 10% NaOCl application would not provide the ABRZ formation was also rejected, because the self-etch adhesive system formed ABRZ even with NaOCl application after etching.

Secondary caries and restorative material fractures have been considered as major causes for the failure of composite restorations[1] and the same adhesive procedures seemed not providing restoration margins free of gaps and microleakage[21]. Regarding caries progression in the dentin margins around adhesive restorations, the presence of ABRZ below the hybrid layer could protect dentin against recurrent caries attack. The ABRZ formation is related to penetration of adhesive monomers into the mineralized dentin, in which chemical interaction occurs between the 10-MDP functional monomer and hydroxyapatite[22].

In this study, etch-and-rinse adhesive systems used according to the manufacturers' instructions showed external lesions adjacent to hybrid layer and no ABRZ formation was detected at dentin-resin interface after the acid-base challenge, which corroborates previous studies[11-13]. However, the hybrid layer disappeared and no erosion lesion was found at the interface after 10% NaOCl. The hybridization produced by etch-and-rinse adhesives is not a uniform layer, especially with respect to the existence of nanospaces within the hybrid layer, which correspond to dentin etched without monomer infiltration and filled with water or organic solvents[23]. When 10% NaOCl was applied to etched dentin, the exposed collagen fibrils were removed and the adhesives were unable to penetrate into underlying dentin, which resulted in no hybrid layer formation[11-13]. However, it was possible to note that there was a good contact without gaps between adhesives and dentin.

For self-etch adhesive, a very thin hybrid layer is formed and after acid-base challenge, Clearfil SE Bond presented ABRZ approximately 2 mm-thick. When the SE primer is applied on the dentin surface, this acidic primer demineralizes the smear layer on the surface and next the underlying dentin. MDP-containing self-etch primer is able to create two layers of demineralized dentin, one totally demineralized and another partially demineralized. Thus, when it is applied on mineralized dentin surface, a superficial demineralization

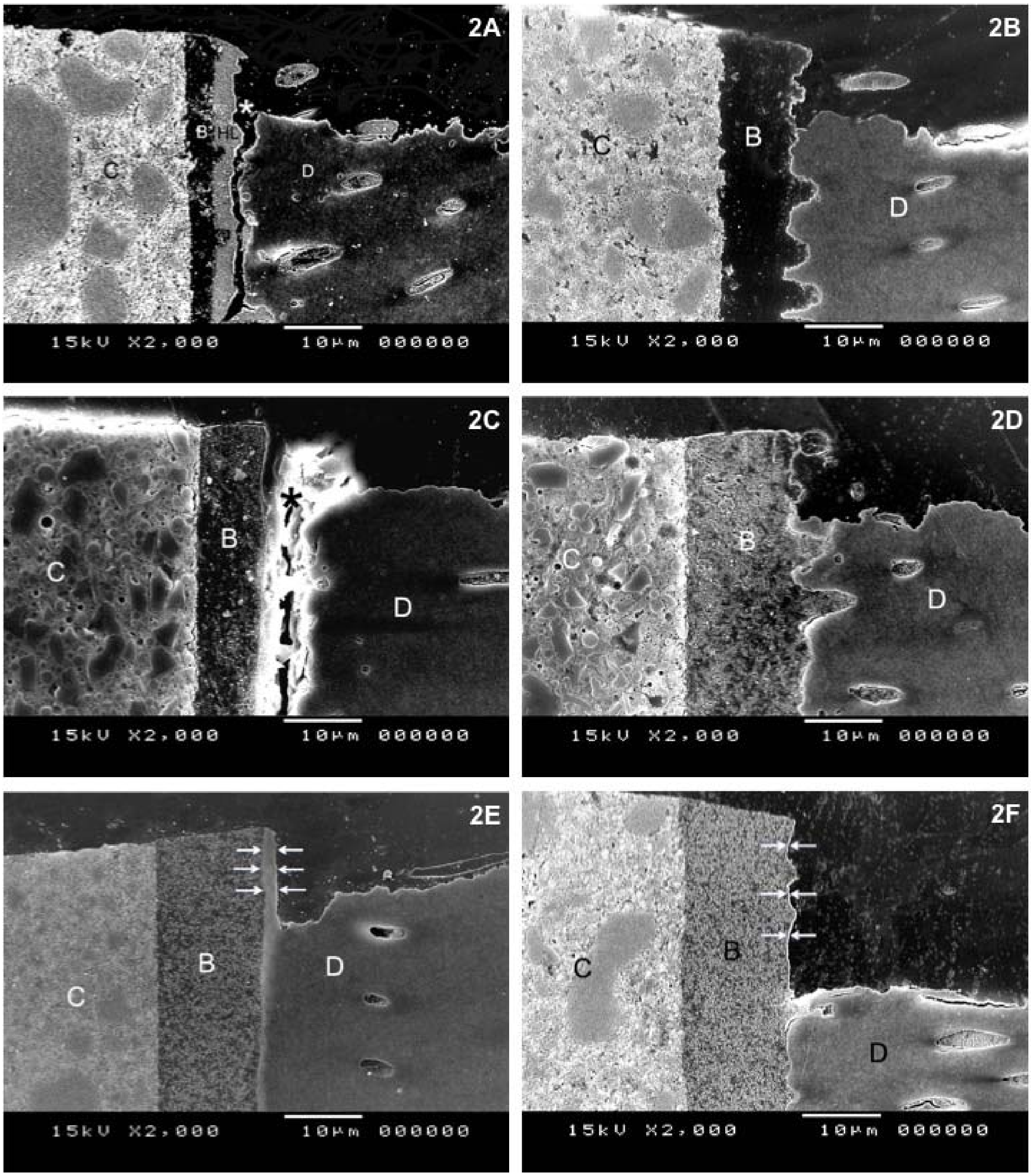

Fig. 2. SEM observations of the adhesive-dentin interfaces after acid-base challenge (x2.000). A- Clearfil Photo Bond, B- Clearfil Photo Bond applied after 10% NaOCl, C- One-Step Plus, D- One-Step Plus applied after 10% NaOCl, E- Clearfil SE Bond and F- Clearfil SE Bond/10% NaOCl (C: composite; B: adhesive layer; HL: hybrid layer; D: dentin; *: hybrid layer destroyed; arrows: acid-base resistant zone).

around 1 mm deep is formed and adjacent dentin at the bottom is also affected, but it is partially demineralized and contains hydroxyapatite, where the MDP chemical interaction occurs[24-25].

When dentin surface was treated with SE Primer, rinsed with acetone/water to remove the excess primer on the surface and treated with 10% NaOCl to deplete the exposed collagen, a very thin, approximately 0.5 mm-thick. ABRZ was observed. This very thin ABRZ formation at bottom (sub-surface region) was due to MDP-hydroxyapatite interaction with the remaining hydroxyapatite after collagen removal[8-10]. Nurrohman, et al.[26] (2012) using transmission electron microscopy detected 5 mm hybrid layer formation on demineralized dentin for Clearfil Photo Bond etch-and-rinse adhesive. They also evaluated the interaction between specific functional groups and apatite crystals and showed chemical bonding potential of 10-MDP with the remaining crystals, which produced the ABRZ zone. Another study detected strong affinity of 10-MDP-based self-etch system to deproteinized dentin by formation of 10-MDP-Ca salt[19].

The acid-base challenge destroyed the hybrid layer formed by One-Step Plus, while the hybrid layer remained intact for Clearfil Photo Bond. These results suggested that different qualities of hybridization promoted by etch-and-rinse adhesive systems maybe caused by difference in the monomer compositions. One-Step Plus contains BPDM and HEMA monomers, while Clearfil Photo Bond presents HEMA and MDP, which contribute to monomer penetration into etched dentin and chemical interaction with hydroxyapatite at the bottom of the hybrid layer, respectively.

The application of 10% NaOCl decreased the dentin bond strength for the tested etch-and-rinse adhesives. However, the results are rather controversial on this matter. While the findings of this study agree with the outcomes from some authors[16-18], other studies reported no changes in dentin bond strength when 10% NaOCl was used[11,19]. Conversely, some reports showed that the treatment of etched dentin with 10% NaOCl is beneficial, depending on the adhesive system evaluated[12-15]. These inconsistent outcomes in the literature hindered a widespread use of this technique.

Besides, deproteinization with 5% NaOCl removes collagen from demineralized dentin and also exposes lateral secondary tubules at the intertubular region and peritubular area[12]. According to Prati et al.[13] (1999), treatment of etched dentin with NaOCl produced an unusual type of resin infiltration of mineralized dentin, which may explain the mechanism of resin bonding to NaOCl-treated dentin. A previous study also reported significant reduction in bond strength to dentin after thermocycling for some adhesives, independent whether they were applied on deproteinized dentin or not[19].

Because 10% NaOCl removes collagen fibrils[8-10], the bonding is formed in absence of collagen fibrils and only by the contact between adhesive monomer and dentin[11-13]. Thus, it was suggested that the only artificial material at the interface, the polymer formed after light activation of adhesives, could undergo degradation depending on monomeric composition, resulting in decrease of bond strength. Some hydrophilic monomer resins, such as those in the current adhesives are highly prone to absorb water[27]. Since adhesives used in the present study contain high concentration of hydrophilic monomers such as HEMA, water sorption of these monomers could contribute to bond strength reduction. In addition, residual water entrapped at the deepest regions of demineralized and/or deproteinized dentin forms poorly polymerized polymer chains[28], which are weaker and less stable over time than those formed in absence of moisture.

The failure patterns related for each group depended on the adhesive system used and NaOCl treatment. After thermocycling, a slightly higher percentage of cohesive dentin failure was reported, suggesting degradation of the collagen matrix of dentin or monomeric components of the adhesive systems infiltrated in dentin.

Secondary caries can initiate at defects, like gaps in the marginal areas of restorations. The self-etch primer system used in this study demonstrated good dentin bonding performance and sealing ability, and also resistance against the acid-base challenge[6,20,22,29-30]. Therefore, formation of an ABRZ is important for the control of secondary caries around restoration, but this effect depends on the type of adhesive system[7,22]. The application of 10% NaOCl changed the demineralization pattern around composite restoration, because there was no hybrid layer formation. The bond strength of One-Step Plus etch-and–rinse adhesive to sodium hypochlorite-treated dentin did not reduce with thermocycling even without the hybrid layer. However, the use of 10% NaOCl represents an extra step and may not assure a superior bonding performance when dentin deproteinization is performed after acid etching.

Within the limitations of this study, the following conclusions were drawn: 1- the application of 10% NaOCl after acid etching did not improve the bond strength to dentin neither after thermocycling; 2- ABRZ formation is material-dependent.

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