Effect of dentin dehydration and composite resin polymerization mode on bond strength of two self-etch adhesives

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Abstract

Background: Dual-cured composite resins are similar to self-cured composite resins in some of their clinical applications due to inadequate irradiation, lack of irradiation, or delayed irradiation. Therefore, incompatibility with self-etch adhesives (SEAs) should be taken into account with their use. On the other, the extent of dentin dehydration has a great role in the quality of adhesion of these resin materials to dentin. The aim of this study was to investigate the effect of dentin dehydration and composite resin polymerization mode on bond strength of two SEAs. **Materials and Methods:** A total of 120 dentinal specimens were prepared from extracted intact third molars. Half of the samples were dehydrated in ethanol with increasing concentrations. Then Clearfil SE Bond (CSEB) and Prompt L-Pop (PLP) adhesives were applied in the two groups. Cylindrical composite resin specimens were cured using three polymerization modes: (1) Immediate light-curing, (2) delayed light-curing after 20 min, and (3) self-curing. Bond strength was measured using universal testing machine at a crosshead speed of 1 mm/min. Data were analyzed with two-way ANOVA and Duncan *post hoc* tests. Statistical significance was defined at $P < 0.05$. **Results:** There were no significant differences for CSEB subgroups with hydrated and dehydrated dentin samples between the three different curing modes ($P > 0.05$). PLP showed significant differences between subgroups with the lowest bond strength in hydrated dentin with delayed light-curing and self-cured mode of polymerization. **Conclusion:** Within the limitations of this study, a delay in composite resin light-curing or using chemically cured composite resin had a deleterious effect on dentin bond strength of single-step SEAs used in the study.

Keywords: Chemically cured, composite resins, dental adhesives, dental bonding, light-curing

Introduction

There have been great advances in restorative dentistry recently as a result of new formulations in composite resins and adhesives. Immediate bonding agents that involve several complex steps, exhibit high technique sensitivity, and are time consuming are gradually becoming obsolete and replaced with simplified and less technique-sensitive self-etch adhesive (SEA) resin systems.[1] These new systems have acidic monomer concentrations and water in their chemical structure, with four categories in relation to their pH: (1) Strong SEAs with a pH value <1 and 3-4 μm-deep fully demineralized hybrid layer similar to etch-and-rinse adhesives; (2) moderately strong SEAs with a pH value of approximately 1.5 and 1-2 μm hybrid layer, with some hydroxyapatite remaining at the bottom; (3) mild SEAs with a pH value around 2, with a hybrid layer <1 μm in thickness that is only partially mineralized; and (4) ultra-mild SEAs that are available with a primer pH value of over 2.5, do not eliminate the smear layer and interact with the smear layer-covered dentin up to a few hundredths of a nanometers.[2]

Dentin is composed of about 50 vol% of minerals in the form of a carbonate-rich apatite, 30 vol% of organic matter consisting mainly of type I collagen, and approximately 20 vol% of water.[3] Since dentin is intrinsically moist, bonding to dentin is more difficult than that to enamel, especially with adhesive systems that require a moisture-free environment for optimal bonding.[4] Furthermore, water content affects the polymerization efficacy of composite resins, depending on their chemical structure and initiator mechanisms.[5] A study showed that the dentin wetness and relative humidity

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did not exert any effects on the bond strength of one-step SEA systems.[6] Another study showed that the adhesive agent and the presence of moisture before the application of adhesive agents and the air drying duration affected the bond strength.[7]

On the other, the monomers remaining in the uncured adhesive resin layer directly contact the dual-cured resin that undergoes a chemical curing process for reasons such as the presence of inadequate light, delayed light-curing, and judicious light-curing; as a result, the amine accelerator in the composite resin is deactivated.[8] Based on some previous studies, there is an incompatibility between SEAs and chemically cured composite resins.[9,10] In addition, it has been concluded that most hydrophilic adhesives exhibit less compatibility with chemically cured composite resins.[11] A study showed that chemical incompatibility does not occur only in the self-cured composite resins and dual-cured composite resins that are cured with a delay exhibit this problem, too.[10]

Little research has been undertaken to evaluate the effects of these variations on bonding interfaces. The present study was undertaken to evaluate the effect of dentin moisture and composite resin curing modes on the microshear bond strength of composite resins.

The null hypotheses tested ran as follows: Differences in dentin moisture and composite resin polymerization mode do not affect the bond strength of composite resins.

Materials and Methods

Sample collection and preparation

A total of 120 intact third mandibular molars were collected, thoroughly cleaned, and stored in 1% chloramine T solution at 4°C for a maximum of 2 months. The occlusal enamel and half of the coronal dentin were removed with a high-speed headpiece under air and water cooling. The dentin surfaces were polished with 180-, 340-, and 600-grit silicon carbide papers to achieve bonding surfaces covered with the smear layer.

In order to dehydrate dentin, the roots were sectioned parallel to occlusal bonding surfaces in half of the specimens to eliminate the contents of the pulp chamber. Then the samples were dehydrated in ascending concentrations of ethanol (70%, 80%, and 95%) for 2 h in each. Then, the samples were immersed in absolute ethanol for another 48 h.[10] Finally, all the samples were mounted in self-cured acrylic resin blocks.

Composite bonding and mode of curing

The samples were divided into hydrated and dehydrated groups. Each group was subdivided into six subgroups based on the type of the bonding agent and the light-curing mode. Clearfil SE Bond (CSEB) (Kuraray Medical, Inc., Japan) and Prompt L-Pop (PLP) (3M ESPE, Seefeld, Germany) adhesives were applied on the sample surfaces according to the manufacturers' instructions. Then, using a Tygon tube, composite resin (Biscore, A3 Shade, Bisco, USA) was applied at a height and diameter of 0.9 mm × 1 mm and cured using three different curing modes as follows.

Light-curing mode

For light-curing mode, the base syringe of the composite resin was used. Hand mixing was performed for the base resin for 20 s under ambient light. Then inserted and cured for 40 s at curing intensity of 500 mW/cm^2 immediately upon placement by means of LED light curing units (Dentsply DeTrey, Konstanz, Germany).

Delayed light-curing mode

Similar to the previous group, using Tygon tube, the hand-mixed composite resin of the base syringe was applied over the cured adhesive and left in complete darkness for 20 min before light activation.

Chemical curing mode

Composite resins of the base and catalyst syringes were hand-mixed for 20 s as described above. Composite resin was applied to the bonded surface and polymerized in complete darkness for 20 min before re-exposure to ambient light.

All the samples were incubated at 37°C for 24 h and then tested for microshear bond strength using a universal testing machine (Zwick Ltd., Herefordshire, UK) at a crosshead speed of 1 mm/min. Bond strength data were analyzed by two-way ANOVA and *post hoc* Duncan tests. Statistical significance was set at $P < 0.05$.

Sample preparation for transmission electron microscopy

Composite core buildups measuring 1 mm in height were prepared on dentin surfaces similar to the study Groups 7, 8, 9, and 12. Transmission electron microscopy (TEM) images were obtained for four specimens: Two groups with lower and two with higher bond strength values. Then, dentin slices measuring 1 mm in thickness were sectioned in an occlusogingival direction. These slices were coated with two layers of nail varnish up to 1 mm from the bonded interfaces. The samples were immersed in ammoniacal silver nitrate (Dako, Glostrup, Denmark) solution for 24 h. The samples were processed according to the TEM protocol introduced by Tay *et al*.[12]

Results

Two-way ANOVA showed statistically significant differences between all the groups ($P \leq 0.05$). *Post hoc* tests revealed no significant differences between CSEB subgroups ($P < 0.05$). Application of PLP on hydrated dentin highlighted the effect of polymerization mode on the shear bond strength so that

Group 11 exhibited the lowest average of bond strength. Groups 7, 11, and 12 exhibited statistically significant differences from other groups ($P < 0.001$) [Table 1].

TEM ultrastructural morphology for Groups 7, 8, 9, and 12 revealed four patterns of silver deposition within the adhesive layer [Figure 1]:

- Reticular silver deposition in the hybrid layer, indicating nano leakage or remaining free water in this portion
- Isolated silver grains, revealing hydrophilic functional domains of adhesive
- Water blisters demonstrating water transmission from dentin to composite-adhesive interface
- Water trees probably created from adjoining isolated silver grains. These canals were water transmission paths.

Discussion

Contrary to enamel bonding, achievement of a successful and predictable dentin bonding to resist polymerization shrinkage is still a challenge. Factors associated with dentin bonding that are responsible for such difficulty include a higher organic content of dentin, fluid pressure exerted from the dentinal tubules, and presence of the smear layer.[13]

In recent decades, SEA systems have been introduced, simplifying the procedure, and minimizing the adhesive steps. Another advantage of SEAs is the absence of or the low incidence of postoperative sensitivity compared to that with etch-and-rinse systems.[7,14] It is important for both the investigators and clinicians to elucidate the effect of moisture on the bond strength of adhesive agents.[7]

This study evaluated the effects of dentin moisture, acidity of two SEAs, and different composite polymerization techniques on the bond strength of composite resin to dentin. Based on the results, CSEB exhibited higher bond strength compared to PLP. A previous study showed that as viscosity decreases with an increase in 2-hydroxyethyl methacrylate (HEMA) content, there is an increase in the supply of oxygen to the radicals through diffusion. The oxygen binds to the reactive site of the growing radicals until the radical formation rate matches or exceeds that of the oxygen diffusion.[15] CSEB has HEMA in its chemical composition and due to its low thickness, it might have less oxygen-inhibited layer compared to PLP, which is highly viscous without HEMA as oxygen does not easily penetrates into viscous materials. In addition, the CSEB, which contains 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), only superficially interacts with enamel and dentin and cannot properly dissolve apatite crystals, but

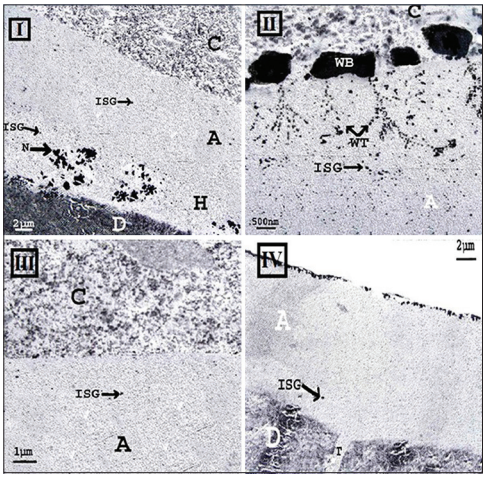

Figure 1: Transmission electron microscopy observation of different groups: (I) Group 9 (hydrated dentin bonded with Clearfil SE Bond and chemically cured composite resin); reticular silver deposition in the hybrid layer (H) demonstrates nanoleakage (N) or the remaining free water. (II) Group 7 (hydrated dentin bonded with Prompt L-Pop bond and delayed light-curing of composite resin); water blister demonstrates water transmission from dentin to composite-adhesive interface. Water trees are created from adjoining isolated silver grains. (III) Group 8 (dehydrated dentin bonded with Prompt L-Pop bond and delayed light-curing of composite resin); composite-adhesive interface was intact and demonstrated complete polymerization of adhesive and composite. (IV) Group 12 (dehydrated dentin bonded with Prompt L-Pop bond and chemically cured composite resin); continuous line of silver deposits could be seen along the adhesive-composite resin interface. The composite resin separated from the composite resin-adhesive interface due to incomplete composite polymerization. In all the figures the following abbreviations were used: A - Adhesive layer; C - Composite resin; D - Dentin; ISG - Isolated silver grain

Table 1: Mean±standard deviation of microshear bond strength in the study groups

Self-etch adhesive	Substrate	Curing mode and group number					
		Group number	Light cured	Group number	Delay light cured	Group number	Chemical cured
Clearfil SE bond	Hydrated dentined	1	27.16±4.16[a]	5	26.86±4.31[a]	9	27.03±3.87[a]
	Dehydrated dentine	2	28.87±5.01[a]	6	28.20±5.08[a]	10	28.82±4.95[a]
Prompt L-Pop	Hydrated dentine	3	27.28±4.18[a]	7	12.67±2.67[b]	11	2.00±0.50[c]
	Dehydrated dentine	4	27.97±4.69[a]	8	25.40±4.75[a]	12	7.00±1.95[d]

Different superscripts (a, b, and c) indicate mean values that are significantly different

rather keeps them in place within a thin submicron hybrid layer. X-ray diffraction technique showed that 10-MDP continuously forms a regular multi-layered structure on the apatite surface. Each layer of this nano-layered structure is composed of two 10-MDP molecules with their methacrylate groups oriented toward each other and their functional hydrogen phosphate groups oriented away from each other.[16]

Molecules such as phosphoric acid in PLP and functional monomers in SEAs will initially bond to Ca of hydroxyapatite, but then will readily de-bond. The negatively charged phosphate ions will remove the positively charged (and thus electrostatically attracted) Ca ions from the surface up to a certain depth, proportional to the application time, leading to a profound decalcification or "etching" effect, similar to the use of phosphoric acid used as a chemical etchant in the etch-and-rinse technique.[14] Use of a separate hydrophobic adhesive resin after the application of a hydrophilic primer resulted in a more hydrophobic interfacial area, providing more efficient seal in the CSEB groups and resulting in a more durable bond than that with one-step SEA (PLP groups).[17]

Based on a literature review, the interface created by SEAs relies greatly on the interactions between the functional monomers and the tooth structure.[18] In addition, the interaction depth is proportional to the pH value of the adhesive.[19] The adhesive pH should be adequately low to properly demineralize enamel or dentin; furthermore, its acidity should not exceed a certain range in order to avoid excessive hydrophilicity.[20] Very low pH and high hydrophilicity of PLP (pH = 1.17) might have a detrimental effect on the mechanical stability as a result of excessive water sorption. A decrease in microshear bond strength of self-cured composite resin to dentin exhibited an inverse relationship with the acidity of PLP. The adverse chemical interaction between unpolymerized acidic adhesive resin monomers and the basic tertiary amine catalyst in the composite resin was attributed to the observed incompatibility.[9] CSEB has lower acidity (pH = 2) compared to PLP (pH = 1.17).[9,21,22] It has been reported that the acidity and aggressive nature of PLP is similar to 32–37% phosphoric acid.[23] CSEB is considered a two-step system compared to PLP, which is one-step. Therefore, a hydrophobic resin layer, with lower acidity, which is placed between the primer and the composite resin, prevents diffusion of water.[9,10] Higher hydrophilicity of PLP and its porous hybrid layer facilitate diffusion of water through the adhesive layer, as confirmed by Nagayassu *et al*., who showed much lower microshear bond strength of PLP compared to CSEB.[24] However, they did not observe different modes of polymerization or delayed activation in their study.

Despite various advantages and applications of light-cured composite resins, there are indications for the use of chemical curing modality of many dual-cured composite resins and resin cement in areas with limited light penetration, for cementation of endodontic posts, bulky ceramics, and metallic restorations.[22] Based on the results of this study, there was a decrease in the mean bond strength of chemically cured composite resins with the use of PLP as an adhesive resin. Given the low pH of PLP, such decrease might be attributed to the adverse chemical interaction between catalytic components of chemically cured composite resin and PLP (Groups 11 and 12). It seems the permeability of the adhesive had a minor role in the decrease in bond strength. Furthermore, the synergistic effect of adhesive permeability and chemical interaction between catalytic components of chemically cured composite resin and PLP decreased the bond strength significantly in Group 11 (chemically cured composite resin and PLP bonded to hydrated dentin).

In dehydrated dentin substrata the mean bond strengths of light-cured and delayed light-cured PLP composite resin (Groups 4 and 8) were 27.9 and 25.4 MPa, respectively. In contrast, in Group 12 (chemically cured PLP composite resin), the bond strength exhibited a decrease. TEM observation for Group 12 showed that the composite resin separated from the composite-adhesive interface due to incomplete composite resin polymerization [Figure 1].

Adverse chemical interactions might take place between uncured acidic monomers on the surface layer of the adhesive and the tertiary amines of composite resin. N, N diethanol P-toluidine (base syringe tertiary amine or chemical accelerator) is much more stronger than ethyl N, N dimethyl 4-aminobenzoate (photo accelerator), which reacts easily with uncured acidic monomers.[10] Therefore, in Group 8, the chemical accelerator was consumed while the photo accelerator remained intact. Therefore, after 20 min, when composite resin was light-cured, polymerization proceeded as well and bond strength approached 25.4 MPa.

Excess moisture might result in phase separation between hydrophobic and hydrophilic monomers, leading to irregular resin infiltration and formation of blisters and voids at the interface. Furthermore, the presence of excess water or other solvents decreases monomer conversion.[25] The consequences of poor resin infiltration and conversion consist of the low durability of the interface, increased enzymatic degradation of the exposed collagen fibers, and hydrolysis of the poorly polymerized adhesive.[17,26]

Based on the results of this study, in hydrated dentin substrate, the mean bond strength of delayed light-cured PLP composite resin (Group 7) decreased significantly, which was confirmed in TEM studies. In the presence of an osmotic gradient, due to the oxygen-inhibited layer of adhesive and adequate delay, water diffuses from the dentin substrate to the adhesive-composite resin interface, giving rise to the view of water trees and blisters [Figure 1]. Exodus of water from the dentinal tubules during etching was reported to be the major reason of vertically oriented water trees in a study on this type of adhesive.[21]

A synergistic effect was observed in Group 11, where water and acidic pH coexisted, forcing free radicals to react with each other rapidly.

Under clinical conditions, delayed light curing might occur in many situations such as when the operator forms the composite resin and sculptures it, removes excess composite resin, there is a low intensity of curing light or when soft-start polymerization technique is used. In case of deep cavities with more hydrated dentin, it appears as it is preferable to apply two-step SEAs with ternary catalyst systems. Moreover, it is recommended that dual-cured resin-based materials be used with an efficient light-curing system immediately subsequent to insertion, particularly with more acidic SEAs.

Conclusion

Within the limitations of this study, it was concluded that a delay in composite resin curing diminished dentin bond strength of the single-step SEA evaluated.

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Conflicts of interest

There are no conflicts of interest.

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