The Effect of Sodium Hypochlorite on Microleakage of Composite Resin Restorations Using Three Adhesive Systems

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Purpose: The aim of this study was to evaluate the effect of three different adhesive systems on microleakage of Class V restorations after the use of sodium hypochlorite.

Materials and Methods: One-hundred eighty bovine incisors were selected and randomly divided into 9 groups (n = 20): G1: Single Bond (SB); G2: 10% NaOCl solution (NS) + SB; G3: 10% NaOCl gel (NG) + SB; G4: Prime & Bond NT (PB); G5: NS + PB; G6: NG + PB; G7: Gluma One Bond (GOB); G8: NS + GOB; G9: NG + GOB. Standardized Class V cavities were prepared. All teeth were etched with 37% phosphoric acid for 15 s. In groups 2, 5, and 8, a 10% NaOCl solution was applied for 60 s to the dentin, and in groups 3, 6, and 9, a 10% NaOCl gel was applied to dentin for 60 s. All cavities were restored with composite resin Definite. The specimens were thermocycled for 1000 cycles (5°C to 55°C) and then immersed in 2% buffered solution of methylene blue for 4 h. The specimens were sectioned and analyzed according to a ranking score (0 to 4). Kruskal-Wallis and Mann-Whitney non-parametric tests ($p \leq 0.05$) were used for statistical analysis.

Results: The NaOCl treatment significantly increased microleakage at the dentin margin ($p = 0.0129$) as shown by the following sums of ranks: G1 = 1008.0^{a}; G4 = 1301.5^{ab}; G3 = 1687.0^{ab}; G7 = 1744.0^{bc}; G2 = 1802.0^{c}; G9 = 1880.0^{c}; G5 = 1889.0^{c}; G8 = 1950.0^{c}; G6 = 1963.0^{c} (different superscripts indicate significant differences). For enamel, there were no statistically significant differences among the groups ($p > 0.05$).

Conclusion: Depending on the adhesive system used, the application of NaOCl increased microleakage along dentin margins.

Key words: sodium hypochlorite, microleakage, dentin.

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Bonding to dentin is a challenge in adhesive dentistry. It is difficult to obtain stable adhesion between dentin and resin due to the complex structure and biological activity of dentin.[6,13,25] Unlike enamel, which is an easily dried substrate, dentin is a vital and wet substrate where dentinal tubules are connected to the pulp.[4,6,13,26,27]

The high organic content of dentin, along with its tubular structure and outward flow of fluid, make dentin bonding difficult to attain.[6,9,12,13,31] It is clinically important to enhance the adhesion between dentin and adhesive resin. Such improved adhesive strength not only leads to better retention of restorations but also prevents marginal leakage, thus reducing the chance of developing secondary caries.[13,32] Penetration of bacterial products, acids, and even oral fluids at the interface between a restoration and a tooth have been regarded as detrimental to the longevity of the restorations.[20]

The adhesion between dentin and resin is described as micromechanical,[13,27,32] and it is generated by resin impregnation of the exposed collagen of demineralized superficial dentin. This transitional zone of resin-rein-

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Table 1 Sequence of the procedure for each group

Groups	37% phosphoric acid	10% NaOCl solution	10% NaOCl gel	Adhesive system	Composite resin
G1	15 s	–	–	Single Bond (3M, St. Paul, MN, USA)	Definite
G2	15 s	60 s	–	Single Bond	Definite
G3	15 s	–	60 s	Single Bond	Definite
G4	15 s	–	–	Prime & Bond NT (Dentsply, Petrópolis, RJ, Brazil)	Definite
G5	15 s	60 s	–	Prime & Bond NT	Definite
G6	15 s	–	60 s	Prime & Bond NT	Definite
G7	15 s	–	–	Gluma One Bond (Heraeus-Kulzer, Hanau, Germany)	Definite
G8	15 s	60 s	–	Gluma One Bond	Definite
G9	15 s	–	60 s	Gluma One Bond	Definite

forced dentin sandwiched between cured resin and unaltered dentinal substrate forms the hybrid layer.[17] However, a structurally weak area which could provide a microscopic space for leakage may exist between the resin and dentin, if the monomers failed to penetrate to the full depth of the demineralized dentin.[17,22,28] The phosphoric acid treatment of dentin can cause the collapse of exposed collagen fibers, making this surface more difficult to impregnate with adhesive monomers.[9,12,29,32]

Laboratory studies have evaluated the morphology and bond strength of adhesive systems to dentin which had been acid etched and subsequently treated with NaOCl or collagenase.[2,24,25] Investigators believe that the removal of collagen fibers results in a dentin surface similar to that of etched enamel.[2,25,29,30]

According to Cobb et al,[5] 5% sodium hypochlorite has been used to remove the exposed, unsupported collagen layer in order to increase resin penetration into the remaining partially demineralized dentin. Moreover, it facilitates resin encapsulation of exposed hydroxyapatite crystals to provide the micromechanical retentive properties of dentin adhesives needed to bond resin restorations to tooth structure.[5] Dissolution of collagen by deproteinization prior to adhesive bonding has been found to produce excellent bonding efficacy.[2,5,7,25,30,31,32]

Different application times and sodium hypochlorite concentrations have been used in in vitro studies. It has been reported that the higher the concentration of NaOCl, the greater the dentin bond strengths, until a plateau is reached at a concentration of 10% and an application time of 60 s.[19] In this study, we examined both forms of 10% NaOCl delivery systems – solution and gel – as the latter facilitates its clinical use.

The aim of the present study was to evaluate the effect of three different adhesive systems on microleakage of Class V composite resin restorations after the use of 10% sodium hypochlorite (gel and solution). The null hypothesis was that the presence of collagen in the demineralized dentin does not qualitatively contribute to the bonded tissue/resin assembly.

MATERIALS AND METHODS

Specimen Preparation

One-hundred eighty freshly extracted bovine incisors were selected, cleaned, and stored in distilled water prior to the study. Each tooth was horizontally sectioned approximately 7 mm from the cemento-enamel junction toward the crown using a double-face diamond disk (KG Sorensen, São Paulo, SP, Brazil). The contents of the pulp chamber and root canal were then removed with a dental explorer. A standardized Class V cavity (2.0 mm in diameter and 2.0 mm in depth) was prepared in the cemento-enamel junction on the buccal surface. A special diamond bur #2294 (KG Sorensen, São Paulo, SP, Brazil) in a high-speed handpiece (Kavo do Brasil S/A, Joinville, SC, Brazil) with constant water-spray cooling was used to prepare the cavities.

Bonding Procedure

The specimens were randomly assigned to 9 groups (n = 20). Before restoration, the cavities were sprayed with water in order to remove any detritus left from cavity preparation.

In all groups, the dentin was etched with 37% phosphoric acid (Dentsply, Petrópolis, RJ, Brazil) for 15 s, rinsed with water for 10 s, and lightly air dried for 2 s. In groups 2, 5, and 8, a 10% NaOCl solution (Proderma Farmácia de Manipulacao, Piracicaba, SP, Brazil) was applied with a dwell time of 60 s, rinsed with water for 30 s, and dried with absorbent paper. In groups 3, 6, and 9, a 10% NaOCl gel (FGM Produtos Odontológicos, Joinville, SC, Brazil) was applied following the same procedure as in groups 2, 5, and 8.

The two forms of 10% NaOCl delivery systems (solution and gel) and the respective adhesive systems are shown in Table 1. The adhesive systems were applied according to manufacturer’s instructions (Table 2).

The composite resin (Definite, Degussa, Hanau, Germany) was condensed in a bulk increment and light cured (Optilux 500/Demetron-Kerr, Danbury, CT, USA) for 40 s.

Table 2 Manufacturer's instructions for the adhesive systems

Adhesive system	Directions
Single Bond	Etched for 15 s, rinsed, lightly dried to leave moist surface, adhesive applied in two consecutive coats, dried for 5 s, light cured for 10 s.
Prime & Bond NT	Etched for 15 s, rinsed, lightly dried to leave moist surface, adhesive applied and left on for 20 s, dried for 10 s, light cured for 20 s.
Gluma One Bond	Etched for 20 s, rinsed, lightly dried to a moist surface, adhesive applied of two consecutive coats, dried for 5 s, light cured for 20 s.

Table 3 Microleakage values for cervical margin dentin

Groups	Median	Sum of ranks*
1. SB/control	1	1008.0[a]
2. SB/NaOCL solution	4	1802.0[c]
3. SB/NaOCl gel	3	1687.0[ab]
4. PB/control	1	1301.5[ab]
5. PB/NaOCL solution	4	1889.0[c]
6. PB/NaOCL gel	4	1963.0[c]
7. GOB/control	4	1744.0[bc]
8. GOB/NaOCl solution	4	1950.0[c]
9. GOB/NaOCL gel	4	1880.0[c]

* Different superscripts indicate significant differences.

Then the specimens were stored in a humidifier at 37°C ± 1°C for 24 h, and the composite resin restorations were polished with a graded series of Sof-Lex disks (3M Dental Products, St Paul, MN, USA).

Microleakage Test

The specimens were subjected to a thermocycling regimen (Instrumental MCT-2, São Paulo, SP, Brazil) of 1000 cycles in water between 5°C ± 2°C and 55°C ± 2°C with a dwell time of 60 s in each bath. After thermal cycling, all external surfaces of each tooth were coated with two layers of acid-resistant varnish, leaving a 1-mm-wide varnish-free margin around the restoration. The teeth were immersed in a 2% buffered solution of methylene blue for 4 h.

The teeth were then thoroughly rinsed under tap water for 10 min and sectioned longitudinally through the center of the restorations with a slow-speed diamond disk (KG Sorensen, São Paulo, SP, Brazil). Two independent observers analyzed the margins separately with an optical microscope (EMZ-TR Meiji Techno, Shanghai, China) at 50X magnification to determine the dye penetration at incisal and gingival margins. The following criteria were used to score dye penetration (Fig 1):

- 0 = no dye penetration
- 1 = dye penetration to 1/3 of the occlusal or gingival wall
- 2 = dye penetration to 2/3 of the occlusal or gingival wall
- 3 = dye penetration to full length of the occlusal or gingival wall
- 4 = dye penetration including axial wall

The scores (0 to 4) for each group were analyzed using Kruskal-Wallis and Mann-Whitney non-parametric statistical tests ($p \leq 0.05$).

RESULTS

The results of the Kruskal-Wallis and Mann-Whitney tests showed that dentin deproteinization with 10% NaOCL (solution and gel) significantly increased microleakage at dentin margins, depending on the adhesive system used ($p = 0.0129$). For enamel, there were no statistical differences among the groups ($p > 0.05$). The median microleakage scores, the sum of ranks for each treatment, and pairwise comparisons for the adhesive systems used on dentin are presented in Table 3.

For Single Bond (water/ethanol-based) and Prime & Bond NT (acetone-based), 10% NaOCL treatment as both solution and gel produced a significant increase in microleakage scores. For Gluma One Bond (acetone-based), 10% NaOCL treatment did not significantly affect microleakage values.

DISCUSSION

The use of phosphoric acid is widely accepted as a pretreatment procedure for improving the adhesion between enamel and resin.[14] However, resin adhesion is not as easily achieved to dentin as it is to enamel.[6,13] The adhesion mechanism for dentin bonding agents is generally believed to be micromechanical in nature, resulting from the penetration of resin into exposed collagen on an acid-demineralized dentin surface.[1,17]

Several researchers have considered the resin-impregnated hybrid layer as a weak link in dentinal bonding.[1,15,23,30] Thus, the role of collagen fibers in dentin adhesion has been questioned.[7,10,13,17,23,24,25,29-32] Some authors have reported that dentin collagen does not contribute to dentin adhesion and may even interfere with the bonding mechanisms because of the fragile structure of collagen fibers after etching.[1,10-12,14]

Gwinnett demonstrated that the collagen-rich zone offers no direct contribution to the interfacial bond

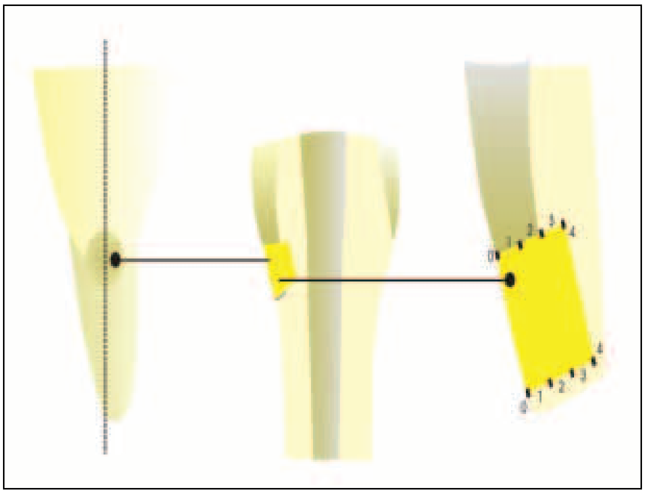

Fig 1 Microleakage scores for enamel dental margins.

strength.[11] The unprotected fibers may undergo hydrolysis after long-term exposure to water, leading to deterioration of adhesion between resin and dentin and thus to decreased bond strength.[17] There appear to be ways to promote adhesion to dentin. The first is to improve monomer impregnation into the substrate, and the second is to increase the diffusivity or penetrability of the dentinal substrate itself.[17,29]

The findings of this study have demonstrated that the application of 10% NaOCl, in gel or solution, increased the microleakage of composite resin restorations using Single Bond and Prime & Bond NT. With Gluma One Bond, 10% NaOCL treatment did not significantly affect microleakage values. Those results were not expected, because 10% NaOCL treatment alters the dentin surface, changing the hydrophilic properties that might influence the intimate attachment at the interface.[19]

In the present study, three one-bottle adhesive systems were used. They did not necessarily require an extra dentin-deproteinizing step, which is considered to be detrimental to bonding. Similar results were found by Frankenberger et al: sodium hypochlorite treatment after etching dentin exhibited detrimental effects on marginal adaptation of totally-bonded direct composite resins, notwithstanding the adhesive system tested.[8] Another study[7] showed that the application of NaOCl for at least 2 min improved the bond strength, but did not improve the seal of the restoration.

Uno et al showed that the removal of the collagen layer with NaOCl enhanced the bond strength between the adhesive resin and dentin for All-Bond 2 but not for Scotchbond Multi-Purpose. This may indicate more efficient penetration of SBMP primer compared to AB2 primer.[30]

In accordance with the results obtained in the present paper, it should also be mentioned that some substances may affect resin polymerization or compete for space inside the hybrid layer. These substances may be water, either present or injected when a wet-bonding technique is used, and residual amounts of solvents such as ethanol and acetone.[13] One hypothesis is that the application of 10% NaOCl considerably influences adhesive systems containing ethanol and acetone by interfering with the wettability of dentin surfaces. Dental tissues are inherently moist, and this state should be preserved and recognized as an important part of the bonding protocol.

According to Pioch et al, the tensile bond strength measurements indicate that a reduction of bond strength is not generally a consequence of pretreatment with NaOCl.[21] Conversely, another study[16] found that the presence of the reactive residual free-radicals in dentin treated with sodium hypochlorite may compete with the propagating vinyl free-radicals generated during light activation of the adhesive. This results in premature chain termination and incomplete polymerization. It might explain the increase in microleakage of restorations observed in the present study.

The removal of the collagen layer using NaOCl can jeopardize the quality of the hybrid layer, perhaps increasing the microleakage.[18,30,34] Young's modulus of elasticity in the hybrid layer is lower than the one in restorative resin. For this reason, it acts as an inherent elastic buffering layer that is able to absorb the resin composite's curing contraction stress.[30] The absence of a hybrid layer could thus interfere with the integrity of marginal adaptation. Another hypothesis for the increase in microleakage is that specimens were subjected to thermocycling, which may have accelerated the degradation of the marginal seal, independent of the NaOCl treatment.

The presence of the hybrid layer has been described previously and has been shown to have a significant effect on the strength of the adhesive.[6] Some investigators believe that the permeability or porosity of the demineralized dentin surface is critical to the creation of resin-infiltrated zones by modifying the depth to which resins can penetrate.[17,34] However, several researchers have demonstrated that collagen layer removal using NaOCl enhanced the bond strength between the resin and dentin.[2,24,25,32] It is necessary to determine whether or not the absence of collagen improves resin infiltration, and in long-term evaluations, whether or not it promotes adhesive strength and marginal seal at the interface between the resin and dentin.

Other studies should be developed to confirm the exact function and longevity of the hybrid layer on marginal seal. The use of NaOCl is controversial; therefore, it is necessary to consider the adhesive system, the dentin wettability, and the ability of the resin to penetrate the substrate. Dentin bonding systems are particularly sensitive to technique and treatment of substrate. According to our results, the quality and/or integrity of the hybrid layer may be of fundamental importance to the integrity of marginal adaptation.

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Clinical relevance: The integrity of the hybrid layer is of fundamental importance to obtaining high-quality marginal adaptation. The application of 10% NaOCl – which removes collagen – on dentin substrates is questionable in clinical use.