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Effect of sodium hypochlorite as dentinal pretreatment on bonding strength of adhesive systems

Anelise Fernandes Montagner[1], Jovito Adiel Skupien[1], Marciano Freitas Borges[1], Ivo Krejci[2], Tissiana Bortolotto[2], Alexandre Henrique Susin[1],
[1] Department of Restorative Dentistry, Federal University of Santa Maria, Santa Maria, RS, Brazil
[2] Department of Cariology and Endodontology, University of Geneva, Geneva, Switzerland

Correspondence Address:
Alexandre Henrique Susin
Department of Restorative Dentistry, Federal University of Santa Maria, Santa Maria, RS
Brazil

Abstract

Aim: This study aimed to evaluate the effect of sodium hypochlorite (NaOCl) pretreatment on bond strength of adhesive systems to different dentin regions. **Materials and Methods:** Forty human molars were randomly assigned according to different adhesive systems (Adper Single Bond 2; Clearfil SE Bond; Adper SE Plus; G-Bond), pretreatments (control and NaOCl - deproteinization) and dentin regions (proximal, superficial occlusal [SO] and deep occlusal [DO]), $n = 5$. Cylindrical cavities were performed at the proximal and occlusal surfaces of each sample. For deproteinization, 10% NaOCl was applied on conditioned dentin for 60 s before the adhesive systems application. Two occlusal and one proximal slice were obtained from each sample and submitted to the push-out test. The mode of fracture was analyzed. The data were subjected to three-way ANOVA and Tukey test ($P < 0.05$). **Results:** There was statistically significant difference between the adhesive systems ($P < 0.01$) and dentin regions ($P < 0.01$); however, the pretreatment did not significantly affect the bond strength values ($P > 0.05$). The bond strength values were higher for the proximal surface, followed by SO and later by DO, being influenced by the adhesive system. The two-step self-etch adhesive systems presented the highest bond strength results. **Conclusion:** The deproteinization pretreatment showed similar bonding effectiveness to the conventional adhesive technique. The dentin region plays a rule on the bond strength values.

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Full Text

Studies have shown that incomplete adhesive monomer infiltration on demineralized dentin zone could result in exposed collagen network not impregnated by the monomer, and this could occurs regardless of the system applied, with both etch-and-rinse or self-etch.[1] The exposed collagen fibrils are susceptible to hydrolysis, resulting in an irreversible process of adhesive interface degradation. Nanochannels are produced by retrograde flux from nonsupported fibrils to inside the hybrid layer which had the collagen fibrils decayed by hydrolysis activity. This process is associated to the nanoleakage and interferes on the longevity of adhesive interface long-time.[2] Scientific evidences suggest the removing of exposed collagen fibrils as an alternative to the conventional adhesive protocol.[3],[4] Substances (sodium hypochlorite [NaOCl] and collagenase) are able to dissolve collagen after dentin etching promote the dentin deproteinization by removing collagen from the etched dentin surface. Further, this technique leaves the dentin substrate rich in apatite, likely to condition enamel morphologic characteristics.[5],[6] The NaOCl is a nonspecific proteolytic agent that breaks down organic compounds in a clinical coherent time.[4] Collagenase is a specific enzyme for dissolving the collagen, therefore, removes only collagen fibers and not proteoglycans and noncollagenous proteins, which are also present on the etched dentin surface.[7]

Removing unsupported collagen fibers can be beneficial by easing the spread of primer and adhesive through the demineralized dentin. Pretreatment with NaOCl gives the surface microporous and irregular characteristics, resulting in a more permeable substrate that facilitates the diffusion of adhesive monomer.[3] Moreover, the use of NaOCl alters the ultrastructural morphology of etched dentin increasing the wettability of the substrate, the tube penetration length, the number of tags and lateral branches,[3],[5],[7] widens the opening of the tubules, from 1.8 to 4.0 μm, due to loss of peritubular dentin and reduction of intertubular dentin spaces.[3],[8],[9]

With the deproteinization protocol, a reverse hybrid layer is formed, which presents different characteristics from the traditional.[3],[5],[6] Since, the interaction between deproteinized dentin and adhesive system affects the hybrid layer formation, the effects of this protocol could be different among the current adhesive systems. Thus, the objective of this study was to evaluate the influence of 10% NaOCl application on the bond strength of current adhesive systems, at different dentin regions.

Materials and Methods

This in vitro study was approved by the Local Ethics Committee in Research (protocol n o 0248.0.243.000-09). Forty extracted human third molars caries-free were cleaned and stored in a 0.5% thymol aqueous solution (5°C), until the used in this study. The teeth were divided into 24 experimental groups (n = 5), according to the adhesive system: A etch-and-rinse (Adper ™ Single Bond 2 [ASB2], 3M ESPE, St. Paul, MN, USA) and three self-etch (Clearfil ™ SE Bond [CSE], Kuraray Medical Inc., Osaka, Japan; Adper ™ SE Plus (ASE), 3M ESPE, St. Paul, MN, USA; G-Bond ™ (GB), GC Corp., Tokyo, Japan) [Table 1], to the pretreatment: Control or deproteinization (NaOCl application) and to the dentin region: Proximal surface or superficial and deep occlusal (DO) surface. Random allocation of the 40 teeth into 24 groups (n = 5) was guarantee using of Random allocation software (Version 1.0, University of Isfahan, Isfahan, Iran). It was generated a table with a random sequence, numbered from 1 to 40, and the completion of the steps followed that sequence.{Table 1}

The roots of the teeth were embedded, approximately 3 mm below the cement-enamel junction, in PVC tubes, 25 mm diameter and 1.5 cm height, with acrylic resin. Two circular cavities, an occlusal and a proximal, were performed on each specimen with a cylindrical diamond bur 2094 (KG Sorensen Ind. e Com Ltda., Kotia, SP, Brazil), resulting in cavities of 2 mm in diameter, 4 mm in depth on the occlusal surface and 3 mm on proximal surface [Figure 1]. Each bur was used to perform 10 cavities. The diamond bur was adapted on an apparatus with high speed under running water to perform standardized cavities and to reduce the interference of the operator's handling. One previously trained operator performed all the cavities. {Figure 1}

Immediately after cavities preparation, the surface was treated following the dentin pretreatments. Control: The adhesive systems were applied on the dentin surface following the manufacturers' instructions. Deproteinization: 35% phosphoric acid gel (Scotchbond ™ Etchant, 3M ESPE, St. Paul, USA) was applied on the surface for 15 s, washing was performed for 30 s, drying with absorbent paper, and an aqueous solution of 10% NaOCl (Novaderme Pharmacy, Santa Maria, RS, Brazil) was applied, remaining 60 s in contact with the surface. Subsequent washing was performed with abundant air/water spray for 30 s, and them the adhesive technique proceeded as recommended by the manufacturers.

The restorative procedure was performed with a composite resin Filtek ™ Z250, shade A2, (3M ESPE, St. Paul, MN, USA), which was incrementally inserted. Each increment was individually light-cured for 20 s with a light emitting diode with 800 mW/cm 2 (LED, Olsen Ind. e Com SA, Palhoca, SC, Brazil). Afterward, the specimens were stored in distilled water, at 37°C temperature, for 24 h.

In order to obtain the dentin slices, the specimens were fixed in a metallic base, and two longitudinal cuts were performed on the proximal surface. The first slice was discarded, and then the second slice was achieved with 1 mm thick. To obtain samples from occlusal surface, the first slice was also discarded; the second was obtained from a section of dentin-resin of superficial dentin and the third one from a section of dentin-resin of DO [Figure 1]. The cuts were made with diamond disc, 0.3 mm thick, at 170 rpm, in the cutting machine Labcut 1010 (ERIOS, Technical and Scientific Equipment Ltd., São Paulo, SP, Brazil), under running water.

Each slice was positioned on a metallic device with a central opening (Ø =4 mm), larger than the restorations diameter. For the push-out test, a metallic cylinder (Øextremity = 1.05 mm) induced load in a central portion of the restoration. The test was performed in a universal testing machine (DL 2000, EMIC, São José dos Pinhais, SP, Brazil) with the load cell of 100 kN, at 1 mm/min of speed until failure occurred.

The bond strength (σ) in MPa was obtained with the formula σ = F/A, where F = load for specimen rupture (N) and A = bonded area (mm 2). To determine the area, the formula to calculate A = 2.π.r.h where, A = interfacial area, π =3.14, R = radius, and h = thickness of slices. The value of R was standardized by half the cavity diameter, equal to 1 mm. While, the height (h) was measured prior to extrusion test with a digital caliper (King Tools, Electronic Digital Caliper, Mooca, SP, Brazil).

The specimens were examined using a ×25 magnification light stereomicroscope (Carl Zeiss do Brazil Ltda., Rio de Janeiro, RJ, Brazil), and each fractured surface was allocated to predominant fracture mode type occurred, which were classified as adhesive failure-fracture occurred at the bond line between the dentin and resin; cohesive failure in dentin; cohesive failure in composite resin.

Some representative fractured specimens were selected to scanning electron microscope (SEM) analysis. The specimens were chemically fixed by immersion in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer for 6 h, and then dehydrated in an ascending series of ethanol: 50%, 75%, and 90% for 5 min, and 100% ethanol for 3 h. After that, the specimens were dried at room temperature. The specimens were sputter-coated with gold (Desk II Denton Vacuum, Moorestown, NJ, USA) and evaluated in a SEM JEOL A110 (JEOL Inc., Tec., Tokyo, Japan) at ×15 to ×300 magnifications, according to the interesting area.

Statistical analysis

The nominal values of bond strength, in MPa, were tabulated in and analyzed using SPSS Program (Statistical Package for Social Sciences, version 18.0, Chicago, IL, USA). Normality of distribution was verified with the Shapiro–Wilk and Levene's testing to the homogeneity of intervals. Data were compared among the different experimental groups by three-way ANOVA and a Tukey post-hoc test, considering a significance level of 5% ($P < 0.05$). Cohesive failures were not included on the analysis.

Results

The three-way ANOVA showed that dentin region ($P < 0.01$) and adhesive system factors ($P < 0.01$) significantly affected the bond strength values. However, the pretreatment factor ($P = 0.06$) did not show statistically significance influence on the bond strength values. The interaction dentin region/adhesive ($P < 0.01$) significantly affected the bond strength, since the other interactions dentin region/pretreatment ($P = 0.59$), adhesive/pretreatment ($P = 0.21$), and region/adhesive/pretreatment ($P = 0.32$) did not significantly affect it [Table 2].{Table 2}

Regarding the dentin regions, the proximal dentin showed the highest bond strength values, superior to occlusal surface, which obtained the higher values in superficial than in deeper surface, but these results were influenced by the adhesive systems. Regarding the adhesive systems, the two-bottles self-etch adhesive systems (CSE and ASE) showed the better results while the all-in-one self-etch adhesive (GB) showed the lower bond strength values.

After testing, three specimens (2.5%) showed resin cohesive failures [Figure 2]a in resin, 8 specimens (6.8%) showed dentin cohesive failures [Figure 2]b in dentin; and most of the specimens, 109 (90.8%), had adhesive failures [Figure 2]c and [Figure 2]d.{Figure 2}

Discussion

In the present study, deproteinized dentin region showed no difference in bond strength values when compared to control. This finding diverging from some studies that indicated that NaOCl increased the bond strength of certain adhesive systems, reaching similar values to enamel bond strength.[6],[10] However, in none of those studies the mechanical test was push-out. Nevertheless, the results of this study support others, laboratory [9] and clinical,[11] studies.

Some studies have shown a strong interference of the type of adhesive system on the bond strength values, with NaOCl protocol use.[3],[7] An explanation for those results can be attributed to factors intrinsic of adhesive systems. Saboia et al.[12] tried to establish the association between the solvents and bonding to the deproteinized substrate, since the acetone-based adhesives can be favored by this technique. The high diffusion rate of this solvent could promotes the volatilization of the residual NaOCl, besides the greater ability to displace water.[6],[11],[12] The trend of the ethanol-based adhesive not responds similarly when applied to deproteinized surface, suggesting that these systems can be adversely affected by the collagen removal.[3],[12] However, in this study, for the ASB, an ethanol-based adhesive system, the pretreatment did not affect its bonding performance.

The formation of the hybrid layer has been considered the most efficient mechanism of interaction between the dental structure and adhesive systems.[13] However, the results of this study demonstrate that the presence of a classical hybrid layer may not be crucial for an adequate resin-dentin adhesion,[6],[13] as the deproteinized dentin leads to a structure different from conventional, creating a reverse hybrid layer.[3] The deproteinized dentin presents similar characteristics to the intact dentin. Nonetheless, this treatment changes the dentin properties by eliminating the exposed collagen, which is responsible by providing toughness and elasticity to dentin, and it decreases the elastic modulus and hardness of dentin in about 75%.[14] When the adhesive interface is stressed, the hybrid layer regulates the peak of stress concentration, thus, speculation has been made regarding the influence of classical hybrid layer absence in the forces absorption.[14],[15] In this study, the number of cohesive fractures in control and NaOCl groups were similar, indicating that the stress concentration at the interface was possibly similar for both pretreatments.

Dentin region represented a factor that significantly affected the bond strength. The proximal surface showed higher bond strength values than the occlusal surface; however, it was adhesive dependent. These findings corroborated a previous study,[16] indicating that the dentinal tubules direction is an important variable in determining the dentin bond strength.[17] Dentin is a hydrated biological complex with the microstructural design, which can represent one of the reasons why the bond strength is not uniform inside the cavity walls.[16],[18] The present study confirms that the different regions of substrate are important factors in the adhesive performance, since for some adhesive systems, superficial occlusal dentin showed higher bond strength values than deeper dentin, but smaller than the proximal dentin. It was shown that the bond strength may decreases up to 50% from superficial to deep dentin.[19]

Several structural components and properties of dentin can affect the resin-dentin adhesion. The difference in the bond strength values trends to decrease when the smear layer is left intact, since this layer is able to decrease the permeability,[20] which was noticeable with the use of self-etch adhesive systems, particularly with the GB that provided similar bond strength values among the dentin regions, however, showed the lowest values among the adhesive systems.

Based on the conception of the interaction between the adhesive and deproteinized dentin could be speculated that the effect of deproteinization would be more evident in deeper areas. As in those regions, the intertubular dentin is available in minor quantities, and the tubules are more numerous, spacious and opening. Thus, the contribution of resin tags and lateral branches in reticular adhesive matrix formation could be more significant in the resin retention.[3],[5],[8],[19] However, this aspect was not verified in this study, as the interaction dentin region/pretreatment did not influence the bond strength, and the deeper dentin showed lower bond strength values for both pretreatments.

In the push-out test, the load compression results in shear at the bond interface, whose failure is observed by extrusion of the resin-restoration, producing a more clinical relevant condition. The NaOCl technique as dentin pretreatment is still a controversial topic, mainly as the results could be dependent on the adhesive system used. The adhesive degradation has been attributed to combine deterioration of resin polymer and collagen.[3] As the hydrolytic deterioration is inherent to the polymeric material; it is promising an intervention action on collagen deterioration. Therefore, the dentin deproteinization could be a way to reduce the technique sensitivity by eliminating the collagen network without compromising the adhesion, but more studies should be carried out. The surface treated with NaOCl can demonstrate greater,[10],[12],[14] lower,[3],[19] or similar [9] performance, as in this study, the values obtained with the application of same adhesive on dentin with collagen.

Within the limitations of this study, it is possible to conclude that the deproteinization pretreatment did not show any beneficial presenting similar bonding effectiveness to the conventional adhesive technique. The dentin region straight influences the dentin bond strength and suggest that resistance is not uniform inside the cavity walls, and the region of application influenced the adhesive systems bond effectiveness.

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Conflicts of interest

There are no conflicts of interest.

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