The importance of dentin collagen fibrils on the marginal sealing of adhesive restorations: An in vitro study

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Abstract

Aim: This study is aimed to evaluate the importance of collagen fibrils in the adhesion of different self-etching adhesive materials to dentin.

Methods: Sixty Class V restorations were prepared on the buccal and lingual surfaces of 30 recently extracted human premolars, with the cervical margins in dentin and the occlusal margins in enamel. These restorations were distributed to three groups of 20 cavities each based on the employed adhesive system used: Group A: G-Bond (GC, Corporation Tokyo, Japan); Group B: Xeno V (Dentsply Caulk Milford, DE, USA); Group C: Adper Easy One (3M/ESPE). Each group was subdivided according to dentin treatment: Subgroup 1 - manufacturer’s adhesive protocol and Subgroup 2 - Removal of the collagen fibers (total etch + sodium hypochlorite 5% for 2 min) + adhesive protocol. After the restorations, the teeth were subjected to thermal cycling, washing and scoring according to dye penetration. Dye penetration was evaluated using stereomicroscope.

Results: When the dentin microleakage scores were compared in subgroups A1VsA2, B1VsB2 and C1VsC2, then Mann–Whitney test revealed significant differences between Subgroup A1 and Subgroup A2 (P < 5%). There was no significant difference in Groups B and C.

Conclusion: The results revealed that the collagen fibrils are not required for adhesion, and their removal improves the marginal seal of G-Bond, an acetone based dentin bonding system.

Keywords

Adhesion, collagen fibril, NaOCl, solvent

Introduction

A major problem in restorative dentistry is that we are yet to invent dental materials that adhere efficiently to the natural tooth structure. Non-adherence of restorative materials leads to marginal leakage can allow the ingress of bacteria and salivary components, causing pulp damage, marginal staining, and secondary caries. The adhesive bonding of resin materials to etched enamel is a proven technique with long-term clinical success for the prevention of microleakage and the retention of esthetic restorative materials. However bonding to dentin remains less reliable and less predictable. Even with the introduction of more advanced dentin bonding systems, microleakage has been reduced, but not eliminated. Marginal discoloration, recurrent carious lesions and post-operative sensitivity are the frequent outcomes of the penetration of oral fluids and bacteria through gaps formed within the restoration/cavity interface toward the pulp.

The mechanism of dentin bonding with most adhesive systems depends on hybridization. In this course of action, the dentin surfaces are acid etched that promote the elimination of the smear layer, dentin demineralization and disclosure of the collagen fibril network. Penetration of the adhesive agent into the exposed collagen network and subsequent hybrid layer formation makes adhesion possible. Any collapse of the collagen matrix as a result of over-drying might prevent monomers from penetrating into deeper areas, increasing the risk of adhesive failures. The dentin surface should also not be too wet, as excess water limits penetration and behavior of the adhesive systems. Incomplete diffusion of the adhesive systems acts on the quality of dentin adhesion and might cause porosities and sub-micrometrics spaces. This would create collagen exposure...
in the tooth/restoration interface, resulting in continued degradation.[8–10]

Several authors have reported that the exposed collagen fibrils, which are in a disestablished and vulnerable stage to the proteolytic degradation,[11,12] have questionable durability over time.[13]

Vargas et al. concluded that the collagen layer may not be crucial to adhesion between resin/dentin, and its removal eliminates failures in the tooth restoration interface.[14] In order to avoid negative consequences related to the organic content of this tissue, the use of proteolytic substances on etched dentin has been suggested.[14,15] The use of deproteinizing solutions (NaOCl) modifies the ultra-morphology of demineralized dentin surface by dissolving the exposed collagen fibrils. The action of NaOCl boosts the exposure of a lateral runway network and amplifies the dentin tubules,[16,17] rendering a dentin similar to etched enamel, which is a favorable characteristic for adhesion. This surface has shown multiple irregularities, with good mechanical retention of the adhesive in modified dentin substratum.[14,17]

This study verified the influence of collagen in the bonding of dentin to different adhesive materials by studying the effect of its removal on the marginal seal of simulated restorations in cavities on buccal and lingual surfaces.

Materials and Methods

This study was conducted in the Department of Conservative Dentistry and Endodontics.

Thirty recently extracted human non-curious premolars without enamel fractures were cleaned and stored in a saline solution of NaCl (0.9%) at room temperature until use. The specimens were collected from the Department of Oral and Maxillofacial Surgery.

Two Class V cavities, one on the lingual and other on the buccal surfaces (width - 3 mm, deep - 1.5 mm, high - 2 mm), with the cervical margin in dentin, were prepared in each tooth, with buccal surfaces (width - 3 mm, deep - 1.5 mm, high - 2 mm), with running water, cleaned, dried and sectioned into two faces, mesial and distal, using diamond disks. The hemi-section which has the clearest chemical marker micro-leakage from each section was evaluated with a stereoscopic microscope with ×10 magnification.

Micro-leakage was scored according to the criteria described in Table 2. Leakage was scored beginning at gingival cavo surface margins. Results are tabulated and analyzed using Mann–Whitney test and evaluated for significance.

Results

This study assessed and compared the microleakage at the cemental cavosurface – restoration junction for the composite resin restorations using three adhesive systems with and without NaOCl application. The microleakage was compared on a scale of 0-3 [Table 2]. The Data were analyzed using Mann–Whitney test and evaluated for significance.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Number of restorations</th>
<th>Adhesive system</th>
<th>Resin composite</th>
<th>Presence of collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>10</td>
<td>G-Bond</td>
<td>Z100</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>GC, Corporation Tokyo, Japan</td>
<td>3M/ESPE</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>10</td>
<td>Xeno V</td>
<td>Z100</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>Dentsply Caulk, Milford, DE, USA</td>
<td>3M/ESPE</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>10</td>
<td>Adper</td>
<td>Z100</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>3M/ESPE, St. Paul, MN, USA</td>
<td>3M/ESPE</td>
<td>No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absence of penetration of chemical marker</td>
</tr>
<tr>
<td>1</td>
<td>Penetration of chemical marker on cervical wall</td>
</tr>
<tr>
<td>2</td>
<td>Penetration of chemical marker on cervical and axial wall</td>
</tr>
<tr>
<td>3</td>
<td>Penetration of chemical marker into pulp wall</td>
</tr>
</tbody>
</table>
Data showed application of NaOCl while using G-Bond reduces the microleakage significantly [Table 3], whereas Xeno V and Adper showed no difference in microleakage scores with or without NaOCl application [Tables 4 and 5].

**Discussion**

The mechanism of dentin bonding to most adhesive systems depend on hybridization. In this course of action, the dentin surfaces are acid etched (total etch technique) that promote the elimination of the smear layer, dentin demineralization and disclosure of the collagen fibril network. Penetration of the adhesive agent into the exposed collagen network and subsequent hybrid layer formation makes adhesion possible.[19] Penetration of the adhesive resin into the exposed fibrillar collagen network of the demineralized dentin zone results in the formation of a micromechanical interlock known as the hybrid layer.[18]

Scientific works demonstrating the enhancement of adhesion after removal of the collagen fibrils, or even revealing a similar statistics between hybridization and deproteinization, resulted in many authors coming to the same conclusion: collagen is not necessary for obtaining effective adhesion.[13,19,21]

The dissolution of organic dentin content promoted different behaviors, based on the adhesive system employed.[3] Previous studies[22,23](Saboia et al., 2000; Saboia et al. 2002) have showed that the use of sodium hypochlorite to remove collagen may decrease microleakage at the resin/dentin interface and increase bond strength between resin and dentin when using an acetone-based adhesive system. Hence, an acetone-based adhesive system and two water-ethanol based adhesive systems were used in this study.

Wakabayashi et al. (1994) indicated that bond strengths after long-term water immersion were significantly higher for those specimens where the collagen was removed after acid conditioning, suggesting that degradation of the bond was due to the hydrolysis of unprotected collagen fibers.[24] In the combined phosphoric acid and sodium hypochlorite treatment technique used herein, the dentin surface was first decalcified to expose the collagen. The exposed collagen was then dissolved with NaOCl, and adhesive resin was directly applied to the dentinal apatite exposed on the dentin surface as first described by Uno and Finger (1995).[25] The hypothesis verified on this work was that the collagen removal would result in increased dentin permeability, and the adhesive could penetrate more into the substrate, resulting in a more homogeneous adhesive interface of uniform thickness. In this way, the whole band of demineralized and deproteinized dentin would be impregnated by adhesive.[22]

Collagen removal by NaOCl treatment increases the surface roughness of dentin and its wettability.[26] Inai et al. (1998) showed that deproteinization exposes a labyrinth of lateral secondary tubules that were not observed on etched dentin surfaces, which could cause some increase in wettability.[27] After deproteinization, dentin turns into a porous structure with multiple irregularities that allows for good mechanical retention.[28] This substrate is also rich in hydroxyapatite crystals, and from a crystallographic viewpoint, it is similar to enamel.[29] It can give a reliable interface over time because of its high mineral content. The polymerization of the resin inside the porous surface ensures a solid anchorage for resin composite restorations and probably ensures for an adequate sealing of the dentin.[26]

In contradiction, several authors have reported detrimental effects NaOCl pretreatment on bond strength and marginal adaptation. Frankenberger et al. (2000) revealed that Sodium hypochlorite treatment of etched dentin gave destructive effects on marginal adaptation of totally bonded direct composite resins, irrespective of the adhesive system tested.[19] Perdigao et al. (2000) reported that the intermingling of the adhesive monomers with the filigree of collagen fibers or hybrid layer should still be considered the paramount dentin bonding mechanism.[30]

Hence, the present study was conducted to know the importance of dentin collagen fibrils on the marginal sealing of adhesive restorations by using 5% NaOCl for 2 min for removal of collagen fibrils from dentin.

The results of the present study showed that removal of collagen significantly reduced the scores of microleakage for the acetone-based adhesive system G-Bond (Mean = 1.4000).

This can be explained by three factors:

1. The higher diffusibility of the acetone as well as its higher ability to displace water (Jacobsen and Soderhold, 1995). These factors could improve the contact of the monomer with the irregular intertubular dentin structure exposed by NaOCl treatment, resulting in a homogeneous interface with no voids.[23]

2. After the placement of composite resin there were no collagen fibers directly exposed to the oral environment. Consequently, the degradation of adhesive interface by
hydrolysis, which probably would start by exposed non hybridized collagen, (Sano et al., 1999) in which the dentin bonding agents do not fully diffuse through; which is potentially be a weak link in the long term adhesion of dentin to resin.[23]

3. The higher acetone level in G-Bond would be in affecting the solvent’s ability to promote the volatilization of free radicals of oxygen released by NaOCl, which could interfere with the bonding agent polymerization process.[31]

The decrease in microleakage observed when the G-Bond adhesive system was applied on collagen-depleted dentin (A2) compared to the control group (A1) can reinforce the hypothesis that NaOCl treatment would promote better contact between bonded surfaces. This results in an interface that resists the challenge of the oral environment simulated herein by thermal cycling and water storage. When the collagen layer was left intact (A1), all specimens restored with G-Bond/Z100 showed some degree of microleakage, and in 50% of the specimens, the dye reached into the axial wall. These findings could be related to the degradation of exposed collagen, as suggested by Sano et al.[32]

In the present study, water-ethanol based adhesive systems (Xeno V and Adper) had no influence on microleakage when collagen is removed. This result may be related to the inability of solvents of this adhesive (ethanol/water) to promote residual hypochlorite volatilization.[17] Under the experimental conditions of this study, there were no statistical differences between groups treated or not treated with NaOCl. The short dwell time of the water-ethanol based adhesive systems (Xeno V and Adper EasyOne) might have been insufficient to permit a full diffusion of the monomer into the substrate. In this way, nanometric porosities of intertubular dentin created by NaOCl treatment were not reached by monomer, leaving the adhesive interface with voids. These voids are similar to those left in the hybrid layer after hydrolysis.[35]

The present study is in accordance with the study done by Nikaido et al.[33] the NaOCl oxidized some of the dentin matrix components that may interfere with the polymerization initiation of some adhesive systems, including Xeno V and Adper Easy One. Residual free radicals of sodium hypochlorite in dentin may compete with free vinyl radicals generated during adhesive photactivation, resulting in incomplete polymerization due to a premature terminal of polymeric interactions.[34] For the ethanol-water adhesive systems (Xeno V and Adper Easy One) the presence or absence of collagen, voids would form at the adhesive interface, resulting in marginal defects. Similar results obtained by these techniques can reinforce this hypothesis.[35]

Therefore, this study verifies that the technique of the fibril collagen removal can represent a valid source for the optimization of adhesive protocol. Even though it results in an extra clinical step, the use of fibril collagen removal in the restorative practice would be justified for acetone based bonding systems, since adhesion longevity and effectiveness were definitely enhanced, depending on the kind of adhesive system employed. However, in vivo studies are required to prove the efficacy of this technique. Conclusion

Within the limits of the present in vitro study, it can be concluded that:

1. Removal of dentin collagen fibrils by NaOCl application improves the marginal seal between resin and dentin when using an acetone-based adhesive system.
2. Application of NaOCl is not necessary to improve the marginal seal between ethanol/water-based adhesive system and dentin.

References
