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The Importance of Dentin Collagen Fibrils on the Marginal Sealing of Adhesive Restorations

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Clinical Relevance

The results of this research showed that, with regard to marginal seal, collagen fibrils were not essential to obtaining an optimal adhesive performance. Deproteinization should be considered as a bonding pre-treatment.

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SUMMARY

This study evaluated the importance of the union between dentin collagen and three different adhesive materials. 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: Single Bond; Group B: Prime&Bond NT; Group C: One Coat Bond. Each group was subdivided according to dentin treatment: 1) manufacturers' adhesive protocol and 2) removal of the collagens fibers (total etch + sodium hypochlorite 5% for two minutes) + adhesive protocol. After the restorations were completed, the teeth were stored in saline solution (24 hours/37°C), subjected to thermal cycling, washing and scoring according to dye penetration. Dye penetration was evaluated, with the numbers ranging from 0 (no infiltration) to 3 (greatest infiltration). When

the dentin microleakage scores were compared in subgroups A1XA2, B1XB2 and C1XC2, the Mann-Whitney Test revealed significant differences between groups B and C ($p<5\%$). The better results were shown in subgroups B2 ($p=0.0345$) and C2 ($p=0.0029$). The results showed that the collagen fibrils were not necessary for adhesion, and their removal positively influenced the marginal sealing of Prime&Bond NT and One Coat Bond.

INTRODUCTION

The mechanism of dentin bonding to most adhesive systems is based on hybridization. In this process, the dentin surfaces are treated with acid etching agents that promote removal of the smear layer, dentin demineralization and exposure of the collagen fibril network. Adhesion is possible due to penetration of the adhesive agent into the exposed collagen network and subsequent hybrid layer formation.

The total acid etch technique modifies the collagen matrix structure, contributing to the sensitivity of this technique with regard to adhesive procedures. In order to avoid collapse of the collagen fibrils, the greatest sensitivity comes from the fact that the etched surface should not be too dry. The dentin surface should also not be too wet, as excess water limits penetration and behavior of the adhesive systems.[1] 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.[2] Incomplete diffusion of the adhesive systems acts on the quality of dentin adhesion and might cause porosities and sub-micrometrics spaces.[3] This would create collagen exposure in the tooth/restoration interface, resulting in continued degradation.[3-5]

Several authors have reported that the exposed collagen fibrils, which are in a disestablished and vulnerable stage to the proteolytic degradation,[6-7] have questionable durability over time.[8] Vargas and others[9] concluded that the collagen layer may not be crucial to adhesion between resin/dentin, and its removal eliminates failures in the tooth restoration interface.

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.[8,10] The use of deproteinizing solutions (NaOCl or collagenase) alters the demineralized dentin surface ultramorphology by dissolving the exposed collagen fibrils. The action of NaOCl promotes the exposure of a lateral runway network and amplifies the dentin tubules,[11-12] 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.[9,12]

This study verified the importance of collagen in the union 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.

METHODS AND MATERIALS

Thirty recently extracted human non-carious premolars without enamel fractures were cleaned and stored in a saline solution of NaCl (0.9%) at room temperature until use. The specimens were obtained from the Federal University of Pernambuco Bank of Human Teeth—Ethics Committee sight CCS-CEP 150/2004. Two cavities on the lingual and buccal surfaces (width—3 mm x deep—1.5 mm x high—2 mm), with the cervical margin in dentin, were prepared in each tooth, with the treatment assigned according to Table 1.

The restorative systems were utilized according to the manufacturers' specifications. The conventional adhesive protocol was performed on subgroup 1. Subgroup 2 received the conventional adhesive protocol after collagen removal. This protocol was performed with an application of phosphoric acid (37%) for 15 seconds, rinsing for 15 seconds, blot-drying for two seconds, applying sodium hypochlorite (Phormula Ativa) for two minutes, then washing for two additional minutes prior to application of the adhesive system and resin composite.

The restored specimens were stored for 24 hours in a saline solution of NaCl (0.9%) (37% with air relative humidity of 100%) to allow the resin composite to expand hygroscopicly.[13] Finishing and polishing of the restorations was accomplished with the assistance of

Table 1: *Groups of Teeth According to the Dentin Treatment Techniques and Employed Adhesive Systems*

Group	Subgroup	# of Restorations	Adhesive System	Resin Composite	Presence of Collagen
A	1	10	Single Bond (3M/ESPE St Paul, MN, USA)	Z250 (3M/ESPE)	yes
	2	10			no
B	1	10	Prime & Bond NT (Dentsply Caulk Milford, DE, USA	Esthet X (Dentsply)	yes
	2	10			no
C	1	10	One Coat Bond (Coltene Whaledent Mahway, NJ, USA)	Fill Magic (Coltene)	yes
	2	10			no

abrasive discs (Sof-Lex/3M ESPE, St Paul, MN, USA). The specimens were subjected to a thermocycling regimen of 500 cycles between 5(+/-5)°C and 55(+/-5)°C water baths (Digital Thermocycling Machine).

The specimens were rubber-coated except for around the prepared cavities, leaving only the tooth/restoration occlusal and cervical interfaces exposed. After coating, the specimens were immersed in a chemical marker (basic fuchsine solution 0.5%) for 24 hours at 37°C, washed under running water, cleaned, dried and sectioned into two faces, buccal and lingual, using diamond disks. The hemi-section showing the clearest chemical marker microleakage from each section was evaluated with a stereoscopic microscope with 20x magnification (Nova Ética, Vargem Grande Paulista, Brazil). Three examiners, previously calibrated on the scoring system, evaluated all specimens (Table 2).

RESULTS

The Kappa test demonstrated the effectiveness of the examiner calibration—Kappa = 0.51 between examiners 1 and 3, Kappa = 0.60 between examiners 1 and 2 and Kappa = 0.78 between examiners 2 and 3.

Evaluating the dentin microleakage scores per subgroups (A1XB1XC1 and A2XB2XC2), the Kruskal-Wallis test showed a significant difference ($p<5\%$) for the C1 subgroup (55.6% of the restorations with a score of 3) related to A1 and B1, both with 70% of the restorations having a score of 1. Comparing subgroups A1XA2, B1XB2 and C1XC2, the Mann-Whitney test revealed a significant difference in groups B and C ($p<5$). The best results were found in the B2 ($p=0.0345$) and C2 ($p=0.0029$) subgroups (Figure 1).

DISCUSSION

According to Pioch and others,[10] the use of sodium hypochlorite is one of the possible strategies to optimize dentin adhesion. Removal of the previously etched dentin surface collagen via a deproteinizing agent can be considered as a way to decrease hybrid layer technique sensitivity without compromising effectiveness of the adhesion.

Scientific works demonstrating the enhancement of adhesion after removal of the collagen fibrils, or even revealing a similar statistic between hybridization and deproteinization, resulted in many authors coming to the same conclusion: collagen is not necessary for obtaining effective adhesion.[8,14-18] With regard to marginal seal analysis, the results obtained in this study are consistent with this theory. However, the dissolution of organic dentin content promoted different behaviors, based on the adhesive system employed.

Generally, in non-vital teeth or in *in vitro* situations, dentin treatment with sodium hypochlorite completely removes the collagen-demineralized network, thus altering the dentin surface and modifying hydrophilic properties. This enables the dentin to be more compatible with hydrophobic resins compared to dentin etched by acid, alone.[2,8] This deproteinization results in enlarged resin tag formation and numerous sidelong resin projections.[9]

It is possible for the correlation between adhesion and surfaces without collagen to present a higher relation with the type of adhesive agent employed due to the composition or possibility of interaction with residual hypochlorite. In accordance with Souza and others,[19] the chemical characteristics of the bonding agent (pH, type and amount of solvent, monomer, presence of inorganic particles) may exercise decisive influence over adhesion to deproteinized surfaces.

In this study, dentin treated with NaOCl had reduced microleakage scores when the acetone-adhesive agent was used (Prime&Bond NT-PB, Dentsply Caulk, Milford, DE, USA). The high rate of diffusion of this solvent and its ability to displace water contributed to the results. According to Pioch and others[2] and Inai and others,[20] agents that present acetone in their composition show better behavior in dentin-deproteinized surfaces. Hence, there was better monomer interaction with the intertubular dentin structure exposed through sodium hypochlorite treatment, enabling penetration of the monomer into substratum porosities. Another consequence of the higher acetone level in Prime&Bond would be in affecting the solvent's ability to promote the volatilization of free radicals of oxygen released by

Table 2: *Microleakage Evaluation Scores*

Score	Description
0	Absence of penetration of chemical marker.
1	Penetration of chemical marker on cervical wall
2	Penetration of chemical marker on cervical and axial wall.
3	Penetration of chemical marker into pulp wall.

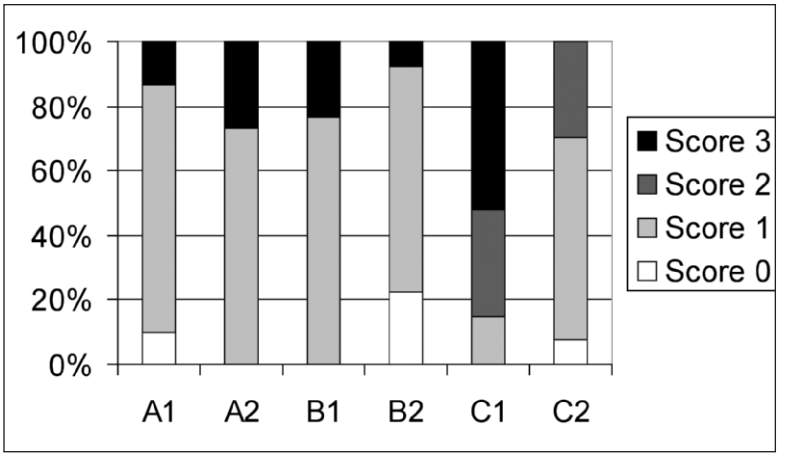

Figure 1: *Score distribution of microleakage per group in dentin.*

NaOCl, which could interfere with the bonding agent polymerization process.[21]

Moreover, the acidic resin monomers of PB (pH=2.3) would be able to re-etch the mineral phases of the dentin surface, further reducing the amounts of collagen with a depth no greater than 0.3—0.5 nm, thus producing a nanohybrid layer large enough to ensure an optimized adhesion.[8] In addition, the presence of phosphate terminals from a phosphoric acid ester (PENTA) in the composition of PB were verified. Phosphate terminals may establish some kind of interaction with calcium ions left over after collagen removal of the dentin surface.[20]

When sodium hypochlorite etching was done, the results observed with the One Coat Bond (Coltene/Whaledent, Mahwah, NJ, USA) adhesive system also exhibited a favorable marginal seal. It is important to emphasize that this agent does not have an organic solvent. As a result, One Coat Bond presents a gel consistency that could make its diffusion on only demineralized dentin difficult. As a result, in restorations where the collagen was maintained, the gel consistency would justify the level of leakage found. These leakage levels were greater than the other leakage scores found with the other adhesives in this study.

The removal of collagen fibrils by Single-Bond (SB) did not have a significant influence on microleakage scores. This result may be related to the inability of solvents of this adhesive (ethanol/water) to promote residual hypochlorite volatilization.

In accordance with Nikaido and others,[22] the action of NaOCl oxidized some of the dentin matrix components that may interfere with the polymerization initiation of some adhesive systems, including Single Bond (3M/ESPE, St Paul, MN, USA). Residual free radicals of sodium hypochlorite in dentin may compete with free vinyl radicals generated during adhesive photoativation, resulting in incomplete polymerization due to a premature terminal of polymeric interactions.[23]

Also, according to Sabóia and others,[24] slow diffusing adhesives, such as Single Bond, probably inhibit the effectiveness of nanometric porosities caused by NaOCl. This could explain the similarity in microleakage scores. In addition, SB co-monomers do not present high acidity (pH=3.5-4.2) and are unable to promote a second etching.[19]

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

CONCLUSIONS

Within the limitations of this *in vitro* study, the collagen fibril network was not important with regard to the marginal seal of the tested adhesive systems. After dentin collagen fibril removal, the bonding agents Prime&Bond NT and One Coat Bond showed improved results on marginal seal; whereas, when compared to the conventional adhesive protocol, Single Bond did not show significant alteration.

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