Effect of cariogenic challenge on the stability of dentin bonds

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ABSTRACT

Objective: The oral environment is subject to biofilm accumulation and cariogenic challenge, and few studies exist on the effect of these factors on the bond strength of adhesive systems. The aim of this study was to test if the exposure of adhesive interfaces to cariogenic challenge under biofilm accumulation could promote higher degradation than the exposure to biofilm accumulation alone. Material and Methods: Five molars were ground until exposure of medium dentin and then restored (Single Bond 2 and Z250 3M eSPe). The tooth/resin sets were cut to obtain beam-shaped specimens, which were distributed according to the aging conditions (n=20): water for 24 h (control); biofilm under cariogenic challenge for 3, 5 or 10 days; biofilm without cariogenic challenge for 10 days; and water for 3 months. Microcosm biofilms were formed from human saliva and grown in a saliva analogue medium, supplemented or not with sucrose to promote cariogenic challenge. Specimens were tested for microtensile bond strength, and failure modes were classified using light microscopy. Bond strength data were analyzed using ANOVA and failure modes were analyzed using ANOVA on ranks (α=0.05). Results: No significant differences in bond strength were detected among the aging methods (P=0.248). The aging period was associated with an increase in the frequency of adhesive failures for the groups aged for 10 days or longer (P<0.001). Conclusion: Aging leads to a higher prevalence of interfacial adhesive failures, although this effect is not associated with cariogenic challenge or reduction in bond strengths.

Keywords: Biofilms. Dental caries. Dentin-bonding agents.

INTRODUCTION

Secondary caries is one of the most important etiologic factors in restoration failure and the most common reason for replacing adhesive fillings, particularly in high caries risk patients10,26,28,32. However, the relationship between caries adjacent to restorations, adhesive/restoration bond strength, and marginal leakage remains relatively unexplored given the existence of few experimental and prospective studies designed to address this aspect5-7,17.

The instability of the adhesion of restorative biomaterials to dentin has been demonstrated in vitro1,13-16,24,25,30. Degradation is known to possibly occur in the dentin substrate and/or in the polymer component of the restoratives3,4,13. Proper hybridization has been considered a key factor for obtaining durable and strong dentin bonds protected from bacteria and hydrolytic action of oral fluids.

Studies on the longevity of restorative materials bonded to tooth structures usually simulate the clinical aging of the adhesive interfaces using mechanical/thermal cycling7,15,19,20,24 or static protocols of water storage1,8,9,13,14,16,20,25,33. However, studies on the quality and longevity of bonding to dentin should be carried out simulating the actual conditions of the oral environment. Attempts to simulate the cariogenic challenge have been performed using pH-cycling models18,22,23,27, which fail to mimic the actual in vivo conditions with
biofilm accumulation.

Considering that biofilm accumulation and cariogenic challenge are conditions to which the oral environment is exposed, and that the effect of these factors on the bond strength of adhesive systems to dentin has been seldom evaluated, this study was designed to investigate whether the cariogenic challenge would interfere with the dentin bond stability of an adhesive system. The null hypothesis is that there are no statistically significant differences in the bond strength of an adhesive system subjected to different aging protocols.

MATERIAL AND METHODS

Experimental design

In a completely randomized and blind study, microcosm dental biofilm originated from saliva of one donor was grown on resin-dentin beam-shaped composite-dentin specimens in 24-well microplates using a previously described method adapted to promote cariogenic challenge in dentin and enamel. The research protocol was approved by the local Ethics Committee (protocol 064/2008); written informed consent was obtained from the saliva donor. Biofilm was grown in a chemically defined saliva analogue with mucin (DMM) supplemented or not with sucrose according to the experimental group. The factor under evaluation was aging condition at 6 levels, as shown in Table 1. Therefore, 6 experimental subsets were obtained and each subset comprised 20 individual resin-dentin beam-shaped specimens. Biofilm acidogenicity was determined daily through pH measurements of the saliva analogue supernatant. After growth media replacements, the pH was individually recorded from each well of the discarded plate (Quimis 50w, Quimis, Diadema, SP, Brazil; V621 electrode, Analion, Ribeirão Preto, SP, Brazil) once a day for batch (without cariogenic challenge) and twice a day for semi-continuous groups (with cariogenic challenge: one pH reading for the pure DMM and one reading for the DMM supplemented with sucrose).

Preparation of the specimens

The occlusal faces of five human third molars were wet-ground to create a flat surface in medium dentin. The surfaces were wet-polished with 600-grit SiC papers to standardize the smear layer. The teeth were then restored using 35% phosphoric acid (15 s), and the two-step, etch-and-rinse adhesive system Single Bond 2 (3M ESPE, St. Paul, MN, USA) was applied to dentin according to the manufacturer’s instructions and then light cured for 20 s. A composite restoration was built-up on each dental surface using 2 mm increments of a resin composite (Filtek Z250; 3M ESPE); each increment was photoactivated for 20 s using a LED unit (Radii, SDI, Bayswater, Victoria, Australia) with 800 mW/cm² irradiance. After storage in distilled water at 37°C, for 24 h, the specimens were sectioned perpendicular to the bonded interfaces into resin-dentin beam-shaped specimens with a cross-sectional area of approximately 0.5 mm². For each tooth, 24 beams were obtained. The beams were separated according to tooth origin, protected with nail varnish (except the adhesive interface region) and randomly assigned into six groups (n=20) according to the aging conditions. Each group had beams from every tooth proportionally distributed according to the randomization procedure.

Cariogenic challenge

Saliva was used as inoculum to provide a multispecies microcosm biofilm. Approximately 9 mL of stimulated saliva (Parafilm ™, American National CanTM, Chicago, IL, USA) was collected from a healthy donor in the morning, 2 h after the last meal, and the volunteer abstained from oral hygiene 24 h before collection. An aliquot of 0.1 mL of fresh and homogenized saliva was inoculated on each specimen, except for the groups aged in distilled water. After 1 h, the saliva was gently aspirated and growth media (1.8 mL) was added according to each group condition. The groups under cariogenic challenge received 1.8 mL DMM with 1% of sucrose (DMM+s) for 4 h and, after the sugar challenge, the discs were dip washed for 10 s in sterile saline solution and transferred to a new plate with pure DMM for 20 h, whereas the group without cariogenic challenge received DMM for 24 h, replaced daily.

Bond strength test and failure analysis

After the experimental period of each group, the specimens were removed from the wells, cleaned, and prepared for the bond strength test. The beam-shaped specimens were subjected to a microtensile test in a mechanical testing machine (DL500, EMIC, São José dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min until failure. The cross-sectional area at the site of the fracture was measured with a digital caliper (Mitutoyo; Suzano, Brazil) with an accuracy of 0.01 mm. The load (in Kgf) and the bonding surface area of each specimen were recorded. The microtensile bond strengths were calculated in MPa, using the formula: R=F/(Kgf)A (cm). Pretest failures were not included in the statistical analysis. Data were submitted to a one-way ANOVA (P<0.05). After testing, the fractured specimens were carefully removed from the testing device and analyzed under optical microscopy at 100 and 500× magnifications by a blinded calibrated examiner. The modes of
failure were classified as adhesive failure (on the interface) or mixed failure (involving dentin and/or resin). Cohesive failures within dentin and prematurely debonded specimens were discarded. Failure data were submitted to a Kruskal-Wallis one-way ANOVA on ranks. All pairwise multiple comparison procedures were performed using Dunn’s method (P<0.05).

RESULTS

Means±standard deviations for pH throughout the experiment were 7.4±0.12 for the group without cariogenic challenge, 7.0±0.15 for the DMM without sucrose addition in the cariogenic challenge groups, and 4.4±0.13 after 4 h exposure to DMM supplemented with sucrose in the cariogenic challenge groups. Table 1 shows the results for the bond strength test. The statistical analysis showed no significant differences among the aging conditions (P=0.248). Table 1 also shows the results for the failure analysis. In contrast, the statistical analysis showed significant differences among the groups (P<0.001). The aging period was associated with an increase in the frequency of adhesive failures. For the control group and the groups submitted to cariogenic challenge for 3 or 5 days, a predominance of mixed failures was detected, whereas a predominance of adhesive failures was detected for the other groups.

DISCUSSION

Previous studies showed that long-term storage in water and other aging conditions may affect the durability of the dentin bonds. The breakdown of the adhesive interfaces was related to loss of stability of the polymer components of the adhesive assembly. Polymer degradation may gradually take place because of water penetration through nano-leakage channels, resulting in lower bond strengths and interfacial failure. Degradation of the collagen matrix from proteolytic activity of dentin intrinsic matrix metalloproteinases was also raised as one of the mechanisms for bonding breakdown. Moreover, it was shown that cariogenic bacteria could degrade dental resin composites and adhesives. However, in the present study, no significant differences in bond strength were observed among the groups tested, i.e., the cariogenic challenge did not promote higher degradation of the adhesive interfaces as compared with accumulation of biofilm alone or aging under water storage. Therefore, the null hypothesis tested was accepted.

Under normal conditions, human saliva meets all chemical requisites for remineralization of the dental hard tissues, and the saliva analogue medium used is supersaturated in relation to hydroxyapatite. This condition automatically facilitates the precipitation of calcium and phosphate, which could protect the collagen network and adhesive components from further hydrolysis. Moreover, the storage time under cariogenic challenge may not have been long enough to promote significant damage to the adhesive interfaces by acid penetration into the interfacial region, which could presumably affect bond strength. In fact, we simulated a low to moderate cariogenic challenge, providing short periods of demineralization followed by longer periods of remineralization, similar to what happens in the oral cavity. To promote a higher cariogenic challenge, a sucrose exposure time in the biofilm model ≥6 h should be used, but a higher cariogenic challenge could also promote fast, unsought demineralization of the dentin tissue in the beam-shaped specimens.

In contrast to the bond strength results, the failure analysis provided evidence of a detrimental effect imposed by the aging conditions on the dentin bonds. A shift from predominance of mixed failures to predominance of adhesive failures was detected for the groups aged for 3 months in water or 10 days in the biofilm model, irrespective of the exposure to cariogenic challenge. For the group stored in water for 3 months, almost all of the failure modes were adhesive, likely an effect of the water uptake leading to hydrolytic degradation of the polymer.
component. Degradation of dental crosslinked networks has been linked to mechanisms involving oxidation, attack of functional groups, and chain scission; the extent of these processes is related to the composition of the monomers producing the network and is expected to be material-dependent. Therefore, the performance of bonding agents under cariogenic challenge may vary according to their formulation.

The present results suggest that storage conditions comprising cariogenic challenge may not promote higher degradation of the dentin adhesive interfaces than static water storage alone. However, although the bond strength test by itself was unable to detect significant differences among groups, the failure analysis provided evidence that a hydrolytic effect took place as a function of the aging time. This finding reinforces the need to associate bond strength data with failure analysis. In addition, increasing the time of cariogenic challenge or the cariogenic challenge by using longer demineralization periods would be a valid approach, although these conditions could potentially increase the frequency of premature debonding or occurrence of cohesive failures within the bonding substrate attributable to the extended mineral loss. Nevertheless, in situ aging of beam-shaped specimens could present the possibility for further assessing the effects that the condition state in an oral environment may have on dentin bond stability.

The conditions of the present in vitro study took into account the effects that the demineralization and remineralization processes may have in the oral cavity, and this model could be used to reproduce the clinical aging conditions for adhesive interfaces. Similar models have been used in the literature to test hypothesis related to materials with antimicrobial activity, development of secondary caries, and other phenomena. Nonetheless, it is important to acknowledge that in vitro studies have limitations because they cannot simulate all the complexity of an in vivo environment, such as exposure to food intake and salivary flow. Additionally, it is important to note that the aging processes in the present study were applied to beam-shaped specimens to allow comparisons with previous studies, which have also aged beam-shaped specimens. Inducing aging in these specimens with reduced dimensions would accelerate the degradation process, reducing the length of the experiment, which is an advantage for in vitro biofilm models. However, future studies should be carried out aging in vitro or in situ restorations where dentin is surrounded by enamel for longer periods, in order to truly simulate the clinical conditions present in the mouth.

CONCLUSION

Aging of dentin bonds leads to a higher prevalence of adhesive failures, although this effect may not be associated with a cariogenic challenge or a reduction in bond strengths.

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