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Bonding of Adhesive Luting Agents to Caries-affected Dentin Induced by a Microcosm Biofilm Model

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Clinical Relevance

Remaining caries-affected dentin after the cavity preparation for indirect restorations results in reduced bond strength of adhesive luting agents.

SUMMARY

Objectives: To evaluate the bond strength of adhesive luting agents applied to caries-affected dentin (CAD).

Methods: Thirty-six noncarious human third molars were abraded to expose an occlusal dentin surface. Caries lesions were induced in half of the samples using a microcosm biofilm model. Biofilm was cultivated under an anaerobic atmosphere for 14 days in a medium enriched with mucin. The same medium containing 1% sucrose was alternated for 4 hours per day. Cylinders of resin cement (RelyX ARC, RelyX U200, or BisCem) were built up over the dentin substrate and submitted to shear bond load. The samples were then longitudinally sectioned. The hardness and elastic modulus of dentin were measured at different depths from the occlusal surface. A three-dimensional finite element simulation was performed to analyze the residual stress distribution during the shear bond strength test. Bond strength data were analyzed by two-way analysis of variance (ANOVA) and hardness and elastic modulus by split-plot ANOVA. Multiple comparisons were performed with the SNK test (α=0.05).

Results: For all cements, the highest bond strengths were observed in sound dentin. Re-

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lyx ARC bond strength was similar to that of RelyX U200 for both substrates; BisCem had the lowest values. CAD had lower hardness (above a depth of 100 μm) and elastic modulus (above a depth of 150 μm) values than sound dentin. Stress distribution during the bond strength test was similar under all experimental conditions.

Conclusion: Impairment of the mechanical properties of dentin promoted by carious lesions reduced the bond strength of adhesive luting agents.

INTRODUCTION

Preparations for adhesively bonded dental restorations are conservative, with tissue removal almost entirely limited to carious structures.[1,2] In some cases, caries-infected dentin is removed and the underlying remineralizable dentin (caries-affected dentin, CAD) is preserved to avoid pulpal complications.[3,4] In clinical practice, it is possible that CAD may be left unintentionally in some areas of the cavity. The performance of composite indirect restorations is related to their adhesion to tooth structures, and proper bonding to CAD is required for their longevity.[5,6]

In the past, luting procedures for indirect restorations were routinely performed using resin cements with bonding agents. More recently, self-adhesive resin cements (SARCs) were introduced to facilitate these procedures.[7-9] The bonding mechanism of SARCs is attributed to both a chemical reaction between phosphate methacrylates and hydroxyapatite and the infiltration of these materials into tooth tissues.[10-13] Several studies have demonstrated reduced bonding ability of dental adhesives to CAD compared with sound tissue.[14-18] By contrast, few studies have evaluated the bond strength of SARCs applied to CAD, and their results have been conflicting.[19,20] A recent study found no difference in SARC bond strength between natural CAD and sound dentin,[20] whereas another study using a pH-cycling model to produce CAD found reduced bond strength to this substrate.[19]

A difficulty of evaluating the bond strength of adhesive materials applied to CAD is standardizing the dentin substrate. Use of natural CAD is difficult because different areas of the substrate can exhibit significant differences in mineral content.[17] The technique used to remove caries and to determine the extent and location of CAD is also controversial and might influence the results.[14,21] Several studies have used the pH-cycling method to artificially induce CAD formation.[19,22,23] The use of a microcosm biofilm model is another approach for *in vitro* formation of CAD.[24] Thus, the aim of this study was to evaluate the bond strength of adhesive luting agents applied to CAD induced by a microcosm biofilm model. The study hypothesis was that the luting agents would exhibit reduced bond strength when applied to CAD.

METHODS AND MATERIALS

Study Design

This *in vitro* investigation was conducted using a 2 × 3 factorial study design to evaluate the factors "substrate" in two levels (sound dentin or CAD) and "adhesive luting agents" in three levels (three different materials). The luting agents evaluated were the regular resin cement RelyX ARC (3M ESPE, St Paul, MN, USA) and the self-adhesive resin cements RelyX U-200 (3M ESPE) and BisCem (Bisco, Schaumburg, IL, USA). CAD was produced using a microcosm biofilm model cultivated for 14 days over the sound dentin. The hardness and elastic modulus of the sound dentin and CAD were evaluated at various depths from the occlusal surface of the specimens (n=5). The bond strength of luting agents applied to sound dentin and CAD was evaluated by the shear bond strength test followed by failure-mode analysis (n=6). Three-dimensional (3D) finite element analysis (FEA) was used to calculate the corresponding residual stress in the tooth during the shear bond strength test.

Sample Preparation

For this study, 36 noncarious human third molars stored in 0.05% thymol saline solution at 4°C for no more than three months were used in this study. The occlusal surfaces were ground flat with a plaster trimmer under running water, followed by the use of 100 grit SiC papers to remove the enamel and expose a flat medium dentin surface. Samples were sectioned parallel to the occlusal surface 2 mm below the cemento-enamel junction using a water-cooled slow-speed diamond saw (#7020, KG Sorensen, Barueri, Brazil); the roots were discarded.

The specimens were ultrasonically cleaned with distilled water, and the dentin surfaces were wet-polished with 600 grit SiC paper for one minute to standardize the smear layer. Surfaces were inspected with an optical stereomicroscope at 40× magnification to ensure the absence of enamel. The specimens were placed in Falcon tubes immersed

Table 1: *Description of Cements Used in the Study and Their Application Protocols*

Cement	Classification	Application Protocol[a]
RelyX ARC	Regular resin cement	The dentin was etched with phosphoric acid for 15 seconds followed by rinsing for the same time. Excess water was removed using absorbent paper. The primer of Scotchbond Multipurpose Plus (3M ESPE) adhesive system was applied and the solvent volatilized with a gently air-stream. The bonding agent was applied in a single layer and light-cured for 10 seconds. The resin cement RelyX ARC was mixed and inserted into the orifices of the elastomer mold.
RelyX U200	Self-adhesive resin cement	The dentin surface was rinsed, and excess water was removed with absorbent paper. The cement was mixed and inserted into the orifices of the elastomer mold.
BisCem	Self-adhesive resin cement	The dentin substrate was rinsed, and all surface water was removed with a strong air stream for five seconds. The cement was mixed and inserted into the orifices of the elastomer mold.

[a] *According to manufacturers' instructions.*

in distilled water and sterilized with gamma radiation at 15 kGy for 13.3 seconds. After sterilization, the specimens were stored at 4°C.

Induction of Caries

Half of the specimens were randomly assigned to caries induction using a microcosm biofilm model. The surfaces were covered with two coats of an acid-resistant, fast-drying nail varnish leaving only the occlusal surface exposed. For the cariogenic challenge, 20 mL of fresh saliva stimulated by paraffin film was collected from a healthy volunteer (a 48-year-old woman) who had not taken any antibiotics for at least six months previously. The volunteer abstained from oral hygiene for 24 hours and from food ingestion for two hours before collection. Then, 400 μL of the collected saliva was inoculated over the exposed occlusal surfaces in Falcon tubes. After one hour, 4 mL of defined medium enriched with mucin (DMM)[25] containing 1% sucrose was added, and the tubes were incubated at 37°C under an anaerobic atmosphere (80% N_2, 10% CO_2, 10% H_2) for four hours. The samples were then rinsed with 10 mL of sterile saline solution, inserted into a Falcon tube containing DMM without sucrose and incubated for 20 hours under an anaerobic atmosphere. The biofilms were cultivated for 14 days in this alternating sucrose regimen, and the medium was replaced twice per day. The period needed for CAD to be produced was determined in a pilot experiment.

Bond Strength Evaluation

All samples, including sound dentin specimens, were embedded in acrylic resin cylinders to facilitate handling. Elastomer molds with four cylindrical orifices (1.5 mm diameter × 0.5 mm thickness) were placed onto the dentin substrate. The adhesive resin luting agents were used to fill the orifices. The classification of and application protocol for the luting agents are described in Table 1. After filling the orifices, a polyester strip and a polymerized composite resin disc (Filtek Z350 XT, shade CT, 5 mm diameter × 2 mm thickness) were placed over the filled mold. A load of 750 gf was applied over the composite disc for 5 minutes. The load was then removed, and the resin cement cylinders were light-cured for 40 seconds through the composite disc, simulating the clinical situation in which the resin cement is indirectly light-activated. Photoactivation was performed using a light-emitting diode curing unit (Radii, SDI, Bayswater, Australia) with an irradiance of 1400 mW/cm^2.

After cement polymerization, the elastomer molds were removed, and the samples were stored in distilled water at 37°C for 24 hours. For the shear test, a stainless steel wire (0.2 mm diameter) was looped around each cylinder and aligned with the bonded interface. The test was conducted on a mechanical testing machine (DL500, EMIC, São José dos Pinhais, Brazil) at a crosshead speed of 0.5 mm/min until failure. Fractured specimens were observed under magnification of up to 500× on an optical microscope to classify the failure mode: type I = adhesive failure, or type II = mixed failure.

Mechanical Properties of Sound Dentin and CAD

After the shear test, the tooth samples were longitudinally sectioned through their center with a water-cooled diamond saw. Half of the specimens were embedded in polymethyl methacrylate and wet-polished with 600, 1200, 1500, and 2000 grit SiC abrasive papers, followed by a final polishing with a 1 μm polycrystalline diamond suspension. Cross-sectional hardness measurements were performed with a Vickers indenter (CSM Micro-Hardness Tester; CSM Instruments, Peseux, Switzerland). Indentations were made in the dentin at depths of

Table 2: *Means (95% Confidence Intervals) for Bond Strength, MPa (n=6)[a]*

Cement	Substrate		Pooled Average
	Sound Dentin	Caries-affected Dentin	
RelyX ARC	4.8 (4.3 – 5.3)	3.1 (2.4 – 3.8)	4.0 (3.5 – 4.5)[y]
RelyX U200	5.9 (5.3 – 6.5)	3.4 (2.7 – 4.1)	4.7 (4.1 – 5.3)[y]
BisCem	3.2 (2.2 – 4.2)	2.3 (2.1 – 2.5)	2.8 (2.3 – 3.3)[z]
Pooled average	4.6 (4.1 – 5.1)[y]	3.0 (2.7 – 3.3)[Z]	

[a] For pooled averages, distinct letters indicate significantly statistical differences (α = 0.05).

Table 3: *Ratios Between Type I and Type II Failure Modes for All Groups[a]*

Cement	Substrate		*P* Value
	Sound Dentin	Caries-affected Dentin	
RelyX ARC	11	3	0.245
RelyX U200	3.8	11	0.416
BisCem	2	5	0.318
P value	0.103	0.301	

[a] Type I indicates adhesive failure; Type II indicates mixed (failure partially adhesive and partially cohesive within dentin).

10, 20, 30, 40, 50, 100, 150, and 200 μm from the occlusal surface of each specimen. Indentation was performed with a controlled force, the test load being increased or decreased at a constant speed and ranging between 0 and 500 mN at 60-second intervals; the maximal load was maintained for 15 seconds. The load and penetration depth of the indenter were measured continuously during the load-unload hysteresis. Hardness was defined as the applied force divided by the apparent area of the indentation at maximal force. Elastic modulus was calculated from the slope of the tangent to the indentation depth curve at maximal force.

Residual Stress Calculation by 3D FEA

To calculate the corresponding residual stress in the tooth, a 3D finite element simulation was conducted using a resin cement cylinder of the same dimensions as that used in the bond strength test bonded to a dentin disc of 5-mm diameter. Boundary conditions were achieved by fixing the dentin cylinder at all external lateral and bottom surfaces. The elastic modulus of sound dentin and CAD was simulated as calculated from the indentation test and Poisson's ratio was obtained from the literature.[26] The elastic modulus of the resin cement was calculated using a Knoop indentation method as described previously, and Poisson's ratio for the resin cement was obtained from a previous study.[27] The model was meshed using linear, eight node, isoparametric, and arbitrary hexahedral elements. A 100 N load was applied through the simulated steel wire as a rigid body, using a dynamic loading process with the same parameters as the experimental test.

FEA was performed using MSC.Mentat (pre- and post-processor) and MSC.Marc (solver) software (MSC Software Corporation, Santa Ana, CA, USA). Then, von Mises equivalent stress and maximal principal stress were used to express the stress conditions. Stress distribution was analyzed using a model with three-quarters of the geometry showing the external and internal view of the stress distribution. Stress values were obtained for 10 nodes located at the center of the dentin structure where the areas of greatest stress concentration were observed.

Statistical Analysis

Data analysis was performed using the SigmaStat v.3.5 statistical software package (Systat Software Inc. Chicago, IL, USA). Bond-strength data were subjected to two-way analysis of variance (ANOVA). All pairwise multiple comparison procedures were performed by the Student-Newman-Keuls (SNK) method. Hardness and elastic modulus data were separately submitted to split-plot design ANOVA and SNK as a *post hoc* test. Nonlinear regression analysis was used to investigate the relationship between dentin depth and hardness/elastic modulus. Data on failure mode ratio were submitted to χ^2 and Fisher's exact tests. The significance level was set at $\alpha = 0.05$ for all analyses.

RESULTS

Bond Strength and Failure Mode

Regarding bond strength (Table 2), a significant effect was detected for the factors "substrate" ($p<0.001$) and "cement" ($p=0.002$), but not for the interaction between the factors ($p=0.42$). The power of performed tests was 0.98 and 0.87 for the factors "substrate" and "cement," respectively. Irrespective of the cement applied, significantly higher bond strength was observed for sound dentin than for CAD. RelyX U200 and RelyX ARC exhibited similar results for the two substrates, with significantly

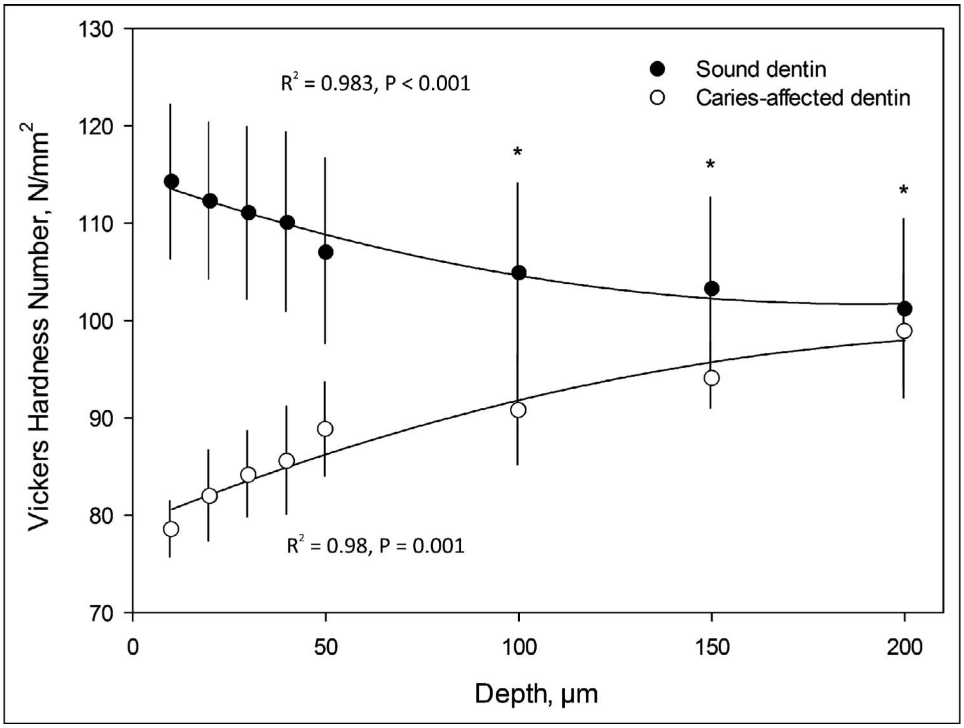

Figure 1. *Results and nonlinear regression plots for hardness (* indicates absence of significant difference between dentin types at the same depth; error bars indicate standard deviation). A logarithmic reduction in hardness was associated with increased depth in sound dentin, whereas a logarithmic increase in hardness was associated with increased depth in caries-affected dentin.*

higher bond strengths than BisCem. Neither cement type nor substrate affected failure mode (Table 3).

Hardness and Elastic Modulus

Regarding Vickers hardness (Figure 1), a significant effect was observed for the factor "dentin type" (p=0.019), but not for the subplot "depth" (p=0.266). The interaction between the factor and the subplot was significant ($p<0.001$). The power of performed tests was 1.00. Except for the depths of 100, 150, and 200 μm, sound dentin was significantly harder than CAD. A significant logarithmic reduction in hardness was associated with increased depth in sound dentin (R^2=0.983; $p<0.0001$), whereas a significant logarithmic increase in hardness was associated with increased depth in CAD (R^2=0.98; $p<0.0001$).

Regarding elastic modulus (Figure 2), significant effects were observed for the factor "dentin type" (p=0.005), the subplot "depth" ($p<0.001$), and their interaction ($p<0.001$). The power of performed tests was 0.75. Sound dentin had a significantly higher elastic modulus than CAD, except at depths of 150 and 200 μm. A significant linear increase in elastic modulus was associated with increased depth in both sound dentin (R^2=0.788; p=0.014) and CAD (R^2=0.967; $p<0.001$).

Residual Stress on FEA

Figures 3 and 4 illustrate the high stress concentration in the inferior region of the resin cement cylinders, corresponding to the contact loading application of the stainless steel wire used to loop the cylinders for the shear test. The peak in stress concentration (expressed in red color) into the dentin substrate near the bonded interface was verified in all groups.

Figure 3 shows the von Mises stress distribution for all experimental conditions tested. No appreciable differences were found between the resin cements or substrates. The stress concentration in the dentin substrate and at the bottom of the resin cement cylinder where the steel wire applied the load was consistent with the most frequent failure mode observed in the experimental tests, irrespective of the resin cement and substrate.

Figure 4 shows the stress distribution inside the bonded dentin area at the interface with the bottom of the resin cement cylinder. No appreciable differences were found between the resin cements or substrates tested. The Von Mises stress and maximal principal stress values obtained at the 10 nodes that coincided with the highest stress levels recorded inside the dentin substrate are shown in Table 4 and are coincident with most of the failure modes observed in the shear test. Similar values were found for both study factors.

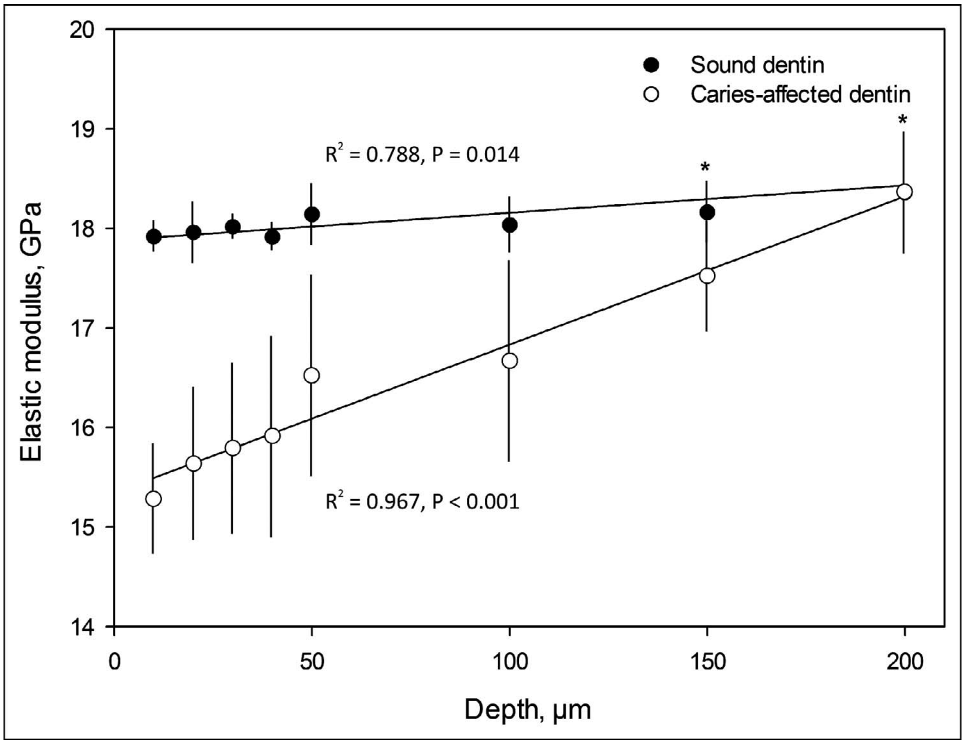

Figure 2. *Results and linear regression plots for elastic modulus (* indicates absence of significant difference between dentin types at the same depth; error bars indicate standard deviation. A linear increase in elastic modulus was associated with increased depth in sound and caries-affected dentin.*

DISCUSSION

The possible presence of CAD after conservative preparation requires bonding protocols that are effective when applied to this substrate. Studies on the bond strength of SARCs applied to CAD are lacking. Several studies have used extracted teeth with carious lesions for this purpose,[2,14,17,20,21,23] but it is difficult to standardize caries removal and to identify regions of dentin that are sufficiently similar for bond strength studies. Therefore, the results of studies using natural caries lesions as substrates for adhesion can be highly variable and may even impair the proper evaluation of adhesive

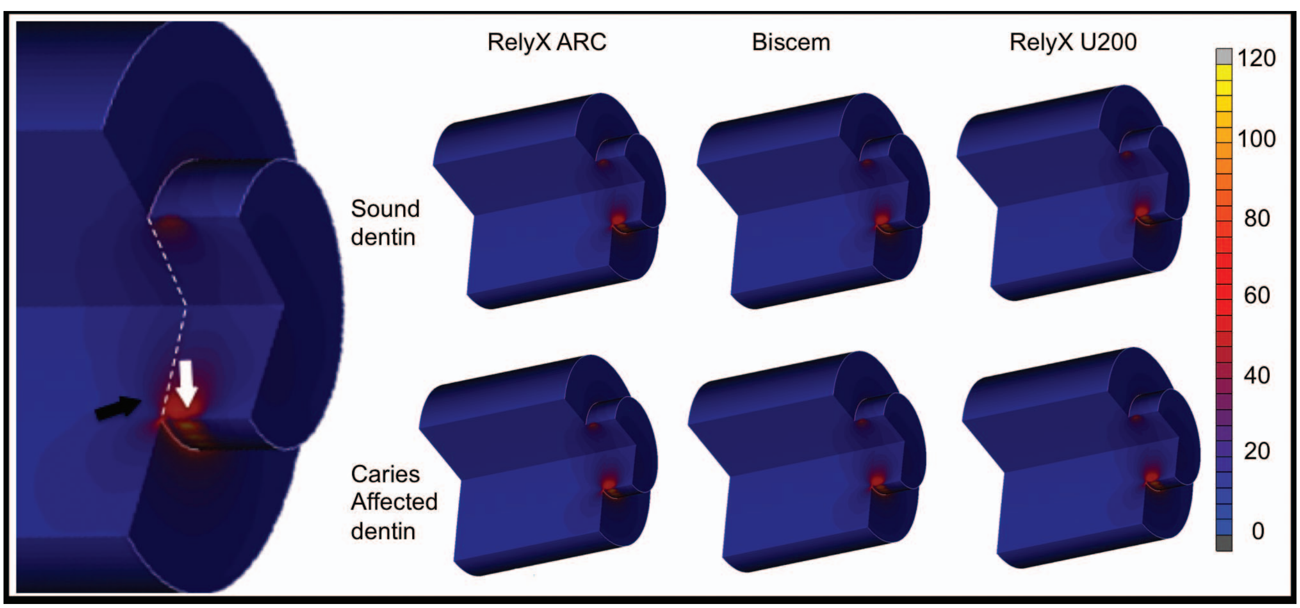

Figure 3. *Equivalent von Mises stress distribution (MPa) for resin cements bonded to sound and caries-affected dentin. The white arrow indicates the stress concentration in the resin cement cylinder; the black arrow indicates the peak in stress concentration (shown in red) into the dentin substrate near the bonded interface.*

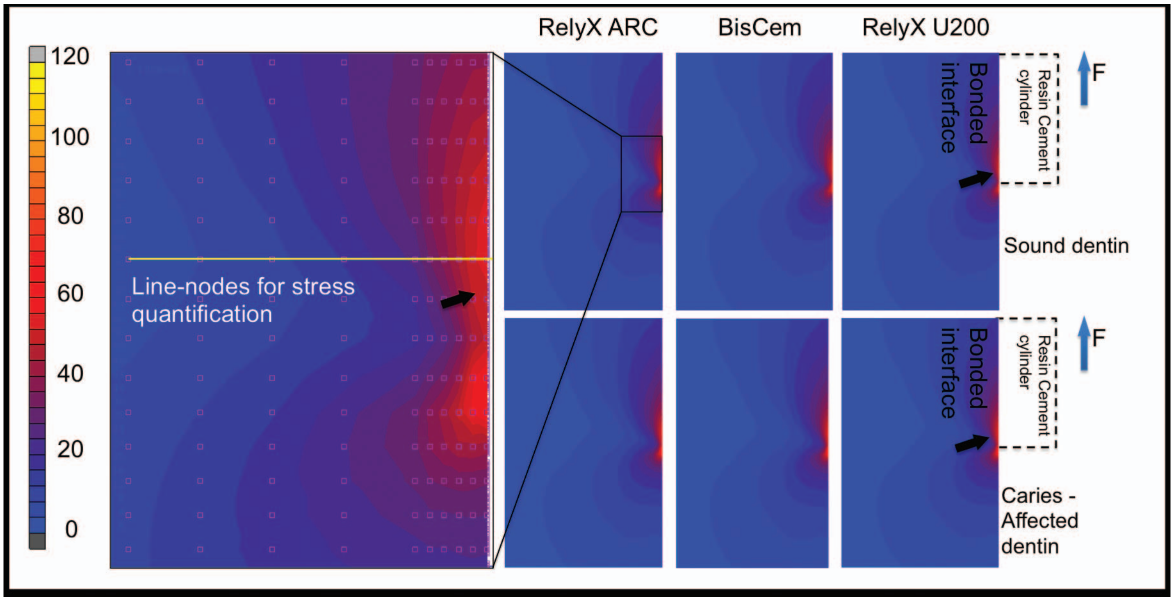

Figure 4. *Equivalent von Mises stress distribution (MPa) extracted into the cut dentin structure where the resin cement was bonded. Line nodes represent the nodes where stress values were extracted for quantitative analysis. Black arrows indicate the peak in stress concentration (shown in red) in the dentin substrate near the bonded interface.*

materials applied to CAD.[14,21] The present study used a microcosm biofilm model to induce carious lesions in dentin. This model simulates the complex interaction that occurs in the tooth substrate during the caries process and thus might be considered a more clinically relevant method than other artificial caries-induction methods, such as pH-cycling.

Evaluation of the mechanical properties of the substrates showed that the protocol used for caries induction resulted in lesions 100 μm deep. Above this depth, CAD exhibited reduced hardness compared with sound dentin. A similar trend was observed for elastic modulus, except that the substrates differed above a depth of 150 μm. The impaired mechanical properties of CAD near the surface where the biofilm was cultivated are due to a reduction of mineral content promoted by acids produced by bacterial metabolism, as occurs *in vivo*.[28,29] An important observation was the reduction of the hardness of sound dentin toward its deeper regions. This finding can be explained by the increased number and diameter of dentinal tubules toward the pulpal chamber, which reduces the area of inter- and peritubular dentin and consequently makes the substrate softer.[30]

Table 4: *Results of Maximum Principal Stress (σmax) and Von Misses Stress (vm) in MPa Versus Dentin Depth*

Distance to the Bonded Surface (mm)	RelyX ARC				BisCem				RelyX U200			
	Caries-affected Dentin		Sound Dentin		Caries-affected Dentin		Sound Dentin		Caries-affected Dentin		Sound Dentin	
	σmax	vm	σmax	vm	σmax	vm	σmax	vm	σmax	vm	σmax	vm
0.00	32.5	52.9	33.5	53.9	32.6	53.5	33.7	54.9	32.4	52.8	33.4	54.3
0.01	33.1	48.6	35.9	51.2	33.3	49.2	36.2	52.2	32.6	48.3	35.5	51.2
0.02	31.1	42.6	33.0	44.3	31.2	43.2	33.3	45.1	30.7	42.5	32.7	44.4
0.03	29.0	37.1	29.8	37.6	29.2	37.5	30.0	38.2	28.8	37.0	29.6	37.7
0.04	26.6	31.9	27.1	32.1	26.7	32.2	27.2	32.6	26.4	31.8	26.9	32.2
0.05	22.6	24.9	22.8	24.9	22.7	25.2	22.9	25.3	22.5	25.0	22.7	25.1
0.10	16.9	16.4	16.7	16.2	17.0	16.6	16.8	16.4	16.9	16.4	16.7	16.3
0.15	12.2	10.8	11.8	10.5	12.2	10.8	11.8	10.5	12.2	10.8	11.9	10.5
0.20	9.6	8.4	9.2	8.0	9.6	8.4	9.2	8.0	9.7	8.4	9.3	8.0
0.25	7.6	6.5	7.2	6.2	7.6	6.5	7.2	6.2	7.7	6.5	7.3	6.2

The bond strength of all of the evaluated resin luting agents was lower on CAD than on sound dentin. Thus, the study hypothesis was accepted. It could be expected that the reduced elastic modulus of CAD would result in a different stress distribution during the shear test compared with sound dentin. In this study, the difference in bond strength between the substrates could be partially explained by stress distribution during testing. The high stress concentration into dentin verified in all groups (Figures 3 and 4) suggests that the dentin pullout may be due to the biomechanics of the shear bond test.[31] The stress concentration into dentin located near the load application was similar for all groups (Figure 4). The peak in stress verified in the dentin may also contribute to failure of the bonded interface. Although the stress concentration was similar for sound and CAD, the strength of the CAD is lower; thus, the adhesive interface is more affected. Furthermore, the differences observed between the substrates might be explained by the dentin bonding mechanism.

The regular resin cement RelyX ARC is used in a three-step, etch-and-rinse adhesive system. This system forms a homogeneous and continuous hybrid layer in sound dentin, resulting in higher bond strength.[32] In CAD, possible collagen degradation promoted by carious lesions could result in the formation of a more heterogeneous hybrid layer with reduced cohesive strength.[22,23] Additionally, the reduction in mineral content allows deeper acid etching, increasing the extent of noninfiltrated exposed collagen.[23] A larger area of noninfiltrated collagen may act to concentrate stress during bond strength testing.[33]

The main bonding mechanism of SARCs, in contrast to regular resin cements, is chemical chelation between functional acid methacrylates and calcium from tooth tissues.[11,12] Thus, the reduced mineral content of CAD results in a less effective interaction with SARCs. In addition, a thicker and more organic smear layer is produced in CAD than in sound dentin, impairing the infiltration ability of SARCs.[34] However, it is important to emphasize that RelyX U200 showed higher bond strengths than BisCem. Other studies evaluating these two SARCs applied to sound dentin have had similar findings.[19,35,36] Differences in the composition of these two materials may explain these results. BisCem contains the highly hydrophilic monomer HEMA. Despite the absence of pulpal pressure simulation in the present study, this monomer is able to attract the intrinsic water of dentin, which may impair cement polymerization.[37] Impaired mechanical properties have been demonstrated for HEMA-based materials when stored in water for 24 hours.[38] In addition, greater interlocking within the underlying dentin was demonstrated for RelyX Unicem (which has a composition similar to that of U200) compared with BisCem using a Masson trichrome technique.[39]

The microcosm biofilm model used in the present study is a method that produces reproducible CAD. Despite the limitations of *in vitro* studies, our results show that the impaired mechanical properties (ie hardness and elastic modulus) caused by caries affect the bond strength of adhesive luting agents applied to dentin. However, clinical trials are necessary to confirm whether bonding to CAD during the cementation of indirect restorations may affect the survival rates of these restorations.

CONCLUSIONS

The mechanical properties alteration produced by the biofilm model reduced the bond strength of adhesive luting agents to caries-affected dentin.

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Human Subjects Statement

This study was conducted at the Federal University of Sergipe in Brazil and was approved by the local research ethics committee (protocol #128.227/2012).

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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