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Evaluation of Novel Plant-derived Monomers-based Pretreatment on Bonding to Sound and Caries-affected Dentin

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Clinical Relevance

Pretreatment with novel, plant-derived monomers is promising to reinforce the hybrid layer, since they preserved the resin–dentin bond strength and improved dentin bonding, especially to caries-affected dentin.

SUMMARY

This study evaluated the influence of new monomers derived from cashew nut shell liquid (CNSL) applied for dentin biomodification on resin–dentin bond strength, nanoleakage, and micropermeability to sound and artificially-created caries-affected dentin. Human dentin specimens were assigned to five groups, according to the following dentin pretreatment solutions: Absolute ethanol (control), 2 wt% grape seed extract *(Vitis vinifera)*, 2 wt% cardol [from cashew nut shell liquid (CNSL)], 2 wt% cardol-methacrylate or 2 wt% cardanol-methacrylate applied on sound and artificial caries-affected dentin. Specimens were analyzed after 24 hour or 1 year of water storage. Microtensile bond strength (μTBS) (n=6), interface micropermeability (n=3), and silver nanoleakage (n=6) were assessed using a universal testing machine, confocal laser scanning microscope, and scanning electron microscope, respectively. In sound dentin, no difference in bond strength was observed between the groups in either storage period. In artificial caries-affected dentin, pretreatment with cardol-methacrylate resulted in statistically higher bond strength than all the other treatments in both storage periods. Cardol-methacrylate treatment resulted in less nanoleakage, along with improved interfacial integrity, compared to further treatments in artificial caries-affected

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dentin. Regarding micropermeability analysis, all treatments depicted deficient sealing ability when applied on artificial caries-affected dentin, with the presence of gaps in the control group. In conclusion, cardol-methacrylate is a promising plant-derived monomer to reinforce the hybrid layer, since it preserved resin–dentin bond strength and improved dentin bonding, especially to caries-affected dentin, a well-known harsh substrate for adhesion longevity.

INTRODUCTION

The resin–dentin interface, in particular, the hybrid layer formed after phosphoric acid etching and the use of etch-and-rinse adhesives, seems to be the vulnerable zone for resin composite restoratives.[1] Dentin bonds are prone to degradation, mainly by polymer hydrolysis and collagen breakdown accelerated by enzymes, such as matrix metalloproteinases (MMPs) and cysteine cathepsins (CTPs).[2-5]

Additionally, current minimally invasive dentistry determines the conservative removal of caries, keeping part of the tissue that is affected by caries in the cavity.[6,7] Adhesion to caries-affected dentin is challenges the creation of a uniform and homogeneous hybrid layer.[8] With the widespread popularity of minimally invasive dentistry, direct restorations are often bonded to caries-affected dentin, which is preserved due to its likelihood to remineralize.[9–11] Nevertheless, caries-affected dentin is porous, with areas of partially demineralized collagen that allow deeper demineralization during phosphoric acid etching. Indeed, with a thicker layer of etched dentin, the infiltration of monomers is compromised and more resin-sparse collagen fibrils are exposed to degradation.[8,12,13]

The use of MMP and CTP inhibitors has been demonstrated to be an alternative to improving dentin bond durability.[8,14] The recent strategy of dentin biomodification by using collagen cross-linkers[15] aims to increase the mechanical properties of the hybrid layer and unprotected collagen fibrils, thereby preventing interface degradation and providing long-lasting dentin bonds.[4,16,17] However, the use of synthetic agents, such as glutaraldehyde, can present great cytotoxicity.[18] Therefore, the demand for natural collagen cross-linkers has increased in recent years,[15,19,20] with proanthocyanidins (PACs) from grape seed extract (GSE) from *Vitis vinifera* showing improvements in dentin's ultimate tensile strength,[15] hardness,[21] elastic modulus,[22] and resistance against biodegradation.[23] However, a great disadvantage of PACs is the pigmentation of the dentin substrate.[24]

Other natural compounds, such as cashew nut shell liquid (CNSL), extracted from plants (*Anacardium occidentale* L), also have the potential for dentin biomodification, thanks to the long carbon chain (15 carbons) and terminal polyphenols, similar to PACs. The application of these substances as dentin pretreatments has been effective in promoting crosslinking and increasing the modulus of elasticity of demineralized collagen.[24,25] However, the long-term effects on dentin bonding, particularly, to caries-affected dentin, has not been studied to date.

Furthermore, the addition of methacrylate groups to the cardol and cardanol molecules allowed for the development of novel functional monomers that can interact both with the exposed collagen and with other methacrylate-based monomers present in the adhesive blend, enabling a reinforcement in bond strength and better durability of the interface.[26,27] This study is a pioneer in synthesizing and evaluating the effectiveness of cardol-methacrylate and cardanol-methacrylate on dentin adhesion.

Therefore, the aim of this investigation was to evaluate the influence of pretreatment using novel monomers derived from CNSL on dentin bonding, micropermeability, and silver nanoleakage. The two study hypotheses were: (1) There are no differences among the new monomers tested in terms of bond effectiveness (bond strength and nanoleakage) to sound and artificially created, caries-affected dentin over the two different storage periods, and (2) the sealing ability promoted by the adjunctive use of different biomodification agents is better than that of the control adhesive.

METHODS AND MATERIALS

Experimental Design

The factors investigated were: (1) Dentin pretreatment (five levels): absolute ethanol (negative control), 2 wt% GSE (*Vitis vinifera*, Mega-Natural Gold; Polyphenolics, Madera, USA), 2 wt% cardol (separated and purified from CNSL), 2 wt% cardol-methacrylate (synthesized from cardol), and 2 wt% cardanol-methacrylate (synthesized from purified cardanol extracted from CNSL). Two dentin substrates were assessed: sound dentin and artificially created caries-affected dentin. The micropermeability test was only performed after 24 hours; experiments were performed with two different storage periods—24 hours and 1 year. All reagents were diluted in EtOH/H_2O (1:1 volume ratio) with 5 minutes agitation at 25°C. The control group used absolute ethanol (≥99.8% ethanol, Sigma–Aldrich, St. Louis, MO, USA). The experiments undertaken were the microtensile bond strength (μTBS) test, dentin

micropermeability, and silver nanoleakage, with the latter two being qualitatively evaluated using confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM), respectively. Six bonded teeth were used in each group (n=6) for the μTBS and nanoleakage assessments, whereas, an additional three teeth per group (n=3) were prepared for the micropermeability evaluation.

Synthesis and Purification of New Monomers

Cardol and cardanol were obtained from industrial CNSL supplied by Amendoas do Brasil Ltda (Fortaleza, Brazil), separated by column chromatography (silica gel 60) and characterized by gas chromatography–mass spectroscopy.[28] The synthesis and purification of cardol-methacrylate and cardanol-methacrylate were undertaken according to the protocol of Ogliari and others,[27] by means of esterification of the phenolic compounds with methacryloyl chloride in order to attach the polymerizable methacrylate functionality. Synthesis of cardol-methacrylate and cardanol-methacrylate occurred by replacing a hydroxyl present in the aromatic ring with a methacrylate (Figure 1).

Preparation of Artificial Caries-affected Dentin

Sixty teeth were prepared from extracted human third molars stored in 0.1% thymol solution at 4°C for 1 month or less, or until use.

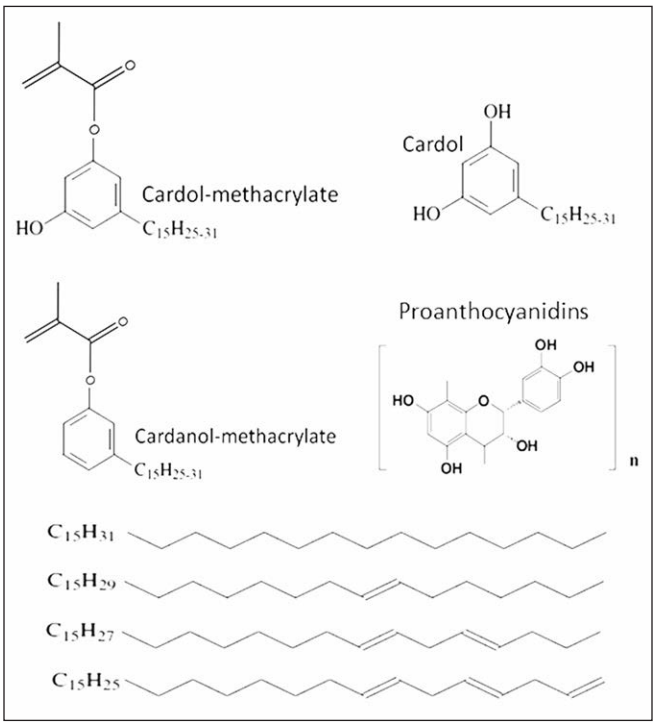

Figure 1. *Chemical structures of the biomodification agents tested.*

Each tooth was sectioned to expose a flat, middle dentin surface by using a slow-speed water-cooled diamond saw (Isomet 4000; Buehler, Lake Bluff, IL, USA), thereby removing the occlusal enamel crown and roots. Exposed dentin surfaces were ground using 320-grit SiC abrasive papers under constant water irrigation for 30 seconds to create a standardized smear layer.

Half of the specimens were subjected to pH cycling to create artificial caries-affected dentin. The occlusal dentin surface was polished with 1200-grit SiC paper to create a smooth surface. All further surfaces were protected with acid-resistant nail varnish. A layer of partially demineralized dentin approximately 200-μm thick[29] was created on the uncoated surface by pH cycling using the demineralizing solution containing 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 50 mM acetic acid, and 5 mM NaN_3 adjusted to pH 4.8. The remineralizing solution consisted of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.13 M KCl, and 5 mM NaN_3 buffered to pH 7.0 with HEPES buffer. Each specimen was immersed in 10 mL demineralizing solution for 8 hours, followed by immersion in 10 mL of remineralizing solution for 16 hours, with fresh solutions used for each cycle. This procedure was performed for 14 days at room temperature.[29]

Microtensile Bond Strength Testing

All specimens (with sound dentin and caries-affected dentin) were etched using a 37% phosphoric acid gel (Condac 37%, FGM, Joinville, Brazil) for 15 seconds, followed by a copious water rinse for 30 seconds. The etched dentin surfaces were gently air-dried for 2 seconds to remove excess water. Each pretreatment (control, GSE, cardol, cardol-methacrylate, and cardanol-methacrylate) was actively applied for 60 seconds, followed by rinsing with distilled water for 30 seconds. The treated dentin was dried with absorbent paper to remove excess water, thus leaving a moist reflective surface. All specimens were bonded using the two-step etch-and-rinse adhesive Optibond Solo Plus (Kerr Corporation, Orange, CA, USA). The bonding agent was actively applied for 30 seconds, gently air-dried and light-cured for 20 seconds using the LED light-curing unit DB-685 (1100 mW/cm^2; Dabi Atlante, Ribeirao Preto, SP, Brazil). Five 1-mm-thick composite increments were built up (TPH Spectrum, Dentsply Caulk, Milford, DE, USA), each increment was light cured for 20 seconds.

All bonded teeth (n=6) were immersed in distilled water for 24 hours at 37°C and subsequently attached to an acrylic device with 90° rotation and sectioned with an Isomet saw to obtain sticks with cross-sectional areas

approximately 1.0 mm^2 (±0.04). The cross-sectional area of each stick was measured with a digital caliper (Absolute Digimatic, Mitutoyo Corporation, Tokyo, Japan). Half of these sticks were tested immediately, and the remainder were stored in 3 mMol/L sodium azide solution for 1 year with exchanges performed every 15 days. The sticks were attached to a modified Geraldeli test apparatus[30] (Odeme Biotechnology; Joaçaba, SC, Brazil) with cyanoacrylate glue (Super Bonder Gel, Loctite, São Paulo, SP, Brazil) and tested to failure under tension in a universal testing machine (DL 2000; EMIC, Sao Jose dos Pinhais, PR, Brazil) with a crosshead speed of 0.5 mm/min. Bond strengths of the sticks from the same tooth were averaged, and the mean of each tooth was used as one statistical unit. A Shapiro–Wilk test was applied to all groups to analyze the normal distribution of errors, and the Bartlett test was used to determine homoscedasticity. After proving normal data, the results were statistically analyzed using two-way ANOVA (pretreatment and storage time) and Tukey test ($p<0.05$) individually for each dentin substrate.

Nanoleakage Evaluation

One stick from each restored tooth (n=6) was selected at random using Excel software (Excel 16.0, Microsoft Corporation, Redmond, WA, USA) for the nanoleakage test (n=6). The sticks were collected before microtensile testing. The nanoleakage test was performed, as previously described by Tay and others,[31] by using 50 wt% ammoniacal silver nitrate solution. The specimens were immersed in the tracer solution for 24 hours and then immersed in a photo-developing solution for 8 hours under a fluorescent light to reduce the silver ions into metallic silver grains. Thereafter, the specimens were rinsed with distilled water, embedded in epoxy resin stubs, and polished using successive 600-, 1200-, and 2000-grit wet SiC papers and 1-μm diamond paste (Buehler). The specimens were cleaned for 5 minutes in an ultrasonic bath after each abrasive/polishing step. The specimens were dehydrated in silica gel for 24 hours, coated with carbon, and examined using a field-emission SEM (Quanta FEG 450, FEI, Amsterdam, Netherlands) in the backscattered electron mode with 1000× and 3000× standardized magnifications.

Micropermeability Characterization

To perform the micropermeability test, three additional teeth per group were restored (n=3). The teeth were bonded, as previously described, with the adhesive resin doped with 0.1 wt% rhodamine-B (Sigma–Aldrich) and assessed using CLSM, according to a previously published protocol.[32] The micropermeability of the resin–dentin interfaces was evaluated using a 0.3 wt% aqueous fluorescein (Sigma–Aldrich) solution. This dye was perfused for 3 hours under 15 cm H_2O simulated pulpal pressure to test the sealing ability of the adhesive after different pretreatments.[32] The specimens were subsequently cut into 1-mm-thick slabs, slightly polished with 2000-grit polishing paper, and sonicated for 2 minutes. All steps were performed in environments with minimal light at room temperature (25°C).

The specimens were evaluated immediately after cutting using a CLSM instrument (LSM 710; Carl Zeiss, Munich, Germany) equipped with a 63× 1.4 NA oil immersion lens by using 488-nm and 568-nm laser illumination. CLSM fluorescence images were acquired with a 1-μm z-step to optically section the specimens up to 20 μm below the surface. The z-stack scans were compiled into single projections. Each resin–dentin interface was entirely characterized, and the images were captured along the bonded interfaces, representing the micropermeability characteristic from each group.

RESULTS

The means and standard deviations of μTBS on sound dentin are presented in Table 1. No difference was observed between the groups in this substrate after both storage periods ($p>0.05$). However, the control group did not maintain bond strength after 1 year ($p<0.001$). All experimental biomodification agents kept the resin–dentin bond strength stable after 1-year aging ($p>0.05$).

The results of μTBS on caries-affected dentin are presented in Table 2. Pretreatment with cardol-methacrylate resulted in significantly higher bond strengths than all other pretreatments in both the storage periods. In the immediate period, cardol and GSE presented statistically significant higher bond strength than the control group ($p<0.05$). However, no difference was observed between cardanol-methacrylate and the control groups ($p>0.05$). In addition, the resin–dentin bond strength was preserved after 1-year aging with pretreatments using cardol ($p=0.325$) and cardol-methacrylate ($p=0.103$).

Representative images of silver-infiltrated specimens of sound and caries-affected dentin substrates are illustrated in Figures 2 and 3, respectively. All biomodification agents on sound dentin resulted in great reduction of silver impregnation when compared to the control group in a 24 hour period (Figure 2), except cardanol-methacrylate (Figure 2-B1), which resulted in more nanoleakage even reaching the adhesive layer (Figure 2-B3). After 1-year aging, the cardol-methacrylate and GSE groups presented less silver infiltration (Figures 2-D2 and E2).

Table 1: *Mean (SD) Microtensile Bond Strengths in MPa for Sound Dentin*[a]

Sound Dentin	**Storage Period**[b]	
	24 Hour	**1 Year**
Control	48.6 (7.9) [47] Aa	26.1 (6.1) [41] Ab
Cardanol-MA	45.6 (3.5) [55] Aa	38.3 (5.7) [46] Aa
Cardol	42.5 (2.7) [49] Aa	36.5 (5.4) [40] Aa
Cardol-MA	36.2 (8.1) [58] Aa	36.9 (8.5) [50] Aa
GSE	37.1 (10.1) [54] Aa	36.2 (7.9) [42] Aa

Abbreviations: GSE, grape seed extract; MA, methacrylate.

[a] *Mean values with the same uppercase letters (column) and lowercase letters (row) are not significantly different (p>0.05).*

[b] *Numbers in brackets give the number of sticks tested per group in each period.*

Table 2: *Mean (SD) Microtensile Bond Strength in MPa for Artificially created Caries-affected Dentin*[a]

Caries-affected Dentin	**Storage Period**[b]	
	24 Hour	**1 Year**
Control	9.2 (7.2) [39] Ca	6.2 (0.7) [29] Cb
Cardanol-MA	10.0 (3.3) [41] Ca	5.7 (2.9) [32] Cb
Cardol	19.6 (5.1) [48] Ba	16.6 (6.8) [37] Ba
Cardol-MA	33.5 (7.3) [54] Aa	28.3 (8.9) [43] Aa
GSE	21.3 (3.8) [42] Ba	7.0 (3.1) [35] Cb

Abbreviations: GSE, grape seed extract; MA, methacrylate.

[a] *Mean values with the same uppercase letters (column) and lowercase letters (row) are not significantly different (p>0.05).*

[b] *Numbers in brackets give the number of sticks tested per group in each period.*

With caries-affected dentin (Figure 3), the porous zone of partially demineralized dentin 5-50 μm beneath the hybrid layer was fully infiltrated by silver deposits (Figures 3-A1 and E1). The presence of cracks and gaps was often noted in the control, cardanol-methacrylate, and GSE interfaces (Figures 3-A2, -B1, and -E2, respectively). Cardol-methacrylate pretreatment yielded less nanoleakage and better interface integrity than all further pretreatments (Figure 3-D). In cardol-methacrylate (Figure 3-D1) and GSE (Figure 3-E1) interfaces, a silver-free separation region between the hybrid layer and porous caries-affected dentin was observed, demonstrating some protection of dentin collagen. This same zone was noted in the cardol-methacrylate group after 1 year of aging (Figures 3- D2 and D3).

On the micropermeability analysis, all treatments showed deficient sealing ability when applied on caries-affected dentin, with the presence of interfacial gaps in the control group (Figure 4-A2). More fluorescein uptake was observed in the hybrid layers created using cardanol-methacrylate (Figure 4-B2) and GSE pretreatments (Figure 4-E2). In sound dentin, the control group showed intense fluorescein uptake in the hybrid layer, thereby indicating high micropermeability, while the pretreatment using cardol (Figure 4-C1) and cardol-methacrylate (Figure 4-D1) attained improved dentin sealing ability.

DISCUSSION

The development of new monomers derived from cardol and cardanol could be attributed to the proven ability of these molecules to improve the mechanical properties of collagen.[24] These molecules are extracted from CNSL—a natural renewable source obtained from the cashew industry—and exhibit three important advantages. (1) They are easy to obtain, since several thousands of tons of CNSL are produced yearly by cashew nut companies, which results in low production costs. (2) Finding various applications of CNSL and its compounds has environmental benefits, since industrial CNSL exhibits slow biodegradation and

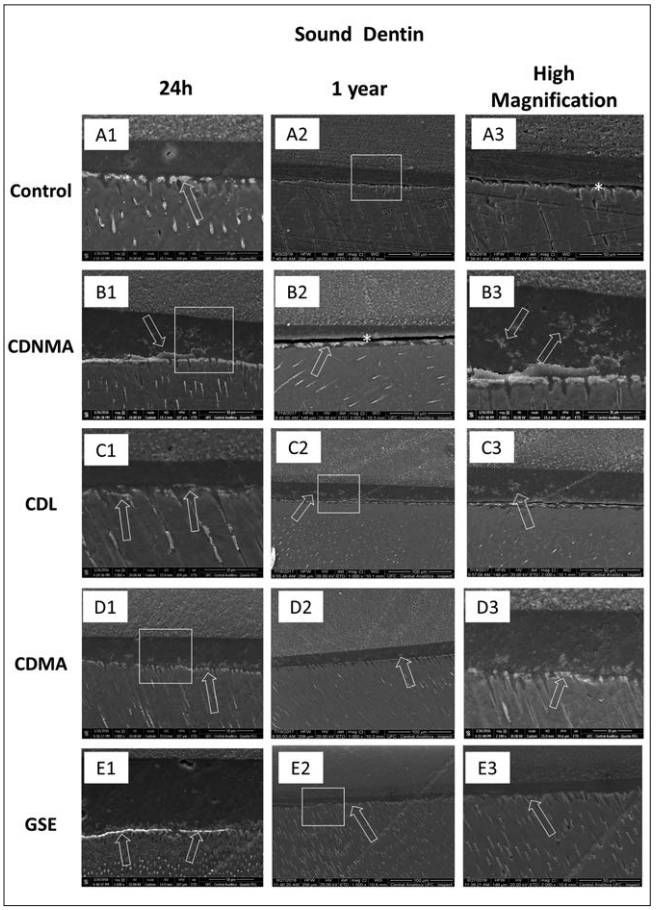

Figure 2. *Scanning electron microscopy (SEM) micrographs of resin–dentin interfaces illustrating the most common nanoleakage characteristics observed after silver uptake. Open arrows indicate silver deposits. The asterisks indicate gaps. CDL, cardol; CDMA, cardol-methacrylate; CDNMA, cardanolmethacrylate; GSE, grape seed extract.*

cannot be discarded in the environment. Moreover, large concentrations of cardol and cardanol can be obtained from CNSL after processing cashew nuts at high temperatures. (3) Cardol and cardanol are noncytotoxic compounds in low concentrations.[33,34] CNSL contains 60%-75% cardanol and 15%-20% cardol. Cardanol has been widely studied and has various chemical applications in the polymer, mineral oil, and ferrofluid industries.[28,35,36] Recently, a new polymer derived from cardanol has been demonstrated to be effective for tubular occlusion and possible treatment of dentin sensitivity.[37] Conversely, cardol has been poorly studied.

The presence of the methacrylate group in cardol-methacrylate and cardanol-methacrylate molecules allows for an interaction between these new monomers with monomers present in the formulation of bonding agents (e.g., triethylene glycol dimethacrylate and 2-hydroxyethyl methacrylate). However, before the copolymerization reaction, the new monomers are applied as a pretreatment in order to produce a chemical reaction with dentin collagen.[24] In fact, these reactions, promoted by cardol-methacrylate, increased the dentin bonding properties as observed in present outcomes (Tables 1 and 2), especially in caries-affected dentin, which has more exposed collagen. Pretreatment using cardol-methacrylate showed the best overall results with respect to bond strength and silver nanoleakage at both the substrate evaluations. Therefore, the first hypothesis was rejected.

According to Moreira and others,[24] the interaction between cardol/cardanol and collagen occurs by two mechanisms: first, forming hydrogen bonds by the presence of phenolic hydroxyl and, second, the hydrophobic bonds promoted by the long carbon chain. On the other hand, grape seed extract is unlikely to induce the formation of hydrophobic interactions.

Cardanol-methacrylate has a very similar chemical structure to cardol-methacrylate, but without the phenolic hydroxyl functionality attached to the aromatic ring. According to reports in the literature, the presence of the hydroxyl group attached to the aromatic ring allows strong interaction with collagen fibrils.[16,38] Therefore, the lack of this group allows only a hydrophobic interaction between cardanol-methacrylate and collagen fibrils. The comparison between the results obtained with cardol-methacrylate and cardanol-methacrylate indicate that the absence of a hydrophilic phenolic hydroxyl group (–OH) seems to compromise the penetration of cardanol-methacrylate in the water-rich demineralized collagen network. The lack of hydroxyl functionalities in cardanol-methacrylate, unlike in cardol-methacrylate, is an adequate explanation for its lower bond strength in caries-affected dentin. In addition, a remarkable difference was observed in silver nanoleakage between these two monomers (Figures 2 and 3).

Although the microtensile results were statistically lower than those observed with cardol-methacrylate, the cardol pretreated group showed stability of bond strength in dentin affected by caries, which indicates a potential effect of forming stable crosslinks, as previously demonstrated in the literature.[20] The integrity of the hybrid layer after storage represents a more relevant clinical outcome than high values of bond strength[39]; therefore, the results of both substances are promising for use in adhesive dentistry.

Nanoleakage images acquired at 24 hours in caries-affected dentin showed an intense concentration of silver deposits a few micrometers below the hybrid layer (Figure 3). This occurs due to caries-affected dentin

having areas of organized but partially demineralized collagen,[40] but this zone is closer to the adhesive layer in the control group than in the GSE group. Silver impregnation was not observed in the groups pretreated with cardol-methacrylate (Figure 3-D1), suggesting the crosslinking of collagen by means of hydrophobic interactions (through long carbon chains) was effective. After 1-year aging, the presence of silver near the hybrid layer was observed in all the experimental groups. However, the cardol-methacrylate application showed no silver infiltration into the hybrid layer (Figures 3-D2 and D3). Furthermore, little infiltration of silver after 1 year revealed a permanent protective effect on collagen, which seems to explain the favorable results of bond strength in caries-affected dentin (Table 2).

The control group showed a large infiltration of silver in both the substrates and the storage times; this is probably due to the incomplete penetration of monomers in the layer of demineralized collagen and

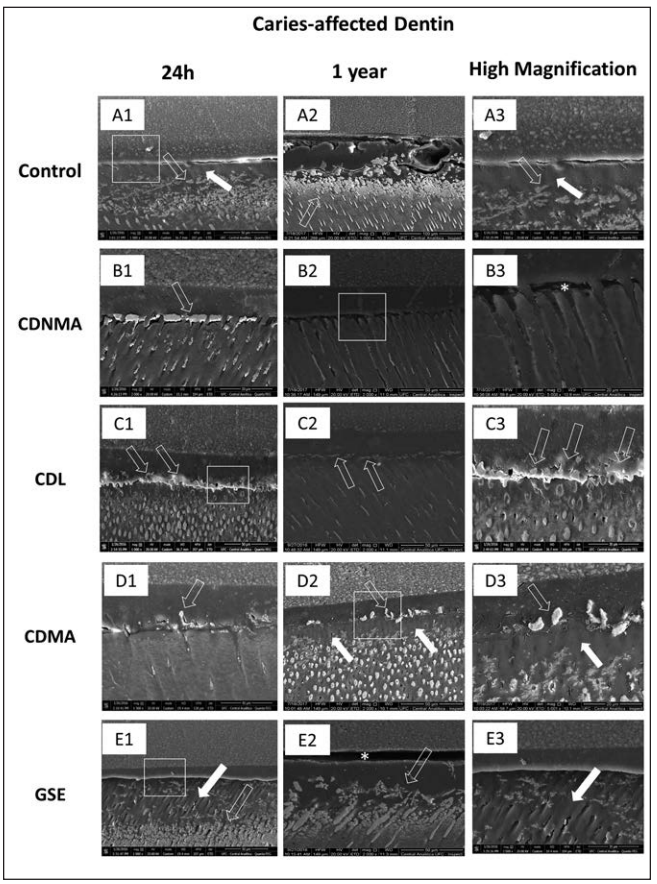

Figure 3. *Scanning electron microscopy (SEM) micrographs of resin–dentin interfaces illustrating the most common nanoleakage characteristics observed after silver uptake. Open arrows indicate silver deposits. White arrows indicate the protective zone between the hybrid layer and silver deposits. The asterisks indicate gaps. CDL, cardol; CDMA, cardol-methacrylate; CDNMA, cardanolmethacrylate; GSE, grape seed extract.*

the action of collagenolytic enzymes.[39,41] The structural reinforcement of collagen with the use of crosslinking agents and the use of amphipathic monomers, such as cardol-methacrylate and cardanol-methacrylate, could reduce these effects.

In caries-affected dentin, the decrease in bond strength and the increase in the amount of silver in the GSE group after 1 year seems to have occurred due to the reversibility of the bonds formed, which can be broken through hydrolysis, since these molecules are polar.[25,42] This outcome has been corroborated with other studies in the literature that also observed this effect.[24,25,43]

The micropermeability outcomes (Figure 4) indicated intense dye accumulation in the control interfaces for both the substrates. Dye penetration at the composite–dentin interface indicates incomplete adhesive infiltration into dentin, thereby resulting in a deeply demineralized dentin zone with exposed collagen fibrils.[44] Nevertheless, with experimental biomodification agents, the presence of fluorescein was notably reduced. This can be explained by the occupation of intrafibrillar and interfibrillar spaces by the plant-derived compounds, which were able to induce collagen cross-linking. The reduced uptake of fluorescein near the hybrid layer observed in the experimental groups indicates better sealing ability. Therefore, the second hypothesis should be accepted.

Indeed, the optimal outcomes of bond strength attained by using cardol-methacrylate with both sound and caries-affected dentin highlight such a monomer as a promising agent for dentin biomodification. However, one limitation of the present investigation and research design comprises the use of a simplified artificial caries model. Therefore, more studies are needed to evaluate other properties of this novel monomer in situations closer to clinical reality, its effects on resin polymerization, MMP/CTP inhibition capacity, cytotoxicity, and its use with self-etching adhesives.

CONCLUSIONS

Cardol-methacrylate is a promising monomer when used as an etch-and-rinse adhesive pretreatment. It might be used to reinforce the hybrid layer, as it preserves resin–dentin bond strength in both sound and caries-affected dentin; it also improves dentin sealing ability.

Acknowledgments

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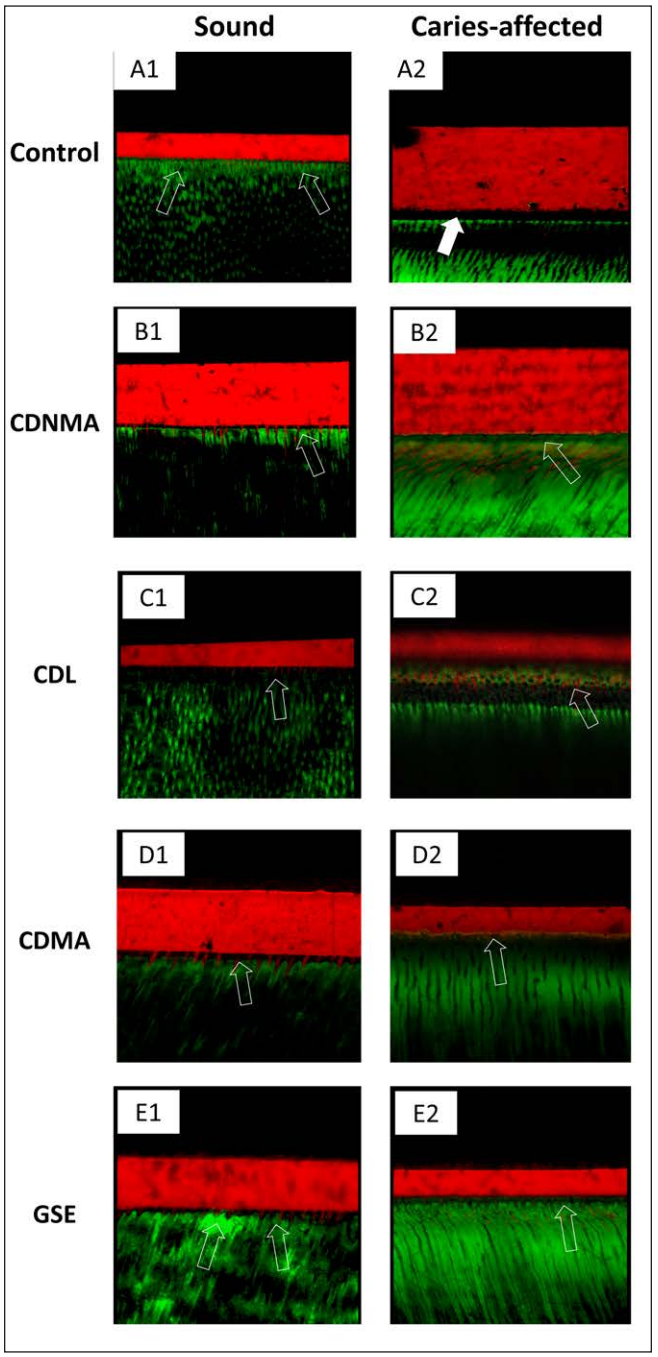

Figure 4. *Confocal micrographs showing the main features of fluorescein micropermeability. Open arrows indicate fluorescein infiltration at and near the hybrid layer. White arrow in A2 indicates a gap. HL in D3 indicates hybrid layer. CDL, cardol; CDMA, cardol-methacrylate; CDNMA, cardanolmethacrylate; GSE, grape seed extract.*

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects' oversight committee guidelines and policies of the Research Ethics Committee on Investigations Involving Human Subjects of Federal University of Ceara. The approval code issued for this study is 1482602.

Conflict of Interest

The authors of this article certify that they 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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