©Operative Dentistry, 2018, **43-5,** E253-E265

Chemical Interaction and Interface Analysis of Self-Etch Adhesives Containing 10-MDP and Methacrylamide With the Dentin in Noncarious Cervical Lesions

BMB Oliveira • ALM Ubaldini • ML Baesso • LHC Andrade
SM Lima • M Giannini • L Hernandes • RC Pascotto

Clinical Relevance

The chemical interaction and morphology at the interface of self-etch adhesives and the dentin of noncarious cervical lesions depend on the functional monomer present in the adhesive. This fact is essential to evaluate the requirement for additional substrate preparation before commencing adhesive procedures.

SUMMARY

Objectives: To characterize the chemical interactions and analyze the interface of adhesive systems containing 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) and N-methacryloyl glycine (methacrylamide) functional monomers with the dentin in noncarious cervical lesions (NCCLs) compared with artificial defects (ADs).

Methods and Materials: Twenty human teeth with natural NCCLs on the buccal surface were used. Class V cavities, similar to NCCLs, were created on the lingual surface to serve as

Bruna M B Oliveira, DDS, MSc, State University of Maringá, Dentistry, Av Mandacaru, 1550, Maringá, Paraná 87010-060, Brazil

Adriana L M Ubaldini, DDS, MSc, Av Mandacaru, 1550, Maringá, 87.080-000, Brazil

Francielle Sato, PhD, State University of Maringá, Physics, Av Colombo, 5790, Maringá, 87020900, Brazil

Mauro L Baesso, PhD, State University of Maringá, Physics, Av Colombo, 5790, Maringá, 87020900, Brazil

Luis H C Andrade, PhD, State University of Mato Grosso do Sul, Physics, Cidade Universitária de Dourados, Dourados, Mato Grosso do Sul 79804-970, Brazil

Sandro M Lima, PhD, State University of Mato Grosso do Sul, Physics, Cidade Universitária de Dourados, Dourados, Mato Grosso do Sul 79804-970, Brazil

Marcelo Giannini, DDS, MSc, PhD, Piracicaba Dental School, University of Campinas, Department of Restorative Dentistry, Av Limeira, 901, Piracicaba, SP 13414-903, Brazil

Luzmarina Hernandes, PhD, State University of Maringá, Morphological Sciences Department, Av Colombo, 5790, Maringá, Paraná 87020900, Brazil

*Renata C Pascotto, DDS, MSc, PhD, State University of Maringá (UEM), Dentistry, Maringá, PR 87.080-000, Brazil

*Corresponding author: Av Mandacarú, 1550, Maringá, PR 87.080-000, Brazil; e-mail: renatapascotto@gmail.com

DOI: 10.2341/17-366-L

controls. Teeth were randomly allocated to two groups according to the functional monomer in the adhesive (N=10): G1, 10-MDP; and G2, methacrylamide. NCCLs and ADs were characterized by their mineral composition (MC) and degree of demineralization (DD) using micro-Raman spectroscopy, adhesive/dentin chemical interactions (CIs) were assessed with infrared photoacoustic spectroscopy, and interface morphology was evaluated with scanning electron and light microscopy. MC, CI, and DD data were submitted to Shapiro-Wilk and Student *t*-tests ($p<0.05$).

Results: Compared with ADs, dentin in NCCLs was hypermineralized ($p<0.05$). In G1, CI, and DD in the first 2 μm, and adhesive projections in NCCLs and ADs interfaces were similar. Additionally, a thin layer of dentin collagen was observed in ADs, while it was hardly present in NCCLs. In G2, although CI could not be identified, changes in the mineral components were observed. The DD in the ADs and NCCLs were statistically similar, while SEM showed a lack of adhesion at NCCLs interface. DD and collagen exposure in the ADs and NCCLs were more pronounced than in G1.

Conclusions: Results suggest that the G1 adhesive could be applied directly on the superficial sclerotic layer in NCCLs. In contrast, previous cavity preparation should be conducted to improve the micromechanical interaction of G2 with the dentin.

INTRODUCTION

Noncarious cervical lesions (NCCLs) form a group of lesions difficult to characterize in the dental practice because of their multifactorial etiology.[1,2] NCCLs result from the slow and progressive loss of mineralized dental structure caused by the association of different phenomena such as erosion, abrasion and abfraction.[3]

Laboratory studies have demonstrated that adhesion to dentin affected by NCCLs may lead to adhesive failures and compromise the longevity of restorations.[4-6] The main reason for this phenomenon is the molecular/chemical structural changes that occur at the interface, which result in less favorable adhesion to the substrate.[7] Because the dentin in NCCLs is sclerotic, the formation of a hybrid layer in the dentin/adhesive interface is compromised by irregular primer diffusion and reduced adhesive infiltration.[8]

Although the main adhesive mechanism to the dental substrate is based on the micromechanical retention resulting from the formation of a hybrid layer and resin tags, attention has recently been focused on the benefit of additional chemical interactions between the functional monomers present in adhesive systems and the components of the dental substrate.[9-12]

Chemical interactions can occur through ionic bonds established by acid monomers such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) that react with the hydroxyapatite, forming monomer-Ca salts that are stable to degradation.[9,10] On the other hand, the interaction of adhesive systems containing methacrylamide can result in bonds with dentin collagen fibrils through the additional reactive groups present in monomeric acids. Since dentin collagen contains reactive groups such as amino or hydroxyl, the aldehyde or anhydride groups of the adhesive system can establish covalent bonds with collagen fibrils.[13]

The aim of this study was to analyze the chemical interactions of two self-etching adhesive systems, one containing the 10-MDP and the other methacrylamide functional monomers with the dentin in NCCLs and artificial defects (ADs) so that we could evaluate the requirement for additional substrate preparation before undertaking adhesive procedures.

METHODS AND MATERIALS

This *in vitro* study was approved by the local ethics committee (CAAE: 47305015.7.0000.0104). Teeth with natural NCCLs extracted for periodontal or orthodontic reasons were used. All teeth presented grade 4 of dentin sclerosis, according to the scale modified by Ritter and others.[14] Grade 4 is attributed to NCCLs with significant presence of sclerosis, in which the dentin is dark-yellow or brownish with a petrified appearance, significant translucency, or evident transparency.

Specimen Preparation

A total of 20 teeth with natural NCCLs located in the cervical region of the buccal surface were used in the experiment. They were randomly divided into two groups (N=10); G1, to be restored with an adhesive system containing 10-MDP, and G2, to be restored with an adhesive system containing methacrylamide. After extraction, the teeth were cleaned with sterile gauze and saline solution. Any remaining periodontal tissues were removed with the aid of

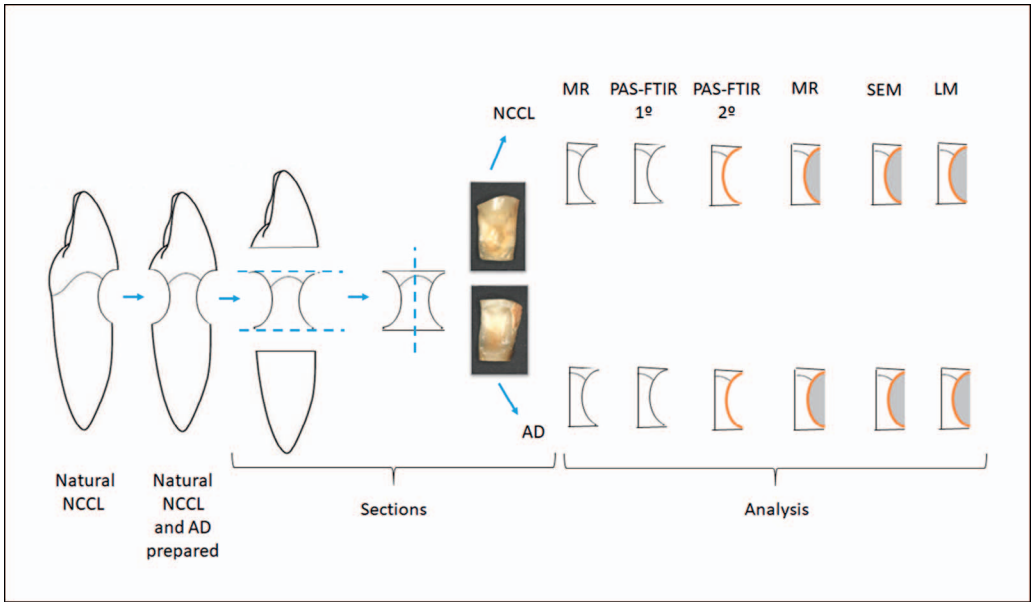

Figure 1. *Diagram illustrating preparation of the specimens and analysis sequence.*

periodontal curettes. After cleaning, the teeth were stored in saline at 4°C.

Artificial defects (ADs) in the shape of Class V cavities were created in the lingual surface of the same tooth with a CVDentus (C1, 1.0 × 4.0 mm) cylindrical diamond tip coupled with an ultrasound device (CVDent 1000, CVDVale, São Carlos, Brazil) under continuous water cooling. ADs were prepared in sound dentin with dimensions and shape approximately the same as those of the corresponding NCCL, serving as a control.[15]

Then, dental specimens containing the NCCLs and ADs were obtained from each tooth. The teeth were sectioned with a diamond disk at low speed under water cooling in the following sequence: first, just above the lesion to remove the crown, and then, just below the lesion to remove the remaining two-thirds of the root. Finally, a section was made along the long axis of the tooth to separate specimens containing the NCCLs from the ADs. Once the dental specimens were obtained, they were ready to be employed in the sequence of analyses described below (Figure 1).

Adhesive System Application

The composition of the adhesive systems used in this study and the recommended mode of application are described in Table 1. Light-curing was done with the Translux Power Blue unit (Heraeus Kulzer, Hanau, Germany) at 1000 mW/cm^2. The light-curing time for each adhesive system was according to the manufacturer's instructions.

Dentin Mineral Composition

Mineral composition analysis of the dentin in ADs (control) and NCCLs was performed with micro-Raman (MR) spectroscopy. The analysis was conducted with a micro-Raman spectrometer (Bruker Optik GmbH, Ettlingen, Germany), equipped with a Senterra confocal microscope, whose operation is based on infrared light scattering, ie, the source of light irradiation (laser with invisible wavelength) excites the studied matter. In this interaction, the Raman effect is obtained, which allows studying vibrations at the molecular level.

Specimens' spectra were measured at three different points on the dentin surface. All measurements were collected at a resolution of 4 cm^{-1} in the spectral region between 3500 and 450 cm^{-1}. Each spectrum was obtained from an average of 60 readings to decrease the signal-to-noise ratio, with a laser wavelength of 785 nm, power of 100 mW, and objective gain of 100×. In addition to the high number of readings, the signal pattern was improved by decreasing detector temperature to −84°C. In all readings, the surface area selected for measurement, the support mirror used, and manual focusing followed by autofocusing were performed following the same standardized procedures.

All spectra were placed on the same baseline and normalized with the aid of Opus spectroscopy soft-

Table 1: *Composition and Mode of Application of Adhesive Systems Used in the Experiment*

Group	System	Composition	Application Mode
G1	Clearfill SE Bond 2 (Kuraray Noritake Dental Inc., Tokyo, Japan)	*Primer:*	Apply primer for 20 s
		MDP	Gently air dry for 5 s
		HEMA	Apply adhesive
		Dimethacrylate	Photopolymerize for 10 s
		Camphorquinone	
		Water	
		Adhesive:	
		MDP	
		Bis-GMA	
		HEMA	
		Dimethacrylate	
		Camphorquinone	
		Initiators	
		Accelerators	
		Silanized colloidal silica (pH 2)	
G2	Xeno V+ (Dentsply Sirona, York, PA, USA)	Bifunctional acrylate	Actively apply adhesive for 20 s
		Acrylate acid	Gently air dry for 5 s
		Esters of phosphoric acid	Photopolymerize for 10 s
		Water	
		Tertiary butanol	
		Initiators	
		Stabilizers	
		(pH 1.38)	

Abbreviations: MDP, 10-methacryloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol-A Bismethacrylate; HEMA, 2-hydroxyethylmethacrylate.

ware (Bruker Optics, Ettlingen, Germany). Additionally, origin software (OriginPro 8 Corp, Northampton, MA, USA) was used to obtain numerical quantifications of MR spectra by integrating each curve of the band at 961 cm^{-1} (phosphate) to calculate the respective area and mean of the three distinct points measured in the sound dentin of the AD specimens and in the sclerotic dentin of the natural NCCLs.

Adhesive/Dentin Chemical Interactions

Specimens' spectra were measured with Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) both before and after being submitted to the adhesive treatment. The technique provides the optical absorption bands of the sample, which are considered the fingerprint of specific molecules. Information on the chemical modifications within the specimen is expressed by means of changes and/or emergence of new peaks.

The experiments were performed with a Nicolet Spectrometer (MTEC Photoacoustics, Ames, IA, USA) equipped with a MTEC 200 photoacoustic cell model. This equipment allows monitoring the absorption of the substance of interest at specific specimen depths, providing the distribution profile of the substances along the thickness studied. All spectra were collected at a resolution of 8 cm^{-1}, with scanning speed of 0.5 cm/s. The spectral region of the measurements lies in the energy range between 4000 and 400 cm^{-1}. After the specimen was inserted, the photoacoustic cell was filled with helium to minimize interference on the optical absorption spectra of oxygen and water molecules present in the air and on the surface of the specimen.

To determine the depth of the analysis in the present experimental condition, FTIR-PAS test measurement depth was defined by the thermal diffusion length. The thermal diffusivity of the adhesive was measured by the thermal lens technique[16] as described by Oliveira and others.[15] The inspection depth of the technique for the readouts taken in this study was about 6 μm for G1 and 4 μm for G2. Since the adhesive film depth can range from 2-3 μm,[17,18] this technique enabled reading not only the hybrid region, but also the dentin under the adhesive.

To evaluate the spectrum of the adhesive system, a disc of pure adhesive was prepared, applying 1 mL of

the material on a histological glass slide. After photoactivation for 20 seconds, the disc was inserted into the measuring equipment. This is an important step to differentiate the composition of the adhesive from that of the dental structure and to verify the differences between photoacoustic absorption peaks of the adhesive and dentin.

Collected data were transferred to the origin software. Graphs were generated for each tooth individually, from which the average spectrum of ADs and natural NCCLs dentin specimens were calculated. Bands identified as chemical interactions between the dentin and the adhesive system were selected, and the respective intensities in the ADs and natural NCCLs before and after the application of the adhesive were compared.

Degree of Demineralization

After FTIR-PAS analysis, all cavities were filled with composite resin (Filtek Z250, 3M ESPE, St Paul, MN, USA) and subsequently sectioned for MR scanning analysis. Dental specimens were cut longitudinally with a sectioning machine (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) using a diamond disc (Diamond Wafering Blade, Series 15LC, Arbon size ½, 12.7 mm, Buehler) under water cooling.

MR spectra were obtained by scanning the inner region of the composite resin toward the deeper layers of the dentin in NCCLs and ADs. All spectra were obtained under the same conditions as previously described for mineral composition analysis. Raman spectra were acquired at 1-μm intervals in 20-μm long lines at a distance of 10 μm between lines. Scans were individually analyzed, and spectra reading presenting any flaws, possibly due to the presence of bubbles, were excluded from the analysis. All spectra were placed on the same baseline and normalized with the aid of the Opus spectroscopy software, and analyses were conducted with the origin software. Degree of demineralization (DD), as a function of location, was determined at the bands at 961 cm^{-1} (PO_4 of the dentin) in relation to the band at 1458 cm^{-1} (CH_2), according to the equation:

$$DD = \left(1 - \frac{961cm^{-1}intensityinterface/1458cm^{-1}intensityinterface}{961cm^{-1}intensitydentin/1458cm^{-1}intensitydentin}\right) \times 100\%$$

Interface Morphology

Analysis of the adhesive system/dentin substrate interface morphology was performed with scanning electron microscopy (SEM). Sample treatment was performed according to the protocol suggested by Monticelli and others.[19] After MR analysis, the samples were embedded in acrylic resin and polished with a wet sandpaper sequence (220, 400, 600, 1200, 1800, 2000 grit). Then the specimens were submitted to demineralization with 37% phosphoric acid for 10 seconds, deproteinization with 2% sodium hypochlorite for 1 minute, and dehydration with 100% alcohol for 2 minutes in an ultrasonic vat (Bio-Free-Gnatus, Ribeirão Preto, Brazil) and air jets. Specimens were sputter-coated with gold and evaluated with SEM (Shimadzu, Model SS-550 Superscan, Kyoto, Japan) with a magnification of 1000×.

Collagen Fiber Exposure

To examine collagen fiber exposure at the adhesive system/dentin interface, four additional teeth were prepared according to the protocol described above, up to the application of the adhesive system, and histologically analyzed under light optical microscopy.

Immediately after application of the adhesive system, samples were fixed in Karnovsky's solution (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.3) for 48 hours and washed in running water for 4 hours. The samples were placed in a decalcifying solution (20% sodium citrate and 50% formic acid) for 25 days. After that, the specimens were washed in running water for 4 hours, dehydrated in an increasingly concentrated alcohol sequence, and embedded in paraffin. Histological sections, 6 μm thick, were serially made using a tungsten carbide blade coupled with a microtome (Leica RM2265, Leica Microsystems, Wetzlar, Germany).

Goldner-modified Masson Trichrome[19-21] was used to stain the specimens. Before staining, the samples were prepared according to the protocol proposed by Wang and Spencer.[20] The sections were first stained with Weigert's iron hematoxylin solution for 5 minutes, immersed in Masson solution for 10 minutes, and rinsed twice in 0.2% acetic acid solution. Afterward, they were kept in mordant solution for 5 minutes and washed again with 1% acetic acid. Finally, they were stained with a light green solution for 5 minutes and rinsed twice with 0.2% acetic acid. For sample dehydration, the sections were immersed in 96% and 100% alcohol twice for 1 minute. Specimens were immersed twice in xylol for 3 and 5 minutes and mounted within histological slides. Sections were examined and photographed at a magnification of 100× under a light optical microscope (Olympus BX41, Tokyo, Japan).

Statistical Analysis

Statistical analysis was performed using the R i386 3.0.2 software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria). The areas of the band at 961 cm^{-1} (PO_4) for the phosphate present in the dentin, the intensities related to the chemical interactions found with FTIR-PAS, and DD were submitted to the Shapiro-Wilk and Student *t*-test ($p<0.05$).

RESULTS

Adhesive/Dentin Chemical Interactions

Figure 2A shows the spectrum of the pure adhesive system containing the 10-MDP monomer (G1) with its organic and inorganic functional groups: methacrylate monomer (carbonyl C=O [1720 cm^{-1}], CH_2CH_3 [1457 cm^{-1}]), BIS-GMA (C=C [1638 cm^{-1}], C-O-C [1140 cm^{-1}], $[CH_3]_2$-C [1300 cm^{-1}], C_6H_4 [840 cm^{-1}]), and load (SiO_2 [1105 cm^{-1}]). Figure 2B shows the dentin spectra of the natural NCCLs and ADs. The bands associated with mineral and organic composition were observed at the following wavenumbers: phosphate (1179 cm^{-1}), amide I (1650 cm^{-1}), amide II (1550 cm^{-1}), amide III (1240 cm^{-1}).

Figure 3A and B illustrate the NCCL spectra before and after application of the adhesive system containing the 10-MDP monomer (G1). Circles and bars indicate changes in spectra, suggesting chemical interactions between the dentin and the adhesive system. Table 2 shows the bands and functional groups identified as possible chemical interactions between the dentin and the adhesive system containing 10-MDP monomer and the means and standard deviations of the intensities obtained from the ADs and natural NCCLs characterizing chemical interactions.

Although no statistically significant differences were observed between the two groups, mean values for natural NCCLs in all band intensities were numerically higher than those of ADs.

Figure 4A shows the photoacoustic absorption spectra of the pure adhesive containing the methacrylamide monomer (G2), with the following functional groups: CH_2 (1456 cm^{-1}), CH_3 (1373 cm^{-1}), PO_2 (1265 cm^{-1}), CH (1139 cm^{-1}), C-O-C (1040 cm^{-1}), and Al-OH (920 cm^{-1}). Figure 4B shows the dentin in the natural NCCLs before and after application of the adhesive system, suggesting a change in the inorganic components such as phosphate and calcium. However, no indication of chemical interactions can be observed.

Dentin Mineral Composition

Means and standard deviations of the measured spectra obtained from the integrated areas at the band at 961 cm^{-1} (PO_4) on the surface of the ADs and natural NCCLs are shown in Table 3. The Student *t*-test showed that the mineral content in dentin in natural NCCLs was significantly higher than that found in the ADs ($p<0.05$).

Degree of Demineralization (DD)

MR scanning analysis of the natural NCCLs and ADs with the adhesive systems used are represented by Figures 5 and 6. It can be observed that, in G1, the behavior in natural NCCLs (Figure 5A) and ADs (Figure 5B) was similar. However, in G2, the dentin in the ADs (Figure 6A) underwent deeper demineralization than did the natural NCCLs (Figure 6B).

Means of the DD (%) of the dentin using the systems G1 and G2 are presented in Table 4. The DD in G1 had similar behavior in the first 2 μm of the hybrid layer, whereas from 3 μm, a statistically significant difference ($p<0.05$) between the ADs and natural NCCLs was observed. In G2, DD presented no statistically significant differences between ADs and natural NCCLs.

Interface Morphology

Scanning electron microscopy analysis showed differences in the adhesive system/dentin substrate interface between G1 and G2 (Figure 7). In G1, images demonstrated similar projections of the adhesive within the demineralized dentin in ADs (Figure 7A) and natural NCCLs (Figure 7B), although the sclerotic aspect of the dentin with greater mineralization can be identified in the natural NCCLs. In G2, when applied to ADs (Figure 7C), the methacrylamide-containing adhesive provided greater inter- and peritubular demineralization, with well-defined projections within the dentin. On the other hand, the interface in the natural NCCLs (Figure 7D) was indefinite and more obliterated because of the higher degree of mineralization, compromising the formation of a hybrid layer due to irregular primer diffusion and reduced adhesive infiltration, leading to failure of the restoration (indicated by the arrow in Figure 7D).

Collagen Fiber Exposure

Photomicrographs of the samples stained with Goldner's Modified Masson's Trichrome are shown in

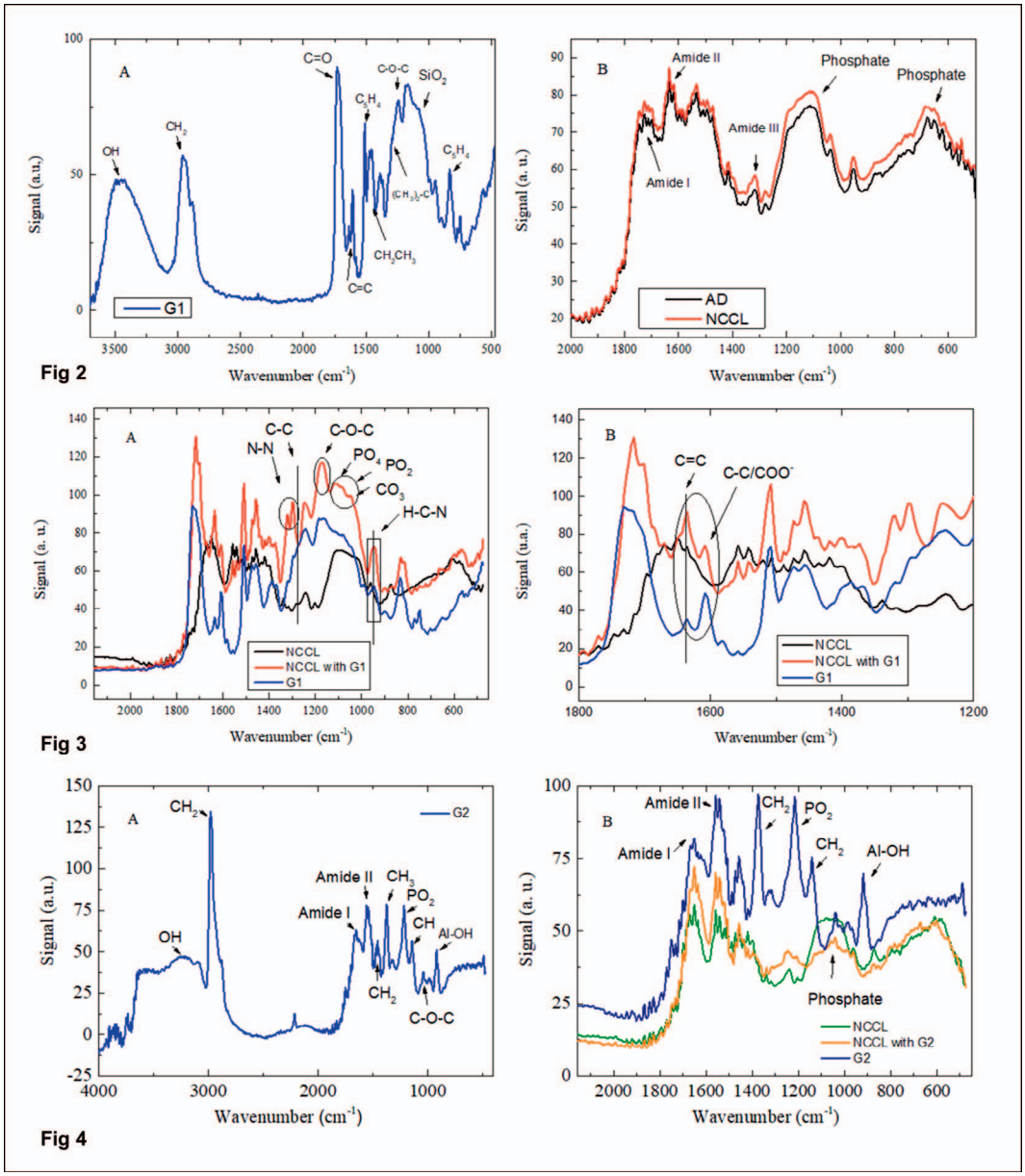

Figure 2. *Photoacoustic absorption spectra: (A) pure adhesive system containing 10-MDP (G1), and (B) dentin in ADs and natural NCCLs.*

Figure 3. *Photoacoustic absorption spectra obtained from the adhesive and the NCCL before and after application of the adhesive system containing the 10-MDP monomer (G1). (A) Spectral region 2100 to 550 cm^{-1}, and (B) spectral region between 1800 and 1200 cm^{-1}.*

Figure 4. *(A) Photoacoustic absorption spectra obtained from the pure adhesive containing the methacrylamide monomer (G2), and (B) NCCL before and after application of the adhesive system. Arrow indicates structural modifications in the region of the inorganic component of dentin.*

Figure 8. The dentin appears stained in green, the exposed unprotected collagen is evidenced in red/pink, while pure adhesive is unstained.[22,23]

In G1, histomorphological analysis demonstrated a thin layer (pink) of exposed collagen in the dentin of the ADs (Figure 8A). In the natural NCCLs (Figure 8B), collagen was hardly evident. In G2, the photomicrographs illustrate deeper demineralization of the dentin and more pronounced collagen exposure (dark red) in the ADs (Figure 8C) as well as natural NCCLs (Figure 8D).

DISCUSSION

Self-etching adhesive systems with various functional monomers were used in this study in an attempt to

Table 2: *Means and Standard Deviations (SDs) of the Intensities of the Bands That Characterize Chemical Interactions in ADs and Natural NCCLs With the Adhesive Containing the 10-MDP Monomer (G1)*

Assignment	Band	Group	Mean	SD	p*
C=C	1635 cm^{-1}	AD	60.82	3.83	0.25
		NCCL	63.24	3.8	
C-C/COO^-	1608 cm^{-1}	AD	49.66	3.67	0.19
		NCCL	52.47	3.92	
N-N stretching	1323 cm^{-1}	AD	59.86	6.22	0.52
		NCCL	62.45	8.26	
C-C stretching	1295 cm^{-1}	AD	64.28	7.23	0.58
		NCCL	66.82	9.55	
C-O-C	1169 cm^{-1}	AD	76.7	8.67	0.58
		NCCL	79.93	12.34	
PO_4	1112 cm^{-1}	AD	68.94	7.86	0.41
		NCCL	72.77	9.23	
PO_2 symmetric stretching	1081 cm^{-1}	AD	67.4	7.55	0.36
		NCCL	71.56	8.77	
CO_3	1045 cm^{-1}	AD	64	6.73	0.25
		NCCL	68.52	7.4	
H-C-N bending	946 cm^{-1}	AD	49.26	5.07	0.36
		NCCL	52	5.67	

** Student t-test ($p<0.05$).*
Abbreviations: AD, artificial defect; NCCL, noncarious cervical lesion

Table 3: *Means and Standard Deviations of the Integrated Areas of the Band at 961cm^{-1} (PO_4) of ADs and Natural NCCLs in arbitrary units (a.u.)*

Group	Mean (a.u.)	Standard Deviation	p
ADs	297,364.6	43,571.92	0.002*
NCCLs	387,726.7	80,574.17	

** Student t-test ($p<0.05$)*

identify strategies that might increase the longevity of esthetic restorations in natural NCCLs.

Controversy on the best strategy to restore NCCLs still exists. A fairly recent systematic review of the literature failed to find sufficient evidence to support a particular adhesive system or adhesive strategy for the restoration of natural NCCLs,[24] highlighting the necessity of investigating the adhesive interface in these situations.

In the present study, MR analysis showed that the areas corresponding to the phosphate band (961 cm^{-1}) in the dentin of ADs and NCCLs were statistically different, clearly demonstrating that the dentin in NCCLs was more mineralized than in ADs. This analysis was performed in our study with the objective of confirming the presence of sclerotic dentin and ensuring that specimens with natural NCCLs were in similar conditions. These findings corroborate previous studies that evaluated the molecular and structural differences in the mineral/organic components in the dentin of natural NCCLs and ADs using MR[7,15] and FTIR-PAS.[25]

It has already been demonstrated that the 10-MDP functional monomer present in G1 is capable of forming chemical bonds with the hydroxyapatite (calcium salts-MDP), improving the adhesion and longevity of restorations.[10,15,26,27] In the present study, FTIR-PAS analysis demonstrated that the peak intensities related to the chemical interactions (Table 2) in ADs were similar to those in natural NCCLs. The DD (Table 4) of natural NCCLs and ADs was also similar for the first 2 μm of the hybrid layer, becoming statistically different below the depth of 3 μm. However, SEM images (Figure 7A,B) demonstrated a similar behavior in terms of adhesive infiltration, despite the higher degree of dentin mineralization in natural NCCLs.

Histomorphological photomicrographs of the adhesive systems tested in this study revealed the presence of exposed and unprotected collagen (Figure 8). For the formation of an ideal hybrid layer, collagen should be fully protected by the monomer in the adhesive system, preventing any collagen labeling.[23] Studies show that fully exposed collagen is marked by a strong red color, and when partially coated by the adhesive, is marked by lighter colors.[22] In the present study, it is possible to observe a thin layer of light red/pink collagen in G1, indicating that the adhesive partially enveloped the demineralized dentin in the ADs (Figure 8A). In the natural NCCLs, the system was able to encapsulate collagen more closely to the ideal and was very little evidenced by the dye (Figure 8B).

The molecular/chemical alterations of the hypermineralized sclerotic dentin in NCCLs may result in a less favorable substrate for adhesion.[7] Some studies advocate removal of the superficial sclerotic layer to increase intratubular retention.[5,28-30] However, according to Tay and Pashley,[8] removal of the hypermineralized layer in NCCLs may not increase adhesive strength, since the sclerotic dentin might still contain crystals capable of preventing the infiltration of the adhesive into the dentinal tubules. Another systematic review failed to determine any differences in survival rates

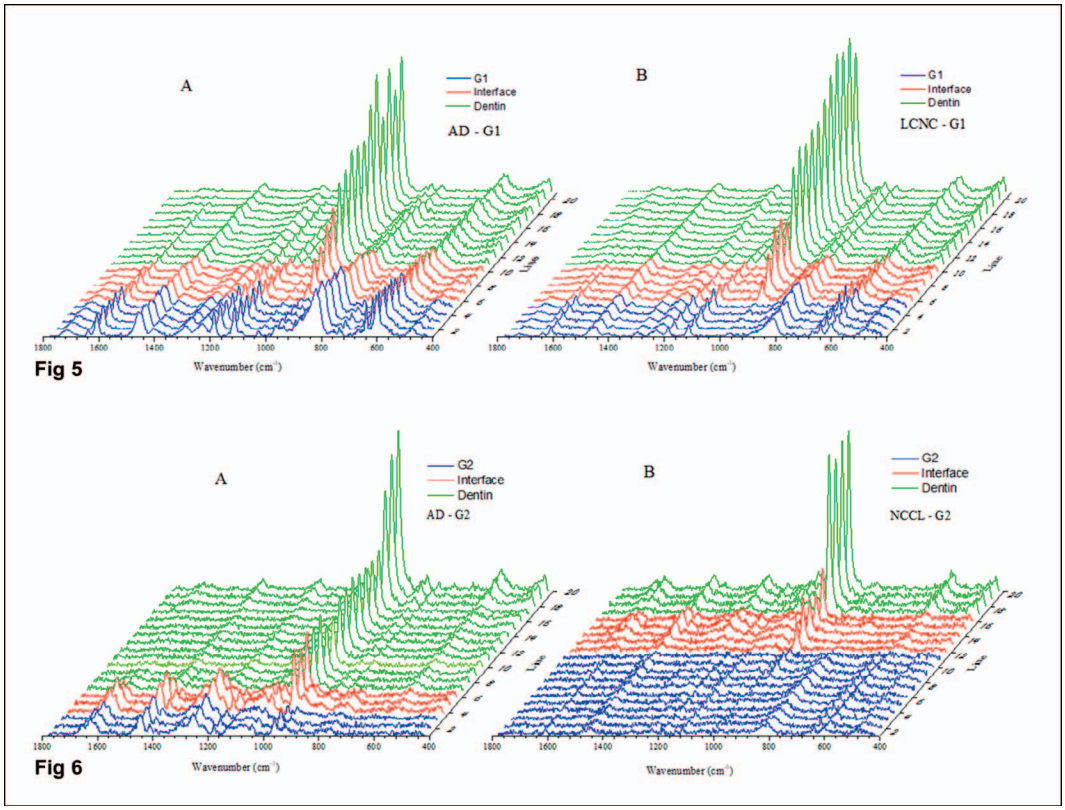

Figure 5. *MicroRaman spectra of the dentin/adhesive system interface in G1: (A) artificial defects (ADs), and (B) natural noncarious cervical lesions (NCCLs).*

Figure 6. *MicroRaman spectra of the dentin/adhesive system interface in G2: (A) artificial defects (ADs), and (B) natural noncarious cervical lesions (NCCLs).*

because of the small number of studies comparing the influence of dentin roughness on the retention of restorations in NCCLs.[31]

However, Luque-Martinez and others[32] demonstrated that the adhesive strength of self-etching systems containing 10-MDP in unprepared sclerotic bovine dentin was superior to the same surface prepared with diamond burs. Corroborating these findings, Oliveira and others,[15] using human teeth with natural NCCLs, also observed that adhesion of a self-etching system containing 10-MDP was stronger in natural NCCLs than in ADs, probably due to the chemical affinity of the monomer with the hydroxyapatite.

The results of the present study suggest that the adhesive system containing 10-MDP functional monomer (G1) can be applied directly on the superficial sclerotic layer in NCCLs since the intensity of chemical interactions and the degree of demineralization of natural NCCL and ADs are similar. This perception is reinforced by the fact that G1 was able to involve collagen in natural NCCLs more completely. As a

Table 4: *Degree of Demineralization (%) in ADs and NCCLs in Groups G1 and G2*

Group	Dentin	Degree of Demineralization							
		1 μm, %	*p*	2 μm, %	*p*	3 μm, %	*p*	4 μm, %	*p*
G1	AD	93	0.557	91	0.242	87	0.005*	81	0.016*
	NCCL	94		87		75		70	
G2	AD	91	0.514	91	0.515	89	0.202	83	0.076
	NCCL	89		87		80		67	

** Student t-test ($p<0.05$).*

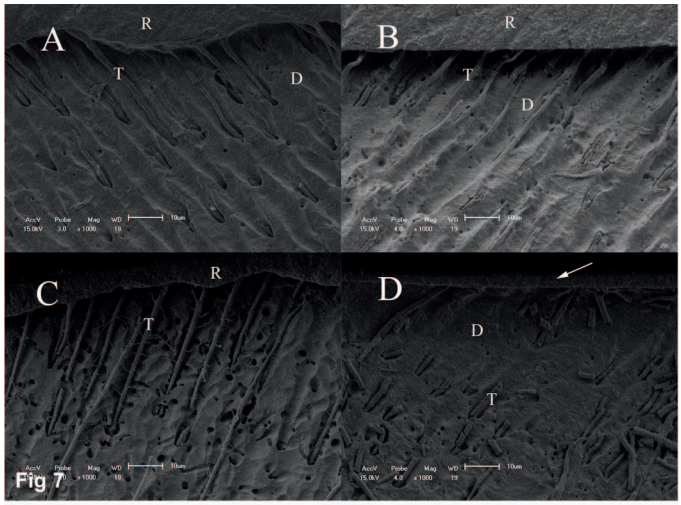

Figure 7. *The dentin/adhesive interface observed with scanning electron microscopy: (A) G1 in AD, (B) G1 in natural NCCL,* ***(C)*** *G2 in AD, and* ***(D)*** *G2 in natural NCCL. Arrow in Figure 7D indicates restoration failure. R: restoration; T: tags; D: dentin.*

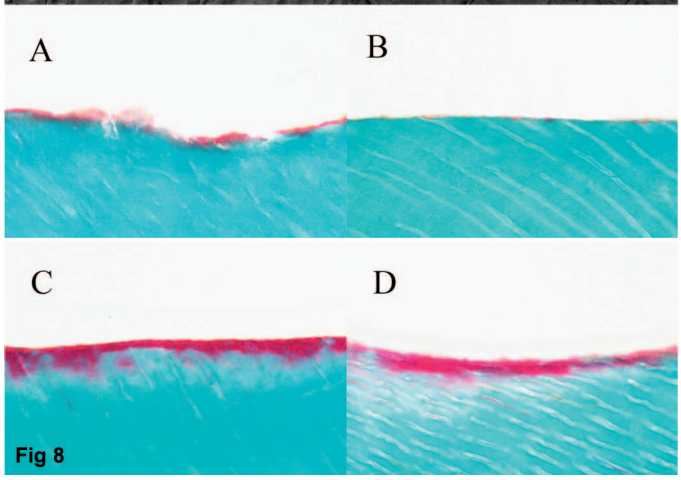

Figure 8. *Photomicrographs representative of natural NCCLs and ADs stained with Goldner's modified Masson's Trichrome, showing the dentin (green) and exposed collagen (red/pink): (A) G1—AD, (B) G1—natural NCCL, (C) G2—AD, and (D) G2—natural NCCL.*

result, apart from promoting unnecessary dental structure wear, surface preparation could actually hinder adhesion because of the presence of debris.

The methacrylamide monomer present in the G2 system interacts with the collagen present in the dentin,[33] showing high hydrolytic stability of the amide portion[34] as well as the ability to demineralize the dentin,[35] providing good long-term adhesive strength in healthy dentin compared with methacrylate-based adhesive systems.[36]

In G2, MR analysis demonstrated that the DD in ADs (Figure 6A) was probably caused by the high demineralizing power of the system (pH 1.38) and the absence of a smear layer. In contrast, in natural NCCLs, the demineralization was less intense due to the hypermineralized characteristic of the surface (Table 4). SEM images (Figure 7C,D) confirmed these findings and demonstrated adhesive failure in the NCCL restoration. A clinical study using the Xeno Select adhesive system, which has the same functional monomer in G2, demonstrated that the adhesive was not able to fulfill the ADA criteria for the restoration failure rate of less than 5% after 6 months of clinical performance in natural NCCLs.[37]

In the natural NCCLs of G2, the DD in the first 4 microns of the hybrid zone was similar to that of the ADs (Table 4). Furthermore, FTIR-PAS analysis demonstrated changes in the dentin spectra after adhesive application. These modifications suggest a change in the inorganic components, such as phosphate and calcium, but do not indicate the occurrence of chemical interactions (Figure 4). Zhou and others,[38] when testing adhesives with 10-MDP (Clearfil S3 Bond), 4-META (GBond), and methacrylamide (Xeno V) in sound and deproteinized dentin, also observed that the -C=C-COO- chemical bonds could not be identified in the spectrum of the methacrylamide-based adhesive. The absence of a signal in the infrared spectrum of the Xeno V adhesive group indicated low affinity with the

dentin. The band at 1718 cm^{-1} of Xeno V was much weaker than that of the other adhesives, which may explain the absence of a signal after being applied to the dentin surface.[38]

In the present study, a thick band of dark red collagen could be observed in the histomorphometric analysis of specimens in G2, indicating that the adhesive system demineralized dentin at a greater depth than in G1, both in the ADs and natural NCCLs. Additionally, they revealed that the functional monomer did not involve the collagen in either dentin substrates tested with G2. This result confirms the FTIR-PAS and SEM findings, which also demonstrated that no hybrid layer was optimally formed to provide the expected chemical interaction between the collagen in the natural NCCLs and the ADs with G2.

Thus, the results of the present study demonstrated that infiltration of the methacrylamide-based adhesive in the NCCLs was limited because of the presence of a more obliterated dentin, compromising hybrid layer formation at the interface. Considering that in the present study, G2 demineralized the dentin more deeply in the ADs than did the natural NCCLs, we recommend that the superficial sclerotic layer be removed by means of cavity preparation to provide micromechanical interaction and effectively bond to the dentin in the NCCLs.

In vitro studies, which attempt to simulate actual clinical conditions, present some important limitations. Despite that, the cavities were tested in a paired way, ie, the same tooth received the control cavity on the surface opposite the natural NCCLs to allow direct comparison between groups and avoid possible differences in the permeability between teeth, which could interfere with the results obtained from the dentin in NCCLs and ADs. Further studies testing adhesive systems with various chemical compositions and dentin surface preparation modes are required to enable dentists to increase the longevity of esthetic restorations in natural NCCLs.

CONCLUSIONS

The use of a self-etch adhesive system in NCCLs requires different substrate preparation strategies according to the functional monomer present in its composition. Because the G1 adhesive containing the 10-MDP monomer was shown to react chemically with the mineral component present in the sclerotic dentin, it can be applied directly to the surface of NCCLs. On the other hand, the G2 adhesive containing methacrylamide demonstrated that the superficial sclerotic layer of NCCLs needs to be removed before adhesive application in order to obtain improved micromechanical interaction.

Acknowledgements

The authors thank the Brazilian funding agencies FINEP (Financiadora de Estudos e Projetos) and CAPES (Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior) for their support.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of approval of the local permanent research ethics committee. The approval code for this study is CAAE: 47305015.7.0000.0104.

Conflict of Interest

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

(Accepted 28 February 2018)

REFERENCES

1. Levitch LC, Bader JD, Shugars DA, & Heymann HO (1994) Non-carious cervical lesions *Journal of Dentistry* **22(4)** 195-207.
2. Perez CR, Gonzalez MR, Prado NA, de Miranda MS, Macêdo MA, & Fernandes BM (2012) Restoration of noncarious cervical lesions: When, why, and how *International Journal of Dentistry* 687058.
3. Grippo JO, Simring M, & Schreiner S (2004) Attrition, abrasion, corrosion and abfraction revisited: A new perspective on tooth surface lesions *Journal of the American Dental Association* **135(8)** 1109-1111.
4. Tay FR, Kwong SM, Itthagarun A, King NM, Yip HK, Moulding KM, & Pashley DH (2000) Bonding of a self-etching primer to non-carious cervical sclerotic dentin: Interfacial ultrastructure and microtensile bond strength evaluation *Journal of Adhesive Dentistry* **2(1)** 9-28.
5. Kwong SM, Cheung GS, Kei LH, Itthagarun A, Smales R, Tay FR, & Pashley DH (2002) Micro-tensile bond strengths to sclerotic dentin using a self-etching and a total-etching technique *Dental Materials* **18(5)** 359-369.
6. Zimmerli B, De Munck J, Lussi A, Lambrechts P, & Van Meerbeek B (2012) Long-term bonding to eroded dentin requires superficial bur preparation *Clinical Oral Investigations* **16(5)** 1451-1461.
7. Xu C, Karan K, Yao X, & Wang Y (2009) Molecular structural analysis of noncarious cervical sclerotic dentin using Raman spectroscopy *Journal of Raman Spectroscopy* **40(12)** 1780-1785.

8. Tay FR, & Pashley DH (2004) Resin bonding to cervical sclerotic dentin: A review *Journal of Dentistry* **32(3)** 173-196.
9. Yoshida Y, Van Meerbeek B, Nakayama Y, Snauwaert J, Hellemans L, Lambrechts P, Vanherle G, & Wakasa K (2000) Evidence of chemical bonding at biomaterial-hard tissue interfaces *Journal of Dental Research* **79(2)** 709-714.
10. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, & Van Meerbeek B (2004) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **83(6)** 454-458.
11. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, & Vanherle G (2003) Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges *Operative Dentistry* **28(3)** 215-235.
12. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, & Van Landuyt KL (2011) State of the art of self-etch adhesives *Dental Materials* **27(1)** 17-28.
13. Moszner N, Salz U, & Zimmermann J (2005) Chemical aspects of self-etching enamel-dentin adhesives: A systematic review *Dental Materials* **21(10)** 895-910.
14. Ritter AV, Heymann HO, Swift EJ, Sturdevant JR, & Wilder AD (2008) Clinical evaluation of an all-in-one adhesive in non-carious cervical lesions with different degrees of dentin sclerosis *Operative Dentistry* **33(4)** 370-378.
15. Oliveira BMB, Ubaldini ALM, Sato F, Baesso ML, Bento AC, Andrade LHC, Lima SM, & Pascotto RC (2017) Chemical interaction analysis of an adhesive containing 10- methacryloyloxydecyl dihydrogen phosphate (10-MDP) with the dentin in noncarious cervical lesions *Operative Dentistry* **42(4)** 357-366.
16. Snook RD, Lowe RD, & Baesso ML (1998) Photothermal spectrometry for membrane and interfacial region studies *Analyst* **123(4)** 587-593.
17. Ubaldini AL, Baesso ML, Sehn E, Sato F, Benetti AR, & Pascotto RC (2012) Fourier transform infrared photoacoustic spectroscopy study of physicochemical interaction between human dentin and etch-&-rinse adhesives in a simulated moist bond technique *Journal of Biomedical Optics* **17(6)** 065002.
18. Yoshihara K, Yoshida Y, Nagaoka N, Hayakawa S, Okihara T, De Munck J, Maruo Y, Nishigawa G, Minagi S, Osaka A, & Van Meerbeek B (2013) Adhesive interfacial interaction affected by different carbon-chain monomers *Dental Materials* **29(8)** 888-897.
19. Monticelli F, Osorio R, Mazzitelli C, Ferrari M, & Toledano M (2008) Limited decalcification/diffusion of self-adhesive cements into dentin *Journal of Dental Research* **87(10)** 974-979.
20. Wang Y, & Spencer P (2005) Evaluation of the interface between one-bottle adhesive systems and dentin by Goldner's Trichrome *American Journal of Dentistry* **18(1)** 66-72.
21. Shin TP, Yao X, Huenergardt R, Walker MP, & Wang Y. Morphological and chemical characterization of bonding hydrophobic adhesive to dentin using ethanol wet bonding technique *Dental Materials* **25(8)** 1050-1057.
22. Spencer P & Swafford JR (1999) Unprotected protein at the dentin-adhesive interface *Quintessence International* **30** 501-507.
23. Wang Y, & Spencer P (2003) Hybridization efficiency of the adhesive/dentin interface with wet bonding *Journal of Dental Research* **82(2)** 141-145.
24. Chee B, Rickman LJ, & Satterthwaite JD (2012) Adhesives for the restoration of non-carious cervical lesions: A systematic review *Journal of Dentistry* **40(6)** 443-452.
25. Mixson JM, Spencer P, Moore DL, Chappell RP, & Adams S (1995) Surface morphology and chemical characterization of abrasion/erosion lesions *American Journal of Dentistry* **8(1)** 5-9.
26. Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, & Van Meerbeek B (2010) Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching *Dental Materials* **26(12)** 1176-1184.
27. Yoshida Y, & Inoue S (2012) Chemical analyses in dental adhesive technology *Japanese Dental Science Review* **48(2)** 141-152.
28. van Dijken JW (2010) A prospective 8-year evaluation of a mild two-step self-etching adhesive and a heavily filled two-step etch-and-rinse system in non-carious cervical lesions *Dental Materials* **26(9)** 940-946.
29. Van Landuyt KL, Peumans M, De Munck J, Cardoso MV, Ermis B, & Van Meerbeek B (2011) Three-year clinical performance of a HEMA-free one-step self-etch adhesive in non-carious cervical lesions *European Journal of Oral Sciences* **119(6)** 511-516.
30. van Dijken JW (2013) A randomized controlled 5-year prospective study of two HEMA-free adhesives, a 1-step self etching and a 3-step etch-and-rinse, in non-carious cervical lesions *Dental Materials* **29(11)** 271-280.
31. Santos MJ, Ari N, Steele S, Costella J, & Banting D (2014) Retention of tooth-colored restorations in non-carious cervical lesions—A systematic review *Clinical Oral Investigations* **18(5)** 1369-1381.
32. Luque-Martinez IV, Mena-Serrano A, Munoz MA, Hass V, Reis A, & Loguercio AD (2013) Effect of bur roughness on bond to sclerotic dentin with self-etch adhesive systems *Operative Dentistry* **38(1)** 39-47.
33. Nishiyama N, Suzuki K, Asakura T, Komatsu K, & Nemoto K (2001) Adhesion of N-methacryloyl-omega-amino acid primers to collagen analyzed by ^{13}C NMR *Journal of Dental Research* **80(3)** 855-859.
34. Nishiyama N1, Suzuki K, Takahashi K, & Nemoto K (2004) The pKa effects of the carboxylic acid in N-methacryloyl-omega-amino acid on the demineralization and bond strengths to the teeth *Biomaterials* **25(23)** 5441-5447.
35. Yoshida H, & Nishiyama N (2003) Development of self-etching primer comprised of methacrylamide, N-methacryloyl glycine *Biomaterials* **24(28)** 5203-5207.
36. Salz U, & Bock T (2010) Adhesion performance of new hydrolytically stable one-component self-etching enamel/

dentin adhesives *Journal of Adhesive Dentistry* **12(1)** 7-10.

37. Lopes LS, Calazans FS, Hidalgo R, Buitrago LL, Gutierrez F, Reis A, Loguercio AD, & Barceleiro MO (2016) Six-month follow-up of cervical composite restorations placed with a new universal adhesive system: A randomized clinical trial. *Operative Dentistry* **41(5)** 465-480.
38. Zhou L, Wang Y, Yang H, Guo J, Tay FR, & Huang C (2015) Effect of chemicial interaction of the bond strengths of self-etching adhesives to deproteinised dentine. *Journal of Dentistry* **43(8)** 973-980