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Laboratory Research

Chemical Interaction Analysis of an Adhesive Containing 10-Methacryloyloxydecyl Dihydrogen Phosphate (10-MDP) With the Dentin in Noncarious Cervical Lesions

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Clinical Relevance

The results suggest that it is unnecessary to remove the hypermineralized layer with burs, as this may decrease the chemical bonding potential of 10-MDP.

SUMMARY

The purpose of this study was to evaluate the chemical bonds of a self-etch 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) adhesive to natural noncarious cervical lesions (NCCLs) and compare them with those occurring in sclerotic dentin in artificially prepared defects (APDs). Four human teeth with natural NCCLs on the buccal surface were selected. Artificial defects matching the natural lesions were prepared on the lingual surface of the same teeth serving as control. Micro-Raman (MR) spectroscopy was used to quantify mineral content in natural NCCLs and in APDs. Fourier transform infrared-photoacoustic spectroscopy (FTIR-PAS) readouts were taken before and after adhesive application to analyze the protein matrix/mineral (M:M) ratio and chemical interactions between 10-MDP adhesive and dentin. The MR and FTIR-PAS spectra collected from natural NCCLs demonstrated a larger area of the band (961 cm^{-1},

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PO_4) and lower M:M ratio, respectively, characterizing a hypermineralized dentin, compared with APDs. FTIR-PAS demonstrated emergence of a peak (1179 cm^{-1}, P=O) in spectra after adhesive treatment, demonstrating a more intense chemical interaction in natural NCCLs. The results demonstrated that chemical bonding of 10-MDP adhesive to natural NCCLs is more intense, due to the hypermineralized surface, and suggest that it is unnecessary to remove the hypermineralized layer with burs, as this may decrease the chemical bonding potential of 10-MDP.

INTRODUCTION

Noncarious cervical lesions (NCCLs) are a challenge in dental practice, and multifactorial etiologies are involved in their development.[1,2] Noncarious cervical lesions usually form as a result of 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 sclerotic dentin in NCCLs is compromised, resulting in reduced bond strength compared with artificial lesions created in sound cervical dentin.[4,5] This reduction in bond strength in NCCLs occurs due to molecular/chemical structural alterations that may result in dentin less favorable to bonding.[6] The presence of a sclerotic layer in NCCLs could make it difficult for the hybrid layer to form, because of the lower degree of primer diffusion and adhesive infiltration.[7]

Recent studies have, however, turned their attention to the additional chemical bonding potential between the functional monomers present in some adhesive systems and the components of dentin,[8-12] which seems to be particularly important for restorations placed in NCCLs.[10,13-15] The functional monomer 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) is present in some commercially available adhesive systems and has demonstrated good chemical bonding potential to hydroxyapatite through the formation of a "nanolayer" capable of enhancing the effectiveness and longevity of bonds.[11,16] Studies have shown this bond to be more stable to hydrolytic degradation than other functional monomers and to have a low solubility of calcium salts and long and hydrophobic spacer carbon chain, such as 4-methacryloyloxyethyl trimellitic acid (4-MET), and 2-methacryloxyethyl phenyl hydrogen phosphate (phenyl-P).[11,17] Based on the survival rates of restorations placed in NCCLs with a mild self-etch adhesive containing 10-MDP, a recent review of the literature has suggested that chemical bonding to NCCL not only occurs, but it would also be more intense due to its hypermineralized surface.[13] However, this improved chemical interaction between the hypermineralized dentin in the natural NCCLs and adhesive systems containing 10-MDP has yet to be demonstrated.

Some studies have suggested the need for roughening the surface of NCCLs to remove the hypermineralized layer to achieve dentin with characteristics closer to those of prepared cavities and better results in the clinical retention of composite restorations.[18,19] Thus, to assess the necessity of cavity preparation in NCCL surfaces before bonding procedures, it is appropriate to evaluate whether the chemical interaction between an adhesive system containing the 10-MDP and dentin in natural NCCLs is reduced compared with the dentin in prepared artificial defects of the same tooth.

Therefore, the objective of this study was to evaluate the chemical interactions that characterize the bond of a self-etch adhesive containing 10-MDP as the functional monomer to hypermineralized dentin in natural NCCLs and compare these chemical interactions with those in sclerotic dentin in artificially prepared defects (APDs).

METHODS AND MATERIALS

This *in vitro* study was conducted after obtaining approval from the local Institutional Review Board (CAAE:25006614.3.0000.0104). The four incisor teeth used in this study were extracted for periodontal or orthodontic reasons and were donated by patients through signature of a term of informed consent. After extraction, the teeth were cleaned and stored in physiologic solution at 4°C. All the teeth presented grade 4 dental sclerosis, according to the modified sclerosis scale defined by Ritter and others.[20] Grade 4 is attributed to NCCLs with the presence of significant sclerosis. The dentin is dark yellow or brownish with a petrified appearance and significant translucence or evident transparency.[20] Noncarious cervical lesions were located in the cervical region of the buccal surface.

Dental Fragment Preparation

Artificial defects (class V cavities) were prepared in the cervical region of the sound lingual surface of the same teeth, with the use of CVDentus cylindri-

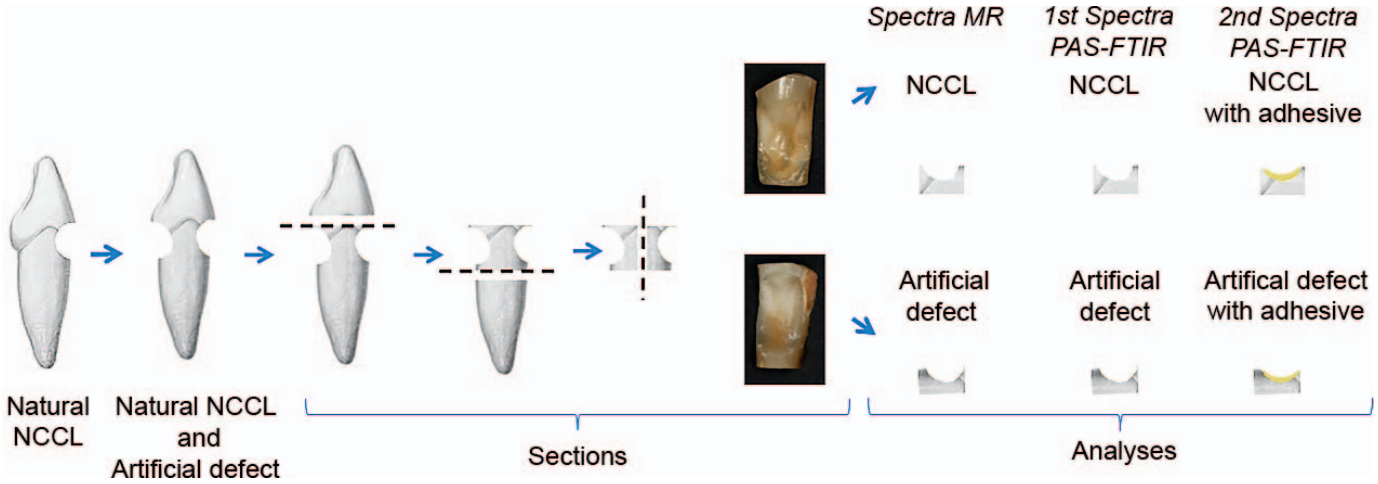

Figure 1. *Illustration showing the natural NCCL, artificial defect preparation, and tooth sectioning for MR and FTIR-PAS spectra acquisition.*

cal diamond burs (C1 1.0 × 4.0 mm) mounted in an ultrasound device (CVDent1000, CVDVale, São Carlos, Brazil), under constant water cooling. The APDs were created in dentin, with approximately the same size, shape, and location as those of their corresponding natural NCCL and served as controls.

After this, dental fragments were obtained from the buccal and palatal surfaces of each tooth, containing the natural NCCLs and the APDs, respectively. The teeth were sectioned with a low-speed diamond saw under water cooling. First, the occlusal crown and apical root were removed from the teeth, immediately above and below the lesions, respectively. Then, the teeth were sectioned in the occlusal-apical direction to separate the buccal and lingual surfaces of the specimens containing the natural NCCLs and the APDs (Figure 1).

Adhesive System Application

The composition of the self-etch adhesive containing the functional monomer 10-MDP used in this study is presented in Table 1. The adhesive was actively applied on the cavities for 20 seconds, by using a microbrush slightly impregnated with the product, without excesses, in accordance with the manufacturer's recommendations. A light stream of air was then applied over the liquid for approximately five seconds, until no movement was seen and the solvent had evaporated completely. Finally, the adhesive system was light polymerized (LED Radii, SDI, Bayswater, Australia; 1200 mW/cm^2) for 10 seconds. After polymerization, the specimens were immediately analyzed by Fourier transform infrared-photoacoustic spectroscopy (FTIR-PAS). No composite resin was applied on the surface.

Mineral Composition Analysis of Dentin

The sclerotic dentin mineral composition in the natural NCCLs and in the APDs was analyzed by means of micro-Raman (MR) spectroscopy. The experiments were carried out in a Raman Bruker spectrometer, equipped with a Senterra confocal microscope (Bruker Optik GmbH, Ettlingen, Germany).

The spectra of specimens containing the natural NCCLs and the APDs were collected in three different points along the surface. All the data were collected with a resolution of 4 cm^{-1} in the spectral range of 3500-450 cm^{-1}. To improve the signal-to-noise ratio, each spectrum was obtained from the

Table 1: *Composition and Lot Number of Self-Etching Adhesive Systems Used in This Study*

Product	Composition	Lot no.
Scotchbond Universal Adhesive (3M/ESPE Dental Products, St. Paul, MN, USA)	MDP ($C_{14}H_{27}O_6P$) TEDGMA ($C_{14}H_{22}O_6$) Bis-GMA ($C_{29}H_{36}O_8$) Polyalkenoic acid copolymer ($C_{21}H_{33}O_8$) HEMA ($C_6H_{10}O_3$) Modified methacrylate ($C_5H_8O_2$) Filler load (SiO_2) Water (H_2O) Silane (SiH_4) Alcohol (C_2H_6O) Primers	507329

Abbreviations: MDP, 10-methacryloxydecyl dihydrogen phosphate; TEDGMA, triethyleneglycol dimethacrylate; Bis-GMA, bisphenol-A glycidyl methacrylate; HEMA, 2-hydroxyethylmethacrylate.

average of 1000 scans at a laser wavelength of 1064 nm, power of 150 mW, and 40× magnification. Apart from the high number of scans, the detector temperature was also reduced to −84°C to improve the signal obtained. All readouts were standardized with the use of a support mirror, selection of the area to be measured, and manual and automatic focusing.

All the spectra collected were placed in the same baseline and normalized with the assistance of the OPUS spectroscopy software (Bruker Optics, Ettlingen, Germany). Origin software (OriginPro 8 Corporation, Northampton, MA, USA) was used to obtain the numerical quantifications of the MR spectra by integration of each curve of the absorption band at 961 cm^{-1} (phosphate) to calculate the respective area. Final area values were obtained from the mean result of the three different preset points (determined before measuring) used for MR spectra acquisition.

NCCL sample homogeneity after adhesive treatment was proved by the calculation of dentin surface matrix:mineral ratio (M:M = the ratio of the intensities of the photoacoustic absorption bands of the amide I [1650 cm^{-1}] divided by the intensities of the phosphate band at 1040 cm^{-1}). These ratios were submitted to a Shapiro-Wilk normality test (R statistic software, R Foundation for Statistical Computing, Vienna, Austria), and were represented by means and SD.

To assess the differences in the M:M ratio between the sclerotic dentin in the natural NCCLs and the control dentin in the APDs, the relative intensities of the absorption bands attributed to amide I and phosphate obtained with FTIR-PAS were compared. To calculate the M:M ratio of the samples, the intensities of the photoacoustic absorption bands of the amide I (1650 cm^{-1}) were divided by the intensities of the phosphate bands (560, 600, and 1040 cm^{-1}). Calculations were conducted using the spectrum obtained using the mean spectrum obtained from the dentin in the natural NCCLs and APDs after the application of the adhesive. These ratios were submitted to Shapiro-Wilk normality and Student *t*-tests at the 5% significance level (R statistic software).

Analysis of the Chemical Interaction Between the Adhesive and Dentin

The optical absorption bands obtained with FTIR-PAS work as fingerprints of specific molecules contained in the specimens, and as a result, provide information on the chemical interactions, expressed through alterations and/or appearance of new peaks.[21]

To determine the depth of the analysis, it is important to consider that the reading depth with FTIR-PAS technique is defined by the thermal diffusion length (cm) as $\mu = [D\lambda/(2\pi v)]^{1/2}$, in which D is the thermal diffusivity of the sample (cm^2/s), v is the speed of the mirror (cm/s), and λ is the wavelength of the incident radiation (cm). This parameter reflects the attenuation of the thermal wave amplitude and was used to analyze the approximate depth at which the photoacoustic signal is generated in the sample.

In this experimental condition, the sample treated with the adhesive was composed of two surfaces with a hybrid interface, both with effective values for thermal diffusivity. The value of μ depends on the value of these parameters, according to the wavelength of the incident radiation. Thermal diffusivity for dentin in the tubular direction has been defined as $D = 2.5 \times 10^{-3}$ cm^2/s.[22] For the adhesive, thermal diffusivity readouts were taken by using the thermal lens technique,[23,24] which provided $D = (1.58 \pm 0.07) \times 10^{-3}$ cm^2/s. As there was exciting radiation incident on the adhesive placed in the cavities, μ was estimated using the value of the thermal diffusivity obtained for the adhesive. Thus, using v = 0.63 cm/s, the measurement depth calculated for the energy of 1176 cm^{-1} ($\lambda = 8.5 \times 10^{-4}$ cm) was μ = 5.8 μm. In other words, the inspection depth of the technique for the readouts taken in this study was about 6 μm. Considering that the adhesive film varies between 2 and 3 μm,[24,25] it may be affirmed that technique was capable of taking the readouts on the dentin in the hybrid region beneath the adhesive system.

The FTIR-PAS spectra were recorded by a Nicolet spectrometer, equipped with a MTEC Photoacoustic Model 200 photoacoustic cell (MTEC Photoacoustics, Inc. Ames, IA). This equipment allows the absorption bands of the matter of interest to be monitored at a specific depth of the sample, providing the distribution profile of the matter throughout the sample. All spectra were collected at a resolution of 8 cm^{-1}, with a scan speed of 0.63 cm/s, in the spectral range of 4000-400 cm^{-1}.

After placing the specimen into the device, the photoacoustic cell was filled with helium gas to minimize interference in the optical absorption spectra caused by molecules of oxygen and water present in the air and on the specimen surface. The

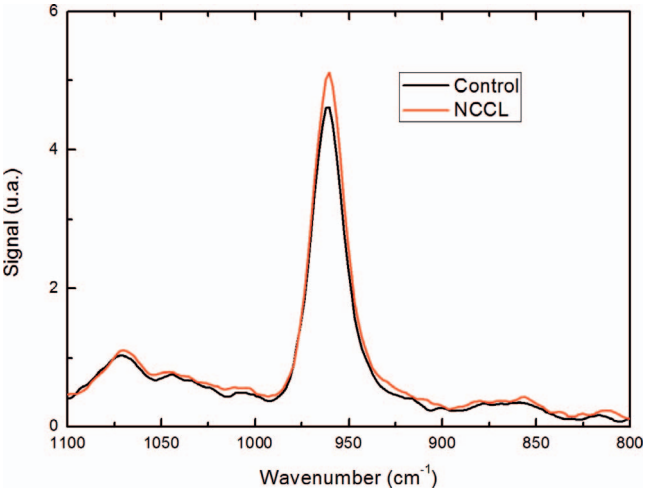

Figure 2. *Mean of the absorption spectra at 961 cm^{-1} (phosphate), demonstrating different intensities between the dentin in the natural NCCLs and the dentin the APDs (control).*

spectra of specimens were collected before and after they were submitted to treatment with the adhesive system. A disk of pure adhesive was prepared by placing 1 mL of the material on a glass slide that was light activated for 20 seconds (LED Radii, SDI; 1200 mW/cm^2). The slide was submitted to MR, and the spectrum of the pure adhesive was collected. This was an important methodologic step to differentiate the composition of the adhesive alone from that of the dental structure, so that the differences in the photoacoustic absorption peaks obtained from the adhesive and from the dentin could be verified.

The FTIR-PAS spectral data obtained were transferred to Origin software (OriginPro 8 Corporation). The spectral interval measured was divided into two spectral ranges (2180-400 and 1800-550 cm^{-1}). For each spectral range, a specific wavelength was established for the normalization of the data at 1460 cm^{-1} (CH stretch). This particular band was selected because it was present simultaneously with constant intensity in the spectra collected from the dentin in the natural NCCLs and in the APDs. Graphs were generated for each individual tooth, from which the average spectrum of the dentin in the natural NCCLs and in the APDs was calculated.

With the objective of observing the chemical interactions between the dental substrate and the adhesive system, the spectrum collected from the pure adhesive was subtracted from the spectrum acquired from the natural NCCL dentin with adhesive and the APD with adhesive. Possible overlaps of the bands associated with the adhesive with the spectral bands of the dental substrate could then be eliminated. As a result, small alterations or appearance of peaks in the spectra associated with the dentin due to the application of adhesive could be visualized. In addition, numerical quantifications of the spectra were obtained by integrating the curve of the band at 1179 cm^{-1} (v1 P=O) in the specimens with those of the natural NCCL and APD.

Table 2: *Means Values and SDs of the Organic M:M Ratio From the Dentin in the Natural NCCLs and in the Artificial Defects (Control) Before and After the Application of the Adhesive Containing 10-MDP*

Treatment with adhesive	Substrate	M:M	SD
Before	Control	1.11	0.06
	NCCL	1.09	0.11
After	Control	0.74	0.07
	NCCL	0.68	0.06

RESULTS

Dentin Mineral Composition Analysis

Spectra of the four NCCL dentin surfaces after adhesive treatment showed homogeneity in organic and inorganic composition (0.68 ± 0.06).

Mean values and SDs obtained from the integrated areas of the MR scans of the band at 961 cm^{-1} (phosphate) performed at three different points (total of 12 scans for each group) on the surface of the dentin in the natural NCCL and APD (control) were 140 ± 7 and 130 ± 10, respectively. Figure 2 illustrates the difference in the intensities of the phosphate absorption band at 961 cm^{-1} between the two studied groups.

The M:M ratios obtained with FTIR-PAS after the application of the adhesive are presented in Table 2. Dentin in natural NCCL cavities treated with adhesive presented a lower M:M ratio than dentin in APD ($p<0.05$).

Analysis of the Chemical Interaction Between the Adhesive and Dentin

The photoacoustic absorption spectra of the pure adhesive dentin in the natural NCCLs and dentin in the APDs were mapped, and they identified the functional groups in their composition (Figure 3A,B). The 1800-500 cm^{-1} spectral range represents the region of interest in this study. Figure 3B illustrates the spectra of the dentin in the natural NCCLs and in the APDs. The observed spectra revealed vibrations of both organic and inorganic components. The absorption bands indicated the major organic contributions at 1650 cm^{-1} (amide I, C=O), 1550 cm^{-1} (amide II, N-H and C-N), and 1240 cm^{-1} (amide III,

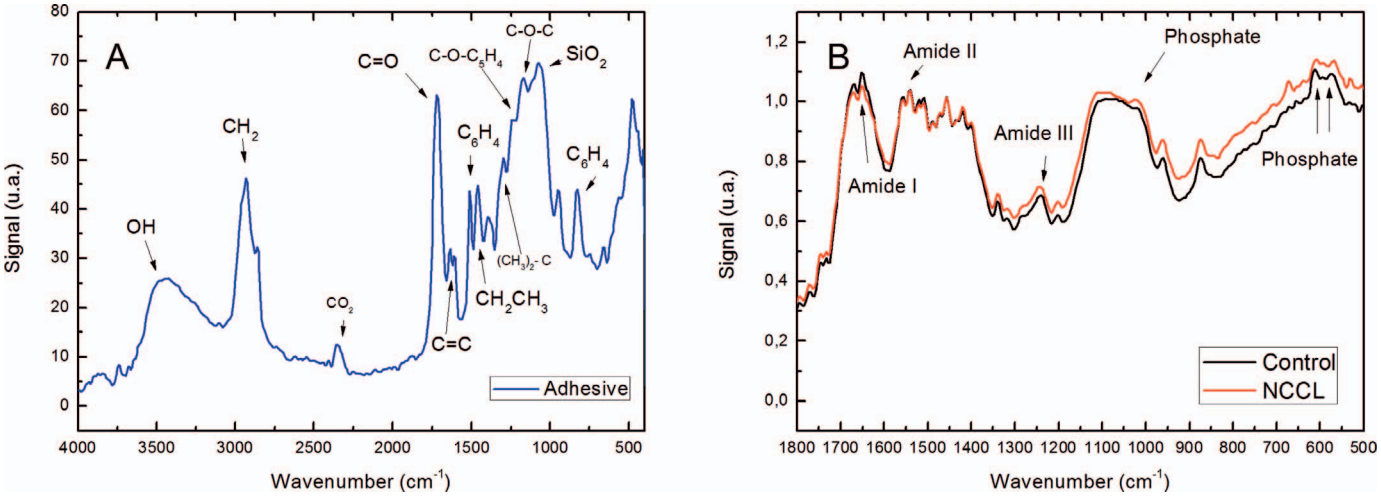

Figure 3. *(A) Photoacoustic absorption spectrum acquired from pure adhesive with the functional groups identified. (B) Photoacoustic absorption spectra acquired from the dentin in the natural NCCLs and in APDs (control).*

C-N and N-H). Contributions from the mineral phase were the orthophosphate bands (PO_4) at 600-560 and 1200-900 cm^{-1}.

After the adhesive was applied on the specimen surfaces (Figure 4), the main contributions from the functional groups with reference to the adhesive system were the methacrylate monomer bands at 1720 cm^{-1} (carbonyl, C=O) and 1457 cm^{-1} (CH_2CH_3); the bisphenol-A glycidyl methacrylate (Bis-GMA) bands at 1638 cm^{-1} (C=C), 1140 cm^{-1} (C-O-C), 1300 cm^{-1} [$(CH_3)_2$], 1260 cm^{-1} (C, C-O-C_5H_4), and 840 cm^{-1} (C_6H_4); and filler load band at 1105 cm^{-1} (SiO_2). The contributions from the

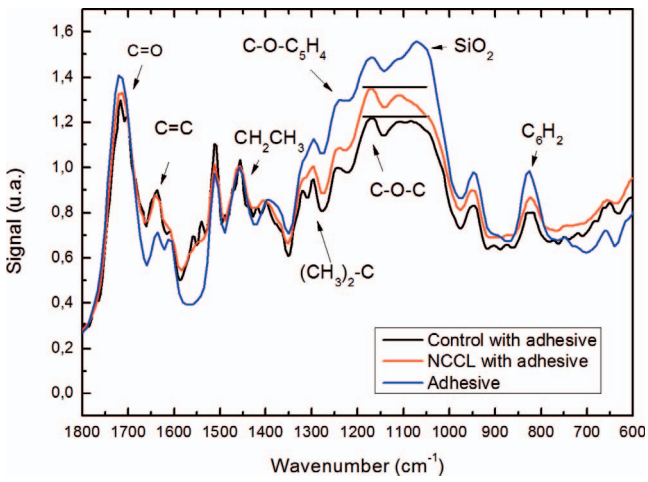

Figure 4. *Mean photoacoustic absorption spectra acquired from the adhesive, dentin in the natural NCCLs with adhesive, and in the APDs (control with adhesive) after treatment. The horizontal bars demonstrate higher relative intensity of the peak at 1179 cm^{-1}, in comparison with the band at 1100 cm^{-1}, for the dentin in natural NCCLs compared with dentin in APDs (control).*

mineral and organic composition in the spectra of dentin in the natural NCCLs and APDs were at 1179 cm^{-1} (P=O), 1650 cm^{-1} (amide I), 1550 cm^{-1} (amide II), and 1240 cm^{-1} (amide III). The horizontal bars in Figure 4 show the appearance of a new band at 1179 cm^{-1} that was more intense compared with the band at around 1100 cm^{-1}, indicating a (more intense) chemical interaction of the adhesive with the natural NCCLs in comparison with that of the APDs. This interaction was more intense for the dentin in the natural NCCLs.

Figure 5A displays the spectra of dentin in the natural NCCLs with adhesive and the control dentin in the APDs with adhesive after subtracting the spectrum acquired from the pure adhesive, to eliminate all the bands of the pure adhesive and show alterations on the spectra. This figure illustrates the new emerging behavior of the band at 1179 cm^{-1} (P=O). The peak in this band was more intense and narrow for the hypermineralized dentin in the natural NCCLs with the adhesive than in the APDs with adhesive. In Figure 5B, the dentin spectra were corrected, so that subtraction would coincide with line zero and allow us to calculate the areas of the band at 1179 cm^{-1}. Mean values and SDs for the integrated areas of the band at 1179 cm^{-1} (v_1 P=O) were 18 ± 4 in the dentin in the NCCLs with adhesive and 12 ± 5 in the APDs with the adhesive, numerically confirming the relatively higher intensity of this band in the natural NCCLs.

DISCUSSION

This study demonstrated differences in the intensity of chemical interactions of a self-etch adhesive

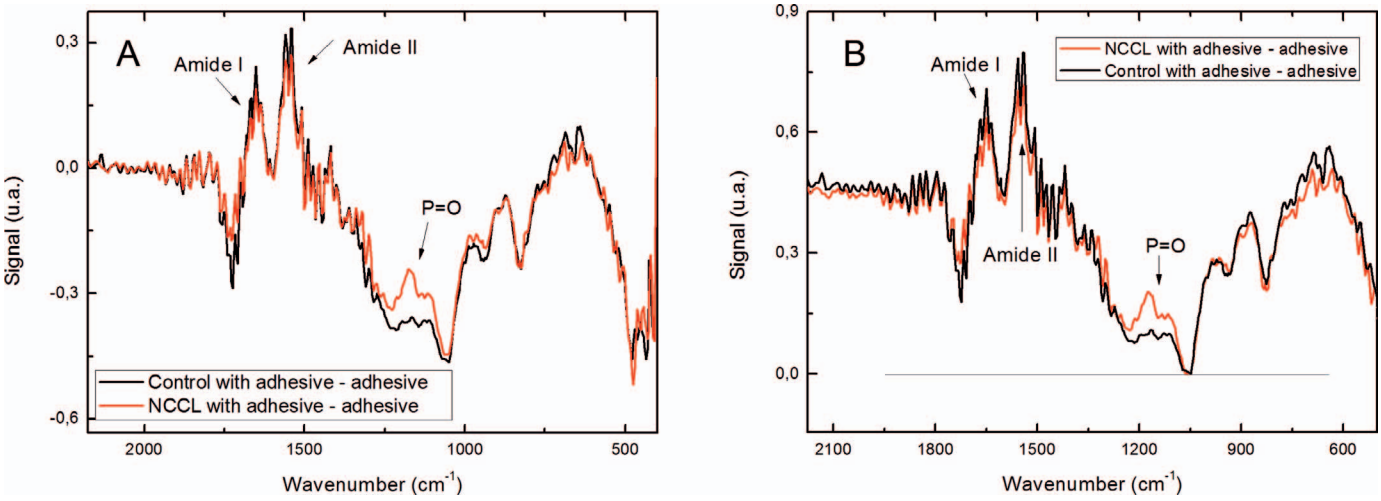

Figure 5. *Photoacoustic absorption spectra obtained after subtracting the adhesive system spectrum from the spectra acquired from the dentin in the natural NCCLs with adhesive and from the dentin in APDs (control) with adhesive. (A) Spectra after subtraction normalized at 1460 cm^{-1}. (B) Spectra after subtraction corrected to coincide with line zero.*

system containing the functional monomer 10-MDP between the sclerotic dentin in natural NCCLs and the control dentin in APDs.

When spectroscopy is used to assess whether there is a chemical bond between an adhesive system and the organic or inorganic portions of the dentin, changes in the absorption bands attributed to the dental substrate must be disclosed.[26] After treatment with the adhesive system (Figure 4), it may be observed that the silica band (SiO_2) at 1179 cm^{-1} in the mean spectra of the hypermineralized dentin in the natural NCCLs presented higher intensity compared with the normal dentin in the APDs, as illustrated by the parallel bars.

However, an overlap of the bands of the adhesive system over the dental substrate could also be observed. Wang and others,[27] using the FTIR-PAS technique, also observed that the bands attributed to the phosphate in the dentin (1200-900 cm^{-1}) overlapped with the band attributed to the SiO_2 in the adhesive, whereas the region of the amide I (1650 cm^{-1}) overlapped with the band at 1680-1620 cm^{-1} in the adhesive. As a result, in the univariate analyses, the limitation caused by the overlapping hindered the disclosure of minimal changes in the spectra.[27] Taking the overlapping issue into consideration, the analysis in the present study was enhanced by subtracting the spectrum of the pure adhesive from the spectra collected from the dentin after applying the adhesive system (Figure 5), thereby allowing the alterations/ appearance of peaks to become more evident. This procedure allowed us to improve visualization of what happened to the band at 1179 cm^{-1}, characteristic of optical absorption of the phosphoric group that was more intense and narrower in the dentin (v1 P=O) of the natural NCCL samples than in that of the APDs samples.

The increase in intensity of the band at 1179 cm^{-1} was related to the chemical interaction of the adhesive with the inorganic matrix of the dentin, more specifically between the ester group in the adhesive and the molecules of calcium and phosphate in the dentin, leading to the formation of a calcium/phosphate ester complex.[11,24] Thus, with the objective of quantitatively assessing possible differences between the sclerotic dentin in natural NCCLs and in the control dentin of APDs impregnated with the adhesive, in the present study, the integrated areas of the band at 1179 cm^{-1} were calculated in the 1218-1052 cm^{-1} band interval. The higher mean value found for the sclerotic dentin in the natural NCCLs (18±4) in comparison with the control dentin in the APDs (12±5) demonstrated that the chemical interaction with the hypermineralized dentin was more intense.

The literature has suggested that the functional monomer 10-MDP that is present in the self-etch adhesive system used in this study is capable of forming chemical bonds with hydroxyapatite (calcium-MDP salts) and therefore improves the performance and longevity of the bond in adhesive restorations.[8,11,16] Studies have justified this process by the formation of a "nanolayer," composed of two molecules of 10-MDP with their methacrylate groups directed toward each other and their functional hydrogen phosphate groups directed away from each

other, in which the methacrylate binds to the calcium ions, and the hydrogen phosphate group binds to the dentin.[17] The chemical bonds between the ester group in the adhesive (methacrylate) and the molecules of calcium and phosphate in the dentin shown in this study (Figure 5) may be attributed to the formation of calcium-MDP salts in the nanolayer as a response to the chemical interaction between the adhesive system and the dental structures.

To promote an effective chemical bond with the dentin, the functional monomer 10-MDP needs to be present in high concentrations. Studies have speculated that the addition of other compounds to the formulation of adhesive systems may decrease the formation of a nanolayer, and as a result, affect the potential chemical interaction of self-etch adhesive systems with the dentin.[28] The presence of 10-MDP in the adhesive and chemical bonding to dentin is modulated by 2-hydroxyethylmethacrylate (HEMA) that is part of the adhesive composition. Yoshida and others[29] demonstrated that HEMA significantly reduced nanolayering, because it reduced the hydroxyapatite (HAp) demineralization rate, a prerequisite to the formation of MDP-Ca salts. HEMA does interfere with, but does not completely inhibit, MDP from interacting chemically with HAp. When Yoshida and others[16] investigated the effect of commercially available adhesive systems containing 10-MDP on the formation of a nanolayer by means of X-ray diffraction, X-ray energy dispersive spectroscopy, and transmission electron microscopy, they observed that the same adhesive system used in the present study presented a less prominent nanolayer in normal human dentin compared with Clearfil SE Bond (Kuraray, Tokyo, Japan). This was attributed to the lower concentration of 10-MDP and the presence of polyalkenoic acid (copolymer) in the Scotchbond Universal Adhesive (3M/ESPE Dental Products, St. Paul, MN, USA), which may have competed with the 10-MDP for the same Ca-bond site in hydroxyapatite. In the present study, however, the relatively low concentration of 10-MDP of the Scotchbond Universal Adhesive (3M/ESPE) was sufficient to produce more intense changes in the spectra of the hypermineralized sclerotic dentin in the natural NCCLs compared with control dentin. Furthermore, when the adhesive system is applied actively (as recommended by the manufacturer), contact of the 10-MDP molecules with dentin is improved, increasing the concentration of 10-MDP in contact with the hydroxyapatite.[28] Considering these factors, when using adhesive systems with higher concentrations of 10-MDP, it would be expected that an enhanced chemical interaction would occur in the hypermineralized dentin in natural NCCLs.

Analysis of the mineral content on the surface of the dentin tested indicated that the sclerotic dentin in natural NCCLs is hypermineralized in comparison with the APDs. The MR analysis demonstrated the areas corresponding to the band at 961 cm^{-1} (v1 phosphate) were more intense in the dentin in the natural NCCLs than in the APDs, characterizing a zone of hypermineralized dentin (Figure 2). This was confirmed by calculating the integrated areas of the bands, which demonstrated that the numerical value of the mineral content found in the dentin in natural NCCLS (140 ± 7) was higher than that found in the control dentin of APDs (130 ± 10). Furthermore, FTIR-PAS analysis also demonstrated that the dentin in natural NCCLs presented a lower M:M ratio than the APDs after adhesive treatment ($p=0.04$). It is very difficult to obtain extracted anterior teeth with NCCL to analysis. Although a sample size of four incisors were used for chemical interaction analysis, the results showed homogeneity among specimens and also were able to demonstrate statistically significant differences. This analysis was carried out in this study to confirm the presence of sclerotic dentin and ensure that the specimens of the group of natural NCCLs were in similar condition. These findings corroborate previous studies on the molecular/structural differences between the organic and inorganic phases of the dentin in the natural NCCLs and APDs by means of Raman spectroscopy[6] and FTIR-PAS.[30]

Due to the fact that the sclerotic dentin is hypermineralized, resulting in a substrate that is less favorable to bonding,[6] studies have recommended roughening of natural NCCLs surfaces.[18,19,31] Some studies have advocated removal of the superficial sclerotic layer to increase intratubular retention to the adhesive system.[5,18] However, according to Tay and Pashley,[7] removing the hypermineralized layer in the natural NCCLs before the adhesive procedure may not result in increased bond strength, as the sclerotic dentin may still contain crystallites capable of preventing the adhesive from infiltrating into the dentinal tubules. Moreover, in a recent systematic review of the literature, it was not possible to test the null hypothesis that there would be no difference in the survival rate of restorations placed in natural NCCLs submitted to different surface treatments, due to the reduced number of studies that compared the influence of dentin roughing on the level of retention of restorations in natural NCCLs.[32] On the other hand, a recent study demonstrated that the

adhesive performance of a self-etch adhesive system containing 10-MDP in the sclerotic dentin of bovine teeth (control group) was superior compared with the same surface prepared with diamond burs.[33]

In the present study, APDs were prepared in the control dentin with ultrasound diamond burs to obtain a smear layer-free surface. A recent study demonstrated that complete absence of the smear layer improved the effectiveness of the bond to dentin of moderate and ultra-moderate self-etch adhesive systems.[34] Despite this, in the present study, chemical bonding occurred with greater intensity in the sclerotic dentin in natural NCCLs than in that of the APDs. Taking into consideration that the adhesive system containing the functional monomer 10-MDP used in this study demonstrated chemical affinity for hydroxyapatite, removing the hypermineralized layer with burs or previous acid conditioning seems to be unnecessary, as this may decrease its chemical bonding potential.

In vitro studies have some limitations regarding the simulation of the real clinical condition. Moreover, there are differences in the composition and degree of mineralization among teeth. To minimize this condition and allow a more direct comparison of the tested groups, cavities were tested pairwise, ie, the APDs were prepared in the same teeth on the surface opposite to the natural NCCL.

CONCLUSION

The results demonstrated that chemical bonding of 10-MDP adhesive to natural NCCLs is more intense than to APDs, due to the hypermineralized surface, and suggests that it is unnecessary to remove the hypermineralized layer with burs, as this may decrease the chemical bonding potential of 10-MDP. Further studies are necessary to test other adhesive systems, with different 10-MDP concentrations (not only in natural NCCLs, but also in other types of substrates with inactive cavitated carious lesions).

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Institutional Review Board of State University of Maringa. The approval code for this study is 25006614.3.0000.0104.

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, and/or company that is presented in this article.

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