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Abstract. The purpose of this study was to provide the physicochemical interactions at the interfaces between two commercial etch-&-rinse adhesives and human dentin in a simulated moist bond technique. Six dentin specimens were divided into two groups ($n = 3$) according to the use of two different adhesive systems: (a) 2-hydroxyethyl-methacrylate (HEMA) and 4-methacryloxyethyl trimellitate anhydrate (4-META), and (b) HEMA. The Fourier transform infrared photoacoustic spectroscopy was performed before and after dentin treatment with 37% phosphoric acid, with adhesive systems and also for the adhesive systems alone. Acid-conditioning resulted in a decalcification pattern. Adhesive treated spectra subtraction suggested the occurrence of chemical bonding to dentin expressed through modifications of the OH stretching peak (3340 cm^{-1}) and symmetric CH stretching (2900 cm^{-1}) for both adhesives spectra; a decrease of orthophosphate absorption band (1040 to 970 cm^{-1}) for adhesive A and a better resolved complex band formation (1270 to 970 cm^{-1}) for adhesive B were observed. These results suggested the occurrence of chemical bonding between sound human dentin and etch-&-rinse adhesives through a clinical typical condition. © *2012 Society of Photo-Optical Instrumentation Engineers (SPIE).* [DOI: 10.1117/1.JBO.17.6.065002]

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1 Introduction

Micro-mechanical retention is established as the principal adhesion mechanism of dental adhesives.[1] However, earlier dentin bonding studies suggest that specific functional monomers from adhesives can chemically interact with dental tissue components.[2–4] The potential benefit of additional chemical interaction has been shown to contribute to adhesive performance,[5,6] prolonging bond longevity of adhesive restorations.[7,8]

Detection of chemical bond between adhesives and dentin has shown to be a controversial topic in literature.[9–11] As a consequence, several techniques have been used in order to provide a better understanding of the involved chemical processes like x-ray photoelectron spectroscopy (XPS),[7] micromechanical tests,[12] and especially those working in the infrared spectral region.[2,13,14]

It is well known that techniques based on infrared radiation are considered the most important route to access chemical bonding information of biological tissues.[15–17] Among them, Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) has the special characteristics of allowing depth profile studies to detect the penetration and interaction of substances through biological tissues.[9,18] It can be applied for measurements at clinical conditions because minimal sample preparation is needed. It is a non-destructive method and provides the inspection in opaque and highly scattering samples. This technique has been used before to investigate the occurrence of adhesive chemical bonds in dentin; exploring the finger print characteristics of the optical absorption bands in the infrared spectral region.[9]

Therefore, considering that there are no surfaces studies about the chemical interactions between human moist dentin blocks and etch-&-rinse adhesives in a clinical typical condition, the aim of this study was to apply FTIR-PAS spectroscopy to investigate the physicochemical structural surface changes of healthy human intact dentin after treatment with two commercial etch-&-rinse adhesives.

2 Materials and Methods

Three sound human premolars were collected from patients who needed extraction for orthodontic reasons in conformity with an informed consent protocol reviewed and approved by the local ethics committee in human research. From the central part of the buccal and lingual faces, two specimens ($3 \times 3 \times 3$ mm) were cut off per tooth using a low-speed Isomet 1000 diamond saw. Therefore, a total of six paired specimens (2×3) were produced.

Through the use of paired specimens both adhesives could be tested on the same dentin substrate, minimizing the influence of dentin composition on the obtained results. Control sample homogeneity was proved by the calculation of dentin untreated surface mineral:matrix ratio ($M:M$ = the ratio of the integrated

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areas of the phosphate, v_1 and v_3, in relation to the amide I peak). These ratios were submitted to Shapiro-Wilk normality test (SPSS 10.0 statistical package), and were represented by means of standard deviation.

According to the use of different adhesive systems: A—Gluma Comfort Bond + Desensitizer (Heraeus Kulzer, Hanau, Germany); B—Single Bond 2 (3M-ESPE, St Paul, MN, USA), the fragments were divided into two groups ($n = 3$). The compositions of the adhesives are shown in Table 1. They present distinct functional monomers, as: A) 2-hydroxyethylmethacrylate (HEMA) and 4-methacrylox-yethyl trimellitate anhydride (4-META), and B) HEMA.

All the specimens were acid-etched for 15 s with 37% phosphoric acid, water rinsed for 5 s and blotted-dry, leaving the dentin surface moist. The adhesives were applied following the manufacturer's instructions. There were some differences in the treatments of each group: Group A received application of three adhesive layers and was polymerized for 20 s using a blue LED light source of 1.2 W/cm^2, while Group B received an application of two layers and was polymerized for 10 s. The final thickness of each film was calculated taking into account the initial adhesive volume and the sample superficial area. This was made neglecting both volume change during polymerization and the amount of interpenetrated adhesive in the dentin hybrid layer. Then, the values for groups A and B were found to be about 3 and 2 μm, respectively. To verify that, the samples were inspected with electron scanning microscopy (MEV) technique, model Shimadzu-SS-550 superscan system. It was noted that the films did not present constant thicknesses along the dentin, varying in an interval of about 1 to 4 μm.

The thermal lens (TL) technique, described elsewhere,[19] was used to measure the thermal diffusivity values of the adhesive films. To do so, the films were deposited in a microscope slide and polymerized as described above. After polymerization, it was gently removed and the TL measurements performed.

Sequential analysis mode was used as an experimental approach, so that the chemical modifications of the same dentin surface were verified by the FTIR-PAS technique before and after the procedures of acid conditioning and adhesives application. Moreover, the spectra of adhesives alone were taken after polymerization of 10 μl of each liquid, according to the manufacturer's instructions. For each measurement, either the selected dentin surface or the adhesive sample was placed in the sample holder and inserted into a MTEC 300 photoacoustic cell. Before sealing the cell, the chamber was purged with helium gas to avoid spectral interference by loosely bound water.[20,21] In addition, the use of helium gas in the PAS cell improved the signal/noise ratio significantly. All spectra were recorded in the rapid scan mode on a FTIR-PAS

spectrophotometer, Varian 7000, between 500 and 4000 cm^{-1} at a mirror speed of 0.64 cm/s (10 kHz) and a resolution of 8 cm^{-1}. Five hundred to 1000 scans were collected in order to increase the signal-to-noise ratio, and all spectra were compared to a carbon black reference provided by MTEC.

To specify the technique ability to provide the depth of analysis in our experimental condition, it is important to consider that in the rapid scan mode, the FTIR-PAS method provides the thermal diffusion length (cm) as $\mu = [D\lambda/(2\pi v)]^{1/2}$, in which D is the sample thermal diffusivity (cm^2/s), v is the mirror velocity (cm/s), and λ the incident radiation wavelength (cm). This parameter is the dimension over which the thermal wave decays to $1/e$ of its original amplitude and has been used in the analysis as approximately the sampling depth, where the PAS signal is generated. In our experimental condition, the sample can be approached as comprised of two layers with a hybrid interface, resulting in effective values for the thermal diffusivity. It depends on each μ values, according to the incident radiation wavelength. For the dentin in the tubular direction, $D = 2.5 \times 10^{-3}$ cm^2/s,[22] while for the adhesives, the TL measurements performed in this work provided similar values, $D = (1.20 \pm 0.04) \times 10^{-3}$ cm^2/s. Since the exciting radiation was incident on the adhesive side of the samples, we estimate μ using the adhesives thermal diffusivity values. Thus, using $v = 0.64$ cm/s, we calculate, for example, the penetration depth at 1176 cm^{-1} ($\lambda = 8.5 \times 10^{-4}$ cm) to be higher than 5.0 μm.

Analyses of adhesives-treated spectra were done through spectra baseline and subtraction.[23] For each group, the etched-dentin spectra were subtracted from the adhesive-treated dentin spectra obtained from the same specimen surface, and the spectra of the difference were compared with that of the original adhesives. Subtracted spectra Gaussian fittings were made in order to help the identification of absorption bands. Furthermore, some absorption bands modified after the adhesives treatment were also fitted by Gaussian functions in order to better identify the spectra alterations. It is well known that the absorption bands in the infrared permit the chemical bonding modifications to be traced, expressed by peak shifting and/or new peak formations. Due to the qualitative characteristic of the data resulting from the FTIR-PAS, statistical analysis was not carried out in this study.

3 Results

Spectra of the six control dentin surface showed homogeneity in organic and inorganic composition (M:M = 1.80 ± 0.02). The etching with 37% phosphoric acid resulted in a decalcification pattern; presenting increased relative intensities and/or better resolved absorption bands of amide groups centered around

Table 1 Composition and manufacture specification of the studied adhesives.

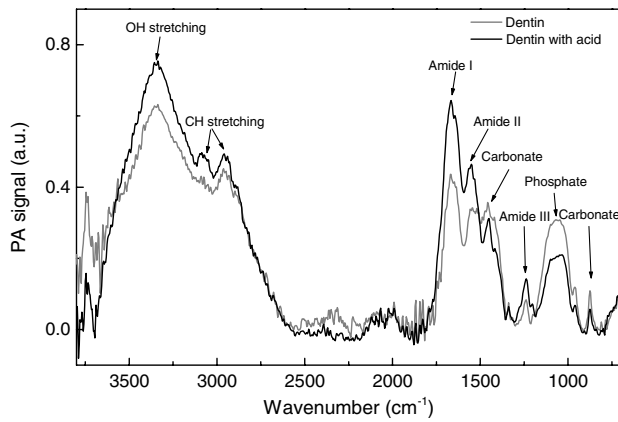| Adhesive	Manufacturer	Composition
Gluma Comfort Bond + Desensitizer	Heraeus Kulzer Hanau, Germany	2-HEMA, 4-META, UDMA, glutaraldehyde, maleic acid, ethanol, water
Single Bond 2	3M-ESPE, St Paul, MN, USA	2-HEMA, BisGMA, dimethacrylates, copolymer of acrylic, itaconic acids, ethyl alcohol, water

Data provided by the manufacturers.

Fig. 1 FTIR-PAS spectra: demineralization pattern verified through the comparison between acid-etched dentin and control dentin surface.

1665 cm^{-1} (amide I), 1550 cm^{-1} (amide II), and 1240 cm^{-1} (amide III), and decreased intensities of absorption bands at 1455 cm^{-1} (carbonate stretching vibrations—ν_3), 1067 cm^{-1} (orthophosphates stretching vibrations—ν_3) and 874 cm^{-1} (carbonate stretching vibrations—ν_2), as shown in Fig. 1. Because of the dentin spectra modifications caused by the etching procedure, the acid-conditioning spectra were considered as negative control for the analysis of adhesives interactions.

The spectra subtraction evidenced that some dentin peaks were modified after the application of both adhesives. In Figs. 2(a) and 3(a), in the interval between 3800 and 2200 cm^{-1}, a slight peak decrease of the OH stretching (OH-bonding) occurred at 3340 cm^{-1} as a result of adhesive treatment, and also a significant reduction of the band at 2900 cm^{-1} (symmetric C-H stretching) took place when comparing spectra of original adhesives with that of the dentin-treated adhesives. Furthermore, the ratio of 2900/2980 bands of the subtracted spectra decreased for adhesive A and increased for adhesive B.
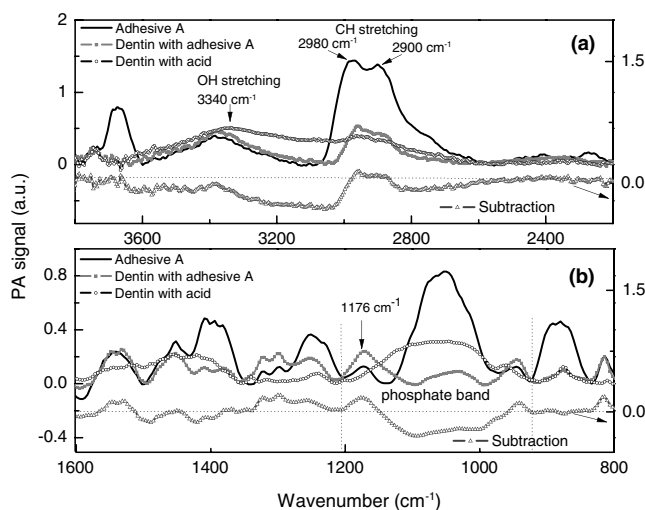

Fig. 2 FTIR-PAS spectra: adhesive A, dentin with acid, dentin treated with adhesive A and subtracted spectra: (a) spectra range between 3600 and 1800 cm^{-1} showing modifications due to adhesive interaction; (b) spectra 1800 to 700 cm^{-1} range, indicating a orthophosphate optical absorption band (1040 to 970 cm^{-1}) reduction because of chemical reaction with adhesive.
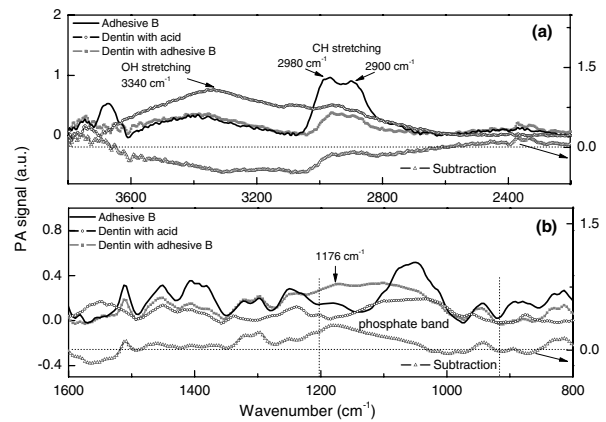

Fig. 3 FTIR-PAS spectra: adhesive B, dentin with acid, dentin treated with adhesive B and subtracted spectra: (a) spectra wavelength between 3600 and 1800 cm^{-1} showing modifications due to adhesive interaction; (b) spectra 1800 to 700 cm^{-1} range, suggesting the occurrence of chemical bonding of adhesive attributed to the calcium/phosphate ester complexes formation.

In addition, differences in spectra subtraction at the interval from 1600 to 800 cm^{-1} can be seen in Figs. 2(b) and 3(b). The subtracted curve for group A, in Fig. 2(b), shows both a relevant decrease of orthophosphate absorption band (1040 to 970 cm^{-1}), and an increase of the peak related to phosphoric group $\nu_{P=O}$ (1176 cm^{-1}). Nevertheless, in Fig. 3(b), another pattern of modification can be noticed in the dentin treated with adhesive B and on the subtracted spectra. These alterations suggest the formation of a calcium-phosphate-ester complex (1270 to 970 cm^{-1}), as observed before by Penel et al.[24] Gaussian fittings were made at the interval of 1400 to 850 cm^{-1} of the dentin-treated adhesives to identify the compound absorption bands, as shown in Fig. 4. The band deconvolution of adhesive B-treated spectrum provides the identification of a better resolved band at 1176 cm^{-1}.

4 Discussion

To the best of our knowledge, this is the first analysis reporting chemical interactions of etch-&-rinse adhesives with human intact and moist dentin treated with commercial adhesive system. After demineralization with 37% acid etching (Fig. 1), mineral components dissolution was evidenced from the reduction of the orthophosphate-carbonate peak, and collagen exposure was verified by the increased intensities of the amide groups absorption bands. The spectra alterations after treatment with adhesives A and B indicated their chemical interactions with dentin (Figs. 2–4).

Detecting true chemical bonding information of the interface depends on the exclusive analysis of the bonded layer.[25] Acid conditioning of sound dentin can result in a demineralization depth around 6 to 8 μm,[26,27] while the hybrid layer thickness is believed to be approximately 6 μm.[28] Since the FTIR-PAS average depth profile in the experimental condition of this work was around 5 μm, and the adhesive surface layer thicknesses smaller than 3 and 2 μm, for adhesives A and B, respectively, this technique characterized the structure, including the chemical interactions at the interface region, between the dentin and the adhesive.

Both adhesives selected for this study have HEMA as a functional monomer. However, only adhesive A contains the monomer 4-META. Previous studies have suggested that HEMA
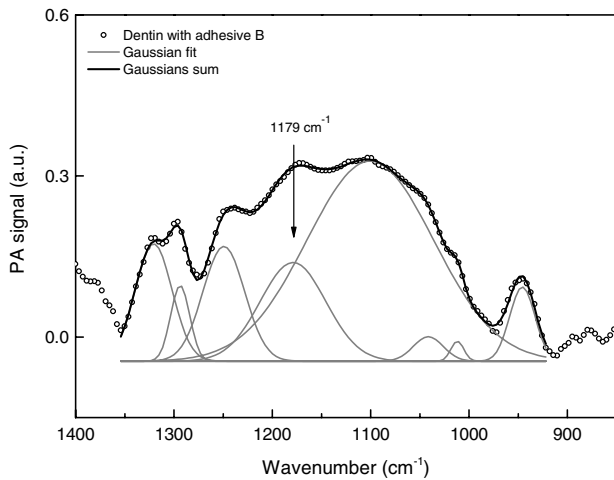
Fig. 4 Evidence of the peak at 1176 cm^{-1} after the Gaussian adjustments and theoretical fit analyses of adhesive B treated spectra between 1400 and 850 cm^{-1}.

reacts with the collagenous fraction of dentin,[11,29] and also that it is associated with the formation of calcium/phosphate ester complexes in the hybrid layer.[3,24] In the case of 4-META, its ability to chemically bond to calcium hydroxyapatite has been described.[7,30] According to the manufacturers, adhesive A also contains poly(methacrylic-oligoacrylic) acid and glutaral, and adhesive B methacrylate functionalized copolymer of acrylic and polyitaconic acids. These compounds are also active and may bond to the dentin as well. These reinforce the complexity to determine the type of adhesive chemical bonding to the dentin.

The modifications found after the adhesive treatment at spectra between 3800 and 2200 cm^{-1} were presented for both adhesives [Figs. 2(a) and 3(a)], evidencing their capacity to chemically interact with the collagenous fraction of dentin. Moreover, despite the fact it is also present in the adhesive spectra, the increase of the absorption band around 1176 cm^{-1} (phosphoric group $\nu_{P=O}$) for group A [Fig. 2(b)] and the occurrence of a wide band at this spectral region in group B [Figs. 3(b) and 4] (probably revealing the formation of a calcium/phosphate ester complexes) are further indications of adhesives bonding to the inorganic dentin matrix.

The reduction of the OH stretching absorption bands around 3340 cm^{-1} is the evidence of functional group formation via transesterification. This reaction occurs when the ester portion of HEMA reacts with the protein hydroxyl group, resulting in the formation of ester and alcohol and the consumption of OH^{-}.[11] The peak at 2900 cm^{-1} on the adhesives spectra can be attributed to symmetric C-H stretching. Thus, the decrease of this 2900 cm^{-1} band, when the original adhesives and dentin-treated adhesives were compared, may be assigned to the reaction of part of the adhesive molecules to the available protein sites. In addition, the occurrence of an absorption band around 1176 cm^{-1} (phosphoric group $\nu_{P=O}$) may also be an indication of chemical interaction between ester of the adhesive and calcium and phosphate dentin molecules.[3,24]

Functional monomers of adhesive system A appears to have more chemical affinity to the dentin inorganic matrix (calcium ion) than adhesive B since its spectra did not show evidence of complex formation, presenting only an increase of the 1176 cm^{-1} peak (phosphoric group $\nu_{P=O}$). Moreover, the

significant reduction of the phosphate absorption band (1040 to 970 cm^{-1}) after treatment with adhesive A [Fig. 2(a)] indicates the chemical reaction of its functional monomer to dentin. This modification suggests the occurrence of ionic binding between carboxylic groups of adhesive to Hap ions;[31] a mechanism in which those carboxylic groups replace and extract the phosphate ions of the dentin substrate.[7] Although the chemical interaction is dependent on the adhesive composition,[32] the third layer of adhesive A applied on dentin could have increased the adhesive performance of this system.

The use of sound human dental tissue in blocks was advantageous because the organic-mineral matrix and water junction of the dentin tissue remained with its natural structure.[4,32,33] However, *in vitro* studies may be limited to simulate the clinical conditions. The dentin specimens used were non-vital; therefore, the dentinal fluid flow produced by the intrapulpal pressure was not present in our experimental approach. In order to minimize this limitation, the dentin blocks were kept in saline solution and gently dried before starting the treatment.

Another disadvantage found in these analyses consisted of bands overlapping in the 1785 to 1500 cm^{-1} spectral region.[13] Because of the interaction of the neighboring bands, it was preferable to disregard the modifications of the amide group absorption bands after adhesive treatment. However, in terms of the tentative to detect the presence of chemical bonding, the use of PAS-FTIR technique permitted the evaluation of these non-homogeneous samples in a procedure in which little sample preparation was required. Especially, the method was able to make depth profile inspection down to about 5 μm from the sample surface, including the region where adhesive dentin interaction was supposed to take place.

To conclude, according to FTIR-PAS investigation, the spectra alterations suggested that both adhesives presented chemical bonding to dentin, a study performed using a typical clinical treatment. The results indicated chemical evidence of the transesterification reaction through adhesive molecules and collagen hydroxyl group, chemical interaction of adhesives to inorganic portion of dentin forming calcium/phosphate ester complexes and, also ionic bonding between carboxylic groups of adhesives and calcium hydroxyapatite. The results reinforced that the adhesive's chemical interaction with dentin depends on their compositions. Since adhesive restorations have limited durability in the mouth and chemical-bonding adhesives are expected to extend bond longevity, the observations on this work may help with the actual goal of the dental material industry for improvements of adhesives that chemically bond to dentin available sites.

Acknowledgments

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