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Characterization of Interfacial Chemistry of Adhesive/Dentin Bond Using FTIR Chemical Imaging With Univariate and Multivariate Data Processing

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Abstract

Fourier transform infrared (FTIR) chemical imaging can be used to investigate molecular chemical features of the adhesive/dentin interfaces. However, the information is not straightforward, and is not easily extracted. The objective of this study was to use multivariate analysis methods, principal component analysis and fuzzy c-means clustering, to analyze spectral data in comparison with univariate analysis. The spectral imaging data collected from both the adhesive/healthy dentin and adhesive/caries-affected dentin specimens were used and compared. The univariate statistical methods such as mapping of intensities of specific functional group do not always accurately identify functional group locations and concentrations due to more or less band overlapping in adhesive and dentin. Apart from the ease with which information can be extracted, multivariate methods highlight subtle and often important changes in the spectra that are difficult to observe using univariate methods. The results showed that the multivariate methods gave more satisfactory, interpretable results than univariate methods and were conclusive in showing that they can discriminate and classify differences between healthy dentin and caries-affected dentin within the interfacial regions. It is demonstrated that the multivariate FTIR imaging approaches can be used in the rapid characterization of heterogeneous, complex structure.

Keywords

FTIR; chemical imaging; multivariate analysis; dentin; adhesive

Introduction

Bonding of current adhesives to dentin is usually through the formation of the interfacial hybrid layer between adhesives and dentin. 1–3 This layer that connects the adhesive to the subjacent dentin is believed to be both chemically and structurally heterogeneous, since the formation of it relies on many processes such as acid etching to remove mineral phase, adhesive penetrating into the demineralized collagen network in the presence of water and photo-polymerization of the adhesive, etc. 1,3–11 The characterization of the heterogeneity of the adhesive/dentin interfacial layer has thus been a topic of great interest. Fourier transform infrared (FTIR) microspectroscopy has proven to be a good imaging technique to investigate the physicochemical interactions at the dentin/adhesive interface. 12 Using FTIR microspectroscopy, the degree of cure, relative chemical composition and homogeneity across the length and breadth of the adhesive/dentin interface can be determined. 12

FTIR microspectroscopy represents the combination of spectroscopy with microscopy and allows the exploration of molecular chemistry of small specimens at the microscopic level. It has been used widely in biology, chemistry and many other disciplines to monitor chemical features within the microstructures of a material, but the microscope mapping usually takes many hours or even days to complete. Recently, a new generation of infrared imaging instruments based on array detectors has changed this situation. Imaging spectrometers capable of collecting large arrays of spectra in a short amount of time are now available. This analytical technique can be used to collect a mosaic of FTIR images, which providing different types of information such as material/tissue chemistry, composition, structure, simultaneously. However, extracting chemical composition from a specimen area has been time-consuming and difficult.

Although FTIR imaging technique permits high-throughput and spatially resolved measurements and can generate a large volume of data which contains rich spectral information, the information is not straightforward, and is not easily extracted. From the point of view of measurement, FTIR imaging technique produces data “hyper cubes” consisting of four dimensions: x and y spatial dimensions, a spectral dimension and an intensity dimension. The term hyper cube reflects the fact that raw imaging data cannot be graphically displayed in three-dimensional space. One challenging task during the application of the imaging technique is how to effectively extract information from the hyperspectral imaging data. The most straightforward method of FTIR spectral data analysis is to generate functional group maps based on band intensities, band areas, or band ratios. In this univariate mode, the FTIR maps/images represent the inherent contrast associated with the unique chemical bonds of the components within the analyzed specimen. A chemical map/image of each component is created by plotting the unique frequencies as a function of spatial position and spectral intensity. A color scheme can be applied to the intensity values to permit the creation of a “false-color composite” image. Previous FTIR imaging studies on the adhesive/dentin interfaces have used univariate methods of analysis to arrive at the spatial relationships and distributions of the desired functional groups in the specimen.¹² While these methods can provide information on the distribution and relative concentration of a particular functional group, they are not very useful in terms of classifying chemical and histopathological features within the specimen matrix. In addition, using these univariate methods, it becomes very difficult to keep track of minor changes in spectra across the specimen. These subtle spectral differences may be the key to the chemical differentiation of areas such as carious dentin regions.

Under these conditions, multivariate methods may be more appropriate. Multivariate analysis techniques serve to analyze the hyperspectral data by treating each spectrum (pixel) as a whole, rather than considering individual bands in each spectrum. Several authors have applied multivariate pattern recognition methods such as hierarchical cluster analysis, K-means clustering, principal components analysis, fuzzy c-means clustering, and artificial neural networks to FTIR imaging analysis for various medical diagnostics.^{13–18} These methods are aimed at classifying spectra based on similarity, and thus are utilized to discriminate chemical features based on underlying differences in the chemistry within the microstructures of a specimen. Bonding adhesive to dentin relies on the extraction of mineral phase and infiltration of adhesive monomers into the voids left by the mineral. Since various dentin substrates will produce very different, complex matrices with variable composition after acid etching, which could be detrimental for adhesive penetration and polymerization, it is expected that the interface structure of adhesive bonded to healthy and caries-affected dentin is both chemically and structurally heterogeneous. The purpose of this study was to apply univariate and multivariate spectral analyses in FTIR imaging to reveal structural/chemical features of the adhesive/dentin interface and to demonstrate the importance of multivariate imaging analysis in the heterogeneous interface studies.

Materials and Methods

Specimen preparation

Three extracted unerupted human third molars and three extracted human molars with coronal caries were collected from the Oral Surgery Clinic at the University of Missouri Kansas City (UMKC) School of Dentistry. The teeth were collected after the patient's informed consent was obtained under a protocol approved by the UMKC adult health sciences institutional review board. Following extraction the teeth were placed in separate vials containing phosphate buffered saline (PBS) and 0.002% sodium azide and stored at 4°C. For healthy teeth, initial specimen preparation proceeded as follows: the occlusal one-third of the crown was sectioned perpendicular to the long axis of the tooth by means of a water-cooled low speed diamond saw (Buehler, Lake Bluff, IL, USA). The exposed dentin surfaces were inspected with a microscope to ensure that no enamel remained. A uniform smear layer was created by abrading the exposed dentin surface with 600 grit SiC under water for 30 s. 19:20

The preparation of caries-affected (c-a) dentin specimens followed the technique described by Nakajima et al. 21:22 Characteristically, carious dentin is described as consisting of infected and affected layers. The affected layer is generally not removed during treatment. The carious material could be identified by visual inspection following staining with a caries detector solution. The occlusal surface was ground perpendicular to the long axis of the tooth until a flat surface composed of the carious lesion surrounded by normal dentin is exposed.

Prepared healthy dentin as well as prepared c-a dentin substrates were selected for treatment with a current commercial dentin adhesive Single Bond Plus (3M/ESPE Dental Products, St. Paul, MN). The application of the adhesive followed the manufacturer's instructions. The different dentin surfaces were etched for 15 s with 35% phosphoric acid. After acid etching, the teeth were rinsed with water for 10 s and blotted dry, leaving the dentin surface moist. Two consecutive coats of Single Bond Plus were applied with a fully saturated brush. The surface was gently dried for 5 s and light-cured for 20 s. These specimens were stored for a minimum of 24 h in H₂O at 37°C before proceeding with the sectioning. The treated dentin surfaces were cut perpendicular to the bonded surface at 2 mm increments by means of a H₂O-cooled low-speed diamond saw. The specimens were then cut at a depth of ~2 mm below the interface to create a number of resin bonded beams or slabs. The final dimensions of these slabs were 10 × 2 × 2 mm.

Sectioning

The rectangular beams were mounted on a methacrylate support with cyanoacrylate adhesive and three-micron-thick sections were cut perpendicular to the adhesive-dentin face of the beam using a tungsten carbide knife mounted on a Leica Polycut S microtome (Leica, Deerfield IL, USA). The knife edge and beams were moistened with distilled water while cutting. As each single section is cut, it is allowed to slide onto the knife, the microtome is stopped, and the thin section was collected on a 13 mm diameter, 1 mm-thick barium fluoride disc with the aid of a fine paintbrush. Most important for high quality image is the flatness of the sections at the end of procedure. For this reason, sections were covered by another BF₂ disc to keep them flat. These sections were placed directly on the motorized stage for FTIR microspectroscopic imaging data acquisition.

FTIR microspectroscopic imaging data acquisition

FTIR microspectroscopic imaging data collection were completed using the Spectrum Spotlight FTIR imaging system (Perkin Elmer, Waltham, MA, USA) with both single point

and imaging mode. The liquid nitrogen cooled detector incorporates a narrow band MCT (mercury-cadmium-telluride) array detector and single point medium band MCT detector on a single substrate. It offers the wavelength range from 7800 cm^{-1} down to 720 cm^{-1} , supplying the mineral fingerprint detail missing from all other FT-IR imaging systems. This system provides $25\text{ }\mu\text{m}$ and $6.25\text{ }\mu\text{m}$ pixel resolution, and could generate chemical image size up to 1.64 cm^2 . Images were scanned between 4000 and 720 cm^{-1} at 8 cm^{-1} spectral resolution, with 16 scans per pixel. Image size was ~ 100 by ~ 150 micrometers, using $6.25\text{ }\mu\text{m}$ pixel resolution. An atmosphere correction was applied to the raw image, subtracting the contribution of atmosphere absorbance, i.e., water vapor and carbon dioxide.

Univariate Imaging Analysis

Univariate methods consider one wave number at a time thereby providing information about the characteristic functional group corresponding to the wave number/band concerned. Wave numbers corresponding to the adhesive monomer, collagen and mineral were used to characterize the distribution of these components in the interface. Images were created using Spectrum Spotlight software. The data can be displayed as a collection of spectra obtained at each pixel in the image. In some cases, individual spectra were extracted from selected areas for more detailed analysis.

Multivariate Imaging Analysis

The same FTIR data were also processed with multivariate procedures. The spectral data were imported into Hyperview v.3.0 software (Perkin Elmer) and spectra were converted to first derivative spectra using a Savitsky-Golay algorithm. The process of conversion into the first derivative spectra essentially eliminates the need for baseline correction of the normal FTIR spectra. In addition, derivatives are very common transformations applied in spectroscopy to emphasize small differences in spectra. Multivariate analyses of the FTIR spectral data, principal component analysis and fuzzy c-means clustering analysis, were carried out on the first derivatives of the FTIR spectra using the algorithms available in Hyperview.

Principal Component Analysis (PCA)—PCA is a multivariate data analysis technique that is now the data processing method of choice to unravel complex spectroscopic data for many applications.²³ It is a method of viewing the data in terms of a set of uncorrelated spectra which are arrived at in the decreasing order of their contribution to the variance in the data. This new set of uncorrelated spectra is composed of factors. The number of principal components or factors to be chosen in order to represent the data without noise can either be left to the software or decided by the user based either on observation of the factors or on previous experience. Hyperview uses a number of tests like F-tests of varying significance and Malinowski's indicator function in two flavors. Each factor has a particular weight associated with it at each pixel, called a score. The score at each pixel represents the contribution of the associated factor to that pixel. The PCA performed by Hyperview uses an iterative NIPALS algorithm, which calculates one factor and the associated score per iteration. PCA helps us to determine the number of components in the specimen, apart from denoising the data and reducing the dimension. The data is now ready for clustering.

Fuzzy C-Means Clustering—Clustering techniques give us the option of being able to divide the specimen into chemically different regions in successively increasing detail.^{15,17} Hyperview follows the procedure of fuzzy c-means clustering. Fuzzy c-means clustering has been used for its ability to display overlap of different clusters representing various chemical constituents at a single region. It is a procedure in which the data is grouped into clusters based on the distance of spectra from the average spectrum of the cluster. Based on the distance from each cluster, a pixel is allocated a membership function inversely proportional

to the distance from the cluster. The data in its reduced dimensions is used for clustering i.e. the scores of the factors chosen to represent the data are used for clustering. The pseudo color images of the individual clusters as well as the entire clustered data can be observed. In this study, the fuzzy c-means clustering algorithm uses the scores given by PCA as the basis data. The use of PCA results as an input to the cluster analysis provides chemical and structural classifications at successively greater cluster resolutions.

Results

Univariate Analysis

Thin sections of the adhesive/dentin interface specimens were analyzed using FTIR imaging. In the images, each pixel contains one FTIR spectrum. Representative FTIR spectra for Single Bond Plus adhesive as well as dentin are shown in Fig. 1. The spectra of the specimens show most of their major features in the 1800–800 cm^{-1} region. For Single Bond Plus, the major bands observed in the spectra are due to IR absorptions from methacrylate monomers (carbonyl 1720 cm^{-1} , CH_2CH_3 1457 cm^{-1}) and SiO_2 filler (1105 cm^{-1}) in adhesive liquid. The most intense band at 900–1100 cm^{-1} range is associated with the inorganic component in dentin. Collagen features are present at 1650 (amide I), 1550 (amide II) 1450 (CH) and 1240 cm^{-1} (amide III).

Fig. 2A shows the visible image of the microtomed adhesive/healthy dentin interface slice. Two layers are visible, in which the top half is adhesive and the bottom is dentin. Fig. 2B presents the total absorbance image of the interface specimen. There is no morphologic/chemical information which can be observed in this full absorbance image. Initial images were constructed by simply color coding the intensities of the bands arising from the distinct chemical components present. Figs. 2C–2E show the univariate images based on color coded areas of the three spectral bands (1718, 1656 and 1068 cm^{-1}), which provide information about the spatial distribution of the adhesive, collagen and mineral (apatite) components across the adhesive/healthy dentin interface. Blue represents the lowest absorbance, while red represents the highest. By comparing the spectra in Fig. 1, it can be seen that the band at 1718 cm^{-1} does not overlap with other bands, and the univariate images based on this parameter provide reliable information on the spatial distribution of the adhesive. However, it is noted that the phosphate band (900–1200 cm^{-1}) of the dentin overlaps with the band of SiO_2 filler in the adhesive. In addition, the amide I region more or less overlaps the 1620–1680 cm^{-1} region of the adhesive. Hence, the univariate images do not give accurate pictures of the description of either mineral or collagen without spectral subtraction.

Representative visible and microspectroscopic images across the adhesive/caries-affected dentin interface are shown in Fig. 3. Visible image (Fig. 3A) shows two layers. Figure 3B shows the total absorbance image of the adhesive/c-a dentin interface collected from a $100 \times 150 \mu\text{m}^2$ area. While there is no chemical information in the total absorbance image, it is noted that a more complicated absorbance pattern is observed in this image. FTIR images were generated with the use of the univariate analysis methods. As shown in Figs. 3C–3E, three images of spectral parameters (1718, 1656, and 1068 cm^{-1}) were generated, representing the spatial distribution of the adhesive, collagen and mineral (apatite) components across the adhesive/c-a dentin interface. Although the images and interface are slightly complicated as compared to the images of the healthy dentin/adhesive interface, the differences between these two types of interfaces are not obvious. Again, this may be due to spectral overlapping from neighboring bands and limitations of univariate approach to reveal minor changes.

Principal Component Analysis

The major outcomes of PCA analysis are the number of components that have detectable signal, the elements that are related to the spectra (known as PCs or factors), and the elements that relate to the concentrations (known as scores). The scores images show the spatial distribution of each factor. PCA analysis was carried out on the first derivatives of the FTIR spectra. For comparison, the first derivative spectra of the dentin and adhesive are shown in Fig. 4. The first six factors and scores from the PCA on the FTIR imaging data collected from the adhesive/healthy dentin interface are shown in Fig. 5. It is shown that the first three or four factors are the ones which contribute to the part of the spectrum comprising the dentin and/or adhesive. The other factors could represent minor changes in the chemical structure.

The score of the first factor (score 1) shown in Fig. 5B represents the dentin mineral distribution, demineralized dentin and partially demineralized dentin layer. The distribution of mineral in mineralized dentin is relative uniform. The score of the second factor (score 2) represents the distribution of the adhesive. It can be seen that the adhesive has penetrated into the demineralized dentin layer. However, the distribution of adhesive is not uniform in the region on the top of the dentin. Score 3 clearly indicates the presence of an adhesive/healthy dentin interface. Interestingly, it can be seen that even within the interface, the distribution or structure is different. The middle part of the interface region is different from the regions close to both adhesive and dentin layers. The above information is difficult to obtain by univariate methods.

The first six factors and scores from the PCA on the FTIR imaging data collected from the adhesive/carious-affected dentin interface are shown in Fig. 6. In contrast to the images shown in Fig. 5B, the score of the first factor (score 1) represents the distribution of both the adhesive and dentin mineral components, score 2 represents the dentin mineral distribution, demineralized dentin layer, and score 3 describes the interfacial region. The distribution of the adhesive is not clearly represented in the first few scores. Since the first few factors/scores account for the most of the variance, these results indicate that the structural/chemical variations in adhesive and healthy dentin interface are different from those in the interface of the adhesive with caries-affected dentin.

In addition, the interpretation of scores 4–6 in both Figs. 5 and 6 is not obvious. In PCA analysis, the factors of each data are arranged in the decreasing order of their contribution to the variance. The latter factors are perceived to be either the minor changes in the chemical structure or noise and their scores are therefore not considered here in extracting information. Clustering analysis will be considered for further detailed analysis.

Clustering Analysis

Cluster analysis separates the group of spectra into clusters with clear similarities within each cluster and distinctions between the clusters. Fig. 7A shows the false color image of the adhesive/healthy dentin interface where the data have been split into three clusters. All spectra in a cluster are assigned the same color. The mean spectrum of each cluster represents all spectra in a cluster, is also shown in the same figure. These mean spectra can be used to interpret the chemical differences between clusters. This is a low level of resolving the data where it can be seen that the image is divided into three regions which represent three major components, the adhesive, the interface and the dentin. Different regions have different spectra clusters. In order to observe further detail, the number of clusters is increased successively. The four-cluster image shows a new, separate region in addition to the details observed in three-cluster (Fig. 7B). The cluster appears in the region between the adhesive and interface. Careful comparison of the spectra of the clusters

indicates that the new cluster region contains more adhesive and almost no mineral (Fig. 7B). In the five-cluster image, the interface is split into two cluster regions. The lower region contains partially demineralized dentin and lesser concentration of the adhesive, while the upper region contains more adhesive and lesser or no mineral (Fig. 7C). In addition, the adhesive can be seen to be split into two regions in which the lower region has a slightly lesser concentration, as indicated in the mean spectrum of the cluster (Fig. 7C). As shown in the six-cluster image, further clustering results in the formation of duplicate clusters and inclusion of more factors is not found to produce significant changes in the clusters (Fig. 7D). The number of clusters as well as factors is fixed at six.

Cluster images of three, four, five and six clusters based on the data from the adhesive/caries-affected dentin interface are shown in Fig. 8. The mean spectra extracted for the cluster analysis are also presented in the same figure. In the false color three-cluster image, three layers are apparent, the interfacial region between adhesive and dentin appears as a separate cluster (Fig. 8A). In the four-cluster image, the interface is split into two. The new cluster appears in the region between the interface and dentin (Fig. 8B). This is different from the four-cluster result for the adhesive/healthy dentin interface, in which the new cluster appears in the region between the adhesive and dentin (Fig. 7B). However, the similar, new cluster appears in the five-cluster image (Fig. 8C). It is noticed that there is further differentiation of the interface at high cluster numbers. The six-cluster image delineates more areas of difference, especially in the caries-affected dentin substrate, with the delineation arising from spectral difference shown in Fig. 8D. The regions of mineralized dentin, slightly demineralized dentin with little or no adhesive contribution, partially demineralized dentin with adhesive contribution and demineralized dentin with more adhesive contribution are clearly observed (Fig. 8D). Given that caries-affected dentin is more complicated, this is what one would expect. The six-cluster is sufficient to delineate the major components and layers within the specimen. The inclusion of further clusters in the analysis resulted in differentiation based mainly on baseline variation and artifacts introduced by the mosaic nature of the images.

Discussion

The strengths of bonding to dentin, especially bonding to the clinical relevant substrates such as caries-affected dentin, continue to drop as a function of time.^{24–28} Durability of this bond relies on the quality of the interface between adhesive and dentin. Understanding of the interfacial structure and chemistry is critical to reveal reasons for the low durable bond. However, obtaining such information has been a formidable challenge. Currently, the widely used techniques for characterization of the adhesive/dentin are bond strength tests in combination with morphologic observations. These techniques measure fracture resistance of bulk adhesive/dentin specimens, and are not sensitive enough to identify interfacial defects that lead to crack initiation or aqueous degradation.^{29–30} FTIR imaging technique used in this paper allow direct nondestructive, in situ visualization of the spatial distribution of variable composition and inhomogeneity across the length and breadth of the adhesive/dentin interface. The FTIR images contain thousands of high quality spectra which include rich chemical information. However, the information is complicated, and is not easily obtained. Both univariate and multivariate analyses were utilized and compared to extract chemical information from the interfaces of the adhesive with health dentin and caries-affected dentin.

In comparison with multivariate methods, univariate analysis is the simplest and most frequently used method. If individual component can be uniquely identified, analysis of absorbance band height and/or area is the most straightforward method to identify its location. For example, the band at 1718 cm^{-1} (C=O) shows little interference with any other

bands and thus, it is a unique band for adhesive distribution. However, the bands sometimes partially overlap due to interference from spectra of other components. For example, the mineral band at 1068 cm^{-1} (P-O) somewhat overlaps with SiO_2 band at 1105 cm^{-1} . In this case, the images based on the partially overlapped wavenumbers are only tentative; when the band completely overlap with other bands, it is difficult to bring out the information using univariate methods. Apart from this, univariate analysis fails to discriminate differences in interfacial structure between healthy dentin and caries-affected dentin. This might be due to limitations of the univariate approach to reveal spectral changes.

These problems can be resolved using multivariate methods via PCA and clustering as demonstrated in this paper. Multivariate methods are performed on the whole spectral region ($1800\text{--}800\text{ cm}^{-1}$). The PCA method enables effective extraction and classification of a large number of component spectra. Although information about molecular structure is suppressed with this multivariate analysis, the capability to identify spatial regions in the interface specimens is enhanced. Through this technique, we were able to show clearly the presence of adhesive, interface and dentin layers; to display non-uniform distribution within the complicated interface; and started to discriminate interfacial differences between healthy dentin and caries-affected dentin (Figs. 5, 6). For example, the dentin, adhesive and interface are reliably imaged through the univariate wavenumbers or score images for adhesive/healthy dentin interface. Both types of images tend to be visually very comparable. This is not true for images of adhesive/caries-affected dentin interface, indicating structural differences exist between the two types of interfaces.

While the PCA is suitable for classifying the regions in the interface specimens, it becomes less effective in distinguishing the minor changes in the chemical structure. As shown in Figs. 5 and 6, the interpretation of scores 4–6 is not obvious. In addition, although we could see some differences between healthy and caries-affected dentin interface specimens, it is not clear how they are related to structural differences. To solve the above problems and differentiate chemically different regions in successively increasing resolutions, fuzzy c-means clustering analysis was applied. In this cluster analysis, a measure of similarity is established for each class of related spectra and a mean characteristic spectrum can be extracted and compared for each class. It is shown that the chemically and structurally heterogeneous interface of the adhesive/dentin bond is clearly classified. The chemical distinctions between healthy and caries-affected dentin interface specimens are also visible. At lower cluster numbers, i.e., 3 clusters, the dentin, adhesive and interface are distinctly imaged. With increasing in the number of clusters, detailed information about the substrates and their interaction with the adhesive is observed. A minimum number of clusters divide the image into regions of different chemical makeup (i.e., the adhesive, dentin, interface) while further clusters tend to reveal differences in the concentrations of the chemical constituents present within basic clusters.

The cluster analyses of the spectra reveal the detailed differences in the adhesive/dentin interfaces between healthy dentin and caries-affected dentin, which could not be easily seen using univariate and/or PCA analyses. As compared to healthy dentin substrate, more areas of difference are delineated in the caries-affected dentin substrate, indicating the interaction of the c-a dentin with adhesive is more complicated (Figs. 7 and 8). In both interfaces, the adhesive is split into two regions in which the new, lower region in contact with dentin substrate has a slightly lesser absorption. The filler contents in the new regions are also slightly lower. However, the distribution patterns of two regions are different in the two interfaces. In the adhesive/healthy dentin interface, the distribution of the new region was seen along the surface of the interface that extended perpendicularly into the adhesive. In the adhesive/caries-affected dentin interface, the new region formed a thin layer along the top of the interface.

The differences in the distribution patterns of two adhesive regions within the specimens may be caused by different substrates during wet bonding processes. When bonding to healthy dentin, the tubules perpendicular to the surface are filled with water after acid etching and water rinsing. Since the wet bonding technique is used, water outflows from tubules during the adhesive penetrating. The new region that perpendicularly extended into the adhesive probably represents sites of incomplete water removal that leads to lower concentration and lower filler content, as conformed by the mean spectrum from this region. Interestingly, this similar pattern was also seen by Tay and Pashley, 31 who observed this pattern in the form of interconnecting, dendritic silver deposits using transmission electron microscopy. They assigned the observed phenomenon as water trees that are associated with water channels present along the adhesive/dentin interface. 5·31·32 The similar pattern was not observed in the adhesive/c-a dentin interface. When bonding to caries-affected dentin, most of tubules are filled with acid-resistant minerals and are not opened during acid etching. 10 However, acid etching produces much thicker, more complicated demineralized layer, which contains more water. The layer of the new region along the interface shown in Fig. 8 is likely due to incomplete removal of water outpouring from the substrate during the adhesive penetrating. The information about the differences between healthy dentin and caries-affected dentin within the interfaces is critical in understanding bonding performance of these different substrates.

In summary, the application of multivariate methods to analyze the adhesive/dentin interface provides a very good technique to bring out the chemical detail in the adhesive/dentin specimens as compared to traditional univariate methods. The above results indicate that these multivariate image processing capabilities can be used to elucidate very clear chemical differentiation of heterogeneous interfaces, which is not obvious by looking at univariate images. Specifically, from the univariate images, it is not possible to draw a chemical distinction between healthy and caries-affected dentin substrates, a detail which is clearly indicated in the infrared images after multivariate analysis. Such detailed chemical maps of molecular information provide a reliable and powerful means of identifying flaws or defects in the patterns of adhesive/dentin interfaces.

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Fig. 1.
Representative FTIR spectra of the Single Bond Plus adhesive and dentin in the region
1800–800 cm^{-1}

Fig. 2.

Visible and infrared spectroscopic images of the microtomed slice of the adhesive/healthy dentin interface; A) visible image, the regions associated with the adhesive and dentin are indicated on the figure; B) total absorbance image; (C) 1718 cm^{-1} image (carbonyl); (D) 1656 cm^{-1} image (amide I); (E) 1068 cm^{-1} image (phosphate).

Fig. 3. Visible and infrared spectroscopic images of the microtomed slice of the adhesive/caries-affected dentin interface; A) visible image; B) total absorbance image; (C) 1718 cm^{-1} image (carbonyl); (D) 1656 cm^{-1} image (amide I); (E) 1068 cm^{-1} image (phosphate).

Fig. 4. Representative FTIR first derivative spectra of the Single Bond Plus adhesive and dentin in the region 1800–800 cm⁻¹

Fig. 5.
The first six factors (A) and scores (B) from the PCA analysis on the FTIR imaging data collected from the adhesive/healthy dentin interface.

Fig. 6.
The first six factors (A) and scores (B) from the PCA analysis on the FTIR imaging data collected from the adhesive/caries-affected dentin interface.

Fig. 7A

Fig. 7B

Fig. 7C

Fig. 7D

Fig. 7. The Clustered images show the division of the adhesive/healthy dentin interface specimen into chemically distinct regions.

Fig. 8A

Fig. 8B

Fig. 8C

Fig. 8D

Fig. 8.
The Clustered images show the division of the adhesive/caries-affected dentin interface specimen into chemically distinct regions.