Using Raman spectroscopy to determine adhesive distribution in hybrid layer of dental bonding

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Abstract

Light-cured adhesive systems have been widely used in dental clinical practice. Bonding to dentin remains poorly understood and unreliable. Previous studies have shown that quality of hybrid layer (HL: interface between adhesive layer and dentin) is very important to the reliability of whole bonding. Raman spectroscopic studies of the adhesive distribution in HL indicate that monomers do not completely infiltrate into etched dentin, thereby contributing to early failure of whole bonding.

Introduction

Polymers have been used in dentistry for nearly half a century. Currently, dimethacrylate-based polymers that can cross-linked networks are widely used in clinical dentistry as resin-based composite (RBC) restoratives and dental adhesives. Although the bond to enamel has been proven to be highly successful,[1] the bond to dentin remains poorly understood and unreliable.

Dentin adhesive systems and RBCs are cured with visible blue light sources, which give the dentist control of the photopolymerization process. In a typical adhesive system, the adhesive monomers penetrate into the demineralized dentin surface, intermingle with the collagen network, and are photopolymerized to form a bond between the dentin and composite layer. The interface between adhesive resin layer and the intact dentin is termed the hybrid layer. Although the thickness of hybrid layer is only several microns,[2] previous study shows that the quality of this layer plays a very important role in the stability and longevity of the bond.[3] Imperfect infiltration into the collagen matrix and low conversion of the adhesive monomers in the hybrid layer may result in premature failure of dentin-adhesive bond. Many studies have focused on the acquisition and analysis of mechanical or morphological properties of the resin bond;[4,5,6,7,8] however, a qualitative and quantitative chemical analysis of adhesive resin in the hybrid layer will lead to a better understanding of the dentin-adhesive bond and improvement of its performance.

In this study, Raman microscopy, a non-destructive method, was used to obtain chemical information about the hybrid layer on the surface of filled tooth samples. This scattering technique is based upon the rotational and vibrational transitions in molecules and is particularly well suited for the detection of chemical bonds. The combination of microscopy and Raman spectroscopy greatly improves the spatial resolution of the measurements. Raman spectroscopy requires little sample preparation and is not affected by water content in the samples. This is an advantage in this system because actual filled tooth samples can be analyzed in a moist environment, simulating *in-vivo* conditions.

Materials and Methods

Materials

Chemicals used in these Raman studies are components of Scotchbond™ MultiPurpose Plus (3M ESPE), a commercially available adhesive system. 2-Hydroxyethyl methacrylate (HEMA), bisphenol A glycidyl methacrylate (bis-GMA), camphorquinone (CQ) as photoinitiator, and ethyl 4-dimethylaminobenzoate (EDMAB) as co-initiator were purchased from Aldrich and used as received. Scotchbond™ Multi-Purpose Plus was used to prepare the dentin-adhesive bonds in tooth samples; however, the commercial primer (water, HEMA and polyalkenoic acid copolymer) was replaced with an experimental primer containing only acetone and HEMA. Adhesive formulations used in this research are listed in Table 1.

Table 1: Adhesive system formulations

Materials	Components	Weight percentage
Experimental Adhesive	HEMA	30
	Bis-GMA	68
	CQ	~1
	EDMAB	~1
Adhesive (3M ESPE)	HEMA	30-40
	Bis-GMA	60-70
	CQ	<1
	EDMAB	<1
	DHEPT	<1

Dentin-Adhesive Bond Preparation

Filled teeth samples were prepared using intact, non-carious, non-restored, human molars. The teeth were prepared using an experimental primer, Scotchbond™ Multi-Purpose Plus dental adhesive system and Z100™ resin composite restorative available from 3M. A dental light curing unit was used to photopolymerize the adhesive and composite resins. The restored teeth were sectioned into 2-mm × 2-mm sticks and placed in distilled water for 24 hours.

Raman Microscopy

The Raman scattering effect was induced by a 785-nm laser to minimize fluorescence in the biological system. Spectra were collected using a Leica DMLP optical microscope attached to the HoloLab5000R modular research Raman spectrograph (Kaiser Optical Systems, Inc) via a 15-μm collection fiber. The exposure time for these spectra was 60 s. Laser output was ~9 mW with a 1- μm spot size.

Raman spectra were processed using the second derivative method in GRAMS AI/32 (Thermo Electron Corporation). This method effectively eliminates baseline shifts and separates overlapped bands. Integrated peak areas calculated from these processed spectra were used in subsequent calculations.

Results and Discussion

Characteristic Raman peaks of monomers were used to identify components, and the peak area was used to determine the amount of each component. The Raman spectra of the two monomers are shown in Figure 1 with characteristic peaks highlighted. Here, the peak at 1610 cm^{-1} indicates the aromatic ring found in bis-GMA, and the peak at 605 cm^{-1} indicates the CC=O functional group contained in both HEMA and bis-GMA.[9] The ratio of these two peak areas was used to calculate the relative concentration of bis-GMA in the hybrid layer.

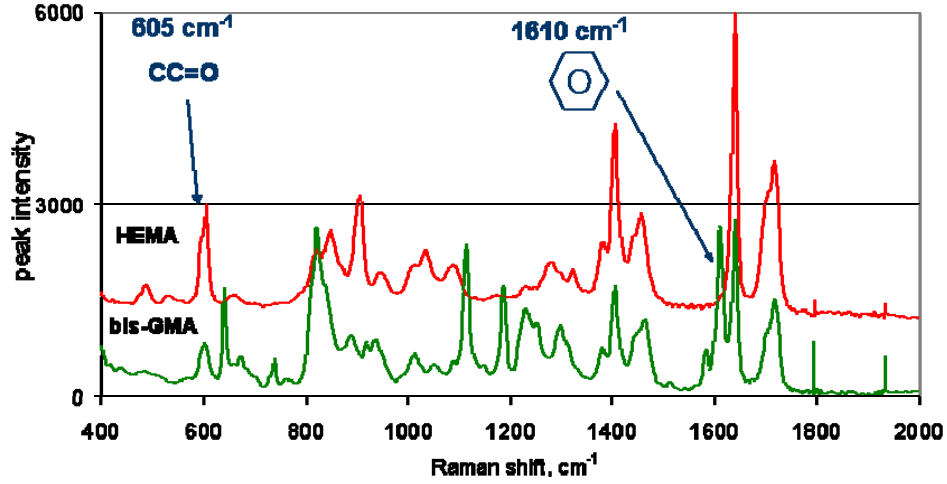

Figure 1. Raman spectra of HEMA and bis-GMA.

Qualitative Study

Monomer diffusivity can be related to the chemical-physical properties of the collagen network, surface tension, capillary pressure, and application technique, as well as monomer size and structure.[10] In this system, it is reasonable to expect that the bis-GMA cannot penetrate into the prepared tooth surface as successfully as HEMA since bis-GMA larger and bulkier. In addition, the HEMA-based primer is applied to the tooth surface before the commercial adhesive resin to prevent the collapse of the exposed collagen network.

A series of Raman spectra were acquired across the hybrid layer of the dentin-adhesive bond, starting from adhesive layer with 1-μm step size (see Figure 2). The 960 cm^{-1} peak representing the mineral component of dentin is highlighted along with the 1610 cm^{-1} indicating the adhesive monomer bis-GMA. The intensity of 1610 cm^{-1} peak decreases across the hybrid layer, finally disappearing at the bottom of the hybrid layer. This suggests that the bis-GMA is not able to penetrate deep enough into the hybrid layer. Unlike HEMA, bis-GMA has two reactive sites (i.e., carbon-carbon double bonds) and can form a cross-linked polymer network. Generally, crosslinking restricts chain mobility and forms three dimensional polymer networks, which can make the formed polymer stiffer. This implies that the bottom of hybrid layer could be a weaker point of the dentin-adhesive bond in the absence of sufficient bis-GMA.

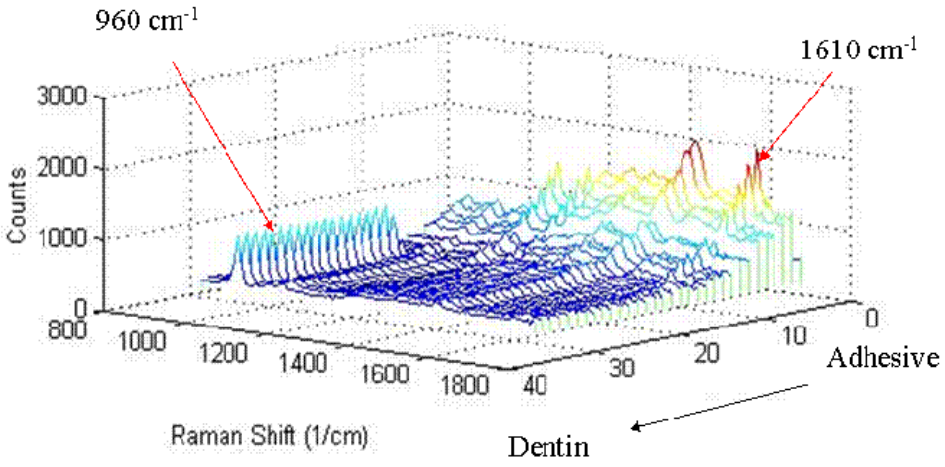

Figure 2: Raman spectra profile across hybrid layer

Quantitative Study

Because the acid-exposed collagen is Raman inactive, only the concentration of bis-GMA relative to the HEMA concentration was determined in the hybrid layer. To achieve this, a calibration curve of bis-GMA and HEMA was constructed the peak area ratio of 1610 cm^{-1} and 605 cm^{-1} (see Figure 3). Data points were obtained by mixing increasing amounts of bis-GMA in HEMA and photopolymerizing the homogeneous samples with a photoinitiator system containing 1wt% each of CQ and EDMAB.

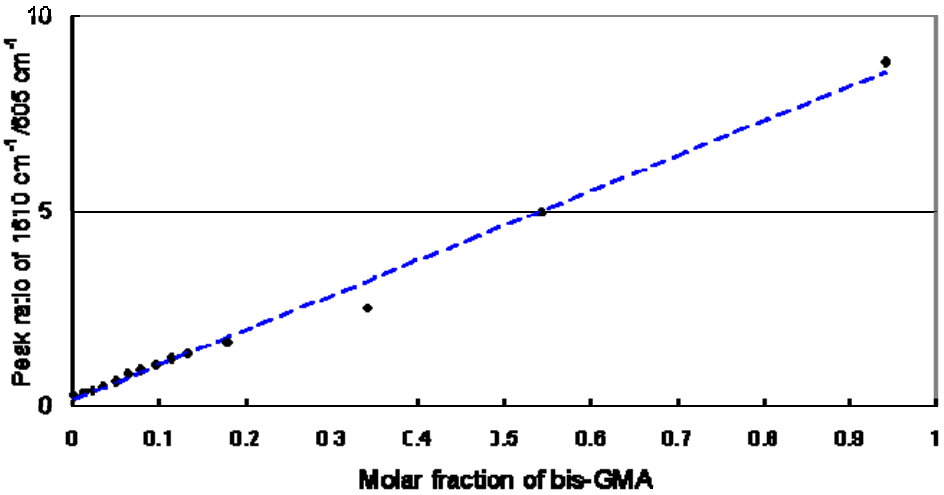

Figure 3. Calibration curve for bis-GMA and HEMA

Tooth specimens were tested using Raman microscopy across the hybrid layer at the following locations: in the adhesive resin, at the top of the hybrid layer, 1/4 into hybrid layer, at the center of the hybrid layer, 3/4 into hybrid layer, and at the bottom of the hybrid layer. For each specimen, three line scans were acquired across the hybrid layer. Acquired spectra were first processed using second derivative method to eliminate the effect of environmental and instrumental factors, and then the calibration curve was applied to determine the relative concentration of bis-GMA across the hybrid

layer. Since the intensity of Raman peaks at 1610 cm^{-1} and 605 cm^{-1} decreased dramatically past the center of the hybrid layer (see Figure 2), only data from first four locations are shown in Figure 4.

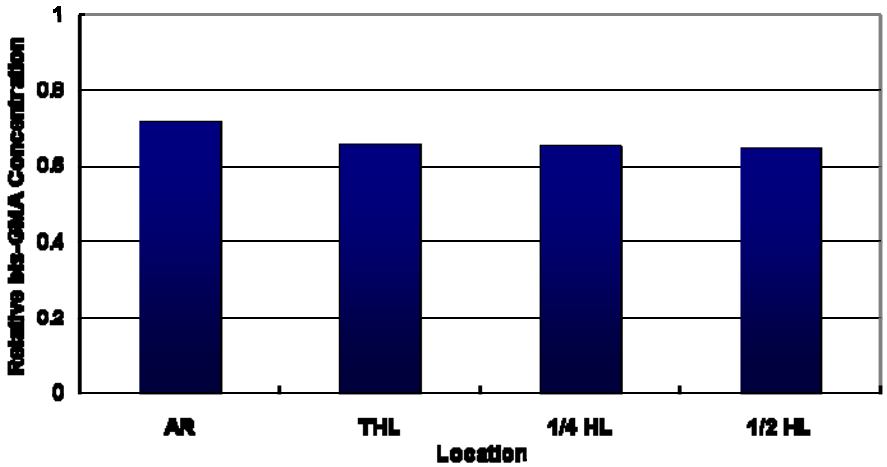

Figure 4. Relative bis-GMA concentration in hybrid layer for samples (24 hr water storage after bond preparation)

The relative concentration of bis-GMA decreased from 72wt% in the adhesive layer to 63wt% at the center of the hybrid layer. This information supports what was seen in the qualitative study: bis-GMA does not penetrate into hybrid layer as effectively as HEMA. Thus, the bottom of the hybrid layer is HEMA-rich and does not benefit from the cross-linked network provided by the bis-GMA. Since there are lots of small tubules traversed the dentin. Recent study also demonstrated that the dentin bonding agent may not able to prevent fluid moving from those tubules to adhesive interfaces.[11] This means the exposed HEMA polymer chains could be hydrolyzed by tubule fluids in the dentin.

Although the bis-GMA concentration decreased throughout the hybrid layer, a much lower relative bis-GMA concentration in the hybrid layer was expected than measured above. This can be explained by the elution of uncured monomer (mainly HEMA), which was detected in complementary GC/MS experiments, as a result of the method used to prepare the filled tooth specimen. The resulting depletion of HEMA in the sample leads to an artificially high measurement of the bis-GMA concentration. Further studies accounting for the Raman inactive collagen in the hybrid layer are underway, and these combined results will provide a better understanding of the bis-GMA distribution across the hybrid layer.

Conclusions

This study has demonstrated that Raman microscopy is an effective technique to obtain chemical information about the dentin-adhesive bond, both qualitatively and quantitatively. Since the bis-GMA molecules cannot penetrate as deeply into the hybrid layer as the HEMA molecules, HEMA-rich areas at the bottom of the hybrid layer result. These areas may contribute to the early failure of whole bonding seen in clinical applications. Future work includes determination of absolute concentrations of the adhesive components and other dental materials in the hybrid layer and promises to present a more complete picture of this bonding issue.

Acknowledgements

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References

1. Buonocore,M,G.; *J. Dent Research, 1955, 34(6), 849-53*

2. Nakabayashi, N.; Shika Zairyo Kikai*, 1982, 1,78-81*

3. De Munck, J.; Van Meerbeek, B.; Satoshi, I.; Vargas, M.; Yoshida Y.; Armstrong, S.; Lambrechts, P.; Vanherle, G. *Amer. J. Dent. 2003, 16, 414.*

4. Armstrong, S.; Vargas, A.; Qian, F.; Laffoon J. *J. Adhesive Dent. 2003, 5, 47.*

5. Ozturk, A. N.; Usumez, A.; *J. Oral Rehabilitation. 2004, 31(9), 905-910*

6. Ceballos, L.; Camejo, D. G.; Fuentes, M. V.; Osorio, R.; Toledano, M.; Carvalho, R. M.; Pashley, D. H.; *J. Dent 2003, 31(7), 469-477*

7. Hashimoto M; De Munck J; Ito S; Sano H; Kaga M; Oguchi H; Van Meerbeek B; Pashley D H.; *Biomaterials, 2004, 25(25), 5565-74*

8. Spencer, P.; Wang, Y.; *J. Biomedical Mat Res 2002, 62(3), 447-456*

9. Nyquist, A, *Interpreting infrared, Raman, and Nuclear Magnetic Resonance Spectra*, Vol.1&2, *Academic Press*, San Diego, 2001

10. Eliades, G; Vougiousklakis,G; Palaghias, G.; *Dent Mater 2001, 17, 277-283*

11. Elgalaid, T. O.; Youngson, C. C.; McHugh, S.; Hall, A. F.; Creanor, S. L.; Foye, R. H.; *J Dentistry. 2004, 32. (5), 413-421*