Chemical interaction of 4-META with enamel in resin-enamel bonds

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X-ray photoelectron spectroscopy (XPS) is used to analyze 4-META resin and enamel that are debonded at an adhesive interface. The XPS spectra showed two chemical states for Ca: one resulted from Ca of hydroxyapatite and the other, an unknown chemical state, suggested that Ca was chemically bonded with 4-META. We postulate that for a chemical reaction of 4-META and hydroxyapatite, the chemical structure of carboxyl groups will resemble that of calcium phthalate. Hence, calcium phthalate was used as a reference material. Additionally, the spectra obtained from the adhesive interface and the mixture of calcium phthalate with hydroxyapatite particles were compared using peak deconvolution analysis. XPS analysis revealed that the chemical bond of 4-META with enamel resembled the chemical state of Ca in calcium phthalate. Consequently, we suggest that Ca of the enamel and the carboxyl group of 4-META were chelate-bonded at the interface.

Keywords: Chemical reaction, Enamel, 4-META, Calcium phthalate, XPS

INTRODUCTION

Numerous studies have provided structural evidence of resin-tooth bonds using optical microscopy and scanning microscopy analyses. Microscopy analyses have clarified the structural information concerning the hybrid layer, micro resin tag structure, nanoleakage pattern, and water tree or bubble formation. A large part of this adhesion is thought to be attributable to mechanical retention.

Xu et al. reported a chemical interaction between 2-hydroxyethyl methacrylate (HEMA) and collagen through either hydrogen bonding or the formation of a new bond to the ester group of HEMA using micro-Raman analysis. However, Fourier transform infrared spectroscopy (FT-IR) has also been conducted for resin-tooth bonds and has shown conformational changes of dentin type I collagen. Some transmission electron microscopy (TEM) studies have identified the deposition of an amorphous phase on top of the hybrid layer created by the resin adhesive or resin-modified glass ionomer cement. This amorphous phase contains a polyalkenoic acid co-polymer component owing to the precipitation of polycarboxylate salts on the dentin surface, which yields calcium through a chemical reaction. Although such studies have provided morphological evidence of bonding around the adhesive joint, the exact mechanism of the chemical reaction remains unclear.

X-ray photoelectron spectroscopy (XPS) is a candidate to clarify the chemical reaction between an adhesive resin and a tooth. Although some XPS studies have been performed on the chemical interaction between phosphoric acid esters or carboxylic acids and hydroxyapatite, the chemical reaction has not been elucidated in detail. This study is the first to evaluate the adhesion of commercially available 4-META resin to enamel using XPS. Here, we study the adhesion interface of 4-META resin to bovine enamel using XPS. From the chemical reaction of 4-META and hydroxyapatite, we postulate that the chemical structure of the two carboxyl groups will resemble that of calcium phthalate. Hence, calcium phthalate was used as a reference material. Additionally, the spectra obtained from the adhesive interface and the mixture of calcium phthalate with hydroxyapatite particles were compared using peak deconvolution analysis. XPS analysis revealed that the chemical bond of 4-META with enamel resembled the chemical state of Ca in calcium phthalate. Consequently, we suggest that Ca of the enamel and the carboxyl group of 4-META were chelate-bonded at the interface.

MATERIALS AND METHODS

Synthesis of calcium phthalate as a model for hydroxyapatite reacting with 4-META

We synthesized calcium phthalate as follows. A mixture of 20 g of phthalic acid (Wako Chemicals, Osaka, Japan) and 0.89 g of calcium hydroxide (Wako Chemicals) was dissolved into 200 mL of water at 75°C. The solution was stirred for 2 h at 75°C and the solution was then cooled to 40°C. The precipitates were discarded by filtering to remove the excess phthalic acid. The filtrate was cooled to room temperature. Subsequently, the precipitates were filtered and rinsed with 100°C distilled water. Phthalate was dissolved into water at 75°C and hydroxyapatite particles (Wako Chemicals) were added to synthesize calcium phthalate. Crystal phases of the specimens were identified using an XRD powder diffractometer (Rint-2000, Rigaku, Tokyo, Japan).
Specimen preparation of fractured surface of resin-enamel bonds
A bovine tooth was used to prepare a flat 10-mm-diameter specimen for XPS. Roots were removed and crowns were sectioned mesiodistally using a low-speed diamond saw (Isomet, Beuhler, Lake Bluff, IL, USA) under water cooling. To eliminate any mechanical factor on the adhesion interface, the enamel surface was polished to a mirror-like finish using silicon carbide paper (#240, 400, and 600) followed by alumina slurry (3.0 and 0.05 μm). The specimens were water-rinsed and air-dried. The enamel surfaces were covered with a 6-mm-inner-diameter and 0.25-mm-thick adhesive tape to restrict the bonding area to the prepared enamel. Subsequently, a 4-META/MMA-TBB resin (Super Bond C&B, Sun Medical, Moriyama, Japan) was applied to the enamel bonding substrate according to the manufacturer’s instructions. The chemical composition of the adhesive resin is shown in Table 1. The specimens were kept in a desiccator at 37°C for 24 h and immersed in 37°C water for 24 h (n=3). After this, the bonded specimens were subjected to 20 thermal cycles from liquid nitrogen (−196°C) to water (40°C). Because thermal stress was applied at the adhesion interface, owing to the difference between the thermal expansion coefficients of the resin (80×10−6/°C) and enamel (11.4×10−6/°C), the resin-enamel bonded specimens became debonded at the adhesion interface\(^1\). The resin side of the debonded interface was investigated using XPS.

Table 1  Chemical formulation of the adhesive resin (4-META/MMA-TBB)

| Adhesive resin | Components |
|----------------|------------|
| Liquid         | 4-META, MMA, poly-functional methacrylate |
| Powder         | PMMA, TMPT, filler, pigment |
| Catalyst       | TBB, TBB-O, hydrocarbon |

4-META: 4-methacryloxyethyl trimellitate anhydride, MMA: methyl methacrylate, PMMA: polymethyl methacrylate, TMPT: trimethylolpropane trimethacrylate, TBB: Tri-n-butylborane, TBB-O: partially oxidized tri-n-butylborane

XPS analysis
The debonded surfaces were examined using XPS (ESCA-850, Shimadzu, Kyoto, Japan) with Al Kα irradiation (1.486.6 eV) at 8 kV and 30 mA under a pressure of 5.0×10−7 Pa. The specimen surface was subjected to argon ion sputtering for 0.1 nm/s at 2 kV and 20 mA under a pressure of 5.0×10−4 Pa to remove the surface contaminator in the XPS spectrometer. An argon ion sputtering rate of 0.1 nm/s was used to determine the chemical compositions of the surface and subsurface layers. The binding energy of the measured photoelectron peaks was calibrated by the C 1s peak of hydrocarbon contamination at a binding energy of 285.0 eV. To clarify the changes in the calcium chemical states (chemical shifts), the data analysis system in the XPS...
spectrometer was used to separate the calcium states in the Ca 2p spectra by assuming that the spectra could be approximated using a Gaussian function.

RESULTS

Figure 2 shows the Ca 2p spectra obtained from the resin-side specimen of the 4-META resin debonded from the adhesive interface of bovine enamel. Spectra with no argon ion etching (Fig. 2(a)) and with argon ion etching for 1.2 min (Fig. 2(b)), 2.6 min (Fig. 2(c)), and 3.8 min (Fig. 2(d)) are shown. The spectrum without argon ion etching clearly showed two peaks (Fig. 2(a)). With increasing the argon ion etching time, the spectrum changed to a broad spectrum with a large half-width value, the two peaks disappeared, and another chemical state appeared on the lower binding energy side.

Figure 3 shows the Ca 2p spectra of the reference materials of hydroxyapatite (Fig. 3(a)), calcium phthalate (Fig. 3(b)), mixture components of 50 mass% hydroxyapatite and 50 mass% calcium phthalate (Fig. 3(c)), and the adhesive interface of the enamel with 4-META resin shown in Fig. 2(c) (Fig. 3(d)). The recorded spectrum without argon-ion etching shown in Fig. 2(a) was almost the same as that of hydroxyapatite in Fig. 3(a). As shown in Fig. 3(a) and (b), the binding energies of Ca 2p for hydroxyapatite and calcium phthalate were 349.4 eV and 347.2 eV, which showed that the chemical shift was 2.2 eV. Figure 3(c) shows a broad spectrum that resulted from the mixture of the two chemical states, hydroxyapatite and calcium phthalate. The Ca 2p spectrum obtained from the fractured surface on the resin side at an etching time of 2.6 min shown in Fig. 2(c) (red line) also appeared in Fig. 3(d), which implied a mixture of some chemical states.

Figure 4 shows the separated spectra of the Ca 2p spectra shown in Fig. 3(c) and (d). Figure 4(a) (orange line) shows the Ca 2p spectrum separated into the two components indicated by the black thin solid (chemical state I) and blue lines (II) in Fig. 3(c). The binding

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**Fig. 3** Ca 2p spectrum obtained from (a) the standard specimen of hydroxyapatite, HAp, (b) calcium phthalate, (c) a mixture (1:1) of hydroxyapatite and calcium phthalate, Ca-phthalate, and (d) the adhesive interface enamel with 4-MET resin. The spectrum shown in Fig. 3 (d) is the same as that in Fig. 2(c).

**Fig. 4** (a) Separated spectra of the Ca 2p spectrum obtained from the mixture (1:1) of hydroxyapatite and calcium phthalate shown in Fig. 3(c), which indicates two chemical states, I (Ca-phthalate) and II (HAp); (b) separated spectra of the Ca 2p spectrum obtained from the adhesive interface of enamel with 4-META resin shown in Fig. 3(d). The spectrum (red line) in Fig. 4 (b) separates the two chemical states, I and II.
Table 2 Binding energy (eV) of Ca 2p$^{3/2}$ and chemical shifts

| Materials and specimen | Binding energy (eV) of Ca 2p$^{3/2}$ | Chemical shifts (eV) |
|------------------------|---------------------------------------|---------------------|
| Hydroxyapatite (HAp)   | 349.4                                 | 2.2 (349.4–347.2)   |
| Calcium phthalate (Ca-ph) | 347.2                               |                     |
| Mixture of hydroxyapatite and calcium phthalate (1:1) | HAp 348.9 | 2.1 (348.9–346.8) |
|                        | Ca-ph 346.8                           |                     |
| Adhesive interface of enamel with 4-MET resin | State II 349.2 | 2.1 (349.2–347.1) |
|                        | State I 347.1                          |                     |

Binding energy (eV) of Ca 2p$^{3/2}$ spectrum, obtained from the standard specimen of hydroxyapatite (HAp), calcium phthalate (Ca-phthalate), obtained from mixture (1:1) of hydroxyapatite and calcium phthalate, and chemical states (I and II) of adhesive interface enamel with 4-MET resin (Fig. 4).

DISCUSSION

A gel layer, which is a chemical reaction layer, can be observed using TEM in the upper part of the hybrid layer at the resin-dentin interface formed by a bonding resin system containing polycarboxylic acid$^{11,12}$. However, because the layer is fragmentarily formed on the upper part of the hybrid layer, it is difficult to analyze the spots on the chemical reaction layer from the sagittal plane. Because of the difficulties for morphological analysis to demonstrate chemical bonding, several analytical tools, such as micro-Raman or FT-IR, have been considered for detecting the chemical reaction. A study has shown that 4-META molecules are hydrolyzed into 4-methacryloyloxethyl trimellitate (4-MET) molecules within the resin-dentin bonds created by a 4-META resin adhesive$^{17}$. However, the chemical bonds between calcium and the carboxyl group have not been detected, even using Raman analysis. In most cases, the distribution of the monomer-permeating resin in the collagen layer is the main result of this type of research$^{18,20}$. However, the chemical reaction with the resin-tooth bonds has rarely been captured.

Analysis of the resin-tooth adhesive interface by scanning electron microscopy (SEM) or TEM are the most popular methods to observe the adhesive interface. For interfacial analysis, an FT-IR resolution greater than 10 μm cannot be used to evaluate a few micrometers of resin-dentin bonds or more than a thin layer of resin-enamel bonds. A resolution limit of approximately a few microns for Raman analysis enables evaluation of the hybrid layer in the case of etch-and-rinse adhesives. However, it has been observed that the analysis of the hybrid layer with a self-etching adhesive or bond structure of the resin-enamel bonds has revealed little, even when using micro-Raman analysis.

SEM is routinely used to observe the fractured surface. However, if Raman or FT-IR is used to analyze fractured surfaces, it detects the information from underlying substance to some depth from the top fractured surface. Conversely, the ultra-short sampling depth (0.5 to 5 nm) of XPS can capture data between the two materials on a thin layer to the order of an atom within the fractured surfaces. Therefore, it is possible to determine the chemical reaction layer inside debonded surface samples using XPS. Additionally, comparison of reference materials and a chemical reaction substance is a novel idea put forth in this study.

Although COO$^-$ chemical ionic adhesion to Ca$^{2+}$ in terms of polyalkenoic acids of glass-ionomer cement is well known, clear evidence of this type of bond for a resin monomer and tooth substrate remains unclear. Yoshida et al.$^{14}$ provided direct evidence of chemical bonding between the functional groups of polyalkenoic acids bonded to the calcium of hydroxyapatite using XPS. The chemical bonding of COO$^-$ to Ca$^{2+}$ was confirmed using XPS, which showed that the peak at 288.6 eV shifted to 288.2 eV. Furthermore, a similar shift has also been shown to occur when 4-META reacts with enamel$^{15}$. The shift in the carboxyl peak to a lower binding energy (288.6 eV to 288.2 eV) suggests the formation of ionic bonds between the carboxyl groups of the acids or monomers with the Ca of the enamel or dentin. Some studies$^{21,22}$ have shown chemical bonds between 10-methacryloyloxycetyl dihydrogen phosphate (MDP) and flat enamel using XPS. The narrow-scan spectra show a peak at 288.6 eV that represents -COO- binding, which suggests the existence of chemical bonding.

In this study, a spectrum similar to that obtained for a mixture of calcium phthalate and hydroxyapatite was found within the debonded surface of the resin-enamel interface (Figs. 2(c) and 5(d)). Their similarities were verified by waveform separation (Fig. 4). As shown
in Table 2, the chemical states of I and II obtained from the adhesion interface between 4-META and enamel coincided with those of peaks I and II, which were obtained from the mixture of hydroxyapatite and calcium phthalate. From the above, the origins of the Ca chemical states shown in Fig. 3(c) could be concluded as follows. State I arises from Ca bonding with 4-META and state II arises from hydroxyapatite, that is, the chemical bond of 4-META with hydroxyapatite forms a chelate compound.

The depth information that can be evaluated using XPS is in the region of several nanometers from the top of the fractured surface. If the remaining fractured resin over the chemical reaction layer is thick, surface sputtering can be performed using argon ion sputtering. From the spectral information obtained by alternately repeating sputtering and measuring, it is possible to obtain a depth profile for the elemental composition or chemical bonding state, as shown in Fig. 2. Even when there is no chemical reaction layer on the top surface, there is an advantage in that it can be detected using sputtering. There are reports with XPS that the chemical state is rarely changed depending on the specimen because of argon ion sputtering\textsuperscript{23,24}. Although the data are not shown in this study, no chemical changes were observed with hydroxyapatite and calcium phthalate because of argon ion sputtering in a preparatory experiment. The experimental methods and results of this study differ greatly from those of previous XPS studies\textsuperscript{18} and there have been no similar research reports. As similar chemical phenomena have now been found for human enamel and dentin with 4-META resin, these results will be discussed in future papers.

CONCLUSION

The XPS spectra showed two chemical states for Ca: one was derived from Ca of hydroxyapatite and one was an unknown chemical state, which suggested that the Ca was chemically bonded to 4-META. We used calcium phthalate as the chemical bond model. The Ca 2p spectrum of the adhesive interface was similar to that obtained for the mixture of calcium phthalate and hydroxyapatite. The unknown spectrum obtained from the adhesive interface resembled that for Ca of calcium phthalate. This suggested that the Ca of bovine enamel and the carboxyl group of 4-META were chelate-bonded at the resin-enamel interface.

ACKNOWLEDGMENTS

The authors thank Edanz Group (https://en-author-services.edanzgroup.com/ac) for editing a draft of this manuscript.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with the publication of this study.

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