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Fluorine analysis of human dentin surrounding resin composite after fluoride application by μ -PIGE/PIXE analysis

Katsushi Okuyama^{a,b}, Hisanori Komatsu^a, Hiroko Yamamoto^c, Patricia N R Pereira^b, Ana K. Bedran-Russo^d, Masaharu Nomachi^e, Takahiro Sato^f, Hidehiko Sano^a

^a Graduate School of Dental Medicine, Hokkaido University, Department of Restorative Dentistry, Kita-13, Nishi-7, Kita-ku, Hokkaido, Sapporo 060-8586, Japan;

^b School of Dentistry, University of North Carolina, Department of Operative Dentistry, 302 Brauer, CB#7450, Chapel Hill, North Carolina 27599-7450, United States;

^c Graduate School of Dentistry, Osaka University, 1-8 Yamada-Oka, Osaka, Suita 565-0871, Japan;

^d University of Illinois at Chicago, College of Dentistry, Department of Restorative Dentistry, 801 S. Paulina St., Chicago, Illinois 60612, United States;

^e Graduate School of Science, Osaka University, 1-1 Machikaneyama, Osaka, Toyonaka 560-0043, Japan;

^f TARRI, JAEA, Advanced Radiation Technology, 1233 Watanuki-machi, Gunma, Takasaki 370-1292, Japan.

Abstract

The use of fluoride for the prevention of caries is based on the transformation of hydroxylapatite to fluoroapatite in the presence of fluoride ions, thereby strengthening tooth structure. Adhesion of dentin and resin composite (tooth-colored restoration

material) requires a dentin bonding system, since resin composite is not able to adhere to dentin directly. Demineralization of dentin by acid etching is an important step in the dentin bonding system; however, demineralization also introduces weaknesses in tooth structure. If the demineralized dentin could be strengthened by the application of fluoride, then the dentin-resin composite bond strength might also improve. To test this hypothesis, the present study evaluated the influence of fluoride applications on the strength of the dentin–resin composite bond by (1) tensile strength testing analyses, (2) SEM analyses of tooth structure, and (3) detection of calcium (Ca) and fluorine (F) distribution patterns by micro proton-induced X-ray emission (μ -PIXE) and micro proton-induced gamma emission (μ -PIGE) analyses, conducted at the Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) at the Takasaki Advanced Radiation Research Institute (TARRI).

In this study, the dentin in extracted human molars was exposed by grinding and the dentin was etched with 35% phosphoric acid. Fluoride was applied at two concentrations, 0.022% (100 ppmF) and 2.21% (10,000 ppmF) NaF solution, for two time periods, 30 s and 60 s, prior to bonding the resin composite with the treated dentin.

Controls were prepared in the same manner, but without the fluoride application. Bond strength was measured with a micro-tensile testing unit, and the fluorine and calcium distributions at the interface between dentin and resin composite were detected by μ -PIGE and μ -PIXE analysis, respectively.

Results indicate that the 10,000 ppmF applications resulted in higher bond strengths than observed in either the 100 ppmF applications or the control group. In addition, PIGE analyses showed high concentrations of fluorine in the hybrid bonding layer of the 10,000 ppmF samples, suggesting that the fluorine contributes to the strength of the dentin–resin composite bond. Detection of fluoroapatite within the hybrid bonding layer suggests that bond strength involves remineralization processes.

INTRODUCTION

The use of fluoride for the prevention of caries is based on the transformation of hydroxylapatite to fluoroapatite in the presence of fluorine, and the resulting strengthening of tooth matrix [1]. Fluoride supplements have been used for many years, applied by a variety of methods including drinking water fluoridation, fluoride

mouth rinses, topical applications to tooth surfaces, and the use of fluoride dentifrices [2-4]. Several studies have reported that fluoride applications cause remineralization of dentin surrounding cavity walls [5-8], as well as the inhibition of secondary caries [5-10]. Thus, fluoride contributes to dental hygiene by improving tooth structure and by inhibiting primary and secondary caries.

Adhesion of resin composite (tooth-colored restoration compounds) to dentin requires special bonding techniques because resin composite does not directly adhere to dentin. Bonding is facilitated by the demineralization of dentin via acid etching, leaving the collagen matrix of the tooth. After demineralization, a dilute resin with adhesive qualities is applied to the dentin surface. The adhesive resin penetrates the collagen matrix of the dentin, forming an intercalated layer of resin–collagen in the demineralized area. This layer is called the “hybrid layer” [11]. Itota et al. [12] reported that the application of fluoride to demineralized dentin surrounding artificial caries improved the bond strength between dentin and resin composite. However, the existence of fluorine within the hybrid interface between dentin and resin composite was not reported.

The goal of this study was to evaluate the influence of fluoride applications on the bond strength between dentin and resin composite. Proton-induced gamma emission (PIGE) analysis permits the detection of fluorine uptake into the enamel surrounding fluoride-containing dental compounds during the pH cycling process [13, 14]. A PIGE technique for teeth sample was developed at the Japan Atomic Energy Research Institute (JAERI) [15-18] to detect fluorine in dentin and in resin composites; the F distributions observed for a range of conditions surrounding were previously reported [19-23]. Analyses were conducted at the Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) at the Takasaki Advanced Radiation Research Institute (TARRI).

MATERIALS AND METHODS

1. Analysis of bond strength between dentin and resin composite

Analyses of bond strength were performed on extracted human molars, stored at 4°C in 0.5% thymol in distilled water. The occlusal enamel and pulp tissue were removed, and the occlusal surface was ground with 600 grit SiC paper under running

water to expose dentin. All dentin surfaces were etched with 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE) for 15 seconds and then rinsed with water for 10 seconds. The samples were immersed in 0.022% (100 ppmF) or 2.21% (10,000 ppmF) NaF solution for 30 or 60 seconds, and then rinsed with distilled water for 10 seconds. The dentin was then blot dried with absorbent paper, leaving a moist surface. After fluoride treatment, Scotchbond Etchant (3M ESPE) was applied twice to the dentin surface with a micro-brush, gently air-dried (oil-free air), and then light-cured for 10 seconds using a light curing unit (Astralis 5; Vivadent Ets, Schaan, Liechtenstein). Finally, three layers of a Z100 resin composite (3M ESPE) were built up to a height of 4–5 mm; each layer was light-cured for 40 seconds prior to the application of the next layer. Specimens were stored in distilled water at 37°C for 24 hours prior to testing. Controls were prepared in the same manner, but without the fluoride treatment.

Samples were sectioned in a direction parallel to the long axis of the tooth into 4–6 slabs, (0.70 ± 0.05) mm thick, using a low speed diamond saw (Isomet; Buehler, Lake Bluff, IL, USA) under water irrigation. The slices were trimmed with a diamond bur under water, yielding a surface area of (1.0 ± 0.1) mm² at the interface between

dentin and resin composite. Specimens were fixed to a Ciocchi's jig with cyanoacrylate cement (Loctite Super Glue; Henkel, Avon, OH, USA) and tested for tensile strength using a desktop micro-tensile testing unit (EZ-Test; Shimadzu Co., Kyoto, Japan) at a cross-head speed of 1 mm/min. The micro-tensile bond strength (MTBS), expressed in megapascals (MPa), was calculated as the maximum load at failure (kgf) divided by the cross-sectional area of the surface (mm²).

2. SEM observations and measurements of hybrid layer thickness

Hybrid layer thickness was determined by scanning electron microscope (SEM) observations, for an additional set of tooth samples. First, the dentin surface was exposed and ground flat. After acid etching, some of the specimens were stored in fluoride solution, followed by the application of the bonding agent on the dentin surface. Next, resin composite was built up on the dentin surface, followed by sectioning in a direction parallel to the long axis of the tooth. After trimming, the specimens were embedded in epoxy resin, polished with 1,200 grit SiC paper, and then polished with a diamond paste with 1 µm particle size. The polished specimens were treated with 10%

phosphoric acid for 3–5 seconds, followed by immersion in 5% sodium hypochlorite for 5 minutes to enhance the hybrid layer. The specimens were gold sputter-coated and examined by SEM (JOEL-6300). The thickness of the hybrid layer was determined as the distance between resin tags, measured from SEM photos (Fig. 1). All MTBS and hybrid layer thickness data were subject to one-way ANOVA analysis; multiple comparisons were performed by Fisher's PLSD test ($p < 0.05$).

3. Fluorine analyses at the interface between dentin and resin composite

As previously reported [14, 15], a 1.7 MeV proton beam, accelerated by the TIARA single-ended accelerator at TARRI, was captured from an ion micro-beam apparatus. Specimens were attached directly to the window at the end of the micro-beam system [18] and bombarded in ambient air conditions. The beam spot diameter was about 1 μm , and the beam current, about 100 pA. The maximum scanned area was 1 mm $\times$ 1 mm.

Fluorine concentrations were measured with PIGE by the nuclear reaction $^{19}\text{F}(p, \alpha \gamma)^{16}\text{O}$; gamma-rays were detected with a 4 inch (10.16 cm) NaI detector located 5

mm behind the sample. Calcium concentrations were measured with proton-induced X-ray emission (PIXE), which was simultaneously detected with a Si(Li) detector placed in vacuum [18]. Beam intensity was determined by the X-ray yield from a copper foil [19]. Samples for F and Ca analyses were prepared in the same manner as for SEM observations prior to embedding the specimens in epoxy resin. Fluorine and Ca concentrations were measured in $400\ \mu\text{m} \times 400\ \mu\text{m}$ sampling areas, with data converted to a graphical resolution of 128×128 pixels [18]. We used the analysis program “PIXEana” for conversion PIXE raw data to concentration [18].

RESULTS

MTBS results are presented in Fig. 2: 10,000 ppmF samples (10,000 ppmF-30 seconds, 10,000 ppmF-60 seconds) showed higher bond strengths than samples exposed to lower F concentrations. The thickness of the hybrid layer showed no significant correlations with either fluoride concentration or the duration of fluoride immersion (Fig. 3).

Fluorine and Ca distributions in the dentin, hybrid, and resin composite layers

are presented in Figs. 4–6. Calcium concentrations were highest in dentin, intermediate in the hybrid layer, and lowest in the resin composite. Fluorine distributions varied with the concentration of applied fluoride. Fluorine was nearly absent in the control group (Fig. 4). In the 100 ppmF samples, F concentrations were low in all areas (dentin, hybrid layer, and resin composite) (Fig. 5). In the 10,000 ppmF samples, fluorine concentrations in the dentin and resin composite were the same as in the 100 ppmF samples. In the hybrid layer, however, fluorine concentrations in the 10,000 ppmF samples were higher than those in the 100ppmF samples (Fig. 6).

DISCUSSION

The bonding of dentin and resin composite requires initial demineralization of the dentin surface by phosphoric acid. Acid etching removes the smear layer and demineralizes the underlying dentin matrix, exposing the tubule apertures, the collagen fibrils, and the interfibrillar spaces. In the bonding process, adhesive resin monomers diffuse into the etched dentin, and polymerization of these monomers results in the formation of a hybrid layer.

Fluoride applications increase the adhesion of resin composites to dentin. However, the increased adhesion is apparently unrelated to the thickness of the hybrid layer, which showed no significant correlation to the concentration of applied fluoride. The concentration of applied fluoride also had no effect on the structure of the hybrid layer or on the infiltration patterns of resin into the demineralized area.

Strong bonding between dentin and resin composite depends on the thorough penetration of adhesive into the dentin substrate [24], facilitated by demineralization by phosphoric acid etching. The application of fluoride to demineralized dentin does not appear to impede the penetration of adhesive.

Applications of high concentrations of fluoride (10,000 ppmF) resulted in higher bond strengths than those observed in control groups. Bond strength is apparently unrelated to fluorine levels in the dentin, which remained low in both low- and high-concentration fluoride applications. It is possible that fluorine in dentin is removed by post-fluoride rinsing, but the details of this process are unknown.

High bond strengths between dentin and resin are correlated with high concentrations of fluorine in the hybrid layer. Because high concentration of fluorine

was detected into hybrid layer, we guessed deposits of fluoroapatite in the collagen network of the hybrid layer; this remineralization of dentin occurs by precipitation onto residual crystals [25]. Thus, the hybrid layer might be reinforced by the remineralization process. Our results indicate that bond strengths between dentin and resin depend on the threshold levels of applied fluoride, since the MTBS was significantly greater in 10,000 ppmF samples than in both the 100 ppmF and control samples.

Itota et al. [12] reported that fluoride ions were detected on artificially demineralized dentin (caries dentin); the fluoride was introduced without a post-application rinse, by means of EDS. However, their research indicated that dentin-resin bond strength did not increase when the fluoride application was followed by water rinsing; our results do not conform with their findings, probably because of differences in (1) the substrate used for the bonding tests, and (2) the type of etching that preceded the application of adhesive. Itota et al. applied a self-etching primer to artificial caries, whereas in our research protocol, a more aggressive total-etching technique (using phosphoric acid) was applied to undeteriorated dentin. Because

phosphoric acid has a lower pH than self-etching primer, phosphoric acid penetrates the dentin more deeply and causes greater demineralization of dentin as compared with self-etching primer. Thus, the total-etching technique affords a greater region for adhesive bonding, as well as structural strengthening due the crystallization of fluoroapatite within the hybrid bonding layer.

CONCLUSION

The results of this study indicate that high concentrations of topical fluoride applied to demineralized dentin after etching improve the bond strength between dentin and resin composite. μ -PIGE analyses detected enhanced fluorine levels on the interface between dentin and resin composite (hybrid layer), suggesting that fluorine (fluoroapatite) enhances structural integrity and bond strength. The research clarifies the relationships between topical fluoride applications, fluorine uptake in dentin and resin composite compounds, and the resulting strengthening of dentin–resin composite bonds.

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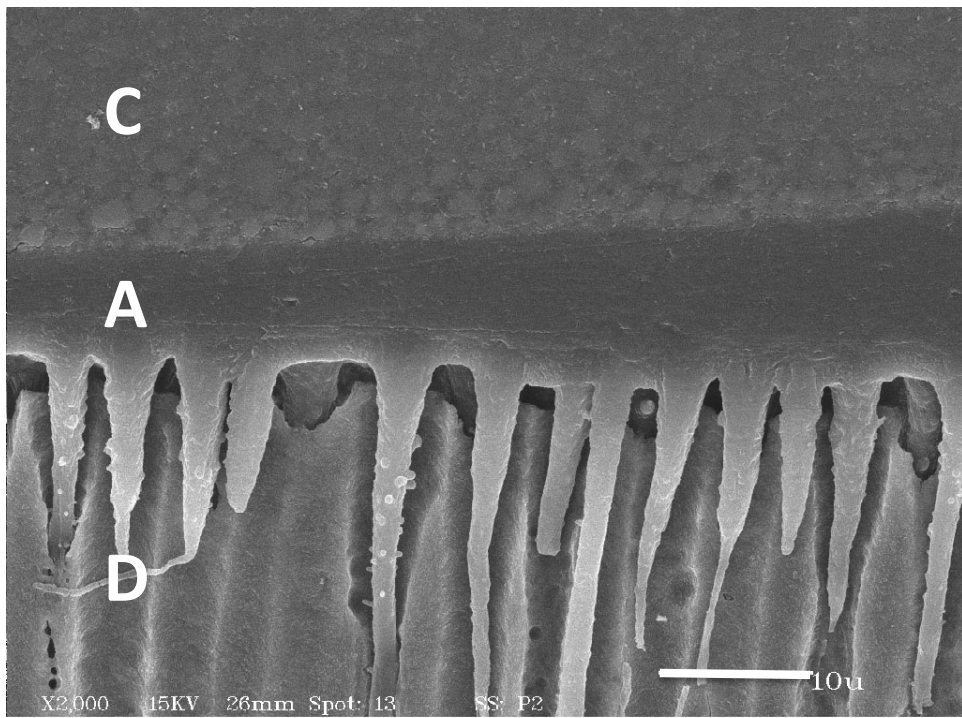
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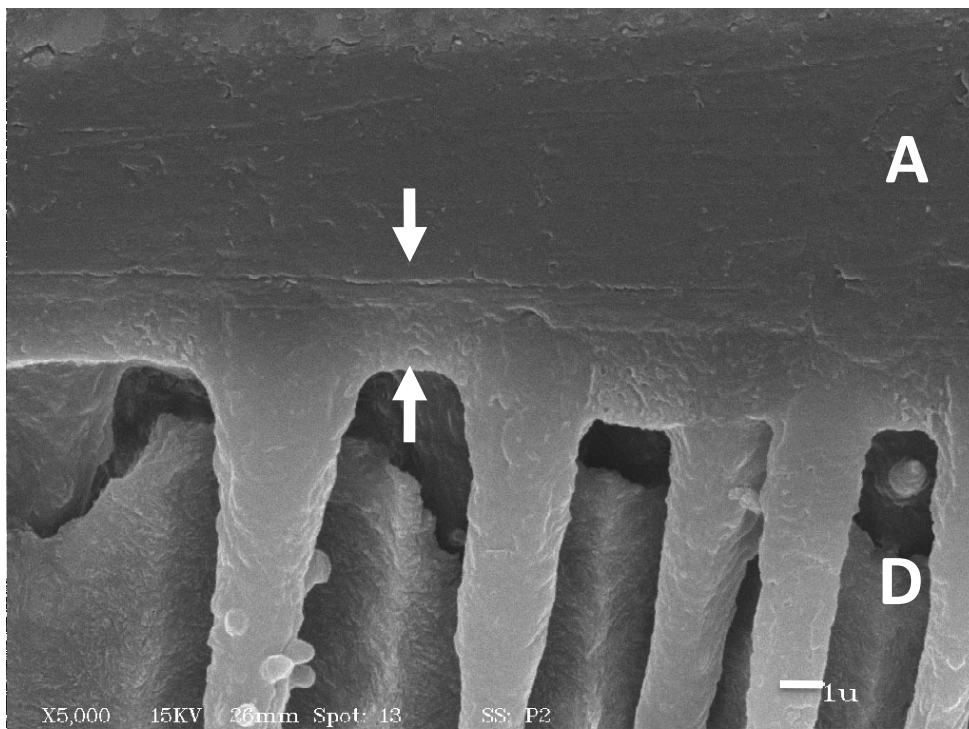
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