An in vivo evaluation of bonding ability of comprehensive antibacterial adhesive system incorporating MDPB

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ABSTRACT

Objectives. This study examined the in vivo bonding ability to sound dentin of antibacterial adhesive systems incorporating an antibacterial monomer MDPB based on morphological evaluation of the resin–dentin interface.

Methods. Class V cavities were prepared on the buccal surfaces of the teeth of a beagle dog and a composite filling performed using (1) commercial self-etching system Liner Bond 2 (LB primer + LB bond), (2) experimental primer containing 5% MDPB and LB bond, (3) LB primer and experimental bonding-resin containing 2.5% MDPB, or (4) combination of experimental primer and bonding-resin. After 7 days, the tooth crown was cut and fixed in half-Karnovsky’s solution, and the sectioned surface observed under scanning electron microscopy (SEM) after treatment with phosphoric acid and NaOCl. The ultrastructure of the bonding interface was also examined by transmission electron microscopy (TEM). Microtensile bond strengths (μTBS) of each group were measured using extracted teeth.

Results. SEM demonstrated that all groups produced a 1–2 μm thick hybrid layer with funnel shaped resin tags, although the length of tags was shorter for the group in which MDPB-containing bonding-resin was used. TEM examination supported good adhesion of the comprehensive adhesive system employing MDPB-containing primer/bonding-resin, showing integrity between resin and dentin. There were no significant differences in μTBS among the four groups tested (p > 0.05, ANOVA).

Significance. This study confirmed that the experimental antibacterial adhesive systems employing MDPB-containing primer and/or bonding-resin could produce an effective bond under in vivo conditions.

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1. Introduction

The challenge in the clinical treatment of dentinal caries is the absence of a universally acceptable regime for the diagnosis of carious lesions. Active bacteria may inadvertently be left behind by incomplete caries removal. Thus, restorative materials that exhibit antibacterial activity are useful for eliminating the harmful effects caused either by residual bacteria or bacterial microleakage.

The authors have previously reported that the incorporation of an antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB) was effective in providing...
a dentin primer and bonding-resin with antibacterial activity [1–4]. The uncured dentin primer incorporating MDPB demonstrates a bactericidal effect based on the strong antibacterial activity of unpolymerized MDPB [1,3,5]. The ability of this monomer to kill residual bacteria in the prepared cavity has been also reported [6]. Bonding-resin containing MDPB could inhibit the growth of bacteria on its surface by means of the action of an immobilized bactericide after the resin is polymerized, without adversely affecting its bonding characteristics [4]. Therefore, it is considered that the use of a comprehensive antibacterial adhesive system that employs MDPB-containing primer and bonding-resin should be highly effective in achieving the successful restorative treatment of caries. These favorable in vitro results await in vivo confirmation. Thus, the purpose of this study was to investigate the bonding ability of an antibacterial adhesive system incorporating MDPB in vivo by morphological evaluation of bonding interface using a beagle dog model. The microtensile bond strength of an experimental antibacterial adhesive system was also measured under in vitro conditions. The null hypothesis tested was that there is no difference in the bonding characteristics of MDPB-containing primer/bonding-resin in vitro or in vivo.

2. Materials and methods

2.1. Adhesive systems

An experimental adhesive system was prepared by the addition of the antibacterial monomer MDPB to a commercial product (Clearfil Liner Bond 2, Kuraray Medical Inc., Tokyo, Japan) consisting of two-liquid type self-etching primer (LB primer) and a one-bottle bonding-resin (LB bond). For the experimental primer, 10% (w/w) of MDPB was added to the B-liquid of LB primer to give 5% (w/w) after mixing with A-liquid. The experimental bonding-resin was prepared by incorporating 2.5% (w/w) MDPB into LB bond and used in combination with the MDPB-containing primer (Table 1).

2.2. In vivo bonding procedures

A beagle dog (female, 13 months old, weight 10 kg) was housed in the Osaka University Animal Facility, and used according to the protocol approved by the ethical guidelines for animal care at Osaka University. The dog was subjected to general anesthesia by intramuscular injection of 30 mg/kg ketamine and intravenous injection of 10 mg/kg sodium pentobarbital. The teeth were cleaned with 3% hydrogen peroxide and 5% tincture of iodine. Class V cavities (3 mm - 4 mm, 1 mm depth) were prepared on the buccal surfaces of molars, canines or incisors using a high-speed diamond bur (D1, Shofu, Kyoto, Japan) under water spray. The cavosurface margin was but-jointed and surrounded by enamel. The cavities were then treated in four different ways:

Group 1 (control): The cavity was treated with Clearfil Liner Bond 2 according to the manufacturer’s instruction. A- and B-liquids of LB primer were mixed and applied to the cavity for 30 s. After drying with a gentle stream of air, LB bond was applied and cured with a light-activation unit (Quick Light, Morita, Kyoto, Japan) for 20 s. Then, a flowable composite (Clearfil Protect Liner F, Kuraray Medical Inc.) was placed in the cavity and light-cured for 40 s. The excess material beyond the cavosurface margin was removed with a finishing point (#60, Shofu).

Group 2: The cavity was restored in the same manner as group 1 using the experimental primer instead of LB primer.

Group 3: The cavity was restored using the control primer and the experimental bonding-resin.

Group 4: The cavity was restored in the same manner as the above three groups using the experimental primer and the experimental bonding-resin.

The animal was sacrificed after 7 days. Crowns of the restored teeth were cut and immersed in half-Karnovsky’s solution (pH 7.4) for 5 h at 4°C. The bonding interfaces were examined by scanning electron microscopy (SEM) or transmission electron microscopy (TEM).

2.3. SEM examination

The specimens were sectioned longitudinally through the center of the restoration using a slow-speed saw equipped with a diamond-impregnated disk (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling. The sectioned surface was polished with silicon carbide papers of increasing fineness, and finally on linen with 0.3 μm aluminum oxide.

The polished surfaces were treated with 50% phosphoric acid for 30 s, then with 10% NaOCl for 2 min, followed by rinsing with a copious amount of water after each treatment. The fixed specimens were dehydrated in ascending...
After being sputter-coated with gold, the resin–dentin interfaces were examined under a scanning electron microscope (JSM-5310LV, JEOL, Tokyo, Japan) at 20–25 kV. Three restorations were examined for each group.

2.4. TEM examination

The specimens of groups 1 and 4 were prepared according to the TEM protocol reported by Tay et al. [7]. Briefly, the specimens were completely demineralized in ethylenediaminetetraacetic acid (pH 7.0), post-fixed in 1% osmium tetroxide, and dehydrated in ascending grades of ethanol (30–100%). After embedding in epoxy resin, 90–100 nm thick sections were prepared with an ultramicrotome. Sections were double-stained with uranyl acetate and Reynolds’ lead citrate to examine the ultrastructural characteristics of the resin–dentin interfaces, using a transmission electron microscope (Philips EM208S, Eindhoven, Netherlands) operating at 80 kV.

2.5. Microtensile bond strength tests

Forty extracted human third molars were employed for microtensile bond testing. The teeth were collected after the patients’ informed consents were obtained under a protocol reviewed and approved by the institutional review board from the Medical College of Georgia, USA. These teeth were stored in 0.5% chloramines T until use. Occlusal enamel was removed from each tooth using the Isomet saw under water cooling, creating flat surfaces for bonding in mid-coronal dentin. Each surface was further abraded with 180-grit silicon carbide papers to create clinically relevant smear layers for dentin bonding. The four groups of commercial/experimental two-step self-etch adhesives were used in the manner previously described, with 10 teeth employed for each group. Incremental composite build-up was performed on each bonded tooth surface using a light-cured micro-hybrid composite (Clearfil AP-X, Kuraray Medical Inc.) to a height of 4 mm.

After 24 h of water storage, two 0.9-mm thick slabs were obtained from each bonded tooth using the Isomet saw under water cooling. These slabs were further sectioned to produce 0.9-mm × 0.9-mm beams containing the resin–dentin interfaces. The four longest beams were selected from those acquired from each tooth. Each beam was fixed to a modified Bencor Multi-T testing assembly (Danville Engineering, San Ramon, CA, USA) using cyanoacrylate adhesive (Zapit; DVA, Corona, CA, USA). The beams were pulled to failure under tension using a universal testing machine (Model 4440, Instron Inc., Canton, MA, USA) at a crosshead speed of 1 mm per minute. The exact dimension of each fractured beam was then individually measured using a digital caliper (Model CD-6BS; Mitutoyo, Tokyo, Japan), from which the tensile bond strength was calculated. Means were taken from the four beams derived from the same tooth and the bond strength data for each group was expressed with the tooth instead of an individual beam as the testing unit (N = 10). Data from the four groups were statistically analyzed using one-way analysis of variance and Tukey’s post hoc multiple comparison test with α = 0.05.

3. Results

3.1. SEM observation

Representative bonded interfaces of each group are shown in Figs. 1–4. Groups 1 and 2 demonstrated interfaces with approximately a 1–2 μm thick hybrid layer and resin tags extending 5–8 μm into the dentin (Figs. 1 and 2). A filigree pattern of the resin-infiltrated layer and cone shape of resin tags, were clearly observed.

The thickness of the hybrid layer produced for groups 3 and 4 was similar to those of groups 1 and 2. However, the resin tags extended up to only 2–3 μm for these groups and were shorter compared with those with LB bond (groups 1 and 2), although a funnel-shaped configuration of the tag necks was apparent (Figs. 3 and 4).

3.2. TEM observation

TEMs of resin–dentin interfaces of groups 1 and 2 demonstrated interfaces with approximately a 1–2 μm thick hybrid layer and resin tags extending 5–8 μm into the dentin (Figs. 1 and 2). A filigree pattern of the resin-infiltrated layer and cone shape of resin tags, were clearly observed.

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Dental tubules were invariably occupied by smear plugs in both groups.

As the experimental bonding-resin was prepared by hand-mixing of MDPB with LB bond, many voids were observed in the bonding-resin layer for group 4 due to the entrapment of air bubbles (Fig. 6a).

3.3. Microtensile bond strength tests

Fig. 7 indicates the results of the microtensile bond strength test. There were no significant differences among the four groups ($p > 0.05$). The mean tensile bond strength values ranged from 30.8 to 34.9 MPa.

4. Discussion

The microtensile test performed on human teeth demonstrated that usage of 2.5% MDPB-containing bonding-resin in combination with the control or a 5% MDPB-containing primer did not adversely affect the dentin bonding ability of the parent commercial system without MDPB under in vitro conditions. These results supported the previous findings obtained by conventional tensile bond strength tests [4]. The addition of extra components such as free, non-polymerizable antimicrobials frequently interrupts the physical form of the polymers, leading to a reduction in their physical properties [8]. However, the synthetic monomer MDPB is a polymerizable bactericide and does not adversely affect curing of the primer and bonding-resin [4]. Curing ability of bonding-resin is one of the important factors for obtaining a strong bond to dentinal substrate [9,10]. Hence, incorporation of MDPB is advantageous over the addition of free antimicrobials such as chlorhexidine.

The pH value of the primer in the control self-etching system Liner Bond 2 is about 1.4. The thickness of the hybrid layer produced in vivo for all groups in the beagle dog model was 1–2 μm when examined under SEM. This value was similar to that reported for Liner Bond 2 by in vitro tests [11]. Since the addition of MDPB does not affect the pH value of the primer (data not shown), it is speculated that the same degree of demineralization of the smear layer and intertubular dentin as the control primer was obtained for the MDPB-containing primer. The fact that there were no differences in the appearance and thickness of the hybrid layers among all groups indicates that the experimental bonding-resin containing MDPB could penetrate well into the demineralized dentinal surface to produce optimal hybridization with exposed collagen. However, resin
tags formed in the tubules showed an apparent difference between the control (groups 1 and 2) and the MDPB-containing experimental bonding-resin (groups 3 and 4), the latter being shorter than the former. This discrepancy was not observed when the experimental bonding-resin was examined under in vitro condition using extracted human teeth [4]. In this study, cavities were prepared in sound dentin and it seems that the dentinal fluid in the tubule caused a disturbance of the infiltration of bonding-resin for groups 3 and 4. One of the possible reasons for this is the greater viscosity of MDPB-containing bonding-resin compared with the control. Incorporation of MDPB increases the viscosity of Bis-GMA based resin probably due to molecular interaction. The concentration of MDPB added to bonding-resin was set at 2.5% as this was the maximum amount of MDPB which would not to cause an adverse influence on handling properties. However, a slight increase in the viscosity was obtained even at 2.5% MDPB. In addition, MDPB is more hydrophobic than HEMA, so that the hydrophobicity of the experimental bonding-resin is greater than that of the control. Although infiltration into the demineralized smear layer and intertubular dentin was not affected, it is likely that penetration of MDPB-containing bonding-resin into the dentinal tubule was limited under in vivo conditions due to outflow of dentinal fluid.

TEM images of the bonding interface demonstrated continuity of resin and dentin with the production of hybridized dentin and hybridized smear layers for both the control and the experimental antibacterial adhesive systems employing MDPB-containing primer/bonding-resin (Figs. 5 and 6). As depicted by SEM, formation of 1–2 μm thick hybridized layers with irregular surfaces was clearly observed. Occlusion of dentinal tubules was manifested by the SEM observation of funnel-shaped resin tags even for the experimental system. This morphologic feature is indicative of the creation of tight seals at the tubule orifices. Thus, it may be concluded from the SEM and TEM evaluation that ultrastructure of the interface of the experimental adhesive system containing MDPB was not different from that of the control, with the exception that shorter resin tags were identified for experimental systems with the MDPB-containing bonding-resin.

It is well known that in vitro evaluation of the bonding ability of adhesive systems using extracted teeth sometimes does not reflect reality. For example, smear layers produced by 600-grit abrasive papers in vitro does not simulate what
dentists produce in a clinical setting, and bond strengths of mild self-etching primers measured under such in vitro conditions are suggested to be overestimated [12]. Most in vitro bond strength tests, including the one in the present study, were performed on flat tooth surfaces that are highly compliant and with minimal polymerization shrinkage stresses [13]. Recent studies showed that bond strengths of the adhesives were substantially reduced when testing was performed on cavities with low compliance such as Class I and Class II cavities [14,15]. Therefore, observation of resin–dentin interfaces under in vivo conditions provides important information on the clinical efficacy of bonding. Although we have to reject the null hypothesis, since in vivo resin infiltration into dentinal tubules was slightly affected for MDPB-containing bonding-resin, the present results using a beagle dog model demonstrated that experimental adhesive systems employing MDPB-containing primer/bonding-resin were able to exhibit satisfactory performance in vivo. Self-etching systems are claimed to have advantages over etch-and-rinse systems as there is little discrepancy between the depth of decalcification and bonding-resin penetration [16]. This is also expected to be valid for the experimental adhesive systems.

It has been reported that polymerized resin matrices are vulnerable to hydrolytic degradation after water sorption [17,18], and bonding agents that contain hydrophilic monomers exhibit lower bond durability compared with those containing hydrophobic monomers [19]. Incorporation of MDPB, which increases the hydrophobicity of the adhesives, may result in greater hydrolytic stability of the bonding interface. It would be of interest in the future to examine the durability of resin–dentin bonds made by the MDPB-containing primer/bonding-resin. Further investigations should be also aimed at assessing the antibacterial effects of this adhesive system against microorganisms that are capable of penetrating these interfaces by microleakage, as well as its clinical performance.

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