抗菌性レジンモノマーMDPB を含有する窩洞殺菌材の開発

Development of a Cavity Disinfectant Containing the Antibacterial Resin Monomer MDPB

Osaka University Graduate School of Dentistry
Course for Oral Science
(Department of Restorative Dentistry and Endodontology)
Supervisor: Professor Satoshi Imazato

Nanako Hirose

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GENERAL INTRODUCTION

Dental caries is a prevalent oral disease that is caused by bacterial infection. In a carious lesion, dental hard tissue is destructed by acidic products produced by bacteria, mainly *Streptococcus mutans*. Caries is treated by surgically removing the infected tooth structure, but it is not possible to eliminate bacterial infection completely in the clinical situation. Bacteria that are present on the cavity surface constitute a danger to the pulp beneath the filling material[1)]. Therefore, cavity disinfection is important for the successful treatment of caries. In addition, disinfection of the prepared tooth is also useful for indirect restorative treatment such as crown and bridge restorations or core build-up. Even after tooth preparation for indirect restorations, infected dentin may exist and salivary infection can occur in the prepared tooth[2)]. During provisional restoration, bacterial invasion occur at the prepared dentin surface through leakage at the cement-dentin interface[3)].

For disinfection of the tooth surface, sodium hypochlorite, hydrogen peroxide or chlorhexidine digluconate solution are often used. However, treatment of the tooth with some cavity disinfectants adversely affects the bonding ability of adhesive materials[4-6)]. In addition, *in vitro* tests using an infected cavity model demonstrated that antibacterial effects of commercially available disinfectants are not enough to achieve complete eradication of bacteria[7)]. Therefore, it is important to develop a novel cavity disinfectant that has reliable disinfecting effects but shows no negative influences on bonding ability of subsequently-applied adhesives.

The antibacterial resin monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB, Fig. 1) is a polymerizable bactericide[8)]. MDPB is a molecule that is synthesized by combining a quaternary ammonium compound (QAC), dodecylpyridinium bromide, with a methacryloyl group. Because MDPB is in a liquid state before being polymerized, it acts on bacterial cells, similar to conventional water soluble QACs at the unpolymerized stage. Several studies have demonstrated that unpolymerized MDPB shows strong bactericidal activity against cariogenic

and endodontic pathogens[8-11]. It has also been confirmed that MDPB can kill bacteria in biofilm form within a short period[10, 12-14]. Because of its rapid bactericidal activity at the unpolymerized stage, MDPB has been incorporated into a self-etching primer to provide cavity-disinfecting effects [15, 16]. Thus, the world's first self-etch adhesive system for composite restorations with antibacterial effects was successfully commercialized in 2004.

The polymerizable bactericide MDPB is unique and can be converted into the polymer by opening C=C bonds, similar to other dental resin monomers[8, 15]. Therefore, after curing of resins containing MDPB, the antibacterial component of MDPB is immobilized in a polymer network. This immobilized bactericide does not leach out from the cured resins but inhibits bacteria that come into contact with the surface[16, 17]. To date, approaches to immobilize the antibacterial component by incorporation of MDPB into various resinous materials, such as composite resins[8, 18, 19], pre-polymerized resin fillers[20], bonding resins[21], or coating resins[22], are available. This ability of MDPB to polymerize is advantageous because it does not influence the bonding ability of adhesives. Imazato *et al*.[15, 21, 23] and Kitagawa *et al*.[11] reported that incorporation of 5% MDPB to provide a self-etching primer with antibacterial effects showed no harmful influences on bonding ability to dentin.

Focusing on the unique characteristics of MDPB mentioned above, an experimental cavity disinfecting solution containing MDPB was fabricated. This disinfecting solution is intended to be used for various direct/indirect restorative procedures such as crown and bridge restorations, core build-up or composite resin filling. It is expected that the experimental MDPB-containing disinfectant will show disinfecting effects on the surface of the prepared tooth without causing adverse effects on bonding ability of the resinous adhesive materials, which will overcome the problems of proprietary cavity disinfectants that are commercially available. To examine this hypothesis, the antibacterial activity of the experimental cavity disinfectant was investigated *in vitro* and the influences on bonding ability of the resin-based adhesives were evaluated by conducting bond strength tests.

Chapter 1

Evaluation of the antibacterial effects of an experimental cavity disinfectant against bacteria related to caries and endodontic infections

1.1 Materials & methods

1.1.1 Bacteria

Two gram-positive cocci, *Streptococcus mutans* NCTC10449 and *Parvimonas micra* GIFU7745, and two gram-positive rods, *Lactobacillus casei* ATCC4646 and *Actinomyces naeslundii* ATCC19246, were used as bacteria related to caries. One gram-positive coccus, *Enterococcus faecalis* SS497, and two gram-negative rods, *Fusobacterium nucleatum* 1436 and *Porphyromonas gingivalis* ATCC33277, were used as bacteria related to endodontic infections.

For culturing *S. mutans*, *A. naeslundii* or *E. faecalis*, Brain Heart Infusion (BHI) broth (Becton Dickinson, Sparks, MD, USA), and BHI agar (Becton Dickinson) were used. For *F. nucleatum*, Todd Hewitt broth (THB; Becton Dickinson) supplemented with 0.05% l-cysteine-hydrochloride monohydrate (NACALAI TESQUE INC., Kyoto, Japan), and THB containing 1% agar (Becton Dickinson) and 0.1% l-cysteine-hydrochloride monohydrate were used. *L. casei* was cultured in Lactobacilli Inoculum Broth (Nissui, Tokyo, Japan), and on Lactobacilli Inoculum Broth (Nissui) supplemented with 1% agar (Becton Dickinson). For culturing *P. micra* or *P. gingivalis*, Gifu Anaerobic Medium broth (Nissui) containing 1% hemin (Sigma Chemical Co., St. Louis, MO, USA) and 0.02% vitamin K3 (Wako Pure Chemical Industries Ltd., Osaka, Japan), and Trypto-Soya agar supplemented with 0.1% BHI broth, 0.02% l-cysteine-hydrochloride monohydrate, 1% hemin and 0.02% vitamin K3 were used.

1.1.2 MIC and MBC measurement of unpolymerized MDPB

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the unpolymerized MDPB against seven bacterial species were measured using a

microdilution assay that was previously described[8, 10]. The antibacterial monomer MDPB was synthesized as described elsewhere[18]. Briefly, 12-bromo-l-dodecanol and methacrylic acid were reacted and then converted to MDPB by reaction with pyridine at 100°C, followed by further purification. The configuration of the final product was confirmed using ^{1}H-nuclear magnetic resonance (NMR).

Unpolymerized MDPB was dissolved in sterile distilled water at 1.6 mg/mL, and added to the wells of a 96-well microplate containing broth that was suitable for each bacterium. Serial two-fold dilutions were made and 50-μL volumes of MDPB solution at 0.2 - 400 μg/mL were prepared. A bacterial suspension incubated for 12 hr from the stock culture was adjusted to 2 x 10^6 colony-forming units (CFU) /mL, and 50 μL of this suspension was inoculated into each well containing MDPB solution. The microplates were incubated anaerobically at 37°C for 48 hours, and the MIC value was determined as the lowest concentration at which turbidity was not observed by visual examination.

From the wells that showed no visible growth, bacterial suspensions were inoculated on agar plates suitable for each bacterium, as described above. After subculture for 48 hours, the MBC value was determined as the lowest concentration that showed no colony formation on the plates. For comparison, the MIC and MBC values of chlorhexidine digluconate (Wako Pure Chemical Industries Ltd.; CHX) and cetylpyridinium chloride (Wako Pure Chemical Industries Ltd.; CPC) were measured. Tests were repeated five times for all bacterial species.

1.1.3 Disinfectants

The experimental cavity disinfectant (ACC) was prepared by dissolving MDPB at 5 wt% in 80% ethanol. For comparison, a commercial cavity disinfectant, Consepsis (Ultradent, South Jordan, UT, USA; CPS), containing 2% CHX was tested. The 80% ethanol solution (Wako Pure Chemical Industries Ltd.; Et), in which the MDPB was dissolved, was also included in the study (Table 1).

1.1.4 Assessment of antibacterial activity of experimental disinfectant

To compare the antibacterial activity of ACC, CPS and Et, agar-disc diffusion tests and MIC and MBC measurements were conducted.

a) Agar-disc diffusion tests

Each of seven bacteria was cultured from the stock culture for 12 hours, and 300 μL of the suspension was spread on agar plates using the culture media described above. Twenty microliters of ACC, CPS, or Et was impregnated into a sterilized filter paper disc (diameter, 6 mm; thickness, 1.5 mm; ADVANTEC, Tokyo, Japan) and placed on agar plates that were left for 15 min after inoculation of bacteria. The plates were incubated anaerobically at 37°C for 48 hours and production of inhibition zones was determined. The size of inhibition zones were calculated using the following equation:

Size of inhibition zone = $(I\text{-}F)/2$

where I = inhibition halo diameter (mean of 3 measurements), and F = filter paper diameter (6 mm).

All procedures were performed under anaerobic conditions (85% N_2, 10% CO_2, 5% H_2). Tests were repeated five times for all bacterial species.

b) MIC and MBC measurements

The MIC and MBC values of ACC, CPS, and Et against *S. mutans* NCTC10449 were measured. Serial two-fold dilutions of each disinfectant were made in a 96-well microplate wells containing BHI broth, and the tested samples (50 μL) at the concentration of 0.000095 – 25% of the original solution were prepared. *S. mutans* suspension (50 μL) at approximately 2×10^6 CFU/mL was inoculated into each well. The plates were incubated anaerobically at 37°C for 48 hours. The MIC value was determined as the lowest concentration in the well at which turbidity was not observed by visual examination. Suspension from the wells that showed no

bacterial growth was inoculated on BHI agar plates. After subculture for 48 hours, the MBC value was determined as the lowest concentration that showed no colony formation on the plates. Tests were repeated five times.

1.1.5 Assessment of bactericidal effects using an infected dentin model

The effectiveness of ACC, CPS, and Et to kill bacteria in dentinal tubules was evaluated using the dentin model infected with *S. mutans*. The infected model was prepared according to the method described by Haapasalo and Ørstavik [24] with some modifications, as described below.

a) Preparation of the infected dentin model

Extracted human sound molars were obtained from patients at Osaka University Dental Hospital under a protocol approved by the Ethics Committee of the Osaka University Graduate School of Dentistry (No. H25-E23). The teeth were cut with a low-speed diamond saw (Isomet 2000, Buehler, Lake Bluff, IL, USA) under water cooling, and rectangular parallelepiped blocks were obtained from the coronal dentin. The blocks were adjusted using a grinder (EcoMet 3000, Buehler) to give 4 mm × 5 mm × 2 mm sized dentin samples.

A smear layer was removed by placing the specimens in 40% phosphoric acid for 1 min followed by 5.25% sodium hypochlorite (NaOCl) for 10 min with ultrasonication. Opening of the dentinal tubules was confirmed using a scanning electron microscopy (SEM; JSM-6390LV, JEOL, Tokyo, Japan; Fig. 2). The specimens were autoclaved and incubated in BHI broth at 37°C for 48 hours under anaerobic conditions to confirm sterilization.

The bottom surface and side surfaces up to 1 mm from the bottom of the dentin block were covered with double layers of nail varnish. The specimens were then incubated in 5 mL of *S. mutans* NCTC10449 suspension at 37°C anaerobically to obtain infection in the dentinal tubules.

After incubation, the surface of the infected dentin block was scraped with a sterile micro brush (Microbrush Fine, Shofu, Kyoto, Japan) to remove the bacterial clamp (Fig. 3).

b) Bacterial culture protocols

To finalize the culture protocol and obtain the model with different levels of bacterial infection, the blocks were incubated under the following four conditions:

Group 1: culture in 1×10^8 CFU/mL bacterial suspension for 7 days

Group 2: culture in 1×10^8 CFU/mL bacterial suspension for 12 hours

Group 3: culture in 1×10^8 CFU/mL bacterial suspension for 6 hours

Group 4: culture in 1×10^5 CFU/mL bacterial suspension for 6 hours

To count the number of bacteria in the model, sample was collected by drilling a 1-mm thick part from the surface with the sterile round bur (ISO031, Beldenta Supply Inc., Hyogo, Japan) mounted on a low-speed hand-piece (Fig. 3). A new sterile bur was used to collect dentin sample from each block, taking care not to generate heat that could damage bacterial cells. Collected samples were placed into a sterile tube containing 5 mL BHI broth. After vigorously agitation for 20 sec, the suspension was 100- or 10000-fold diluted with BHI broth and inoculated onto BHI agar plates. The number of viable bacteria recovered was counted after culturing the plates anaerobically at 37°C for 48 hours. Tests were repeated three times.

c) Bactericidal activity tests

Based on the results obtained in 1.1.5 b), two culture conditions were selected as the finalized protocol, as follows: 1) culture in 1×10^8 CFU/mL bacterial suspension for 12 hours to prepare a heavily-infected model; and 2) culture in 1×10^5 CFU/mL bacterial suspension for 6 hours for a lightly-infected model.

The 12 infected dentin specimens were prepared for each culture condition and divided into four groups (n = 3). ACC, CPS, or Et was applied to the upper side of the infected dentin and

left for 30 sec. Application of no solution served as the control. After drying with a gentle air blow for 5 sec, collection of the sample to a depth of 1 mm from the surface was conducted, as described above. The collected sample was put into 5 mL of BHI broth, and the number of viable bacterial was counted by culturing on BHI agar plates.

The condition of bacterial infection for each model was confirmed by SEM observation and Brown and Brenn staining. For SEM, the whole dentin block or vertically fractured block was fixed with half-strength Karnovsky's solution (2% paraformaldehyde and 2.5% glutaraldehyde, pH 7.4) for 2 hours at 4°C, and dehydrated in an ascending ethanol series (50, 70, 80, 90, 95 and 100%). After being freeze-dried and sputter-coated with platinum, the top surface of the whole block and the cleaved surface of the fractured specimen were observed. For Brown & Brenn staining, the whole dentin block was fixed with 4% paraformaldehyde and decalcified by storage in 4% EDTA for 30 d. The sample was dehydrated in an ascending ethanol series (50, 70, 80, 90, 95 and 100%), dealcoholized, and then embedded in paraffin. The 8-μm thick sliced sections were cut and stained with crystal violet staining solution, Gram's iodine solution and basic fuchsin working solution. The slices were observed under an optical microscope (Eclipse Ni, Nikon, Tokyo, Japan).

d) Assessment of bactericidal effects using smear layer-covered model

The infected dentin model with a smear layer-like structure was fabricated, and the bactericidal effects of ACC application were assessed.

The lightly-infected dentin model was prepared as previously described. The top surface of the infected block was treated with an ultrasonic scalar (Suprasson Pmax, Acteon Satelec, Mérignac, France) at low power of the root planning mode with a light touch, and reciprocating 10 times on the surface. SEM observation revealed that the surface was covered with a layer of smeared dentin. ACC was applied to the surface for 30 sec, and the number of viable bacteria in dentin was counted, as described above. Tests were repeated three times.

1.1.6 Statistical analysis

The results of MIC and MBC measurements of unpolymerized MDPB or ACC, agar-disc diffusion tests and the bactericidal activity test using heavily- and lightly-infected dentin models were statistically analyzed using an analysis of variance and Tukey's honesty significant difference (HSD) test, with a significance level of $p < 0.05$. The results of the test using the smear layer-covered model were statistically analyzed using Welch's *t* test, with a significance level of $p < 0.05$.

1.2 Results

1.2.1 MIC and MBC measurement of unpolymerized MDPB

The MIC and MBC values of unpolymerized MDPB, CHX, and CPC against seven bacterial species are shown in Table 2. The same results were obtained for five repetitions of the tests. The MIC values of MDPB ranged from 6.4 to 51.2 μg/mL, and the MBC ranged from 51.2 to 102.4 μg/mL. The MIC and MBC values of CHX were 3.2 - 6.4 μg/mL and 6.4 - 25.6 μg/mL, respectively, and those of CPC were 0.8 - 25.6 μg/mL and 1.6 - 25.6 μg/mL, respectively.

1.2.2 Assessment of antibacterial activity of experimental disinfectant

ACC and CPS produced inhibition zones against all bacteria, while Et did not show inhibition against any bacteria except against *L. casei* (Fig. 4). Significantly greater inhibition zones were observed for ACC against all bacterial species compared with CPS ($p < 0.05$).

The MIC values for ACC and CPS against *S. mutans* were expressed as the percentage of the original solution, and the results are shown in Table 3. The same results were obtained for five repetitions of the tests. The MIC values were identical for ACC and CPS. The MBC value of ACC was greater than that of CPS, but the difference was a one-step dilution level. Et did not show any antibacterial activity in the measurable range.

1.2.3 Assessment of bactericidal effects using an infected dentin model

a) Preparation of infected dentin model

Fig. 5 shows the number of *S. mutans* collected from the dentin samples infected using four different culture conditions. No significant differences in bacterial number were observed between Group 1 and Group 2, and significantly less bacterial recovery was obtained for Group 3 compared with Groups 1 and 2. Group 4 demonstrated significantly smaller number of bacteria than Group 3 ($p < 0.05$).

Based on these results, Group 2 and Group 4 were selected as the heavily- and lightly-infected model, respectively, and they were used for subsequent tests.

b) Assessment of bactericidal effects

Fig. 6 shows SEM images of the top and vertically-fractured surfaces of heavily- and lightly-infected dentin models. Both models presented bacterial penetration into the dentinal tubules, but the number of tubules containing bacteria was greater for the heavily-infected model.

Fig. 7A and B show microscopic images after Brown & Brenn staining of a section obtained from the *S. mutans*-infected dentin model. Bacterial penetration into the dentinal tubules, up to approximately 50 μm, was confirmed for both models. For the heavily-infected dentin model, there was bacterial invasion into most of the dentinal tubules, while in comparison, the number of the dentinal tubules penetrated by bacteria was lower for the lightly-infected model.

For the heavily-infected dentin model, no significant reduction in bacterial number was observed for CPS treatment compared with the control (Fig. 8A). ACC and Et application resulted in recovery of significantly less bacteria than the control, and ACC demonstrated significantly greater reduction than Et ($p < 0.05$) (Fig. 8A). When ACC was applied to the lightly-infected model, complete killing of bacteria was observed, while CPS showed no

significant reduction in bacterial number compared with the control (Fig. 8B). Et application significantly reduced viable bacterial number compared with the control, although reduction in bacteria by ACC was significantly greater ($p < 0.05$).

c) Assessment of bactericidal effects using smear layer-covered model

The surface of lightly-infected dentin treated with an ultrasonic device was observed using SEM, and confirmed to be covered with a smear layer (Fig. 9A). When ACC was applied, bacteria in the model were completely killed (Fig. 9B).

1.3 Discussion

1.3.1 Antibacterial activity of unpolymerized MDPB

To examine the effectiveness of unpolymerized MDPB in inhibiting and killing oral bacteria that are frequently isolated from caries lesions or from an infected root canal, MIC and MBC values for *S. mutans, L. casei, A. naeslundii, P. micra, E. faecalis, F. nucleatum* and *P. gingivalis* were measured. *S. mutans* and *Lactobacillus* spp. represent a higher proportion of the microflora in the infected dentin[25-29]. Lactobacilli have been detected in high numbers in both superficial and deep caries, and *L. casei* is a lactobacillus that is frequently isolated from caries lesions[30, 31]. The number of *A. naeslundii* and *P. micra* are higher in initial root carious lesions[32, 33], and *A. naeslundii* originating from root caries lesions are able to synthesize significant amounts of glycogen at low pH, especially from glucose[34, 35]. *E. faecalis, F. nucleatum* and *P. gingivalis* are associated with an infected root canal[36-44]. *E. faecalis* alone has the potential to maintain root canal infection and periradicular lesions[45-49], and has also been detected in retreatment cases of root canal [44, 45, 47, 48, 50]. *F. nucleatum* is frequently isolated from primary-infected root canals with periapical pathologies[51, 52]. *F. nucleatum* biofilm formation is significantly enhanced by *P. gingivalis*[53], and *P. gingivalis* has been found to be associated with symptomatic cases, including abscessed teeth[44, 54, 55]. In this study, the MIC and MBC values of

MDPB against seven species were determined to be 6.4 - 51.2 μg/mL and 51.2 –102.4 μg/mL, respectively. Several studies are available that examined the MIC and MBC values of MDPB against *S. mutan*s. The MIC values ranged from 7.81–15.6 μg/mL and the MBC from 50–250 μg/mL[8, 9, 14, 56]. Yoshikawa *et al*.[56] reported that the MIC and MBC values of MDPB for *A. naeslundii* were 25 and 50 μg/mL. For *E. faecalis* and *F. nucleatum*, the MIC and MBC values of MDPB have been reported to be 31.25 and 62.5 μg/mL, respectively[10]. Thus, the values determined in this study were similar to those in the previous reports, and MDPB was confirmed to have strong antibacterial activity against various oral bacteria.

QACs are membrane active agents[57], and they kill bacteria via the following sequence of events: (i) adsorption and penetration of the agent into the cell wall; (ii) reaction with the cytoplasmic membrane (lipid or protein) followed by membrane disorganization; (iii) leakage of intracellular low-molecular-weight material; (iv) degradation of proteins and nucleic acids; and (v) wall lysis caused by autolytic enzymes[58]. MDPB, the derivative of QAC, is considered to disrupt bacteria using the same mechanism. However, the MIC and MBC values of typical water soluble antimicrobials CHX and CPC were smaller than those of MDPB for all bacteria tested. Combining a methacryloyl group to form MDPB may have an influence on some of interacting functions that are mentioned above, thus decreasing its ability to inhibit bacteria compared with the conventional cationic biocides.

1.3.2 Antibacterial effects of experimental cavity disinfectant

The experimental cavity disinfectant ACC was fabricated by adding MDPB at 5 wt% into an ethanol solution. Imazato *et al*. investigated various properties of HEMA-based, light-cured self-etching primer containing 5% MDPB for composite restorations[15, 23, 59-62]. They reported that this primer shows strong antibacterial activity against caries-related bacteria[15, 60-63], and that it causes no adverse influences on bonding abilities *in vitro* and *in vivo*[15, 23]. It has been also reported that MDPB has superior biocompatibility[64] and the cytotoxicity towards human pulpal

cells were not altered by incorporation of MDPB at 5% to the HEMA-based primer[59, 61]. In addition, Türkün *et al.*[7] reported that Clearfil Protect Bond, which uses an antibacterial primer containing 5% MDPB, was able to inactivate bacteria in the cavity more effectively than other disinfectants such as a chlorhexidine digluconate-based Consepsis, Tubulicid Red containing benzalkonium chloride, and 3% hydrogen peroxide. Thus, the MDPB concentration added to achieve a new cavity disinfectant was identified as 5%.

To confirm the sensitivity of seven oral bacteria to ACC, agar-disc diffusion tests were conducted. This test has been widely used to screen antibacterial activity of various dental materials *in vitro*. Both ACC and CPS showed inhibition of all bacteria, but Et produced no inhibition zones for six species and only small zones that were less than 1 mm for *L. casei*. It is known that 80% ethanol, used as the solvent of MDPB to fabricate ACC, is also used as a disinfectant and shows antibacterial activity against oral bacteria[65]. The reason that almost no inhibition zones were produced by Et is probably because ethanol quickly evaporated and, thus, could not diffuse into the agar.

In the agar-disc diffusion tests, ACC produced significantly greater inhibition zones than CPS against all seven bacteria. However, it is not possible to precisely compare antibacterial activities of different materials by this method because the size of inhibition zones produced depends on diffusivity of antimicrobial components in the agar in addition to their activity to inhibit bacteria. Therefore, to compare intrinsic antibacterial activity of ACC and CPS, the MIC and MBC values against *S. mutans* were determined. The results confirmed that ACC has similar antibacterial activity as CPS. The concentrations of active component in ACC, *i.e.* MDPB that is calculated from MIC and MBC, are shown in Table 4. For the MIC results, the value corresponded well with that obtained when the MDPB aqueous solution was tested. The concentration of MDPB in ACC at the MBC value was not the same as that measured using MDPB aqueous solution, but the difference was only a one-step dilution level. As the procedure for MIC and MBC determination in the present study, the original solution was initially diluted

four times and 20% ethanol was tested as the highest concentration for Et. Because this concentration is too low to show antibacterial activity[65], Et did not show any inhibition in MIC and MBC measurements. Thus, MDPB was confirmed to maintain its activity to act on bacteria even when incorporated into 80% ethanol to fabricate ACC.

Evaluation using the infected dentin model is useful for assessing the possible clinical effectiveness of cavity disinfectants[66-69]. However, most of the previous studies used demineralized dentin to mimic caries lesions, and there is no appropriate model to simulate infection of non-demineralized dentin, which is one of the targets of ACC. On the other hand, Haapasalo and Ørstavik[24] fabricated an infected root canal dentin model by incubating a dentin specimen in the suspension of endodontic pathogens. Kitagawa *et al*.[11] examined the bactericidal effects of experimental MDPB-containing primer using a model similar to the one reported by Haapasalo and Ørstavik[24]. In the present study, therefore, an original infected dentin model was established by modifying the methods used by Haapasalo & Ørstavik[24] and Kitagawa *et al*.[11].

The level of bacterial infection in the tooth is diverse clinically. Considering possible bacterial infections in various situations such as after crown preparation, post space preparation, or caries removal, two types of infected models were prepared. The dentin block was incubated in an *S. mutans* suspension at 1×10^8 CFU/mL for 12 hours to achieve heavily-infected model, or at 1×10^5 CFU/mL for 6 hours for the lightly-infected model. Both models showed bacterial penetration into the dentinal tubules up to approximately 50 μm from the surface, but the number of tubules that were penetrated by bacteria was greater for the heavily-infected model (Fig. 7). SEM observation also showed that the amount of bacteria invading each tubule was larger for the heavily-infected model (Fig. 6). The mean bacterial number in the heavily-infected dentin block was 2.3×10^7 CFU and the lightly-infected model contained 1.6×10^4 CFU per dentin block. Kidd *et al*.[70] found that the residual amount of bacteria in dentin after removing the caries lesion based on the consistency is about 10^3 CFU per tungsten carbide bur.

Accordingly, bacterial levels in the lightly-infected model appear to be suitable to mimic the condition where caries removal is insufficient or infection occurs after tooth preparation.

Although the results of MIC and MBC measurement indicated that ACC and CPS have the same level of antibacterial activity, *S. mutans* in the dentin block could be eradicated more effectively by application of ACC than CPS. Complete killing of bacteria in the block was obtained by ACC treatment in the lightly-infected model. There are two possible reasons for the greater effects of ACC. One reason is that ACC may have had greater permeability into dentinal tubules. Chlorhexidine easily adsorbs to organic matter such as dentin collagen[71], and can be trapped on the tooth surface (Fig. 10). It has been reported that antibacterial activity of chlorhexidine was significantly reduced when applied to dentin[72]. Chlorhexidine in CPS may not penetrate enough to kill bacteria deep in dentinal tubules. On the other hand, Schmalz *et al*.[73] reported that the commercial Clearfil Protect Bond self-etching primer containing 5% MDPB penetrated through 500-μm-thick dentin, showing better penetration than chlorhexidine solution. ACC is composed only of MDPB and ethanol, so that its viscosity is lower than the Clearfil Protect Bond primer, which contains other resin monomers. Production of greater inhibition zones by ACC than by CPS in the agar diffusion tests in this study also support better permeability of MDPB than CHX. In addition, permeability of MDPB may have been further enhanced by ethanol in ACC because infiltration of resin components into dentin can be promoted using ethanol[74]. The other reason for greater effectiveness of ACC is that 80% ethanol demonstrated additive effects to destroy bacteria. Although not as effective as ACC, applying Et alone significantly reduced bacterial recovery in both models. The combination of MDPB, which shows rapid killing activity[9-11, 14, 75], and 80% ethanol resulted in effective killing of bacteria.

Complete killing of bacteria by application of ACC to the lightly-infected model indicates that ACC penetrated into dentinal tubules up to 50 μm. In the heavily-infected model, the applied MDPB was thought to be consumed for killing large amounts of bacteria in the shallow

parts and certain amount of bacteria survived. However, this does not seem to be a problem because the heavily-infected model is almost like the outer part of a severe caries lesion without any drilling[76, 77]. To confirm ACC's penetration depth and its ability to kill bacteria deeply in dentinal tubules, direct observation using a confocal laser scanning microscope after LIVE/DEAD staining could be useful.

After preparation of dentin or drilling caries, a smear layer is formed on the dentin in the clinical situation. Therefore, the antibacterial effect of ACC against the smear layer-covered infected dentin was also examined, and the same effect as for the model without smear layer was obtained. The thickness of the smear layer formed with fine grid diamond bur or tungsten carbide bur is approximately 1.2 - 2.0 μm[78, 79]. The smear layer of the infected dentin model used in this study is considered to be thinner (approximately 1 μm) and uneven (Fig. 9A) compared with the one formed by bur cutting. However, ACC passes thorough the smear layer and penetrates into dentinal tubules, showing antibacterial activity. Thus, ACC is expected to exhibit beneficial antibacterial effects in the clinical situation.

It is important to know whether ACC can be used without causing harm to the pulp. Toxic effects of MDPB on viability and function of host cells, such as human pulpal cells or odontoblasts, have been thoroughly investigated. MDPB has been reported to show similar cytotoxicity as other monomers that are routinely used for dental resins[9, 64, 80]. In *in vivo* tests using animals, an MDPB-containing primer for restoration showed no detrimental effects on pulpal tissue[81], even when directly applied to the pulp[80]. In addition, Scheffel *et al.*[82] reported that the application of 100% ethanol does not cause pulpal damage by *in vivo* tests. Therefore, ACC comprised of 5% MDPB in 80% ethanol is considered to be safe for clinical use, while a clinical study to confirm biocompatibility of ACC remains to be conducted before commercialization.

1.4 Conclusions

The experimental cavity disinfectant containing 5% MDPB was shown to have a strong antibacterial effect against caries-related and endodontic pathogenic bacteria. Using the *in vitro* dentin model infected with *S. mutans*, application of the experimental cavity disinfectant was confirmed to be effective in killing bacteria in dentinal tubules.

Chapter 2

Evaluation of influences of the experimental cavity disinfectant on bonding abilities of various adhesives

2.1 Materials & methods

2.1.1 Influences on bonding ability of resin cements

To evaluate the influences of ACC application on bonding ability of resin cements to the tooth structure, a self-adhesive resin luting cement (Clearfil SA Cement Automix, Kuraray Noritake Dental; SA) and a dual-cure resin cement used with a primer (PANAVIA F2.0, Kuraray Noritake Dental; PA) were used (Table 5).

The crown of bovine incisors was embedded in chemically cured acrylic resin (TRAY RESIN, Shofu) with the buccal surface facing upward. The surface was planed with 120-grit silicon carbide paper to expose flat enamel or dentin, and polished with 600-grit silicon carbide paper using a grinding machine (EcoMet 3000). ACC or CPS was applied for 30 sec to the enamel or dentin exposed, and dried with a gentle air blow.

A sandblasted stainless steel rod (3 mm in diameter) was bonded using SA. After light irradiation using an LED light-curing unit (Pencure, Morita, Kyoto, Japan) for 3 sec, the surplus cement was removed and the specimen was light-cured for an additional 20 sec under a force of 5 N. For PA, the tooth surface was treated with ED primer II (Kuraray Noritake Dental) for 30 sec, and dried using a gentle air blow. The A and B pastes of PA were mixed for 20 sec and applied to the sandblasted stainless steel rod, and the rod was bonded as described above.

The bonded specimens were stored at room temperature for 1 hours, and then placed in distilled water at 37°C. After 24 hours, the shear bond strength test was conducted with a tabletop testing machine (EZ Test, Shimadzu, Kyoto, Japan) with a crosshead speed of 1.0 mm/min (Fig. 11). Application of no disinfectants served as the control.

The shear bond strength value was calculated by dividing the load by the bonded area (7.07 mm^2). The fracture mode was observed using a stereoscopic microscope (SMZ-U, Nikon) at ×20 magnification. Ten specimens were tested for each material.

2.1.2 Influences on bonding ability of one-step self-etch adhesives

To evaluate the influence of ACC application on the bonding ability of one-step self-etch adhesives to dentin, two commercial products, Clearfil Bond SE ONE (Kuraray Noritake Dental; SE) and ScotchBond Universal Adhesive (3M ESPE, St Paul, MN, USA; SU), were used (Table 6).

In total, six non-carious human molars were randomly divided into two groups to test each adhesive. The occlusal enamel of the crown was removed using a slow-speed diamond saw (Isomet 2000) and the dentin surface was polished with 600-grit silicon carbide paper. To eliminate the influence of differences among the teeth, one tooth specimen was divided into three pieces, allocating one piece from each tooth for the ACC, CPS and control groups. For the experimental group, ACC or CPS was applied for 30 sec, and dried using a gentle air blow. Application of no disinfectants served as the control.

The dentin surface was treated with SE for 10 sec or SU for 20 sec using an applicator brush, dried with a gentle air blow for approximately 5 sec to evaporate the solvent, and light-cured with an LED light-curing unit (Pencure) for 10 sec. A resin composite (Clearfil AP-X, Kuraray Noritake Dental) was then built up in 3 - 4 layers to a height of 5 - 6 mm. Light-curing was performed using an LED light-curing unit (Pencure), and the specimens were immediately placed in distilled water at 37°C. After storage for 24 hours, the bonded specimens were sectioned perpendicular to the bonding surface using a slow-speed diamond saw to obtain rectangular sticks (1 mm × 1 mm; 8 - 9 mm long). From each piece, 3 - 4 specimens were obtained. Each specimen was attached to a jig with an adhesive (Model Repair Pink, Dentsply

Sankin, Tochigi, Japan), and the microtensile bond strength test was conducted with a tabletop testing machine (EZ Test) with a crosshead speed of 1.0 mm/min (Fig. 12).

The cross-sectional area of each specimen was measured using a digital caliper (Absolute Digimatic Caliper, Mitutoyo, Kanagawa, Japan), and the bond strength value was calculated by dividing the load by the bonded area. The fracture mode was observed using a stereoscopic microscope (SMZ-U, Nikon) at ×20 magnification.

2.1.3 Statistical analysis

The results were statistically analyzed using analysis of variance and Tukey's HSD test, with a significance level of $p < 0.05$.

2.2 Results

2.2.1 Influences on bonding ability of resin cements

No significant differences in the shear bond strength of SA to enamel were observed among the three groups (Fig. 13A). For the bond strength of SA to dentin, there were no significant difference between ACC and the control, but application of CPS resulted in significantly lower bond strength than ACC or the control ($p < 0.05$; Fig. 13B). For the failure modes, adhesive failures were observed in most of specimens, and the rest of the specimens exhibited mixed failures, involving a combination of adhesive failure and cohesive failure within the cement (Table. 7).

For PA, there were no significant differences in the bond strength to enamel and dentin among the three groups (Fig. 14). Most of the specimens exhibited adhesive failure, and a few specimens demonstrated mixed failures involving a combination of adhesive failure and cohesive failure within the cement (Table 8).

2.2.2 Influences on bonding ability of one-step self-etch adhesives

Microtensile bond strengths of SE and SU to dentin after treatment with ACC or CPS are shown in Fig. 15. For both adhesives, no significant differences in the bond strength were observed among the three groups. For the failure modes of SE, adhesive failures were observed in most of the specimens, and a few specimens demonstrated mixed failure by a combination of adhesive failure and cohesive failure within the dentin when used with ACC. For SU, adhesive failure between the dentin and the composite resin was seen in all specimens for all groups (Table 9).

2.3 Discussion

Influences on bonding ability of resin cements

To test the influence of ACC application on the bonding ability of SA and PA, shear bond strength tests that are commonly used to measure the bond strength of resin cements were conducted[83-85]. For the self-adhesive resin cement SA, application of ACC showed no negative influences on the bonding abilities to enamel and dentin. MDPB, the antibacterial component in ACC, is a resin monomer and able to copolymerize with other monomers[16]. Because of this polymerizable property, there was no decrease in bond strength by incorporation of 5% MDPB into the self-etching primer[15]. It is suggested that MDPB applied to the tooth surface in the form of ACC could polymerize with resin components of SA, causing no negative influences on its bonding ability.

On the contrary, application of CPS was found to reduce bond strength of SA to dentin, although there was no difference in the failure mode between the ACC and CPS groups. Several reports have examined the effects of application of chlorhexidine-based disinfectants on the bonding ability of commercial self-adhesive resin cements[6, 86, 87]. Similar to the present study, Hipólito *et al.*[6] reported that pre-treatment of dentin with 0.2% or 2% chlorhexidine solutions adversely affected the bonding efficacy of both RelyX U100 and Multilink Sprint. A possible

reason for this decrease in bond strength of SA by CPS application is hindrance of cement curing by chlorhexidine. The chlorhexidine molecule has been reported to influence the polymerization of resins and decrease the degree of polymer conversion[88-90]. Because this chemical can easily bind with collagen[71], certain amounts of chlorhexidine are left on the dentin surface after drying CPS. Disturbance of the etching effects of adhesives by chlorhexidine can be another reason for reduction in bonding of SA. Meiers and Kresin[91] reported that chlorhexidine-treated smear layers were made acid resistant. SA, a self-adhesive resin luting cement, causes mild tooth etching and interacts with very thin superficial dentin, producing neither hybridized layer nor resin tags[92, 93]. Therefore, SA is susceptible to adverse effects shown by chlorhexidine, such as hindrance of resin polymerization and etching, and impairment of these properties can be critical.

Self-adhesive cements contain no water in their composition, and moisture on the tooth surface is essential for the bonding mechanism to induce demineralization based on ionization of acidic monomers such as MDP[94]. Guarda *et al*.[95] and Türker *et al*.[96] reported that the degree of residual moisture significantly affected the adhesion of self-adhesive resin cements to radicular dentin, and less moisture decrease the bonding ability. Because ethanol volatilizes water in dentin, it was anticipated that application of ACC based on 80% ethanol may reduce the bond strength. However, application of ACC showed no negative influences on the bonding abilities of SA to dentin or enamel. Acidic monomer MDP in SA may be able to quickly interact with the tooth surface to show etching effects before water is removed by spontaneous evaporation of ethanol, and therefore, 80% ethanol is acceptable as a component of the experimental cavity disinfectant.

For PA, neither ACC nor CPS showed negative influences on the bonding abilities to enamel and dentin. PA is used with ED primer II, which is a self-etching primer containing MDP. The pH value of ED primer II is about 2.1, and it can dissolve the smear layer on the dentin surface, similar to the primer of two-step self-etch adhesives for composite fillings.

Combining PA with ED primer II allows PA to impregnate into dentin deeper than SA and form the hybridized layer[93]. Therefore, the bonding mechanism of PA was not affected by residual chlorhexidine on the surface of dentin after application of CPS.

Influences on bonding ability of one-step self-etch adhesives

Microtensile bond strength tests are frequently used to investigate the bonding ability of self-etch adhesives for composite filling[97-99]. Therefore, the influence of ACC and CPS application on the bond strength of the one-step self-etch adhesives SE and SU to dentin was examined using this test method. The results indicate that the bond strength of both adhesives were not affected by application of ACC and CPS, and all groups, including the control group, demonstrated similar failure modes. A lack of reduction in bond strength by ACC indicates the advantage of tooth disinfection using polymerizable MDPB, as in the case of SA or PA.

Application of CPS also did not show any adverse effects on bonding of SE and SU to dentin. The influence of using commercial cavity disinfectants on the bonding ability of one-step self-etch adhesives was different dependent upon the materials. Saber *et al*.[5] reported that the irrigation with 2% chlorhexidine gluconate solution or 2.5% sodium hypochlorite solution followed by application of Clearfil S^3 bond resulted in a reduction in the shear bond strength values. On the other hand, Agrawal *et al*.[100] reported that chlorhexidine application did not significantly affect the sealing ability of Xeno V or Adper Easy One. In general, the etching ability of one-step self-etch adhesives is mild. The pH value of SE is 2.3 and that of SU is 3.0. A thin layer of dentin is etched by one-step self-etch adhesives, fabricating a thinner hybridization with dentin compared with two-step self-etch adhesives[101]. However, one-step self-etch adhesives have lower viscosity and higher permeability into dentin than self-adhesive cements. Therefore, SE and SU could demineralize dentin deep enough regardless of residual chlorhexidine, and the harmful action of chlorhexidine may have a smaller effect.

Because MDPB copolymerizes with other monomers, it is expected that the ACC application will have little to no influence on the bonding ability of any other adhesives. However, each product has different compositions, and shows different etching capacity and curing ability. The lack of influence by ACC on bonding with different types of adhesives that use other acidic monomers and catalysts for curing needs to be confirmed.

2.4 Conclusions

Application of the experimental cavity disinfectant containing MDPB did not demonstrate any adverse influences on the bonding abilities of various resin-based adhesives for direct or indirect restorations. However, application of Consepsis was found to reduce the bond strength of self-adhesive resin luting cements to dentin. Thus, the experimental cavity disinfectant consisting of the antibacterial monomer MDPB is advantageous compared with commercial chlorhexidine-based disinfectant.

GENERAL CONCLUSIONS

Disinfection of the cavity is important for direct or indirect restorations. This *in vitro* study confirmed that an experimental cavity disinfectant containing 5% MDPB is more useful than the commercially available chlorhexidine solution to eradicate bacteria in dentin, and causes no adverse influences on the bonding ability of resinous luting cements and one-step self-etch adhesives to tooth structure.

To use the new cavity disinfectant containing MDPB clinically, further investigation into antibacterial effects using the models of dentin infected with multiple bacterial species, which simulates actual infectious conditions in the oral environment, is needed. Moreover, *in vivo* examination using animals is also important to show whether the MDPB-containing cavity disinfectant can be used without causing harm to pulp. In addition to these experiments, further bond strength tests using other commercial adhesives may be useful.

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TABLE LEGENDS

Table 1. Disinfectants used

Table 2. MIC and MBC values (μg/mL) of MDPB, CHX and CPC against seven bacterial species

Table 3. MIC and MBC values of ACC, CPS and Et against *S. mutans*, expressed as the percentage of the original solution

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Table 5. Resin cements used

Table 6. One-step self-etch adhesives used

Table 7. Failure mode of SA to enamel or dentin

Table 8. Failure mode of PA to enamel or dentin

Table 9. Failure mode of SE and SU to dentin

FIGURE LEGENDS

Fig. 1. Chemical structure of the antibacterial monomer MDPB

Fig. 2. SEM image of the dentin block surface after placing in 40% phosphoric acid for 1 min followed by 5.25 % NaOCl for 10 min with ultrasonication

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Fig. 5. Number of *S. mutans* collected after incubation with four different culture conditions

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Fig. 6. SEM images of the top and fractured surfaces of *S. mutans*-infected dentin model

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Fig. 7. Microscopic images of a section obtained from the *S. mutans*-infected dentin model with Brown and Brenn staining

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Fig. 8. Number of viable *S. mutans* recovered after treatment with each solution

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Fig. 10. Schematic diagram of the infected dentin model and the effects of ACC or CPS

Infected dentin model (A) before application of disinfectant, (B) after application of ACC, and (C) after application of CPS. ACC penetrated deeper into dentinal tubules, and demonstrated greater bactericidal activity against *S. mutans* than CPS.

Fig. 11. Test method of shear bond strength measurement

Fig. 12. Test method for microtensile bond strength measurement

Fig. 13. Shear bond strength of SA to enamel (A) and dentin (B) after treatment with ACC or CPS

Control received no application of disinfectants. The bar represents the standard deviation of 10 specimens. No significant differences between the same letters (*i.e.*, a-b, analysis of variance and Tukey's HSD test; $p < 0.05$).

Fig. 14. Shear bond strength of PA to enamel (A) and dentin (B) after treatment with ACC or CPS

Control received no application of disinfectants. The bar represents the standard deviation of 10 specimens. No significant differences between the same letters (*i.e.*, a, analysis of variance and Tukey's HSD test; $p < 0.05$).

Fig. 15. Microtensile bond strength of SE (A) and SU (B) to dentin after treatment with ACC or CPS

Control received no application of disinfectants. The bar represents the standard deviation of 10 specimens. No significant differences between the same letters (*i.e.*, a, analysis of variance and Tukey's HSD test; $p < 0.05$).

Table 1. Disinfectants used

	Manufacturer	Code	Components
Experimental cavity disinfectant		ACC	MDPB (5 wt%), 80% ethanol
Consepsis	Ultradent	CPS	CHX (2%), 16% ethanol
Ethanol solution	Wako Pure Chemical Industries Ltd.	Et	80% ethanol

MDPB: 12-methacryloyloxydodecylpyridinium bromide

CHX: chlorhexidine digluconate

Table 2. MIC and MBC values (μg/mL) of MDPB, CHX and CPC against seven bacterial species

	MIC			MBC		
	MDPB	CHX	CPC	MDPB	CHX	CPC
S. mutans	6.4	3.2	0.8	102.4	25.6	1.6
L. casei	12.8	6.4	1.6	51.2	25.6	1.6
A. naeslundii	25.6	3.2	1.6	51.2	6.4	3.2
P. micra	51.2	6.4	25.6	51.2	6.4	25.6
E. faecalis	25.6	6.4	1.6	51.2	12.8	3.2
F. nucleaum	25.6	3.2	3.2	51.2	6.4	3.2
P. gingivalis	25.6	6.4	6.4	102.4	12.8	25.6

Table 3. MIC and MBC values of ACC, CPS and Et against *S. mutans*, expressed as the percentage of the original solution

	MIC	MBC
ACC	0.012	0.098
CPS	0.012	0.049
Et	—	—

—: No antibacterial activity in the measurable range

Table 4. MDPB concentration in ACC at MIC and MBC

at MIC	at MBC
6.1 μg/mL	48.8 μg/mL

Table 5. Resin cements used

	Manufacturer	Code	Components
Clearfil SA cement automix	Kuraray Noritake Dental	SA	Paste A: Bis-GMA, TEGDMA, MDP, hydrophobic aromatic dimethacrylate, silanated barium glass filler, silanated colloidal silica, dl-camphorquinone, benzoyl peroxide, initiator Paste B: Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, silanated barium glass filler, silanated colloidal silica, surface treated sodium fluoride accelerators, pigments
PANAVIA F2.0	Kuraray Noritake Dental	PA	Paste A: MDP, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated silica filler, silanated colloidal silica, l-camphorquinone, catalysts, initiators Paste B: sodium fluoride, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated barium glass filler, catalysts, accelerators, pigments, others
ED Primer II	Kuraray Noritake Dental		Liquid A: HEMA, MDP, N-methacryloyl-5-aminosalicylic acid, water, accelerators Liquid B: N-methacryloyl-5-aminosalicylic acid, water, catalysts, accelerators

Bis-GMA: bisphenol-A-diglycidyl methacrylate
TEGDMA: tri-ethyleneglycol dimethacrylate
MDP: 10-methacryloxydecyl dihydrogen phosphate
HEMA: 2-hydroxyethyl methacrylate

Table 6. One-step self-etch adhesives used

	Manufacturer	Code	Components
Clearfil Bond SE ONE	Kuraray Noritake Dental	SE	MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, camphorquinone, initiators, accelerators, ethanol, water, silanated colloidal silica, sodium fluoride
Scotchbond Universal Adhesive	3M ESPE	SU	MDP, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane

MDP: 10-methacryloxydecyl dihydrogen phosphate
HEMA: 2-hydroxyethyl methacrylate
Bis-GMA: bisphenol-A-diglycidyl methacrylate

Table 7. Failure mode of SA to enamel or dentin

	Enamel			Dentin		
	Control	ACC	CPS	Control	ACC	CPS
Adhesive (tooth/cement)	6 (60%)	8 (80%)	10 (100%)	6 (60%)	8 (80%)	9 (90%)
Cohesive (tooth)	0	0	0	0	0	0
Cohesive (cement)	0	0	0	0	0	0
Mixed (tooth/cement & tooth)	0	0	0	0	0	0
Mixed (tooth/cement & cement)	4 (40%)	2 (20%)	0	4 (40%)	2 (20%)	1 (10%)

Failure modes: Adhesive (tooth/cement), adhesive failure between the tooth and the cement; Cohesive (tooth), cohesive failure within the tooth; Cohesive (cement), cohesive failure within the cement; Mixed (tooth/cement & tooth), mixed failure by a combination of adhesive failure and cohesive failure within the tooth; Mixed (tooth/cement & cement), mixed failure by a combination of adhesive failure and cohesive failure within the cement

Table 8. Failure mode of PA to enamel or dentin

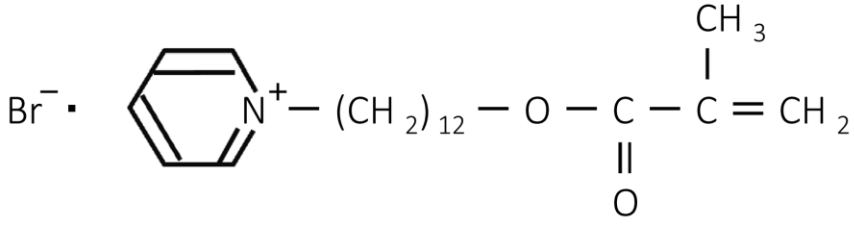
	Enamel			Dentin		
	Control	ACC	CPS	Control	ACC	CPS
Adhesive (tooth/cement)	10 (100%)	10 (100%)	8 (80%)	10 (100%)	10 (100%)	10 (100%)
Cohesive (tooth)	0	0	0	0	0	0
Cohesive (cement)	0	0	0	0	0	0
Mixed (tooth/cement & tooth)	0	0	0	0	0	0
Mixed (tooth/cement & cement)	0	0	2 (20%)	0	0	0

Failure modes: Adhesive (tooth/cement), adhesive failure between the tooth and the cement; Cohesive (tooth), cohesive failure within the tooth; Cohesive (cement), cohesive failure within the cement; Mixed (tooth/cement & tooth), mixed failure by a combination of adhesive failure and cohesive failure within the tooth; Mixed (tooth/cement & cement), mixed failure by a combination of adhesive failure and cohesive failure within the cement

Table 9. Failure mode of SE and SU to dentin

	Enamel			Dentin		
	Control	ACC	CPS	Control	ACC	CPS
Adhesive (dentin/CR)	10 (100%)	8 (80%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Cohesive (dentin)	0	0	0	0	0	0
Cohesive (CR)	0	0	0	0	0	0
Mixed (dentin/CR & dentin)	0	2 (20%)	0	0	0	0
Mixed (dentin/CR & CR)	0	0	0	0	0	0

Failure modes: Adhesive (dentin/CR), adhesive failure between the dentin and the composite resin; Cohesive (dentin), cohesive failure within the dentin; Cohesive (CR), cohesive failure within the composite resin; Mixed (dentin/CR & dentin), mixed failure by a combination of adhesive failure and cohesive failure within the dentin; Mixed (dentin/CR & CR), mixed failure by a combination of adhesive failure and cohesive failure within the composite resin



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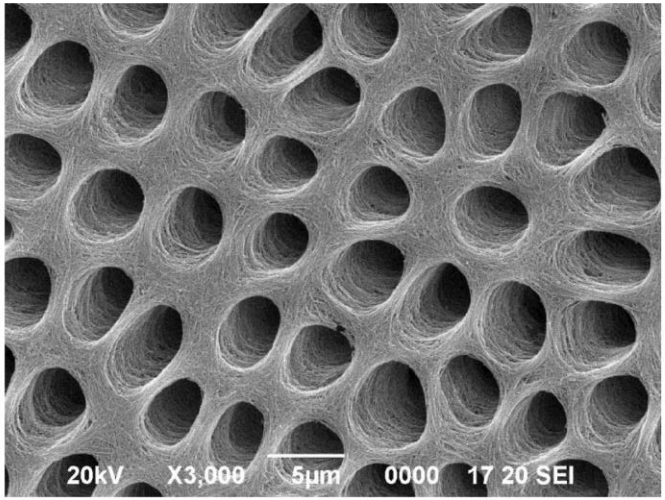

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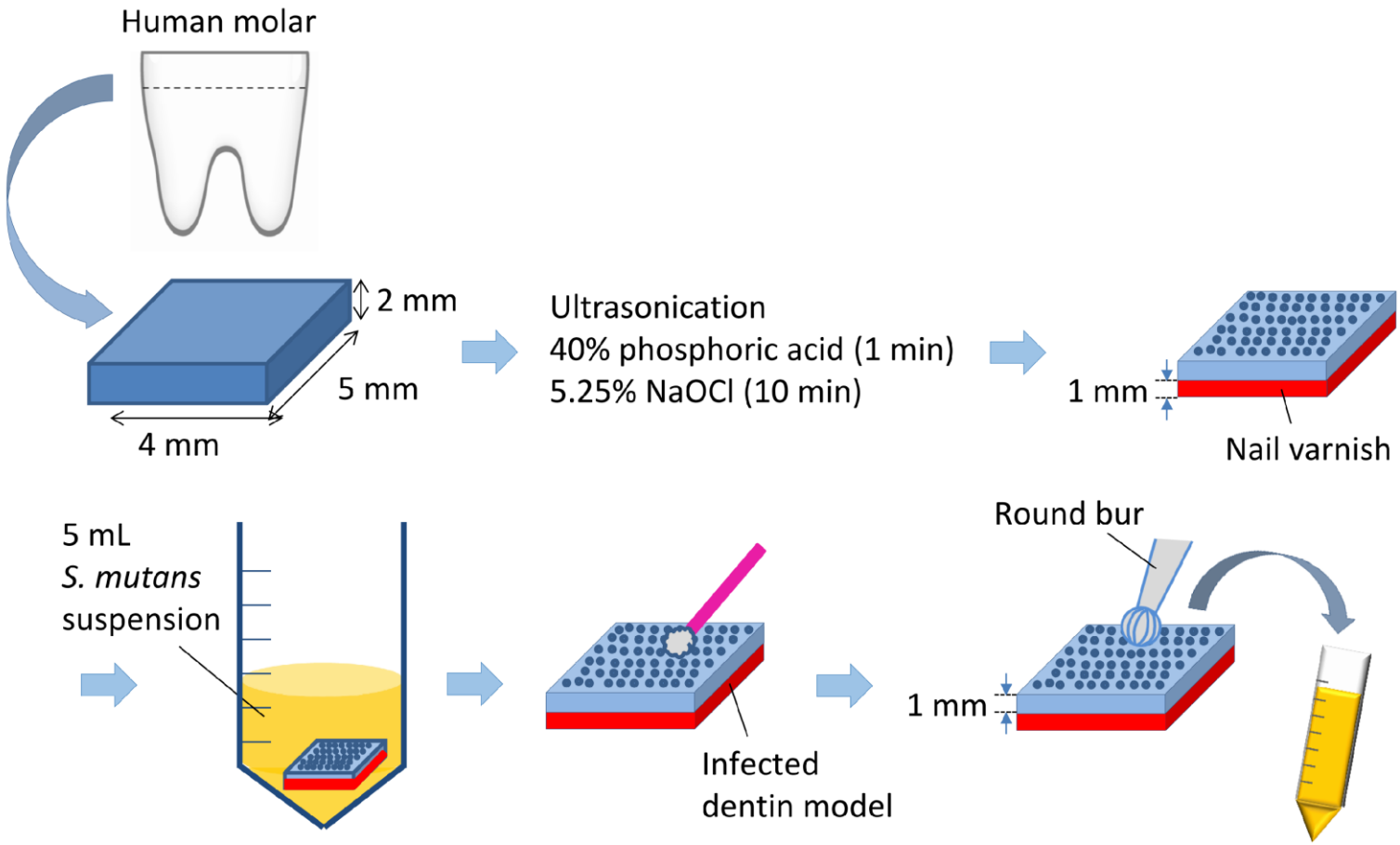
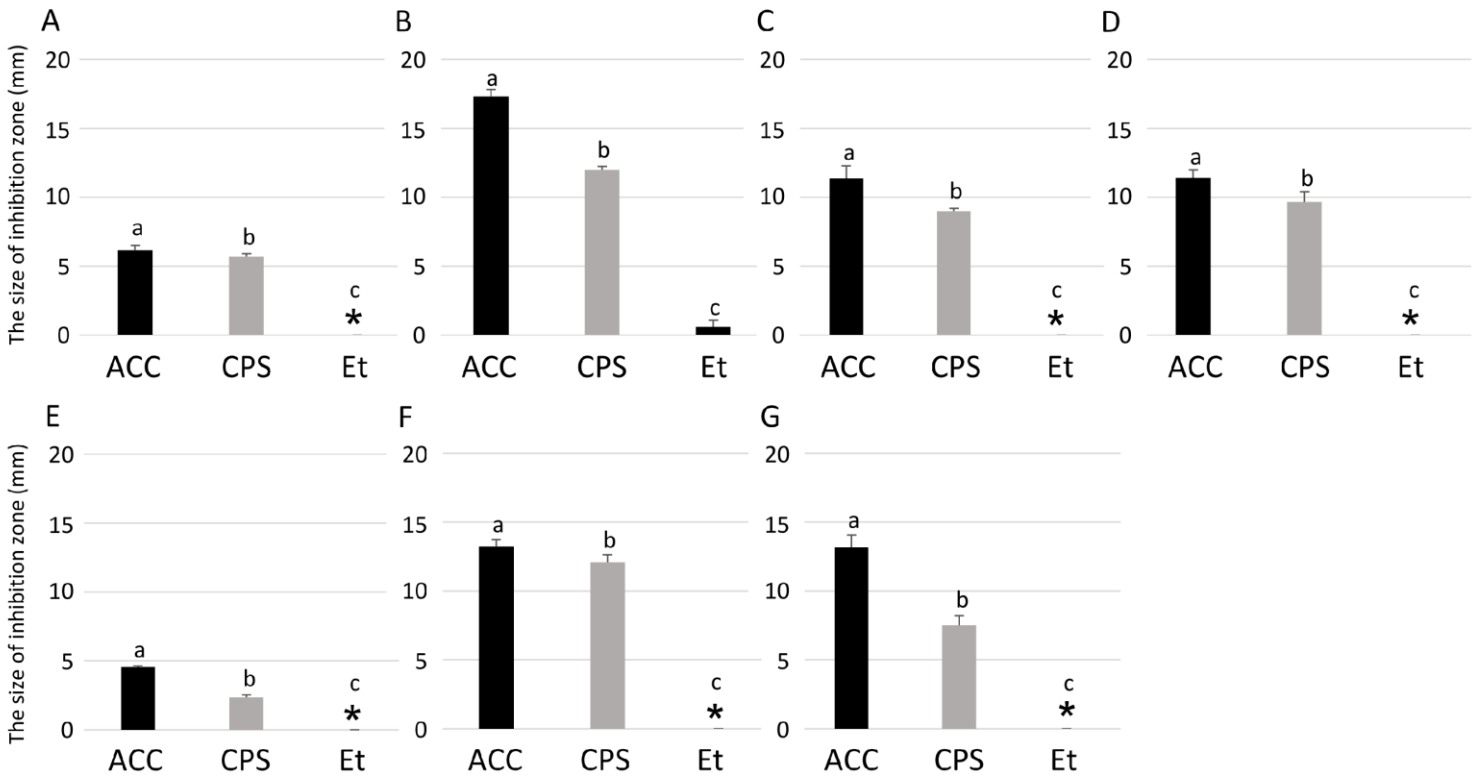


Fig. 3. The method of fabricating the infected dentin model and collecting the dentinal samples



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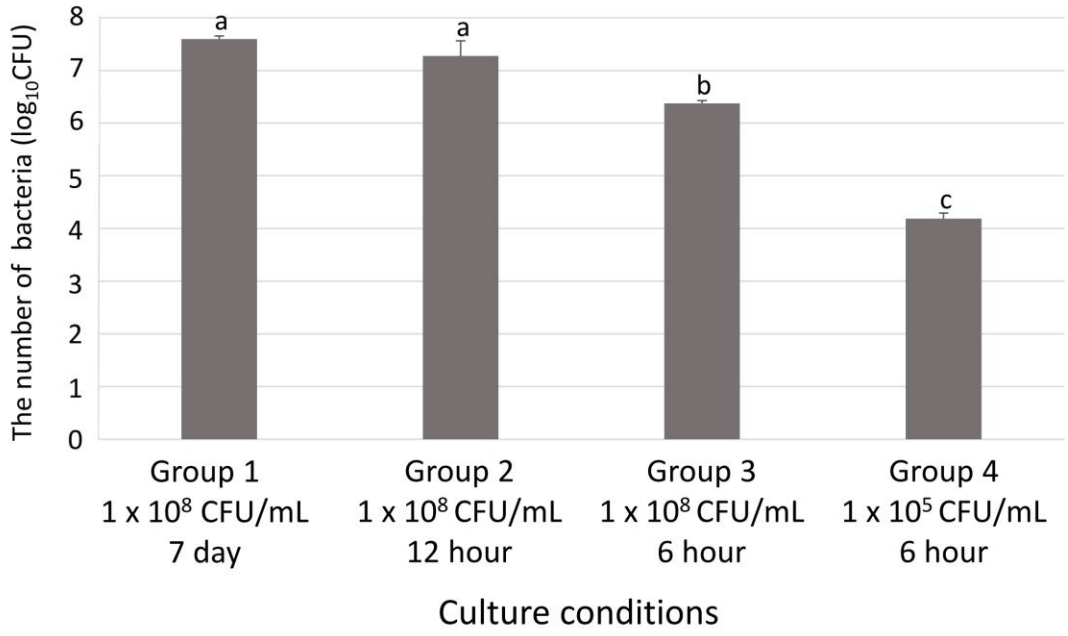

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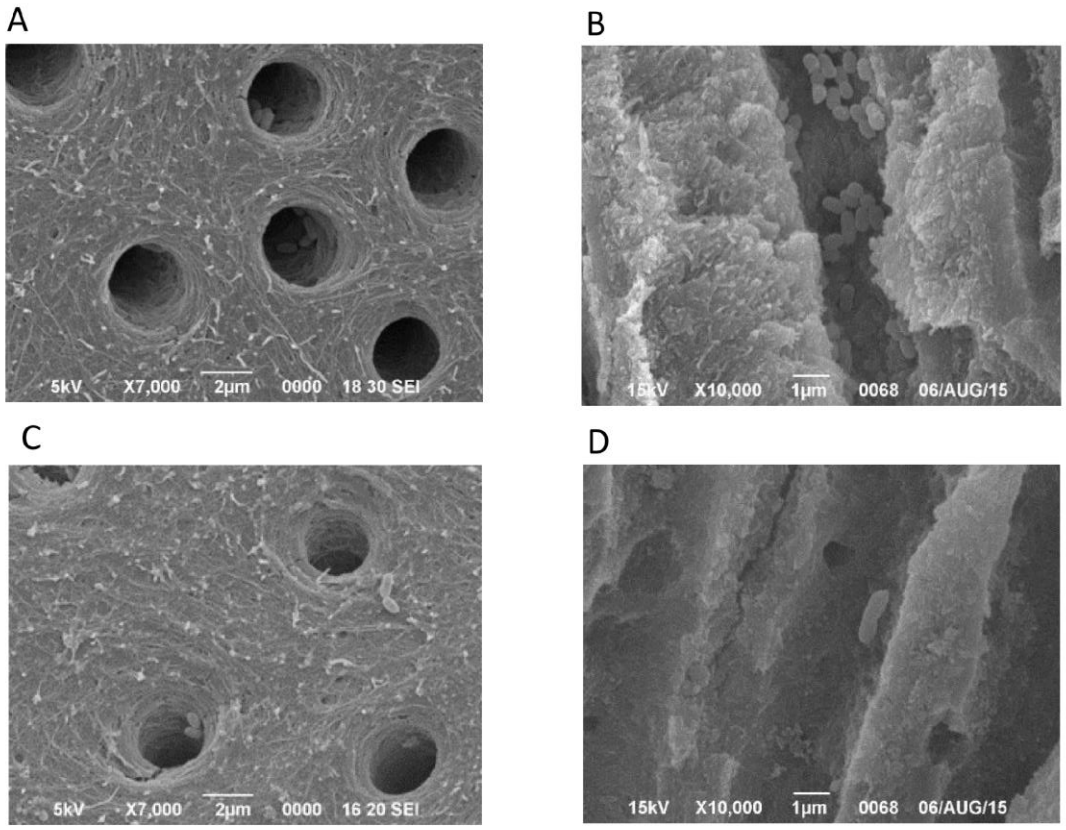

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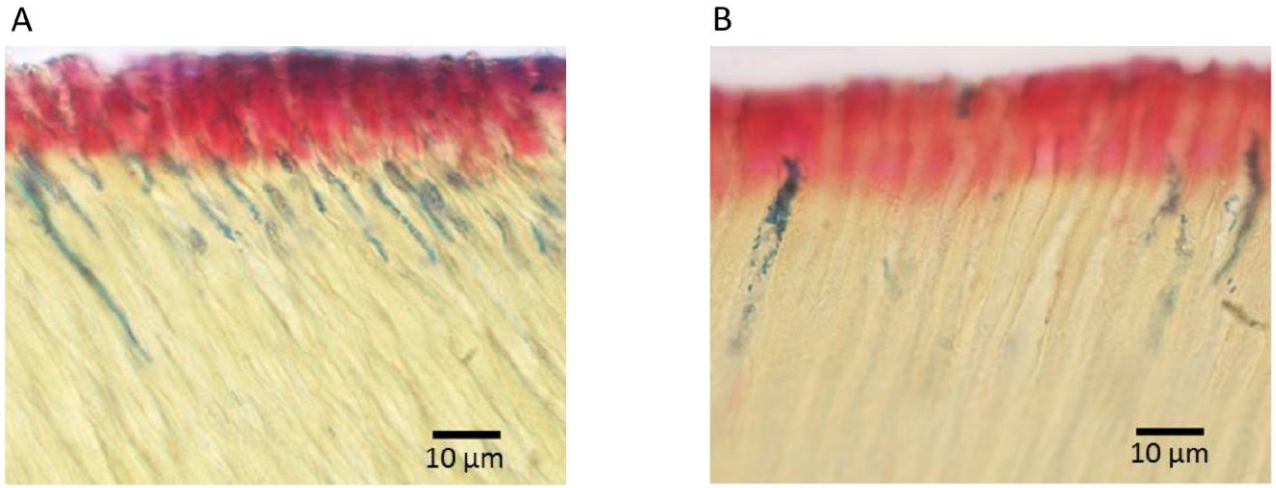

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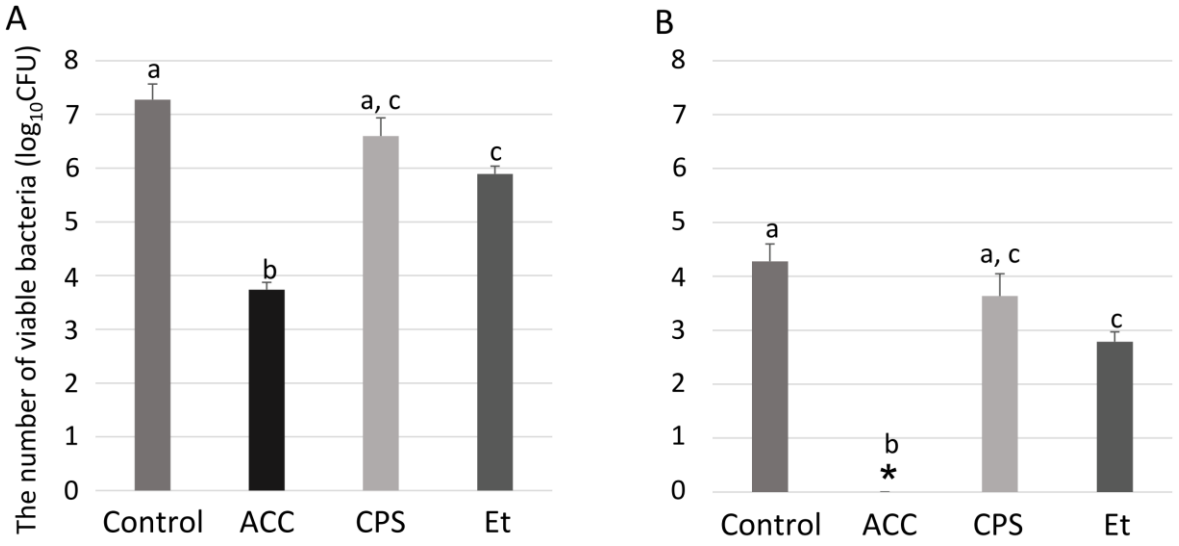

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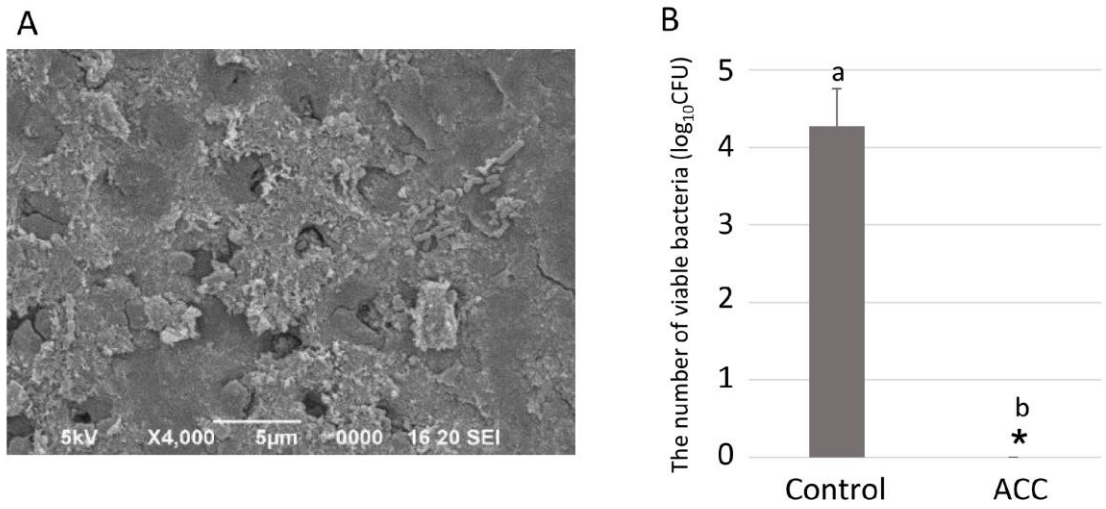

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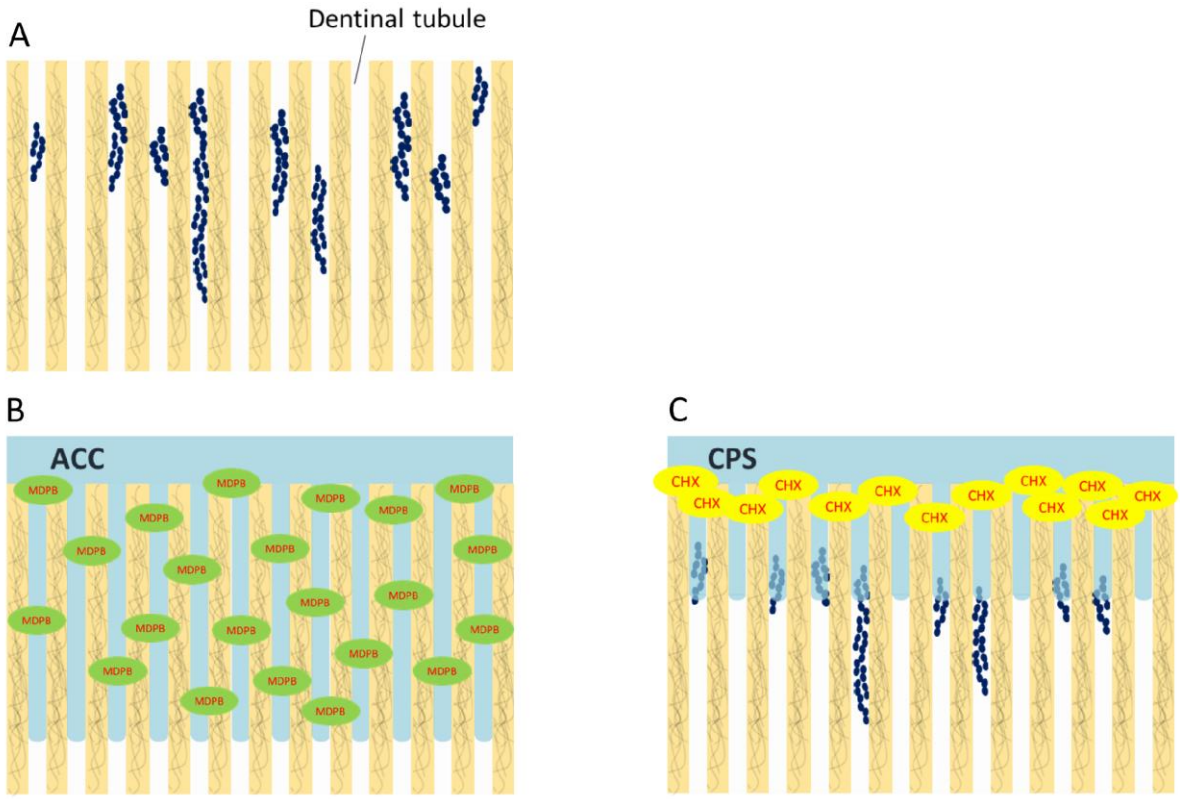

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Infected dentin model (A) before application of disinfectant, (B) after application of ACC, and (C) after application of CPS. ACC penetrated deeper into dentinal tubules, and demonstrated greater bactericidal activity against *S. mutans* than CPS.

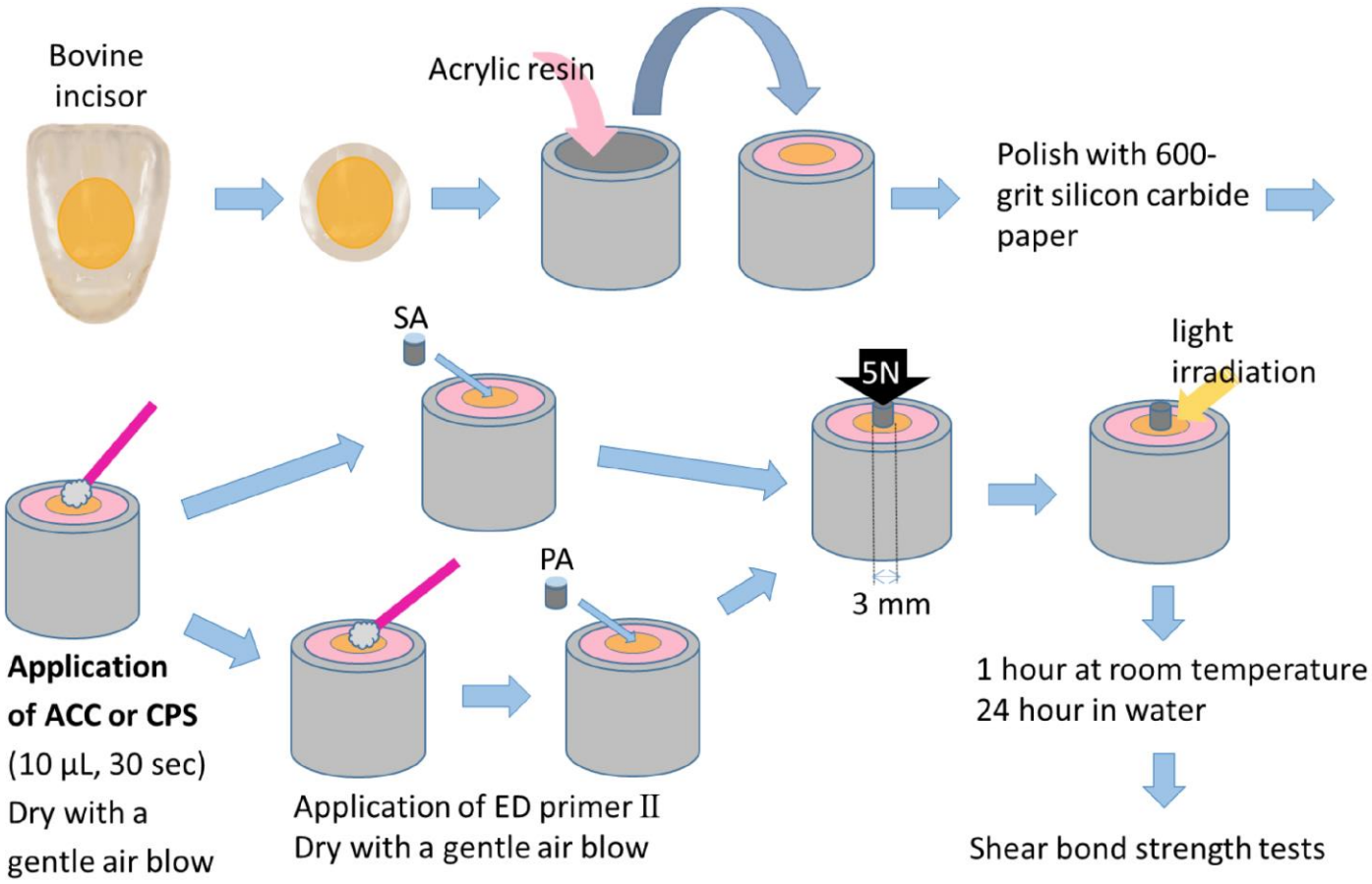


Fig. 11. Test method of shear bond strength measurement

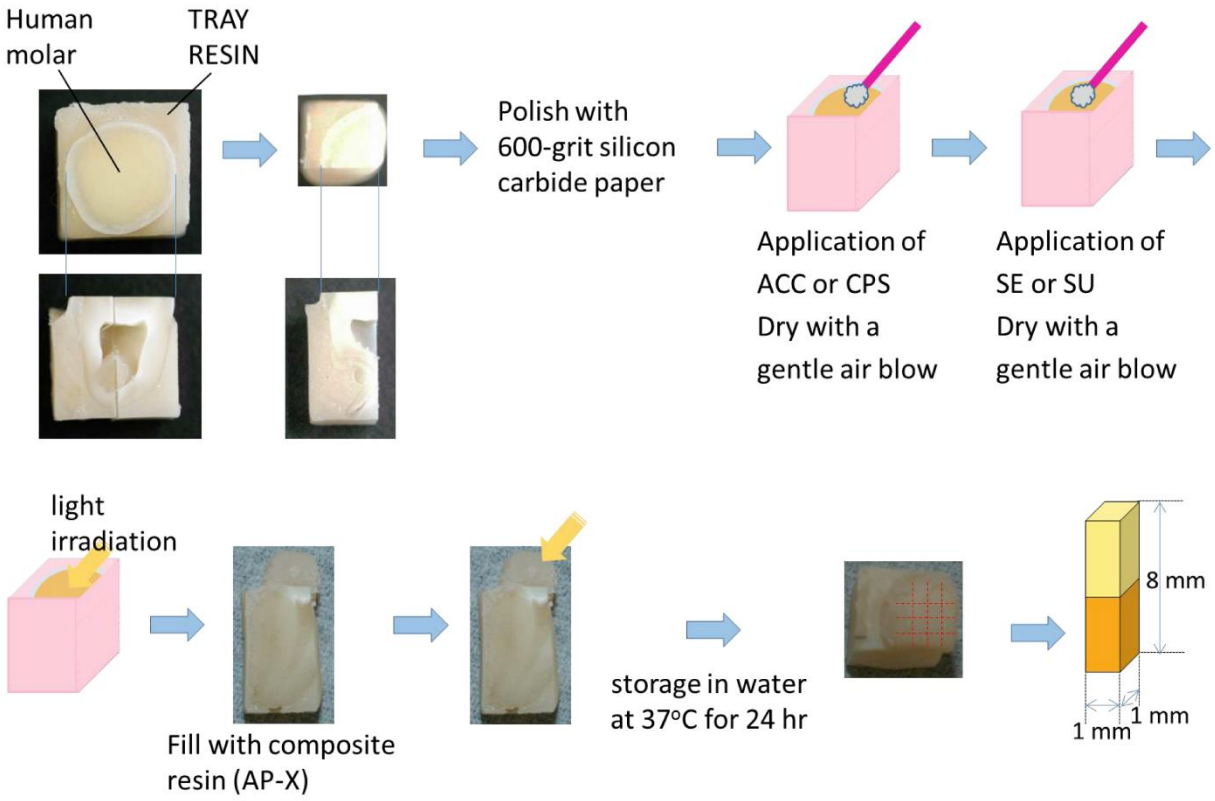
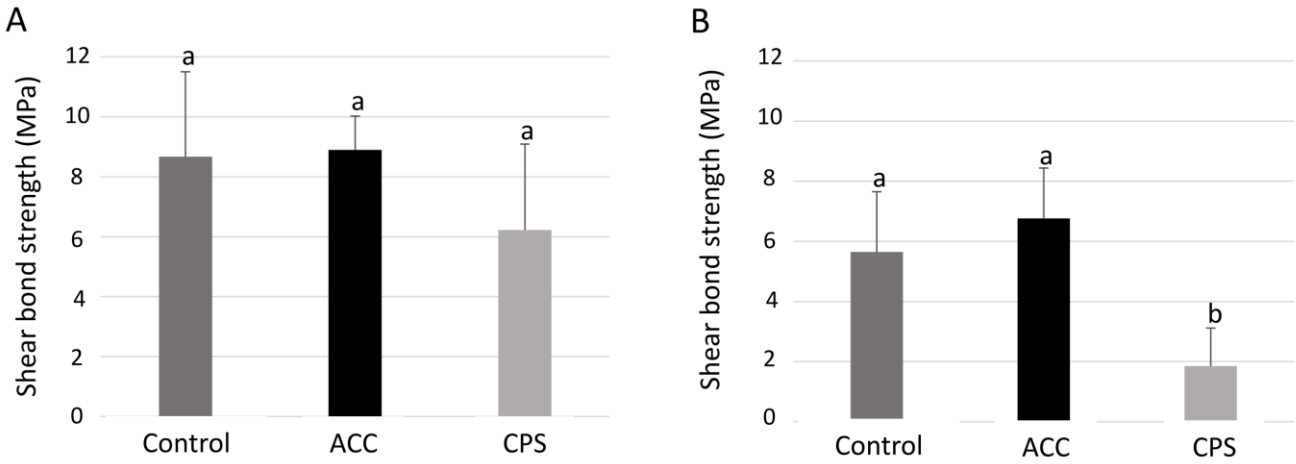


Fig. 12. Test method for microtensile bond strength measurement



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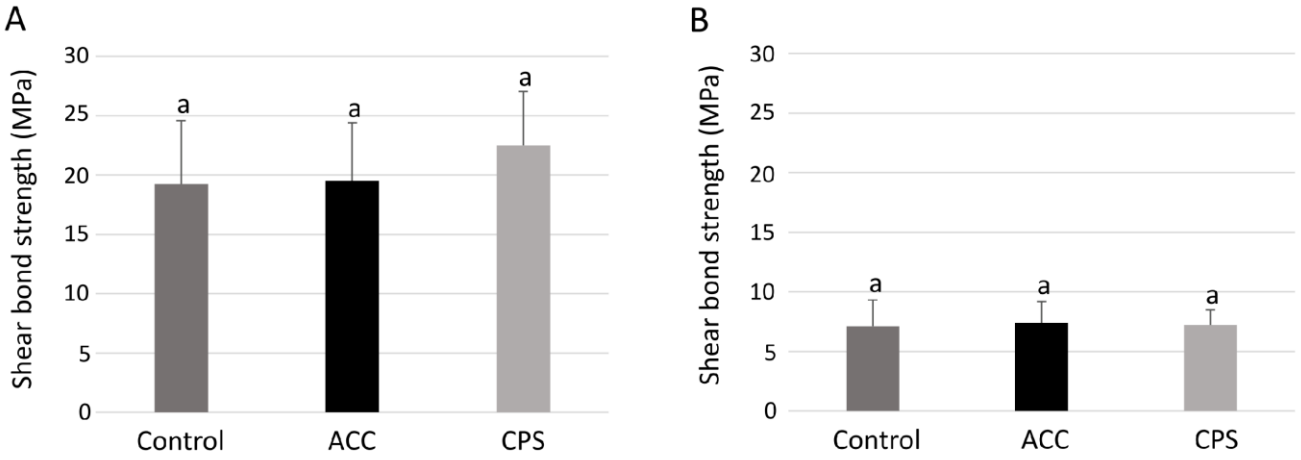
Control received no application of disinfectants. The bar represents the standard deviation of 10 specimens. No significant differences between the same letters (*i.e.*, a-b, analysis of variance and Tukey HSD test; $p < 0.05$).



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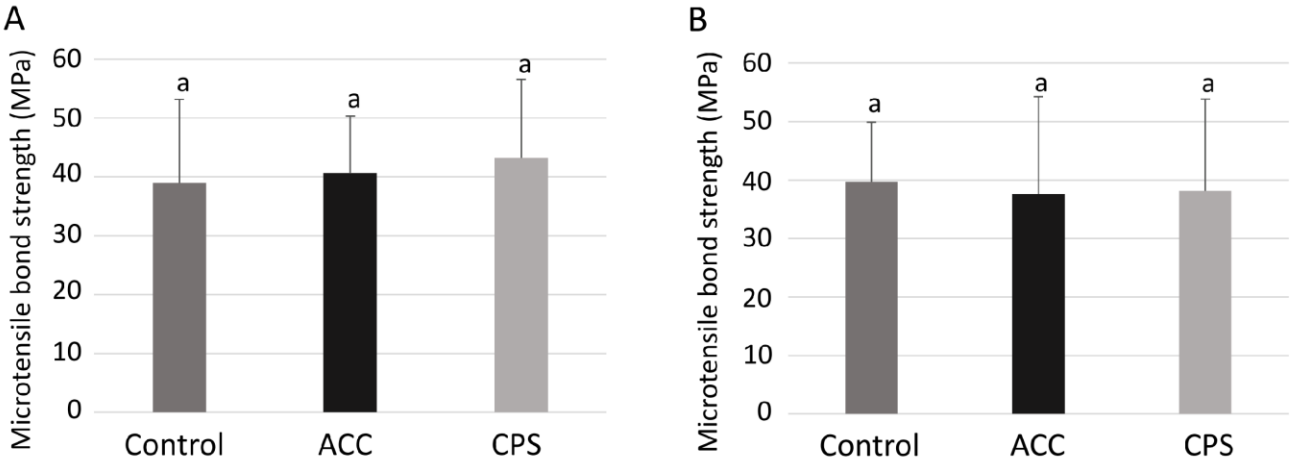

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