

R. Kitagawa¹, H. Kitagawa¹,
N. Izutani¹, N. Hirose^{1,2}, M. Hayashi¹,
and S. Imazato^{2*}

¹Department of Restorative Dentistry and Endodontology, Osaka University Graduate School of Dentistry, Osaka, Japan; and ²Department of Biomaterials Science, Osaka University Graduate School of Dentistry, Osaka, Japan; *corresponding author, imazato@dent.osaka-u.ac.jp

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ABSTRACT

An antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB)-containing experimental, chemically cured primer was prepared to develop a new resin-based root canal filling system. This study investigated the antibacterial effects of the MDPB-containing primer (experimental primer [EP]) against *Enterococcus faecalis* and assessed the *in vitro* bonding and sealing abilities of the filling system, consisting of EP and a Bis-GMA-based sealer resin. Antibacterial effects of EP were evaluated by contact with planktonic or adherent bacteria for 30 or 60 sec, and the viable bacterial number was counted. The antibacterial effects against *E. faecalis* in dentinal tubules were also assessed, according to a root canal infection model. Bonding and sealing abilities of the experimental filling system were examined by microtensile bond strength tests and leakage tests based on fluid filtration methods. Significantly greater reduction in viable bacteria in planktonic and adherent form was obtained by short-period contact with EP compared with the control primer (without MDPB) or with the proprietary (Epiphany) primer ($p < .05$). Significantly greater bactericidal effects of the EP inside the dentinal tubule of root, as opposed to the control primer or Epiphany primer, were confirmed according to a root canal infection model ($p < .05$), and 100% killing of *E. faecalis* could be obtained by the application of EP after irrigation with a 5% sodium hypochlorite solution. The experimental endodontic filling system demonstrated significantly greater bond strength to root dentin than Epiphany sealer system (Epiphany primer and Epiphany Root Canal Sealant; $p < .05$), showing formation of resin tags and a hybridized layer. Leakage tests clarified that the experimental system provided excellent sealing. This study confirmed that the MDPB-containing experimental antibacterial primer has the ability to effectively disinfect the root canal. Additionally, the experimental root canal filling system employing this primer and the Bis-GMA-based sealer resin is useful for achieving good sealing, suggesting its possible benefit for successful endodontic treatments.

KEY WORDS: root canal filling materials, antibacterial agents, resins, quaternary ammonium compounds, endodontics, *Enterococcus faecalis*.

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Development of an Antibacterial Root Canal Filling System Containing MDPB

INTRODUCTION

For successful endodontic treatment, eradication of bacteria inside the root canal is fundamental. However, complete elimination of bacterial infection in the root canal is difficult, even by mechanical instrumentation, irrigation (Matsuo *et al.*, 2003), or medication (Siqueira & Lopes, 1999). Therefore, it is considered to be beneficial to provide endodontic filling materials with antibacterial activity.

The antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB) is a resin monomer synthesized by combining a quaternary ammonium compound with a methacryloyl group (Imazato *et al.*, 1994). MDPB has a strong antibacterial activity against various caries-related bacteria before polymerization (Imazato *et al.*, 1994, 1999). In addition, the antibacterial component is immobilized in a polymer network by polymerization of MDPB, and such immobilized antimicrobial does not leach out from cured resins. Employing an MDPB-containing antibacterial self-etching primer, the world's first adhesive system for restoration with antibacterial effects was successfully commercialized (Imazato, 2009).

Izutani *et al.* (2010) investigated antibacterial activity of MDPB against endodontic pathogenic bacteria in biofilm form and found that these bacteria were killed by application of MDPB in a short period. Given such findings, we hypothesized that the MDPB-containing root canal filling system demonstrates an antibacterial effect against endodontic pathogens and provides excellent sealing as endodontic filling materials. Therefore, a new MDPB-containing primer was developed for disinfection of root canal dentin and filling of root canals in combination with a sealer resin. In this study, *in vitro* antibacterial effects of this experimental primer (EP) and its sealing effectiveness in combination with a Bis-GMA-based sealer resin were assessed to test the hypothesis.

MATERIALS & METHODS

Primers and Bacteria Used

The EP is a chemically cured, 2-bottled-type self-etching primer based on HEMA. Liquid A contains 10% MDPB, and the concentration of MDPB in EP after mixing liquids A and B is 5%. MDPB-free control primer (CP)—with similar components to EP, except for inclusion of MDPB—and the proprietary primer (PP; Epiphany Primer, Pentron, Wallingford, CT, USA) were included for comparison (Table 1).

As representative bacteria isolated from the root with periapical lesion (Gomes *et al.*, 2004), *Enterococcus faecalis* was used. *E. faecalis* SS497 was cultured for 12 hr in brain-heart infusion (BHI; Becton Dickinson, Sparks, MD, USA) broth and used for the experiments.

Table 1. Materials Used by Group

Material (Code): Manufacturer	Components
EP-SA sealer	
Primer: Experimental primer containing 5% MDPB (EP) ^a	Liquid A: HEMA, MDP, MDPB (10%) Liquid B: methacrylate monomer, water
Sealer: SA cement (SA sealer); Kuraray Noritake Dental (Tokyo, Japan)	Paste A: Bis-GMA, TEGDMA, methacrylate monomer, MDP, filler (barium glass, silica), dl-camphorquinone, benzoyl peroxide, initiator Paste B: Bis-GMA, methacrylate monomer, filler (barium glass, silica, sodium fluoride), surface treated sodium fluoride, accelerators
Point: Resilon; Pentron (Wallingford, CT, USA)	Polyester difunctional methacrylate resin, bioactive glass, radiopaque filler
CP-SA sealer	
Primer: control primer (CP) ^a	Liquid A: HEMA, MDP Liquid B: methacrylate monomer, water
Sealer: SA cement (SA sealer); Kuraray Noritake Dental	
Point: Resilon; Pentron	
PP-Epiphany sealer	
Primer: Epiphany Primer (PP); Pentron	HEMA, AMPS, camphorquinone, water
Sealer: Epiphany Root Canal Sealant (Epiphany sealer); Pentron	UDMA, PEGDMA, EBPADMA, Bis-GMA, barium borosilicate glasses, barium sulfate, silica, calcium hydroxide, bismuth oxychloride with amines, peroxide, photo initiator
Point: Resilon; Pentron	
ZOE sealer	
Sealer: Pulp Canal Sealer EWT (ZOE sealer); SybronEndo (Orange, CA, USA)	Powder: zinc oxide, silver powder, thymol iodide, dimeric resin Liquid: 4-allyl-2-methoxyphenol, balsam resin, water
Point: Gutta Percha Points (gutta-percha point); GC (Tokyo, Japan)	Gutta-percha, zinc oxide, wax, resin, sulfate

AMPS, 2-acrylamide-2-methylpropane sulfonate; Bis-GMA, 2,2-bis[4-(3-methacryloxy-2-hydroxypropoxy)phenyl] propane; EBPADMA, ethoxylated bisphenol A dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloxydecyl dihydrogen phosphate; MDPB, 12-methacryloxydodecylpyridinium bromide; PEGDMA, poly (ethylene glycol) dimethacrylate; TEGDMA, triethyleneglycol dimethacrylate; UDMA, urethane dimethacrylate.

^aAll components of EP and CP were given from Kuraray Noritake Dental.

Bactericidal Effects against Planktonic Bacteria

E. faecalis was adjusted to approximately 1×10^6 colony-forming units (CFU) per milliliter, and 10 μ L of each primer or distilled water for the control was added to 90 μ L of bacterial suspension. After agitation for 30 or 60 sec to keep the bacteria in contact with each primer, the suspension was diluted 100 times by adding 0.01 M phosphate-buffered saline (pH 7.4) to reduce the concentration of the primer far below the minimum inhibitory concentration values. The diluted suspensions were inoculated onto BHI agar plates, and the number of viable bacteria (CFU) was counted after 48 hr of incubation at 37°C in an anaerobic chamber.

Bactericidal Effects against Adherent Bacteria

A collagen disc (\varnothing 13.5 mm, Sumilon cell tight C-1 cell disc LF, Akita Sumitomo Bake Co., Akita, Japan) was placed in a well of a 12-well microplate, and 3 mL of *E. faecalis* suspension at approximately 1×10^3 CFU/mL was inoculated. The microplate was incubated anaerobically for 6 hr at 37°C to enable bacteria attach to the disc.

Fifty microliters of each primer or distilled water for the control was applied for 30 or 60 sec to the disc with adherent

bacteria. The primer solution was removed gently after adding small amount of water, and the disc was put into a tube containing 10 mL of broth. The tube was ultrasonicated in an ice-cold bath for 10 min to collect bacteria. The suspensions were inoculated onto BHI agar plates, and the viable bacterial number was counted.

Assessment of Bactericidal Effects Based on a Root Canal Infection Model

The effectiveness of the EP inside the dentinal tubule of root was evaluated with the method described by Haapasalo and Ørstavik (1987), with some modification.

Extracted human, sound, single-rooted teeth were obtained from patients at Osaka University Dental Hospital under the protocol approved by the Ethics Committee of the Osaka University Graduate School of Dentistry (No. H21-E19). The teeth were cut with a low-speed diamond saw under water cooling, and 4-mm-thick slices were obtained from the upper part of the roots. The slices were prepared into an approximate external diameter of 6 mm using a grinding polisher (Ecomet III, Buehler). The canals of the specimens were then enlarged with round carbide burs with a 2.3-mm diameter

(ISO 023, Beldenta Supply Inc., Hyogo, Japan), mounted in a low-speed hand piece. A smear layer was removed by placing the specimens in 18% EDTA, followed by 5% sodium hypochlorite (NaOCl), for 10 min with ultrasonication. The absence of a smear layer and the presence of open dentinal tubules were confirmed *via* a scanning electron microscope (SEM; JSM-6390LV, JEOL, Tokyo, Japan).

The specimens were autoclaved and incubated in BHI broth for 48 hr to confirm sterilization. Then, 1 root specimen was placed into 5 mL of *E. faecalis* suspension (approximately 1×10^8 CFU/mL) and incubated for 7 d, replacing 2 mL of suspension with fresh broth every 2 d. Bacterial penetration into the dentinal tubules was confirmed by Brown and Brenn staining.

The 12 root specimens with bacterial contamination were divided randomly into 4 groups ($n = 3$). The intracanal dentin of the infected specimen was treated by each primer for 30 sec or left untreated for the control. After drying with sterile paper points, the dentin chips were harvested from the canal lumens by drilling with the sterile round burs mounted to a low-speed hand piece. Sequential sampling of dentine to a depth of 400 μ m was performed through burs with increasing diameters (ISO 025, 027, 029, and 031; Beldenta Supply Inc.). The dentin chips obtained (approximately 18 mg) were collected into a tube containing 5 mL of BHI broth, and the viable bacterial number was counted.

Another 15 infected specimens were divided into 5 groups and treated as follows:

Group 1: no treatment (control)

Group 2: irrigation with 5% NaOCl for 10 sec

Group 3: irrigation with 5% NaOCl for 10 sec, drying, and treatment with EP for 30 sec

Group 4: irrigation with 5% NaOCl for 30 sec

Group 5: irrigation with 5% NaOCl for 30 sec, drying, and treatment with EP for 30 sec

After each treatment, the bacteria were collected as described above, and the viable bacterial number was counted.

Bonding Ability of Experimental Sealer System

For the sealer resin to be combined with EP, dual cured Bis-GMA-based resin (SA Cement, Kuraray Noritake Dental, Tokyo, Japan; *i.e.*, SA sealer) was employed (Table 1). Human incisors within 6 mo of extraction were sectioned at 9.5 mm from the apical end. A canal was prepared to ISO size 60 and rinsed with 5 mL of 5% NaOCl and 18% EDTA, followed by 5 mL of distilled water, and dried with paper points. Sixteen roots were divided randomly into 2 groups:

Group 1: apply EP for 30 sec, dry, and fill with SA sealer

Group 2: apply PP for 30 sec, dry, and fill with Epiphany Root Canal Sealant (Pentron; *i.e.*, Epiphany sealer)

After obturation, the roots were light irradiated for 40 sec and stored in 100% humidity at 37°C. After 24 hr, from the cervical half of the roots, 3 disc-shaped slabs with a thickness of approximately 1.0 mm were obtained by slicing perpendicular to the

tooth axis. An hourglass-shaped specimen, which included the 2 sides of the bonding interfaces, was trimmed from each slab as described previously (Wu *et al.*, 2009). The specimen was then tested with a tabletop materials testing machine (EZ Test, Shimadzu, Kyoto, Japan) with a crosshead speed of 1.0 mm/min. The microtensile bond strength (MTBS) was calculated according to a unit of stress (megapascal [MPa]) based on the area of the fracture surface. The interfacial fracture surface area was calculated according to the formula used in the previous study (Bouillaguet *et al.*, 2003).

To observe the interfacial morphology, the root canal prepared as described above was filled with a resin point Resilon (Pentron), with EP and SA sealer by the single-cone obturation technique ($n = 3$). The coronal surface of the root filling was irradiated for 40 sec, and the specimens were stored in 100% humidity at 37°C for 24 hr. The surface of specimens sectioned longitudinally into halves was polished and observed through SEM after dehydration in an ascending ethanol series, freeze-drying, and platinum sputter coating. For micromorphologic analysis of the bonding interface, the polished surfaces were treated with 50% phosphoric acid for 2 min, then 12% NaOCl for 4 min, and observed by SEM.

Leakage Tests Based on Fluid Filtration Methods

Twenty-four roots were prepared as described and divided randomly into 4 groups. Groups 1, 2, and 3 were obturated with EP-SA sealer, CP-SA sealer, or PP-Epiphany sealer, respectively. Filling with gutta-percha point (Gutta Percha Points, GC Co., Tokyo, Japan), based on zinc oxide eugenol sealer (Pulp Canal Sealer EWT, SybronEndo Co., Orange, CA, USA; *i.e.*, ZOE sealer), was employed as the fourth group.

After 1 or 4 wk of storage in water at 37°C, microleakage of the filled roots was evaluated by a fluid filtration method (Raina *et al.*, 2007). A pressure of 0.0689 MPa (10 psi) was applied, and the fluid flow rate was expressed in microliters per minute based on the air bubble movement.

Statistical Analysis

The results for bacterial counts and leakage tests were statistically analyzed by analysis of variance and Student-Newman-Keuls's *post hoc* test, with a significance level of $p < .05$. The results for MTBS tests were statistically analyzed by the Student's *t* test with a significance level of $p < .05$.

RESULTS

Bactericidal Effects

For both planktonic and adherent bacteria, significantly greater reduction in viable bacteria was obtained by EP than CP or PP ($p < .05$). By contact with EP, 99.9% killing of planktonic bacteria was obtained after 30 sec and 100% killing after 60 sec (Fig. 1A, 1B). For adherent bacteria, EP killed more than 98% after 30 sec and 99% after 60 sec (Fig. 1C, 1D). For bacteria in dentinal tubules, EP demonstrated significantly greater reduction than CP or PP ($p < .05$) (Fig. 1E), showing 99.5% killing.

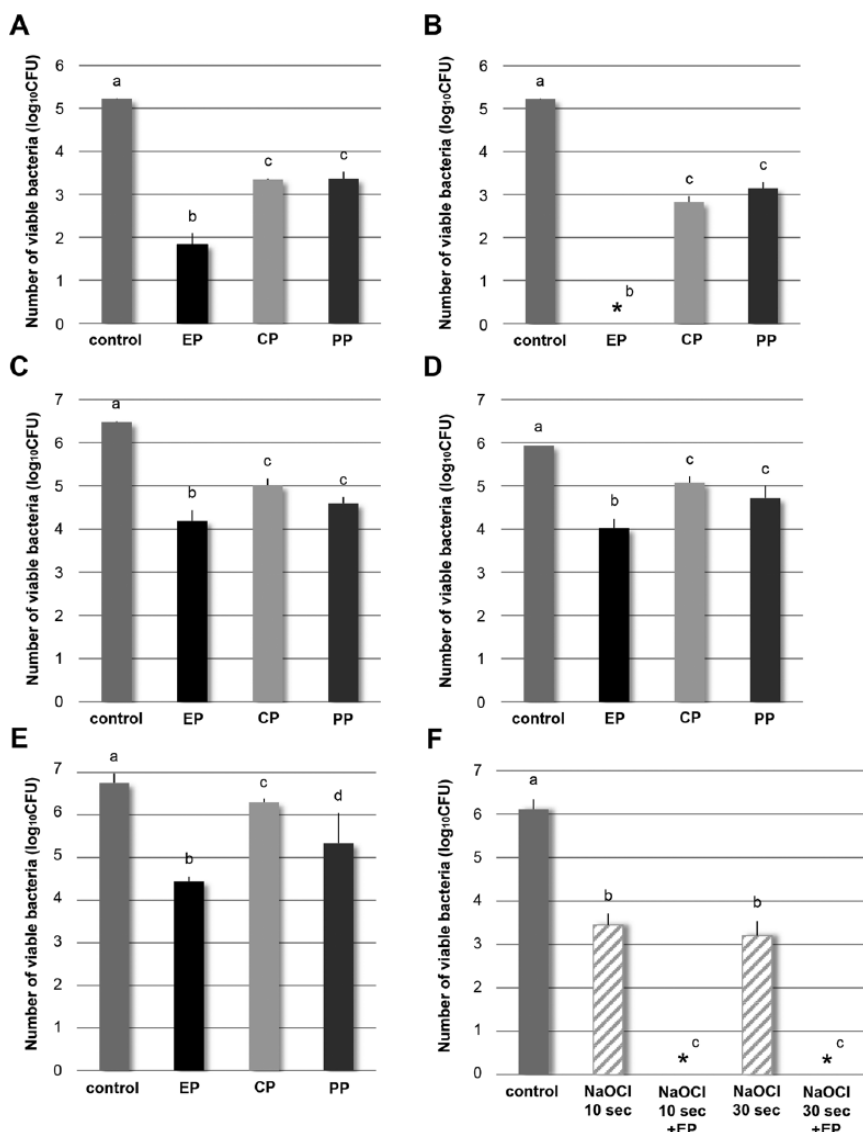


Figure 1. Number of viable *Enterococcus faecalis* after contact with the primer. (A, B) Planktonic bacteria and (C, D) adherent bacteria. Contact times: 30 sec (A, C), 60 sec (B, D). (E, F) Infected root model. (F) Treatment with 5% NaOCl alone or 5% NaOCl + EP. *No bacteria were recovered. The bar represents the standard deviation of 3 replicates. No significant differences between the same letters (i.e., a-d; analysis of variance and Student-Newman-Keuls's test; $p < .05$). CP, control primer; EP, experimental primer; PP, proprietary primer.

Irrigation with NaOCl reduced viable bacteria in the dentinal tubules, but complete elimination could not be obtained. However, application of EP after NaOCl irrigation resulted in 100% killing for both irrigation times by NaOCl (Fig. 1F).

Bonding Ability

The EP-SA sealer group demonstrated significantly greater MTBS than the PP-Epiphany sealer group ($p = .001$; Fig. 2A). By SEM observation of the interface, close contact of SA sealer with root dentin was observed, and no gap was found in any part of the root (Fig. 2B). Resin tags penetrated inside the dentinal tubules for a depth of up to approximately 10 μ m. Formation of

a thin hybridized zone, less than 0.5 μ m thick, was seen (Fig. 2C).

Fluid Filtration Tests

After 1 wk, the fluid flow rate was null for the EP-SA sealer group, and those of the EP-SA sealer and CP-SA sealer groups were significantly lower than the PP-Epiphany sealer and ZOE sealer groups ($p < .05$) (Fig. 3A). After 4 wk, although the values of all groups increased, EP-SA sealer and CP-SA sealer groups showed significantly lower values than the PP-Epiphany sealer group ($p < .05$) (Fig. 3B).

DISCUSSION

Many studies on various properties of the HEMA-based, light-cured self-etching primer containing 5% MDPB for composite restoration are available, including demonstration of its strong antibacterial activity against caries-related bacteria (Imazato *et al.*, 1997, 2004, 2014; Özer *et al.*, 2003; Carvalho *et al.*, 2012). It has also been reported, through *in vitro* and *in vivo* studies, that incorporation of MDPB into the light-cured primer up to 5% had no adverse influences on the bonding ability to dentin (Imazato *et al.*, 1997, 2007). Therefore, an experimental chemically cured primer containing 5% MDPB for root canal filling (EP) was developed, expecting maximum antibacterial effects and high sealing ability. All 3 primers tested showed antibacterial effects due to their acidic composition to some extent. However, EP showed the highest antibacterial activity of all against planktonic and adherent bacterial during a short-period contact. This indicates that MDPB incorporated in chemically cured

primer can attack bacteria in a rapid manner based on the killing mechanism of quaternary ammonium compounds (McDonnell and Russell, 1999). It has been reported that the light-cured MDPB-containing primer for composite restorations had the ability to penetrate deeply into dentin *in vitro* (Schmalz *et al.*, 2004) and *in vivo* (Imazato *et al.*, 2004). The ability of MDPB-containing EP to penetrate and kill bacteria in root canal dentin was clarified by *via* the infected root model.

E. faecalis is relevant to secondary and persistent root canal infection (Siqueira and Rôças, 2005). Complete elimination of *E. faecalis* in the infected root model was not achieved by irrigation with 5% NaOCl. In the model used, bacterial penetration into the dentinal tubules reaching up to approximately

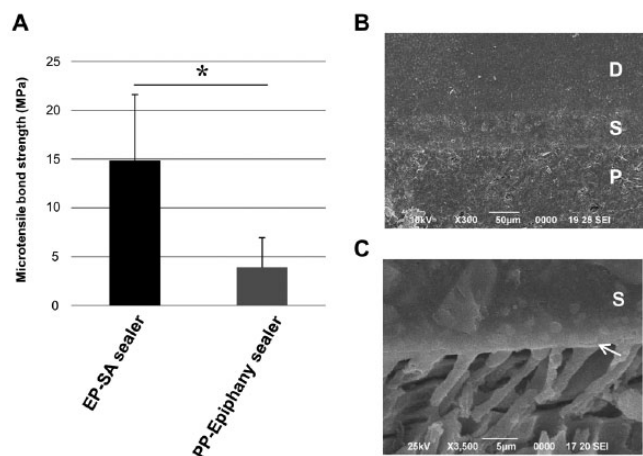


Figure 2. Bonding ability of experimental sealer system. **(A)** Microtensile bond strength of EP-SA sealer and PP-Epiphanly sealer. The bar represents the standard deviation of 24 specimens. Three dish-shaped slabs (approximately 1.0 mm thick) were obtained by slicing filled root perpendicular to the tooth axis, and an hourglass-shaped specimen was trimmed from each slab with a diamond point. Microtensile bond strength tests were conducted with a crosshead speed of 1.0 mm/min. Pretesting failure, resulting in 0 MPa, was seen in 1 of 24 specimens of the EP-SA sealer group and 10 of 24 specimens of the PP-Epiphanly sealer group. For the EP-SA sealer group, adhesive failure at the interface between dentin and sealer was seen in 15 of 24 specimens, and 6 of 24 specimens showed mixed adhesive failure and cohesive failure within the dentin or within the sealer. In the PP-Epiphanly group, 21 of 24 specimens showed adhesive failure, and the rest showed a combination of adhesive failure and cohesive failure within the sealer. *Significant differences (Student's *t* test; $p = .001$). **(B)** Scanning electron microscope image of a section of root filled with EP-SA sealer. **(C)** Scanning electron microscope image of the bonding interface of the EP-SA sealer to root dentin observed after treatment with 50% phosphoric acid for 2 min and 12% NaOCl for 4 min. Formation of resin tags and a hybridized zone (arrow) can be seen. D, dentin; EP, experimental primer; MPa, megapascal; P, resin point; PP, proprietary primer; S, SA sealer.

300- μ m depth was observed by Brown and Brenn staining (Appendix Figure), which is consistent with the previous report (Haapasalo & Ørstavik 1987). NaOCl may not have been able to reach the deeper part of tubules even when application time was prolonged. However, application of EP was effective to kill residual bacteria after NaOCl irrigation, and all *E. faecalis* could be eradicated. Elimination of bacteria in the shallow part by NaOCl irrigation enabled complete killing of bacteria by EP treatment in the deeper part of the dentinal tubules. Therefore, the new treatment strategy to use MDPB-containing primer for root canal filling is expected to contribute to successful endodontic treatment. To visualize the antibacterial effects of EP against the bacteria in dentinal tubules, further observation of bacterial viability in infected root is under progress through confocal laser scanning microscopy as reported previously (Ma *et al.*, 2011).

Many studies indicated the presence of interfacial gaps between dentin and the Epiphany system (Tay *et al.*, 2005; Costa *et al.*, 2010). The MTBS value of the EP-SA sealer group was 3 times higher than that of PP-Epiphanly sealer. In addition, the EP-SA sealer group demonstrated a lower

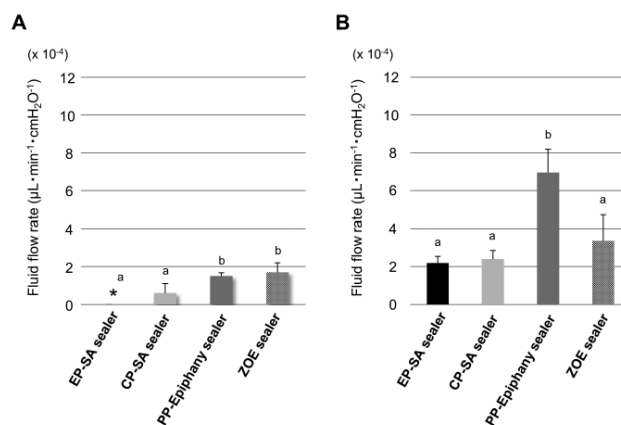


Figure 3. Fluid flow rates of EP-SA sealer, CP-SA sealer, PP-Epiphanly sealer, and ZOE sealer. **(A)** After 1 wk of storage and **(B)** after 4 wk of storage. The bar represents the standard deviation of 3 replicates. *No fluid flow was observed. No significant differences between the same letters (*i.e.*, a, b; analysis of variance and Student-Newman-Keuls's test, $p < .05$). CP, control primer; EP, experimental primer; PP, proprietary primer.

chance of adhesive failure than PP-Epiphanly sealer and cohesive failure within the sealer or dentin. These findings support high bonding ability of the EP-SA sealer group to root dentin. By observing the interfacial morphology, close contact of the filling with root dentin was found when EP and SA sealer were used. The effective bonding of EP-SA sealer to root dentin is clear from the results of observation of the interface with SEM to show formation of resin tags and a hybridized zone. The self-etching primer EP contains MDP (10-methacryloxydecyl dihydrogen phosphate), and chemical binding of MDP to hydroxyapatite (Yoshida *et al.*, 2004) in addition to hybridization is considered to contribute to the high bonding ability of EP-SA sealer.

Many recent studies used the fluid filtration method to assess the sealing ability of root fillings (Biggs *et al.*, 2006; Paqué & Sirtes, 2007). After both 1 and 4 wk of storage, the EP-SA sealer and CP-SA sealer groups showed better sealing abilities than the PP-Epiphanly sealer group. It has been reported that the sealing ability of root canal sealer correlates with bonding ability (Neelakantan *et al.*, 2011). The superior bonding ability of EP-SA sealer, as demonstrated by MTBS tests, was effective at improving the sealing ability. Epiphany sealer was reported to show extensive calcium release and high solubility because of the erosion of filler particles (Versiani *et al.*, 2006). Even when the SA sealer in combination with EP or CP showed deterioration of sealing after 4 wk, it was found to have better sealing ability than others.

Antibacterial monomers, including MDPB, have been shown to demonstrate bacteriostatic effects after polymerization (Imazato *et al.*, 1994, 2014; Li *et al.*, 2009; Antonucci *et al.*, 2012). Therefore, it is possible that EP inhibits bacterial growth after being cured by immobilized MDPB. The long-term antibacterial effects shown by endodontic filling materials contribute to better clinical results, and such usefulness of the experimental MDPB-containing primer for root canal filling is of further interest.

CONCLUSIONS

This *in vitro* study demonstrated that an experimental antibacterial root canal filling system has the ability to effectively disinfect the root canal and is useful for achieving good sealing, indicating its possible benefit for successful endodontic treatments.

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