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Effects of immersion in 4-methacryloyloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butyl borane resin-activated liquid on microtensile bond strength of root canal dentin

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Abstract *Background/purpose:* 4-methacryloyloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butyl borane (4-META/MMA-TBB) resin is used for indirect restorations. We aimed to evaluate effects of immersion in 4-META/MMA-TBB-activated liquid on the bond strength of root canal dentin.
Materials and methods: We used freshly extracted single-rooted human teeth. After decoronation, each root was vertically sectioned into halves; their dentin walls were polished and flattened. The control group underwent dentin treatment with Green Activator. The immersion group was treated with Green Activator and Teeth Primer and immersed in 4-META/MMA-TBB-activated liquid. After bonding the resin blocks with Super-Bond, microtensile bond strength (μTBS) tests were performed (n = 6), and fracture surfaces were analyzed. Before surface treatment, dentin was immersed in a sodium fluorescein solution for 3 h, and resin blocks were bonded with Super-Bond with rhodamine B as in the bond strength test. The bonded cross section was observed using confocal laser scanning microscopy (CLSM).
Results: μTBS was significantly higher in the immersion group than in the control group (61.5 [51.3–66.7] vs. 33.0 [20.4–57.8] MPa; $P < 0.05$). Fracture mode analysis showed that, compared with the control group, the immersion group had a significantly lower rate of adhesive failure at the dentin interface and a significantly higher rate of cohesive failure in Super-

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Bond ($P < 0.01$). CLSM showed a water droplet-like accumulation of fluorescein dye above the hybrid layer in the control group, not in the immersion group.
Conclusion: Immersion in a 4-META/MMA-TBB-activated liquid inhibited water exudation from the root canal dentin and improved the bond strength.

Introduction

Recently, fiber-reinforced composite resin post-and-cores have increasingly replaced cast metal post-and-cores to reconstruct endodontically treated teeth. The clinical advantages of the composite resin foundation restoration include their ability to fabricate more esthetic restorations owing to the favorable translucency of the composite resin.[1] Their use could also reduce the incidence of metal allergic reactions.[2] Fiber-reinforced composite resin post-and-cores are expected to improve the long-term effectiveness of restorative treatments through prosthesis and tooth structure integration.[3]

There are two types of composite resin foundation methods: direct and indirect. In the indirect method, resin cements such as chemically cured and dual cured cements (light and chemical) are used. Chemically cured 4-methacryloyloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butyl borane (4-META/MMA-TBB resin; Super-Bond) contains tri-n-butyl borane (TBB) as the polymerization initiator. Water generally inhibits chemically cured resin cement polymerization. However, TBB reacts in the presence of a small amount of water.[5,6] In addition, 4-META/MMA-TBB has a longer curing time than light-cured resin cement, possibly reducing polymerization shrinkage stress at the bond interface.[4] Therefore, the 4-META/MMA-TBB resin is advantageous when bonding to dentin with a high moisture content.

Dentinal tubule arrangement differs based on location.[7] Dentinal tubules are perpendicular to the bond cross-section when bonding to root canal dentin and increase in diameter the closer they are to the pulp cavity.[8–10] Therefore, the percentage of dentinal tubule openings on the bonding surface is expected to be higher for root canal dentin than for crown dentin. The smear layer formed after dentin is cut inhibits the permeability of the dentinal tubules,[11] which prevents the exudation of water from the tubules.[12] When the percentage of dentinal tubule openings is high, bond strength is significantly affected by exudation of water from dentinal tubules on smear layer removal.[13] When bonding on dentin, such as for crown placement, self-etching primers are often used without completely removing the smear layer.[14] After root canal treatment, a thick smear layer forms over root canal dentin due to the influence of various agents,[15,16] and the penetration of resin monomers can be inhibited by retention of the smear layer.[17] Hence, even when a self-etching primer is used, the bond strength of the root canal dentin is higher when the smear layer is removed.[18,19] However, smear layer removal can cause water exudation from dentinal tubules into the adhesive layer, reducing the bond strength.

Although the adhesion of 4-META/MMA-TBB resin cement has been studied extensively, most studies have focused on crown dentin. Root canal dentin is more sensitive to water in the dentinal tubules than crown dentin. However, few studies have used human root canal dentin.[20,21] Investigating techniques of bonding to root canal dentin is crucial for improving the prognosis of the restored tooth. Thus, in this study, we aimed to evaluate effects of different surface treatments on root canal dentin bond strength after smear layer removal. The null hypothesis was that effects of moisture in dentinal tubules do not change with different surface treatment methods for root canal dentin and that the microtensile bond strength (μTBS) of surface treatment methods is similar.

Materials and methods

Specimen preparation

We used nine caries-free fresh human single-rooted teeth extracted owing to periodontal disease. These were used within six months after extraction. Teeth were collected with the approval of an appropriate ethics committee, and informed consent was obtained from the patients. The teeth were stored in a normal saline solution at 4 °C.

Fig. 1 presents a schematic diagram of the experimental procedure for the μTBS test. Materials used are listed in Table 1. To fabricate grip for the μTBS test, outer root surfaces were cut and built with composite resin (Clearfil DC Core Automix ONE; Kuraray Noritake Dental Inc., Tokyo, Japan). Crowns were separated from the tooth at the cementoenamel junction using a low-speed saw (Isomet; Buehler, Lake Bluff, IL, USA). The pulpal tissue was removed with K-files up to No. 40 (GC Corporation, Tokyo, Japan), and an RTP reamer (#2; Dentech Corporation, Tokyo, Japan) was used to prepare spaces for the post up to two-thirds of the root length. After preparing the root canal, each root was vertically cut into halves with a low-speed saw and polished with 600 grit wet SiC paper to flatten the pulp side dentin. Half of each root was divided into the immersion and control groups, respectively.

Bonding procedure

Dentin surfaces of the immersion group were treated with a mixed aqueous solution of 10 % citric acid and 3 % ferric chloride (Green Activator; Sun Medical Co., Ltd., Shiga, Japan) for 10 s, followed by rinsing and drying according to the manufacturer's instructions. The dentin was treated with Teeth Primer (Sun Medical Co., Ltd.) for 30 s, dried,

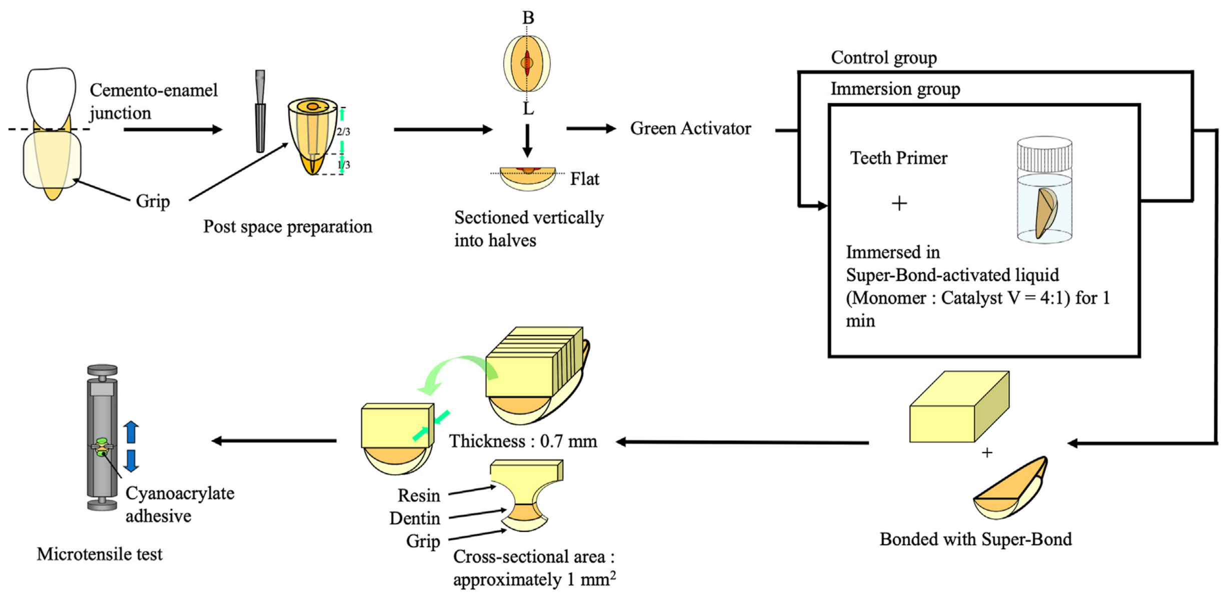

Figure 1 Schematic of the experimental procedure for microtensile bond strength testing.

and immersed in a large volume of Super-Bond-activated liquid (Monomer:Catalyst V = 32:8 drops; Sun Medical Co., Ltd.). The dentin immersion time was set at 1 min based on preliminary experiments, considering the clinical application. Dentin surfaces of the control group were only treated with Green Activator.

Bonding surfaces of resin blocks (10 × 5 × 7 mm) prepared with Clearfil DC Core Automix ONE were sandblasted with aluminum oxide (0.1 MPa) from 10 mm for 20 s, followed by the application of PZ Primer (Sun Medical Co., Ltd.). After surface treatment, resin blocks were bonded to dentin using 4-META/MMA-TBB resin cement (Super-Bond

Table 1 Composition of the materials used.

	Material	Manufacturer	Lot number	Composition
Resin cement	Super-Bond Monomer	Sun Medical	TW2	MMA, 4-META
	Super-Bond Catalyst V	Sun Medical	TX1	TBB
	Super-Bond Polymer (Bulk-mix Clear)	Sun Medical	TX1	PMMA
Dentin conditioner	Super-Bond C&B Green Activator	Sun Medical	TV1	Citric acid, $FeCl_3$, water
	Teeth Primer	Sun Medical	TW1	4-META, water, acetone
Composite resin block	Clearfil DC Core Automix ONE	Kuraray Noritake Dental	2J0377	Paste a: monomers (Bis-GMA, other methacrylic acid-based monomers), fillers (surface-treated glass powder, surface-treated silica-based microfillers, silica-based microfillers), photopolymerization catalysts, chemical polymerization catalysts, colorants Paste b: monomers (TEGDMA, other methacrylic acid-based monomers), fillers (surface-treated glass powder, surface-treated silica-based microfillers, alumina-based microfillers), chemical polymerization accelerators
Silane coupling agent	PZ Primer	Sun Medical	VK1	Liquid a: MMA, phosphate ester monomer Liquid b: MMA, silane compound

MMA: methyl methacrylate, 4-META: 4-methacryloyloxyethyl trimellitate anhydride, TBB: tri-n-butyl borane, PMMA: poly methyl methacrylate, Bis-GMA: bisphenol A-glycidyl dimethacrylate, TEGDMA: triethyleneglycol dimethacrylate

C&B [Bulk-mix clear]; Sun Medical Co., Ltd.). After bonding, specimens were stored at 100 % humidity and 37 °C for 1 h and in 37 °C water for 24 h.

Microtensile bond strength test

We used six teeth for the μTBS test. Perpendicular to the bonding surface, specimens were cut into 0.7-mm-thick slabs. Each slab was shaped with a diamond point (K3ff; GC Corporation) mounted on an air turbine handpiece under copious water into a dumbbell-shaped specimen with a cross-sectional area of approximately 1 mm^2. Dumbbell-shaped specimens were fixed with cyanoacrylate glue (Model Repair II Blue; Alteco Inc., Osaka, Japan) and tested on a universal testing machine (Autograph AGS-H; Shimadzu Corporation, Kyoto, Japan) at a crosshead speed of 1.0 mm/min.[4,22] μTBS values of three or four specimens from the same half of a root were averaged to obtain the μTBS value for that tooth.

Fracture analysis

After the μTBS test, fracture surfaces were analyzed under a 200 × digital microscope (VHX-900; Keyence Corporation, Osaka, Japan). The fracture mode was classified by calculating the fracture surface area using Image J, an image processing program.[23]

Scanning electron microscopy

Scanning electron microscopy of fracture surfaces

Scanning electron microscopy (SEM) of fracture surfaces was performed after the μTBS test. Specimens were mounted on a specimen holder with carbon tape, sputtered with platinum (ESC-101; Elionix Inc., Tokyo, Japan) and examined by SEM (JCM-6000Plus; Jeol Ltd., Tokyo, Japan).

Scanning electron microscopy of dentin surfaces after surface treatment

We used one tooth for SEM of dentin pre- and post-surface treatment. The dentin surface was treated in the same procedure as for the μTBS test. Samples were dried using the *t*-butyl alcohol freeze-drying method after graded dehydration with ethanol. Dentin was fixed and sputtered, similar to that for SEM of fractured surfaces, and examined using SEM (JSM-6701F; Jeol Ltd.).

Confocal laser scanning microscopy

We used two teeth for confocal laser scanning microscopy (CLSM). Before surface treatment, dentin was immersed in 0.005 % sodium fluorescein solution (F6377-100G; Sigma-Aldrich, St. Louis, MO, USA) for 3 h for labeling. Super-Bond Monomer was labeled with 0.00025 % Rhodamine B (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan). Using the same bonding procedure as that for the μTBS test, resin blocks were bonded to the root canal dentin. After embedding in resin (Palapress Vario; Kulzer GmbH, Hanau, Germany), specimens were sliced with a low-speed saw perpendicular to the bonding surface to a 0.1-mm thickness. Specimens were polished with 4000grit wet SiC paper and mounted on glass slides with mounting medium (Vectashield Mounting Medium; Vector Laboratories, Inc., Newark, CA, USA). Adhesive cross sections of each group were observed with CLSM (TCS SP8; Leica Microsystems, Wetzlar, Germany) and image analysis software (Leica Application Suite X; Leica Microsystems). CLSM was performed in the double fluorescence mode using a 63 × /1.40 OIL objective with optically pumped semiconductor lasers at excitation wavelengths of 488 and 552 nm.

Statistical analysis

Wilcoxon signed-rank test was used to analyze μTBS values. The minimum number of samples required for quantitative testing was determined using a power calculation of 0.8 with a confidence level of 95 %. The mean values (25.3) and standard deviations (12.8) used for estimation were obtained from the pilot study. Fracture modes were analyzed using the chi-square test and multiple comparisons using Bonferroni correction. SPSS (version 26.0; IBM, Chicago, IL, USA) was used to perform statistical analyses. The significance level was set at $P < 0.05$.

Results

Microtensile bond strength

A box plot of μTBS results is shown in Fig. 2. The median μTBS (61.5 [51.3–66.7] MPa) was significantly higher for the immersion group than (33.0 [20.4–57.8] MPa) for the control group ($P < 0.05$).

Fracture mode

Fracture modes are listed in Table 2. The rate of adhesive failure was significantly lower and cohesive failure within Super-Bond was significantly higher in the immersion group than in the control group ($P < 0.01$). Scatter plots of μTBS values and the dentin and cohesive failure rates within Super-Bond are shown in Fig. 3. Specimens with low bond strength tended to fracture at the interface between dentin and Super-Bond. Representative SEM images of fracture surfaces are shown in Fig. 4. Numerous bubble-like morphologies were observed on SEM images of specimens with adhesive failure with dentin (Fig. 4a1, a2). In SEM images of cohesive failure within Super-Bond, polymethyl methacrylate and unstructured Super-Bond were observed (Fig. 4b1 and b2).

Scanning electron microscopy findings

SEM images of the dentin surface pre- and post-surface treatment are shown in Fig. 5. Before surface treatment, a smear layer was observed on the dentin surface (Fig. 5a1, a2). Post-treatment with Green Activator, no smear layer was observed, and numerous dentinal tubule openings were present (Fig. 5b1, b2). After immersion in Super-Bond-activated liquid, reticulated structures were observed on the dentin surface in peritubular and intertubular dentin (Fig. 5c1, c2).

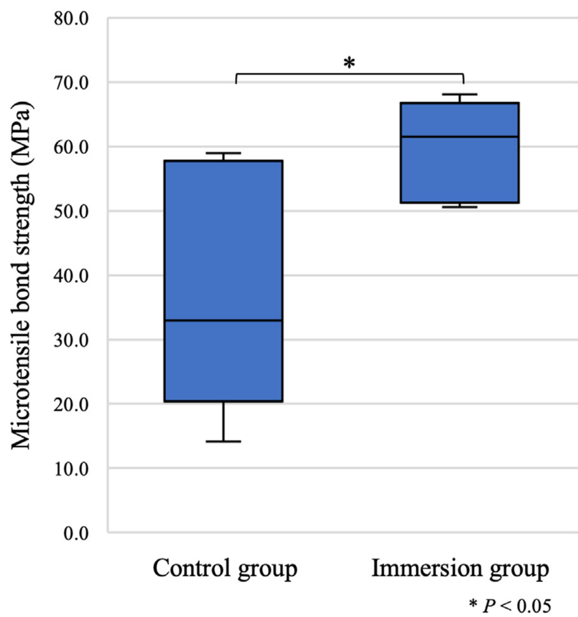

Figure 2 Box plot of microtensile bond strength. The median microtensile bond strength of 61.5 (51.3—66.7) MPa for the immersion group was significantly higher than that of 33.0 (20.4—57.8) MPa for the control group ($P < 0.05$). The μTBS values of three or four specimens from the same half of a root were averaged to obtain the μTBS value for that tooth (n = 6).

Confocal laser scanning microscopy findings

The bonded cross section observed on CLSM is shown in Fig. 6. Fluorescein dye accumulation was observed directly above and below the hybrid layer in the control group (Fig. 6a), but not in the immersion group (Fig. 6b).

Discussion

In this study, the μTBS values were observed to be significantly higher for the immersion group, which was treated with Green Activator and Teeth Primer followed by immersion in Super-Bond-activated liquid, than for the control group, which was treated with Green Activator alone. The null hypothesis that the μTBS value does not differ between surface treatments of root canal dentin was rejected. An analysis of the relationship between μTBS values and fracture mode showed that specimens with low bond strength tended to fracture at the interface between dentin and Super-Bond. SEM observations of fractures at the dentin interface revealed numerous bubble-like structures that resembled dentinal tubule openings. CLSM revealed water droplet-like accumulations of fluorescein dye just above the hybrid layer in the control group and film-like accumulations of dye just below the hybrid layer. Thus, fluorescein-stained water in dentinal tubules was present immediately above and below the hybrid layer. Thus, smear layer removal by treating the root canal dentin with Green Activator may cause water from the dentinal tubules to exude into the adhesive layer, which easily causes an interfacial fracture in the adhesive layer and low μTBS.

After smear layer removal by Green Activator treatment, immersion in Super-Bond-activated liquid resulted in a significantly lower rate of adhesive failure with dentin. CLSM revealed no fluorescein-dye accumulation immediately above the hybrid layer. Thus, immersion in Super-Bond-activated liquid may have suppressed water exudation from dentinal tubules, reducing adhesive failure and increasing bond strength. Therefore, the null hypothesis that the effect of water on the adhesive layer does not change with different dentin surface treatments was rejected.

Herein, dentin was treated with Teeth Primer followed by immersion in Super-Bond-activated liquid. 4-META (4-MET), a Teeth Primer component, promotes monomer penetration[24] and was used here to allow the resin monomer to penetrate more deeply into the dentin. Previous studies have reported no difference in bond strength between Teeth Primer and Green Activator treatments.[25] In the pilot study, no obvious change in fracture surface properties was observed with the Teeth Primer treatment alone after smear layer removal. Therefore, in this study, only the group immersed in Super-Bond-activated liquid after the Teeth Primer treatment was considered the experimental group.

Pre-surface treatment SEM images of the dentin showed that it was covered with a thick smear layer. Green Activator treatment resulted in smear layer removal and dentinal tubule opening. Therefore, smear layer removal with citric acid may increase dentin permeability and decrease bond strength, and the effect of water in dentinal tubules should be considered when bonding to root canal dentin. After immersion in the Super-Bond-activated liquid, a reticulated structure was observed on the dentin surface, indicating that the resin monomer penetrated deeply into the demineralized dentin during the impregnation process, filling dentinal tubules with simultaneous exudation of water and thereby occluding tubules.

Table 2 Fracture mode in the specimens.

	Control group	Immersion group	Total
Cohesive failure in dentin	$1^{a, b}$	$2^{a, b}$	3
Adhesive failure at the luting-dentin interface	10^{b}	2^{a}	12
Cohesive failure in luting cement	6a	16^{b}	22
Adhesive failure at the luting-resin interface	$4^{a, b}$	$2^{a, b}$	6
Cohesive failure in resin block	$2^{a, b}$	$0^{a, b}$	2
Total	23	22	45

Groups identified by different superscripts have significant differences ($P < 0.01$). The immersion group had a significantly lower rate of adhesive failure with dentin and a significantly higher rate of cohesive failure within Super-Bond than the control group.

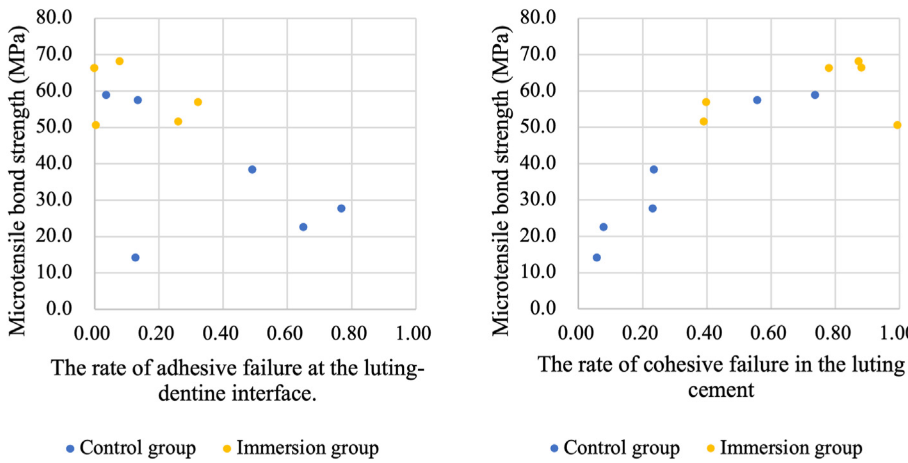

Figure 3 Scatter plots of the rate of each fracture mode and microtensile bond strength. Rate of each fracture mode in the total sample area (three or four samples) for each tooth (n = 1). Specimens with low bond strength tended to fracture at the interface between dentin and Super-Bond.

Super-Bond is chemically polymerized and has a longer curing time than light-cured resin cement. In the case of delayed light curing, water droplets are observed at the bond interface with dentin, and bond strength is reduced compared with that of immediate light curing.[26] Herein, the bond strength of the control group decreased owing to the penetration of dentin-derived water into the adhesive layer during the curing of Super-Bond.

The method and duration of adhesive application are reported to improve resin impregnation in dentin and improve adhesion.[27] Resin impregnation techniques for biological tissues have been applied in studies on tissue sections; various resins and impregnation techniques have been studied depending on the condition of the observation target.[28–31] In this study the intertubular dentin showed a more reticulated structure after immersion in the Super-Bond-activated liquid than in the control group, suggesting that the dentin surface has a structure that can be easily penetrated by the resin and that the monomer acts on the dentin surface for a longer time than usual, which in turn promotes resin impregnation and reduces the water content in the dentin.

This study elucidated the behavior of water in root canal dentin during bonding and its effect on bonding. Furthermore, immersion in 4-META/MMA-TBB-activated liquid has been suggested as an effective method to suppress water

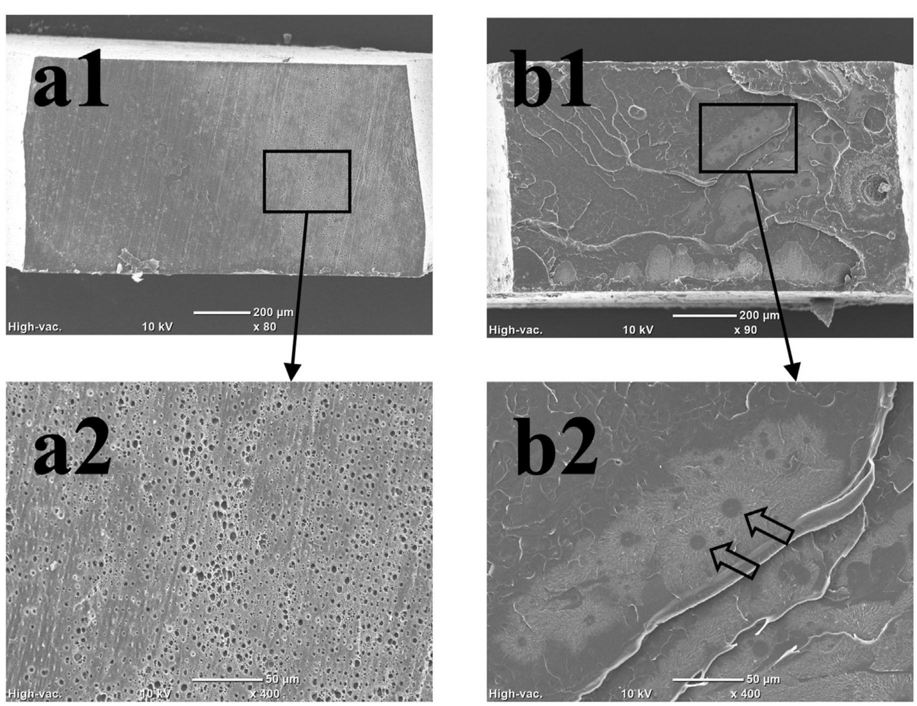

Figure 4 Representative scanning electron microscopy images (a1: 80 ×, b1: 90 ×, a2, b2: 400 × magnification) of fracture interfaces. (a1) and (a2) Adhesive failure at the luting-dentin interface. Numerous bubble-like structures are observed. (b1) and (b2) Cohesive failure within the Super-Bond. Polymethyl methacrylate (arrow) and unstructured Super-Bond are observed.

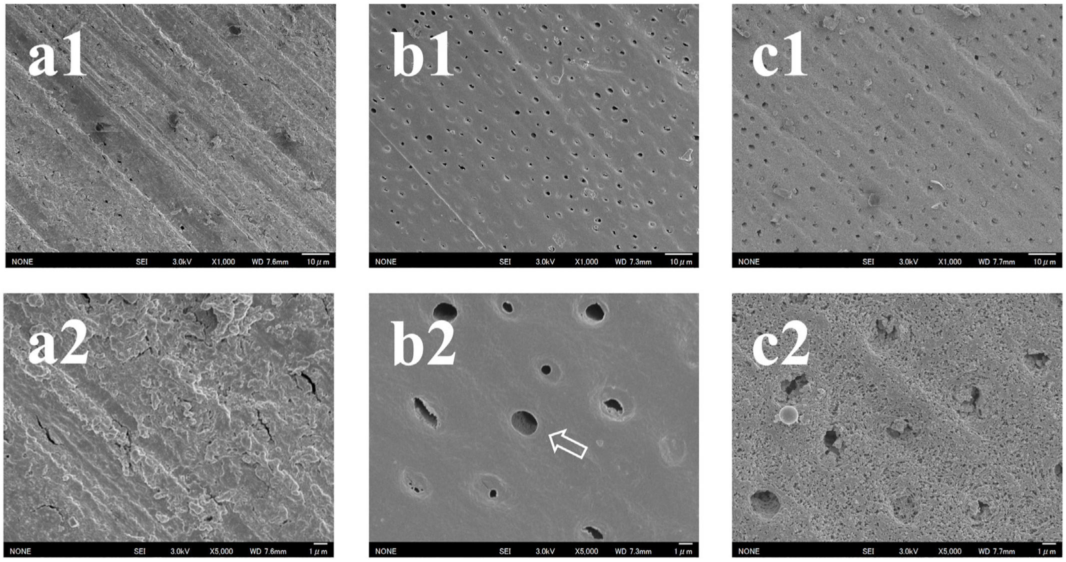

Figure 5 Representative scanning electron microscopy images (a1, b1, c1: 1000 ×, a2, b2, c2: 5000 × magnification) of the dentin surface after surface treatment. (a1) and (a2), after polishing with #600. A thick smear layer is observed. (b1) and (b2), after treatment with Green Activator. The smear layer has been removed, and the openings of the dentinal tubules are observed (arrow). (c1) and (c2), after immersion in the Super-Bond-activated liquid. A reticulated structure is observed in the peritubular and intertubular dentin.

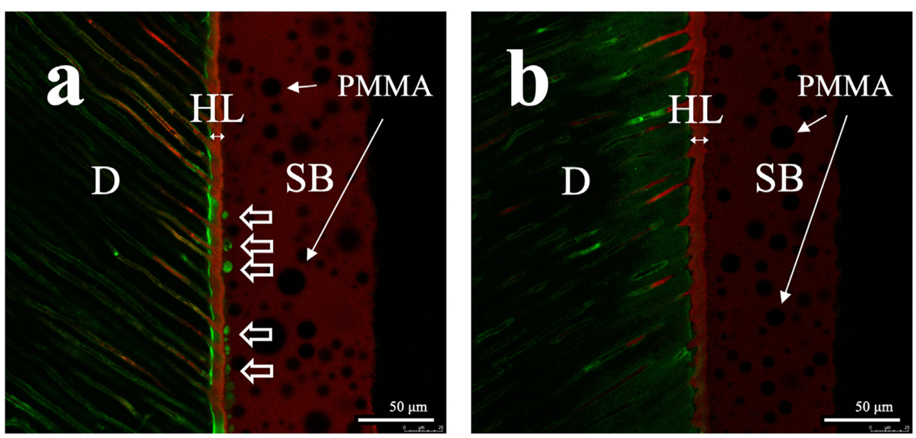

Figure 6 Representative confocal laser scanning microscopy images of the hybrid layers. (a) Control group. A water droplet-like accumulation of fluorescein dye is observed directly above the hybrid layer and a film-like accumulation of fluorescein dye is observed directly below it (arrow). (b) Immersion group. The accumulation of water droplet-like fluorescein dye observed in (a) is absent here. D: dentin. HL: hybrid layer. SB: Super-Bond. PMMA: polymethyl methacrylate.

exudation and increase bond strength. This approach leads to a better outcome of the restored teeth.

Herein, one tooth was cut into half: one half was assigned to the control group and the other to the immersion group. Therefore, the influence of differences between individual teeth on the experimental results was considered small. The fact that experiments were performed on a flat plate, which is different from the conditions in the oral cavity, is a limitation of this study. Clinically, it is important to note that the shape of the root canal, such as indicated by the C-factor (configuration factor), also affects results. We require research on root canals, incorporating methodologies such as injection of 4-META/MMA-TBB-activated liquid into the root canal instead of immersion. Furthermore, this study only assessed immediate μTBS, prompting future studies to explore the effects of thermal loading and coronal leakage. Additionally, evaluating the biocompatibility of 4-META/MMA-TBB-activated liquid and its long-term effect on surrounding tissues is considered necessary for a comprehensive understanding.

In conclusion, immersion in 4-META/MMA-TBB-activated liquid after smear layer removal inhibited water exudation from the root canal dentin, suggesting an improvement in the bond strength of dentin.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

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