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博 士 論 文

Effect of sodium hypochlorite on bonding performance of universal adhesives to pulp chamber dentin

（各種ユニバーサルアドヒーシブの髄腔内象牙質接着に対する３％次亜塩素酸ナトリウムの影響）

令和６年３月申請

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Introduction

Endodontically treated teeth (ETT) are more susceptible to biomechanical failure when compared to vital teeth.[1] This is mostly owing to the quantity of internal tooth structure being lost during endodontic treatment and the loss of coronal hard tissue.[2] ETT's long-term success depends on both the quality of endodontic treatment and the subsequent restorative techniques.[3] With an immediate and proper restoration following ETT, functional stresses can be transmitted across the bonded interface, eventually reinforcing the weaker tooth structure.[4] Despite the wide range of restoration options available, choosing the most appropriate treatment choice for a patient's clinical circumstance can be difficult because of the structural differences in the root filled teeth.[5-7]

Recent attention has been focused on methods of acquiring an effective coronal seal after endodontic therapy, despite the fact that coronal leakage is still a critical feature in endodontic failures and jeopardizes root canal treatment prognosis.[8] In addition, an effective coronal seal can be achieved with the use of bonded restorations, which were proven to be one of the most successful treatment options for preventing coronal microleakage.[9-11] Nevertheless, dentin-resin bonding can only be achieved if the resin is able to penetrate the pretreated dentinal surface and initiate micromechanical interlocking between the dentin collagen and resin, resulting in a hybrid layer formation.[12] Moreover, to ensure a successful bonded restoration, a strong bonding and sealing are necessary between the restoration material and dentin structure.[13]

During endodontic treatment, the use of irrigation solutions can influence the bonding performance between the pulp chamber dentin walls and adhesive material.[14] Sodium hypochlorite (NaOCl) is the most commonly used irrigation solution in endodontic

treatment. However, NaOCl has been shown in numerous investigations to decrease the bond strength between dentin and adhesive.[15-17] This could possibly be related to the existence of residual oxygen in dentin, which has an adverse effect on the polymerization of adhesive resin.[18-20] With the current dentin bonding system, numerous studies have focused on the surfaces of the occlusal, proximal, and root areas of the dentin with or without NaOCl treatment,[21-23] while the pulp chamber dentin adhesion has not been extensively investigated. This is a significant information gap since the pulp chamber dentin differs from other part of the coronal dentin, consisting of predentin as well as regular and irregular secondary dentin. Moreover, a typical smear layer has not often been formed owing to root canal therapy, usually without contacting pulp chamber dentin.[14] Consequently, bonding of the adhesive material to pulp chamber dentin is different from bonding to other dentinal surfaces covered with smear layers.[24] Therefore, it is very important to achieve a good bond to pulp chamber dentin, to enhance retention, and to maximize coronal seal. As a result, it is necessary to optimize bonding to pulp chamber dentin.

Therefore, the objective of this study was to evaluate the effects of NaOCl on the bond strength of four universal adhesives and one two-step self-etch adhesive to pulp chamber dentin. The null hypothesis was: (1) the bond strength would not be influenced by different adhesives on pulp chamber dentin; and (2) the bond strength would not be influenced by NaOCl application on pulp chamber dentin.

Materials and methods

Hokkaido University, Graduate School of Dental Medicine, research ethics committee approved this study (approval number 2018-7). One hundred sixteen extracted human third molars were kept in 0.5% chloramine-T at 4°C and used within 6 months

following extraction. The schematic diagram of this study is shown in Figure 1.

Teeth preparation and bonding procedures

One hundred extracted non-carious human third molars were used for the μTBS test. The pulp chamber was exposed by using a gypsum model trimmer under water coolant, and the root of the tooth was cut with model trimmer from the crown approximately 2 mm below the cementoenamel junction. Then the pulp tissue was removed carefully by a spoon excavator without touching pulp chamber dentin, followed by rinsing with distilled water. All the teeth were divided into two main groups: (1) with NaOCl treatment (2) without NaOCl treatment as a control group (Cont). In the NaOCl treatment group, all the teeth were immersed in 3% NaOCl for 20 minutes[15] followed by rinsing with distilled water using a syringe with a needle, whereas in the control group, all the teeth were rinsed with distilled water in the same manner. All the specimens of these two groups were randomly subdivided into five adhesive groups (n=10/group). The bonding groups were as follows: (1), G-Premio Bond (GP); (2), Beautibond Xtreme (BBX); (3), Scotchbond Universal (SBU); (4), Clearfil Universal Bond Quick (UBQ); (5), Clearfil Megabond 2 (MB2). Each adhesive was applied to the pulp chamber dentin according to the manufacture's instruction. Then the Clearfil AP-X resin composite was filled into the pulp chamber incrementally in 2 mm layers and light cured for 20 seconds until the cavity was filled. All the specimens were stored in distilled water at 37℃ for 24 hours. All the materials used in this study, along with their compositions and their application modes, are shown in Table 1.

μTBS test and fracture mode analysis

After storage in distilled water at 37℃ for 24 hours, each bonded specimen was vertically sectioned into resin/dentin slices using a low-speed diamond saw (Isomet

1000, Buehler, IL, USA). The first horizontal cut was made 0.5 mm below the specimen's surface to remove extra resin composite. Then three horizontal cuts were made of slices of 1±0.05 mm^2 and three beams randomly selected from each specimen for the μTBS test. The beams were fixed to a Ciucchi's jig with cyanoacrylate glue (Dentsply, Otahara, Japan) and subjected to tensile forces at a crosshead speed of 1 mm/min in a desktop testing apparatus (EZ-S, Shimadzu, Kyoto, Japan) until failure occurred. Bond strength was expressed in MPa. Each tooth's μTBS was represented by the mean bond strength of three beams obtained from that tooth, yielding 10 values for each tested group.[25]

After testing, the fractured specimens were carefully removed from the jigs and mounted on the aluminum stubs. Failure modes of the specimens were visualized by light microscope (×20 magnification; Magnifier Light, Asone, Osaka, Japan). Then coated with Pt-Pd for 120 seconds and confirmed by scanning electron microscopy (S-4000, Hitachi, Tokyo, Japan) at an accelerating voltage of 10kV. Failure modes on the dentin sides of specimens were taken into consideration and classified as follows: mixed failure, adhesive failure, cohesive failure in dentin, and cohesive failure in resin composite.

Interfacial observation by SEM

One tooth per group was bonded in the same way as described for the μTBS test. After 24 hours, the specimens were sectioned perpendicularly by the low-speed diamond bur and obtained two slabs of the lateral wall pulp chamber dentin-resin interface. The slabs were embedded with Epoxy (Struers, Copenhagen, Denmark) and cured at room temperature for 8 hours. Then the slabs were consecutively polished with SiC papers (#600, #800, and #1000) under running water and then with diamond pastes (6, 3 and 1

μm) (Struer, Copenhagen, Denmark). The slabs were cleaned with distilled water in an ultrasonic device after polishing with each size diamond paste. After that, they were treated with 1 mol/L hydrochloric acid for 30 seconds, followed by 5% sodium hypochlorite for 5 minutes, then rinsed with distilled water. All the slabs were coated with Pt-Pd for 120 seconds after drying at room temperature for 24 hours and observed with SEM.

Observation of dentin surface treated with adhesive

An additional six teeth were used and carefully removed pulp tissues as mentioned above. Then every tooth was vertically cut into two segments by a low-speed diamond saw. One segment was assigned as the control group and another segment was assigned as the NaOCl group from the same tooth and each group obtained two discs. Each segment of six teeth was assigned to subgroups according to five adhesives, whereas one tooth was assigned to observe without the application of adhesive. Application time of adhesives were followed according to the manufacturers' instructions without light curing. After applying the adhesive, the specimens were immediately immersed in 100% acetone for 1 minute to remove the applied adhesive, then fixed in 2.5% glutaraldehyde containing 0.1 mol/L sodium cacodylate buffer for overnight at 4℃ and rinsed with the same buffer for 1 minute followed by dehydrated in an ascending concentration series of ethanol (50%, 70%, 80%, 90% for 20 minutes, 95% for 30 minutes and 100% for 1 hour). Finally, the dehydrated segments were dried with hexamethyldisilazane (HMDS, Wako Pure Chemical, Osaka, Japan) for 10 minutes. The segments were mounted on aluminum stubs and coated with Pt-Pd using an ion sputter coating machine (E-1030; Hitachi, Tokyo, Japan) for 120 seconds and observed by SEM.

Statistical analysis

Statistical analysis was achieved using the SPSS software package, Statistical Package for the Social Sciences for Windows (SPSS version 25; Chicago, IL, USA). Normal distribution was checked with the Shapiro-Wilk test and homogeneity of variance was tested by Levene's test. According to those test results, a two-way ANOVA and t-test with Bonfferoni's correction as a post-hoc test were employed to analyze the μTBS (α=0.05).

Results

Microtensile bond strength

The results of the μTBS are summarized in Figure 2. The mean bond strength of five adhesives to pulp chamber dentin in this study were as follows: 28.50±3.38 MPa for GP-Cont, 18.43±5.09 MPa for GP-NaOCl; 31.30±5.30 MPa for BBX-Cont, 28.98±7.44 MPa for BBX-NaOCl; 31.83±5.31 MPa for SBU-Cont, 27.45±4.29 MPa for SBU-NaOCl; 30.83±5.40 MPa for UBQ-Cont, 27.30±4.88 MPa for UBQ-NaOCl; 37.63±4.00 MPa for MB2-Cont, 31.49±4.55 MPa for MB2-NaOCl. Two-way ANOVA revealed significant effects of adhesive ($F=12.182$, $P<0.001$), and irrigation ($F=27.224$, $P<0.001$) on the μTBS. In addition, interaction between adhesive and irrigation was not significant ($F=1.761$, $P=0.144$).

The bond strength of the NaOCl group was significantly decreased compared to control groups in GP and MB2 adhesives ($P<0.05$). Nevertheless, SBU, UBQ and BBX showed no significant difference between control and NaOCl group.

Fracture modes analysis

The percentage of fracture modes analysis are shown in Figure 3. There was no pretest

failure in this study. The fracture modes of all groups were mainly classified as mixed failure. In GP, more adhesive failure was observed with the NaOCl group. No adhesive failure was observed in the SBU-Cont, UBQ-Cont, BBX-NaOCl, MB2-Cont and MB2-NaOCl groups. No cohesive failure was observed in the BBX-Cont group.

Interface observation by SEM

The results of the dentin-resin bonding morphological observations are shown in Figure 4. The adhesive layer with variable thickness was observed in all groups. The GP, BBX and SBU showed thicker adhesive layer in the control group, whereas thinner adhesive layer was observed after NaOCl treatment group. On the other hand, UBQ and MB2 showed almost similar adhesive layer thickness both in control and NaOCl treatment groups. In GP, BBX and SBU control groups showed the shorter resin tags than NaOCl groups. Resin tag branches were observed in the UBQ-NaOCl group. Clear hybrid layer was observed in the MB2-NaOCl group.

Dentin surface observation by SEM

Pulp chamber lateral wall structure after removing pulp tissues is shown in Figure 5. Organic tissues that remained in the pulp chamber consist principally of collagen and line the innermost portion in the control groups. The predentin and exposed collagen on the pulp chamber dentin surface were eliminated after NaOCl treatment. Furthermore, through the action of NaOCl, open dentin tubules were clearly exhibited in all the bonding groups. Both tubular and peritubular dentin were observed in the MB2-NaOCl group.

Discussion

During the endodontic procedure NaOCl has been widely used at a concentrated level of

0.5-5.25% for irrigation which is an effective process removing tissue from the root canals and killing microorganisms.[26] Higher concentrations of NaOCl cause more cytotoxicity, erosion of dentin, collagen degradation, dentin deproteinization, and tooth surface strain while reducing microhardness, elastic modulus, and flexure of dentin.[27,28] In the present study, 3% NaOCl has been used which might be less cytotoxic, less erosive to dentin, less collagen degradation. On the other hand, 3% NaOCl showed 100% *Enterococcus faecalis* growth reduction, therefore might have better antibacterial properties.[29] Moreover, NaOCl acts as a non-specific deproteinizing agent, dissolving organic components on the dentin surface.[30]

In the present study, 3% NaOCl is used as an irrigation solution for 20 minutes on the pulp chamber dentin to evaluate the bond strength with different adhesives. The estimated time used for 15-20 minutes was considered to be the clinically equivalent endodontic procedure which is in accordance with other study.[15] Results of this study indicated that the 3% NaOCl treated group had significantly lower bond strength in GP and MB2 adhesives, when compared with without NaOCl treated group, therefore rejecting the first and second null hypothesis. Noteworthy, all NaOCl groups showed a decreasing trend of μTBS. These findings are in agreement with other reported studies that evaluated the NaOCl effect on pulp chamber dentin.[8,14,20,31] This might be because of NaOCl is an oxidizing chemical substance that necessitates for solid restraint of the adhesive restorative materials' interfacial polymerization.[18] In this contest, the reactive residual free-radicals from the NaOCl in the dentin might complete the vinyl-free radicals' propagation from the resin light activation system, and consequently, a premature chain termination is formed along with an unfinished polymerization.[18,32] Although the use of NaOCl in this study might inhibit the polymerization which may varies depending on the adhesives.

The μTBS of GP was significantly decreased which might be due to the polymerization inhibition of NaOCl treatment and had a higher percentage of adhesive failure in the GP-NaOCl group. GP has the intermediately strong acidic ability which created a more porous dentin surface and enlarged funnel-shaped orifices of dentinal tubules because of the absence of a smear layer in the pulp chamber dentin (Fig. 5). Furthermore, voids were observed in resin-dentin interface in the GP-NaOCl group that might cause inadequate solvent evaporation or phase separation, which can lead to the lower bond strength (Fig. 4).[33,34] On the other hand, BBX did not exhibited any voids and statistically not reduced the bond strength. Although GP and BBX do not contain HEMA, acetone is used as a solvent. This might be because of the absence of filler and the low viscosity characteristics into their adhesive system. The authors speculate that the low viscosity characteristics of BBX with strong air blow might contribute to removing the water moisture easily from the adhesive layer. In addition, BBX does not contain functional monomer 10-MDP which is differ to other tested adhesives in this study. The presence of 10-MDP is identified as capable of establishing a very intensive and stable chemical interaction with hydroxyapatite.[35] Although, the 24 hours bond strength of BBX showed similar results with other tested universal adhesives. This might be because BBX contains different functional monomers into their adhesive system. Moreover, the bonding durability of BBX still needs future study.

In the present study, SBU and UBQ showed a slight decrease in μTBS, but no significant difference was noted. This might be because of their different composition in their adhesive system. This is in accordance with other previous study.[20] On the other hand, the bond strength of MB2-NaOCl group significantly decreased but showed higher bond strength compared to other tested universal adhesives. This might be because MB2 produced a thicker hybrid layer in NaOCl group which may be attributed

to the higher 10-MDP concentration.[36] Another explanation might be the separate application of hydrophilic primer which demineralized the dentin and an additional layer of solvent-free hydrophobic bond agent, creating a stronger adhesive layer than all-in-one adhesive system.[36]

In conclusion the result of this study suggested that the effect of 3% sodium hypochlorite treatment on the bond strength differs depending on the type of universal adhesive. The lower bond strength on pulp chamber dentin may relate to the influence of NaOCl in polymerization inhibition which varies depending on the adhesive components.

Declaration of competing interest

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

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Table. 1 Chemical composition and application mode of the materials used in this study.

Materials	pH	Composition	Application mode
Universal Bond Quick (Kuraray Noritake, Tokyo, Japan) Lot no. CK0239	2.3	10-MDP, Bis-GMA, ethanol, HEMA, hydrophilic amide monomer, colloidal silica, silane coupling agent, sodium fluoride, dl-CQ, water.	1. Apply the adhesive for 10 s. 2. Gentle air blow for 5 s. 3. Light cure for 10 s.
G-Premio Bond (GC corporation, Tokyo, Japan) Lot no. 2002181	1.5	10-MDP, 4-META, MDTP, methacrylate acid ester, fine powdered silica, photoinitiator, synergist, acetone, water	1. Apply the adhesive for 10 s. 2. Strong air blow for 5 s. 3. Light cure for 10 s.
Scotchbond Universal Adhesive (3M ESPE, USA) Lot no. 6697326	2.7	10-MDP, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	1. Apply the adhesive for 20 s with vigorous agitation. 2. Gentle air blow for 5 s. 3. Light cure for 10 s.
Beautibond Xtreme (Shofu; Kyoto, Japan) Lot no. 122012	2.3	Acetone, water, Bis-GMA, carboxylic acid monomer, TEGDMA, organophosphate monomer, acid resistant silane coupling agent	1. Apply the adhesive. 2. Gentle air blow 3 s. 3. Strong air blow the surface. 4. Light cure for 5 s.
Clearfil Mega BOND 2 (Kuraray Noritake, Tokyo, Japan) Lot no. 000086	<2.5	Primer: 10-MDP, HEMA, hydrophilic aliphatic dimethacrylate, dl-CQ, accelerators, water, dyes Bond: 10-MDP, Bis-GMA, HEMA, dl-CQ, hydrophobic aliphatic dimethacrylate, initiators, accelerators, colloidal silica	1. Apply the primer and leave for 20 s. 2. Gentle air-blowing for more than 5 s. 3. Apply the bond. 4. Gentle air blow to make the film uniform. 5. Light cure for 10 s.
Clearfil AP-X (Kuraray Noritake, Tokyo, Japan) Lot no. AEO119		Bis-GMA, TEGDMA, silanated barium glass filler, silanated silica filler, silanated colloidal silica, dl-CQ, initiators, accelerators, pigments	
ChlorCid™ J (Ultradent Products, INC. USA) Lot no. BK6YM		3% sodium hypochlorite, water	

Abbreviations: 10-MDP, 10-methacryloxydecyl dihydrogen phosphate; 4-META, 4-methacryloxyethyl trimellitic anhydride; MDTP, methacryloxydecyl dihydrogen thiophosphate; Bis-GMA, bisphenol A diglycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; dl-CQ, dl-camphorquinone; TEGDMA, Triethylene glycoldimethacrylate

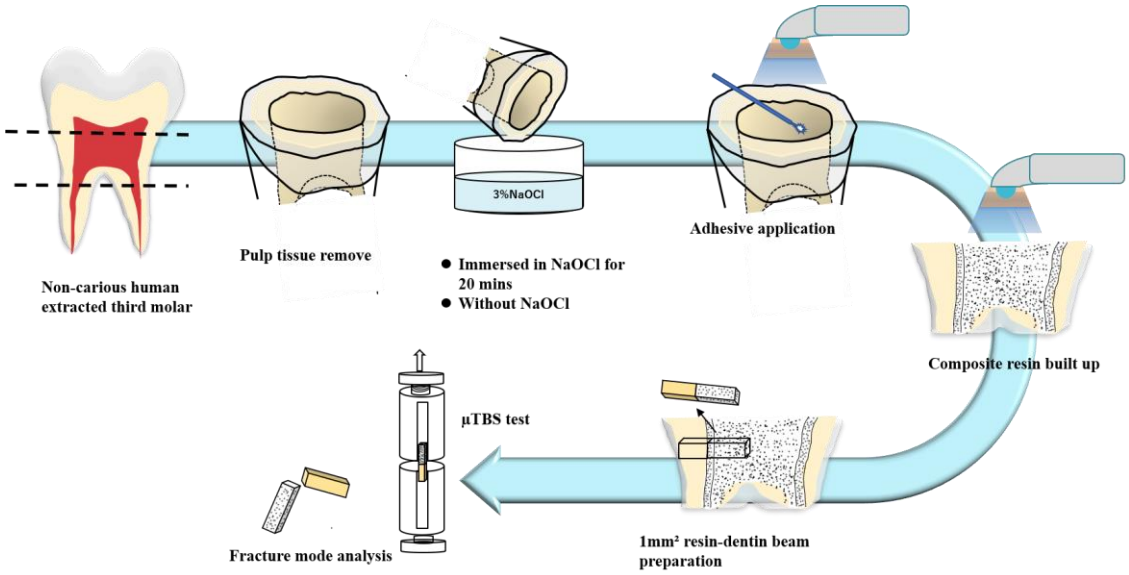

Figure 1. Graphical representation of specimen preparation for evaluating μTBS and fracture mode.

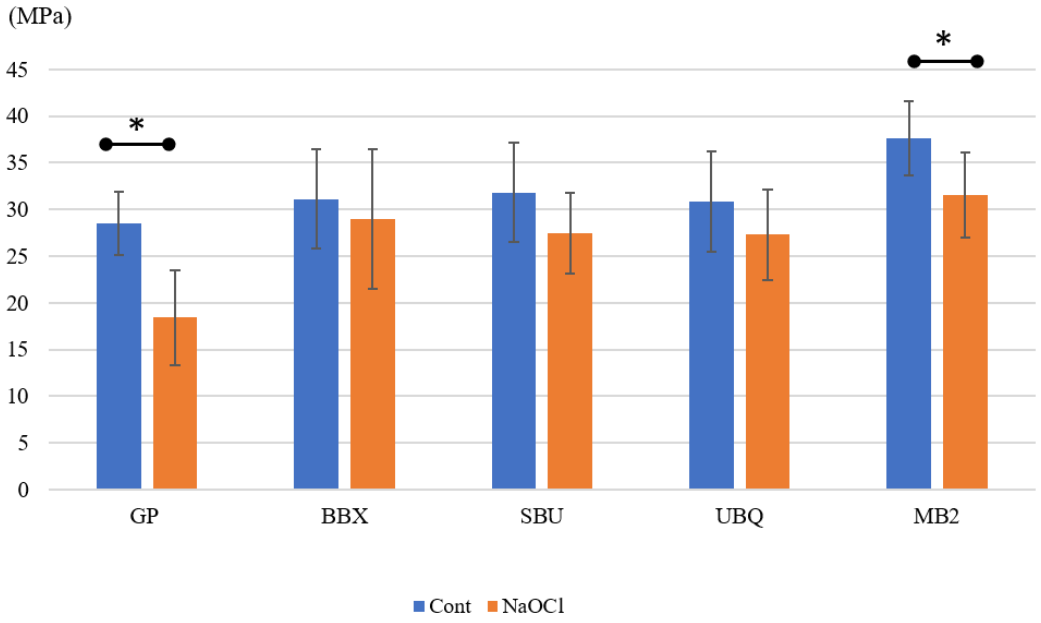

Figure 2. Mean (Standard deviation) of μTBS. *, The mark indicates statistically significant difference ($p < 0.05$).

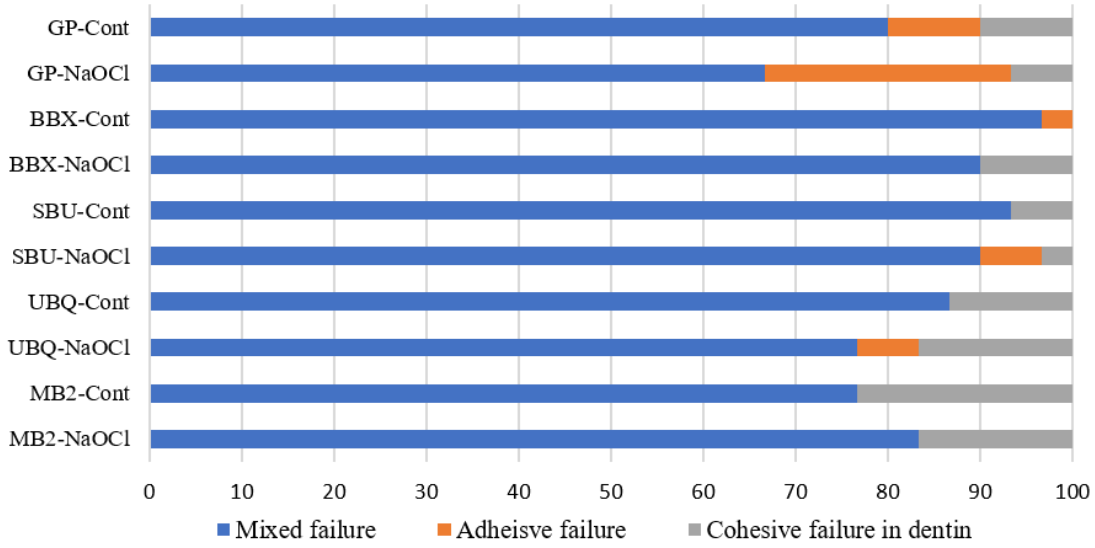

Figure 3. Percentage of failure modes.

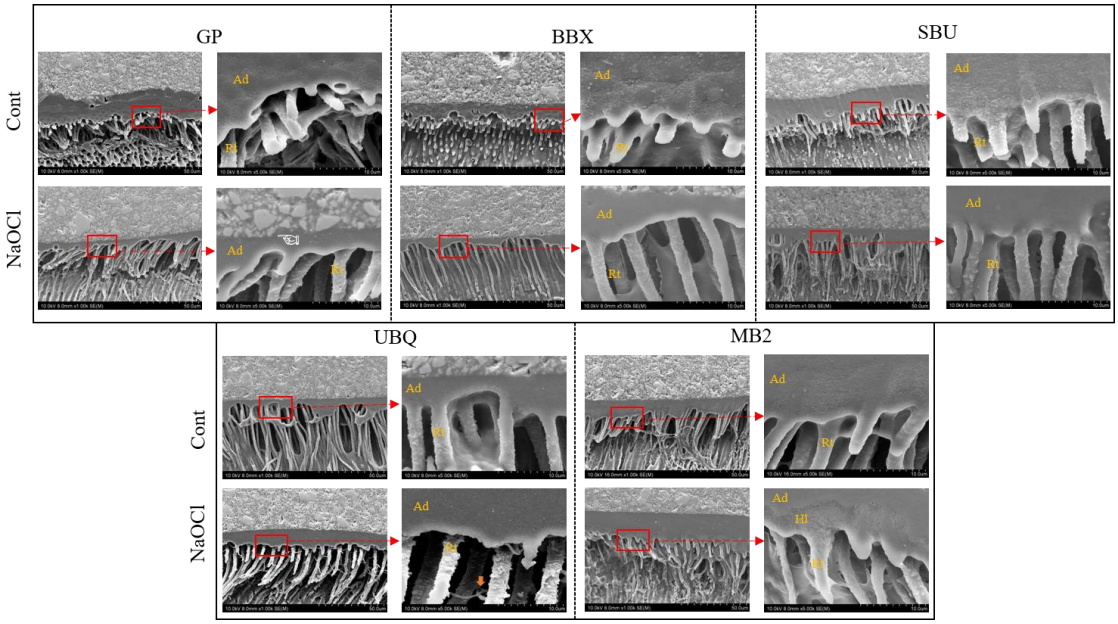

Figure 4. SEM images of dentin-adhesive interface. Hand pointer indicates voids remain in the adhesive layer; Ad indicate adhesive layer, Rt indicate resin tag; Hl indicate hybrid layer; the yellow arrow indicates the branches of resin tags.

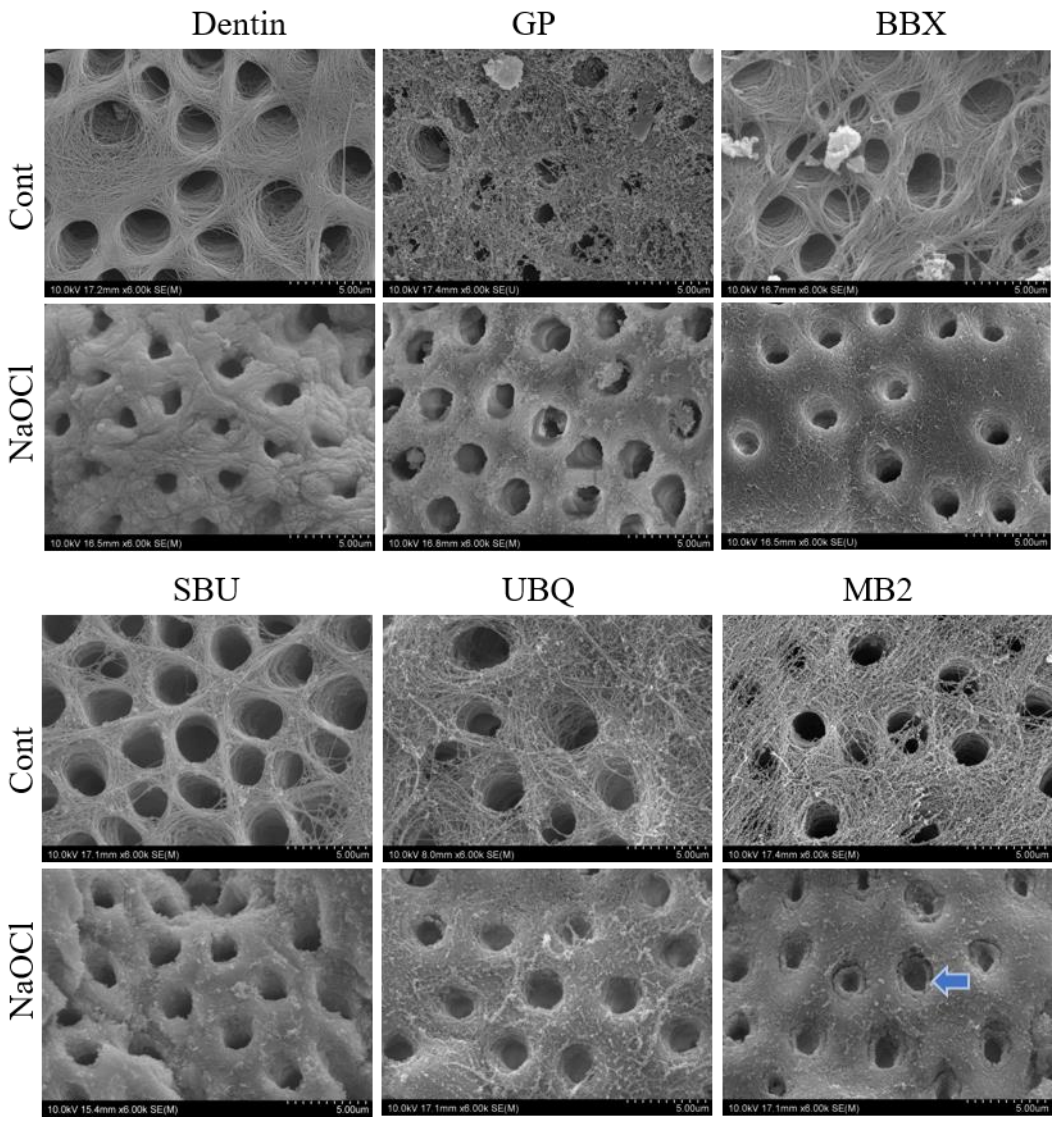

Figure 5. Representative SEM images of pulp chamber dentin surface treated with or without adhesive. Blue arrow indicates peritubular dentin.