Effect of EDTA and Maleic acid on Microtensile bond Strength of Self-etch Adhesive Resin Cement after Temporary Cement Removal

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Abstract

To investigate microtensile bond strength of self-etch adhesive resin cement to dentin after temporary cement removal by using EDTA and maleic acid. Forty-five extracted human molar were included in this study. Forty of the specimens were divided into two groups for temporary cementation on dentin using zinc oxide eugenol cement, and the others with zinc oxide non-eugenol cement. The remaining five specimens were used as a control group. After storing all specimens in distilled water at 37 oC for 24 hours, the specimens in the experimental groups were divided into 4 groups based on cleaning techniques: air water spray, pumice, 17% ethylenediaminetetraacetic acid (EDTA), and 10% maleic acid. To test microtensile bond strength, each specimen was bonded to a composite disc using self-etched adhesive resin cement before being sectioned into square rods with 1 mm2 cross sectional area. The microtensile bond strength was determined with a crosshead speed of 0.5 mm/minute. The data was statistically analyzed using two-way ANOVA with Holm-Sidak multiple comparison and one-way ANOVA on rank with Dunn's multiple comparison versus control (α=0.05).

The fractured surfaces were examined using light microscope to classify the mode of failure. The use of EDTA on the zinc oxide non-eugenol group and maleic acid on the zinc oxide eugenol group resulted no significant difference on microtensile bond strength compared to control group. Removing zinc oxide non-eugenol cement using EDTA and removing zinc oxide eugenol cement using maleic acid provided microtensile bond strength comparable to those of uncontaminated dentin.

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Introduction

The ability of dental adhesive to bond to tooth structures depends upon the infiltration ability of resin into enamel and dentin to form the micromechanical interlock.[1] The ultimate goals of dental adhesive are to form complete integration between dental restorations and tooth structure, to minimize post-operative sensitivity, and to prevent microleakage.[2] However, many factors may impede the bonding efficiency, such as the sensitivity of each technique's adhesive system, the outward movement of dentinal fluid under pulpal pressure, the thickness of the adhesive layer, blood and saliva contamination during bonding, and residual temporary cement on the tooth surface.[3-6]

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Provisionalization using temporary cement is a crucial procedure in fixed restorative treatment. Zinc oxide eugenol is the most commonly used temporary cement because of its advantages over other cements, including its sedative effect, ease of removal, good biocompatibility, and sealing efficiency.[7] However, the eugenol in this cement can penetrate into the deeper dentine and inhibit resin polymerization, resulting in a decrease in the bond strength and an increase in the microleakage of the permanent resin cement.[8-13] Therefore, zinc oxide without eugenol has been introduced into the market.

There is controversy about the reduction of bond strength of resin cement caused by eugenol and the remnants of temporary cement itself. The *in vitro* study suggested that the bond strength of resin cement to dentin was not affected if the surface was thoroughly cleaned prior to adhesive application.[14]

The current recommendation protocol for cleaning tooth surface before permanent cementation is polishing with pumice, since mechanical cleaning using an excavator and a rotary bur has been shown to be ineffective[3,15,16]. Mojon and Grasso et al[17, 18] found that residual pumice and temporary cement remained on the dentin surface when examined under SEM. This residual cement could reduce dentin wetting and interfere with the infiltration of resin monomer into the dentinal tubules.

Using acid or chelating agent is an alternative method of removing smear layer, remnant cement, and eugenol from dentin to increase the bond strength of resin cement.[19-22] Munirathinam et al[23] suggested that applying 17% EDTA for 1 minute was the most effective dentin cleaning technique as compared to polishing with pumice or applying 0.2% chlorhexidine gluconate. However, it has been claimed that maleic acid is more effective in removing smear layer and demineralized dentin than EDTA, but maleic acid has never been studied as a cleaning agent for dentin surface.[24-26] This *in vitro* study aimed to investigate the effectiveness of temporary cement cleaning techniques (air and water spray, pumice, 17% EDTA and 10% maleic acid) on microtensile bond strength of self-etched resin cement.

Materials and methods

This project was approved by the Human Experimentation Committee, No. 11/2019 for using human tissue in research. Forty five carie-free and unrestored extracted human third molars were included in this experiment .They were cleaned and immersed in a solution of 0.5% chloramine-T in distilled water at room temperature. The specimens were prepared according to ISO/TS 11405/2015 specification. Each tooth specimen was mounted in dental stone type IV block with the buccal surfaces upward. The enamel and dentin surface was cut 3 mm from the buccal surface using slow speed cutting machine (Isomet, Buehler Ltd, Lake Bluff, IL, USA) to produce a flat surface, which was then polished with 600 grit silicon carbide paper. Five specimens were reserved as a control group.

The forty specimens were divided equally into 2 experimental groups in which dentin surfaces were contaminated with 1 of 2 types of temporary cement: zinc oxide eugenol (Temp-Bond®, Kerr Corporation, CA, USA, TB group) or zinc oxide non-eugenol cement (Temp-Bond NE®, Kerr Corporation, CA, USA, TN group). Acrylic discs (6 mm in diameter and 3 mm thick) were temporary cemented on the dentin surfaces under 10 N static load for 1 minute. After the cement had set, the specimens were stored in distilled water at 37 ^{o}C for 24 hours. The acrylic discs were removed and the dentin surfaces were mechanically cleaned using a spoon excavator until the dentin appeared macroscopically clean.

The specimens from each group were evenly divided into 4 subgroups, each with a different cleaning technique: air-water spray for 10 seconds (TBW, TNW), polishing with pumice under light pressure for 10 seconds (TBP, TNP), scrubbing with 17% EDTA for 1 minute (TBE, TNE), scrubbing with 10% maleic acid for 15 second (TBM, TNM). The specimens were rinsed for 1 minute with distilled water and gently air dried before bonding with resin cement.

Composite discs (5 mm in diameter, 3 mm thick, Filtek Z350, 3M ESPE, St. Paul, MN, USA) were prepared for permanent cementation to all specimens including the control group. The bonding surfaces were polished using 600 grit silicon carbide paper under running water, sandblasted with 50 μm aluminum oxide particles for 10 seconds, and cleaned in ultrasonic cleanser[27] prior to monobond N (Ivoclar Vivadent, Schaan, Liechtenstein) application according to the manufacturer's instructions. Dentin primer was applied on the prepared dentin surface for 1 minute and dried with air blower. Self-etch adhesive resin cement (Multilink Automix®, dual cure luting, Ivoclar-Vivadent, Schaan, Liechtenstein) was used to cement the composite disc to the dentin surface under 10 N static load for 1 minute. The cement was light polymerized for 40 seconds and allowed to set for 10 minutes.

For microtensile bond strength testing, the specimens were sectioned perpendicular to the bonding surface using a slow speed cutting machine to form three to four rectangular rods with cross-sectional areas of approximately 1 mm^2. Sixteen rods (n=16) were randomly selected from each subgroup and stored in distilled water at 37 ^{o}C for 24 hours before testing. Each rod was glued to the gripping device using cyanoacrylate adhesive (Model repair II blue, Tokyo, Japan), mounted to the universal testing machine (Instron Corp., Canton, MA, USA), and pulled with a cross-head speed of 0.5 mm/min using 500 N load cell until it fractured. The microtensile bond strength (MPa) was calculated from maximum force (N) divided by bonded surface area (mm^2). The fractured specimens were evaluated under a light microscope at 200x magnification and classified as adhesive, cohesive, or mixed failure. The failure modes were expressed in percentages.

In order to evaluate ultrastructure of the dentin surface of each experiment group, two additional third molars were prepared using similar technique as above. The acrylic discs were temporary cemented using zinc oxide eugenol on one tooth and zinc oxide non-eugenol on the other under 10 N static load for 1 minute. The acrylic discs were removed and the remaining cement was cleaned using spoon excavator. Each tooth was sectioned into 4 rods perpendicular to the cut surface and subsequently cleaned using the four techniques included in this study. They were left dry and coated with gold-sputtered technique and examined under SEM (JSM-5910LV; Joel, Tokyo, Japan) at 15.0 kV. The SEM photomicrographs were evaluated at x5000 magnifications.

Two statistical analysis methods were used to compare the difference of microtensile bond strength in this study; two-way ANOVA with Holm-Sidak multiple comparison and Kruskal-Wallis one-way ANOVA on rank with Dunn's multiple comparison versus control group.

Results

The microtensile bond strength (medians, semi-interquartile range) from all groups is presented in Table 1. The bond strengths of the TNE (40.12, 4.28 MPa) and TBM (33.40, 2.74 MPa) groups were not significantly different compared to that of the control group (42.22, 1.91 MPa). The TBW group exhibited the lowest bond strength (17.89, 4.01 MPa) followed by the TBP (19.32, 2.11 MPa) and TNW (23.79, 1.67 MPa) groups.

Cleaning methods	Bond strength (MPa)			
	Median	Upper quartiles	Lower quartiles	Semi- interquatiler range
Control (no temporary cement)	42.22	43.98	40.16	1.91
Zinc oxide eugenol cement (TB)				
TBW) Air water spray	17.89	20.97	12.95	4.01
TBP) Pumice	19.32	21.14	16.92	2.11
TBE) 17% EDTA	29.04	30.2	22.95	3.63
TBM) 10% Maleic acid	33.40*	35.03	29.55	2.74
Zinc oxide non-eugenol cement (TN)				
TNW) Air water spray	23.79	25.48	22.14	1.67
TNP) Pumice	26.66	30.68	25.2	2.74
TNE) 17% EDTA	40.12*	45.19	36.62	4.28
TNM) 10% Maleic acid	28.96	30.09	27.51	1.29

Table 1. The microtensile bond strength of each cleaning method.
* indicated no statistically different with control. (p<0.001).

Analysis of the interaction between type of temporary cement and cleaning techniques on microtensile bond strength using two-way ANOVA suggested that there was a statistically significant difference (p<0.001). Moreover, all pair of Holm-Sidak multiple comparison showed significant differences between temporary cement type and among cleaning techniques.

Further analysis of microtensile bond strength using one-way ANOVA on ranks and Dunn's multiple comparison showed no significant difference between either the TNE or TBM group and the control group, while the bond strength of the other groups was significantly lower than that of the control group (p<0.001).

Cleaning methods	Number of failure (n (%))		
	AD	MI	CO
Control (no temporary cement)	2 (12.5%)	14 (87.5%)	-
TBW	11 (68.75%)	5 (31.25%)	-
TBP	9 (56.25%)	7 (43.75%)	-
TBE	7 (43.75%)	9 (56.25%)	-
TBM	6 (37.5%)	10 (62.5%)	-
TNW	9 (56.25%)	7 (43.75%)	-
TNP	7 (43.75%)	9 (56.25%)	-
TNE	4 (25%)	12 (75%)	-
TNM	5 (31.25%)	11 (68.75%)	-

Table 2. Types and number of failure in microtensile bond strength testing.
AD; adhesive failure at interface, MI; mixed failure at interface and resin cement, CO; cohesive failure of resin cement.

The number of failure modes resulting from microtensile bond strength testing is presented in Table 2. In brief, no cohesive failure was found. The adhesive failure was found more often in the TBW, TBP, and TNW groups, while mixed failure was found more often in the other groups.

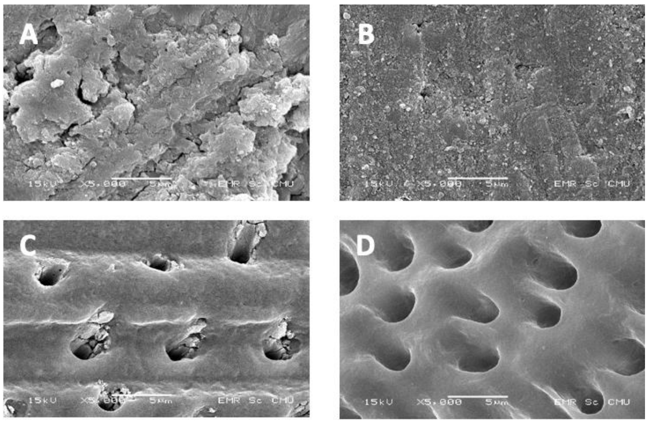

Figure 1. SEM micrograph at 500X magnification of contaminated dentin surfaces with zinc oxide eugenol using different cleaning methods A. air water spray, B. pumice prophylaxis, C. 17%EDTA, D. 10% maleic acid.

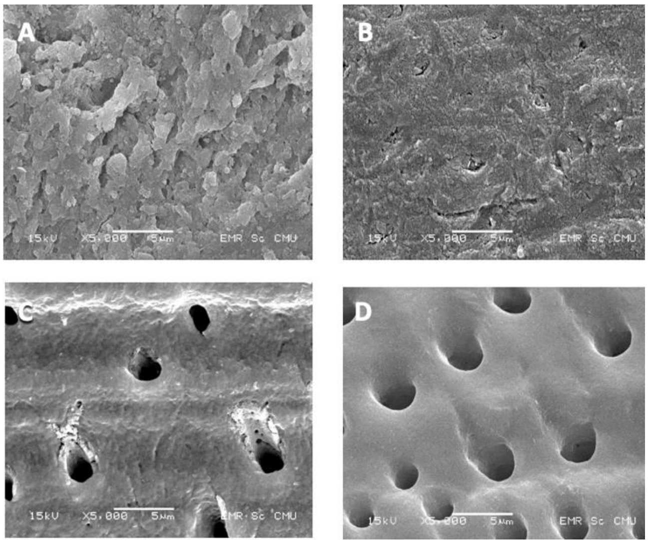

Figure 2. SEM micrograph at 500X magnification of contaminated dentin surfaces with zinc oxide non-eugenol using different cleaning methods A. air water spray, B. pumice prophylaxis, C. 17%EDTA, D. 10% maleic acid.

The SEM images of the dentin surface after cleaning using the various techniques in this experiment are presented in Fig. 1 and 2. In general, remnants of temporary cement were seen when air water spray was used. The smear layer was present after the specimens were polished with pumice, whereas the opening of dentinal tubules was found when 17% EDTA or 10% maleic acid was used to clean the surfaces. However, 10% maleic acid could remove the peritubular dentin and had more demineralized effect than 17% EDTA.[25]

Discussion

The results showed that the presence of residual temporary cement from either zinc oxide eugenol or zinc oxide non-eugenol can reduce the microtensile bond strength of permanent cement, corresponding to other reports.[28-31] Moreover, many studies claimed that cement containing eugenol had adverse effects on the bond strength, surface hardness, and wettability and inhibited the polymerization of methyl methacrylate in definitive resin cement.[10-12, 32-35] The results from this study also suggested that the microtensile bond strength of resin cement after using zinc oxide eugenol cement was significantly lower than the bond strength of resin cement after using zinc oxide non-eugenol cement ($p<0.001$).

The common clinical technique used for cleaning temporary cement-contaminated dentin surface is to use a spoon excavator and air water spray until the dentin surface is visually clean. Many studies[16-18] have reported that this mechanical removal method is not fully effective since the existing cement can be observed under SEM observation. Watanabe et al.[30] suggested that the temporary cement could not be mechanically removed by an excavator or air water spray even when preconditioned by any adhesive resin system. In this study, the microtensile bond strength of permanent cement after cleaning the dentin surface with air water spray was significantly lower than that of the control group for both types of temporary cement. However, the mechanical removal of zinc oxide eugenol cement showed greater significant reduction of microtensile bond strength than did the mechanical removal of zinc oxide non-eugenol cement.

The mechanical cleaning technique recommended by many studies[3,15,16] is polishing the bonding surface with pumice paste using a rubber cup and a slow speed handpiece under light pressure, after using a spoon excavator. This technique provides sufficient cleaning surface to receive the demineralization solution of resin cement.[36,37] However, it prevents the penetration of acidic monomer into the dentinal tubules due to the residual pumice and temporary cement plug caused by the force of the rotary instrument.[17,38] In this study, the tensile bond strength of permanent cement after using this mechanical cleaning technique was greater than that after using the air water spray technique, but lower than that after scrubbing with 17% EDTA or 10% maleic acid.

It has been claimed that the use of chelating agent (EDTA) and acids, such as phosphoric, polyacrylic and maleic acid to treat dentin surface promotes bond strength since they can eliminate smear layer, expose dentin's collagen, and facilitate hybrid layer formation.[19,21,39]

However, strong acid might have some adverse effects, such as post-operative sensitivity and pulpal irritation due to the excessive widening of dentinal tubules.[40,41] Applying 17% EDTA for 1 min or 10% maleic for 15 seconds in this study demineralized dentine and removed smear layer enough to widen the dentinal tubules but still left the deep part of smear plugs to prevent pulpal irritation. As a result, resin was able to penetrate into the dentinal tubules and the bond strength increased.[24,25,42-45]

The result of this study suggests that the microtensile bond strength of the surface after cleaning zinc oxide non-eugenol and zinc oxide eugenol with EDTA and maleic acid was comparable to that of the control group. Munirathinam et al[23] suggested using EDTA to clean dentin surfaces contaminated with temporary cement, either zinc oxide non-eugenol or zinc oxide eugenol. They claimed that dentin surfaces resulting from those cleaning techniques were the same as the uncontaminated surfaces. Chelating agent, 17% EDTA at neutral pH, was able to remove calcium ions from dentin to the depth of 5 µm and demineralize it without alternating the properties of collagen fibrils.[44,46,47] Furthermore, it was claimed that resin-dentin bonding interface was improved, which increased resistance to cement degradation.[43,48] The use of EDTA was more effective in removing the contamination of zinc oxide non-eugenol than zinc oxide eugenol, but maleic acid had the opposite result.

On the other hand, cleaning the surfaces using EDTA resulted in lower bond strength in the zinc oxide eugenol group than in the zinc oxide non-eugenol group. It can be hypothesized that 17% EDTA could not remove eugenol that had diffused through dentin surfaces.

The results of the current study showed that microtensile bond strength of the zinc oxide eugenol-contaminated dentin surface after cleaning with 10% maleic acid was comparable to that of the uncontaminated dentin. From previous studies,[24-26] only 7% maleic acid had the efficiency in removing smear layer and demineralizing dentin similar to 17% EDTA, while 10% maleic acid produced a greater depth of demineralization due to the higher acidic property. Consequently, it could eliminate eugenol that diffused through dentin surfaces and create better resin-dentin boning efficiency. The results in this study suggest that using 10% maleic acid to remove zinc oxide eugenol cement is sufficient to obtain adequate microtensile bond strength.

Adhesive and mixed failures were found in this experiment. The specimens in the control group showed the highest bond strength, and also presented the highest percentage of mixed failure. Specimens cleaned with EDTA or maleic acid showed more mixed failure than adhesive failure. Although, mixed failure was mostly found in specimens that showed high bond strength, Armstrong et al[49] suggested that the failure of fracture substrate cannot be attributed to high tensile bond strength of resin cement or dentin itself. However, the number of each failure mode was consistent to the bond strength in this study.

The SEM images showed that EDTA and maleic acid were more effective techniques for cleaning temporary cement from dentin surfaces, compared to using pumice or air water spray. When the contaminated surface was polished with pumice, a mixture of granules was found over the dentin surface including the dentinal tubules. Moreover, the insufficient cleaning by air water spray showed irregular and amorphous surfaces, in which the tubular structure could not be observed, resulting in a decrease in bond strength between resin and dentin.[16] EDTA and maleic acid resulted in a cleaner dentin surface than air water spray or pumice methods. Additionally, EDTA and maleic acid caused the dentinal tubules to open, and cement particles could not be observed. Therefore, using EDTA and maleic acid allowed for adhesive diffusion into demineralized dentin surface and effectively bonded resin to dentin.

Conclusions

Based on the results of this *in vitro* study, it can be concluded that dentin surfaces contaminated with eugenol or non-eugenol temporary cement affected the resin-dentin bond strength of self-etch adhesive system. Cleaning the dentin surface with 17% EDTA for 1 minute provided higher bond strength than cleaning mechanically, especially when dentin was contaminated with eugenol free cement. When cement containing eugenol was used, 10% maleic acid for 15 seconds is best used to clean the dentin surface and provide bond strength as high as bond strength in the control group.

Declaration of Interest

The authors have no conflicts of interest involve in any organization or entity with any financial interest in materials discussed in this manuscript.

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