

Microtensile Bond Strength of Resin-Modified Glass Ionomer Cement to Sound and Artificial Caries–Affected Root Dentin With Different Conditioning

A Saad • G Inoue • T Nikaido • M Ikeda • MF Burrow • J Tagami

Clinical Relevance

Both self conditioner and cavity conditioner have the potential to improve the bond strength and durability of resin-modified glass ionomer cement (RM-GIC) to sound and carious root dentin; however, ethylenediamine tetraacetic acid conditioning with RM-GIC to root dentin should be avoided.

SUMMARY

In this laboratory study, the microtensile bond strengths (μ TBS) of resin-modified glass ionomer cement (RM-GIC) to sound and artificial caries-affected bovine root dentin (ACAD) using three different conditioning agents were evaluated after 24 hours and three months. The fractured interface was examined with a scanning electron micro-

scope (SEM). Specimens were created on bovine root dentin that was embedded in epoxy resin. For the ACAD specimens, artificial carious lesions were created. The RM-GIC (Fuji II LC) was applied either directly (no treatment), after application of self conditioner, cavity conditioner, or 17% ethylenediamine tetraacetic acid (EDTA) applied for 60 seconds, on sound dentin and ACAD, then light cured. They were stored in artificial saliva for 24 hours or three months. Following this, the specimens were cut into sticks for the μ TBS test, and the failure mode of the debonded

Amr Saad, Cairo University, Tokyo Medical and Dental University, Cariology and Operative Dentistry, Tokyo, Japan

*Go Inoue, DDS, PhD, assistant professor, Tokyo Medical and Dental University, Cariology and Operative Dentistry, Oral Restitution Department, Graduate School of Medical and Dental Sciences, Tokyo, Japan

Toru Nikaido, DDS, PhD, lecturer, Tokyo Medical and Dental University, Cariology and Operative Dentistry, Oral Restitution Department, Graduate School of Medical and Dental Sciences, Tokyo, Japan

Masaomi Ikeda, RDT, BSc, PhD, Tokyo Medical and Dental University, Clinical Oral Science, Department of Oral Health Care Sciences, Faculty of Dentistry, Tokyo, Japan

Michael F. Burrow, BDS, MDS, PhD, MEd, MRACDS(Pros), FRACDS, University of Melbourne, Melbourne Dental School, Carlton, Victoria, Australia

Junji Tagami, DDS, PhD, Tokyo Medical and Dental University, Cariology and Operative Dentistry, Tokyo, Japan

*Corresponding author: 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113-8549, Japan; e-mail: inoue.ope@tmd.ac.jp

DOI: 10.2341/16-375-L

specimens was examined by using SEM. Pre-test failures were excluded from the statistical analysis of the μ TBS values because of their high incidence in some groups. Results showed that the μ TBS values were significantly affected by the dentin substrate as well as the conditioning agent. Self conditioner provided the highest and most stable μ TBS values, while cavity conditioner showed stable μ TBS values on sound dentin. Both self conditioner and cavity conditioner had significantly higher μ TBS values than the no treatment groups. EDTA conditioning reduced the μ TBS after three months to sound dentin, while it showed 100% pretest failure with ACAD for both storage periods.

INTRODUCTION

Root surface caries is becoming a common clinical phenomenon because of the aging population and increasing number of elderly people maintaining their teeth. This presents new challenges from a restorative perspective, with root surface caries being found to be one of the main risk factors of tooth loss in older adults.¹ Previous epidemiologic studies have shown that the incidence of root caries increases with age.^{2,3} In Japan, for example, Imazato and others⁴ showed an incidence of root caries of 53.3% in a selected sample of 287 adults older than 60 years.

Resin-modified glass ionomer cement (RM-GIC) was introduced in 1988 to improve the mechanical and esthetic properties of the conventional GICs by the addition of hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA) as well as other resins and photoinitiators to the fluoroaluminosilicate glass and polyacrylic acid in the conventional glass ionomer cement.^{5,6} Its lower sensitivity to moisture as well as fluoride release made it a successful option for the restoration of root caries lesions, especially in cases adjacent to gingival tissues, which makes complete isolation and access for material placement difficult.

Bonding of the RM-GIC to tooth structure occurs via two mechanisms: chemical bonding between anions of polyalkenoic acid chains and calcium ions in hydroxyapatite⁷ and micromechanical adhesion limited to retention provided by the intrinsic surface roughness of dentin and porosity created by the RM-GIC's self-etching characteristics.⁸ Several conditioning agents have been evaluated with enamel and coronal dentin.

Caries-affected dentin (CAD) is one of the most relevant substrates in clinical practice. For conservative purposes, CAD should be retained and thus become the bonding substrate. Unlike caries-infected dentin, CAD has much fewer bacteria and can be remineralized^{9,10} because of the presence of cross-linked collagen, a key to remineralization.¹¹ However, CAD has a higher degree of porosity due to mineral loss, greater water content, and typically a thicker smear layer when cut.¹² Previous studies showed lower bond strength values to CAD than to intact dentin with RM-GIC,^{13,14} conventional glass ionomer cement,¹⁴ and resin composite materials.¹⁵

To avoid the great variability in the CAD morphology and also to obtain a flat and uniform surface, an artificial caries-affected model (ACAD) was made in an attempt to simulate natural caries-affected dentin (NCAD). The properties of the ACAD showed a similarity to NCAD in nanohardness and mineral density of the superficial layer (approximately 150 μ m). Similar bond strength values were obtained in NCAD and ACAD with a two-step self-etch adhesive.¹⁶

There is little information about the bond strength of RM-GIC to ACAD; therefore, the aim of this study was to evaluate the microtensile bond strength (μ TBS) to sound and artificial caries-affected root dentin using different conditioning materials after storage in artificial saliva for 24 hours and three months. The null hypotheses of this study were that there are no significant differences in μ TBS among different conditioning, dentin substrates, and storage periods.

METHODS AND MATERIALS

Forty bovine teeth were collected and stored frozen, pulp tissues were removed, and root surfaces were cleaned with periodontal curettes prior to experimental procedures. A low-speed diamond saw (Iso-met, Buehler, Lake Bluff, IL, USA) was used to separate the crown and the apical part of the root under water cooling and then discarded. The remaining root portion was cut along the longitudinal axis and transversally to obtain four dentin blocks from the cervical and middle parts of the root, and each block was then embedded in epoxy resin (Epoxicure2, Buehler) using a cylindrical mold. After curing of the resin, the surface was manually wet polished with 600-grit SiC paper to expose flat root-dentin surfaces. The specimens were divided into two main groups: sound dentin and ACAD. For the sound dentin group, the smear layer was standardized by grinding with 600-grit SiC paper for five

Table 1: Restorative and Conditioning Materials Used in the Study, Manufacturer, Batch Number, Composition, and Method of Application				
Brand Name and Manufacturer	Batch Number	Chemical Composition	Method of Application	Code
Cavity conditioner (GC Corp, Tokyo, Japan)	1508101	77% distilled water, 20% polyacrylic acid, 3% aluminum chloride hydrate pH 1.9	Applied to the surface for 10 seconds Rinsed thoroughly with water and dried without desiccation	CC
Self conditioner (GC Corp, Tokyo, Japan)	1411111	20%-30% HEMA, 5%- 10% 4-META, 30%-35% distilled water, 28%-40% ethanol pH 1.8	Applied to the surface and left undisturbed for 10 seconds Air dried for 5 seconds	SC
EDTA (Dojindo Molecular Technologies, Japan)		0.5 M 2NA(EDTA·2Na) in distilled water pH 7	Applied to the surface for 60 seconds Washed with water for 10 seconds and air dried gently	EDTA
Fuji II LC capsule shade A2 (GC Corp, Tokyo, Japan)	1505151	Fluoro-alumino-silicate glass, polyacrylic acid, HEMA, urethane dimethacrylate, camphorquinone, water	Capsule mixed for 10 seconds and applied on dentin surface, then light cured for 20 seconds	
Abbreviations: EDTA, ethylenediamine tetraacetic acid; HEMA, 2-hydroxyethylmethacrylate; 4-META, 4-methacryloxyethyltrimellitate anhydride.				

seconds. For the ACAD group, each specimen was immersed in 15 mL of demineralizing solution (1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 50 mM acetic acid, 0.02% of NaN₃ at pH 4.5)¹⁶ at 37°C for 60 hours. Following this, the specimens were rinsed thoroughly with deionized water.

The demineralized surface was ground with 600-grit SiC paper for five seconds to create a demineralized dentin surface with a smear layer, which was checked by optical coherence tomography (Santec OCT-2000, Santec Co, Komaki, Japan) to ensure a standardized demineralized layer approximately 150-μm deep.

Specimen Preparation

The sound and ACAD root dentin surfaces were conditioned with either cavity conditioner (GC Corp, Tokyo, Japan), self conditioner (GC Corp), or 0.5 M ethylenediamine tetraacetic acid (EDTA). For the cavity and self conditioner subgroups, each was applied to the dentin surface according to the manufacturer’s instructions described in Table 1. EDTA was prepared by dissolving 2NA(EDTA·2Na) (Dojindo Molecular Technologies, Kumamoto, Japan) in distilled water to obtain an EDTA solution of concentration 0.5 mol/L at pH 7.0. It was applied as a conditioner for 60 seconds and then washed with an air-water syringe for 10 seconds and gently air dried. The dentin surfaces without conditioning were left as a control group. Following this, the Fuji II LC capsule (RM-GIC) was mixed using a GC Capsule Mixer CM-II (GC Corp) for 10 seconds, applied to the dentin surface in a cylindrical mold 2-mm high, and then light cured for 20 seconds using a halogen light

curing unit (Optilux 501, Kerr Corp, Orange, CA, USA) at 600 mW/cm² output. The bonded specimens were stored separately in artificial saliva at 37°C for 24 hours and three months. Each week, the artificial saliva was replaced with freshly prepared solution at room temperature during the three-month storage period.

Measurement of μTBS

After each storage period, each bonded specimen was longitudinally sectioned in two directions perpendicular to each other across the bonded interface to obtain sticks of approximately 1 × 1 mm for the μTBS test. Digital calipers (Mitutoyo Corp, Kawasaki, Japan) were used to check the cross-sectional area of the sticks. A total of 20 sticks for each subgroup were tested. Each stick was fixed to the test jig with a cyanoacrylate adhesive (Zapit, Dental Ventures of American, Anaheim Hills, CA, USA) and stressed in tension using a universal testing machine (EZ-Test, Shimadzu, Kyoto, Japan) at a cross-head speed of 1 mm/min until failure. The procedures of specimen preparation for the μTBS test are shown briefly in Figure 1.

When pretest failures occurred during cutting of the bonded specimens, these specimens were excluded from the calculation of the μTBS values. However, the discarded specimens were counted, and the survival rate was calculated for each group. Normal distribution of μTBS strength data was assumed after Shapiro-Wilk test. Bond strength values were analyzed by three-way analysis of variance (ANOVA) with repeated measures to determine the

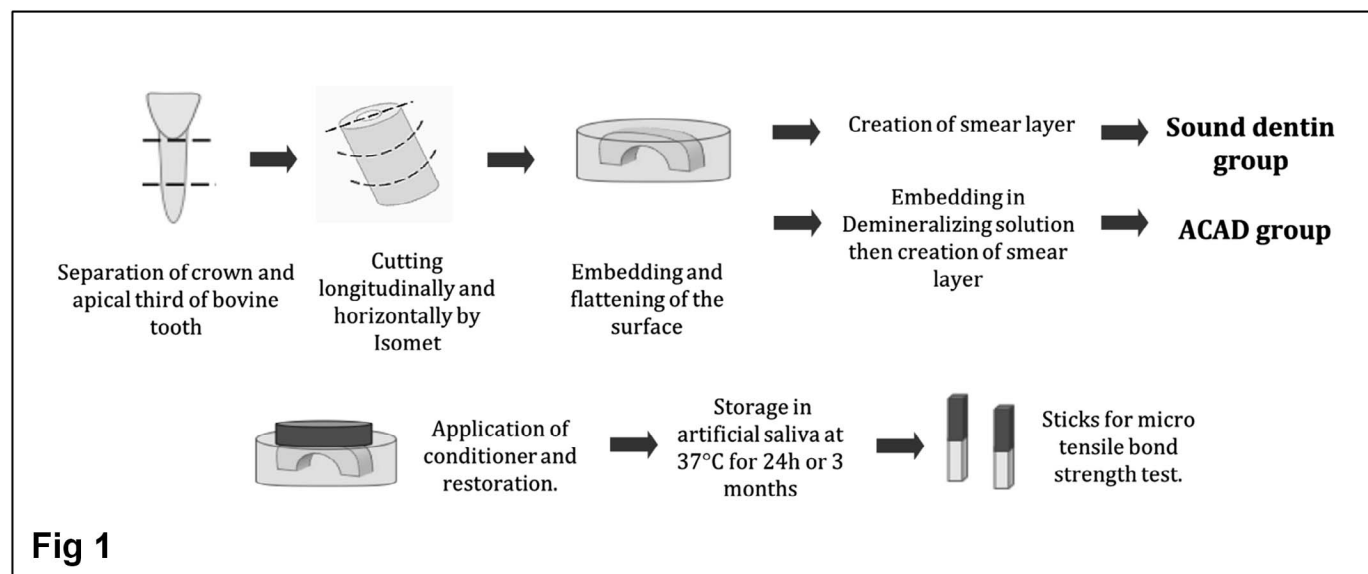


Figure 1. Schematic diagram showing specimen preparation for the μ TBS test.

effect of dentin substrate, conditioning agent, and storage condition.

Two-way ANOVA was then used to test for significant differences in conditioning agent and storage condition with each dentin substrate, followed by *t*-test with Bonferroni correction for pairwise comparison. The *t*-test was used to analyze the significant differences in μ TBS value at 24 hours and three months with each conditioning material under each type of dentin substrate. All statistical calculations were performed using the statistical software package Statistical Package for the Social Sciences for Windows (SPSS, SPSS Inc, Chicago, IL, USA) with $\alpha=0.05$.

For failure mode evaluation after debonding, the debonded specimens were fixed on a specimen holder using double-sided adhesive tape and sputter coated for evaluation under scanning electron microscope (SEM; S-4500, Hitachi, Ibaraki, Japan) at 15 kV accelerating voltage. Failure modes were classified into the following three categories: adhesive failure (failure at the dentin-material interface), cohesive failure (failure within the material or dentin itself), and mixed failure (partially adhesive and cohesive failure).

SEM Observations of Conditioned Dentin Surfaces

To observe the conditioned dentin surfaces, eight sound and ACAD dentin specimens' surfaces were treated in the same manner as described for specimen preparation for the μ TBS test to obtain

two specimens for each condition. The dentin surfaces were fixed by hexamethyldisilane for 10 minutes,¹⁷ dried in a desiccator for 24 hours, and gold coated for SEM observation.

RESULTS

Table 2 shows the mean μ TBS values of the RM-GIC to sound and ACAD after 24 hours and three months of storage in the artificial saliva as well as the survival rate for the different experimental groups. Three-way and two-way ANOVA followed by *t*-test with Bonferroni correction detected that dentin condition, storage period, and conditioning agent have a significant effect of the μ TBS to dentin. For each conditioner, the μ TBS values in sound dentin were significantly higher than those in ACAD ($p<0.05$), except for three months with no treatment "none" group, due to the significant drop in bond strength with sound dentin, which approaches its corresponding value in the ACAD group.

For the sound dentin, the three conditioners significantly increased the μ TBS of RM-GIC to dentin ($p<0.05$) after 24 hours, in which self conditioner provided the highest μ TBS, followed by EDTA and cavity conditioner. The lowest μ TBS was obtained in the "none" group. After storage for three months, the μ TBS of self conditioner remained the highest among the groups. However, a significant reduction in bond strength occurred for the "none" and EDTA groups. The μ TBS of the self conditioner and cavity conditioner groups showed no significant change after three months of storage, although

Table 2: Mean μ TBS Values (MPa) and Percentage of Survived Sticks for Resin-Modified Glass Ionomer Cement to Root Dentin ^a				
Conditioning	Sound Dentin		ACAD	
	24 Hours	3 Months	24 Hours	3 Months
None	13.4 \pm 2.3 (78)	4.3 \pm 1.5 ^C (30)	7.2 \pm 1.9 (55)	4.5 \pm 1.9 ^C (50)
CC	18.5 \pm 3.3 ^A (91)	17.1 \pm 5.5 ^A (89)	11.9 \pm 2.2 (85)	8.9 \pm 2 (85)
SC	26.6 \pm 4.4 ^B (100)	25.3 \pm 3.9 ^B (100)	14.6 \pm 3.3 ^a (100)	14.1 \pm 3.5 ^a (100)
EDTA	21.5 \pm 3.5 ^A (100)	4.6 \pm 1.6 ^C (35)	n.d. (0)	n.d. (0)
Abbreviation: CC, cavity conditioner; SC, self-conditioner; EDTA, ethylenediamine tetraacetic acid; n.d., not detected. ^a Mean values \pm standard deviation. Groups identified by similar large/small superscripts were not significantly different at $p < 0.05$. Numbers in parentheses are survival percentage after sticks preparation.				

slight decreases were recorded. The survival rate decreased markedly in the “none” and EDTA groups but was almost stable with cavity conditioner and self conditioner, both of which had the highest survival rates.

For the ACAD groups, self conditioner also showed the highest μ TBS values after 24 hours, followed by cavity conditioner and then “none.” The difference between them was significant ($p < 0.05$). The EDTA-conditioned group resulted in no specimens surviving during trimming procedures at either time period. After storage for three months, there was a significant reduction in bond strength for cavity conditioner and “none,” whereas the self conditioner group remained the same.

Regarding failure mode (Table 3), after 24 hours, the mixed mode of failure was predominant in all conditioned sound dentin groups except for EDTA, in which adhesive failure was predominant. For the ACAD group, the predominant mode of failure was adhesive for the “none” group, while both adhesive and mixed mode of failures were equally predominant for the self conditioner and cavity conditioner groups. For the ACAD with EDTA conditioning, no sticks could be obtained for mode of failure investigation after the μ TBS test because of debonding during preparation. After storage for three months, adhesive failure decreased for both the cavity conditioner and self conditioner groups but increased for the “none” and EDTA groups with sound dentin. For ACAD, the adhesive failure rate reduced with all groups, and no bonded sticks survived during preparation from the EDTA group.

Representative SEM images of the ACAD and sound dentin surfaces after each conditioner application showed a difference in the action of each conditioner with the smear layer and dentinal tubules openings of each dentin substrate, as shown in Figure 2. Cavity conditioner showed removal of the smear layer to a greater extent and partial

opening of dentinal tubules, whereas EDTA totally unplugged the dentinal tubules and completely removed the smear layer. For the ACAD surfaces, a denser smear layer was observed; however, cavity conditioner managed to expose more open dentin tubules and a less compacted smear layer than the nonconditioned surface, while the EDTA-conditioned surface showed a smooth surface with occluded dentin tubules.

DISCUSSION

Previous studies reported that risk for root caries among individuals increased with the presence of exposed root surfaces, especially in elderly patients, patients with gingival attachment loss, or patients with deep pocket probing depths¹⁸; however, such findings are still disputed. Root dentin was used as the substrate with RM-GIC and conditioners in this study. Although RM-GIC is an excellent restorative choice for root caries treatment, coronal dentin has been routinely used as a bonding substrate in most laboratory studies because of its ease of preparation and use. Root dentin has not generally been used as bonding substrate because of the small region available for bonding, and its anatomical structure varies from coronal dentin.¹⁹ Therefore, no previous published work was noted by the authors on prominent evaluation of the adhesive properties of RM-GIC to human root dentin. Root dentin typically exhibits fewer dentinal tubules and lower perme-

Table 3: Mode of Failure of Specimens (Percentage) ^a				
Conditioning	Sound Dentin		ACAD	
	24 Hours	3 Months	24 Hours	3 Months
None	35/48/17	55/45/0	70/30/0	32/52/16
CC	30/56/14	25/52/24	45/47/8	17/53/30
SC	17/50/33	9/29/62	45/39/16	19/37/44
EDTA	54/13/33	70/30/0	n.d.	n.d.
^a Values are presented as adhesive failure mode/mixed failure mode/cohesive failure mode.				

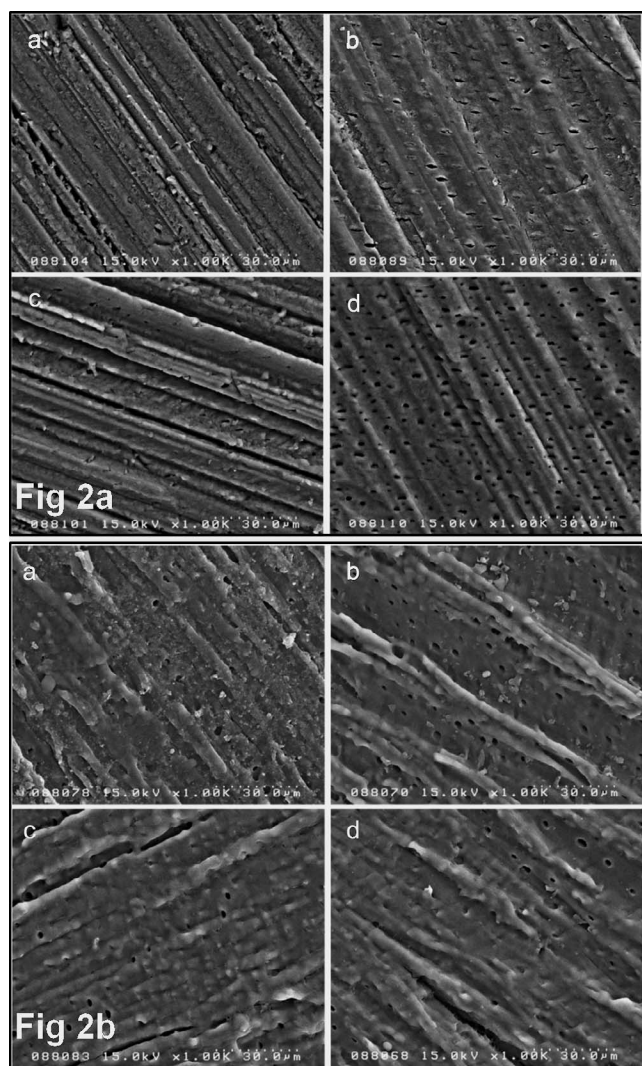


Figure 2. (A): Representative SEM images of sound dentin surface after conditioning. (a): Sound dentin surface without conditioning with smear layer and surface scratches of SiC No. 600 preparation. (b): Sound dentin after cavity conditioner application showing smear layer removal and partial opening of dentinal tubules. (c): Sound dentin after self conditioner application showing partial dissolution of the smear layer. The hybrid layer between the self conditioner and dentin could not be identified. (d): Sound dentin after EDTA application showing total dissolution of the smear layer and patent dentinal tubules. (B): Representative SEM images of ACAD dentin surface after conditioning. (a): ACAD dentin surface with apparently dense smear layer. (b): ACAD surface after cavity conditioner application showing partial removal of the smear layer and more exposed dentinal tubules. (c): ACAD after self conditioner application. Again, this image could not show its mode of action. (d): ACAD after EDTA application showing large smooth areas of collapsed collagen and plugged dentinal tubules not suitable for micromechanical retention of RM-GIC.

ability compared with coronal dentin. It should be noted that there is also a difference in the microhardness and the microstructure between coronal and root dentin because of a variation in the organization of both collagen fibrils and apatite

minerals.²⁰ Fewer dentinal tubules exist, and they often have a circuitous path with a greater amount of intertubular dentin in the root dentin. Studies done with resin adhesives to compare bonding of root and coronal dentin showed variations in bond strength even for those undertaken on the same tooth.^{19,21}

ACAD simulates the NCAD, which is partially demineralized. NCAD has a lower calcium and phosphate content and greater prevalence of exposed unprotected collagen fibrils than sound dentin.¹⁵ The smear layer of CAD is thicker and appears to be enriched with organic components compared with a sound dentin smear layer.²² This fact leads to a decrease in both micromechanical and chemical bonding between the carboxyl groups of the polyalkenoic acid and calcium ion of hydroxyapatite.^{14,23}

ACAD has a mineral density similar to that of NCAD^{16,24}; however, ACAD was created in a short period of time under controlled conditions using a demineralizing solution and is devoid of crystal logs occluding some of the dentinal tubules of the NCAD,²⁵ making the ACAD more standardized among specimens. According to the current results, the μ TBS of the RM-GIC to ACAD was significantly lower compared with sound dentin in all groups. The SEM images of the ACAD groups in Figure 2B showed an apparently denser but diffuse smear layer with fewer open dentinal tubules available for resin-modified glass ionomer retention.

Fuji II LC is a light-cured RM-GIC; its bond strength to the root dentin after 24 hours and three months was evaluated with three conditioning materials (cavity conditioner, self conditioner, and EDTA). To improve RM-GIC bond strength to the tooth structure, the manufacturer recommends dentin conditioning with GC cavity conditioner, which contains 20% polyacrylic acid and 3% aluminum chloride or GC self conditioner, containing 4-META and HEMA. In previous studies,^{7,26} EDTA was used for dentin conditioning because of its calcium chelation action and ability to remove the smear layer while having a neutral pH value.

It was reported that the RM-GIC used has an initially higher bond strength than the conventional GICs.²⁷⁻³⁰ The RM-GIC used contains HEMA, which provides a superior wetting ability of the dentin^{29,31} and improves mechanical interlocking with the dentinal tubules. In addition, the polyacrylic acid in the RM-GIC acts as a mild self-conditioner.⁷ When the smear layer on the dentin surface was not conditioned, this layer possibly blocked the chemical

bonding of the RM-GIC to the intertubular dentin and also limited any micromechanical interlocking to exposed collagen fibers or patent dentinal tubules. Retention from inherent dentin irregularities⁷ was believed to be responsible for short-term bonding strength, but on storage, the bond dropped significantly,³² leading to high failure rates and low bond strength of both sound and ACAD.

When cavity conditioner was used as the conditioning agent, the μ TBSs were significantly higher than those of the no treatment (none) group in both sound dentin and ACAD. The bond strength was maintained after three months of storage in artificial saliva with sound dentin; however, the bond strength to ACAD decreased significantly during storage, but the survival rate remained unchanged.

Cavity conditioner contains 20% polyacrylic acid and 3% aluminum chloride. The SEM images demonstrated that cavity conditioner partially removed the smear layer but did not totally unplug the dentinal tubules. The increase of μ TBS may be due to the removal of the smear layer and partial demineralization of the underlying dentin, which increased the surface area and microporosities.³³ The exposed calcium ions within the hydroxyapatite are available for chemical bonding with the carboxyl groups of the polyalkenoyic acid.⁷ The aluminum chloride is believed to play role in stabilization of collagen matrix during demineralization.⁷ Abdalla³⁴ reported formation of a 2- μ m-thick hybrid-like layer with resin tags of 1 μ m in length when cavity conditioner was used for conditioning of sound dentin. According to Yui and others,³⁵ cavity conditioner increased the dentin permeability, providing an additional source of water for the acid-base setting reaction of the GIC, promoting the maturation of the glass ionomer at the interface. These factors may contribute to the greater resistance against dentin bonding degradation of the RM-GIC over time^{36,37} compared with the "none" group. However, this thin hybrid-like layer or acid-base-resistant layer as described by Tanumiharja and others³⁸ was less resistant to degradation when cavity conditioner was used with ACAD.

Because of the resin component of the RM-GIC, bond strength can be enhanced by incorporating resin bonding mechanisms. Self conditioner contains HEMA as well as 4-META, a functional monomer that is able to chemically interact with hydroxyapatite in dentin³⁹; therefore, by conditioning with self conditioner, dentin wettability should have been improved, allowing better monomer penetration into the dentin and therefore improving the quality of the

hybrid layer, which may be the major source of bond strength, as suggested by Imbery and others.⁷ Self conditioner provided the highest μ TBSs for each substrate, and this outcome corresponds with previous studies.^{7,40} Self conditioner treatment led to the only group in which the μ TBS did not significantly decrease after three months in both sound dentin and ACAD.

EDTA used in this study was adjusted as 0.5 M concentration at neutral pH, which is commonly and effectively⁴¹ used for dentin treatment. EDTA has the ability to chelate calcium ions and dissolve the mineral phase of the dentin without altering the dentin organic phase.⁴² The 24-hour bond with sound dentin was the second highest. The SEM images of the sound dentin treated with EDTA showed complete removal of the smear layer and lack of dentin tubule smear plugs, which may have facilitated the micromechanical retention of the RM-GIC. However, after three months of storage, a significant drop in μ TBS with high failure rates was observed. It is suggested that a microporous scaffold of collagen fibrils exposed when EDTA removed the mineral component of the dentin enabled good immediate bonding via micromechanical interlocking, but there may have been limited chemical bonding, which is the essential factor for dentin bond durability for GIC.

Using EDTA with ACAD, no bonded sticks survived during the trimming procedure for measuring the μ TBS. This might have been due to disadvantages in the chelating effect of EDTA. The low calcium content that remained in the ACAD substrate with an unsupported weak and collapsed collagen fibril network (Figure 2B) was not suitable for the chemical binding of the RM-GIC. It should be noticed that in this study, it was preferred to standardize the number of sticks subjected to a μ TBS test among all subgroups. The high failure rate in some subgroups led to a limitation in the number of tested sticks.

From the failure mode, previous literature reported variability in failure modes for Fuji II LC with different conditioners.^{7,38,40,43,44,45} Therefore, investigation of the fractured beams was undertaken using SEM at high magnification. It showed the presence of a fine film of RM-GIC close to the bonded surface, which was also reported by Yap and others,⁴⁶ who described this as the ion-exchange layer.^{46,47} When calculating the mode of failure, this thin resin layer was considered as part of the RM-GIC. SEM micrographs for some debonded speci-

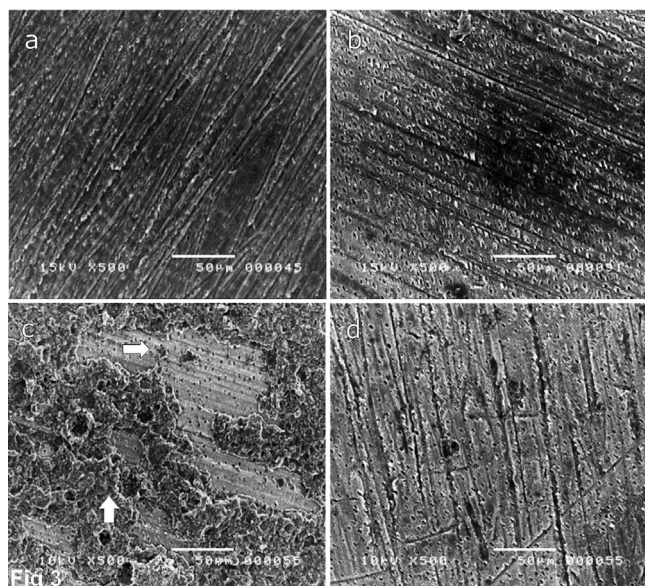


Figure 3. SEM observations of debonded specimens at various magnifications along the dentin side of sound groups. (a): High magnification of adhesive failure in the "none, 24h" group, showing most of the dentinal tubules occluded by the smear layer, and the remaining scratches from the surface grinding are visible. (b): High magnification of adhesive failure of the cavity conditioner, 24-hour specimen. Dentin scratches are clearly visible with almost no smear layer and partially open dentinal tubules. (c): Self conditioner, 24 hours. The upper arrow points to dentin, while the lower arrow points to a resin area covering the dentin surface. (d): EDTA, 24-hour specimen, showing the dentin surface with no smear layer and open dentinal tubules.

mens taken during mode of failure investigation are presented in Figures 3 and 4.

For the ACAD groups, the predominant mode of failure was adhesive failure at 24 hours; however, after three months, adhesive failure was reduced and mixed failure was predominant except for the self conditioner group, in which cohesive failure in the RM-GIC predominated. It was noticed that a few beams with mixed failure in the cavity conditioner and self conditioner groups showed partial cohesive fracture in the demineralized dentin layer after three months of storage. It was notable that most of the cohesive failures occurred in the bulk of the RM-GIC after 24 hours. However, most of the cohesive fractures were at the base of the RM-GIC after three months probably because of the maturation of the cement, leaving a thin resin surface covering the dentin and detected by high magnification (marked with white arrow, Figure 4d). This fact suggested maturation of the interface between the RM-GIC and dentin, especially when self conditioner was used. The high incidence of a thin resin layer in the self conditioner specimens indicated penetration of

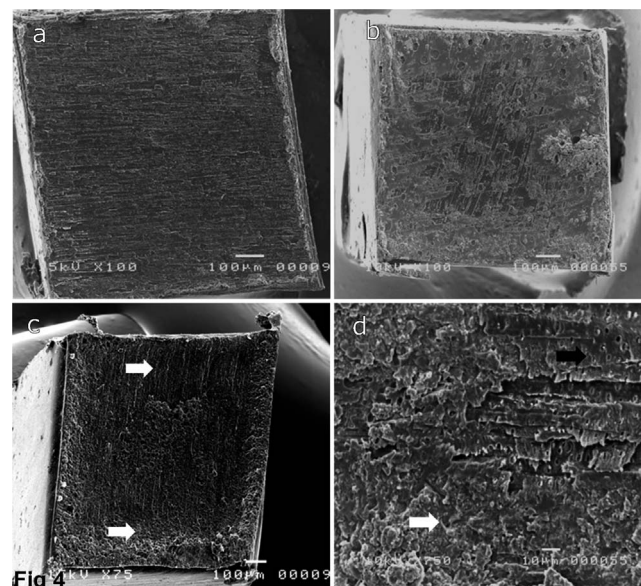


Figure 4. SEM observations of debonded specimens at various magnifications along the dentin side of ACAD groups. (a): Adhesive failure of none, three months (denser smear layer than sound dentin). (b): Mixed failure specimen of cavity conditioner, three-month group. The ACAD surface appears at the center of the specimen. (c): Cohesive failure of self conditioner, 24 hours, with thick resin-modified glass ionomer at the bottom arrow that becomes thinner at the upper arrow. (d): High magnification of self conditioner specimen to show ACAD marked with a black arrow at the top of the image and thin RM-GIC layer marked with a white arrow at the bottom.

RM-GIC into dentinal tubules and also the formation of a true hybrid layer at the RM-GIC–dentin interface. Therefore, the clinical implication of this study is that use of self conditioner or cavity conditioner has the potential to improve the bond strength and durability of RM-GIC to sound and carious root dentin, while using EDTA as a conditioning agent to root dentin should be avoided.

CONCLUSION

According to the outcomes from the current study, self conditioner provided a superior bond strength with the RM-GIC Fuji II LC compared with the other tested conditioning agents when applied to sound and artificial caries-affected root dentin. However, EDTA is not suitable as a conditioner for root dentin with Fuji II LC.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Tokyo Medical and Dental University. In this study, extracted bovine teeth were used. The study was approved by the Human Research Ethics Committee, Tokyo Medical and Dental University. The approval code for this study is 725.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company presented in this article.

(Accepted 27 February 2017)

References

- Locker D, Ford J, & Leake JL (1996) Incidence of and risk factors for tooth loss in a population of older Canadians *Journal of Dental Research* **75**(2) 783-789.
- Griffin SO, Griffin PM, Swann JL, & Zlobin N (2004) Estimating rates of new root caries in older adults *Journal of Dental Research* **83**(8) 634-638.
- Chi DL, Berg JH, Kim AS, & Scott J (2013) Correlates of root caries experience in middle-aged and older adults within the Northwest PRECEDENT *Journal of the American Dental Association (1939)* **144**(5) 507-516.
- Imazato S, Ikebe K, Nokubi T, Ebisu S, & Walls AW (2006) Prevalence of root caries in a selected population of older adults in Japan *Journal of Oral Rehabilitation* **33**(2) 137-143.
- Antonucci JM, McKinney JE, & Stansbury JW (1988) Resin-modified glass ionomer cement. US Patent 7-160 856.
- Zhang L, Tang T, Zhang ZL, Liang B, Wang XM, & Fu BP (2013) Improvement of enamel bond strengths for conventional and resin-modified glass ionomers: acid-etching vs. conditioning *Journal of Zhejiang University Science B* **14**(11) 1013-1024.
- Imbery TA, Namboodiri A, Duncan A, Amos R, Best AM, & Moon PC (2013) Evaluating dentin surface treatments for resin-modified glass ionomer restorative materials *Operative Dentistry* **38**(4) 429-438.
- Coutinho E, Yoshida Y, Inoue S, Fukuda R, Snauwaert J, Nakayama Y, De Munck J, Lambrechts P, Suzuki K, & Van Meerbeek B (2007) Gel phase formation at resin-modified glass-ionomer/tooth interfaces *Journal of Dental Research* **86**(7) 656-661.
- Fusayama T (1991) Intratubular crystal deposition and remineralization of carious dentin *Journal de Biologie Buccale* **19**(3) 255-262.
- Pinna R, Maioli M, Eramo S, Mura I, & Milia E (2015) Carious affected dentine: its behaviour in adhesive bonding *Australian Dental Journal* **60**(3) 276-293.
- Marshall GW Jr, Marshall SJ, Kinney JH, & Balooch M (1997) The dentin substrate: structure and properties related to bonding *Journal of Dentistry* **25**(6) 441-458.
- Inoue G, Tsuchiya S, Nikaido T, Foxton RM, & Tagami J (2006) Morphological and mechanical characterization of the acid-base resistant zone at the adhesive-dentin interface of intact and caries-affected dentin *Operative Dentistry* **31**(4) 466-472.
- Palma-Dibb RG, de Castro CG, Ramos RP, Chimello DT, & Chinelatti MA (2003) Bond strength of glass-ionomer cements to caries-affected dentin *Journal of Adhesive Dentistry* **5**(1) 57-62.
- Choi K, Oshida Y, Platt JA, Cochran MA, Matis BA, & Yi K (2006) Microtensile bond strength of glass ionomer cements to artificially created carious dentin *Operative Dentistry* **31**(5) 590-597.
- Ekambaram M, Yiu CKY, & Matinlinna JP (2015) Bonding of resin adhesives to caries-affected dentin: a systematic review *International Journal of Adhesion and Adhesives* **61** 23-34.
- Joves GJ, Inoue G, Nakashima S, Sadr A, Nikaido T, & Tagami J (2013) Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin *Dental Materials Journal* **32**(1) 138-143.
- Takai T, Hosaka K, Kambara K, Thitthaweerat S, Matsui N, Takahashi M, Kishikawa R, Nakajima M, Otsuki M, Foxton RM, & Tagami J (2012) Effect of air-drying dentin surfaces on dentin bond strength of a solvent-free one-step adhesive *Dental Materials Journal* **31**(4) 558-563.
- Lawrence HP, Hunt RJ, & Beck JD (1995) Three-year root caries incidence and risk modeling in older adults in North Carolina *Journal of Public Health Dentistry* **55**(2) 69-78.
- Korkmaz Y, Gurgan S, Firat E, & Nathanson D (2010) Effect of adhesives and thermocycling on the shear bond strength of a nano-composite to coronal and root dentin *Operative Dentistry* **35**(5) 522-529.
- Wang R (2005) Anisotropic fracture in bovine root and coronal dentin *Dental Materials* **21**(5) 429-436.
- Burrow MF, Sano H, Nakajima M, Harada N, & Tagami J (1996) Bond strength to crown and root dentin *American Journal of Dentistry* **9**(5) 223-229.
- Nakajima M, Kunawarote S, Prasansuttiporn T, & Tagami J (2011) Bonding to caries-affected dentin *Japanese Dental Science Review* **47**(2) 102-114.
- Yoshida Y, Van Meerbeek B, Nakayama Y, Snauwaert J, Hellemans L, Lambrechts P, Vanherle G, & Wakasa K (2000) Evidence of chemical bonding at biomaterial-hard tissue interfaces *Journal of Dental Research* **79**(2) 709-714.
- Marquezan M, Correa FN, Sanabe ME, Rodrigues Filho LE, Hebling J, Guedes-Pinto AC, & Mendes FM (2009) Artificial methods of dentine caries induction: a hardness and morphological comparative study *Archives of Oral Biology* **54**(12) 1111-1117.
- Nakajima M, Kitasako Y, Okuda M, Foxton RM, & Tagami J (2005) Elemental distributions and microtensile bond strength of the adhesive interface to normal and caries-affected dentin *Journal of Biomedical Materials Research Part B, Applied Biomaterials* **72**(2) 268-275.
- Fagundes TC, Toledano M, Navarro MF, & Osorio R (2009) Resistance to degradation of resin-modified glass-ionomer cements dentine bonds *Journal of Dentistry* **37**(5) 342-347.
- Mitra SB (1991) Adhesion to dentin and physical properties of a light-cured glass-ionomer liner/base *Journal of Dental Research* **70**(1) 72-74.
- Torii Y, Iwami Y, Kobayashi K, Iga M, & Tsuchitani M (1991) Studies on light-cured glass ionomer cements, Part

1. Physical properties and marginal sealing abilities *Japanese Journal of Conservative Dentistry* **34** 451-458.
29. Friedl KH, Powers JM, & Hiller KA (1995) Influence of different factors on bond strength of hybrid ionomers *Operative Dentistry* **20(2)** 74-80.
30. Swift EJ Jr, Pawlus MA, & Vargas MA (1995) Shear bond strengths of resin-modified glass-ionomer restorative materials *Operative Dentistry* **20(4)** 138-143.
31. Nakanuma K, Hayakawa T, Tomita T, & Yamazaki M (1998) Effect of the application of dentin primers and a dentin bonding agent on the adhesion between the resin-modified glass-ionomer cement and dentin *Dental Materials* **14(4)** 281-286.
32. Inoue S, Van Meerbeek B, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, & Sano H (2001) Effect of remaining dentin thickness and the use of conditioner on micro-tensile bond strength of a glass-ionomer adhesive *Dental Materials* **17(5)** 445-455.
33. Poggio C, Beltrami R, Scribante A, Colombo M, & Lombardini M (2014) Effects of dentin surface treatments on shear bond strength of glass-ionomer cements *Annali di Stomatologia* **5(1)** 15-22.
34. Abdalla AI (2000) Morphological interface between hybrid ionomers and dentin with and without smear-layer removal *Journal of Oral Rehabilitation* **27(9)** 808-814.
35. Yiu CK, Tay FR, King NM, Pashley DH, Carvalho RM, & Carrilho MR (2004) Interaction of resin-modified glass-ionomer cements with moist dentine *Journal of Dentistry* **32(7)** 521-530.
36. Marquezan M, Fagundes TC, Toledano M, Navarro MF, & Osorio R (2009) Differential bonds degradation of two resin-modified glass-ionomer cements in primary and permanent teeth *Journal of Dentistry* **37(11)** 857-864.
37. Cardoso MV, Delme KI, Mine A, Neves Ade A, Coutinho E, De Moor RJ, & Van Meerbeek B (2010) Towards a better understanding of the adhesion mechanism of resin-modified glass-ionomers by bonding to differently prepared dentin *Journal of Dentistry* **38(11)** 921-929.
38. Tanumiharja M, Burrow MF, & Tyas MJ (2000) Micro-tensile bond strengths of glass ionomer (polyalkenoate) cements to dentine using four conditioners *Journal of Dentistry* **28(5)** 361-366.
39. Giannini M, Makishi P, Ayres AP, Vermelho PM, Fronza BM, Nikaido T, & Tagami J (2015) Self-etch adhesive systems: a literature review *Brazilian Dental Journal* **26(1)** 3-10.
40. Cook NB, Feitosa SA, Patel A, Alfawaz Y, Eckert GJ, & Bottino MC (2015) Bonding ability of paste-paste glass ionomer systems to tooth structure: in vitro studies *Operative Dentistry* **40(3)** 304-312.
41. Serper A, & Calt S (2002) The demineralizing effects of EDTA at different concentrations and pH *Journal of Endodontics* **28(7)** 501-502.
42. Osorio R, Erhardt MC, Pimenta LA, Osorio E, & Toledano M (2005) EDTA treatment improves resin-dentin bonds' resistance to degradation *Journal of Dental Research* **84(8)** 736-740.
43. Dursun E, Le Goff S, Ruse DN, & Attal JP (2013) Effect of chlorhexidine application on the long-term shear bond strength to dentin of a resin-modified glass ionomer *Operative Dentistry* **38(3)** 275-281.
44. Fagundes TC, Toledano M, Navarro MF, & Osorio R (2009) Resistance to degradation of resin-modified glass-ionomer cements dentine bonds *Journal of Dentistry* **37(5)** 342-347.
45. Miyazaki M, Iwasaki K, Onose H, & Moore BK (1999) Resin-modified glass-ionomers: effect of dentin primer application on the development of bond strength *European Journal of Oral Sciences* **107(5)** 393-399.
46. Yap AU, Tan AC, Goh AT, Goh DC, & Chin KC (2003) Effect of surface treatment and cement maturation on the bond strength of resin-modified glass ionomers to dentin *Operative Dentistry* **28(6)** 728-733.
47. Berry EA III, & Powers JM (1994) Bond strength of glass ionomers to coronal and radicular dentin *Operative Dentistry* **19(4)** 122-126.