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Effect of conditioning and 1 year aging on the bond strength and interfacial morphology of glass-ionomer cement bonded to dentin

Shuhei Hoshika^a, Shihchun Ting^a, Zubaer Ahmed^a, Fei Chen^a, Yu Toida^a,
Norihiro Sakaguchi^b, Bart Van Meerbeek^c, Hidehiko Sano^a,
Sharanbir K. Sidhu^{d,*}

^a Department of Restorative Dentistry, Division of Oral Health Science, Hokkaido University Graduate School of Dentistry, Sapporo, Japan

^b Laboratory of Integrated Functional Materials, Center for Advanced Research of Energy and Materials, Hokkaido University, Faculty of Engineering, Sapporo, Japan

^c KU Leuven BIOMAT, Department of Oral Health Sciences, KU Leuven (University of Leuven) & Dentistry, University Hospitals Leuven, Belgium

^d Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

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ABSTRACT

Objective. The purpose of this study was to determine the bond stability and the change in interfacial ultra-structure of a conventional glass-ionomer cement bonded to dentin, with and without pre-treatment using a polyalkenoic acid conditioner.

Methods. The occlusal dentin surfaces of six teeth were ground flat. Glass-ionomer cement was bonded to the surfaces either with or without polyalkenoic acid conditioning. The teeth were sectioned into 1-mm² stick-shaped specimens. The specimens obtained were randomly assigned to two groups with different periods of storage in water: 1 week and 1 year. The micro-tensile bond strength (μ TBS) was determined for each storage time. Additional specimens were prepared for Transmission Electron Microscopy (TEM); they were produced with or without prior polyalkenoic acid conditioning in the same way as in the μ TBS test.

Results. There was no significant difference in μ TBS to conditioned dentin and non-conditioned dentin ($p > 0.05$). The failures appeared to be of a mixed nature, although aging caused more areas of cohesive than adhesive failure in both groups. The TEM observation showed an intermediate layer, a matrix-rich layer and a partially demineralized layer in the polyalkenoic acid conditioned group.

Significance. Aging did not reduce the bond strength of the conventional glass-ionomer cement to dentin with or without the use of a polyalkenoic acid conditioner.

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* Corresponding author at: Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Turner Street, London E1 2AD, UK.

E-mail address: s.k.sidhu@qmul.ac.uk (S.K. Sidhu).

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1. Introduction

With the help of numerous research and clinical evidence, we are now able to accomplish tooth adhesion to enamel and dentin to a satisfactory level by means of dental restorative materials such as resin-based composites and glass-ionomer cement (GIC). Contemporary focus is on ensuring materials are bioactive, tougher and self-reparable. The concept of biocompatibility has evolved to bioactivity, which is now a big trend in restorative dentistry [1]. Dental restorative materials should be called “bioactive” only when they actively stimulate or direct tissue responses, and they can control interactions with microbiological species besides their primary function of restoring or replacing missing tooth structure [2]. In this sense, bioactivity has two major aspects, which are remineralization and anti-microbial properties. Regarding remineralization, bioactive materials containing calcium phosphate [3], hydroxyapatite [4,5], calcium silicate [6,7] etc., were reported to have remineralization ability. Regarding the anti-microbial property, the release of compounds with antibiotic-like efficacy added to dental restorative materials such as quaternary ammonium compounds [8], zinc oxide nanoparticles [9] etc., were used to inhibit oral bacteria and biofilm.

GIC is one example of a dental bioactive material. It has both remineralization and anti-microbial ability [10–13] and has been used for dental restoration and the Atraumatic Restorative Treatment (ART) technique reliably for a long time [14,15]. Although resin composite is the major dental restorative material used nowadays, GICs are often used in clinical situations because of their technique simplicity, cost effectiveness and relative tolerance in the moist oral environment. Additionally, having no conversion shrinkage is an advantage compared with resin composite, and for relatively deep cavities it is still an ideal material for use [16,17]. Moreover, Peumans et al. reported the lowest annual failure rate scores for GIC in vivo [18]. Although the bond strength of GIC may be much weaker compared with resin-based materials, the means by which GICs obtain such clinically satisfactory results is still not fully understood.

Some laboratory studies have reported improvement of the adhesion of GICs to tooth structure in terms of bond strength when surface pre-treatment is carried out [19,20]. In contrast, some other studies have reported certain GICs adhere to tooth structure without pre-treatment [21,22].

The purpose of this study was to assess the tooth-GIC adhesion by means of bond strength and interfacial morphology after 1 week and 1 year of aging, with and without surface pre-treatment. The null hypothesis tested in this study was that pre-treatment of dentin using a polyalkenoic acid conditioner did not affect the long-term durability of a conventional GIC.

2. Materials and methods

2.1. Micro-tensile bond strength (μ TBS) test

The bond strength to dentin was determined using a standard micro-tensile bond strength test [23]. The materials used in this study are shown in Table 1. Six human molars, stored

in a 0.5% chloramine T solution, were used within 1 month of extraction. The protocol of this research was approved by the Commission for Medical Ethics of Hokkaido University. The extracted molars were sectioned at the mid-coronal portion to create a flat dentin surface by using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). A standard smear layer was produced using #600 grit silicon carbide paper. The teeth were randomly divided into two groups of three teeth each. Prior to the application of the GIC, the dentin surface of the specimens in one group was pre-treated with a polyalkenoic acid conditioner (Cavity Conditioner, GC, Tokyo, Japan). This contains 3% Aluminum chloride as well as 20% polyalkenoic acid. The specimens in the other group did not receive any pre-treatment. The dentin surface was subsequently built up free-hand and in bulk with a conventional GIC (Fuji IX GP Extra, GC, Tokyo, Japan) to a height of 5–6 mm, followed by application of a surface sealer (GC Fuji Coat LC, GC, Tokyo, Japan) which was light-cured for 10 s.

After 1 week of storage in distilled water at 37 °C, the specimens were sectioned perpendicular to the bonding surface, to obtain 1-mm² stick-shaped micro-specimens using an Isomet saw. The specimens were then randomly assigned to four groups (10 specimens each) according to age/storage time: 1 week and 1 year, i.e. the 1 week specimens were tested after sectioning while the rest continued in storage to 1 year. This is based on the following power calculation: if the specimen is used as the statistical unit, an absolute 3 teeth per experimental group with appropriate consideration of tooth dependency are required [24]. At the relevant time period, the micro-specimens were fixed to a jig with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Ohtawara, Japan) and stressed in a testing device (EZ-test, Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min until failure occurred. The μ TBS was calculated in MPa, derived by dividing the force applied (in N) at the time of fracture by the bonded area (in mm²). Statistical analysis was performed using one-way ANOVA ($\alpha = 0.05$) and *post hoc* Tukey-Kramer multiple comparisons tests. The mode of failure was determined by examining the fractured surface at a magnification of 50x using a stereomicroscope (Wild M5A, Heerbrugg, Switzerland).

2.2. Transmission Electron Microscopy (TEM) interface characterization

Additional GIC specimens were prepared for examination using TEM (H-800, Hitachi, Tokyo, Japan). For this, a further four teeth were randomly divided into two groups of two teeth each; the dentin was pre-treated with polyalkenoic acid conditioner in one group but not in the other. The procedure of bonding the GIC to dentin was the same as previously described in the μ TBS test, before storage in distilled water for 1 week and 1 year at 37 °C. The GIC-bonded dentin specimens were sectioned perpendicular to the GIC/dentin interface using an Isomet diamond saw. From each tooth, seven or eight rectangular sections, of approximately 1 mm thickness each, were obtained. After storage for each time period, TEM sample preparation was performed in accordance with common procedures used for ultra-structural TEM examination of biological tissues [25]. This involved specimen fixation overnight in 2.5% glutaraldehyde in 0.1M sodium cacody-

Table 1 – The materials used in this study.

Product name	Composition
Cavity conditioner (GC, Tokyo, Japan)	20% Polyacrylic acid, Distilled water, Aluminum chloride hydrate, Food additive Blue No. 1
Fuji IX GP Extra (GC)	Polyacrylic acid, Aluminosilicate glass, Proprietary ingredient
GC Fuji COAT LC (GC)	Multifunctional urethane methacrylate, Aliphatic dimethacrylate, Methyl methacrylate, Tertiary amine

late buffer at pH 7.4 and 4 °C, followed by rinsing in 0.1M sodium cacodylate buffer for 1 min with 3 changes. Dehydration was carried out in ascending grades of ethanol (50%, 75%, 95%, 100%) for 10 min each, with 2 changes. This was followed by immersion in 1:1 absolute ethanol-epoxy embedding resin for 30 min, and then resin infiltration in 100% epoxy embedding resin for another 4 h. Finally, embedding of the resin-infiltrated samples in molds with 100% epoxy resin was carried out. Before being embedded, the specimens were oriented in the molds so that ultra-thin sections through the GIC/dentin interface could be cut from the dentin part from each original tooth. The epoxy blocks were polymerized in an oven at 60 °C for a minimum of 48 h. Subsequently, non-demineralized, 70–90 nm thin sections were cut using a diamond knife (Diatome, Bienne, Switzerland) in an ultramicrotome (Ultracut UCT; Leica, Vienna, Austria). The GIC/dentin interface in each section was observed by TEM.

2.3. Chemical element analysis

To analyze the chemical elements of the GIC/dentin interface, a Field Emission Transmission Electron Microscope (FE-TEM) (JEM-2010F, JEOL, Tokyo, Japan) was used. The same specimens prepared for TEM observation were used for the FE-TEM observation as well. Images were captured and analyzed by scanning mode of TEM (STEM) at 200 kV.

3. Results

3.1. Micro-tensile bond strength (μ TBS)

The mean μ TBSs are presented in Fig. 1. No pre-testing failures (ptfs) were found in this study.

There was no significant difference in μ TBS when Cavity Conditioner was used at each time period ($p > 0.05$). In addition, 1 year water storage did not show significant difference between conditioned and non-conditioned dentin in terms of μ TBS results.

3.2. SEM failure analysis

At 1 week, the failure patterns were generally of a ‘mixed’ nature, involving areas that failed at the interface and areas that failed cohesively within the GIC, for both the conditioned and non-conditioned groups. At 1 year, while the failure was still of a mixed nature, there was a tendency for more areas of cohesive failure. It appeared that aging of both conditioned and non-conditioned specimens caused them to fail slightly more frequently cohesively within the GIC.

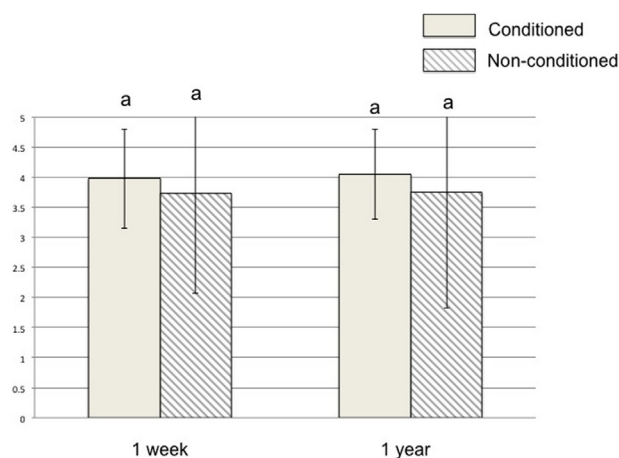


Fig. 1 – Micro-tensile bond strength of GIC bonded to polyalkenoic acid conditioned (Cavity Conditioner) and non-conditioned dentin for 1 week and 1 year. Mean μ TBS are presented in MPa. $n = 10$. The same letters indicate no statistically significant difference ($p > 0.05$).

3.3. TEM interface characterization

Representative TEM photomicrographs of unstained, non-demineralized sections of the GIC/dentin interface with polyalkenoic acid conditioning using Cavity Conditioner stored for 1 week and 1 year are shown in (Fig. 2a&b), while GIC/dentin with non-conditioned interface for 1 week and 1 year are shown in Fig. 3a&b).

A shallow partially demineralized dentin layer (De) of about 0.5–1 μ m was seen at the dentin-conditioned interface (Fig. 2a&b). Hydroxyapatite (HAp) remained within this partially demineralized layer. On top of this layer, a seemingly matrix-rich layer (ML) was seen; this appeared to be of a few hundred nanometers for the 1 week specimens and of about 100 nanometers for the 1 year specimens (Fig. 2a&b). On top of the matrix-rich layer (ML), an intermediate layer (IL) of a few hundred nanometers was noted, and this zone was typically demarcated from the rest of the GIC matrix by small electron-lucent globules (Fig. 2a&b).

Representative TEM photomicrographs of unstained, non-demineralized sections of the GIC/dentin interface without polyalkenoic acid conditioning stored for 1 week and 1 year are shown in Fig. 3a&b. The GIC was closely attached to the dentin surface without any intervening layers detected (Fig. 3a&b). No clear signs of bond degradation were observed after 1 year of water storage.

3.4. Chemical element analysis

The image of GIC/dentin interface with polyalkenoic acid conditioning for 1 week storage as captured by FE-TEM is shown in

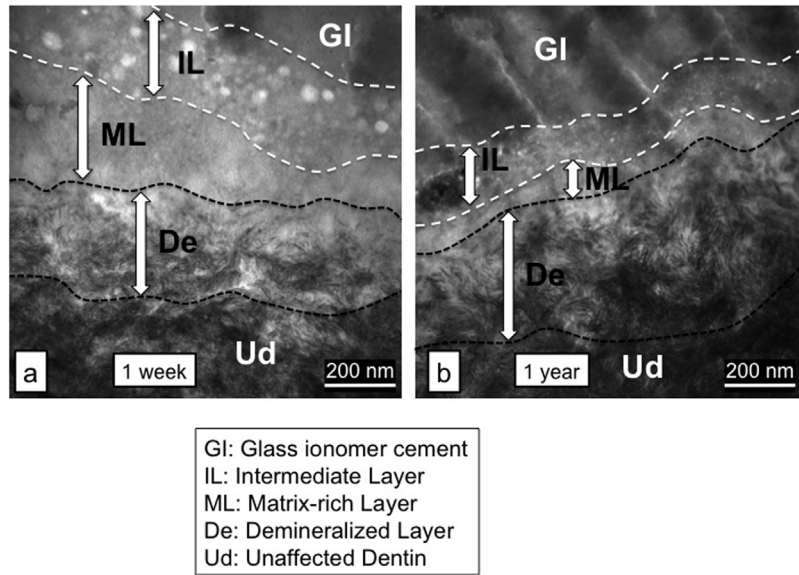


Fig. 2 – Representative TEM photomicrographs of unstained, non-demineralized sections of the GIC/dentin interface with polyalkenoic acid conditioning using Cavity Conditioner stored for 1 week and 1 year. (a,b) A partially demineralized dentin layer (De) of about 0.5–1 μm was seen at the dentin-conditioned interface (a,b). Hydroxyapatite (HAp) remained within this partially demineralized layer. On top of the partially demineralized layer, a matrix-rich layer (ML) of a width of a few hundred nanometers at 1 week and about 100 nanometers at 1 year was seen (a,b). On top of the matrix-rich layer, an intermediate layer (IL) of a few hundred nanometers was deposited, and this zone was typically demarcated from the GIC matrix by small electron-lucent globules (a,b). [GI = Glass ionomer cement; IL = Intermediate Layer; ML = Matrix-rich Layer; De = Demineralized Layer; Ud = Unaffected dentin].

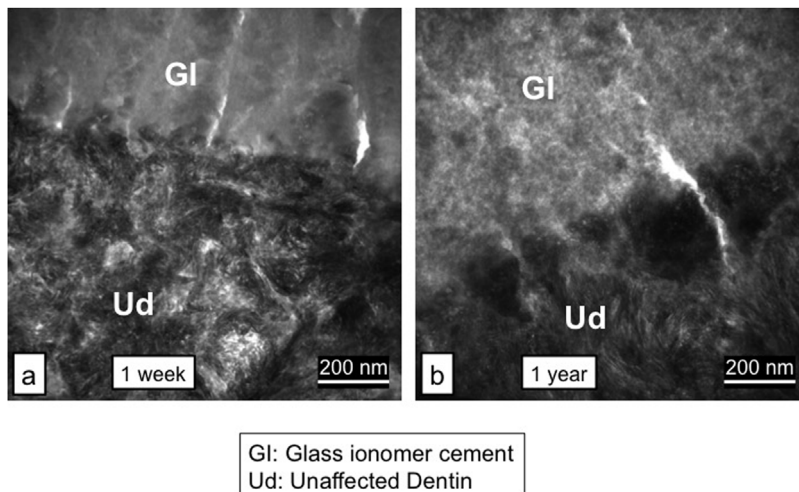


Fig. 3 – Representative TEM photomicrographs of unstained, non-demineralized sections of the GIC/dentin interface without polyalkenoic acid conditioning stored for 1 week and 1 year (a,b). The GIC material was closely attached to the dentin surface without a smear layer and no other layer could be detected (a,b). The bond appeared intact. There were no clear signs of bond degradation after 1 year of water storage. [GI = Glass ionomer cement; Ud = Unaffected dentin].

Fig. 4. There were 3 plots made in this analysis. Plot 1 indicated the GIC area, Plot 2 indicated the IL area and Plot 3 indicated the ML area. Chemical compositions analyzed by STEM mode are shown in Fig. 5. Plots 1 and 2 showed the various components of GIC such as Si, Sr, Al. Plots 1, 2 and 3 showed almost the same tendency although Plot 3 showed more Ca content.

4. Discussion

The use of Cavity Conditioner did not make a significant difference to the μTBS . As cohesive failure within the GIC tends to occur over time, this may be the reason why there was no significant difference in μTBS . The fact is that there was no difference in μTBS even when polyalkenoic acid conditioning



Fig. 4 – The image of GIC/dentin interface with polyalkenoic acid conditioning after 1 week storage as captured by FE-TEM. Plot 1 indicated the GIC area, Plot 2 indicated the IL area and Plot 3 indicated the ML area.

was carried out, although it does not mean that there are no advantages of surface conditioning. Polyalkenoic acid probably facilitates the calcium and phosphate ions from dentin for the ionic reaction with GIC because it removes the smear layer,

increases the contact area and facilitates wetting of the surface [26–28]. Hence, it may be difficult to evaluate the quality of the interface by means of only μ TBS in this case.

From the TEM photomicrographs of Fig. 2a and b, in the conditioned specimens, we can observe different layers moving outwards from the dentin towards the bulk of the GIC: a partially demineralized layer (De), a matrix-rich layer (ML) and a further intermediate layer (IL). The De layer, within which HAp remained, is immediately adjacent to the unaffected dentin. Beyond this, there is a zone (ML) that is reasonably well-defined; it may be a zone that arises due to interaction between the acid-affected dentin layer and the glass component of the glass-ionomer. This interaction was confirmed by the presence of more Ca in the ML as detected by the chemical element analysis. The ML is followed by the next layer, which appears to contain more unreacted glass. The differentiation of layers at the conditioned interface is likely, given the high viscosity of the setting glass-ionomer material.

The widths of the De and IL layers were almost the same in the 1 week and 1 year samples. In contrast, the dimensions of the ML reduced over time. This phenomenon may be ascribed to the maturing effect of GIC, especially as with the use of polyalkenoic acid conditioning, the calcium and phosphate ions' reaction with GIC was activated and the remineralizing effect may have been promoted as well. The increase of apatite formation and mechanical property could be expected, but this has to be confirmed in further work. There were no signs of interface degradation comparing the 1 week and 1 year (Fig. 2a and b) interfaces observed.

From the TEM photomicrographs of un-conditioned specimens (Fig. 3a&b), we can observe the GIC area and dentin area without any intervening differentiation or layers; there was no significant difference in μ TBS compared with the polyalkenoic acid conditioned group. It is possible that there was an ultra-

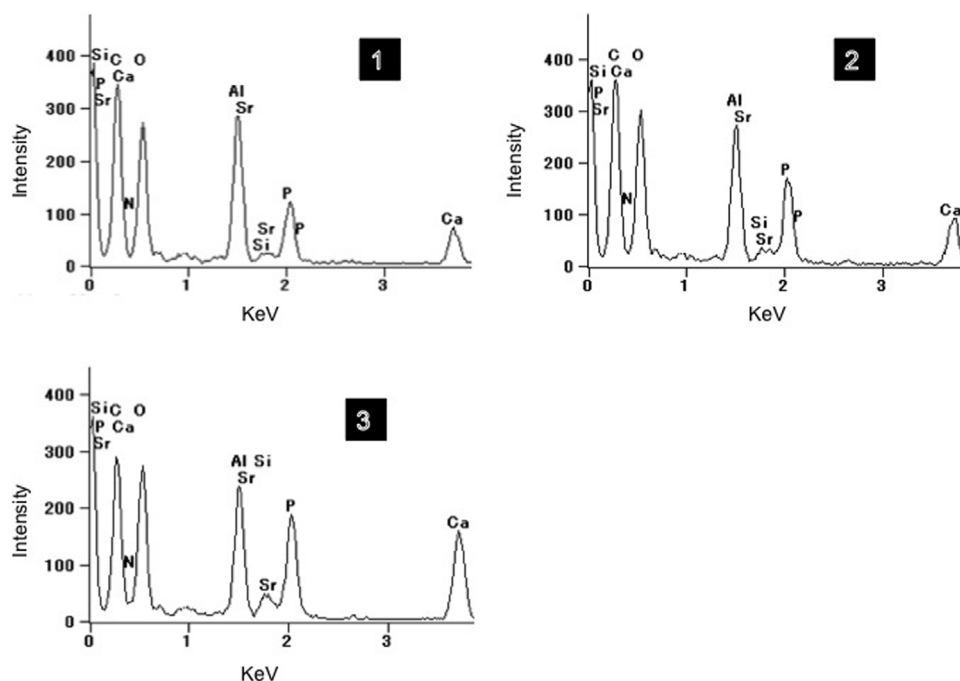


Fig. 5 – Chemical compositions were analyzed by STEM mode. Plots 1 and 2 showed the components of GIC such as Si, Sr, Al. Plots 1, 2 and 3 showed almost the same tendency while Plot 3 showed more Ca content.

thin demineralized layer at the interface which could not be seen in these TEM photomicrographs. There was again no clear sign of degradation between the 1 week and 1 year specimens (Fig. 3a and b).

From the chemical element analysis, 3 areas were chosen, which were estimated areas: De (Plot 1), IL (Plot 2) and ML (Plot 3) areas. Basically, the composition of GIC includes a polymeric water-soluble acid, glass, and water [29]. The glass components were either of the $\text{SiO}_2\text{-Al}_2\text{O}_3\text{-CaF}_2$ system or the more complex $\text{SiO}_2\text{-Al}_2\text{O}_3\text{-P}_2\text{O}_5\text{-CaO-CaF}_2$ system, also calcium has been substituted by strontium [30]. The components of the GIC material such as Si, Al, Sr, Ca were detected from all 3 plots, indicating the presence of GIC components in the IL and ML. The ML appears to be a mixture of GIC and dentin tissue, which has been unknown until now. The IL is a reaction layer which is probably formed by polyalkenoic acid and HAp. Due to the ionic exchange of fluorine and strontium, GIC has a remineralization effect on demineralized tooth in terms of quantitative analysis of the mineral content of the remineralized structures, and their mechanical properties were previously described [31–35].

Partial caries removal or incomplete caries removal is more demanding based on scientific evidence [36–38]. For those situations, using the stepwise removal and selective removal technique, GIC is recommended as it has similar bond strength to both normal and caries-affected dentin [39,40]. GIC has superior clinical survival results for deep dentin and hypermineralized dentin as well [18,41]. This is because of its resilience, low polymerization shrinkage and good sealing ability.

From the results of the μTBS test, pre-treatment of dentin using a polyalkenoic acid conditioner did not affect the long-term durability of a conventional GIC; hence, the null hypothesis should be accepted.

Further research will provide an understanding of the remineralizing effect of GIC on caries-affected dentin using polyalkenoic acid.

5. Conclusion

Aging did not reduce the bond strength of the conventional GIC to dentin whether the surface was pre-treated with a polyalkenoic acid conditioner or not. Conditioning of dentin appears to increase the durability of the GIC to dentin.

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CRedit authorship contribution statement

Shuhei Hoshika: Writing - original draft, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration. **Shihchun Ting:** Methodology. **Zubaer Ahmed:** Methodology. **Fei Chen:** Investigation. **Yu Toida:** Investigation. **Norihito Sakaguchi:** Methodology. **Bart Van Meerbeek:** Supervision. **Hidehiko Sano:** Project administration. **Sharanbir K. Sidhu:** Writing - original draft, Writing - review & editing.

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