Ten-year observation of dentin bonding durability of 4-META/MMA-TBB resin cement—a SEM and TEM study

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This study aimed to assess dentin bond durability of 4-META/MMA-TBB resin cement over ten years, by evaluating the tensile bond strength, and SEM and TEM observations. Tensile bond strength of Super Bond C&B (SB) to bovine dentin was evaluated at 1 day and after 10 years. The mode of failure after debonding was observed by SEM. Interfacial ultrastructure and nanoleakage was observed by TEM at the baseline and after 10 y. The tensile bond strength significantly dropped after 10 y. The failure pattern shifted from cohesive failure in resin towards adhesive failure or cohesive failure in dentin. TEM observation revealed degradation of both resin and collagen networks within the hybrid layer and nanoleakage at the base of the hybrid layer after 10 y. The bond strength of SB to dentin significantly decreased, and the hybrid layer degraded, while the overlying hydrophobic resin layer showed little disintegration over 10 y.

Keywords: Bond durability, Resin cement, Tensile bond test, SEM, TEM

INTRODUCTION

Adhesive resin cements have been preferred over conventional luting cements because of their advantageous mechanical properties and their ability to bond to the tooth. Umino *et al.*¹⁾ revealed that resin cements exhibited significantly lower cement solubility compared to other luting cements, and that fluoride-releasing resin cements such as Panavia F (Kuraray Medical, Tokyo, Japan) could contribute to prevention of secondary caries around restorations.

Nakabayashi *et al.*²⁾ demonstrated that a 4-META/MMA (4-methacryloxyethyl trimellitate anhydride/methyl methacrylate) resin initiated with partially oxidized tri-*n*-butylborane (TBB) provided high bond strength to dentin treated with 10% citric acid and 3% ferric chloride (FC). It was reported that the 4-META monomer, which contained both hydrophobic and hydrophilic structures, increased the molecular weight of the polymer formed and promoted adhesion to tooth structures³⁾. It was also suggested that FC was adsorbed onto dentin collagen and was involved in the promotion of MMA polymerization⁴⁾. The 4-META/MMA-TBB resin cement has been widely used over the past two decades, not only as a luting cement, but also for cementation of orthodontic brackets and temporary fixation as part of the treatment for periodontal disease. According to the manufacturer, the composition formula of the 4-META/MMA-TBB resin cement, Super Bond C&B (SB; Sun Medical Co., Moriyama, Japan), has not changed for over 10 years.

Despite high bond strengths values at early times, several studies reported gradual decrease in the bond strength over time for SB^{5–7)}. Kitasako *et al.*⁶⁾ evaluated the durability of SB adhesion to extracted bovine dentin after 3 y water storage, demonstrating a significantly lower bond strength compared to 1 day and 6 months. They reported that after 3 y water storage, the failure patterns were mainly cohesive within the demineralized dentin or intact dentin.

In vitro tests have been used to evaluate the performance of resin-based cements, and provide useful information on resin cements to help practitioners choose a material for clinical use. The tensile bond strength (TBS) test is one of the conventional methods used over the past decades to evaluate the bond strength. This test is also a suitable choice for *in vitro* studies on bond degradation, as it was reported that the fracture under the tensile load might initiate within the degradation area of bonded samples after long-term storage^{8,9)}. On the other hand, nanoleakage experiment by transmission electron microscopy (TEM) is a relatively recent evaluation technique that was not widely available over a decade ago. This technique has facilitated investigation of the location and pattern of interfacial defects, where silver grains can be deposited at voids within the resin layer or in the hybrid layer¹⁰⁾. However, there is no report on the *in vitro* bond durability of resin cements and morphological changes of hybrid layer in high resolution using TEM over as long period of time as a decade.

Based on this background, the purpose of this study was to evaluate the TBS durability of the 4-META/

Fig. 1a Experimental procedures for the tensile bond strength (TBS) test, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) observations.

MMA-TBB resin cement to extracted bovine dentin over the period of ten years. In addition to scanning electron microscopy (SEM) of the failure mode after the tensile bond test, the ultrastructure of the bonded interface was observed by various TEM techniques, aiming to further clarify the mechanisms involved in the long-term degradation. The null hypothesis was that there were no changes in TBS to dentin and morphological features of SB over 10 y.

MATERIALS AND METHODS

This study involved TBS test, and SEM and TEM observations. The experimental procedures of this study are schematically illustrated in Fig. 1a.

Tooth preparation

Freshly extracted bovine teeth were prepared within 6 months following extraction and used as the test substrate. The root of each tooth was cut 2 mm below the cemento-enamel junction, and a flat surface on superficial

Fig. 1b Nine points for fracture mode were evaluated for each specimen.

Table 1 Chemical formulations of resin cement investigated in this study

Material	Manufacturer	Bath No	Composition	Manufacturer's recommended protocol
Super Bond C&B	Sun Medical (Moriyama, Japan)	Etchant: LV2	10% citric acid with 3% ferric chloride	Apply etchant to dentin for 10 s, wash with water and air-dry.
		Powder: RK11	PMMA	Apply mixture of the polymer powder and monomer-catalyst, using the brush-on technique to the bonded tooth surface.
		Liquid: MK2	MMA, 4-META	
		Catalyst: MS11	TBB	

PMMA: polymethyl methacrylate; MMA: methyl methacrylate; 4-META: 4-methacryloxyethyl trimellitate anhydride; TBB: Tri-n-butylborane.

coronal dentin was created using a model trimmer under copious water flow. The dentin region to be bonded was then finished with 1000-grit silicon carbide paper under running water. To prevent the embedding material from entering the pulp chamber, the pulp canal was sealed with a self-cured conventional glass polyalkenoate luting cement (Ketac-cem, 3M ESPE, Seefeld, Germany). After the cement had set, the teeth were embedded in a self-curing epoxy resin (Epon 815, Nissin EM Co. Ltd., Tokyo, Japan) with the prepared bonding surface placed face down on a glass slab. Care was taken so as not to contaminate the prepared surfaces with the epoxy resin. The tooth surfaces were kept moist throughout specimen preparation. The same experimenter made the samples at both experiment times in the same laboratory and under similar experiment conditions. So the procedure of bonding technique was same between 1 d and 10 y.

Specimen preparation for TBS test

Once the self-curing epoxy resin had hardened, the prepared dentin surfaces were covered with a piece of vinyl tape (0.15 mm thick) in which a 3 mm diameter hole was cut to demarcate the bonding area. The teeth were then bonded with the 4-META/MMA-TBB resin cement (SB) according to the manufacturer's instructions. The resin cement system, batch numbers, manufacturers and compositions are listed in Table 1. Ten percent citric acid with 3% ferric chloride was placed on the dentin surface for 10 seconds, thoroughly washed with air-water spray and gently dried, ensuring the dentin was not desiccated. For Super Bond C&B, the bonded area was covered with a mixture of the polymer powder and monomer-catalyst, using the brush-on technique. For tensile testing, a resin composite rod (Clearfil CR Inlay, Kuraray Medical., Tokyo, Japan) 15 mm long and 4 mm in diameter was cemented perpendicularly to the bonded tooth surface. The resin rods were left undisturbed for 5 minutes to allow the resin cements to set. The bonded assemblies were then placed in tap water maintained at 37°C for up to 10 y. The water was changed weekly and no preservative or antimicrobial agents were used.

Ten teeth were subjected to TBS test at each period (1 d as the baseline and 10 y). The jig used for the tensile test was similar to that described by Fusayama *et al.*¹¹⁾. The jig was mounted on a universal testing machine (Autograph AGS-J, Shimadzu, Kyoto, Japan), and specimens were stressed in tension at a crosshead speed

of 1 mm/min. The means and standard deviations of the bond strengths were calculated for each time period, and statistically analyzed by the student's *t*-test ($p < 0.05$), to find any significant change in TBS after 10 y. All the data were analyzed using the Statistical Package for Medical Science (SPSS Ver.11 for Windows) for statistical procedures.

SEM of the debonded surfaces

After failure, the specimens were trimmed to the debonded area under running water. The surface was then gold sputter-coated and observed under a scanning electron microscopy (SEM, JSM-5310LV, JEOL, Tokyo, Japan). The mode of fracture was observed at nine areas in each specimen^{6,12)}(Fig. 1b). At each area, the failure mode was recorded as one of the three categories as follows: A- cohesive failure within the resin cement or a partial adhesive failure where remnants of resin were observed on the dentin surface; B- complete adhesive failure at the resin-dentin interface; and C- mixed mode of adhesive failure and cohesive failure in dentin or complete cohesive failure in dentin. The percentage of each fracture category was calculated based on the frequency of the mode observed in the total of 90 locations for each experimental group (10 specimens and 9 locations). Results for the mode of failure were statistically analyzed by Pearson's Chi-square test in the SPSS software.

Interfacial evaluations by TEM

Additional specimens, prepared in the same manner as described for tensile bond-strength, were used for TEM evaluations of the interface. Debonded specimens were observed under stereo microscope at $\times 30$ magnification at the baseline and after 10 y, and specimens that showed cohesive failure within resin after loading were selected for the interfacial observation. A block $1 \times 1 \times 1$ mm in size was then sectioned from the widest part of the bonded area on the selected specimens. These blocks were randomly distributed into three groups, each undergoing different preparation procedures for evaluation by TEM, namely; undemineralized unstained, demineralized stained or undemineralized silver-impregnated. Specimens in the first group (undemineralized unstained) were used for morphological evaluation of the resin-dentin interface and observation of thickness of hybrid layer, those in the second group (demineralized

stained) were used to observe the organic phase and collagen after staining, and those in the third group (undemineralized silver-impregnated) were used for nanoleakage evaluation. The laboratory procedures for each preparation are explained later. At least five specimens were observed under TEM in each of these groups. Moreover, five additional specimens were prepared to observe the unloaded and non-aged interface at the baseline. In this group, the cement was applied without attaching a resin composite for tensile test. After 1 d of storage following application of the cement, the specimens, a block was sectioned and prepared similar to undemineralized unstained specimens for TEM observation.

1. Preparation of demineralized and undemineralized specimens

Each specimen in the demineralized group was immersed for seven days in an aqueous solution of 0.1 mol/L ethylene diamine tetra-acetic acid (EDTA) buffered with sodium hydroxide to pH 7.0, to remove the minerals. The demineralized and undemineralized specimens were fixed in Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 mol/L cacodylate buffer, pH 7.3) for a minimum of 1 hour and rinsed thoroughly with 0.1 mol/L sodium cacodylate buffer (pH 7.0) for 1 h at room temperature. The fixed specimens were then rinsed three times in cacodylate buffer with a dwell time of 1 min each, then dehydrated in an ascending ethanol series (30% to 100%), and immersed in propylene oxide as a transition fluid. Finally, the specimens were embedded in epoxy resin (TAAB 812, TAAB Laboratories, Aldermaston, UK) at 60°C for 48 h¹³⁾.

2. Preparation of undemineralized silver-impregnated specimens

Nanoleakage expression was examined on undemineralized silver-impregnated specimens. Ammoniacal silver nitrate was prepared by the dissolution of 25 g of silver nitrate crystals (Wako, Osaka, Japan) in 25 mL of distilled water. Concentrated (28%) ammonium hydroxide (Sigma, Tokyo, Japan) was used to titrate the black solution until it became clear as ammonium ions complexed the silver into diamine silver ions ($[\text{Ag}(\text{NH}_3)_2]^+$). This solution was diluted to 50 mL with distilled water, yielding a 50 wt% solution (pH 9.5).

The tooth blocks were immersed in the tracer solution for 1 d with care being taken to ensure the blocks did not dehydrate prior to immersion, as previously described¹⁴⁾. The silver-impregnated blocks were then rinsed thoroughly in distilled water and placed in photo-developing solution for 8 h under a fluorescent light to reduce the diamine silver ions into metallic silver grains. The specimens were then dehydrated and embedded in epoxy resin, according to the embedding protocol described by Tay *et al.*¹⁵⁾.

3. TEM observation

Ultrathin transverse sections (90–110 nm) were cut from the embedded specimens in each group described earlier. An ultramicrotome (Reichert-Nissei, ULTRACUT N, Leica, Vienna, Austria) with a diamond knife (Diatome, Bienne, Switzerland) was used for ultrathin sectioning.

Staining of the demineralized specimens involved the use of 1% uranyl acetate (UA) for 5 min and Reynold's lead citrate (LC) aqueous solution for 1 min. The former allowed observing the relationship between adhesive resin layer, hybrid layer, and the laboratory demineralized dentin; the latter was a specific TEM staining for collagen used for discerning the fine subfibrillar architecture of the collagen fibrils within the hybrid layer.

The sections were placed on 100 mesh copper grids (Okenshoji Co., Tokyo, Japan). Finally, all sections were examined with a transmission electron microscopy (H7100, HITACHI, Tokyo, Japan) operating at 75 kV.

RESULTS

Bond strength results

The results for the TBS test after debonding are shown in Table 2. The specimen stored in water for 10 y showed a remarkable and statistically significant drop in bond strength compared to the baseline ($p < 0.01$).

Fracture mode and SEM examination

The fracture modes after bond testing were shown in Table 2. It was revealed that at 1 d, the predominant failure pattern was cohesive failure within the resin cement or partial adhesive failure, where remnants of resin were observed on the dentin surface (pattern A,

Table 2 Comparison of dentin tensile bond strengths and fracture mode

	Tensile bond strength (MPa)	Fracture mode (%)		
		A	B	C
1 d	17.2 (3.6) ^a	59	22	19
10 y	6.0 (1.7) ^b	16	15	69

Bond strength: Mean (standard deviation), $n=10$. Values with different superscript letters were statistically different ($p < 0.01$).

Fracture mode categories:

A - cohesive failure within the resin cement only and partial adhesive failure, where remnants of resin were observed on the dentin surface.

B - adhesive failure at the resin-dentin interface.

C - mixed adhesive failure and cohesive failure in dentin and cohesive failure in dentin only.

A significant difference was found between 1 d and 10 y ($p < 0.001$).

59%). On the other hand, after 10 y, mixed adhesive failure and cohesive failure in dentin and cohesive failure in dentin were the dominant observed patterns (pattern C, 69%). There was a statistically significant difference in the distribution of fracture modes between the baseline and 10 y groups ($p < 0.001$).

Representative SEM images of the fractured surface (on dentin side) after 1 d and 10 y were presented in Fig. 2. Figure 2a showed cohesive failure within the resin cement observed at 1 d (pattern A). The micrographs representing adhesive failure at the resin-dentin interface (pattern B) at 1 d and 10 y were presented in Figs. 2b and 2c, respectively. Figure 2d showed cohesive failure in the dentin observed at 10 y (pattern C). In contrast to 1 d, after 10 y many resin tags remained in

the tubules after the adhesive failure indicating that the mechanical properties of resin were affected by the storage (Fig. 2c).

TEM examinations

TEM micrographs of the adhesive interfaces at 1 d water storage were included in Fig. 3. In the interfacial observation of an undemineralized unstained sample not subjected to the tensile bond test (unloaded specimens), an approximately 3 μm -thick hybrid layer could be observed (Fig. 3a). Figure 3b showed TEM micrograph of an undemineralized, unstained specimen after debonding under tensile load. The thickness of hybrid layer beneath the debonded area (Fig. 3b) in this specimen was similar to that in the unloaded specimen (in Fig. 3a), with the

Fig. 2 SEM observations of the fractured surface ($\times 2,000$ magnification). (a) 1 d specimen: the observed mode was cohesive failure within the resin cement. The resin cement remained on the tooth surface; (b) 1 d specimen: the failure was adhesive failure at the resin-dentin interface; (c) 10 y specimen: adhesive failure can be observed with the opening of dentin tubules, fractured tags in the dentin tubules, as well as resin tags being pulled out of the dentin tubules; (d) 10 y specimen: The observed mode was cohesive failure in the demineralized dentin and intact dentin.

Fig. 3 TEM observations of the 1 d specimens. R: resin cement, H: hybrid layer, D: dentin. (a) before tensile bond test, undemineralized, not stained: a hybrid layer about 3 μm can be observed (×20,000 magnification, Bar=500 nm); (b) After tensile bond test, undemineralized, not stained: the resin cement remained on the dentin surface. Cohesive failure within the resin cement was observed (×4,000 magnification, Bar=2 μm); (c) After tensile bond test, demineralized and UA and LC stained: higher magnification of the zone within the hybrid layer. Collagen fibrils with cross banding pattern were observed within the bulk of the hybrid layer. The hybrid layer appeared as a thick structure (×30,000 magnification, Bar=500 nm); (d) After tensile bond test, undemineralized and immersion in ammoniacal silver nitrate: the white arrow points to sporadic existence of the silver deposits within the hybrid layer (×10,000 magnification, Bar=500 nm).

hybrid layer exhibiting a uniform density (Fig. 3b). Figure 3c showed a debonded specimen demineralized and stained with UA and LC. Resin impregnated collagen fibrils were observed within the body of hybrid layer, revealing a cross-banding pattern. TEM micrograph presented in Fig. 3d was from an undemineralised silver-impregnated specimen revealing a nanoleakage pattern with several isolated spots of silver grains dispersed within the body of hybrid layer. No silver uptake was

observed within the cement layer itself.

TEM micrographs of the interface after the tensile bond test at 10 y were presented in Fig. 4. As shown in the SEM observations (Fig. 2c), some resin tags were pulled out of the dentinal tubules together with a part of the hybrid layer (Fig. 4a). On the other hand, some specimens exhibited a defective hybrid layer, with parts of resin missing from the body of the layer (Fig. 4b). In the demineralized stained specimens after 10 y, it was

Fig. 4 TEM observations of the 10 y specimens. R: resin cement, H: hybrid layer, D: dentin, T: resin tag. (a) After the tensile bond test, undemineralized, not stained: resin tags fractured (white arrow head) or pulled out from the dentin tubules (white arrow). The fracture occurred between SB and the hybrid layer. Some parts of the hybrid layer remain attached to the resin tags (black arrow). Partial loss and mechanical disruption within the hybrid layer was observed. ($\times 6,000$ magnification, Bar=2 μm); (b) After the tensile bond test, undemineralized, not stained: at higher magnification of the resin-dentin interface, a partial loss of hybrid layer structure was clearly observed (white arrow). The body of resin layer (R) appears dense and well-integrated ($\times 20,000$ magnification, Bar=500 nm); (c) After the tensile bond test, demineralized and UA and LC stained: a lack of bonding resin was typically observed in the demineralized zone, exhibiting a degenerated hybrid layer. The collagen fibrils within the hybrid layer were hardly seen in the degraded zone compared with the control ($\times 20,000$ magnification, Bar=500 nm); (d) After the tensile bond test, undemineralized and immersion in ammoniacal silver nitrate: in addition to the scattered silver deposits within the hybrid layer, This specimen isolated silver grains could be observed along the resin-dentin interfaces (white arrow) ($\times 10,000$ magnification, Bar=500 nm).

difficult to observe the arrangement of the collagen fibrils in the hybrid layer (Fig. 4c). A partial absence of resin components was also observed within the hybrid layer, creating a large flaw. Figure 4d revealed the nanoleakage pattern after immersion in the ammoniacal

silver nitrate. Silver deposits were observed along the hybrid layer-dentin border, in addition to several silver spots within the hybrid layer.

DISCUSSION

Formulations of commercially available adhesive resin-based systems are usually modified by the manufacturers within a few years after their introduction, and newer versions of the system are introduced into the market. However, SB has not changed its components over the past decade and has been available for clinical usage for well over 10 y. Several studies have shown the stable bond strengths of bulk specimens, even after 1 y of water storage^{5,6}. Therefore, a question has arisen as to whether the resin-dentin bond strength changes with storage in water for longer than 1 y in the case of bulk specimens. Several studies have examined longer periods of storage time, of 5 or 7 y^{5,16}. It is possible that a new degradation phase of resin-dentin bonds might be observed under longer storage periods than those used in previous studies¹⁷. Therefore, the results of the current study can be regarded as long-term findings about a currently available product.

Bovine dentin was chosen as the substrate in this study because of the convenient size of the teeth. It has been demonstrated that bovine teeth are a good substitute for human teeth¹⁸. Moreover, plain tap water was selected as the storage medium as it showed a stable pH value over a long time (pH 7.2–7.5 for up to 3 months)⁶.

TBS test used in the current study was the most common and widely recognized bonding test when the specimens of this decade-long study were bonded. While several advantages have been pointed out for more recent micro-tensile bond tests which have a smaller bonding surface area, Braga *et al.*¹⁹ demonstrated that the number of articles using conventional (or macro) tests published in recent years still remains high, meaning that a lot of the available data on dental adhesion still comes from mechanical tests performed in specimens with larger bonded areas.

A 10 y bond durability test can contribute to the understanding of degradation mechanisms of adhesive materials, and the laboratory results can indicate outcomes within a clinically relevant time scale. However, only few reports regarding adhesive-dentin bond durability over such long periods of time have been published^{5,16,20,21}. The current study confirmed the previous findings from those laboratory reports that resin-dentin interface degraded over time when stored in water²⁰. Moreover, *in vivo* clinical studies have also demonstrated degradation of resin-dentin bonds and increased porosity at the bonded interface after one or three years^{22,23}.

Interestingly, a comparison between TBS results after 10 y water storage to that after up to 3 y, previously reported from similar experiments by our group⁶, indicated that the bond strength which had abruptly decreased within the first year (around 40% loss), continued degradation at a much lower pace after that, reaching to approximately 50% and 65% loss compared to the baseline after 3 y and 10 y, respectively.

In addition to a significant drop in bond strength,

SEM observation of the fractured surfaces after debonding in the present revealed that at the baseline, cohesive failure within resin cement was predominantly observed. This finding could be explained by the fact that SB contained no filler particles and therefore a low mechanical strength, leading to predominant failure within the resin under the load. However, after 10 y of storage, the location of the failure shifted from within the resin cement towards the bottom of hybrid layer and underlying dentin. This finding was also in line with the previous reports, indicating that the hybrid layer was gradually degrading^{5,6}. It was suggested that after applying SB to the acid etched dentin, the adhesive had failed to envelope the collagen fiber network at the bottom of hybrid layer and therefore demineralized dentin remained exposed at this zone. The demineralized and exposed dentin would become the weak-link in the long-term, shifting the failure mode under load from pattern A to pattern C. Furthermore, it has been shown that the mechanical properties of the dental hard tissue decreased when teeth were stored in physiological saline, presumably due to the loss of surface calcium²⁴. Changing the storage solution might induce the loss of calcium from the dentin resulting in further exposure of the dentin collagen, which could have an important effect on the hydrolysis of unprotected collagen fibrils¹². Conversely, an equilibrium of calcium ion transfer between the dentin and unchanged storage solutions would be established in the solution¹². Such changing of the storage solution may also have further accelerated hydrolysis at the interface between the hybrid layer and dentin, and also between hybrid layer and resin cement²⁵. The changes would also result in cohesive failures within dentin during the bond strength test.

Previous studies have revealed the presence nanoleakage within resin-dentin bonds of various bonding systems, particularly those with high amount of hydrophilic monomers and solvents contained in resin adhesives (*i.e.* simplified adhesive two-step etch-and-rinse or all-in-one systems)^{10,26}. In contrast, SB resin cement contains a small amount of 4-META in methyl methacrylate liquid (MMA) mixed with polymethyl methacrylate (PMMA) powder. Basically, the hydrophobic MMA monomer serves as the solvent for 4-META and PMMA. The system is water-free, and exhibits very low water absorption due to hydrophobic nature of the matrix²⁷. It is noteworthy that nanoleakage was not found within the hydrophobic resin cement at the baseline. On the other hand, the existence of sparse spotted silver deposits within the hybrid layer at 1 d water storage in the current study might suggest imperfect penetration of adhesive monomers and imperfect resin polymerization¹⁰. This baseline nanoleakage is particularly notable, as SB monomers, MMA and 4-META were expected to penetrate into the demineralized dentin more easily than dimethacrylates, such as Bis-GMA-based adhesive resins that have higher molecular weight. Moreover, diffusion-induced leakage of this resin cement was low, presumably due to its relatively hydrophobic formulations after

polymerization²⁸). Nevertheless, it was suggested that a discrepancy between the depth of acid-etching and the depth of resin infiltration into the collagen network was thought to be responsible for water movement from the underlying hydrated dentin^{25,26}) and result in nanoleakage in as early as 1 d.

On the other hand, in the long-term, not only a loss of interfacial seal at the bottom of hybrid layer was observed, but also deterioration of resin within the hybrid layer was detected. The impregnating resin was partially missing from the hybrid layer in specimens stored for 10 y, and fracture was observed within the hybrid layer. Therefore, the reduction observed in tensile bond strength may also be attributed to the uptake of water causing changes in the mechanical properties, especially within the resin tag and hybrid layer. In this regard, several studies have reported on the hydrolytic degradation of methacrylate-based resin with time²⁹). The hydrolysis of 4-META/MMA-TBB resin, particularly if incompletely polymerized, and gradual water uptake might cause the loss of resin from the hybrid layer³⁰). However, it should be mentioned that in the current study, nanoleakage or disintegration within the body of resin overlying the hybrid layer was not observed even after 10 y, indicating the advantages of a hydrophobic resin layer, when compared to extremely hydrophobic resins that show remarkable nanoleakage within the bonding layer itself.

At 10 y, it was difficult to observe the arrangement of the collagen fibrils in the hybrid layer. The unprotected collagen fibrils at the hybrid layer undergo degradation marked by disarrangement of collagen web, widening the interfibrillar space, and the thinning diameter of collagen fibrils when exposed to water for several months³¹). On the loss of collagen fibrils at the hybrid layer, it has also been suggested that activation of host-derived matrix metalloproteinase (MMP) within the dentin matrix at the interface may be responsible for the degradation^{17,32}).

In short, the null hypothesis in this study was rejected, as differences in bond durability of SB to dentin over 10 y was clearly observed. The bonded-dentin sample with SB still showed a mean TBS value slightly over 5 MPa after 10 y. The degradation of the adhesive interface and hybrid layer with a micro-porous structure³³) may compromise the durability of adhesive restorations leading to their failure^{5,7}). A bonding procedure that requires separate etching of dentin (namely etch and rinse approach) seems to leave exposed collagen fibrils beneath an incompletely infiltrated dentin matrix^{34,35}), resulting in hydrolysis of collagen, and opening leakage pathways^{20,36}). Moreover, the elution of unconverted resin monomers within the hybrid layer and their replacement by water may cause deleterious effects to bonds^{23,37}). Nevertheless, the overlying hydrophobic resin layer showed little disintegration after 10 y. Further studies are required to evaluate the effect of milder acids and monomers with higher affinity to the mineral substrate on the long-term bond durability of adhesive materials, and clinical implications of the *in*

vitro degradation tests.

CONCLUSIONS

The bond strengths of resin cements to dentin significantly decreased after 10 y, and the long-term degradation of both the resin components and organic phase of dentin was observed at the hybrid layer.

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