Dentin bonding performance using Weibull statistics and evaluation of acid-base resistant zone formation of recently introduced adhesives

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Dentin bonding durability of recently introduced dental adhesives: Clearfil SE Bond 2 (SE2), Optibond XTR (XTR), and Scotchbond Universal (SBU) was investigated using Weibull analysis as well as analysis of the micromorphological features of the acid-base resistant zone (ABRZ) created for the adhesives. The bonding procedures of SBU were divided into three subgroups: self-etch (SBS), phosphoric acid (PA) etching on moist (SBM) or dry dentin (SBD). All groups were thermocycled for 0, 5,000 and 10,000 cycles followed by microtensile bond strength testing. Acid-base challenge was undertaken before SEM and TEM observations of the adhesive interface. The etch-and-rinse method with SBU (SBM and SBD) created inferior interfaces on the dentin surface which resulted in reduced bond durability. ABRZ formation was detected with the self-etch adhesive systems; SE2, XTR and SBS. In the PA etching protocols of SBM and SBD, a thick hybrid layer but no ABRZ was detected, which might affect dentin bond durability.

Keywords: Dentin bonding, Etch-and-rinse mode, Self-etch mode, Weibull statistics, ABRZ

INTRODUCTION

Etch-and-rinse adhesive systems require the use of 30–40% phosphoric acid (PA) etching before application of a primer and bonding resin. Although more effective enamel bonding is achieved by PA etching, the application of PA on dentin surfaces is still problematic requiring a meticulous technique for long-term success. Two-step self-etch adhesive systems (2-SEA) were introduced, in part, to overcome the problems of PA etching of dentin and have demonstrated good bonding performance and sealing ability both in clinical and laboratory studies1-20. The development of self-etch adhesive systems simplified the procedure of resin bonding thus reducing technique sensitivity for clinical use, especially after the introduction of 1-step self-etch adhesives (1-SEA)21-41. Also referred to as ‘one-bottle’ or ‘all-in-one’ self-etch adhesives, 1-SEAs have been tested and regarded as acceptable for immediate bond strengths. Considering the differences in professional judgment amongst clinicians regarding the selection of an adhesive strategy and number of steps needed, some manufacturers have recently released more versatile adhesive systems that have been termed ‘universal’, ‘multi-purpose’ or ‘multi-mode’ adhesives42. This ‘new’ group of adhesives, which can be used in either an etch-and-rinse or self-etch mode, enables general practitioners to decide which specific protocol would be preferable when placing a restoration43. However, concerns exist with respect to both the short- and long-term bonding effectiveness as well as the quality of the dentin-bonding interface of these relatively new systems.

Contemporary dentin-resin bonding is achieved by infiltration and polymerization of monomers within the dentin substrate, chiefly to the collagen fibrils that are exposed during acid etching from PA or acidic monomers in SEA systems. The infiltration of resins creates the well-known hybrid layer (HL), which is regarded as a ‘new’ dentin made up as a composite of resin and collagen5,24.

The acid-base resistant zone (ABRZ) for resin-based systems was first coined by Tsuchiya et al.8. It is characterized as a structure at the resin-dentin interface that is resistant to acid-base challenges and different from the HL. It was detected by using argon-ion etching of bonded specimens of dentin. Recent studies have demonstrated the formation of ABRZ with 1-step and 2-step SEA and it seems to contribute to the sealing of restoration margins and therefore promoting the durability of the restoration. Numerous studies have reported the structure of the ABRZ9-11. The ultrastructure of the HL and ABRZ have been revealed using various treatments and analyzed using both scanning and transmission electron microscopy12-14.

When considering bond degradation, the hydrolytic effects of water on adhesives is of great importance. Micromorphological examination has demonstrated various degradation phases for each adhesive system, both in vivo and also laboratory-based work15. The typical morphological evidence of degradation is apparent by collagen hydrolysis associated with etch-and-rinse adhesive systems, resin elution from the HL of all systems, and hydrolytic degradation at the border between the adhesive/composite junction of 1-SEA. By
simulating the aging progress using water storage in a laboratory-based environment, with thermal cycling and mechanical loading we can more closely replicate the situation during functioning in the oral cavity. This combination of load and temperature cycling will provide a greater depth of information to better predict the durability and clinical performance of resin-based adhesives. The small cross-sectional area of microtensile bond strength (µTBS) specimens used with thermal cycling has been frequently used as the simulation of oral temperature changes as a means to evaluate bonding performance.

Since the immediate bond effectiveness of most current adhesive systems is quite favorable, the long-term durability of the bonds has yet to be established. The longevity of adhesively bonded restorations is, in part, dependent on the quality of the bonded area of approximately 1.0 mm².

Therefore, the purpose of this study was to investigate the bonding properties and the micromorphological features of the ABRZ with SEM and TEM by testing recently-developed 2-SEAs: Clearfil SE Bond 2 (SE2, Kuraray Noritake Dental, Tokyo, Japan) and Optibond XTR (XTR, Kerr, CA, USA) and one 1-step universal or multi-purpose adhesive: Scotchbond Universal (SBU, 3M ESPE, MN, USA) in the self-etch and etch-and-rinse modes. The null hypotheses were: 1, the µTBS of each adhesive is not affected by thermal cycling; and 2, the characteristics of the ABRZ do not differ amongst the tested materials.

MATERIALS AND METHODS

Materials used in this study

Table 1 lists the compositions, manufacturers, and batch numbers of the materials employed in this study. Two 2-step SEAs (SE2 and XTR) and one universal adhesive used in etch-and-rinse and 1-step SEA modes (SBU) were the materials selected for the study. K-etchant gel (Kuraray Noritake Dental), 40% phosphoric acid, was used with SBU when it was used in etch-and-rinse mode. The bonding procedures of each group are listed in Table 1.

Caries-free extracted human third molars were obtained under a protocol approved by the Ethical Committee at Tokyo Medical and Dental University (No.725). Before use, the teeth were cleaned of debris and stored in physiological saline containing 0.1% thymol at 4°C and used within 6 months after extraction.

Experiment 1 — µTBS

1. Tooth specimen preparation

Forty-five human third molars were selected for this part of the study. For each tooth, the coronal portion was removed to expose a flat, mid-coronal dentin surface using a low speed water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). The exposed dentin surface was finished using 600-grit silicon carbide abrasive paper under a water stream for 60 s to produce standardized smear layers.

2. Bonding and restorative procedures

Teeth were randomly divided into five groups, and bonded according to the respective manufacturers’ instructions (Table 1). Clearfil AP-X (A2 shade, Kuraray Noritake Dental) hybrid resin composite was used to build up a block of composite on the bonded surface. Increments approximately 2 mm thick were used. Each increment was light-activated for 40 s using a quartz halogen light curing unit (Optilux 501, 600 mW/cm², Demetron, Danbury, CT, USA).

3. Thermal cycle challenge

After the adhesive procedures, all teeth were stored in distilled water for 24 h at 37°C. The bonded samples were sectioned perpendicular to the adhesive interface into serial slabs with a water-cooled diamond saw. Each slab was further sectioned to obtain beams with an adhesive area of approximately 1.0 mm².

For each adhesive, 20 beams were subjected to either 0, 5,000 or 10,000 thermal cycles, respectively. The beams were cycled between two water baths at 5 and 55°C with a dwell time of 30 s in each bath and a transfer time of 5 s using a thermal cycling device (K178-08, Tokyo Giken, Tokyo, Japan).

4. Microtensile bond test

Immediately after the thermal cycling, the bonded sticks were fixed to a testing apparatus with a cyanoacrylate adhesive (Zapit, Dental Ventures of American, Anaheim Hills, CA, USA) and subjected to µTBS at a crosshead speed of 1 mm/min (EZ Test, Shimadzu, Kyoto, Japan). Each test group comprised 20 specimens. The mean bond strengths were calculated then analyzed using Tukey’s HSD Test and Bonferroni correction (α=0.05). The fractured beams of the debonded specimens were gold-sputter coated and observed under a SEM (JSM-5310LV, JEOL, Tokyo, Japan) to determine the mode of failure. Failure mode of each specimen was classified as: (1) cohesive failure in dentin; (2) adhesive failure between resin adhesive and tooth surface; (3) mixed failure (failure at resin-dentin interface which included cohesive failure of either dentin or composite); and (4) cohesive failure in resin composite. Statistical analysis of the failure mode was undertaken using Pearson’s chi-square test (α=0.05). All the statistical analyses were performed using PASW version 18 (IBM SPSS, Tokyo, Japan).

The bond strength test results were also analyzed using two-parameter Weibull statistics for each material group at 0, 5,000 or 10,000 thermal cycles. From this the Weibull moduli, m, were determined. The 63.2% probability of specimens failing was also determined for each material by number of cycles (Fig. 1).

Experiment 2 — SEM observation after acid-base challenge

Two dentin disks of approximately 1.5 mm thickness were obtained from the mid-coronal portion of human molars with the use of a water-cooled diamond saw (Isomet 1000). The dentin surfaces were ground with 600-grit silicon carbide paper under running water to
| Materials     | Adhesive system       | Manufacturer (batch no.) | Composition (pH value)                                                                 | Self-etch strategy                                                                 | Etch-and-rinse strategy | Dry surface | Moist surface |
|--------------|-----------------------|--------------------------|----------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------|-------------|---------------|
| Clearfil SE Bond 2 | Kuraray Noritake Tokyo, Japan (Primer: #9C0002; Bond: #9B0002) | Primer: MDP, Water, HEMA, Hydrophilic dimethacrylate, CQ, N,N-Diethanol p-toluidine. (pH=2.0) | Bond: MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, CQ, N,N-Diethanol p-toluidine, Silanated filler (pH value N.A) | 1. Apply primer to tooth surface, and leave in place for 20 s 2. Dry with air stream to evaporate the volatile ingredients 3. Apply bond to the tooth surface and then create a uniform film using a gentle air stream 4. Light polymerize for 10 s | N.A N.A         |             |               |
| Two-step, self-etch | Optibond XTR | Kerr, CA, USA (Primer: #4800791; Bond: #4867830) | Primer: GPDM self-etching adhesive monomer, Hydrophilic co-monomers including mono and di-functional methacrylate monomers, Water, Acetone, Ethanol, CQ (pH=2.4) | 1. Apply primer by scrubbing for 15 s 2. Air thin for 5 s with medium air pressure 3. Shake the adhesive bottle and apply adhesive with light brushing motion for 15 s, air thin for 5 s 4. Light curing for 10 s | N.A N.A         |             |               |
| One-step, self-etch | Scotchbond Universal | 3M ESPE, St. Paul, MN, USA (#499467) | MDP Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond Copolymer, Filler, Ethanol, Water, Initiators, Silane. (pH=2.7) | 1. Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. If necessary rewet the disposable applicator during treatment 2. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely 3. Light curing for 10 s | 1. Apply etchant for 15 s 2. Rinse for 10 s 3. Partially dry by blotting with a small piece of cotton pellet, leaving a visibly moist surface 4. Apply adhesive as for the self-etch mode |             |               |
| phosphoric conditioner K-etchant gel | Kuraray Noritake (#00499A) | 40% phosphoric acid | —                                                                                       | —                                                                                 | — —                     |             |               |

Fig. 1  Schematic illustration of the methodology of specimen preparation for μTBS.
standardize the smear layers. Bonding procedures were conducted in the same manner as described for the bond test.

Following application of the adhesive, a thin layer of flowable resin composite was placed between the two disks using Clearfil Majesty Flow (A2 shade, Kuraray Noritake Dental) to produce a ‘dentin disk sandwich’. The bonded specimens were stored in distilled water at 37°C for 24 h. Subsequently, the specimens were sectioned vertically at the dentin-adhesive interface and embedded in epoxy resin (Epoxicure, Buehler). For acid-base challenges, the specimens were initially immersed for 90 min in a buffered demineralizing solution (2.2 mmol/L CaCl₂, 2.2 mmol/L NaH₂PO₄, and 50 mmol/L acetic acid) adjusted to pH 4.5, then rinsed with running water and secondly immersed for 30 min in a 5% sodium hypochlorite solution to remove any demineralized dentin collagen fibers, and then rinsed in running water again for 60 s.

The edge of the adhesives could be easily damaged during specimen polishing, to prevent this, a self-cured adhesive resin, Super-Bond C&B (Sun Medical, Moriyama, Japan), was applied to the surface without any surface preparation. The specimens were sectioned vertically at the dentin-adhesive interface and polished with diamond pastes down to 0.25 µm (DP-Paste, Struers, Ballerup, Denmark). To bring the hybrid layer into sharp relief, the polished specimens were etched with an argon ion beam (EIS-1E, Elionix, Tokyo, Japan) for 6 min under operating conditions: accelerating voltage of 1 kV and an ion current density of 0.2 mA/cm². The specimens were sputter-coated then with gold for examination under a SEM (JSM-5310LV, JEOL) (Fig. 2).

**Experiment 3 — TEM observation after acid-base challenge**

Fifteen human teeth were selected for TEM observation. For each tooth, the coronal portion was removed to expose a flat, mid-coronal dentin surface and finished...
as previously described. The adhesives were applied on the smear-layer finished dentin surfaces of the teeth that were to be bonded using the same protocol as for the other parts of the study (Table 1). After polymerization of the bonding resin, a thin soft polymethyl methacrylate (PMMA) was placed on the bonded surfaces followed by light curing. After 24 h in distilled water the specimens were sectioned into two halves at the center, the crosscut surface on each hemisection was subjected to the acid-base challenge as previously outlined. The hemisections were then trimmed into rectangular blocks (1×1.5×1.5 mm³) using a diamond saw (Isomet), then dehydrated and embedded before being sectioned into ultrathin specimens for TEM observation (H-7100; Hitachi, Tokyo, Japan) operating at 75 kV without any further staining (Fig. 3).

**RESULTS**

The µTBS values are shown in Table 2. Tukey’s HSD Test with Bonferroni correction revealed the 24 h bond strengths of the etch-and-rinse groups were significantly lower than the self-etch groups (p < 0.05). After the 5,000 thermal cycle challenge, the µTBS result in SBM decreased significantly (p = 0.001). After 10,000 thermal cycles, SE2 remained stable, whereas that of XTR was significantly elevated (p = 0.0006), SBS, SBM and SBD groups all decreased significantly (p < 0.05).

The immediate bond strength of SBD group was significantly lower than the other groups (p < 0.05), while the SBM group was only significantly different compared to the SE2 group (p = 0.04). After 5,000 thermal cycles, there was a significant difference between self-etch and etch-and-rinse adhesives; after 10,000 thermal cycles, SBU, no matter whether in self-etch or etch-and-rinse modes, the bond strengths significantly decreased (p < 0.05); there was a significant difference between self-etch mode and etch-and-rinse for SBU. SBS group demonstrated a lesser reduction in bond strengths.

No premature failure was observed in any of the groups. SEM observation indicated the fracture pattern of etch-and-rinse groups were predominately adhesive failure which involved the HL (Figs. 4 A/B), whereas the failure pattern in self-etch groups was mainly mixed as well as cohesive failure either in dentin or resin composite (Figs. 4 C/D). Distribution (%) of failure mode in the failure mode after the thermal cycling challenge for any of the groups (p > 0.05).

The outcomes of the Weibull analysis showed the modulus, m, varied amongst materials (Table 3). At 0

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**Table 2** Means and standard deviations (SD) of micro tensile bond strength values of all groups

| Adhesive system | Application mode | 24 h Bond strength (MPa) | 5000 TC Bond strength (MPa) | 10000 TC Bond strength (MPa) |
|----------------|------------------|--------------------------|-----------------------------|------------------------------|
| SE2 | Self-etch | 67.3±16.7^a (65/35/0/0) | 71.0±7.3^b (30/40/20/10) | 71.3±20.6^a (35/25/20/20) |
| XTR | Self-etch | 64.3±15.6^ab (45/30/15/10) | 68.1±11.2^b (50/30/10/10) | 79.2±12.0^a (50/5/25/20) |
| | Self-etch | 58.9±14.2^ab (30/15/55/0) | 64.7±13.1^b (40/20/40/0) | 49.6±9.9^a (45/20/15/20) |
| SBU | Etch-and-rinse (Moist) | 54.8±10.9^a (5/10/85/0) | 39.9±11.7^b (0/0/100/0) | 20.2±4.8^a (0/5/95/0) |
| | Etch-and-rinse (Dry) | 41.8±10.8^a (0/15/85/0) | 38.0±8.8^b (5/10/85/0) | 30.3±13.2^a (0/0/100/0) |

Mean followed by the same superscript capital letters between columns are not statistically different (p < 0.05).
Mean followed by the same superscript lowercase letters between vertical columns are not statistically different (p < 0.05).
Percentage of failure mode (Cohesive failure in dentin/Mixed failure/Adhesive failure/cohesive failure in composite resin).
cycles the variation was quite small with SBM showing the highest modulus at 5.749, the lowest being 4.246 for SBD. The other SEA systems were quite similar. At 5,000 cycles the moduli for all groups except SBM increased. SE2 $m$ doubled whilst the others saw $m$ increase slightly. The change in $m$ was associated with an increase in bond strength for most groups. The trend for which 63.5% probability of specimen failure showed an increase in bond strengths by number of cycles for SE2 and XTR, but falls for all groups associated with SBU for the different dentin surface etching modes (Table 4). Figure 5 shows the survival probability for all materials.

Figure 6 shows the typical SEM images of the dentin-adhesive interface for each group after acid-base challenge. The outer lesion (OL), created by dissolution of dentin due to the acid-base challenge, was observed in each group. The depth of the OL ranged from 10 to 15 $\mu$m. The hybrid layer (HL) was brought to a sharper contrast after argon-ion etching. ABRZ was observed at the demineralization front of OL only in the self-etch groups. A slope was formed at the bottom OL region in the XTR group. For SBS group, a funnel-shaped lesion was at the bottom of OL. In etch-and-rinse SBM and SBD groups, the HL were about 5 $\mu$m thick without the appearance of an ABRZ.

The representative TEM images are shown in Fig. 7, the morphological features of each adhesive tend to vary. In the SE2 and XTR groups, electron-dense zones were noted beneath the HL with a thickness of 0.5 $\mu$m in challenge.

### Table 3  Weibull moduli $m$ as a function of number of thermal cycles

| Material/Cycles | 0    | 5,000 | 10,000 |
|-----------------|------|-------|--------|
| SE2             | 4.405| 10.988| 3.594  |
| XTR             | 4.565| 6.679 | 7.217  |
| SBS             | 4.451| 5.436 | 5.625  |
| SBM             | 5.749| 3.578 | 4.51   |
| SBD             | 4.256| 4.865 | 2.421  |

### Table 4  Characteristic bond strengths at which 63.5% of specimens may be expected to fail (MPa)

| Materials/Cycles | 0    | 5,000 | 10,000 |
|-----------------|------|-------|--------|
| SE2             | 73.8 | 74.2  | 79.2   |
| XTR             | 70.4 | 72.9  | 84.4   |
| SBS             | 64.6 | 70.1  | 53.6   |
| SBM             | 59.1 | 44.4  | 22.1   |
| SBD             | 46   | 41.5  | 34.3   |

Fig. 5  Weibull analysis survival probability for each of the adhesive groups at 0 (4-a), 5,000 (4-b) and 10,000 (4-c) thermal cycles.

It can be noted that by 10,000 cycles the survival probability of the materials becomes more distinct amongst the tested adhesives.
DISCUSSION

In order to assess the durability of the dentin bond of the adhesives, thermal cycling was chosen as the aging process instead of long-term water storage. Through thermal cycling, thermal shock as well as some plasticization of the resin takes place at the interface that can lead to a change of its mechanical properties. This process is accelerated due to the small surface area (1 mm²) used. It was suggested that the first stage of biodegradation of the interface already begins when dentin is acid-etched to remove the smear layer, then the second stage involves solubilization and leaching of the unreacted monomers of the resins that had infiltrated into the dentin matrix via water-filled nanometer-sized voids within the hybrid layer. The bonded specimens were cut into beams and then subjected to the thermal cycle test to accelerate the effects of the temperature changes and associated water sorption. Aging of the specimen with thermal cycling for 0, 5,000 and 10,000 times can be regarded as a simulation for immediate, 6 months and one year of in vivo functioning with respect to temperature change and the effects of water. Interestingly though, the Weibull moduli for all materials increased from the immediate time to 5,000 cycles. These changes may be associated with post-cure polymerization. In the thermal cycle challenge, especially the heat challenge may accelerate post-cure polymerization of the resin by increasing the degree of...
conversion (DC) of the adhesive, which led to improved moduli and bond strength values. The results of the current study align well with the work by Par et al.\textsuperscript{29} who showed that a temperature increase was associated with a significant increase in the degree of post-cure polymerization. However, by 10,000 thermal cycles, the degradation of dentin-resin interface caused by the thermal stresses of the temperature changes became the dominant factor resulting in a decrease in bond strength. However, this requires further investigation.

The hydrophilic primers in SE2 and XTR are composed of the aqueous solutions of acidic functional monomers, MDP (10-methacryloyloxydecyl dihydrogen) and GPDM (glycero-phosphate dimethacrylate), respectively. SE2 and XTR demonstrated stable dentin bond strengths regardless of the aging process by thermal cycling. The conventional two-step self-etch adhesive, Clearfil SE Bond (SE), has demonstrated excellent clinical performance and bonding durability\textsuperscript{24}. SE2 is the most recent version of SE. The updated version of SE2 has an improved catalytic system with a newly developed dual-cure activator for indirect restorations and core build-up resin composites. The Weibull moduli were similar to those previously reported\textsuperscript{25,26}. Interestingly for XTR, the bond strengths increased after the thermal cycle challenge, which was attributed to further polymerization of the bonding resin due to temperature increases associated with the 55°C bath of the thermocycling process. Also, GPDM is a dimethacrylate monomer, which creates a cross-linked polymer network at the adhesive interface that contributes to an increase of mechanical properties at the interface. XTR also showed a large increase in its Weibull modulus from 0 to 5,000 cycles. The high moduli is a reflection of its reliability which was seen as an improvement in the bond.

SBU contains MDP, HEMA and ethanol as its main components. One-step SEAs have been reported to typically yield lower bond strength values to dentin, regardless of the aging treatment, which demonstrates some deficiencies in the performance of this group of adhesive systems\textsuperscript{27,28}. The Weibull modulus was slightly lower than that previously reported, although the value for SBM is very similar\textsuperscript{29}. This also indicates there may be more defects at the adhesive interface which is reflected in the lower bond strengths. Baracco et al.\textsuperscript{30} compared the dentin bond strengths of immediate and 5,000 thermal cycles of etch-and-rinse, 2-step SEA and 1-step SEA. They reported that 1-step SEA demonstrated lower bonding performance for both immediate and aged samples compared with etch-and-rinse and 2-step SEA. Several research papers have demonstrated 1-step SEA when extended to a multi-step system by using PA etching were able to achieve improved bond effectiveness\textsuperscript{30,31}. Although the immediate bond strengths were satisfactory, it was demonstrated that hybridization of the dentin surface was poor\textsuperscript{30}.

In this study we examined the efficacy of PA etching prior to application of the recently introduced ‘universal’ dentin bonding system. However, the immediate bond strengths to dentin of the etch-and-rinse groups were significantly lower than those of the 2-step SEA or when this system was used as a 1-step SEA. With 5,000 or 10,000 thermal cycles, the bond strengths of etch-and-rinse groups also decreased significantly. Takamizawa et al.\textsuperscript{32} demonstrated PA etch of dentin before application of Scotchbond Universal showed no significant difference in immediate shear-bond strengths between groups with or without PA etching. This outcome fits well with the results of the microtensile bond strengths of the current study. Thus, a thermal cycling challenge seems a useful tool to define the long-term durability of these materials. Reis et al.\textsuperscript{31} demonstrated immediate dentin bond strengths were much better when wet-bonding was used in comparison to the dry-bonding group. Under moist conditions, the nanospaces between the collagen fibrils in the demineralized dentin are preserved, which allows the resin monomers to more effectively infiltrate into the underlying dentin, and polymerize in situ. Water is responsible for carrying resinous monomers into dentin and contributes to supporting the collagen fibrils in an expanded network when the dentin surface remains moist. However, degradation of the bond in the moist-dentin group seems to have been accelerated by the thermal cycling challenge which was seen as a significant decrease in bond strengths. In addition, insufficient evaporation of solvents in bonding systems will compromise the structural integrity of the hybrid layer, leading to lower µTBS and worsen the nanoleakage, meaning a porous bonding interface has been formed\textsuperscript{33}. In addition, the fracture mode analysis indicated that the weakest point was usually located at the top of the HL in SB, and this interface was more easily degraded when subjected to the thermal stress (Fig. 4B).

In general, it would seem that the use of PA on dentin leads to an increased number of defects at the bonded interface. This is reflected by the lower Weibull modulus values recorded for the PA-treated groups compared with the self-etch group for SBU. However, the moduli are much better than those observed by Burrow et al.\textsuperscript{34} indicating current adhesive systems are less technique sensitive and potentially more reliable than older adhesives.

It was reported that the morphology of the HL depends on the functional monomers and other aspects of the adhesive. The current SEM and TEM observations indicated that the thickness of the HL was approximately 1–1.5 µm in SE2 and 2 µm in XTR respectively. However, the HL was almost undetectable for SBU when used in self-etch mode (SBS). On the other hand, a thicker HL was detected in etch-and-rinse mode (approximately 5 µm). Incomplete infiltration of the bonding resin into the deeply demineralized collagen network with PA revealed a clear sign of poor quality hybridization at the dentin-resin interface. In the SBM group, after acid-base challenge, HL in the OL area was partially preserved due to the nano-sized voids of water preserving the region replaced by the infiltrating adhesive thus localizing the cured adhesive. However,
in case of SBD, due to the strong air-drying, the collagen fibril network most likely completely collapsed which left little or no access for the adhesive to infiltrate the collagen fibril network allowing for total dissolution of the HL. Such a change in the HL after acid-base challenge suggested that the HL was permeable to acidic and basic ions, and therefore easily affected by the acid-base challenge. This indicates the HL formed in dry mode was of a poor quality.

ABRZ was detected only in the self-etch groups, but not in etch-and-rinse groups. Inoue et al. demonstrated that no ABRZ could be detected in etch-and-rinse adhesives when bonded to dentin. They believed this was due to the aggressiveness of PA that dissolves the dentin substrate and leaves no remnant apatite crystals at the front that can react with infiltrating monomers. However, Li et al. demonstrated that in self-etch adhesives, the resin monomers penetrate the demineralized dentin and enveloped hydroxyapatite crystals upon curing, thus protecting the crystals from acid attack. SE2 created a partially demineralized hybrid layer with densely packed crystallites of an acid-resistant apatite-rich zone which was approximately 0.5 µm thick. This result is similar to a previous study that evaluated Clearfil SE Bond. The thickness of the ABRZ created in the self-etch adhesive systems was influenced by functional monomers, solvent type, pH of the adhesive, the existence of fluoride releasing component etc. SE2 and SBU contain the functional monomer, MDP. MDP has been rated as the most promising monomer for chemical bonding to hydroxyapatite of enamel and dentin. The characteristics of the ABRZs of SE2 and SBS indicated a similarity in thickness which was approximately 0.5 µm. However, a funnel-shaped lesion adjacent to the ABRZ was observed in SBS, which is similar to that found in previous studies. The reason for the funnel-shaped lesion remains unclear, however, the pH value of SBU is approximately 2.7, mild self-etching may not allow the resin monomers to penetrate into demineralized dentin sufficiently, causing nano-spaces that remain beneath the ABRZ.

A thicker ABRZ was expected in the XTR group, because XTR contains a fluoride-releasing component, sodium hexafluorosilicate, in the adhesive. Fluoride release from adhesives was reported to be a key factor to create thick ABRZs. However, the ABRZ of the XTR group was relatively thinner (0.1 µm) than those of SE2 and SBS. XTR contains the functional monomer, GPDM in both the primer and the bonding resin. According to the manufacturer’s instructions, the pH value of the primer on dentin surface drops from 2.4 to 1.6 after application of mild air stream. According to Van Meerbeek et al., the pH value of XTR is regarded as a “strong” self-etch adhesive, that demineralizes dentin deeper and dissolves more of the hydroxyapatite, resulting in a thinner ABRZ even with the presence of a fluoride component in the adhesive. In the etch-and-rinse groups, no electron-dense zone could be observed. Sparsely arranged crystallites remained at the bottom of the HL in the SBM group.

Considering the relationship between the µTBS result and the ABRZ pattern obtained from each adhesive system, bonding durability to tooth substrates may be predicted by using the morphological pattern of the ABRZ. The ABRZ pattern may become a good indicator of dentin bonding durability and subsequent degradation of the bond. This can be related to the Weibull modulus and possibly serve as a predictor of clinical performance. Further correlations are needed between laboratory and clinical studies to confirm or deny this point.

From the current results, the first hypothesis was rejected: 1. µTBSs of etch-and-rinse groups were affected by thermal cycling, for the universal adhesive, there was a significant decrease in bond strength after 10,000 thermal cycles; and the results from the 2-step SEAs were stable. The second hypothesis was also rejected: Characteristics of ABRZ are verified in all the tested groups giving different composition of adhesives and application procedure of each group.

Within the limitations of this study, it was concluded that the bond strength and durability in all adhesives depended on adequate penetration of resin into the dentin substrate and quality of the HL. In case of prior PA etching with the universal adhesive, regardless of the moisture content of the demineralized dentin surface, it produced a loosely crosslinked HL which was more soluble and susceptible to thermal cycling challenge. Furthermore, specific degradation patterns of long-term water storage of the specimens could be considered in future studies to better illustrate the mechanism of bond degradation.

CONCLUSION

The results of this study suggest that the etch-and-rinse method with a universal adhesive created an inferior interface on the dentin surface which resulted in reduced bond durability.

ABRZ formation was detected with the use of self-etch adhesive systems; SE2, XTR and SBS. In the PA etching protocols of SBM and SBD, thick HL, but no ABRZ was detected, which might affect dentin bond durability.

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