

# The effect of a self-etching primer on the continuous demineralization of dentin

Sofia S. A. Oliveira, Sally J. Marshall, Stefan Habelitz, Stuart A. Gansky, Robert S. Wilson, Grayson W. Marshall Jr

Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, California, USA

Oliveira SSA, Marshall SJ, Habelitz S, Gansky SA, Wilson RS, Marshall Jr GW. The effect of a self-etching primer on the continuous demineralization of dentin. *Eur J Oral Sci* 2004; 112: 376–383. © Eur J Oral Sci, 2004

Self-etching primers (SEP), used for adhesion of resin restorations, contain an acidic monomer that is not rinsed off after application; therefore, residual acid could further demineralize dentin, jeopardizing adhesion. This study evaluated whether dentin demineralization continues after a 20-s application of a SEP and also after polymerization of the adhesive. Surface recession was measured, using atomic force microscopy (AFM), between the masked surface and dentin etched with SEP (Clearfil SE Bond) or phosphoric acid (pH 1.94) immediately, 5 min, 2 h or 12 h after application. AFM-based nanoindentations were performed to determine mechanical property profiles across resin-bonded interfaces of two sequence groups: SG 1, where the adhesive was applied and polymerized immediately after application of the primer and specimens were immersed in Hank's solution after 5 min, 2 h and 12 h; or SG 2, where specimens were placed in an incubator before the application of the adhesive, for the same time periods. Significant surface recession occurred over time for all groups, except for SEP before desiccation. Nanoindentations yielded decreased hardness and elastic modulus below the hybrid layer after application of the initial primer, even after polymerization of the adhesive. The results reveal a demineralized dentin zone below the hybrid layer formed by the SEP, not fully protected by the adhesive, which could jeopardize bond strength.

Grayson W. Marshall, Jr, Department of Preventive and Restorative Dental Sciences, 707 Parnassus Avenue, D2246, University of California, San Francisco, San Francisco, CA 94143-0758, USA

Telefax: +1-415-4760858  
E-mail: graymar@itsa.ucsf.edu

Key words: self-etching primers; dentin adhesion; atomic force microscopy; nanoindentation; elastic modulus

Accepted for publication April 2004

With the introduction of acids as dentin-demineralizing agents in 1979 (1), the bond strength of resin restorations to dentin became more reliable. However, a significant problem with many systems is their technique sensitivity. Demineralized dentin must be sufficiently hydrated to maintain the porosities for the mechanical penetration of resin (2), but it should not be overly wet, because, among other problems, there may be dilution or deterioration of the monomer, reduced final degree of cure, and formation of water-containing defects within the adhesive layer (3–5). Resin penetration must occur throughout the whole demineralized layer, or a layer of unprotected demineralized collagen will be exposed that is prone to hydrolysis and degradation over time (6,7). Unfortunately, studies have shown that a demineralized dentin layer is often left unprotected at the bottom of the hybrid layer (5,8–10).

There is also some concern about the aggressiveness of the acids used. Studies have suggested that some acids used to etch dentin can denature the collagen fibrils (11–

13). This denatured collagen, not protected by the adhesive, can be even more prone to hydrolysis over time, placing the restoration at risk (6).

The development of self-etching primers that use an acidic monomer to demineralize dentin at the same time as it impregnates the dentin matrix should allow the unprotected layer of collagen beneath the hybrid layer to be reduced, leading to higher long-term bond strengths (14). However, the acidic monomer is not rinsed off the tooth, and if any further demineralization occurs after its application, a layer of demineralized dentin would be left to undermine bond strength. SANO *et al.* (15) speculated that the acidic environment in such systems could lead to poor polymerization, and a recent study (16) reported that the acidic groups of an adhesive system would interfere with the polymerization of auto-cure composites.

It has been suggested that the etching process is stopped by three mechanisms:

- (1) The acidic groups are first neutralized by the reaction with the calcium.
- (2) After evaporation of the solvent, the primer's viscosity rises, reducing the diffusion of the monomers.
- (3) Polymerization reduces the concentration of free acidic monomers.

A preliminary report of this study was presented at the IADR 80th General session. Oliveira, SSA; Marshall, SJ; Habelitz S; Gansky, SA; Wilson, RS; Marshall, GW: The effect of an acidic monomer on dentin demineralization.

Atomic force microscopy (AFM) is a powerful tool that has been used to image tooth structure and to study dentin demineralization (17–23). Modified AFM is capable of determining the mechanical properties of dentin by nanoindentations (24,25). The major advantages of this method are the ability to test under wet conditions and the immediate imaging of the sample surface, which allows for the precise positioning of the indentations. There is at least one study (26) published on the mechanical properties of the different bonded layers that did not reveal the existence of any altered dentin layer within the bonded layers. However, this was carried out under dry conditions, which can lead to misleading conclusions as the dried demineralized collagen collapses, presenting different mechanical properties (24).

In the present work, we used AFM to investigate whether the application of an acidic monomer of a self-etching primer can result in deeper demineralization some time after application, and to determine nanomechanical properties across the bonded layers to detect areas of further demineralization.

The null hypotheses in the present study were as follows:

- (1) There is no further demineralization of the dentin structure after applying and drying the primer.
- (2) There is no further demineralization of the dentin structure after polymerization of the monomers.

## Material and methods

The specimens used in this study were prepared from randomly selected human non-carious third molars, as approved by the UCSF Committee on Human Research. All teeth had been recently extracted (< 3 months) from patients in need of extractions as part of their dental treatment. Extracted teeth were gamma irradiated and refrigerated at 4°C in Hank's balanced salt solution (HBSS) prior to use.

The adhesive and restorative materials used in this study are listed in Table 1, along with the compositions, batch numbers and codes provided by the manufacturer. Both the self-etching material and the composite were used according to the manufacturers' instructions.

Occlusal dentin disks were sectioned using a water-cooled low-speed diamond saw (Buehler, Lake Bluff, IL, USA) at approximately the same depth (occlusal surface  $\approx$  2 mm above the pulp horns). The occlusal surfaces were finished with 320 grit abrasive paper to create a standard smear layer (27).

As the analysis of both dentin demineralization after drying the primer, and dentin demineralization after the application of the adhesive and the polymerization of the primer, was the subject of this study, two different methods were used. These methods are described below.

## Surface recession

The AFM study was performed using contact mode (Nanoscope III; Digital Instruments, Santa Barbara, CA, USA), as described previously (28). The occlusal surfaces of 18 dentin disks were further polished through 0.05- $\mu$ m alumina powder slurry (Buehler Micropolish; Buehler) to provide flat, smear-layer free surfaces for imaging in the AFM, as the roughness of the smear-layer would interfere with the tip from the AFM. This allowed comparison of the effects of the surface treatments on intact dentin. A phosphoric acid solution of the same pH as the Clearfil SE Bond [0.0134 M (0.13 wt.%) aqueous phosphoric acid] was prepared from 85 wt.% phosphoric acid (Fisher Scientific, Fair Lawn, NJ, USA). The pH value of both etchants was 1.94, measured using an Accumet 1003 handheld pH meter and a glass electrode microprobe (Fisher Scientific). The etchants were applied to the specimen surfaces after half the surface had been protected with a masking tape to create an unetched reference layer, as previously described (20,29). After application for 20 s, each etchant was dried for 5 s with a gentle air stream from a water- and oil-free air source (Fisher Scientific), and specimens were soaked in 100% ethanol for 5 min immediately, or 5 min, 2 h or 12 h after drying, to remove the resinous components of the etchant for Clearfil SE (28), thus enabling us to assess whether there was a difference in demineralization by leaving the dried primer on the sample surface. The procedure was also used for the phosphoric acid control samples. Samples were then soaked in deionized water for 5 min to reverse any desiccation effects that the ethanol might have caused, as described previously (28). Three specimens were used per group. Specimens were protected from light during the waiting periods to avoid possible polymerization of the monomers.

Table 1  
*Restorative and adhesive materials*

Material	Code	Composition	Batch no.	Function
Clearfil SE bond	SE	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, <i>dl</i> -camphorquinone, <i>N,N</i> -diethanol- <i>p</i> -toluidine, water Resin: 10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, <i>dl</i> -camphorquinone, <i>N,N</i> -diethanol- <i>p</i> -toluidine, silanated colloidal silica	Primer-00101A Adhesive-00103A	Adhesive system
Z-100	Z100	Bisphenol A diglycidyl ether dimethacrylate; silanated zirconium silica synthetic mineral, triethyleneglycol dimethacrylate	OKA	Restorative material
0.13 vol.% phosphoric acid	PA	0.13 vol.% phosphoric acid diluted from 85% phosphoric acid	933812	Acid conditioner

10-MDP, 10-methacryloyloxydecyl-dihydrogen phosphate; Bis-GMA, bisphenol A diglycidyl methacrylate; HEMA, 2-hydroxyethylmethacrylate.

Three 50- $\mu\text{m} \times 50\text{-}\mu\text{m}$  AFM images were taken for each specimen, while under deionized water. Depth changes of intertubular dentin, relative to the reference layer (dentin surface that was protected from the acid with the masking tape), were measured at five randomly selected locations within each of the images. Specimens were desiccated in a desiccator (Bel-Art Products, Pequannock, NJ, USA) containing silica gel desiccant (6–16 Mesh; Fisher Scientific) for 24 h and the same sites were imaged dry and measured again.

### Mechanical properties across the hybrid layer

Two different sequence groups (SG) were used to analyse the effect of polymerization on the acid activity of the primer, as shown schematically in Fig. 1.

**SG 1** – the primer was applied on the occlusal surface of dentin disks (prepared with a 320-grit smear layer) for 20 s and air dried for 5 s. The adhesive of the self-etching primer system was immediately applied and polymerized for 10 s with a curing light (Demetron-Optilux, Kerr model-VCL 401; Demetron Research Corporation, Danbury, CT, USA). The restorative composite (Z100; 3M, St Paul, MN, USA) was applied incrementally (1 mm layer) and polymerized for 40 s, between increments, to a thickness of  $\approx 4$  mm. The intensity of the curing light was monitored periodically with a curing radiometer (acceptable range: 500–600  $\text{mw cm}^{-1}$ ) (Model 100; Demetron Research Corporation). These specimens were then placed in an incubator at 37°C, in 100% humidity and darkness, for 5 min, 2 h and 12 h. Subsequently, specimens were immersed in 10 ml of HBSS in an attempt to limit further acidic reaction.

**SG 2** – the primer was applied and dried in the same way as described above for SG 1 except that specimens were placed in the incubator before application of the adhesive and its polymerization. In this way, longer time periods for eventual acid residues to react with the sample surface were facilitated after drying the primer, but before application and polymerization of the adhesive and composite. Specimens were immersed in HBSS immediately after the restoration was complete.

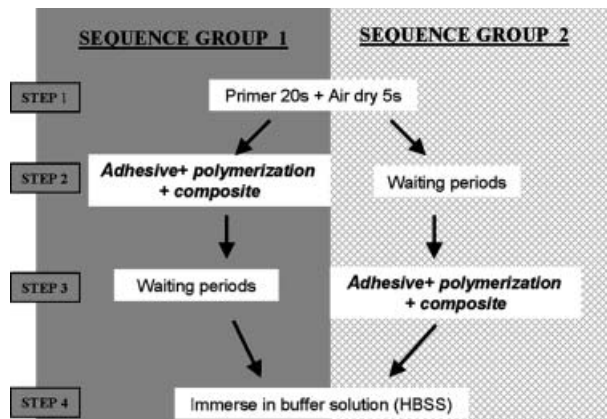


Fig. 1. Mechanical properties of sequence groups. The major difference between sequence groups is in the waiting periods that took place after (SG1) or before (SG2) application and polymerization of both adhesive and composite.

After approximately 24 h, specimens were sectioned with a diamond saw, polished through ascending grades of silicon dioxide paper, and given a final polish with 0.25- $\mu\text{m}$  diamond paste. Three specimens were used per treatment group.

In each specimen, at least three lines of nanoindentations were made across the bonded layers, using a modified AFM where the standard head was replaced by a Triboscope indenter system (Hysitron, Minneapolis, MN, USA), as previously described (24). This system is capable of inducing a vertical force to the diamond tip while measuring the tip force and displacement. A diamond cube corner indenter, with a tip radius of curvature of  $\approx 20$  nm, was used for both imaging and indentation. All images and indentations were made with the sample submerged in water.

The hardness ( $H$ ) and reduced elastic modulus ( $E$ ) were calculated from the load displacement curves generated from each indentation, where  $F_{\text{max}}$  is the maximum force applied,  $a$  is the effective contact area of the indentation and  $S$  is the stiffness determined from the initial portion of the unloading cycle in the force displacement curve:

$$H = (F_{\text{max}} \div a) \quad (1)$$

$$E = [(\sqrt{\pi} \div \sqrt{a}) \times (S \div 2)] \quad (2)$$

$E$  and  $H$  were measured at 1.5- $\mu\text{m}$  intervals along lines of indentations from the adhesive into the dentin, as shown in Fig. 2A. Indentations were made at a maximum force of 300  $\mu\text{N}$ . After each line, an image was taken and the distance of each indentation from the adhesive was measured. As the hybrid layer thickness was difficult to identify in the AFM, specimens were also imaged in the scanning electron microscope (SEM) (Fig. 2B). Therefore, all samples were then immediately immersed in 3 vol% glutaraldehyde with 0.2 M sodium cacodylate buffer solution at 4°C. After 24 h, the hybrid layers were revealed (i.e. demineralization and deproteinization of the dentin unprotected by the resin) by the method used by SCHNEIDER *et al.* (30), so that by the difference in surface height, the hybrid layer could be identified and measured. In this procedure, after immersion in glutaraldehyde, but before desiccation with ethanol, samples were immersed in 0.1 mol hydrochloric acid for 1 min, rinsed in deionized water for 2 min, immersed in 10% sodium hypochlorite for 1 min and rinsed in deionized water for 2 min. Samples were then submitted to desiccation and prepared for the SEM as described previously (28). Using SEM (ISI ABT SX-40 A SEM; Topcon Instruments, Pleasanton, CA, USA), the large reference indentation made in the resin layer near each line with a force of 1000  $\mu\text{N}$  was identified and the position and thickness of the hybrid layer were determined using image analysis software (Ultrascan 2.1.1; Soft Imaging Software, KeveX Sigma, Noran Instruments, Madison, WI, USA). Thus, the location of each indentation from the bottom of the hybrid layer was determined and any change in mechanical properties of the dentin below the hybrid layer could be evaluated.

### Statistical methods

The surface recession data from the AFM measurements were analysed using logarithmic regression analysis over time to determine whether there was any change in the surface recession of dentin after drying of the primer.

Nanoindentations across the resin-bonded interfaces were made to study whether mechanical properties below

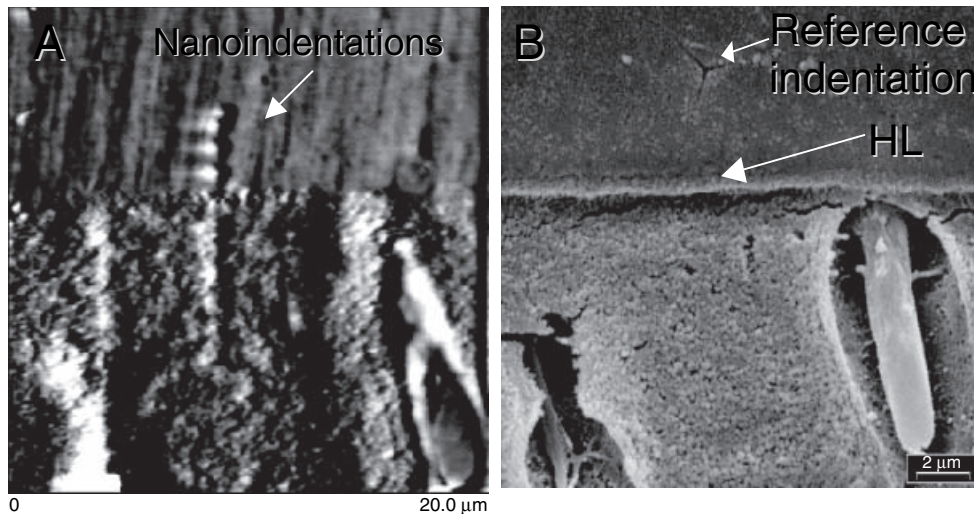


Fig. 2. Cross-section images: (A) atomic force microscopy (AFM); and (B) scanning electron microscopy (SEM). These images are of the same sample in the same zone. After AFM indentation, an SEM image was taken to measure the hybrid layer (HL) thickness at the indentation area.

the hybrid layer decrease as a result of the sample treatment. The width of the dentin zone with altered mechanical properties was determined by analysis of elastic modulus and hardness profiles across the hybrid layer into the normal dentin. The widths were compared for different delay times (5 min, 2 h, 12 h) and sequence groups (delay time before and delay time after polymerization). Two methods were used to detect when mechanical properties started to level off at normal dentin values. One method computes the absolute values of the slope between adjacent measurements, beginning with the first measurements beyond the hybrid layer into the demineralized dentin. Once the slope falls below a cut-off point, demineralization is considered to end at the measurement closest to the hybrid layer. The second method moves from points within the normal dentin towards the hybrid layer, computing the percentage difference between adjacent points. Once this difference falls below a cut-off point, demineralization of the dentin is considered to have begun. Cut-off point values were determined statistically, based on the variances of the cut-off points obtained from hardness and modulus data and the correlation coefficients between these cut-off points. The value of the cut-off point affects the width estimate of the altered dentin below the hybrid layer. Once selected, identical cut-off points were used throughout this analysis. Of the two statistical methods used to analyse the width of altered dentin below the hybrid layer, the slope method minimized the variance, and gave the maximum correlation between hardness and elastic modulus, and therefore was used.

This method of detecting the end of demineralization yields a total of two estimates of the width of demineralized layer: one is based upon hardness and one is based upon elastic modulus measurements. Each was used as the dependent variable in a mixed linear regression model (SAS proc mixed). The fixed factor was sequence group (delay time before and delay time after polymerization).

Hybrid layer thickness data were compared using two-way analysis of variance (ANOVA), where the factors were sequence group (waiting before and waiting after polymerization) and time (5 min, 2 h, 12 h). All pairwise comparisons were performed using Tukey's tests at  $\alpha = 0.05$ .

## Results

### Surface recession

There was a statistically significant surface recession over time ( $P < 0.05$ ) for all groups except for the self-etching primer before desiccation. This recession was observed, in particular, in the 2-h group, where there was a significant increase in surface recession for both etchants, as shown in Fig. 3. Figure 4 shows AFM images of the surface recession caused by the self-etching primer at different time-points, before and after desiccation.

### Mechanical properties across the hybrid layer

In every group, the mechanical properties of dentin in a zone below the hybrid layer were lower than in normal dentin.  $E$ - and  $H$  values increased with distance from the bottom of the hybrid layer until they reached a plateau in normal dentin. The average mechanical properties of this zone varied with sample and group, but were between  $E = 8\text{--}10$  GPa and  $H = 0.36\text{--}0.47$  GPa. Normal hydrated dentin had approximate values of  $E = 20$  GPa and  $H = 1$  GPa, respectively, in accordance with previous observations (31, 32).

From the two statistical methods used to analyse the width of altered dentin below the hybrid layer, the slope method minimized variability, and gave the maximum correlation between hardness and elastic modulus, and therefore was chosen for further application. The value of the cut-off point will affect the mean width estimate of the altered dentin below the hybrid layer. We analyzed the width of this altered dentin zone (Fig. 5) and found a statistical difference between SGs for the hardness measurements ( $P = 0.03$ ), but not for the elastic modulus measurements ( $P = 0.08$ ). The effect of delaying polymerization (SG 2) was to reduce the width of altered dentin to  $1.0\ \mu\text{m}$  (95% confidence interval:  $0.76\text{--}1.25$ ), or by  $0.3\ \mu\text{m}$  (Fig. 5), as compared to SG 1 where the

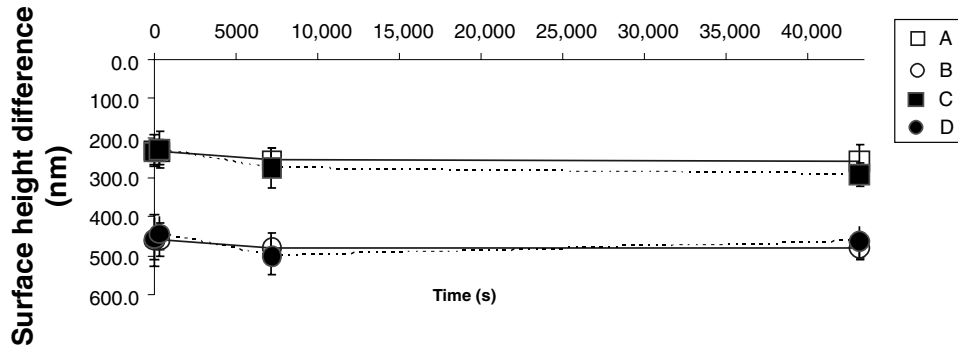


Fig. 3. Surface recession over time, using self-etching primer before (A) and after 24 h (B) of desiccation of the samples, and using phosphoric acid, before (C) and after 24 h (D) of desiccation of the samples.

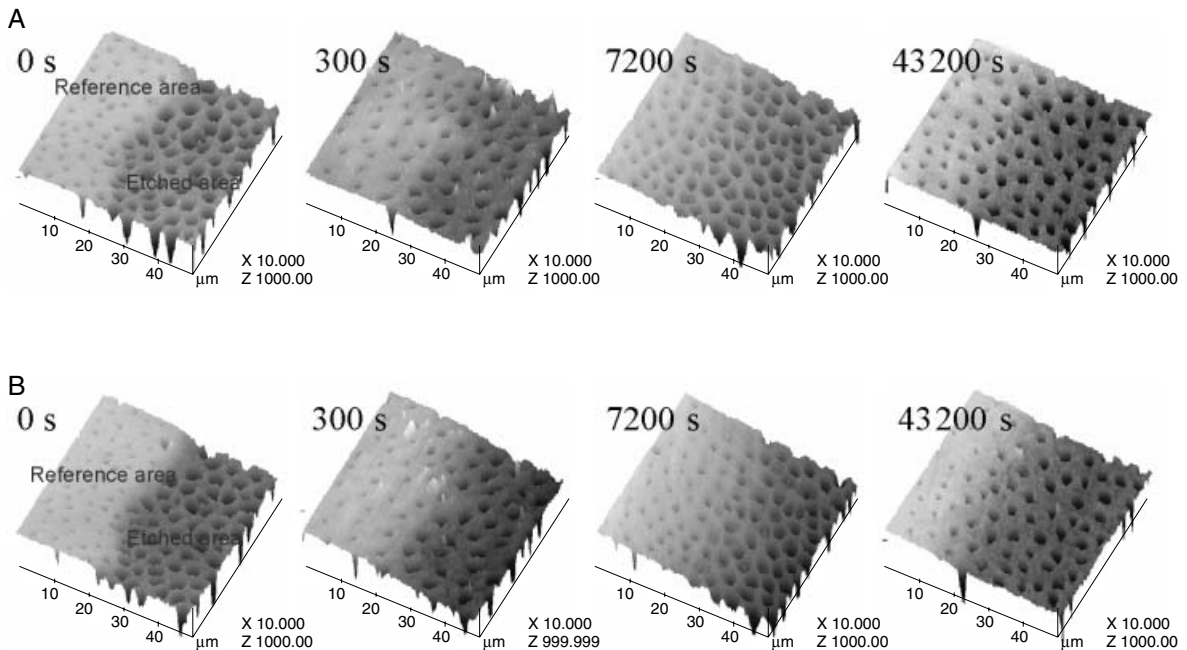


Fig. 4. Atomic force microscopy (AFM) images of surface recession with different times of self-etching primer application. Images show the same samples before (A) and after (B) 24 h of desiccation. Note the reference area at the left and the etched area at the right of each image.

adhesive was applied and polymerized immediately after primer application, and that had a mean value of 1.3  $\mu\text{m}$  (95% confidence interval: 0.97–1.5). This 0.3- $\mu\text{m}$  reduction is based on hardness measurements.

We examined models that considered time as a discrete variable and as a continuous variable, with and without SG, but none of these factors were found to be statistically significant.

Using this method, mechanical properties of the adhesive layer could also be determined. The mean values computed only from the samples that were made following manufacturer’s recommendation, SG 1, were  $E = 3.01$  (standard deviation = 0.44) GPa, and  $H = 0.24$  (standard deviation = 0.04) GPa.

**Hybrid layer thickness**

Analysis of the hybrid layer thickness revealed a two-way interaction. The 12-h group did not react in the same

way as the other groups. In fact, contrary to the other groups, the 12-h group had a thinner hybrid layer when polymerization was delayed (SG 2). When the 12-h

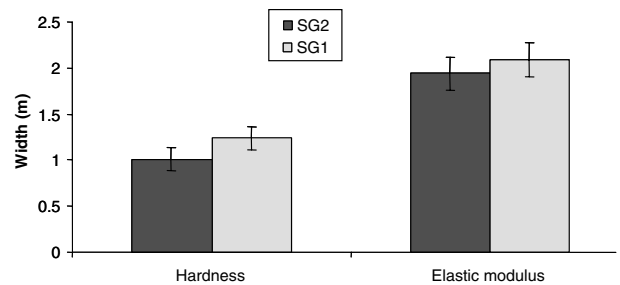


Fig. 5. Width of altered dentin below the hybrid layer. Average width (in  $\mu\text{m}$ ) was based on changes in hardness and modulus for SG1 (waiting periods before polymerization) and SG2 (waiting periods after polymerization); data are combined for all time periods. Error bars represent standard errors.

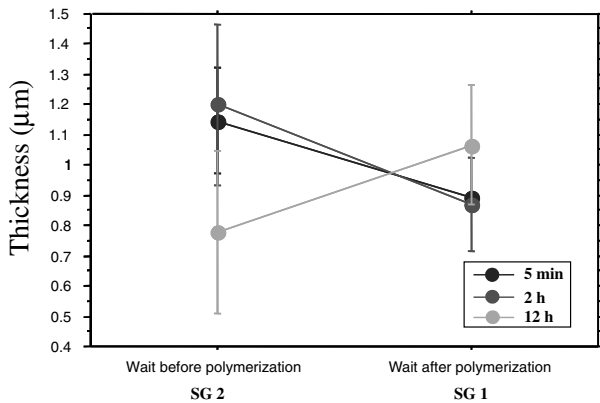


Fig. 6. Hybrid layer mean thickness with different experimental sequence groups (SG 1 and SG 2). The 12-h group did not react in the same way as other groups. Without this two-way interaction, there was a statistically significant ( $P < 0.05$ ) reduction in hybrid layer (HL) thickness ( $\approx 0.3 \mu\text{m}$ ) when application and polymerization of both adhesive and composite was delayed (SG 2), as compared to the sequence group (SG 1) where we waited before applying and polymerizing the adhesive and the composite.

group was removed from the model there was a significant difference between sequence groups. The hybrid layer was thicker by  $\approx 0.3 \mu\text{m}$  in SG 2 ( $P < 0.05$ ), when the application and polymerization of the adhesive was delayed (Fig. 6).

## Discussion

The AFM has proven, on numerous occasions, to be a valuable tool in dentin demineralization studies (33, 34). Surface recession studies have shown that, when hydrated, the demineralized dentin shows some recession (18, 19), probably because the water is less capable of sustaining the 'demineralized mat'. However, when desiccated for 24 h this mat collapses, leaving a larger recession that is a better measurement of the real demineralization depth, even though, as discussed in other studies, we have to account for the mesh of collagen that has collapsed, so this is not the real depth of demineralization, but an approximation (20, 28, 35).

Therefore, we believe that the recession in the self-etching primer hydrated group was not sufficiently large to reveal statistically significant differences, or the lack in recession could be caused by the incomplete removal of the primer by the immersion in ethanol. However, following desiccation for 24 h, the effect of waiting before removal of the etchant showed increased surface recession. This difference can be seen in the samples desiccated for 24 h (Fig. 3), in which the primer was dried according to the procedure recommended by the manufacturer. Phosphoric acid-etched samples also presented a continued demineralization, and this could be detected even in the samples before desiccation. This may have resulted from deeper demineralization as an effect of faster penetration of the phosphoric acid owing to its lower viscosity. This progressive demineralization was more intense in the earlier phase, slowing down with longer incubation

times (Fig. 3), probably ending as the acid was consumed. The authors are aware that this part of the study does not resemble clinical conditions, where the adhesive would be applied immediately after drying the primer, but it was performed to investigate whether the primer alone would cause any further demineralization.

In the second part of this study, we used the modified AFM to test the changes in mechanical properties across the hybrid layer, which revealed an altered dentin zone beneath the hybrid layer. The average elastic modulus values of this zone were between 8 and 10 GPa (depending on the sample). KATZ *et al.* (36) found a layer of partially demineralized dentin below the hybrid layer using a total-etch dentin-bonding system, with values for elastic modulus of  $\approx 13$  GPa. These are slightly higher than ours, but because this test was performed under different experimental conditions, we cannot directly compare results. The presence of this layer using a self-etching system was a surprise, as self-etching systems are thought to improve bonding by impregnating dentin at the same time as they demineralize (14), preventing the creation of an unprotected demineralized layer.

Statistical analysis did not show a difference in the width of this altered dentin zone over time, perhaps because the difference was below the resolution limit of the method, or because the buffered Hank's solution did not stop the acidic reaction in the samples during the time that they were immersed. On the other hand, the width of this altered dentin zone, evaluated based on variations in hardness values, differed for the two sequence groups (Fig. 5). When the application of adhesive and its polymerization were delayed, as in SG 2, dentin continued to be demineralized after the 20-s application period (as determined in the first part of the study). Therefore, when the adhesive is applied it can impregnate the whole demineralized layer, creating a thicker hybrid layer (Fig. 6). As the demineralizing effect must end at some point, the adhesive impregnates almost all the demineralized zone, leaving a thinner altered zone of dentin below the thicker hybrid layer. In SG 1, following the manufacturer's directions, the layer permeated by the adhesive had been demineralized only for 20 s, creating a thinner hybrid layer. However, this group had a wider altered dentin zone below the hybrid layer, suggesting that demineralization did not stop with polymerization. This process is shown schematically in Fig. 7. These results show that the experimental groups (SG 2) gave rise to better proportions between hybrid layer and altered dentin below the hybrid layer than the groups that were used to simulate clinical application (SG 1).

In the samples in which adhesive application was delayed by 12 h, it is conceivable that the dried primer which was left undisturbed for 12 h would decrease the permeability of the dentin surface to the adhesive, leading to a thinner hybrid layer; for this reason, we removed this group from the analysis, which also eliminated the two-way statistical interaction that was found.

The fact that we could detect a difference between SGs with hardness data, but could not with elastic modulus data (Fig. 5), can be related to the statistical methods used as well as to the inherent properties of dentin and

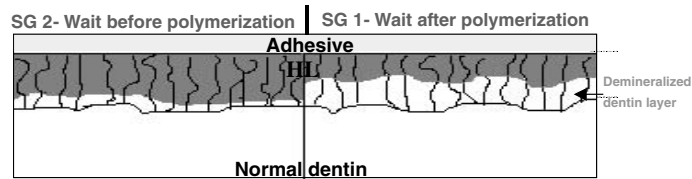


Fig. 7. Schematic drawing of different demineralization and adhesive infiltration with self-etching primer. The width of the hybrid layer (HL) and of the demineralization layer below the HL had different values in different sequence groups. The schematics are exaggerated for clarity and show that SG 1 (which simulated the clinical situation) resulted in a different and less favourable proportion of hybrid layer thickness/demineralized dentin than SG 2 (the experimental group).

the hardness and elastic modulus measurements themselves. Elastic modulus is more sensitive to changes in the inherent structural properties of the substrate, while hardness is probably sensitive to total mineral content without regard to how the mineral is incorporated in the structure. Hardness may also be less variable than elastic modulus. As there is a mineral gradient from demineralized to mineralized zones (34), the change in elastic modulus was probably more gradual than in the hardness. Therefore, the cut-off point for elastic modulus was more difficult to determine, and it may not exactly reflect where fully mineralized dentin begins.

The altered dentin below the hybrid layer is probably related to a partially demineralized dentin, as totally demineralized dentin has been reported to have a lower elastic modulus, of  $\approx 58$  MPa (37), or even lower, as suggested by BALOCH *et al.* (24).

The self-etching primer used in this study is a weaker acid than the etchants normally used; thus, the collagen of this layer may be less affected than with a total-etching system that might denature some of the collagen (11). On the other hand, the partially demineralized zone might be remineralizable (38), and this depends, in part, on the presence of intact collagen fibrils. It would be useful to study whether this partially demineralized zone contributes to the weakness of the bond strength over time. There are a few published studies on the long-term bond strength of self-etching materials. An *in vivo* study (15) in monkeys demonstrated stable bond strength over a period of 1 yr with the same self-etching primer used in this study. Even though it reveals increased porosity in the hybrid layer over time, the 1-yr period may not be sufficiently long to detect an effect on bond strength. BURROW *et al.* (39), using a total etch system, reported a stable bond strength *in vitro* at 1 yr, but those values decreased by almost 50% after 3 yr.

It should be noted that only one self-etching system was used in this study, and although we cannot extrapolate to other self-etching systems that have different compositions, this study is, to our knowledge, the first to demonstrate that self-etching systems do not totally prevent the formation of an unprotected demineralized dentin layer.

In conclusion, our results lead us to reject the null hypothesis and conclude that, within the limitations of this *in vitro* study, there was a slight demineralization after the initial 20 s application of the primer, even after the application of the adhesive and its polymerization. We believe that the residual acid is consumed and neutralized, at some point, by reaction with hydroxyapatite ( $\text{HA} + \text{H}^+ =$

$5\text{Ca}^{2+} + 3(\text{PO}_4)^{3-}$ ), which is in accordance with the observation that the difference in recession depth was smaller at 12 h waiting time. However, the arrest of demineralization does not occur immediately after drying and polymerization, consequently resulting in the formation of a thin layer of partially demineralized dentin below the hybrid layer that remains incompletely protected by the adhesive and could jeopardize the adhesive bond.

*Acknowledgements* – Sofia Oliveira is supported by a PhD fellowship from the Portuguese Ministry for Science and Technology, Praxis XXI program. This study was supported by the National Institutes of Health/National Institute of Dental and Craniofacial Research Grant P01 DE 09859.

## References

1. FUSAYAMA T, NAKAMURA M, KUROSAKI N, IWAKU M. Non-pressure adhesion of a new adhesive restorative resin. *J Dent Res* 1979; **58**: 1364–1370.
2. KANCA J. Improving bond strength through acid etching of dentin and bonding to wet dentin surfaces. *J Am Dent Assoc* 1992; **123**: 35–43.
3. JACOBSEN T, SODERHOLM KJ. Some effects of water on dentin bonding. *Dent Mater* 1995; **11**: 132–136.
4. TAY FR, GWINNETT AJ, WEI SH. The overwet phenomenon: a scanning electron microscopic study of surface moisture in the acid-conditioned, resin–dentin interface. *Am J Dent* 1996; **9**: 109–114.
5. WANG Y, SPENCER P. Quantifying adhesive penetration in adhesive/dentin interface using confocal Raman microscopy. *J Biomed Mater Res* 2002; **59**: 46–55.
6. KATO G, NAKABAYASHI N. The durability of adhesion to phosphoric acid etched, wet dentin substrates. *Dent Mater* 1998; **14**: 347–352.
7. HASHIMOTO M, OHNO H, KAGA M, ENDO K, SANO H, OGUCHI H. *In vivo* degradation of resin–dentin bonds in humans over 1–3 years. *J Dent Res* 2000; **79**: 1385–1391.
8. SANO H, SHONO T, TAKATSU T, HOSODA H. Microporous dentin zone beneath resin-impregnated layer. *Oper Dent* 1994; **19**: 59–64.
9. SPENCER P, SWAFFORD JR. Unprotected protein at the dentin–adhesive interface. *Quintessence Int* 1999; **30**: 501–507.
10. HASHIMOTO M, OHNO H, ENDO K, KAGA M, SANO H, OGUCHI H. The effect of hybrid layer thickness on bond strength: demineralized dentin zone of the hybrid layer. *Dent Mater* 2000; **16**: 406–411.
11. MIZUNUMA T. Relationship between bond strength of resin to dentin and structural change of dentin collagen during etching. Influence of ferric chloride to structure of the collagen. *J Jpn Dent Mater* 1986; **5**: 54–64.
12. ELIADES G, PALAGHIAS G, VOUGIOUKLAKIS G. Effect of acidic conditioners on dentin morphology, molecular composition and collagen conformation in situ. *Dental Materials* 1997; **13**: 24–33.

13. AGEE K, ZAHNG Y, PASHLEY DH. Effects of acids and additives on the susceptibility of human dentin denaturation. *J Oral Rehab* 2000; **27**: 136–141.
14. NAKABAYASHI N, SAIMI Y. Bonding to intact dentin. *J Dent Res* 1996; **75**: 1706–1715.
15. SANO H, YOSHIKAWA T, PEREIRA PN, KANEMURA N, MORIGAMI M, TAGAMI J, PASHLEY DH. Long-term durability of dentin bonds made with a self-etching primer, *in vivo*. *J Dent Res* 1999; **78**: 906–911.
16. SANARES AM, ITTHAGARUN A, KING NM, TAY FR, PASHLEY DH. Adverse surface interactions between one-bottle light-cured adhesives and chemical-cured composites. *Dent Mater* 2001; **17**: 542–556.
17. MARSHALL GW, JR, BALOOCH M, TENCH RJ, KINNEY JH, MARSHALL SJ. Atomic force microscopy of acid effects on dentin. *Dent Mater* 1993; **9**: 265–268.
18. MARSHALL GW, JR, BALOOCH M, KINNEY JH, MARSHALL SJ. Atomic force microscopy of conditioning agents on dentin. *J Biomed Mater Res* 1995; **29**: 1381–1387.
19. MARSHALL GW, JR, INAI N, WU-MAGIDI IC, BALOOCH M, KINNEY JH, TAGAMI J, MARSHALL SJ. Dentin demineralization: effects of dentin depth, pH and different acids. *Dent Mater* 1997; **13**: 338–343.
20. MARSHALL GW, SAEKI K, GANSKY SA, MARSHALL SJ. AFM study of citric acid-ferric chloride etching characteristics of dentin. *Am J Dent* 1999; **12**: 271–276.
21. ROSALES JI, MARSHALL GW, MARSHALL SJ, WATANABE LG, TOLEDANO M, CABRERIZO MA, OSORIO R. Acid-etching and hydration influence on dentin roughness and wettability. *J Dent Res* 1999; **78**: 1554–1559.
22. MARSHALL GW, JR, CHANG YJ, SAEKI K, GANSKY SA, MARSHALL SJ. Citric acid etching of cervical sclerotic dentin lesions: an AFM study. *J Biomed Mater Res* 2000; **49**: 338–344.
23. MARSHALL GW, CHANG YJ, GANSKY SA, MARSHALL SJ. Demineralization of caries-affected transparent dentin by citric acid: an atomic force microscopy study. *Dent Mater* 2001; **17**: 45–52.
24. BALOOCH M, WU-MAGIDI IC, BALAZS A, LUNDKVIST AS, MARSHALL SJ, MARSHALL GW, SIEKHAUS WJ, KINNEY JH. Viscoelastic properties of demineralized human dentin measured in water with atomic force microscope (AFM)-based indentation. *J Biomed Mater Res* 1998; **40**: 539–544.
25. MARSHALL GW, HABELITZ S, GALLAGHER R, BALOOCH M, BALOOCH G, MARSHALL SJ. Nanomechanical properties of hydrated carious human dentin. *J Dent Res* 2001; **80**: 1768–1771.
26. VAN MEERBEEK B, WILLEMS G, CELIS JP, ROOS JR, BRAEM M, LAMBRECHTS P, VANHERLE G. Assessment by nano-indentation of the hardness and elasticity of the resin-dentin bonding area. *J Dent Res* 1993; **72**: 1434–1442.
27. OLIVEIRA SSA, PUGACH MK, HILTON JF, WATANABE LG, MARSHALL SJ, MARSHALL GW. The influence of the dentin smear layer on adhesion: a self-etching primer vs. a total-etch system. *Dent Mater* 2003; **19**: 758–767.
28. OLIVEIRA SSA, MARSHALL SJ, HILTON JF, MARSHALL GW, JR. Etching kinetics of a self-etching primer. *Biomaterials* 2002; **23**: 4105–4112.
29. SAEKI K, MARSHALL SJ, GANSKY SA, MARSHALL GW. Etching characteristics of dentin: effect of ferric chloride in citric acid. *J Oral Rehab* 2001; **28**: 301–308.
30. SCHNEIDER H, FROHLICH M, ERLER G, ENGELKE C, MERTE K. Interaction patterns between dentin and adhesive on prepared class V cavities *in vitro* and *in vivo*. *J Biomed Mater Res* 2000; **53**: 86–92.
31. MARSHALL GW, YUCEL N, BALOOCH M, KINNEY JH, HABELITZ S, MARSHALL SJ. Sodium hypochlorite alterations of dentin and dentin collagen. *Surface Sci* 2001; **491**: 444–455.
32. HABELITZ S, MARSHALL GW, JR, BALOOCH M, MARSHALL SJ. Nanoindentation and storage of teeth. *J Biomech* 2002; **35**: 995–998.
33. KINNEY JH, BALOOCH M, MARSHALL GW, MARSHALL SJ. Atomic-force microscopic study of dimensional changes in human dentine during drying. *Arch Oral Biol* 1993; **38**: 1003–1007.
34. KINNEY JH, BALOOCH M, HAUPT DL, JR, MARSHALL SJ, MARSHALL GW, JR. Mineral distribution and dimensional changes in human dentin during demineralization. *J Dent Res* 1995; **74**: 1179–1184.
35. MARSHALL GW, JR., WU-MAGIDI IC, WATANABE LG, INAI N, BALOOCH M, KINNEY JH, MARSHALL SJ. Effect of citric acid concentration on dentin demineralization, dehydration, and rehydration: atomic force microscopy study. *J Biomed Mater Res* 1998; **42**: 500–507.
36. KATZ JL, BUMRERRAJ S, DREYFUSS J, WANG Y, SPENCER P. Micromechanics of the dentin/adhesive interface. *J Biomed Mater Res* 2001; **58**: 366–371.
37. TAY FR, CARVALHO RM, YIU CK, KING NM, ZHANG Y, AGEE K, BOUILLAGUET S, PASHLEY DH. Mechanical disruption of dentin collagen fibrils during resin-dentin bond testing. *J Adhes Dent* 2000; **2**: 175–192.
38. TATSUMI T, INOKOSHI S, YAMADA T, HOSODA H. Remineralization of etched dentin. *J Prosthet Dent* 1992; **67**: 617–620.
39. BURROW MF, SATOH M, TAGAMI J. Dentin bond durability after three years using a dentin bonding agent with and without priming. *Dent Mater* 1996; **12**: 302–307.