# Effect of the application time of phosphoric acid and self-etch adhesive systems to sclerotic dentin

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## ABSTRACT

Objectives: To evaluate the effect of application time on the resin-dentin bond strength (μTBS) and etching pattern of adhesive systems applied on sclerotic dentine. Material and Methods: A total of forty-two bovine incisors had their roots removed. The 1-step self-etch GO (SDI), the 2-step self-etch Adper SE Bond (3MESPE) and the 35% phosphoric acid (3MESPE) from the 2-step etch-and-rinse Adper Single Bond 2 (3MESPE) were applied on the bovine incisal surfaces according to the manufacturer's instructions or duplicating the recommended conditioning time. After adhesive application, thirty teeth were restored with composite resin, stored for 24 h in distilled water at 37°C, and sectioned into resin-dentin bonded sticks (0.8 mm²) and tested according to the μTBS at 0.5 mm/min. The etching pattern of the remaining twelve teeth (n=4 for each material) was examined under scanning electron microscopy. Each tooth was divided into a buccal-to-lingual direction into three thirds, and each third randomly assigned to the groups: control (no treatment), according to the manufacturers' instructions and duplicating the recommended application time. The μTBS and the relative percentage of the tubule area opening were evaluated by two-way repeated measures ANOVA and Tukey's tests ($\alpha$=0.05). Results: The duplication of the conditioning time favored only the GO adhesive (p<0.05). Both application methods significantly increased the tubule area opening (p<0.05) compared to the controls. Conclusions: The efficacy of duplicating the conditioning time was only effective for the 1-step self-etch adhesive system tested.

Key words: Dentin. Acid etching, Dental. Dentin-bonding agents.

## INTRODUCTION

The primary goal of dentin bonding systems is to provide retention of restorative materials to the dental structure as well as to seal the dentin substrate. Even though the immediate bonding effectiveness of most current adhesive systems is favorable[6], these findings are based upon their ability to bond sound dentin. Although sound dentin may be a common substrate in the daily practice, a variety of pathological dentin substrates are also encountered in clinical scenarios, which includes carious-affected and sclerotic dentin[7,26]. Irrespective of the bonding strategy used, bonding to pathologically altered substrates such as sclerotic dentin led to compromised bonding[17,26]. This has been due to partial or complete obliteration of the dentinal tubules with mineral crystals and due to the presence of an acid-resistant hyper-mineralized layer that acts as an acid resistant substrate[17,26].

As the current bonding strategies [etch-and-

rinse (ER) and self-etch (SE) adhesives] rely primarily on micromechanical retention, the existence of such obstacles may compromise effective infiltration of resin monomers into the dental tissues. Thus, previous studies suggested that bonding to human sclerotic dentin could be improved by changing the adhesive protocol that is typically employed for sound dentin. For ER systems, the duplication of the phosphoric acid conditioning time was suggested; however, the effectiveness of this approach is not unanimous[4,18,19]. As for the SE adhesives, phosphoric acid pre-treatment[8,16] or surface roughening of the sclerotic dentin with diamond burs[8,27], has also been suggested. To the extent of the author's knowledge, no study has so far evaluated the effectiveness of duplication of the conditioning time of SE in sclerotic dentin.

Although phosphoric acid conditioning or surface roughening of sclerotic dentin has shown promising results, they increase the number of clinical steps or may produce thicker smear layers when diamond burs are employed. Thicker smear layers were shown to restrict the penetration of some SE adhesives into the dentin[16,25]. Therefore, other simpler strategies should be investigated. Thus, the aim of this study was to evaluate the micro-tensile resin-dentin bond strength (µTBS) and etching pattern of ER and SE adhesives to sclerotic bovine dentin applied as recommended by the manufacturers or after duplicating the conditioning time. The null hypothesis tested was that bonding to sclerotic dentin will not be affected by the application time of the ER and SE adhesives.

## MATERIAL AND METHODS

The Ethics Committee from the State University of Ponta Grossa (Paraná, Brazil) reviewed and approved this study under protocol number 06289/09. Forty-two bovine incisors, from animals older than 3 years old[2] were obtained from a local slaughterhouse. These teeth exhibit natural dentin exposure in the incisal edges, and therefore, no bur preparation was required to expose the dentin substrate for bonding.

The roots were sectioned with a water-cooled low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). The coronal pulp was removed and the pulp chamber was kept unfilled. The smear-layer free incisal surfaces were cleaned with an anionic detergent rubbed with a disposable sponge for 30 s and rinsed in running water for 30 s.

For the µTBS testing, thirty teeth were randomly selected and divided into six groups according to the combination of the main factors Adhesive (3 levels) and Application time (2 levels) so that 5 teeth were employed in each group.

Before the adhesive application, one third of each crown was longitudinally sectioned in a buccal-to-lingual direction using a water-cooled low-speed diamond saw (Isomet 1000) in order to ensure that the bonding substrate was, in fact, sclerotic (Figure 1). In these thirds, no treatment was performed, and the specimens were mounted on aluminum stubs and desiccated in colloidal silica for 24 h. After this period, they were gold-sputtered (Sputter Coater IC 50, Shimadzu, Tokyo, Japan) and examined under the scanning electron microscope (SEM). The SEM was operated in the secondary electrons mode (SSX-500, Shimadzu, Tokyo, Japan) with an accelerating voltage of 12 kV. In case the sclerotic characteristic was not confirmed, the specimen was discarded.

In the other two thirds (not sectioned), the 1-step SE GO (GO, SDI, Bayswater, Victoria, AU), the 2-step SE Adper SE Bond (ASE, 3MESPE, St. Paul, MN, USA), and the 2-step ER Adper Single Bond 2 (SB2, 3MESPE, St. Paul, MN, USA) were applied according to the manufacturers' instructions or duplicating the conditioning time (Figure 2). A total of 5 tooth specimens were employed for each group. The application mode, composition and batch number of the adhesive systems are shown in Figure 2.

The adhesives were applied by a single and calibrated operator (Figure 2). Composite resin build-ups (Opallis, FGM, Joinville, SC, Brazil) were constructed in three increments of 1 mm, and each one was light-cured for 40 s (Figure 1). The light-curing unit was set at 500 mW/cm$^2$ (VIP, Bisco, Schaumburg, IL, USA) and used throughout the restorative procedure.

After 24 h of storage in distilled water at 37°C, the specimens were longitudinally sectioned in both "x" and "y" directions by means of a water-cooled low-speed diamond saw (Isomet 1000, Buehler) in order to obtain approximately 10-14 resin-dentin bonded sticks *per* tooth, with a cross-sectional area of approximately 0.8 mm$^2$ (Figure 1). All the resin-dentin bonded sticks were tested in a universal testing machine at a crosshead speed
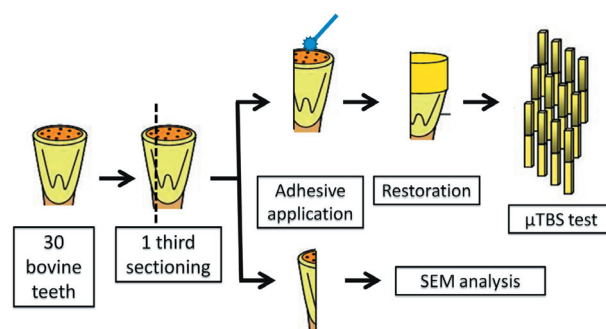


**Figure 1-** Flowchart of the micro-tensile bond strength (µTBS) test

of 0.5 mm/min (Kratos, São Paulo, SP, Brazil). The fracture mode was analyzed at 40X magnification and classified as (1) cohesive within dentin; (2) cohesive within composite resin and (3) adhesive or adhesive/mixed (failure at the resin/dentin interface or mixed with cohesive failure of the neighboring substrates). The number of specimens with premature failures during the specimen preparation was also recorded.

For the etching pattern analysis, the remaining twelve teeth were divided into three groups (n=4 teeth *per* group) according to the material to be used (Figure 2). The crowns of these twelve bovine teeth were longitudinally sectioned in a buccal-to-lingual direction with a water-cooled low-speed diamond saw (Isomet 1000), in order to obtain three crown thirds. One third was used for the evaluation of the sclerotic dentin degree, where no treatment was performed; the second third was treated with one of the adhesives applied according to the manufacturers' instructions and the last third was treated with the same material but duplicating the conditioning time (Figure 3). The allocation of each third to the subgroup was randomly determined.

In the SE groups, the adhesives were applied as described earlier for the µTBS testing, but they were not light-cured. Then, the resin monomers of the self-etch primer were removed by immediately immersing the specimens in acetone for 5 min followed by immersion in deionized water for 5 min. After this, the specimens were immersed in 96% ethanol for 5 min and again in deionized water for 5 min[13]. The specimens treated with phosphoric acid were only rinsed with deionized water for 15 s (Figure 3).

They were mounted on aluminum stubs, ultrasonically cleaned with distilled water for 30 min (Dabi Atlante, Ribeirão Preto, SP, Brazil) and desiccated in colloidal silica for 24 h. After this period, they were gold-sputtered (Sputter Coater IC 50, Shimadzu) and examined under the scanning electron microscope (SEM). The SEM was operated in the secondary electrons mode (SSX-500, Shimadzu) with an accelerating voltage of 12 kV (Figure 3).

Three pictures were taken of each crown third. The relative percentage of the tubule area occlusion of each specimen was measured in all pictures using the UTHSCSA ImageTool 3.0 software (Department of Dental Diagnostic Science at The University of Texas Health Science Center, San Antonio, Texas, USA) by a blinded author.

The µTBS values of sticks from the same tooth half were averaged. Specimens with a cohesive fracture mode and premature failures were excluded from the tooth half mean. The three readings of the relative open tubule area from the same tooth half were averaged for statistical purposes. Data from the µTBS testing and the relative percentage of the open tubule area were evaluated by two-way repeated measures ANOVA

| Material | Composition (batch number) | Application mode |
|---|---|---|
| Adper Single Bond 2 | Etchant: 35% phosphoric acid (997505) Adhesive: dimethacrylates, HEMA, polyalkenoid acid copolymer, 5 nm silane treated colloidal silica, ethanol, water, photoinitiator (7NK) | 1. Apply Scotchbond™ Etchant to dentin for 15 s (control group) or for 30 s (experimental group) 2. Rinse for 10 s. 3. Blot excess water. 4. Apply 2-3 consecutive coats of adhesive for 15 s with gentle agitation. 5. Gently air thin for 5 s. 6. Light-cure for 10 s. |
| Adper Scotchbond SE | Liquid A: water, HEMA, surfactant, pink colorant. (8AP) Liquid B: UDMA, TEGDMA, TMPTMA, HEMA phosphates, MHP, bonded zirconia nanofiller, initiator system based on camphorquinone (8AP) | 1. Apply Liquid A to the entire bonding area. 2. Apply Liquid B scrubbing, for 20 s (control group) or for 40 s (experimental group) 3. Air dry for 10 s. 4. Apply a second coat of Liquid B. 5. Lightly air thin the adhesive layer. 5. Light cure for 10 s. |
| GO | Phosphoric acid ester monomer, dimethacrylate monomer, monomethacrylate monomer, silicon dioxide filler, water, acetone, photoinitiators, stabilizer, sodium (071001) | 1. Apply and leave in place for 20 s (control group) or 40 s (experimental group) 2. Blow air for at least 5 s, leaving the surface glossy. If not, repeat this step. 3. Light-cure for 10 s |

**Figure 2-** Composition (batch number) and application mode of the materials used in this study
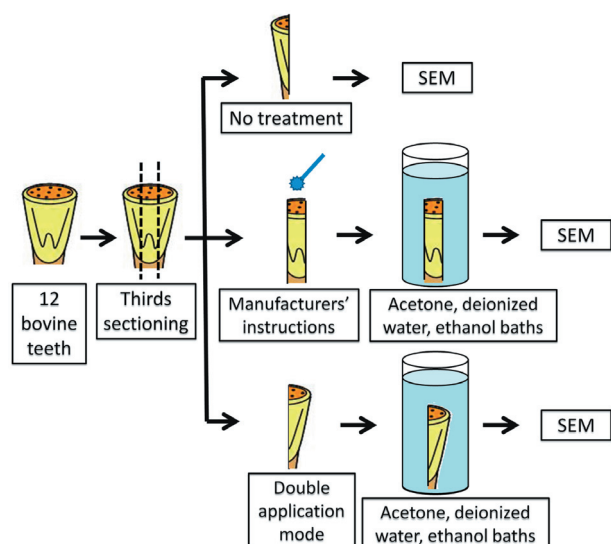
**Figure 3-** Flowchart of the etching pattern examined by scanning electron microscopy (SEM)

(Material *vs*. Application time) and Tukey's tests ($\alpha=0.05$).

## RESULTS

Sclerotic dentin was observed in all specimens used for the µTBS testing and, therefore, no specimen was discarded. The two-way ANOVA revealed that the cross-product interaction Adhesive vs. Application time was statistically significant (p=0.031). Under the manufacturers' instructions, the GO adhesive showed the lowest µTBS values. The duplication of the application time yielded the highest µTBS mean only for the GO adhesive (Tukey's test, p<0.05, Table 1). The fracture pattern of the experimental conditions is shown in Table 2. No significant difference was observed between the groups (data not shown).

As for the percentage of the tubule area opening, only the main factor, Application time was significant (p=0.00003). Both treatments (manufacturer's instructions and duplicating the conditioning time) yielded a similar open tubule area (p=0.89, Table 3), which were significantly higher than the sclerotic dentin surface (no treatment) (Tukey's test, p<0.05). Representative

**Table 1-** Micro-tensile bond strength values (MPa) (means ± standard deviations) obtained for each experimental condition

| Adhesive syste | Application mode | |
|---|---|---|
| | Manufacturer's instructions | Double application time |
| Adper Single Bond 2 | 31.1±3.0[a] | 30.1±5.1[a] |
| Adper Scotchbond SE | 34.0±5.0[a] | 31.9±3.2[a] |
| GO | 18.4±7.0[b] | 30.0±3.3[a] |

Groups with different letters are significantly different (p<0.05). A total of 5 teeth were used per group

**Table 2-** Number and percentage of specimens (%) according to fracture pattern mode and the premature de-bonded specimens from each experimental condition (*)

| Adhesive | Application mode | Fracture pattern | | | |
|---|---|---|---|---|---|
| | | A/M | CD | CR | D |
| Adper Single Bond 2 | Manufacturer's instructions | 46 (82.1) | 1 (1.8) | 4 (7.1) | 5 (8.9) |
| | Double application time | 46 (73.0) | 6 (9.5) | 5 (7.9) | 6 (9.5) |
| Adper Scotchbond SE | Manufacturer's instructions | 45 (57.7) | 17 (21.8) | 12 (15.4) | 4 (5.1) |
| | Double application time | 38 (66.7) | 8 (14.0) | 6 (10.5) | 5 (8.8) |
| GO | Manufacturer's instructions | 43 (81.1) | 2 (3.8) | 4 (7.5) | 4 (7.5) |
| | Double application time | 58 (77.3) | 4 (5.3) | 4 (5.3) | 9 (12) |

(*) A/M – adhesive/mixed fracture mode; CD – cohesive fracture mode in dentin; CR – cohesive fracture mode in resin; PF – premature failures.

**Table 3-** Relative percentage of the tubule area opening (means ± standard deviations) obtained for each experimental condition (*)

| Material | Application mode | | |
|---|---|---|---|
| | No treatment | Manufacturer's instructions | Double application time |
| Phosphoric acid | 7.0±2.5[a] | 12.4±4.7[b] | 12.7±4.9[b] |
| Adper Scotchbond SE | 8.2±6.7[α] | 14.9±5.1[β] | 18.2±9.7[β] |
| GO | 6.5±5.0[A] | 14.8±5.0[B] | 12.1±5.5[B] |

(*) Comparation only between lines. Groups with different letters are significantly different ($p<0.05$). A total of 4 teeth were used per group
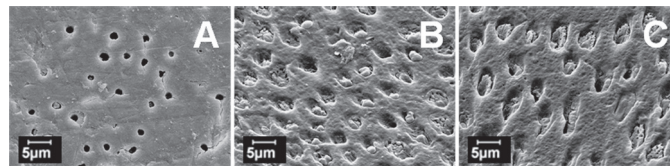


**Figure 4-** Scanning electron micrographs of sclerotic dentin (A - with no treatment) and after phosphoric acid etching (B and C). In B: sclerotic dentin treated with phosphoric acid for 15 s and in C for 30 s. The number of exposed tubules in B and C is higher than A and similar between them
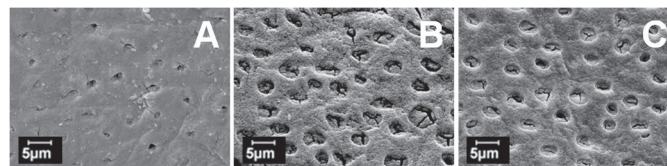


**Figure 5-** Scanning electron micrographs of sclerotic dentin (A - with no treatment) and after application of adhesive system Adper Scotchbond SE (B-C). In B, sclerotic dentin treated for 20 s and in C, sclerotic dentin treated duplicating the conditioning time. The number of exposed tubules in B and C is higher than A and similar between them
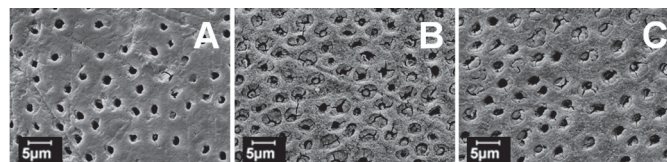


**Figure 6-** Scanning electron micrographs of sclerotic dentin (A - with no treatment) and after application of adhesive system GO (B-C). In B, sclerotic dentin treated for 20 s and in C, sclerotic dentin treated for 40 s. Observe that, the number of exposed tubules after conditioning (B and C) is higher than A and similar between them

images of each experimental condition can be seen in Figures 4 to 6.

## DISCUSSION

Although most *in vitro* studies that evaluated adhesion to sclerotic dentin used cervical lesions of human dentin as bonding substrates[8,19], the present study employed bovine sclerotic dentin. This substrate is morphologically similar to human dentin[3] and, therefore, a suitable substitute for human teeth in bond strength tests[24]. Besides that, bovine specimens are easier to obtain, and the substrate areas for bonding procedures are larger than sclerotic cervical lesions in human teeth[28].

Sclerotic dentin is a common substrate that occurs in response to tooth wear caused by attrition, abrasion, abfraction or erosion[29]. This substrate has demonstrated to be a challenge for bonding procedures[4,7]. The presence of a hyper-mineralized surface layer, bacteria and sclerotic casts obliterates the dentinal tubules and makes the dentin substrate less susceptible to acid demineralization[9].

The results of the present investigation showed that the effect of the experimental treatment on the immediate performance of the adhesive systems is adhesive-dependent, which led us to reject the null hypothesis of this study. The

duplication of phosphoric acid etching was not capable of increasing the removal of the sclerotic casts presented in the hyper-mineralized surface layer of the sclerotic dentin. The recommended and double etching with 35% phosphoric acid resulted in a similar open tubule area and µTBS values.

Cervical sclerotic dentin, unlike sound dentine, exhibit extensive variations in the hybrid layer thickness within the cervical sclerotic lesions[26]. Using the recommended etching times, the thickness of the hybrid layer may change abruptly due to an uneven etching[26]. This fact may be even worse when the phosphoric acid etching time is duplicated and may account for the controversial results observed when the etching time is duplicated in sclerotic dentin[9,26,29]. Besides that, the duplication of the phosphoric acid etching may cause the deepest demineralization of some intertubular and peritubular dentin, which may not be thoroughly infiltrated by resin monomers[10].

With regard to the SE adhesives, it has been reported that the additional layers of un-polymerized acidic monomers from SE adhesives may improve their etching potential by increasing the concentration of acidic reagents and counteracting the buffering capacity of hydroxyapatite[5]. The application of a single coat of a SE adhesive (Adper Prompt L-Pop, 3MESPE) was reported to be not enough to make sufficiently thick hybrid and adhesive layers in sound dentin[1,22]. These literature findings led some to hypothesize that the duplication of the SE application could produce a higher dissolution of the sclerotic casts, which was not observed in the present investigation.

Therefore, increased dissolution of sclerotic cast does not explain the increased µTBS for the GO adhesive when applied by double the recommended time. Therefore other mechanisms, operating simultaneously, may explain such findings. For instance, it is known that as the solvent is evaporated between each coat, the concentration of co-monomers after each coating increases[12], thereby improving the quality of the polymer inside the hybrid layer[10]. This was indirectly demonstrated by Nakaoki, et al.[21] (2005) who observed that the resin that occupied the area of the inter-tubular dentin of fractured dentin surfaces were much denser when the SE adhesive was applied in multiple coatings.

Based on that, one can argue that this technique may be beneficial for adhesive systems that produce weaker polymers. A recent study demonstrated that, among several SE adhesives tested, GO produced the lowest ultimate tensile strength and the lowest µTBS values[11]. Earlier studies reported that the ultimate tensile strength of the adhesive systems is positively correlated with the µTBS values[11,23]. Therefore, any effort to improve the strength of

the adhesive itself may lead to improvements in the resin-dentin µTBS of the adhesives.

It is likely that the double application of the GO adhesive may have improved the resin infiltration into the hybrid layer, contributing for the increase in the ultimate tensile strength of the polymer. This led to the achievement of resin-dentin µTBS values similar to that obtained for ASE and SB under control and experimental conditions.

Besides that, one cannot rule out the fact that the mode of adhesive application might have played a role in the differences between the GO and ASE. In the present study, the materials were applied according to the manufacturer's instructions, varying only the etching time. The recommended application time of the ASE is higher than the GO (Figure 2). Additionally, the former is recommended to be scrubbed on the surface while the latter is recommended to be only slightly applied. Several recent studies have reported that active application produces the highest immediate and long-term µTBS values[2,18], due to the formation of a polymer with increased cross-linking and greater solvent/water evaporation.

There are other features of the adhesive ASE that may have accounted for this difference. Contrary to GO, which is a 1-step self-etch adhesive, ASE is a 2-step self-etch adhesive that takes the additional advantage of having a more hydrophobic and resin filled coating. Thus, even when applied under manufacturer's instructions, the hybridized complex produced by ASE is richer in hydrophobic monomers and fillers making the extra supply of acidic resin by double application useless. The advantages of such hydrophobic resin coating were demonstrated recently by some studies. The application of one coat of a non-solvent containing resin, used to replace the subsequent coat of the hydrophilic adhesives supplied by the manufacturer, was able to increase the µTBS of a SE adhesive to sound dentin[1,15].

Additionally, filled adhesives, such as bottle B from the ASE, produced the thickest adhesive layers, even with a single coat application. Materials similar to ASE produce adhesive layers less sensitive to oxygen inhibition[14], ensuring adequate coverage of the etched dentin and reducing the harmful effects of oxygen inhibition[1,12,22].

## CONCLUSIONS

The efficacy of duplicating the conditioning time was only effective for the 1-step self-etch adhesive system tested.

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