Research article

Effect of thermo-mechanical cycling and chlorhexidine on the bond strength of universal adhesive system to dentin

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ABSTRACT

Objectives: This study evaluated the influence of thermo-mechanical cycling (TMC) on the bond strength (BS) of a universal adhesive system (UAS - Adper Single Bond Universal, 3M ESPE) to dentin treated or not with 0.2% chlorhexidine (CHX).

Methods: Eighty human molars were flattened until reach the dentin and separated into 4 groups according to the bonding protocol: ENR Group: 37% phosphoric acid + 3-step etch-and-rinse adhesive system (ENR); UAS Group: UAS in self-etch mode; ENR + CHX Group: 37% phosphoric acid + CHX + ENR; UAS + CHX Group: CHX + UAS in self-etch mode. After treatments, teeth were restored (Filtek Z350, 3M ESPE). Samples (n = 10) were submitted to aging process: stored in distilled water at 37°C/30 days or TMC (ERIOS - 98N/1.6Hz + thermal cycling 5/37/55°C - 1,200,000 cycles). Specimens were sectioned into sticks (1.0 mm²) and submitted to the microtensile test (Mechanical Test Machine - 0.5 mm/min). Fracture patterns and hybrid layer integrity were analyzed under Stereomicroscope and Scanning Electron Microscopy (SEM).

Results: The BS results (3-way ANOVA, Bonferroni’s test, α = 5%) showed that groups treated with CHX presented higher BS values than control groups; significant in all cases (p < .05), except for ENR submitted to TMC (p > .05). When CHX was applied and samples were cycled, UAS revealed higher BS (p < .05) than ENR. After TMC, cohesive fractures increased for UAS, regardless of CHX application. SEM analysis demonstrated different hybridization patterns for the adhesive systems tested.

Conclusion: The performance of the universal adhesive system used in self-etch mode was better than that of the 3-step etch-and-rinse adhesive system.

Clinical significance: Universal adhesive systems have been developed in order to simplify the dentin hybridization protocol. It is important to determine the longevity of the adhesive interface using these bonding materials after chewing.

1. Introduction

The longevity of an esthetic restoration is related to good sealing and stable bond of the adhesive system used [1], reached by its impregnation through the collagen network for the formation of the hybrid layer [2]. In order to achieve stable hybridization, the choice and use of a suitable bonding protocol on the dental substrate are essential [3].

Whereas original simple bonding agents evolved to multi-step systems, recent development focuses on simplification of adhesive procedures decreasing the technique sensitivity and the clinical application time [4]. Therefore, a new category of dentin adhesive systems, called “universal” or “multi-mode” adhesives, have been developed [5]. They are essentially 1-step adhesive systems, combining acidic primers and bonding agent in a single solution [6], and might be indistinctly applied following either an etch-and-rinse, selective-etch or self-etch approach [7]; adapting to different clinical situations. Despite that, clinical studies employing these adhesive systems have presented controversial results [8], which demonstrates that further research is still needed to assess the performance of these new bonding agents.

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On the other hand, in terms of adhesion durability and strength, it is well known that the 3-step etch-and-rinse adhesive systems are considered the “gold standard” among bonding agents since the application of a solvent-free, neutral-pH, hydrophobic, adhesive resin layer in a separate step results in bonding effectiveness [2, 7]. With this approach, some problems related to simplified adhesives could be avoided; such as phase separation, low degree of conversion, lack of dentin sealing and poor hybridization [9, 10].

Likewise, it is widely acknowledged that the long-term stability and success of adhesive restorations depend not only on the adhesive system chosen, but also on the high quality and durability of the hybrid layer over time [7]. Thus, the demineralized collagen matrix must be completely infiltrated by resin [2, 3]. Even in self-etch strategies, in which micromechanical bonding is achieved by shallow hybridization, and chemical reactions between phosphate groups of functional monomers and residual hydroxyapatite occur, the unprotected collagen may undergo hydrolytic degradation and/or proteolysis by endogenous proteases, such as matrix metalloproteinases (MMPs) [11, 12].

The MMPs 2, 8 and 9 are enzymes mainly activated during acid etching and contribute to the degradation of the dentin collagen matrix and the hybrid layer [13], affecting the longevity of the restoration [14]. The loss of bond to dentin may be quantified by significant reduction in microtensile bond strength (μTBS) immediately and over time [3].

In the attempt to solve this drawback, various MMPs-inhibitors have been proposed as dentin surface pre-treatment [12]. Chlorhexidine (CHX) is considered the non-specific protease inhibitor most investigated and used [13]. Its application may increase the durability of the adhesive interface not only by controlling the activity of MMPs, but also by inhibiting other enzymes in the dentin matrix [15, 16]. Besides that, CHX may have detergent effect that facilitates the impregnation of the resin monomers in the demineralized dentin [17]. The success of CHX together with conventional adhesive systems has already been reported in the literature, and it is known that the use of aqueous primers containing CHX results in the preservation and integrity of the hybrid layer over time [18, 19].

On the other side, recently some studies have evaluated the stability of the resin-dentin interface under simulated oral conditions and reported low levels of MMPs enzymatic activity after stress during chewing simulations [20]; even some possibility of remineralization during the masticatory load [21]. However, there is no evidence of these findings using universal adhesives. Therefore, the purpose of this study was to evaluate the in vitro influence of thermo-mechanical cycling on the bond strength of a universal adhesive system to dentin previously treated or not with 0.2 % chlorhexidine. The null hypothesis tested was that there would be no difference in the bond strength of the adhesive systems to dentin either pre-treated with chlorhexidine or not, and irrespective of thermo-mechanical cycling (TMC).

2. Materials and methods

The materials used in this study are described in Table 1.

2.1. Dental restorative procedures and thermo-mechanical cycling (TMC)

After approval by the Research Ethics Committee, (CAAE #11457812.9.0000.5419), 80 sound-impacted human third molars were collected, disinfected with 0.1% thymol and stored in distilled water at 37 °C. Their roots were embedded in PVC tubes (2 cm in diameter x 2 cm high) using self-polymerizing acrylic resin (VIPI FLASH, Pirassununga, SP, Brazil). Then, the occlusal surfaces were flattened (Polipan-U, Panambra Zwick, Ferrazopolis, SP, Brazil) with SiC abrasive papers (320, 600 and 1200-grit) until reach the dentin surface. After that, the teeth were divided into 4 groups according to the adhesive system used for the dentin hybridization (Table 1) and whether or not treated with 0.2 % CHX [13, 14, 22].

The group which teeth were hybridized with the 3-step etch-and-rinse adhesive system (Adper Single Bond Universal, 3M ESPE, Sumaré, SP, Brazil) and treated with chlorhexidine solution (ENR + CHX Group), 100 μl of 0.2 % CHX were applied on the dentin surface after etching (with 37 % phosphoric acid), using a micropipette (Pipetman P500, Gilson, Villiers-le-Bel, France). After 30 s, excess solution was removed with absorbent paper [22] and afterwards, dentin hybridization was performed according to the protocol described in Table 1.

The group which teeth were hybridized with the universal adhesive system in self-etch mode (Adper Single Bond Universal, 3M ESPE, Sumaré, SP, Brazil) and treated with chlorhexidine solution (UAS + CHX Group), 0.2 % CHX was applied before the application of the adhesive system as explained before. Then, dentin hybridization was carried out as specified in Table 1.

The groups that were treated only with the 3-step etch-and-rinse adhesive system (ENR Group) or the universal adhesive system (UAS Group), without chlorhexidine application, the protocol was the same described in Table 1.

After applied, the adhesive systems were light-activated with a LED device (FlashLite 1401), Discus Dental, Culver City, CA, USA – 1100 mW/cm² during the time stated in Table 1.

Following adhesive procedures, restorations were performed with composite resin (Filtek Z350, 3M ESPE, St. Paul, MI, USA) by the incremental technique and light-activated for 40 s (Flash Lite 1401, Discus Dental). Then, samples from each group were randomly separated into 2.

Table 1. Materials used.

| Materials          | Composition                        | Manufacturer    | Application protocol |
|--------------------|------------------------------------|-----------------|----------------------|
| Universal Filtek Z350 | Bis-GMA, Bis-EMA, UDMA and small quantities of TEGDMA. Non-agglomerated nanoparticles of silica 20nm in size and nanoagglomerates formed of zirconium/silica particles ranging from 0.6 to 1.4 μm in size. | 3M ESPE, Sumaré, SP, Brasil | 1. Acid etching for 15 s; 2. Rinse for 30 s; 3. Dry with absorbent paper; 4. Apply primer and volatization with air dry for 15 s; 5. Apply adhesive. 6. Light activation for 20s. |
| Adper Scotchbond Multi-Purpose (3-step etch-and-rinse adhesive system) | Primer: HEMA, PAMA, GPDM, polyalkenoic acid. Adhesive: Bis-GMA, HEMA, EMAB, polyalkenoic acid copolymers. | 3M ESPE, Sumaré, SP, Brasil | 1. Apply adhesive for 20 s; 2. Volatization with air dry for 5 s; 3. Light activation for 10s. |
| Adper Single Bond Universal (Adhesive system applied in self-etch mode) | 10-MDP, dimethacrylate resins, HEMA, Vitrrebond copolymer, filler, ethanol, water, initiators, silane | 3M ESPE, Sumaré, SP, Brasil | 1. Acid etching for 15 s; 2. Rinse for 30 s; 3. Dry with absorbent paper; 4. Apply primer and volatization with air dry for 15 s; 5. Apply adhesive. 6. Light activation for 20s. |
| Gel etchant | 37 % H3PO4, colloidal silica, surfactant and coloring agent. | Dentsply, Petrópolis, Brasil | |
| 0.2 % aqueous Chlorhexidine solution | 0.2 % Chlorhexidine digluconate | Not applicable | |
subgroups (n = 10), as described in Table 2. Half of them were submitted to thermo-mechanical cycling (TMC) with a load of 98 N applied in the center of the restorations at a frequency of 1.6 Hz for 1,200,000 cycles, simulating chewing for five years [23], with temperatures ranging between 5, 37 and 55 °C for 60 s each, with intervals of 12 s. The other half of the teeth were control groups and were not submitted to any aging process (stored in distilled water at 37 °C for 30 days).

2.2. Bond strength

Samples were sectioned into sticks (1.0 mm²), by means of diamond discs (SYJ-150 Digital Diamond Low Speed Saw 4, MTI Crystal, Richmond, CA, USA); and submitted to the “non-trimming” microtensile test [24] in a mechanical testing machine (Emic, Model 1L-2000, Sao Jose dos Pinhais, PR, Brazil) at a crosshead speed of 0.5 mm/min until failure.

The BS was calculated according to formula $R = (F/A)/10$, where “R” is the strength (MPa), “F” is the load required to rupture the specimen (Kgf) and “A” is the area of the adhesive interface (mm²), measured before the test. Subsequently, the BS values were statistically analyzed (3-way ANOVA, Bonferroni’s test, α = 5 %). The pre-test failures were excluded to avoid increasing the standard deviations [25].

2.3. Fracture type analysis

The fractured sticks were analyzed under a Stereomicroscope (USB Digital Microscope, Shenzhen WOTNG Technology Limited, Guangdong, China) at up to 800x magnification, and classified according to the fracture type as: adhesive (at the dentin-resin interface), cohesive (failure in the body of the sample) in resin or in dentin, and mixed (adhesive and cohesive).

2.4. Hybrid layer evaluation

The remaining specimens were embedded in epoxy resin (Buehler, Lake Bluff, IL, USA) and after 24 h, they were polished with SiC abrasive papers of 600, 1200, 2000-grit and a diamond paste (Metadit II, Buehler, Lake Bluff, IL, USA). Then, the specimens were immersed in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer solution, with a pH of 7.4 (Merck KGaA – Darmstadt, Germany) at 4 °C for 12 h. After fixation, they were rinsed with distilled water for 3 min and then, immersed in distilled water for 1 h, with the water being changed every 20 min. Subsequently, the surfaces were treated with 37 % phosphoric acid for 10 s, rinsed, immersed in distilled water and kept in an ultrasound appliance (T-1449-D, Odontobras, Ribeirao Preto, SP, Brazil) for 10 min, to remove possible residues. Afterwards, the specimens were dehydrated through ascending grades of ethanol (Labyzynth Ltda., Diadema, SP, Brazil): 25 % (20 min), 50 % (20 min), 75 % (20 min), 95 % (30 min) and 100 % (60 min); and kept in HMDS solution (Merck KGaA, Darmstadt, Germany) for 10 min, dried with absorbent paper and then, fixed onto stubs and sputter-coated with gold. Scanning Electron Microscopy (SEM – Philips XL-30 FEG) was performed at 1000x and 2000x magnification. The adhesive interface morphology was analyzed as regards hybrid layer formation, concerning its integrity, homogeneity and thickness; as well as, presence and disposition of resin tags.

3. Results

3.1. Bond strength

The means and standard deviations of the microtensile bond strength values for all groups are presented in Table 3.

In control conditions, TMC significantly increased (p < .05) the bond strength values of the 3-step etch-and-rinse adhesive system (ENR Group) when compared to the group that was not cycled. Under the same conditions, for the universal adhesive system (UAS Group) there was no significant difference (p > .05) between the cycled and uncycled groups.

When chlorhexidine was used as dentin pre-treatment, TMC was a determinant factor for the performance of the 3-step etch-and-rinse adhesive system (ENR + CHX Group), showing significantly (p < .05) lower bond strength than the group not cycled. On the contrary, between the universal adhesive system (UAS + CHX Group), with and without TMC, there was no significant difference (p > .05).

On the other hand, it was also observed that the surface pre-treatment with chlorhexidine positively affected the bond strength values, presenting higher values than the control groups; significant (p < .05) in all cases, except for the 3-step etch-and-rinse adhesive system (ENR Group compared to ENR + CHX Group) submitted to TMC (p > .05).

In Figure 1, it could be observed that when samples were pre-treated with CHX and submitted to cycling, the universal adhesive system (UAS + CHX Group) presented significantly (p < .05) higher bond strength values compared to the 3-step etch-and-rinse adhesive system (ENR + CHX Group) Also, the universal adhesive system (UAS Group) revealed higher values in control conditions and without cycling (p < .05). Finally, there was no significant difference (p > .05) between the adhesive systems tested when compared under the other variables.

3.2. Fracture type analysis

In Figure 2, the percentages of the fracture pattern are presented for the groups without chlorhexidine application (ENR Group and UAS Group). There was predominance of mixed fractures for the 3-step etch-and-rinse adhesive system (ENR Group) compared to the universal adhesive system (UAS Group), regardless of whether the samples were or

| Table 2. Distribution of the groups (n = 10). |
|---------------------------------------------|
| Adhesive system                           | Pre-treatment | Aging          |
| Etch-and-rinse adhesive system (3-step) (n = 40) | Control (n = 20) | No cycling |
|                                           | CHX (n = 20)   | No cycling    |
|                                           | Control (n = 20) | Thermo-mechanical cycling |
|                                           | CHX (n = 20)   | Thermo-mechanical cycling |
| Universal Adhesive system applied in self-etch mode (1-step) (n = 40) | Control (n = 20) | No cycling |
|                                           | CHX (n = 20)   | No cycling    |
|                                           | Control (n = 20) | Thermo-mechanical cycling |
|                                           | CHX (n = 20)   | Thermo-mechanical cycling |

| Table 3. Comparison of the mean (standard deviation) bond strength (MPa) values for the groups tested (3-way ANOVA, Bonferroni, α = 5%), analyzing treatment and aging within each adhesive system. |
|---------------------------------------------|
| Adhesive system                           | Groups | Pre-treatment | Aging |
| Etch-and-rinse adhesive system (3-step)   | ENR    | Control      | 23.9 (±0.7) aA |
|                                           | ENR + CHX | CHX | 25.6 (±5.0) aA |
| Universal adhesive system applied in self-etch mode (1-step) | UAS | Control | 21.3 (±0.8) aA |
|                                           | UAS + CHX | CHX | 36.7 (±6.9) bA |

Different letters, uppercase on the line and lowercase letters in the column, indicate statistically significant difference (p < .05).
not submitted to TMC. On the other hand, adhesive fractures were more frequently identified for the universal adhesive system (UAS Group) than for the 3-step etch-and-rinse adhesive system (ENR Group), also irrespective of whether they were or not cycled.

Likewise, an incidence of cohesive fractures in dentin was observed, for both groups (ENR Group and UAS Group), when subjected to TMC compared to those that were not. Finally, the universal adhesive system (UAS Group), submitted or not to TMC, mainly resulted in cohesive fractures in resin, showing equal percentages for both cases.

In Figure 3, the fracture patterns of the groups pre-treated with CHX (ENR + CHX Group and UAS + CHX Group) are presented in percentages. It was observed that regardless of the adhesive system employed, when samples were submitted to TMC, the cohesive fractures in resin increase and the adhesive fractures decrease compared to uncycled samples. On the other hand, once again it was shown incidence of cohesive fractures in dentin when the substrates were pre-treated with CHX before UAS application (UAS + CHX Group) and submitted to TMC, compared to those that were not cycled. What is more, when chlorhexidine was used as dentin pre-treatment and samples submitted to TMC, the universal adhesive system (UAS + CHX Group) revealed lower percentages of adhesive and mixed fractures compared to the 3-step etch-and-rinse system (ENR + CHX Group).

3.3. Hybrid layer evaluation

Figure 4 exhibits the morphology of the hybrid layers obtained in all the groups. In images A and B, uniform hybrid layers were observed with formation of resin tags throughout the adhesive interfaces. In images C, D and G, the thickness of the hybrid layers was smaller but uniform, and there was no evidence of resin tag formations.

In images E and F, the thickness of the hybrid layers was smaller and less uniform than the other groups, and fewer resin tags were observed. In image H, there were no regions with evident and uniform hybrid layers, and there was no evidence of resin tag formations.

4. Discussion

The aim of this study was to evaluate the influence of thermo-mechanical cycling on the bond strength of a universal adhesive system to dentin previously treated or not with 0.2 % chlorhexidine; starting
with the null hypothesis that irrespective of cycling, there would be no significant difference in the bond strength of this adhesive system to dentin, either pre-treated or not with chlorhexidine, compared to a 3-step etch-and-rinse adhesive system. From the results obtained, the hypothesis was rejected due to the fact that when the dentin surface was pre-treated with chlorhexidine and the samples submitted to TMC, the performance of the universal adhesive system achieved better results than the 3-step etch-and-rinse adhesive system. Furthermore, the universal adhesive system showed significantly higher values when samples were under control conditions and without TMC (Figure 1). It is known that the hybrid is susceptible to degradation by the slow action of matrix metalloproteinases or due to the hydrolytic degradation of resin monomers of the adhesive systems [19]. Several MMPs-inhibitors were proposed [3, 13], highlighting from the beginning the CHX [14, 15, 16, 18, 19, 26].

The CHX application is recommend directly on etched dentin, and without rinsing, prior to the use of adhesive systems [13]. Thus, CHX would be capable of inhibiting the enzymatic activity of endogenous proteases present in the adhesive interface [26].

Previous studies have demonstrated that the hybrid layer formed in CHX-treated dentin exhibits a normal structural integrity of the collagen network, contrary to untreated substrate; however, it is smaller and less consistent [27, 28]. In the present study, these features were not translated in reduction of the bond strength. Conversely, it was observed that CHX positively affected the bond strength values (Table 3), presenting higher values in the pre-treated groups than control groups, regardless of whether the samples were or not submitted to TMC, significant in all cases (p < .05), except for the 3-step etch-and-rinse adhesive system (ENR groups compared to ENR + CHX group) submitted to TMC (p > .05). Results in agreement with previous findings obtained by Breschi et al. [14], in which acid-etched dentin treated with 0.2% CHX showed higher bond strength and higher quality of the hybrid layer compared to control groups. What is more, in a meta-analysis performed by Montagner et al. [29], the authors concluded that both categories of adhesive systems (self-etching and etch-and-rinse) demonstrated to be benefited by 0.2% CHX after aging.

Several studies [14, 15, 30] have demonstrated that low concentration of CHX, as 0.2%, can be equally effective or superior compared to other concentrations (2%), presenting higher substantivity, and ability to form a relatively stable monolayer of retained CHX, with fewer adverse effects [31].

On the contrary, other studies have reported that CHX may interfere with the infiltration ability of the hydrophilic monomers through the dentinal tubules; fact that over time, can lead to decrease in the bond strength and increase in microleakage [27, 28]. Acid etching prior to the use of conventional adhesive systems promotes deeper infiltration into dentin [30]; so, probably CHX could infiltrate more easily through the dentinal tubules and interfere with the bonding procedure, impeding the formation of a uniform hybrid layer and presenting fewer resin tags (as seen in Figure 4E and 4F). Moreover, CHX is water soluble and its long-term efficacy may be compromised [32]. Thus, when TMC was performed, aging negatively affected the bond strength of the 3-step etch-and-rinse adhesive system tested in the study, being significantly (p < .05) lower than the group without cycling (Table 3); presumably due to increase in microleakage and water penetration. Unprotected collagen fibrils are more susceptible to proteolytic degradation, hydrolysis, and functional and thermal stress [33], as was found by Da Silva et al. After CHX application, the bond strength values decreased for samples treated with an etch-and-rinse adhesive system and stored for 15 days in distilled water [34]. On the other hand, for Hashimoto et al. [35] self-etching adhesive systems allow lower degree of nano-infiltration into dentin; obtaining better sealing, less presence of water; and consequently, lower hydrolytic degradation of the hybrid layer; which could increase the longevity of esthetic restorations, corroborating the results of the present study. As seen in Figure 1, the universal adhesive system presented higher bond strength values than the 3-step etch-and-rinse adhesive system when the dentin surface was pre-treated with CHX and the samples subjected to TMC, being statistically significant (p < .05). Previous studies have compared the bonding ability of conventional and self-etching adhesive systems, and it was shown that a successful bond strength is intimately related to the sensitivity of the technique performed by the operator, considering the order and number of clinical steps [36, 37]. Moreover, the presence of humidity in the dentin substrate, interacting with the hydrophobic components of the adhesive systems, presents an important role in achieving efficient bond strength [38].

With the simplification of the technique, diminishing the number of steps and reducing the humidity by eliminating the acid-etching stage, the self-etching systems promote less technique sensitivity and substrate variability [39]. In self etch approach, demineralization and infiltration occur simultaneously, creating a uniform hybrid layer, although smaller when compared to conventional adhesive systems [40], as may be visualized in Figure 4.
Furthermore, the reduced demineralization obtained with self-etching approaches maintains the availability of hydroxyapatite crystals within the hybrid layer for chemical bond between the functional monomers and calcium ions that contributes to the stability and durability of the adhesive interface [3].

Two phenomena – bonding and decalcification – may occur at the time when the functional monomers of the resin, containing carboxyl or phosphate groups, come into contact with the demineralized dentin substrate [41]. Decalcification and release of calcium and phosphate ions subsequently take place after calcium sulphate formation [42].

The universal adhesive systems have functional monomers with phosphate (Methacryloyloxydecil dihydrogen phosphate – MDP) in their composition, which potentiates the chemical bond to hydroxyapatite [42], thus justifying its good performance in the present study, even in the control group without TMC (Figure 1). Beyond that, it has been demonstrated that the chemical bond generated by 10-MDP is not only more effective but also more stable in water, which is valuable in terms of bonding durability [43], as evidenced in the present study. The bonding ability of the universal adhesive system was not affected by TMC (Table 3). Findings in accordance with a previous study by Ionue et al. The authors also noted that after TMC, there was no decrease in the bond strength or ultramorphologic changes for a 10-MDP containing adhesive system [44].

The longevity of the adhesive interface may also be influenced by physical intraoral conditions such as occlusal chewing loads and repetitive expansion and contraction stresses caused by temperature changes within the oral cavity [45]. Long-term clinical trials have been the gold standard for evaluating the success of adhesive procedures [46]. However, its execution is difficult due to operator variability, substrate differences, recall failure, and the time and resources involved [47].

The use of thermocycling with microtensile test method is very common to assess bonding durability, especially for newly introduced
materials [48]. Thermocycling simulates changes that occur in the oral cavity caused by consumption of food, drinks and even by breathing habits. This aging method is expected to recreate temperature variations by exposing samples to repetitive cycles of hot and cold alterations, producing volumetric changes and fatigue of the adhesive interface [49]. In addition to thermal aging, the application of mechanical loads may also alter the interface and provide valuable information about the long-term performance of bonding materials [21, 22]. From the fracture type evaluation (Figures 2 and 3), an incidence of cohesive fractures was observed when the universal adhesive system was used in self-etch mode (UAS Group and UAS CHX Group) and subjected to TMC compared to those that were not cycled. Likewise, under these conditions the percentage of adhesive fractures decreased. While adhesive fractures microscopically represent rupture in the interface between resin and dentin, characterized by open dentinal tubules; cohesive fractures indicate that the hybrid layer is intact [50].

These findings could prove the theory that load cycling promotes the mineralization of degraded areas within the hybrid layer [21]. Remaining crystals in dentin, partially demineralized by the self-etch approach, associated with its functional monomer may have contributed to the remineralization process after thermo-mechanical cycling [20]. What is more, according to Shadman et al., CHX effectively prevents bond strength decrease over time, for samples treated with the same universal adhesive system used in the present study [51]. Therefore, when the dentin surface was pre-treated with this MMPs-inhibitor, and submitted to TMC, the universal adhesive system presented higher bond strength values (p < .05) than the 3-step etch-and-rinse adhesive system (Figure 1).

Moreover, in control conditions, it seems that the resin monomers of the 3-step etch-and-rinse adhesive system (ENR Group) properly infiltrate through the collagen matrix without interference, obtaining a uniform hybrid layer and resin tags throughout the adhesive interface (as seen in Figure 4A); so this group was not negatively affected by thermo-mechanical cycling, on the contrary, there was a significant increase (p < .05) in the bond strength values (Table 1); surely attributable to dentin remineralization by mechanical load.

5. Conclusions

The performance of the universal adhesive system used in self-etch mode was better than that of the 3-step etch-and-rinse adhesive system when the dentin surface was pre-treated with chlorhexidine and the samples submitted to TMC. Also, when samples were under control conditions and no TMC was performed.

TMC contributes to the stability and longevity of the adhesive interface when the universal adhesive system was used on dentin surface pre-treated or not with chlorhexidine, and when the 3-step etch-and-rinse adhesive system was applied on samples under control conditions.

Declarations

Author contribution statement

Rocio Geng Vivanco, Renata Silva Cardoso: Performed the experiments; Wrote the paper. Ana Beatriz Silva Souza, Sergio Augusto de Freitas Vincenti: Contributed reagents, materials, analysis tools or data. Michelle Alexandra Chinellati, Raffaella Tonani-Torrieri: Performed the experiments. Fernanda de Carvalho Panzeri Pires-de-Souza: Conceived and designed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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