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Bonding effectiveness of composite-dentin interfaces after mechanical loading with a new device (Rub&Roll)

Anelise Fernandes MONTAGNER1,2, Niek J OPDAM2, Jan L RUBEN2, Maximiliano Sérgio CENCI1 and Marie-Charlotte HUYSMANS2

1 Graduate Program in Dentistry, School of Dentistry, Federal University of Pelotas, Rua Gonçalves Chaves, 457, Fifth Floor, 96015560, Pelotas, RS, Brazil
2 Department of Preventive and Restorative Dentistry, Radboud University Nijmegen Medical Centre, P.O. Box 9101, NL 6500 HB, Nijmegen, The Netherlands

Corresponding author, Anelise Fernandes MONTAGNER; E-mail: animontag@gmail.com

This study evaluated the effect of mechanical loading with a new device on the microtensile bond strength (µTBS) of adhesive systems to dentin. Forty molars were divided according to adhesive systems: self-etch (Clearfil™ SE Bond —CSE) and etch-and-rinse (Adper Scotchbond™ TMT IX —ASB); and to aging (n=5): control; MC1-250,000; MC2-500,000; and MC3-750,000 mechanical cycles. Microtensile bond strength was measured and fracture modes were analyzed. Data for µTBS were subjected to Kruskal-Wallis and post hoc tests (p<0.05). Mechanical loading (p<0.001) and adhesive systems (p=0.024) affected µTBS values. The adhesive systems showed a similar behavior, except in the MC3 group, which the self-etch CSE showed the highest µTBS. The new device promotes a decreasing of µTBS as the number of cycles increased. Difference between materials was observed only after 750,000 mechanical cycles.

Keywords: Mechanical load, Adhesive systems, Microtensile, Bond strength, Loading

INTRODUCTION

Dentists worldwide spend most of their time placing and replacing direct restorations. Therefore, the long-term survival of clinically placed restorations is an important focus for dental research. The main reasons for failure of restorations on the long-term are fracture and secondary caries1). Considering the high turnover of new materials on the market, it is important to test these materials first in vitro in a clinically relevant way, in order to be able to predict possible clinical pathways of failure2,3). Therefore, aging processes resembling those taking place in the oral environment were introduced in laboratory testing procedures. Simulations of thermal and mechanical stresses have been used to try to reproduce these conditions. However, the popular thermal cycling method has limited effect compared to mechanical aging4).

Mechanical load cycles and forces applied are not standard in most studies, as well as methods and devices used to apply the force5,6). Most of those studies use machines that apply the force on the sample in just one direction, which seems quite different from the continuous process of chewing forces and bruxism clinically present to the tooth-restoration complex. Moreover, forces applied in in vitro experiments show a large variation from 15 to 60 N7-8). A main problem related to the mechanical loading is that when multidirectional forces are applied, devices become more complicated and expensive. Moreover, the process is time-consuming as most devices only have a limited number of sample spaces available.

Numerous in vitro studies to test the performance of dentin adhesives systems focus on its relationship with clinical performance. The potential relationship between marginal adaptation found in vitro of class V restorations and the clinical longevity of class 5 restorations has been demonstrated9). Moreover, Van Meerbeek et al. demonstrated correlation between microtensile bond strength tests on aged samples by storing and the clinical outcome of class V restorations9). In class 2 restorations, aging by mechanical loading may play an even larger role and therefore, it seems useful to include mechanical loading as an aging process in in vitro studies to compare bonding capacities of dental adhesives.

High initial composite-dentin bond strength values are considered desirable, but durability of the bond over time is also of great interest, especially when the adhesive is applied in load bearing restorations. Loading stress seems to be concentrated mostly at the interface between the adhesive and the top of the hybrid layer10). Sano et al.11) introduced the microtensile bond strength test to measure bonding of small areas surface (∼1 mm²), and nowadays this test is accepted as the most useful bond strength test showing a higher discriminative power when compared to other tests12).

So, in order to evaluate the effectiveness of a new device for application of mechanical loading the present study uses the microtensile bond strength (µTBS) test to evaluate the effect of aging with mechanical loading on the adhesive bond between tooth and restoration. The null-hypothesis tested is that the applied mechanical loading does not influence microtensile bond strength values.
MATERIALS AND METHODS

Description of the device
The device for applying mechanical loading (Rub&Roll) is shown in Fig. 1 and described in detail elsewhere12. Basically, the Rub&Roll is a new device with multifunctional characteristics. The machine has one outer cylinder that is mounted fixed on a base, and an inside cylinder that fits centrally in the outer cylinder. The inside cylinder, which is mounted on a rotation axle, contains 16 samples holders (20×14×10 mm dimensions) which are evenly distributed over the outer surface of the cylinder. The inner cylinder is rotating around the axle, which is driven by an engine that can be adjusted according to the required rotation speed. In the space between inside and outside cylinder a loosely fitting rod is placed, consisting of a metal rod inside a silicon tube. When the inside cylinder is rotated, this rod rolls over the samples protruding from the inner cylinder, leading to cyclic loading of the samples. Silicon tubing of 1 mm thickness was used in this study, and the samples protruded 1 mm above the cylinder surface, resulting in a maximum load of 30 N. The actual applied force on the samples was measured with a force sensor mounted in the surface of the outside cylinder.

Specimens preparation
Forty extracted sound human third molars were selected, cleaned and stored in water. Anonymously collected extracted human teeth were used, which does not require ethical committee approval in The Netherlands. Flat occlusal superficial dentin surfaces were exposed using 200-grit SiC paper under running water, and complete removal of enamel was confirmed by stereomicroscopy (M50 Leica Microsystems, Singapore) examination. Teeth were embedded in acrylic resin (ProBase Cold Acrylic Resin, Ivoclar Vivadent, Mississauga, Canada) resulting in samples of 16 mm in height×14 mm width×10 mm length (Fig. 1). After that, dentin surfaces were polished using 600-grit SiC paper to create a uniform smear layer.

Samples were assigned randomly to one of eight groups (n=5), receiving one of two adhesive systems and one of four aging protocols. Dentin surfaces were treated with a self-etch adhesive system ClearfilTM SE Bond–CSE–(Kuraray Noritake Dental, Tokyo, Japan) or an etch-and-rinse adhesive system Adper ScotchbondTM 1XT–ASB–(3M ESPE, St. Paul, MN, USA) which was applied with wet-bonding technique. Both adhesive systems were applied according to the manufacturer’s instructions as indicated in Table 1. Thin layers of resin-based composite (ClearfilTM AP-X, Kuraray Noritake Dental), approximately 1.5 mm in thickness, were bonded incrementally to the cured adhesive, and each increment was individually light-cured for 20 s using a LED curing unit with an intensity of ≈900 mW/cm² (Fusion™ S7 Curing Light, DentLight, Richardson, TX, USA). This resulted in restorations 4 mm in height. Aging protocols were (Fig. 1):

1. Control (no aging): samples were stored in distilled water for 24 h, at room temperature;
2. MC1: mechanical loading for 1 week, 250,000 cycles;
3. MC2: mechanical loading for 2 weeks, 500,000 cycles;
4. MC3: mechanical loading for 3 weeks, 750,000 cycles.

Before the samples were exposed to mechanical aging, they remained in distilled water for 24 h.

Mechanical loading
Samples were mounted into the Rub&Roll device...
Table 1  Study Material

| Material                          | Type                        | Composition*                                                                 | Application procedures                                                                                                                                                                                                 | Lot       |
|----------------------------------|-----------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Adper Scotchbond 1XT (3M ESPE)   | Etch-and-rinse two-step adhesive system | Etching agent: 35% phosphoric acid (pH 0.7) Adhesive: Bis-GMA, HEMA, dimethacrylates, polyalkenoic copolymer, ethanol, water, photoinitiator | 1. Apply phosphoric acid for 15 s, 2. Rinse for 15 s, 3. Blot excess moisture using a cotton pellet, 4. Apply two adhesive coats under pressure for 15 s, 5. Gently air thin for 5 s, 6. Light-cure for 10 s. | 188103    |
| Clearfil SE Bond (Kuraray)       | Self-etch two-step adhesive system | Primer: MDP, HEMA Dimethacrylate, monomer Water. Photoinitiator Bond: MDP, HEMA Dimethacrylate, monomer Microfiller, Photoinitiator | 1. Apply primer and leave it undisturbed for 20 s, 2. Dry thoroughly with mild air flow for 10 s, 3. Apply bond, 4. Gently air thin for 5 s, 5. Light-cure for 10 s. | 041892    |
| Clearfil AP-X (Kuraray) Shade A2 | Hybrid light-cure resin-based composite | Barium glass, silica colloidal, silicon dioxide, Bis-GMA, TEGDMA, photoinitiator | 1. Apply composite 2 mm thick, 2. Light-cure for 20 s. | 1090AA    |

*Bis-GMA (bisphenol glycidyl dimethacrylate); HEMA (2-hydroxyethyl methacrylate); MDP (10-methacyrloyloxydecyl dihydrogen Phosphate); TEGDMA (Triethylene glycol dimethacrylate)

and mechanical loading was applied by the rotation movement of the inner cylinder (Fig. 1). In this study, samples were loaded at 20 rpm, 0.4 Hz, and ±30 N. Mechanical loading took place in distilled water, which filled the outer cylinder. The distilled water was weekly changed.

$\mu$TBS test
After aging, loaded and unloaded (control) samples were sectioned into beams (stick-shaped) with an approximate cross-sectional area of 1 mm$^2$, using a low speed diamond saw under continuous water-cooling, following the nontrimming method (Fig. 1)\cite{13}. This resulted in 50–75 beams for each experimental group. Each beam was measured for its cross-sectional dimensions using a digital caliper (Mitutoyo America, Los Angeles, CA, USA) to calculate the surface area. Beams were tested for microtensile bond strength ($\mu$TBS) by attaching them with superglue gel (Cyanoacrylate Rite-Lok, 3M, Bracknell, UK) adhesive to a movable jig, a modified Gerwaldeli’s jig, which is attached to the Universal Testing Machine (Materials Testing Machine LS1, Lloyd Materials Testing, Hampshire, UK) at 1 kN. The beams were stressed to failure in tension using $\mu$TBS tester at a crosshead speed of 1 mm/min. The fractured beams were removed from the apparatus and the modes of fracture were evaluated.

The bond strength ($\sigma$) in MPa was obtained with the formula $\sigma=F/A$, where $F=$load for specimen rupture (N) and $A=$bonded area (mm$^2$). To determine the area, the formula to calculate was $A=b.h$, where $A=$interfacial area, $b=$base and $h=$thickness of slices. Pre-testing failures were included in the calculation of mean $\mu$TBS as 0 MPa.

Mode of failure determination
All fractured beams were observed under stereomicroscope (M50 Leica Microsystems) and the fracture mode was determined at 75× magnification. Images were captured by camera (Canon EOS 50D, Canon, Melville, LA, USA). The fracture surfaces were classified as: apparently interfacial (fracture occurred within the adhesive interface, between the dentin and composite); cohesive in dentin (fracture occurred at the dentin portion); cohesive in composite (fracture occurred at the resin-based composite portion) or mixed failures (designates a mixture of adhesive and cohesive failure within the same fractured surface).

Subsequently, selected fractured beams of each group ($n=10$), exhibiting a representative failure mode, were processed for field-emission-gun scanning electron microscopy (Fig. 1). Specimens were chemically fixed by immersion in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for 6 h, and dehydrated in an ascending series of ethanol: 50% for 5 min, 90% for 5 min, and finally 100% ethanol for 3 h. After that, specimens were dried at room temperature followed by sputter coating with gold and evaluated in a scanning electron microscope (Feg-SEM, Philips XL30, Eindhoven, The Netherlands).

Statistical analysis
The microtensile bond strength values, in MPa, were subjected to Levene Test to evaluate Homogeneity of Variances, and then, data were analyzed through
Kruskal-Wallis Test (tested variables were: material and aging times conditions) and a post hoc Dunn’s Test. Pre-testing failures were included in the calculation of mean μTBS as 0 MPa. Differences in distribution of failure mode distribution among groups were analyzed using Chi-square test. Additionally, the relationship between μTBS values and the type of fracture mode was analyzed with one-way ANOVA. All tests were conducted using a statistical software package (SPSS, version 19, Chicago, IL, USA). The statistical significance was set at p<0.05.

RESULTS

Bond strength
The results are shown in Table 2. There was a significant difference between the adhesive systems (p=0.024) and among the different aging time according to the Kruskal-Wallis test (p=0.001). MC1 (250,000 cycles) and MC2 (500,000 cycles) showed similar results, but these were different from the control and MC3 (750,000 cycles). For both adhesive systems, the control group showed the highest μTBS values and MC3 showed lower μTBS values when compared to other aging conditions. So with increasing mechanical load cycles, bond strength values decreased. The adhesive systems showed similar results at control, 1 and 2 weeks aging, but after 3 weeks aging CSE showed significantly higher μTBS values than ASB. So, CSE was more stable through time than the ASB adhesive. Mean μTBS varied from 17.9 up to 37.4 MPa.

Mode of fracture
There was no relation between the bond strength values and the type of fracture (ANOVA, p=0.726). A higher frequency of pre-testing failures was observed for the loaded groups, statistically significantly so for ASB along the aging conditions (p=0.004, Chi Square Test) (Table 2). Regarding fracture modes, there was no difference between the materials (p=0.461); however for CSE, the control group showed more cohesive failures than the aged groups (Chi Square Test, p=0.029) (Table 3). Figure 2 shows some representative fracture

| Time | Materials | 24-h Bond Strength (MPa) | MC1 Aging Bond Strength (MPa) | MC2 Aging Bond Strength (MPa) | MC3 Aging Bond Strength (MPa) |
|------|-----------|-------------------------|-----------------------------|-------------------------------|-------------------------------|
|      |           |                         | % Reduction between 24 h and MC1 | % Reduction between 24 h and MC2 | % Reduction between 24 h and MC3 |
| Adper Scotchbond 1XT | 37.4 (14.0) Aa | 24.4 (12.6) Ab | 45.0 | 24.7 (10.5) Ab | 17.9 (9.5) Bc | 63.5 |
| | [2] *60 | [13] *58 | | [14] *60 | | [19] *53 |
| Clearfil SE Bond | 34.0 (12.2) Aa | 25.0 (14.2) Ab | 32.4 | 24.7 (10.5) Ab | 22.7 (10.7) Ac | 37.3 |
| | [3] *60 | [8] *60 | | [9] *60 | | [7] *60 |

For each line, values with different small letters indicate significant difference among the aging times, within the same adhesive system (p<0.05).

For each column, values with different capital letters indicate significant difference (p<0.05) between the adhesive systems, within the same aging time.

Table 3  Number and percentage of mode of failure using different adhesive system and aging conditions

| Aging | Adhesive | 24-h (1 week/250,000 cycles) | MC1 (2 weeks/500,000 cycles) | MC2 (3 weeks/750,000 cycles) |
|-------|----------|-----------------------------|-----------------------------|-------------------------------|
|       |          | AI  | CR  | CD  | M   | AI  | CR  | CD  | M   | AI  | CR  | CD  | M   |
|       | Adper Scotchbond 1XT | 31  | 1   | 0   | 28  | 26  | 3   | 2   | 27  | 22  | 1   | 2   | 35  |
|       | Clearfil SE Bond | 26  | 8   | 3   | 23  | 28  | 0   | 2   | 30  | 2   | 5   | 2   | 26  |
|       |          | 51.7% | 1.6% | 0% | 46.7% | 44.8% | 5.2% | 3.5% | 46.5% | 36.6% | 1.6% | 3.4% | 58.4% | 41.5% | 0% | 1.8% | 56.7% |
|       |          | 43.4% | 13.3% | 5% | 38.3% | 46.7% | 0% | 3.3% | 50% | 45.1% | 8.3% | 3.3% | 43.3% | 35% | 1.6% | 6.7% | 56.7% |

AI=apparently interfacial; CR=cohesive in resin-based composite; CD=cohesive in dentin; M=mixed
DISCUSSION

The present study investigated the influence of aging as applied by a new mechanical loading device on adhesive bond strength and the results demonstrate that the aging method results in a decreasing µTBS. Therefore, the null-hypothesis was rejected. The new device used to apply cyclic mechanical loading, the “Rub&Roll”, is a relatively simple construction compared to other devices and has as a main advantage the high number of samples that can be subject to testing at once. Moreover, the cylindrical construction enables to include liquids, such as erosive drinks and abrasive foodstuffs in the process. This opens a lot of opportunities for further research investigating relations between cyclic loading and wear aspects. Samples are loaded with compressive force from the occlusal restoration in direction to the adhesive interface. As the device operates, the force is not applied exactly perpendicularly to the adhesive interface during the whole cycle, which is probably more clinically relevant. Until now, the device is only described in a technical paper and the present study is the first to establish its functionality. Future research has to confirm the validity of the device, and it should be compared to other devices for cyclic loading on the market. However, a gold standard for mechanical aging of restorations is not available, probably because of the complicated nature of most devices.

In this study, mechanical loading statistically influenced the microtensile bond strength values for all the aged conditions tested. It was observed that with an increasing number of mechanical cycles, the µTBS decreased significantly with 32% up to 63%, depending on the number of cycles applied. Clinically, most bonded interfaces are subject to some degree of cyclic loading due to masticatory function and parafunctional habits, and this may vary with the size and position of the restoration and individual risk of the patient. It is difficult at this moment to establish which kind of cyclic loading protocol is the most clinically relevant. In the present study, 30 N of force was applied up to a frequency of 750,000 cycles. A previous study suggested that 150,000 cycles at 60 N is...
able to simulate six months of oral masticatory stresses\(^5\), which is five times less but at a higher force than applied in the present study. Future research should establish which kind of cycling protocol could be considered as clinically representative, although increased number of cycles is probably favorable for that purpose. Clinical loading force will also show large individual variation, and people with parafunctional habits will probably apply higher and more frequent forces to the tooth restoration complex. A small number of mechanical cycles may not be able to produce a significant decrease in bond strength\(^6,\,7\).

The number of pre-testing failures in the present study confirmed the effect of the mechanical aging on the adhesive interface. A higher frequency of pre-testing failures can be a predictor for diminished bond strength values, which is in accordance with other studies\(^15\). However, in the present study this effect was not statistically significant for the self-etch adhesive system that was more stable on the long term than the etch-and-rinse type.

Apart from mechanical loading, thermocycling and water storage can play a role in the aging process of the tooth restoration interface resulting in a decreased bond strength after combined thermal and mechanical loading\(^15\). This may be due to the degradation of the adhesive interface by combined hydrolytic deterioration of the resin polymer and the exposed collagen\(^16\). This degradation in the hybrid layer has also been described after 6 months water storage\(^17\), also when no cyclic loading was applied\(^16\). In the present study, samples were stored and loaded in water, but the time that the samples remained in contact with water (1 up to 3 weeks) is deemed too short to have a significant effect\(^19\).

It was observed that the number of load cycles plays a significant role in the decrease of adhesion. Both adhesive systems showed a similar performance for the control and 1 and 2 weeks aging groups, but for the 3 weeks aging groups differences between the adhesive systems were found, and the two-step self-etch adhesive-CSE showed higher \(\mu\)TBS values and a more stable behavior when compared to the etch-and-rinse adhesive-ASB, also statistically confirmed by the pre-testing failures. A similar performance for the investigated adhesives tested without aging, both in dentin and enamel, has previously been reported\(^20,\,21\). Moreover, when using different aging protocols such as water storage and thermocycling, the CSE adhesive was also observed to be more stable than ASB\(^18\). In the present study, only after 750,000 cycles this difference appeared. CSE adhesive is based on a functional phosphonate acidic monomer (10-MDP) that is able to establish chemical bonds to calcium ions of hydroxyapatite crystals. This chemical interaction may be an explanation for the differences between the adhesives as found in this and other studies\(^22\).

There was no relation found between the values of the microtensile test and the type of fracture. It is reported in the literature that very high microtensile bond strength values are related to cohesive failures, and because of that, the \(\mu\)TBS values related to cohesive fractures should be viewed cautiously or can even be discarded out of the statistical analyses as they do not represent true interfacial bond strength\(^23,\,24\). In the present study, there was no statistical difference between groups and the mode of fracture, as the most prevalent fractures were apparently interfacial and mixed. This supports a study reporting that the mode of fracture may also be associated with the kind of gripping device\(^25\). The device used in this study was a K.U.Leuven-BIOMAT jig, which is a modified Geraldeli’s microtensile testing jig\(^25\). The jig is designed to ensure that a pure tensile force is applied to the test specimen, but still bending forces may occur during load application due to non-parallel specimen alignment, the bonding surface being non-perpendicular to the specimen gripping surface, and uneven gripping forces. The mixed failures in the present study were predominantly adhesive ones, as fractures commonly started at the borders, occurring from the corners through the center of the sticks-shaped samples, which were predicted by finite element analyses in this geometry of the sample\(^27\).

CONCLUSION

In conclusion, the new Rub&Roll device was able to promote mechanical loading on samples and an increased number of load cycles resulted in decreased \(\mu\)TBS values. Moreover, differences between the materials occurred when a higher number of cycles were applied.

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