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Effect of Intraoral Humidity on Dentin Bond Strength of Two Universal Adhesives: An In Vitro Preliminary Study

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Abstract: This study investigated the effect of intraoral humidity on microtensile bond strength (μTBS) to dentin of two universal adhesives applied in self-etch (SE) mode. Forty extracted human molars were selected for this study. Dentin specimens were allocated into four groups, standardized and then bonded with two commercial multimode adhesives, according to two humidity conditions (50% relative humidity (RH) and 90% RH). Following composite resin build-up and 24 h of water storage, the μTBS of the bonded interface was analyzed. The 50% RH was higher for Scotchbond Universal (SBU) than Prime&Bond Universal (PBU), while for 90% RH, SBU had significantly lower values than PBU. With PBU, the mean bond strength was not significantly different between both humidity settings tested ($p > 0.05$), while for SBU, the mean bond strength was significantly different between both conditions tested ($p < 0.05$); μTBS was significantly higher for 50% RH than for the other group. Within the limitation of this in vitro study, it can be concluded that: 1) the bonding performance of adhesives systems depends on the humidity settings; 2) increased RH exerts a detrimental effect on the bond strength of 2-hydroxyethyl methacrylate (HEMA)-containing adhesive tested. However, this phenomenon was not observed for HEMA-free adhesive tested; 3) further research in this area is needed to investigate different adhesive systems, temperatures and humidity settings.

Keywords: adhesion; bond strength; dentin; humidity; universal adhesive

1. Introduction

Adhesive systems have largely progressed throughout the last 50 years. However, there are still some unsolved problems concerning the durability of the dentinal–resin bond interface [1]. In fact, adhesion to dentin depends on the formation of an appropriate and compact hybrid layer (HL), also called the interdiffusion zone, which forms between the demineralized dentin and the adhesive resin [2,3]. Consequently, this leads to the appearance of the micromechanical retention of restoration [4]. Thus, HL is a mixture of hydroxyapatite, collagen, remaining solvents and resin monomers; its stability relies on the resistance of each component to the degradation process [5].

Bonding to enamel structure has become a monotonous and well-established procedure [6], whereas bonding to dentin, due to its heterogenous composition and histology, was considered challenging [7,8]. During the adhesion process, the mineral constituent was partially or totally removed by acidic monomers in self-etch (SE) or etch-and-rinse (ER) adhesive systems. The ER strategy requires two steps. The first stage is a demineralization process with phosphoric acid on the dental surface, and the second stage is the use of the bonding agent and subsequent application of a light curing device to polymerize the resin in situ, directly inside the surface treated [9]. In an attempt to eliminate the etching step or to include it in another step, the SE strategy was evolved [6,7,9]. Contrary to the prospects, the first SE adhesives did not perform well in clinical situations, presenting a number of shortcomings, including lessened bond strength, enlarged nanoleakage and improved water sorption of HEMA-containing adhesive systems, the monomer from the solvent phase separation (HEMA-free adhesive systems). Further improvements in adhesive technology introduced novel molecules into the composition of adhesive systems: 10-methacryloyloxyidecyl dihydrogen phosphate (10-MDP), 4-methacryloxyethyl trimellitic acid (4-MET) and N-Phenyl-p-phenylenediamine (phenyl-P). They were called functional monomers and were considered to bond chemically to calcium in hydroxyapatite [10].

Currently, universal adhesive systems (UAs), also called multimode adhesives, have become commercially available in the market, providing dental practitioners the choice of selecting the adhesion strategy [11]. This latest generation marketed by dental manufacturers can be used in a SE, ER or selective enamel etching mode (SEE) [12]. Moisture control was considered the biggest dilemma when formulating a new multimode adhesive [13]. Almost all universal adhesives include 10-MDP as an acidic monomer in their formulation, which can be used as an etching monomer and interact with calcium ions in order to promote the chemical bond to tooth structure [13–15]. A previous study proved that this integration may upsurge the durability of the bonds produced with simplified SE adhesives [16].

The clinical achievement of adhesive restorations characterizes an important parameter to evaluate the success of resin-based dental materials [17]. This could be possible by understanding the behavior of each component inside adhesive systems, although in vitro studies provide important information regarding the physical and biomechanical properties of these materials [17,18].

It was suggested that the resin–dentin bond strength may be affected by high oral humidity (between 78% and 94%) and the presence of contaminants (for example, blood, saliva, gingival crevicular fluid or hand-piece lubricant) [19,20]. In order to solve the aforementioned problem, the use of rubber dam has been deemed mandatory in restorative dentistry [21,22]. As a result, the humidity decreases to that of the ambient air, hence promoting stable and optimal adhesion to dentin [23]. Indeed, it is important to mention that the use of rubber dam provides a dry field for enhanced visibility, and it improves visual contrast [24,25]. In addition, it is a low-cost material with a high effectiveness appliance [26].

A lot of difficulty had been noted in evaluating the effect of RH on the bond strength of dentin [27]. Interestingly, Amsler et al. found that increased RH had a detrimental effect on the immediate shear bond strength (SBS) of dentin [28]. Nevertheless, Saraiva et al. proved that intraoral environment was not able to compromise the immediate dentin bond strength [17].

Accordingly, the aim of this study was to assess the influence of oral humidity on the dentinal bond strength of universal adhesives used in an SE mode. The null hypothesis tested was that the intraoral humidity has no effect on the microtensile bond strength (μTBS) of dentin in both adhesives tested after 24 h of storage in distilled water.

2. Materials and Methods

A total of 40 human caries-free wisdom molars recently extracted (n = 40, age range of the patients: 18–30 years) were selected to be used in this study after the approval of the Ethical Committee of Saint-Joseph University (Beirut, Lebanon; ref.USJ-2021-118). Immediately after extraction, the teeth were pumiced, then stored in 0.2% sodium azide solution at 4 ◦C for one month to hinder bacterial growth. The sample size (n = 7) was estimated based on a previous study that evaluated the bond strength of resin composites to dentin under different environment conditions in a comparative study design with 4 independent groups [29], a 6.2 minimum evident difference in means, a 3.2 standard deviation (SD), power of 0.8 and an a = 0.05. The sample size was calculated by means of a graphing software program (SigmaPlot 14.0; Systat Software, Inc., Chicago, IL, USA).

2.1. Development of Environmental Chamber

A plexiglass chamber was constructed to simulate different humidities. The chamber was enclosed with acrylic transparent panel to allow a good vision and control of environmental conditions. A LED light was fixed in the chamber as a source of heat, and a fogger device (277 Stage LED Fogger with LED, Guangzhou, China) filled with water was used to produce humidity. In addition, a sensor element and digital control (Thermo control and humidity incubator 220, Taizhou, China) was implanted in the chamber to control the temperature and humidity to keep it within a set range, which automatically activates the fogger and LED to maintain the preset humidity.

2.2. Bonding Procedures

Two universal adhesives were tested in this study in SE mode: Prime&Bond Universal (Dentsply DeTrey GmbH, Konstanz, Germany) (PBU) and Scotchbond Universal (3M/ESPE, St. Paul, MN, USA) (SBU) (Table 1).

Table 1. Manufacturer and composition of the universal adhesives used.

Adhesive Used	Classification	Composition	Manufacturer
Prime&Bond Universal (PBU)	Mild pH = 2.5	10-MDP, PENTA, isopropanol, water, photoinitiator, Bi-and multifunctional acrylate	Dentsply DeTrey GmbH, Konstanz, Germany
Scotchbond Universal (SBU)	Mild pH = 2.7	10-MDP, 2-HEMA, Bis-GMA, DCDMA, MPTMS, VP-copolymer, fumed silica, ethanol, water, photoinitiators	3M ESPE, St. Paul, MN, USA

The occlusal enamel of each tooth was removed using a slow-speed diamond saw (Isomet 1000; Buehler, Lake Bluff, IL, USA). To standardize the smear layer, the exposed dentin surfaces were grinded for 1 min with 320 grit Silicon Carbide (SiC) polishing papers (Carbimet, Buehler, Lake Bluff, IL, USA) at a motor speed of 70 rpm, under water irrigation. Dentin specimens were randomly allocated into 4 groups (n = 10 per each group) (Table 2).

Table 2. Groups used in this study.

Humidity Used	PBU	SBU
50% RH	PBU RH:50%	SBU RH:50%
90% RH	PBU RH:90%	SBU RH:90%

The adhesive procedure was performed at 37 ± 2 °C and 50 ± 3% RH. Next, the bonding system was applied using a microbrush in two successive layers for 20 s, followed by 5 s air spray to evaporate the solvent, then cured for 20 s using a Curing Pen (Eighteeth, Changzhou, China) at 1000 mW/cm^2. A resin composite Filtek Z250 (3M ESPE, St. Paul, MN, USA) was placed in incremental way with 2 mm thick increments on the dentin surface and cured for 20 s for each layer. The total height of the composite was measured by means of a periodontal probe, obtaining a height of 4 mm. After bonding, the specimens were stored in distilled water for 24 h at 37 °C.

2.3. Specimen Preparation for μTBS Test

The samples were segmented in perpendicular direction to the adhesive surface using a water-cooled cutting machine with high-speed diamond saw (Exakt Technologies Inc., Norderstedt, Germany). Around ten composite dentin beams were acquired, and among them, six central beams were carefully chosen from the mid-coronal dentin formed by 4 mm of resin composite and 4 mm of dentin structure [30]. Next, these beams were kept moist until testing. Extra thin (0.8 mm) and extra thick (1.2 mm) beams were excluded, and each one was examined and measured using a digital caliper (Mitutoyo, Tokyo, Japan) to calculate the bonded area.

2.4. Microtensile Bond Strength Testing

The beams to be tested were attached to a Geraldeli's Jig device by means of a cyanoacrylate glue (Zapit; Dental Ventures of North America, Corona, CA, USA), then stressed in tension by using a universal testing machine (Intron 1165, Instron, Norwood, MA, USA) at a crosshead speed of 1.0 mm/min and a 50 N load cell. Afterward, the cross-sectional area of the fractured specimens was measured by means of a Vernier caliper (CD-6BS; Mitutoyo, Tokyo, Japan). The bond strength (MPa) value was calculated by dividing the maximal load [N] to the bonded surface area [mm^2].

2.5. Statistical Analysis

The statistical analyses were performed using the IBM SPSS Statistics (version 26.0, Chicago, IL, USA). The level of significance was set at $p \leq 0.05$. The primary outcome of the study was the μTBS calculated in MPa. The normality distribution of the continuous variable was assessed using Kolmogorov–Smirnov tests. Two-way ANOVA with two between-subject factors (the first factor—bonding materials: PBU/SBU adhesives; the second factor—the condition of humidity: RH:50%/RH:90%) were used to compare the μTBS among groups. This was followed by univariate analyses due to statistical interaction.

3. Results

Table 3 shows the results of the μTBS of the PBU and SBU adhesives under different humidities.

Table 3. Mean ± SD of μTBS (MPa) for study groups. Groups recognized by dissimilar symbols are statistically different ($p < 0.05$).

Humidity Used	PBU	SBU
50% RH	22.146 ± 4.170^{a}	28.414 ± 12.888^{b}
90% RH	19.613 ± 5.586^{a}	18.800 ± 5.552^{c}

The 50% RH was higher for SBU than PBU, while for 90% RH, SBU had significantly lower values than PBU. With PBU adhesive, the mean bond strength was not significantly different between 50% RH and 90% RH (-p-value = 0.474), while for SBU adhesive, the mean bond strength was significantly different between 50% RH and 90% RH (-p-value = 0.009); it was significantly elevated for 50% RH.

4. Discussion

The bonding of universal adhesive to dentin has been previously revealed to be more durable in the SE strategy [31]. Moisture control during adhesive procedures was imperative to reach a suitable bond to dental tissues [32]. Hence, adequate relative isolation must be reflected in these clinical circumstances [33,34]. Despite the simplified application protocol of universal adhesives, the influence of RH has been a subject of interest for clinicians aiming to increase the longevity of dental restoration [35–37]. Accordingly, in the present study, an analysis was intended to evaluate the effect of RH on the bond strength of two universal adhesives to dentin. There are several important factors that influence adhesion in dentistry. The adhesive interface can be influenced by the properties of the substrate, the components and chemistry of the adhesive, the humidity/oral humidity with other contaminants, such as blood and saliva, and the operator's skill. Dental adhesive systems are commonly characterized by three stages and steps of application of three different substances, known as etching, priming and bonding. One of the most recent inventions in adhesive dentistry is the introduction of "universal" or "multimode" adhesives [1,7,9,11,13,14].

The influence of increased RH on the dentinal bond strength to dentin is controversially deliberated in the literature. One previous report examined the influence of RH of ambient air on the bond strength of six adhesive systems and demonstrated that for four of them, the rises in RH meaningfully lessened the bond strength [35]. This could thus be explained by the fact that insufficient water evaporation from the dentinal substrate and a further diminished concentration of acidic monomers inside the self-etching primer and/or adhesive could be observed, leading to a poor demineralization and thus hindering the process of polymerization [28].

The discovery of this study revealed that the dentin bond strength was not significantly affected by the humidity condition for PBU; however, with SBU, the difference was significant. Thus, the null hypothesis stating that the intraoral humidity does not affect the μTBS after 24 h can be partially rejected. One notable discovery presumed from this analysis was that the bond strength decreased due to an increased RH from 50% to 90%. This could be clarified by the fact that the type of adhesives used and the addition of specific components to their composition may be responsible for controlling whether or not the increased RH affects the dentin bond strength [28].

Considering universal adhesives, the information about their properties and behavior regarding oral humidity was limited in the literature. In the present study, the immediate bond strength was shown to be important with RH 50%. This could be explained by the fact that SBU adhesive was based on the functional monomer 10-MDP responsible for the formation of 10-MDP-calcium salts. This interaction can significantly improve the bond strength to dentin immediately and over time [38]. Furthermore, this adhesive contains Vitrebond™ Copolymer as a component, providing more consistent bond performance to dentin under varying moisture levels [39]. A possible explanation could be the formation of extra ionic bond to hydroxyapatite produced by Vitrebond™ Copolymer, which leads to a reduction in microleakage and enhancement of the sealing capability of dentin [40].

Knowing that approximately all adhesive systems, including universal systems, use HEMA in their formulations [41], it is important to understand the pros and cons of HEMA. The latter is a multipurpose low molecular hydrophilic monomer that is specifically adept at penetrating and "wetting" dentinal substrate. It is tremendously soluble in water, ethanol and acetone, and therefore easy to include into adhesive formulations [42]. The hydrophilicity of this monomer makes it an exceptional adhesion-promoting monomer that has been disclosed to increase immediate bond strengths of adhesive systems by enriching monomer diffusion into dentin and easing the formation of HL [43]. This can support the finding obtained in this study, as SBU adhesive contains HEMA. Presumably, the use of 50% RH with SBU adhesive may increase the bond strength to dentin, hence facilitating better adhesive distribution. In summary, the use of lower humidity was thus essential and mandatory with this type and condition of HEMA-containing adhesive.

In addition, in the opposite direction, SBU under 90% RH showed the lowest bond strength results. Although HEMA has many positive features, it also has drawbacks. HEMA, both in the unpolymerized and polymerized state, certainly absorbs water, jeopardizing the integrity of HL [44]. Additionally, Vitrebond™ Copolymer has a high attraction to water and may lead to phase separation within the adhesive layer and HL [45]. This could be a potential cause of the reduction in bond strength in such high RH condition. The co-solvents might also impact this procedure, as all HEMA-containing adhesives were solvated with ethanol, which develops a higher hydrogen bonding capacity (19.4 $(J/cm^3)1/2$) compared to isopropanol (16.4 $(J/cm^3)1/2$) inside PBU. So, certainly, ethanol hydrogen bonded to water may have persisted in the adhesive layer of HEMA-containing adhesives, similarly to SBU used in this study, and decreased their degree of polymerization to the dentinal structure [46]. Seemingly, the use of 90% RH with SBU adhesive decreases the bond strength to dentin, thus impairing the penetration and complete polymerization of the adhesive. This could be possible with insufficient evaporation of water occurring at higher RH during the evaporation process, leading to poor demineralization and hampering the dentinal polymerization.

The present study noted that with PBU adhesive, the mean bond strength was not significantly different between both humidities tested. Since PBU is a HEMA-free adhesive, this specific composition could be helpful to effectively remove water during air-drying procedure [47]. In addition, methacrylamide monomers were formulated to replace HEMA in PBU, thus preventing phase separation, while reducing water sorption within the adhesive [48]. Methacrylamide monomers were also described to be further hydrolytically stable, permitting an extension of the shelf-life [46]. This could explain the stability of this adhesive under different humidity conditions. Furthermore, it is possible that the solvent (isopropanol) present in this adhesive may facilitate adhesive distribution in between collagen fibers, even in a higher RH condition [49]. A previous study denoted that the isopropanol solvent re-expands the collapsed collagen network, and the low-viscosity resin mixture may sufficiently infiltrate the inter-fibrillar spaces, hence making a suitable HL when affected on both over-dried and moist demineralized dentinal surface [50]. Another potential explanation of the findings obtained was that other variances in the composition, such as the initiators, percentage of water content, presence/absence of different functional monomers, for example, 10-MDP, among others, might be involved in the bonding performance to dentin [50,51]. Although the manufacturers do not state the detailed percentage of each constituent inside adhesive systems, it is probable that the presence of diverse percentages of the 10-MDP monomer can influence the vulnerability of the adhesive to the degradation process [52]. This could maybe describe the difference in the results obtained between both universal adhesives tested. In addition to the aforementioned components presented in the formulation of PBU, this adhesive contains a monomer called dipentaerythritol penta-acrylate phosphate monomer (PENTA), which contains five vinyl groups compared to one in MDP monomer. These four additional vinyl groups make PENTA more resistant to hydrolytic degradation [53,54]. Thus, when hydrolysis appears and breaks one vinyl group off the main structure of the monomer, there are four vinyl groups that are still available for the maintenance of the phosphate group [53]. Consequently, copolymerization to other monomers and adhesion to tooth structure occurs at the same time [53,55,56]. This finding could be explained by Cuevas-Suárez et al. in 2019, who suggested that PENTA included in the formulation of Prime&Bond Elect® (Dentsply Caulk, Milford, DE, USA) was more stable than MDP because the bond strength is preserved even at the end of their shelf life. This phenomenon occurs due to the maintained connection between these four vinyl groups and the phosphate group [53]. In this respect, PBU maintained its stability under 50% and 90% RH. Considering this, HEMA-free tested adhesive seems to not have any negative influence under higher RH condition. More research is mandatory to assess their bonding performance.

The materials intricated in the field of adhesive dentistry are somewhat hydrophobic; their performance and properties could be impeded not only by contamination with proteins, water or blood but also by the intraoral humidity [28]. Based on a previous laboratory study testing numerous classes of adhesive systems, a strong negative influence of RH on the bond strength to enamel and dentin was concluded [57]. In the present analysis, only the humidity variations (50% and 90%) were examined, ignoring the potential effects of temperature. According to Plasmans et al. [58], different environmental temperatures (25 or 37 °C) had no influence on the bonding strength of adhesive systems. There are several restrictions of the present study that should be acknowledged. From this in vitro evidence, two universal adhesives were bonded to dentinal structure under different humidities, 50% RH and 90% RH. The results should be considered with caution, since the presence of an odontoblastic processes through the length of dentinal tubules in clinical reports could not be found in vitro. Therefore, this finding cannot be extrapolated to other adhesive systems, and further studies should be directed, as only two adhesives were tested in the present research. Above all, when an adhesive is examined in vitro, it must be accompanied by clinical examination to evaluate the clinical effectiveness of this adhesive. Furthermore, storage was carried out in distilled water for 24 h; accordingly, a long-term evaluation would be needed in future studies. In addition, research might be directed to test more dental adhesives under the same conditions tested in this study. Moreover, more research is required to evaluate the bond strength to dentin in another adhesion strategy, such as ER, as the adhesive was only tested in SE mode in this research. Next, adhesion to enamel under the same conditions could be of great interest in future examination. Further investigations, including testing the failure mode of these adhesives, the influence of humidity on the microleakage, on the adhesive wetting process and on the flexural strength, are imperative for expanding the knowledge in this area. Additionally, it would be warranted to examine a broader humidity range and, in addition, consider temperature ranges to provide more precise information about the impact of humidity and temperature on bond strength to dentin and/or enamel. Particularly, the temperature used in this study was too high and cannot be considered as a factor for simulating the use of rubber dam. Future work should be carried out to define a golden standard for the best bonding to dentin when different humidity settings are used. Determining a threshold level could be required in the future.

5. Conclusions

Within the limitation of this in vitro study, it can be concluded that:

1. The bonding performance of adhesive systems depends on the humidity settings;
2. Increased RH exerts a detrimental effect on the bond strength of the HEMA-containing adhesive tested. However, this phenomenon was not observed for the HEMA-free adhesive tested;
3. Further research in this area is needed to investigate different adhesive systems, temperatures and humidity settings.

Clinical implications: HEMA-containing adhesives might provide poorer clinical performance when applied in conditions of increased humidity, i.e., without rubber dam protection.

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