Mestrado Integrado em Medicina Dentária

Faculdade de Medicina da Universidade de Coimbra

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EFFECT OF RELATIVE HUMIDITY ON BOND STRENGTH TO ENAMEL

Eliana Elisabete Azevedo Melo

Orientador: Professor Doutor Fernando Guerra

Co- Orientador: Doutor Rui Falacho

Coimbra Julho, 2021

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AZEVEDO MELO E.[1], FALACHO R.[2], GUERRA F[2]

[1] Aluna de Mestrado Integrado, Área de Medicina Dentária, Faculdade de Medicina, Universidade de Coimbra

[2] Instituto de Implantologia e Prostodontia, Faculdade de Medicina, Universidade de Coimbra

Área de Medicina Dentária

Faculdade de Medicina da Universidade de Coimbra

Av. Bissaya Barreto, Bloco de Celas

3000-075 Coimbra, Portugal

Tel + 351 239 249 151/2

Fax +351 239 402 910

E-mail: eliana.melo@live.com

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Resumo

Introdução: Atualmente a grande maioria dos procedimentos restauradores requerem técnicas adesivas. O sucesso clínico destas restaurações está fortemente relacionado com a durabilidade da força de adesão. No entanto, o efeito das condições ambientais intraorais, como a humidade, nos procedimentos adesivos, continua a ser uma preocupação, especialmente se estes procedimentos forem realizados na ausência de isolamento absoluto. Este estudo tem como objetivo avaliar os efeitos da humidade relativa nas forças de adesão ao esmalte.

Materiais e Métodos: Trinta terceiros molares humanos íntegros foram preparados em 4 superfícies (mesial, distal, palatino e vestibular), totalizando 120 superfícies de esmalte para adesão. Os 4 grupos experimentais (Optibond FL ou Prime&Bond Active, combinados com o uso ou a ausência de dique de borracha) foram testados em cada dente. Um dispositivo intraoral adaptado ao maxilar do paciente voluntário foi fabricado. O dispositivo foi concebido contendo uma ranhura cilíndrica no palato, onde a base acrílica previamente produzida para cada dente encaixa. Todos os procedimentos anteriores à aplicação do adesivo foram realizados fora da boca do paciente. Os procedimentos adesivos incluíram a aplicação de um sistema adesivo e subsequente colocação do material restaurador, sob as condições descritas. As amostras foram submetidas a teste de adesão por cisalhamento. As interfaces adesivas foram analisadas para determinação do padrão de fratura. A análise estatística das forças de adesão foi feita com recurso a ANOVA de medidas repetidas e teste post-hoc Dunn-Sidak ($p < 0,05$). A análise das diferenças entre as proporções de cada tipo de fratura foi realizada utilizando o teste de McNemar e correção dos valores de p pelo método Benjamini-Hochberg.

Resultados: Observaram-se diferenças estatisticamente significativas ($p < 0.001$) entre os grupos experimentais relativamente às forças de adesão. Todos os pares de grupos apresentaram diferenças estatisticamente significativas ($p < 0.05$). O grupo OptiBond FL com dique de borracha teve o valor médio de forças de adesão mais elevado. Apenas fraturas adesivas e coesivas no esmalte foram registadas, tendo as últimas sido verificadas exclusivamente nos grupos experimentais com dique de borracha.

Conclusão: Os níveis de humidade relativa intraoral afetam adversamente a resistência ao cisalhamento do esmalte. O isolamento absoluto com dique de borracha é uma técnica indispensável sempre que procedimentos adesivos são necessários, uma vez que se mostra eficaz no aumento da resistência de união ao esmalte, independente do sistema adesivo utilizado. O sistema adesivo total-etch de 3 passos, Optibond FL, obteve melhor desempenho que o outro sistema testado, em ambas as condições experimentais.

Palavras-chave: Adesão, Humidade, Força de adesão por cisalhamento, Dique de borracha

Abstract

Introduction: Nowadays, the great majority of restorative procedures require adhesive techniques. The clinical success of these restorations is strongly related to the durability of the bond strength. However, the effect of the intraoral environmental conditions, such as humidity, on bonding procedures remains a concern, mainly if performed in the absence of absolute isolation. This study aims to evaluate the effect of relative humidity on shear bond strength to enamel.

Materials and Methods: Thirty sound human third molars had the mesial, distal, lingual, and vestibular enamel surfaces prepared, providing a total of 120 surfaces for bonding. All four experimental groups were tested within each tooth (Optibond FL or Prime & Bond Active, combined with the use or absence of rubber dam). A custom upper arch splint was made to fit our volunteer's mouth. The oral device was designed holding a palatal cylindrical slot where the acrylic base previously produced for each tooth tightly fits. All procedures prior to the adhesive application were performed outside of the patient's mouth. Bonding procedures included the application of an adhesive system, followed by restorative material placement under both aforementioned conditions. Specimens were then submitted to shear bond strength testing. In addition, the adhesive interfaces were inspected for fracture pattern assessment. Statistical analysis of bond strength results was carried out using repeated measures ANOVA and Dunn-Sidak post hoc test ($p < 0.05$). The differences between fracture type proportions were analyzed using McNemar testing and p-values corrected by the Benjamini-Hochberg method.

Results: Statistically significant differences were observed ($p < 0,001$) between the experimental groups regarding bond strength. Pairwise comparisons of all study groups revealed statistical differences ($p < 0.05$). The group OptiBond FL with rubber dam presented the highest mean bond strength value. Only adhesive and cohesive within enamel fractures were recorded, with the latter being exclusively verified in rubber dam experimental groups

Conclusion: Intraoral relative humidity levels adversely affect shear bond strength to enamel. Absolute isolation with rubber dam is an indispensable technique whenever adhesive procedures are required, as it proves effective in potentiating bond strength to enamel independently of the adhesive system used. The three-step total-etch system OptiBond FL performed better than the other tested system in both experimental conditions.

Keywords: Adhesion, Humidity, Shear bond strength, Rubber dam

Introduction

Nowadays, the great majority of restorative procedures require adhesive techniques.[1] The clinical success of these restorations is strongly related to the durability of the bond strength as it will prevent marginal gap formation, bacterial microleakage, postoperative sensitivity, recurrent caries, and overall loss of the rehabilitation, therefore having consequences in terms of longevity. [1,2]

To ensure the best clinical outcomes and restorative results, in addition to selecting the best adhesive, it must be applied with the best technique possible. Bonding quality of the resin-adhesive-tooth interface can be influenced not only by the chemical composition of the adhesive resins but also by the environment to which they are exposed, such as temperature and humidity, as well as the polymerization protocol. [3–5]

Proper moisture control is one of the most challenging situations in adhesive dentistry. Previous studies have shown that keeping a dry enamel surface by eliminating remnant moisture prior to adhesive application is crucial for long-term bond durability.[6,7] Bonding surfaces are exposed not only to saliva, blood and crevicular fluid but also to water molecules present in exhaled air. [7,8]

The amount of water saturated in the exhaled air is often ignored. However, it is reported to be about 27 mg/dm3, thus its possible detrimental effects on the bonding interface require careful evaluation. [9] Yoshida et al. reported that the average oral temperature and relative humidity are around 30ºC and 80% respectively while Plasmans et al. stated that without proper rubber dam isolation, humidity can range from 74% up to 94% [1,10]. Factors that may influence relative humidity are the location of the tooth within the dental arches, the patient's nose or mouth breathing and operative procedures such as the application of a rubber dam. [10]

The rubber dam isolation technique was first proposed in 1864 and it offers many advantages such as reduction of humidity, prevention of contamination of the operative field by saliva, blood or crevicular fluid, as well as enhancing safety by preventing injuries to the surrounding soft tissues, leakage, or aspiration of dental materials and cross-infection. [5,8,11] However, the majority of dentists never or only sometimes use rubber dam isolation during operative dentistry procedures. Whether absolute or relative isolation should be used is a commonly asked question among clinicians. [12–14]

When it comes to adhesive systems, these can be divided according to their approach into etch-and-rinse adhesives and self-etching adhesives. The effectiveness of etch-and-rinse adhesive systems on enamel surfaces is well supported by the literature. [7] However, in order to simplify the clinical procedures, universal adhesives have been introduced. The all-in-one adhesives combine etching, priming, and bonding into a single application step, giving the operator the freedom to select the adhesive strategy; etch-and-rinse, self-etch or selective enamel etching. These universal systems are widely accepted by dentists everywhere, although they might not offer the same bond strength and durability as a total-etch. [15,16]

There appears to be a lack of scientific studies that consider the possible effects of oral humidity on bond strength and most fail to provide an accurate experimental model. Although in vitro studies provide great information regarding the physical and biomechanical properties of materials, they do not take into account interoperative variations, patient behaviour and the intraoral environment contributing to dissimilarities in bond strength results between in vivo and in vitro studies. [17,18]

Most often, temperature and relative humidity are simulated in experimental chambers. However, these devices are not capable of replicating the exact oral environment conditions. Testing adhesive restorations performed in the patient's oral cavity may provide additional and more accurate information on the actual effects of relative humidity. [17]

This study aims to evaluate the effect of relative humidity on shear bond strength (SBS) of two different adhesive systems to enamel, with and without rubber dam. The alternative hypothesis tested is that the relative humidity present in the oral environment has a significant effect on bond strength to enamel and that this effect can be minimized by using a rubber dam.

Materials and Methods

Specimen Preparation:

This study was approved by the Ethics Committee of the Faculty of Medicine - University of Coimbra (notification CE001/2013).

Thirty sound human third molars clinically and radiographically free of caries, cracks, restorations or other abnormal features, as well as no previous root-canal treatment, were collected and immediately stored in distilled water for a maximum period of 3 months. After the ideal number of teeth was reached, each tooth was cleaned using periodontal scalers and polished with pumice and water to remove adherent organic material or calculus. The cleaned teeth were transferred and stored in chloramine for a period of 5 weeks, after which they were placed in a cylindrical mould and embedded by auto-polymerized acrylic resin (SCHMIDT laboratory, Madrid, Spain Lot: 47975, Expiration date: 2024/11) up to the cementoenamel junction. Each tooth had the mesial, distal, lingual, and vestibular enamel surfaces carefully flattened using a high-speed conical diamond burr, under water cooling, attached to a parallelometer. All preparations were kept in sound enamel tissue. The surfaces were finished with coarse contouring and polishing discs (Sof-Lex TM, 3M, CA, USA). Bonding procedures were performed immediately after the preparation of the enamel surfaces. Specimen preparation as well as experimental groups are illustrated in Figure 1.

Oral device design:

A custom upper arch splint was made to fit our volunteer’s mouth. Dental impressions were taken by an intraoral scanner (iTero Element 5D, Amsterdam, The Netherlands). The custom splint was designed in Zirkonzahn Software Modellier - Byte Splints Module (version 9071, Zirkonzahn, Gais, Austria) and printed in NexDent Model 2.0 White resin (Vertex-Dental B.V., Soesterberg, The Netherlands), using a NextDent 5100 printer (3D SYSTEMS, Vertex-Dental B.V., Soesterberg, The Netherlands).

The oral device was designed holding a palatal cylindrical slot where the acrylic base previously produced for each tooth tightly fits. Samples could then be supported on the referred slot, exposing only the tooth’s crown and enabling full access to any of the four surfaces (mesial, distal, vestibular, lingual).

Experimental Groups:

Each one of the four prepared enamel surfaces was randomly divided into one of the four experimental groups using a research randomizer, adding up to a total of 120 specimens. All four experimental groups were present in the same tooth to allow direct comparison in identical enamel conditions. Materials used, their chemical compositions, manufacturers, and application procedures are listed in table 1.

The experimental groups were the following:

RD-OFL – Optibond FL adhesive with rubber dam (absolute isolation) nRD-OFL – Optibond FL adhesive without rubber dam

RD-PB – Prime&Bond Active adhesive with rubber dam (absolute isolation)

RD-PB – Prime&Bond Active adhesive without rubber dam

Bonding Procedure:

The teeth were attached one by one to the slot present in the oral device, with the area of interest facing the anterior region, thus simulating the normal positioning and angle of a central incisor being restored on the vestibular side. A thermo-hygroscope was used to record the dental office's environmental conditions. The room's relative humidity was kept at 46% and the temperature was kept at 22 degrees Celsius. An experienced operator performed all experimental procedures under operative microscope (Leica M320, Leica Microsystems, Heerbrugg, Switzerland).

All procedures prior to the adhesive application were performed outside of the patient's mouth to avoid any cross-contamination between fluids that contacted the tooth samples and the patient oral cavity. In all experimental groups, each prepared enamel surface was etched for 30 seconds with phosphoric acid at 37,5% (Gel etchant, Kerr Corporation, CA, USA – Table 1) followed by a thorough rinse with an air-water stream for 30 additional seconds and air dried with a strong air flow until completely dry. Each sample was then placed inside the patient's mouth according to the specificities of the tested groups.

During the bonding procedure of experimental groups performed under relative isolation (nRB-OFL, nRB-PB), the patient was instructed to breathe through the nose and a suction cannula was placed to remove excess moisture. In both groups, the etched enamel surfaces were air-dried one more time to ensure total absence of water and left to rest for 30 seconds without the presence of any oral fluids in order to mimic clinical conditions better. One of the two bonding systems, total-etch (nRB-OFL) or universal (nRB-PB), was actively applied using a microbrush for 20 seconds, after which the surfaces were airdried with a mild air flow free of any oil or water residues and light-cured for 20 seconds with a LED light-curing unit (Bluephase® Style 20i, Ivoclar Vivadent, Schaan, Liechtenstein, 1200 mW/cm^2).

For the bonding procedure of the experimental groups performed under absolute isolation (RB-OFL, RB-PB), a rubber dam sheet (nic tone, Manufacturera Dental Comercial, Jalisco, Mexico) was punctured with a single hole, placed around the tooth's crown and held in place by a clamp and a frame holder. Once the enamel surfaces were completely dried, one of the two adhesive systems, total-etch (RB-OFL) or universal (RB-PB), was actively applied using a microbrush for 20 seconds, after which the surfaces were airdried with a mild air flow free of any oil or water residues and light-cured for 20 seconds.

The primer bottle of the OptiBondFL system was not applied to any surface as all tested samples consisted exclusively of enamel.

Once the application of the bonding systems was concluded, a composite resin (Ceram.X.Soectra™ST Low viscosity, Dentsply Sirona, Konstaz, Germany) was condensed into a soluble translucent cylindrical capsule with 4.39 mm height and 2.54 mm of internal diameter and positioned onto the prepared enamel surface to be polymerized in all sides by the previously used LED light-curing unit for a total of 60 seconds.

After bonding, each specimen was stored in distilled water at 37 degrees Celsius for seven days.

Shear Bond Strength:

The testing sequence was randomly defined for teeth and for groups within each tooth. A calibrated and experienced operator, blind to the groups, performed the shear bond tests.

All 120 specimens were set up in a universal testing machine (model AG-I, Shimadzu Corporation, Kyoto, Japan) and tested to failure using the previously defined shear mode. The compression load resulting in shear bond strength was performed as parallel and as close as possible to the adhesive interface. The shear force was applied at a crosshead speed of 0.5 mm per minute. The values for maximum stress in Newton(N) were obtained and converted into megapascal (MPa) by dividing them by the bonded area in squared millimeters (1MPa= 1N/mm^2).

Fracture pattern analysis:

The fracture surfaces were evaluated by two independent operators, blind to the groups, using a dental operative microscope (Leica M320, Leica Microsystems, Heerbrugg, Switzerland), under x40 magnification. The fracture patterns were classified as follows:

Type 0 - Adhesive fracture

Type 1 - Cohesive fracture within enamel

Type 2 - Cohesive fracture within the composite resin

Type 3 - Mixed fracture within enamel

Type 4 - Mixed fracture within the resin

Statistical Analysis:

Statistical analysis was performed using IBM® SPSS® for Windows version 26.0 (SPSS Inc., Chicago, IL, USA) and MS® Excel® (Microsoft Corporation., Redmond, Washington, USA). The significance level was set at 5%. The shear bond strength results were described using mean, standard deviation, minimum and maximum values. After verifying the normality of data distribution using the Shapiro-Wilk test, repeated measures ANOVA testing was carried out to detect statistically significant differences between the means across the groups. Post-hoc multiple pairwise comparisons were performed using Dunn–Šidák test.

In order to evaluate the differences between the proportions of fracture types, McNemar testing was used between all pairs of groups. The p-value was corrected by the Benjamini-Hochberg method (false discovery rate controlling procedure) for multiple comparisons false positive rate of 0.05.

TABLE 1. The type, brand, chemical composition, batch numbers and application protocols of materials used in this experiment

Type	Brand, Abbreviation, Chemical Composition	Manufacturer and Batch Numbers	Application Protocol
Three-step Total-etch Adhesive	OptiBond™ FL (OFL), Primer: 2-hydroxyethyl methacrylate, Ethanol, 2- [2 (methacryloyloxy) ethoxycarbonyl] benzoic acid, Glycerol phosphate dimethacrylate Bond: Glass, oxide, chemicals, 2-hydroxyethyl methacrylate, Ytterbium trifluoride, 3-trimethoxysilylpropyl methacrylate, 2-hydroxy-1,3-propanediyl bismethacrylate, Alkali fluorosilicates (Na)	Kerr Corporation, California, USA Batch nr: 7831887	1-Dispense and apply OptiBond FL Adhesive (Bottle #2) actively for 20s, over enamel, creating a thin coating. 2-Dry with mild air flow. 3- Light cure
Universal Adhesive	Prime&Bond active™ (PB), Bi- and multifunctional acrylate, Phosphoric acid-modified acrylate resin, Initiator, Stabilizer, Isopropanol, water	Dentsply Sirona, Konstaz, Germany Batch nr: 2011000070	1-Dispense and apply Prime&Bond Active, actively for 20s over enamel, creating a thin coating. 2-Dry with mild air flow. 3- Light cure
Composite Resin	Ceram.X.Soectra™ST Low viscosity, Ethoxylated Bisphenol A Dimethacrylate, Urethane modified Bis-GMA dimethacrylate resin, 2,2´-ethylenedioxydiethyl dimetharcylate, ytterbium trifluoride, 2,6-di-tert-butyl-p-cresol	Dentsply Sirona, Konstaz, Germany Batch nr: 2008000516	Light curing composite
Etching Gel	Gel etchant, 37,5% Phosphoric acid 35-40%, Cobalt alumina blue spinel	Kerr Corporation, California, USA Batch nr: 7831887	1-Apply Kerr Gel Etchant to enamel surfaces for 30s. 2-Rinse thoroughly for 30s with water. 3-Dry with clean, oil-free, and water-free air. 4-Avoid contamination of etched surface during the bonding procedure. Proceed with placement of the bonding agent.
Rubber Dam	Nic tone Dental Dam, latex	Manufacturera Dental Comercial, Jalisco, Mexico Batch nr:11068038	isolate operatory area

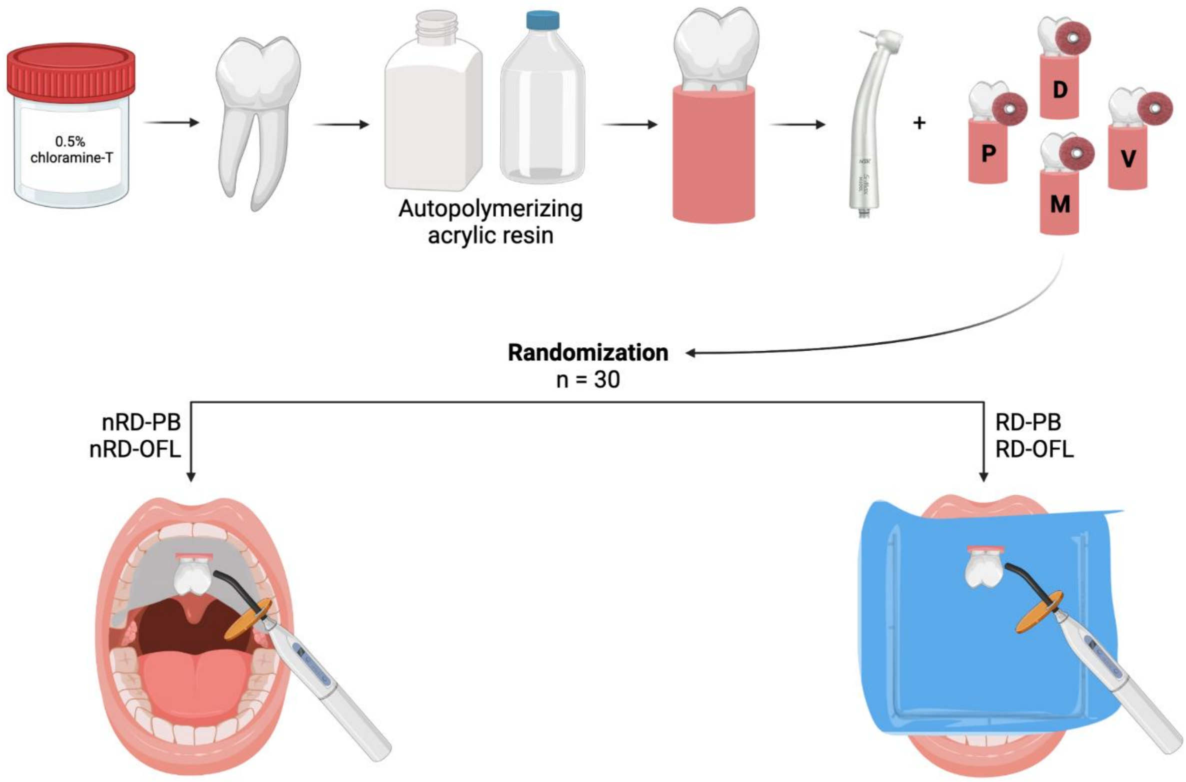

FIGURE 1. Schematic representation of the experimental protocol.

Results

Shear Bond Strength

Statistically significant differences were observed ($F(3.87) = 98.27$; $p < 0{,}001$) between the experimental groups regarding shear bond strength. Multiple pairwise comparisons revealed statistical differences between all study groups (with $p < 0.001$, except for the comparison between nRD-PB and nRD-OFL in which $p = 0.008$).

The highest mean shear bond strength values were obtained in rubber dam experimental groups, regardless of the adhesive system, as shown in Table 2. Optibond FL resulted in greater mean shear bond strength in both conditions (with and without rubber dam).

Fracture Mode/Failure Analysis:

Cohesive fractures within enamel were exclusively verified in rubber dam experimental groups (Figure 3). Fractures were mostly adhesive in RD-PB and RD-OFL groups (56.7% and 66.7%, respectively). All specimens from both no-rubber dam groups exhibited adhesive fracture pattern only. No mixed or cohesive fractures within the composite resin were verified (Table 2).

Prime&bond Active and OptiBond FL show no statistically significant differences in fracture types when tested in the same experimental condition (with or without rubber dam isolation) as shown in Table 3 ($p > 0.05$). However, when comparing the rubber dam groups with groups in which rubber dam was not used, a statistically significant predominance of cohesive fractures is observed in rubber dam groups ($p < 0.05$).

TABLE 2. Mean bond strength (MPa) values and maximum and minimum standard deviation for the four groups studied and the percentage of each fracture type for the different groups.

	RD-PB	RD-OFL	nRD-PB	nRD-OFL
Shear Bond Strenght[1]	23.16 (4.26) 14.31/ 31.82	30.84 (6.31) 19.37/ 49.42	12.57 (4.12) 4.80/ 19.32	16.33 (6.08) 3.00/ 25.94
Fracture[2]	17/13 (56.7%/43.3%)	20/10 (66.7%/33.3%)	30/0 (100%/0%)	30/0 (100%/0%)

[1] mean (standard deviation) minimum/maximum (MPa)

[2] Adhesive fracture / Cohesive fracture in enamel (%/%)

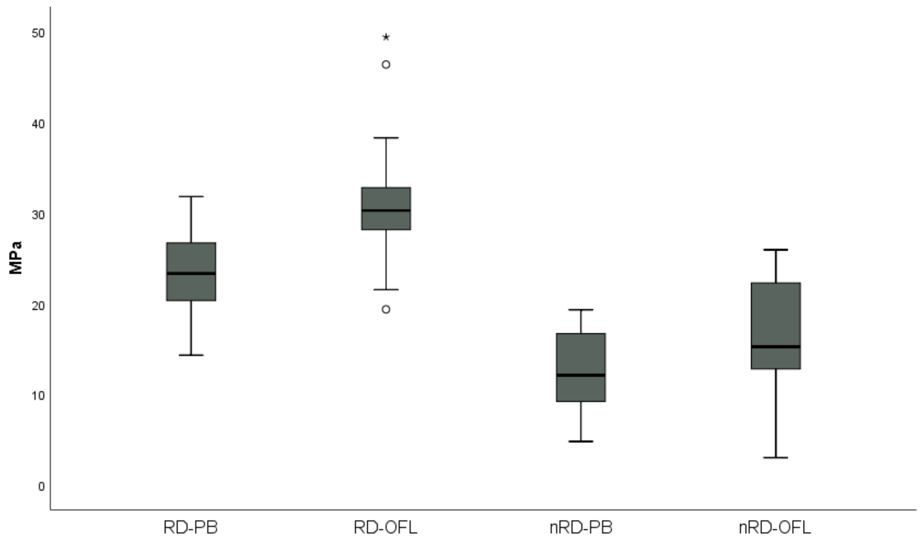

FIGURE 2. Box-plot of shear bond strength (SBS) distribution within the four study groups (RD-PB - Prime&Bond Adhesive Activate with rubber dam; RD-OFL - OptiBond FL adhesive with rubber dam; nRD-PB - Prime&Bond Activate without rubber dam, nRD-OFL - OptiBond FL adhesive and procedure without rubber dam).

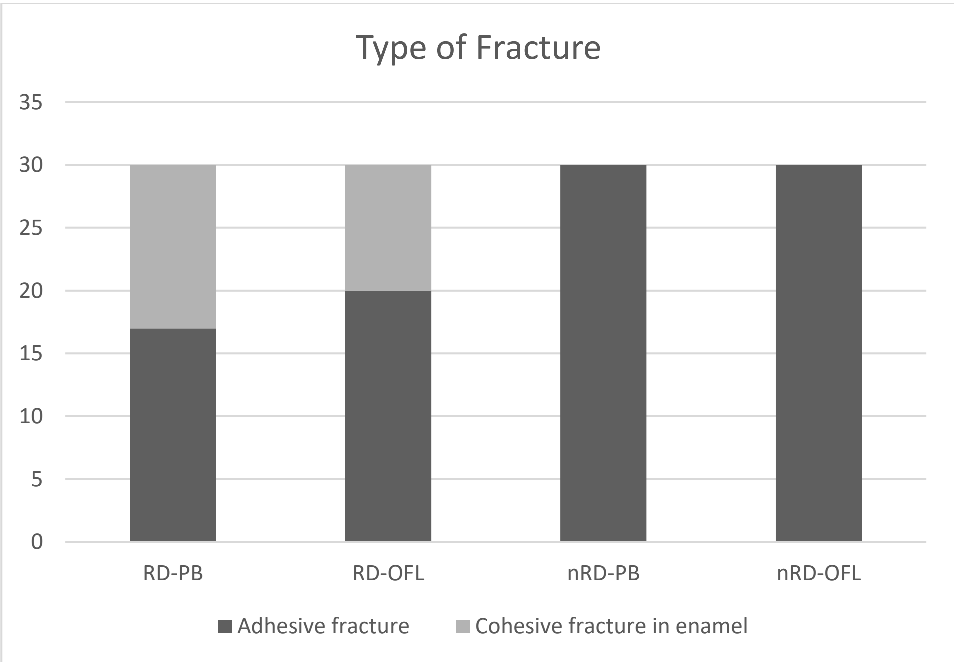

FIGURE 3. Proportion of each fracture type for the four groups studied (RD-PB - Prime&Bond Adhesive Activate with rubber dam; RD-OFL - OptiBond FL adhesive with rubber dam; nRD-PB - Prime&Bond Activate without rubber dam, nRD-OFL - OptiBond FL adhesive and procedure without rubber dam).

TABLE 3. The following table shows the corrected p-value for the McNemar testing, for the comparison of the different types of fracture between all pairs of groups, by the Benjamini-Hochberg method.

Comparison (Type of Fracture)	p_{ajd}
RD-PB vs RD-OFL	0.695
RD-PB vs nRD-PB	0.003
RD-PB vs nRD-OFL	0.003
RD-OFL vs nRD-PB	0.007
RD-OFL vs nRD-OFL	0.007
nRD-PB vs nRD-OFL	1.000

Discussion

This research aims to assess the effect of relative humidity on the bond strength of two different adhesive systems, a three-step total-etch (OptiBond FL) and a universal adhesive (Prime&Bond Active), to enamel in conditions as close to clinical scenarios as possible. For this reason, bonding procedures were conducted on teeth held by an intra-oral custom-designed device fitting our volunteer's oral cavity. To the authors' best knowledge, a similar methodology has not been established to date.

Of the few studies where relative humidity effects are considered, most opt to test the bonding strength to dentin and overlook the importance of adequate enamel bonding. While adhesion to dentin may even benefit from a moist environment, enamel requires the absence of water and moisture to attain peak bond strength. Failure to ensure optimal conditions for proper bonding to enamel will lead to poor marginal sealing, resulting in restorative failure. [(6)]

Statistically significant differences were detected regarding bond strength between all groups, with higher mean shear bond strength values in experimental groups where rubber dam was used. When appropriately used, rubber dam can therefore act as a shield to relative humidity, which in turn seems to play a detrimental role in the adhesive interface. Thus, the alternative hypothesis was accepted.

These findings are in accordance with those reported by Plamans et al., who concluded on the impossibility of achieving a dry working field in the oral cavity without the correct application of a rubber dam.[(10)]

Similarly, Bicalho et al. conducted an in vitro study leading to the deduction that bond strength declines with increasing temperature and humidity of the environmental chamber used. The significantly worse values obtained for the group exposed to 37ºC and 90% relative humidity support Bicalho's recommendation to perform composite resin restorations under absolute isolation.[(3)]

Authors who studied analogous hypotheses, such as Bavbek et al., chose to test under an oral environment simulation using a controlled humidity chamber.[(18)] There are possible disadvantages to this method: the chamber's inability to replicate natural inhalation, down time, and exhalation cycles present in clinical scenarios. In addition, the constant high humidity may impair water evaporation, directly promoting a bias towards an adverse outcome. [(10,17)]

In Bavbek's [(18)] study, the micro shear bond strength of a resin composite to enamel was tested with three different adhesive systems applied at various humidity conditions. However, contrary to what was observed in this present study, Bavbek did not find any significant influence of humidity on bond strength to enamel. The divergence of results might be explained by the differences in the experimental protocols and adhesive systems used. Despite that, this same study stated that total-etch and two-step self-etch adhesive systems exhibited significantly higher µSBS than that of one-step self-etch adhesives to enamel, for all humidity conditions.[(18)] These results are in line with differences found in this research between the three-step total-etch system and the universal adhesive system, with higher mean shear bond strength values invariably found in Optibond FL when both are tested under the same isolation conditions.

It is common understanding that universal systems are associated with lower in vitro bond strength results and poorer in vivo longevity of restorations, most often due to vulnerabilities in the bonding interface. [15,19] These outcomes are almost certainly a result of the numerous molecules in complex formulations within technically simplified adhesive systems, which may impair complete solvent volatilization and lead to poor adhesive polymerization [15,16]

When considering the standard deviation, differences in groups where a rubber dam was used are about 20% of the bond strength, while in the test groups where absolute isolation was not performed, the differences are 33% for the universal system and 42% for the three-step total-etch system, suggesting greater variability in the result.

Even though the three-step total-etch system showed higher mean values of shear bond strength in both experimental conditions, with and without absolute isolation (RD-OFL 30.84 MPa / nRB-OFL 16.33 MPa), it was also the one with greater differences between maximum and minimum values of shear bond strength obtained in the group without rubber dam (min 3.00 MPa/ max 25.94 MPa), hinting that it can be highly unpredictable when the rubber dam is not used and highly susceptible to differences in relative humidity. These differences in behaviour may be attributed to the chemical composition of adhesives used in the study.

HEMA is a hydrophilic monomer found in OptibondFL, absent from Prime&Bond Active. If water absorption takes place before polymerization, it may lead to a reduction in conversion degree due to dilution of the adhesive system.[15,20,21] Increased content of HEMA in adhesives decreases the degree of conversion and may jeopardize the polymer mechanical properties, particularly when aging conditions are applied. [22,23]

In this study, fracture patterns were either adhesive or cohesive in enamel. Out of the two, there was a higher proportion of overall adhesive fractures. However, it is worth mentioning that cohesive enamel fractures were exclusively registered in experimental groups where rubber dam was used. This suggests that in the absence of absolute isolation, there are more vulnerabilities in the bonding interface to enamel caused, most likely by the increase in moisture levels. When adequate absolute isolation techniques are used, either adhesive system tested may provide bond strength values that exceed the cohesive strength of enamel itself.

Fracture patterns were similar among groups where the same experimental conditions were tested, meaning that no significant difference in fracture type between adhesive systems was found independently of whether absolute isolation was present. Not only bond strength values but also failure patterns must be assessed when evaluating adhesion, as the cohesive strength of dental substrates sets the bar for the expected performance of adhesive systems. [18,24]

Although the present study was performed in situ, higher relative humidity values are expected to be found in clinical conditions when rubber dam is not used or improperly placed.[17] The custom-designed oral device used in this experimental work may have led to improved relative isolation compared to the clinical environment, as all humidity from surrounding tissues was utterly blocked (i.e., gingival crevicular fluid, saliva, or blood). In addition, the capsule used to apply the composite resin also shielded the restorative material from humidity immediately after being placed. It is also worth noting that restorative procedures in a clinical environment are more time-consuming and therefore increased exposure to humidity and its effects on adhesion are expected.

In this study, the best-case scenario was simulated, meaning that the tooth slot was in a location equivalent to that of the vestibular surface of an anterior tooth. Other oral locations, such as mandibular teeth or even posterior upper teeth, are exposed to a higher degree of humidity and moisture and will therefore suffer greater effects. A Saraiva et al. study showed that significantly higher temperatures and relative humidity values are found at molar sites when compared to those found in the incisor positions (Incisor 26.2ºC/84.8%RH vs Molar 27.3ºC/90.7%RH).[17]

To standardize intraoral conditions, procedures were performed in one patient only. However, differences between patients' oral environments should be considered in further studies. A study by Pierre Varène et al. stated that during mouth breathing, temperatures are significantly higher, and the amount of exhaled water is higher when compared to nose breathing. Thus, even though our volunteer was instructed to breathe through the nose, differences in patients breathing patterns must be taken into consideration. [1,9,17]

Although intraoral temperature and relative humidity values were not recorded in this study, Kameyama et al. showed that the placement of different isolation methods produces significant alterations in intraoral temperature and relative humidity. The room's environmental conditions also influence these values. [5]

Regarding the use of rubber dam, Haruyama et al. studied how different types, number of exposed teeth and air vents in the rubber dam sheet would influence temperature and relative humidity. The researchers concluded that simple moisture exclusion with cotton rolls is insufficient (100% relative humidity), and that by using rubber dam, relative humidity can be lowered to levels equivalent to those of the room but the same cannot be said about temperature. Additionally, it was concluded that there seems to be no difference in moisture exclusion when a single tooth or multiple teeth are exposed.[5]

The present study demonstrated that moisture control is not easily obtained without using a rubber dam and that intraoral relative humidity plays a vital role in guaranteeing excellent bond strength values in the adhesive interface to enamel. Without adequate isolation, proper marginal sealing cannot be achieved, thus compromising the longevity of restorations and having long-term consequences on our patient's oral health and comfort.

Future studies on this matter should contemplate not only the relative humidity on bond strength but also relate it to the different fracture patterns assessing adhesion quality on both enamel and dentin.[18] Clinical studies should be implemented to correlate the survival of restorative procedures with humidity levels found when bonding procedures took place.

Conclusions

Considering the limitations of this study, we can conclude that:

- Intraoral relative humidity levels adversely affect shear bond strength to enamel.
- Absolute isolation with rubber dam is an indispensable technique whenever adhesive procedures are required, as it proves effective in potentiating bond strength to enamel independently of the adhesive system used.
- The three-step total-etch system OptiBond FL performed better than the other tested system in both experimental conditions.

Acknowledgments

I would like to express my gratitude to my primary advisor, Professor Fernando Guerra, who supervised this project.

I would also like to show my deep appreciation to my co-advisor Doctor Rui Falacho for offering guidance, support, and deep insight into this study.

I thank Drª Joana Marques for her essential contribution, patience, and active participation in this project.

I also wish to acknowledge the precious help provided by Doctor Francisco Caramelo and the promptness with which he shared his knowledge.

Lastly, I would like to thank my boyfriend Ricardo Silva, my family, and friends for providing support in every step of the way and for their valuable help in collecting all the samples used in this study.

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