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Influence of the Flowable Resin Layer on Bond Strength Between Resin Cement and a Universal Adhesive Applied in the Immediate Dentin-sealing Technique

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Clinical Relevance

The immediate dentin-sealing technique is a promising method for the optimization of adhesive procedures in indirect restorations. This clinical protocol benefits from the use of universal adhesive systems associated with a low-viscosity resin layer. Reduction of postoperative sensitivity and better bond strength values are relevant clinical contributions of the technique.

SUMMARY

Objectives: The present study evaluated the influence of a flowable resin layer on bond strength between resin cement and a universal adhesive applied using an immediate dentin sealing (IDS) technique.

Methods and Materials: Coronary portions of bovine teeth were randomly divided into six groups (n=15). In the IDS.U group, the exposed dentin was immediately sealed with the Single Bond Universal adhesive (3M ESPE) following the self-etching protocol. In the IDS.UF group, a layer of Filtek Z350 (3M ESPE) flow resin was applied over the universal adhesive. In the DDS (control) group, the dentin was kept "fresh" and delayed dentin sealing was performed. After 24 hours in distilled water at 37°C, dentin surfaces were treated

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with pumice, phosphoric acid, and the application of the universal adhesive in the IDS.U and IDS.UF groups. The DDS group was treated with pumice and the universal adhesive was applied. The samples received cylinders of resin cement Rely X Ultimate (3M ESPE) made with the aid of starch tubes of 0.96 mm in diameter and 2 mm in length. They were submitted to the microshear bond strength test (μSBS) at 0.5 mm/min, after 24 hours (T1) and 3 months (T2). The fracture areas were evaluated qualitatively using a DSM 300 microscope (KOZO) with 45× magnification and classified as: adhesive, cohesive in cement, cohesive in dentin, or mixed. Samples were analyzed by scanning electron microscopy (SEM). The data were compared statistically between groups using the Kruskal-Wallis test, and intra-groups using the Mann-Whitney test (α=0.05).

Results: There were no significant differences between groups for the bond strength values (*p*>0.05). The IDS.UF group showed higher values at 3 months, when compared to the values of 24 hours (*p*<0.001). All groups showed a predominance of adhesive fracture (86.7% to 100%). SEM showed dentinal tubules exposed in the IDS.U and DDS groups; in the IDS.UF group, the tubules were completely sealed.

Conclusions: The flow resin can be used on the adhesive when using the IDS technique because it increased the bond strength values after 3 months and promoted effective sealing of the dentinal tubules.

INTRODUCTION

Dental preparation for indirect restorations often exposes dentin. The exposed dentinal tubules allow the transmission of mechanical and chemical stimuli to the dental pulp, which can cause sensitivity and even irreversible pulp damage.[1] In the conventional protocol for indirect restorations, the exposed dentin is sealed only at the time of cementation, being subject to contamination throughout the period of provisional restoration.[2]

Immediate dentin sealing (IDS) is an alternative method to the conventional protocol, in which the exposed dentin is sealed with an adhesive system immediately after preparation and prior to the execution of the impression.[3] Thus, dentin and, consequently, dentinal tubules are sealed, providing protection against bacterial contamination and the action of impression materials. The IDS also makes it possible to reduce dentin sensitivity.[3] It is known that "fresh" dentin, freshly prepared, is the ideal substrate for adhesion, as it is less likely to be contaminated, resulting in greater final bond strength.[3] In addition, when IDS is performed, higher values of adhesive strength of resin cement to dentin are obtained when compared to delayed dentin sealing (DDS).[4]

There are several clinical protocols, associated with different materials, that can be used to perform the IDS technique, varying the dentin hybridization from adhesive systems of total acid conditioning (etch-and-rinse),[2-9] self-etch systems,[4,5,10-13] and techniques that apply a layer of flow resin over the previously hybridized substrate.[4,11,13-19] According to Nikaido and others,[14] the application of flow resin on the dental adhesive, in addition to helping to protect the dental pulp, also promotes the greater reduction of dentinal sensitivity at the time of insertion and removal of provisional restorations, which demonstrates a notable clinical advantage.

Recently, IDS has been performed with universal adhesives,[11,13,17] also contributing to higher values of dentin bond strength when compared to the delayed dentin sealing protocol. The use of universal adhesives in IDS can make the technique less susceptible to errors, considering the possibility of applying the adhesive in a single step.[11,13,17] Therefore, the use of universal adhesives reaffirms the advantages generated by not using acid conditioner on the dentin surface initially presented in self-etching systems.[20] Among the main clinical advantages, there is less postoperative sensitivity and also greater bond stability, when compared to the hydrolytic degradation of collagen observed in etch-and-rinse systems.[21] The option for "multi-modal" adhesives thereby is also a relevant alternative in the application of the IDS technique for immediate protection of the dentin substrate.

Based on the scientific evidence that supports the immediate sealing of dentin, as well as the positive results associated with the use of a layer of flow resin on the dental adhesive in IDS, studies that evaluate the applicability of new generations of adhesives and resin cements in this technique are fundamental. Hence, studies are important to contribute to the definition of satisfactory and safe clinical protocols for the performance of the IDS in the cementation of indirect restorations on sealed dentin. The present *in vitro* study evaluated the influence of the flowable resin layer on bond strength between resin cement and a universal adhesive used in an IDS technique. The tested hypotheses were that the flow on the universal adhesive does not interfere with the bond strength results of the resin cement and improves the quality of the dentin seal when used in an IDS technique.

METHODS AND MATERIALS

Tooth Preparation

Bovine lower incisors were used, which were cleaned and packed in neutral 0.1% thymol solution at 6°C to prevent dehydration and bacterial proliferation. The root portions of the teeth were removed with the aid of a double-sided diamond disc (KG Sorensen, São Paulo, SP, Brazil). The vestibular face of the coronary portion was inserted about 1 mm in depth in a utility wax slide (Lysanda, São Paulo, SP, Brazil).

A wooden matrix with 20-mm diameter perforations was placed over the wax, surrounding the coronary portion of the teeth. Epoxy resin (Cristal, Redelease, São Paulo, SP, Brazil) was carefully manipulated and poured in during the sandy phase until it filled the entire wood matrix perforation. After about 4 hours, the time required for resin setting, the epoxy resin cylinder and tooth assembly were removed from the matrix.

The enamel of the labial face of the teeth was removed using a PVV polisher (Teclago, Vargem Grande Paulista, SP, Brazil) associated with silicon carbide sandpaper #100 (Norton, Guarulhos, SP, Brazil) to expose the dentin. Then, for flattening the surface, sandpapers #320 for 10 seconds and #600 for another 10 seconds were used.

The dentin surfaces were cleaned using a pumice paste (Polidental Ind Com Ltda, Cotia, SP, Brazil) manually prepared into a dappen glass with a small amount of water. The pumice was then applied with a rotary brush for 15 seconds, followed by abundant washing with air-water spray for 15 seconds.

Dentin Surface Treatments

Table 1 lists the trade names, manufacturers, and compositions of the main materials used in the study.

The prepared teeth were randomly divided into six groups (n=15). In the IDS.U group, the exposed dentin was immediately sealed with Single Bond Universal adhesive (3M ESPE), following the self-etching protocol. The adhesive was actively applied with microbrush and friction for 20 seconds, followed by the application of a light air jet for 5 seconds, as recommended by the manufacturer, and light curing for 20 seconds at 1000 mW/cm^2 (Emitter D, Schuster, Santa Maria, RS, Brazil).

Table 1: *Materials Used in the Study*

Commercial Name	Manufacturer	Main Components
RelyX Ultimate	3M ESPE, St Paul, MN, USA	Base paste: Methacrylate monomers, radiopacifiers, silanized filler particles, initiator components, stabilizers, rheology additives Catalyst paste: Methacrylate monomers, radiopacifiers, alkaline filler particles, stabilizers, pigments, rheology additives, fluorescent components, light-free polymerization activator for Single Bond Universal
Single Bond Universal	3M ESPE, St Paul, MN, USA	Alcohol, water, silicon-treated silica, 10-MDP, Bis-GMA, 2 hydroxyethyl methacrylate, decamethylene dimethacrylate, acrylic copolymer and itaconic acid, camphorquinone, N, N-dimethylamonoethyl, methyl ketone, silane
Filtek Z350 XT Flow Resin	3M ESPE, St Paul, MN, USA	Bis-GMA, UDMA, TEGDMA, PEGDMA, Bis-EMA, ytterbium trifluoride, silica particles, zirconia
Pumice	Polidental Ind Com Ltda, Cotia, SP, Brazil	Quartz
Alpha Etch Gel	Nova DFL, Rio de Janeiro, RJ, Brazil	37% Phosphoric Acid

Abbreviations: MDP, methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bisphenol A-glycidyl methacrylate; UDMA, urethane dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; PEGDMA, polyethylene glycol dimethacrylate; Bis-EMA, bisphenol A ethoxylated dimethacrylate.

In the IDS.UF group, to cover the universal light-cured adhesive, a thin layer of Filtek Z350 flow resin (3M ESPE) was applied, followed by light curing for 20 seconds (1000 mW/cm^2). In the DDS (control) group, the dentin was kept "fresh."

The teeth were stored in distilled water and placed in an oven at 37°C for 24 hours.

Resin Cement Cylinders

After the 24-hour storage time, the sealed dentin surfaces were cleaned with pumice, as previously described. In the IDS.U and IDS.UF groups, 37% phosphoric acid was applied for 30 seconds, followed by a rinse with an air-water spray for 30 seconds, and air-jet drying for 5 seconds. A new application of the universal adhesive was performed with a microbrush and friction for 20 seconds, followed by the application of a light air jet for 5 seconds to evaporate the solvent, as recommended by the manufacturer, and light cured for 20 seconds at 1000 mW/cm^2 (Emitter D, Schuster, Santa Maria, RS, Brazil). In the DDS group, delayed dentin sealing was performed with a universal adhesive, following the self-etching protocol, as previously described for the IDS.

A double-sided adhesive tape with four circular perforations made with a rubber dike perforator (hole nº 5) was fixed over the sealed dentin surfaces. Starch tubes (Renata, Pastificio Selmi, Londrina, PR, Brazil) were fixed on the areas delimited by the perforations (0.96 mm in internal diameter and 2 mm in height).[22] The starch tubes were filled with Rely X Ultimate resin cement (3M ESPE) followed by light curing for 40 seconds at 1000 mW/cm^2 (Emitter D).

The teeth with polymerized resin cement cylinders inside the starch tubes were stored in closed plastic containers, submerged in distilled water, and placed in an oven at 37°C. Fifteen specimens from each group were stored for 24 hours (T1), and another 15 specimens from each group were stored for 3 months (T2). After times T1 and T2, the starch tubes were carefully removed with the aid of a scalpel blade number 15 (LAMEDID, Osasco, SP, Brazil). The resin cement cylinders then proceeded to the test of micro-shear bond strength. Figure 1 shows the study flowchart.

Micro-shear Bond Strength Test

After the storage times T1 and T2, each cylinder of resin cement was subjected to the micro-shear bond strength test. An active tip in the form of a knife blade was applied in the region of the bond area, parallel to the dental surface, with a speed of 0.5 mm/min, in the Instron 5965 universal testing machine. The values recorded in newtons (N) were divided by adhesion area, to calculate the bond strength (MPa).

Failure Analysis

The cylinder fracture surface areas were qualitatively assessed using a DSM 300 microscope (KOZO) with 45× magnification and classified as: adhesive—failure in the adhesion interface; cement cohesive—cement failure; dentin cohesive—dentin failure; or mixed.

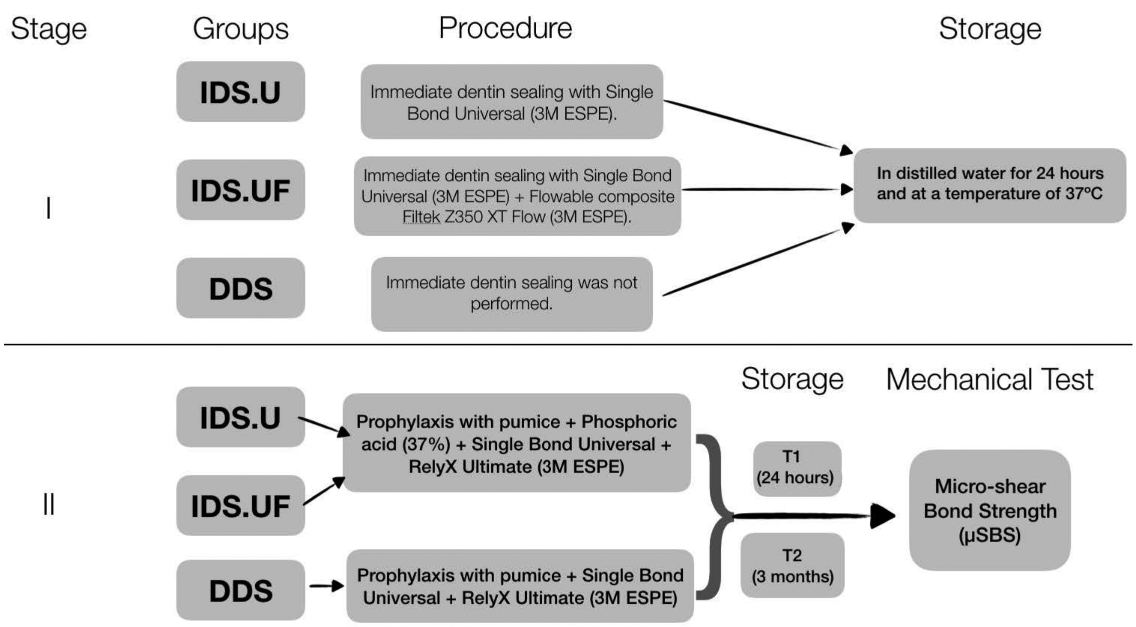

Figure 1. *Study flowchart (n = 15).*

Scanning Electron Microscopy (SEM)

Two samples from each group were analyzed using a scanning electron microscope JEOL JSM-IT300 (JEOL Brasil Inst Científicos Ltda, Sao Paulo, SP, Brazil) with 5000× magnification. For the IDS.U and IDS.UF groups, a sample was separated after the IDS protocol and another after treating the sealed surface with pumice, phosphoric acid, and universal adhesive. For the DDS group (control), a sample of "fresh" dentin was separated; and another after the delayed dentin sealing with universal adhesive.

Statistical Analysis

The data were submitted to statistical analysis using the SPSS 24 software (SPSS Inc, Chicago, IL, USA) at a significance level of 5%. The values of the micro-shear bond strength (MPa) were assessed for homogeneity of variances by the Levene test, and for normal distribution using the Shapiro-Wilk test. Due to the absence of normality and non-homogeneous variances, the bond strength data were compared between groups using the Kruskal-Wallis test. For intra-group comparisons of data obtained at 24 hours and 3 months, the Mann-Whitney test was used. Descriptive analysis of the frequencies observed for the fracture pattern and the SEM images was performed.

RESULTS

The bond strength values obtained in the studied groups (Table 2) were statistically similar to each other, both at 24 hours (p=0.107) and at 3 months (p=0.074). In intra-group comparisons, significant statistical differences were found only for the IDS.UF group (p<0.001), with higher values in 3 months. The IDS.U groups (p=0.526) and the DDS group (p=0.185) did not show significant differences between the times of 24 hours and 3 months. Table 2 presents descriptive statistics, with graphical representation in the Box-plot (Figure 2).

According to the qualitative analysis of the fracture pattern, all groups presented predominantly adhesive-type fractures, as shown in Table 3.

As for the analysis of scanning electron microscopy,the DDS group presented images of "fresh" dentin with the presence of smear layer, occluded dentinal tubules, and irregular surface (Figure 3A); it was possible to observe the exposure of dentinal tubules after prophylaxis and application of the adhesive (Figure 3B). For the IDS.U group, a partially sealed dentin surface was observed (Figure 3C), and the presence of exposed dentinal tubules after treatment of the sealed dentin with prophylaxis, acid conditioning and application of the adhesive (Figure 3D). The IDS.UF group had a smoother and more regular surface than the other groups (Figure 3E), and no exposed dentinal tubules were observed after performing the treatment of sealed dentin with prophylaxis, acid conditioning and application of the adhesive (Figure 3F).

Table 2: *Median, Inter-Quartile Interval (Q25-Q75) and Minimum and Maximum Values (Min-Max) for the Data of the Micro-Shear Bond Strength (MPa) in the Different Groups*

Groups	Storage	Median	Q25-Q75	Min-Max
IDS.U	24 hours	3.71	2.61-7.54	1.83-10.62
	3 months	4.62	2.77-8.71	1.72-13.91
IDS.UF*	24 hours	2.76	2.01-3.75	1.57-4.61
	3 months	5.49	4.01-8.79	2.79-14.34
DDS	24 hours	3.35	2.45-4.25	1.57-5.69
	3 months	4.10	3.19-4.63	1.94-6.27

**Significant statistical differences between times (*p*<0.001, Mann-Whitney test)*

DISCUSSION

The present work evaluated the influence of the application of flow resin on the micro-shear bond strength of resin cement to dentin immediately sealed with a universal adhesive system. The *in vitro* evaluation of this technical IDS emerges as an important precursor to clinical studies, given the relevant benefits generated by the mechanism of action of "multi-modal" adhesives, which can be used without acid etching in dentin, in

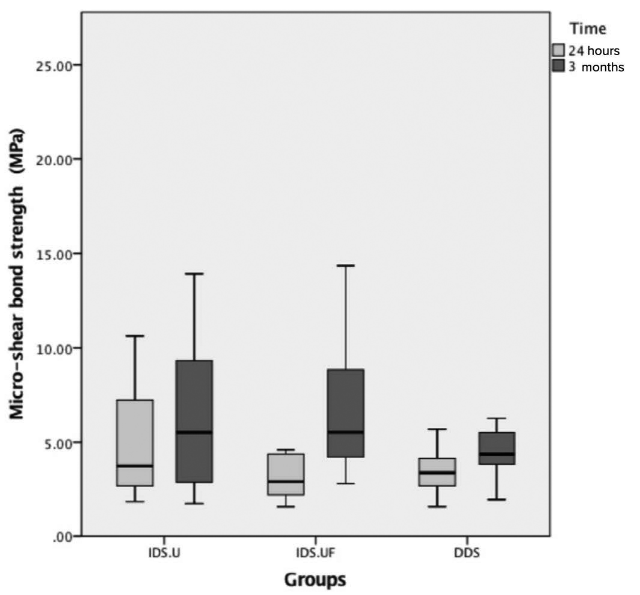

Figure 2. *Box-plot of the values of the micro-shear bond strength (MPa) for the different groups at 24 hours and 3 months.*

Table 3: *Absolute Frequencies (Percentages) for the Fracture Pattern in the Different Groups*

Groups	Storage	Fracture Pattern	
		Adhesive	Mixed
IDS.U	24 hours	14 (93.3%)	1 (6.7%)
	3 months	15 (100%)	0 (0%)
IDS.UF	24 hours	15 (100%)	0 (0%)
	3 months	15 (100%)	0 (0%)
DDS	24 hours	13 (86.7%)	2 (13.3%)
	3 months	15 (100%)	0 (0%)

addition to establishing a chemical interaction with this substrate, due to the incorporation of the acidic molecule 10-MDP.[23] These characteristics are also desirable for obtaining and maintaining a resistant protective layer on the dentin surface that in the future will interact with the cementing agent and the indirect restoration.

Furthermore, the results of this research corroborate with the philosophy that dentin should be sealed immediately and that this seal does not impair the bond strength when using a dual resin cement.[4,13,24] Regarding the use of a flow resin layer over the adhesive, the studies by Knobloch and others[24] and Van den Breemer and others[4] found that the low viscosity resin did not result in increased bond strength for the tested protocols. Nevertheless, other studies[11,19,31,32] found that the use of flow resin increased the bond strength of resin cement to dentin subjected to immediate sealing. In the present work, the application of flow resin did not promote a significant increase in bond strength when compared to the other groups. Therefore, the hypothesis that flow resin does not interfere with the bond strength values of resin cement to sealed dentin was accepted.

Another important aspect refers to the sample aging method carried out in the study, which possibly influenced the non-significant statistical differences in bond strength between the groups tested. The contact with elastomeric impression materials, the use of provisional restorative materials, and the placement of the samples in artificial saliva are procedures that would contribute to better adhesive performance of the immediate sealed groups. In this case, the specimens submitted to the delayed dentin sealing (DDS) would be more affected by this aging method as a result of nonprotection of the exposed dentin. This fact was confirmed in another study[33] and brings a solid explanation for the outcome found in the present study.

Additionally, in intra-group comparisons, the bond strength values obtained after 3 months of storage were

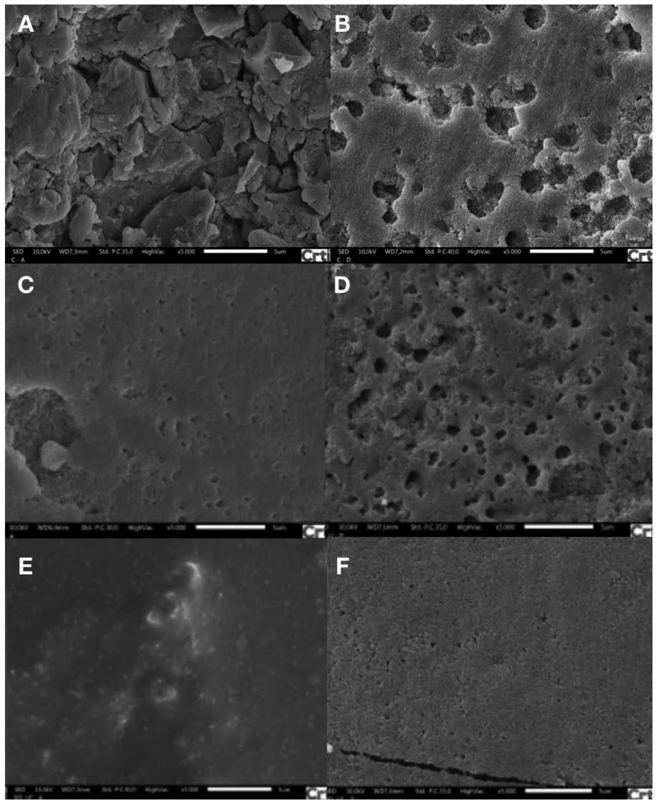

Figure 3. *Photomicrographs of dentin surfaces (5000×): (A) DDS "fresh" dentin; (B) dentin DDS after prophylaxis and application of the adhesive; (C) IDS.U after applying the adhesive; (D) IDS.U after sealed surface treatment; (E) IDS.UF after application of the adhesive and flow; and (F) IDS.UF after sealed surface treatment.*

greater than after 24 hours, with a statistically significant difference only for the IDS.UF group. Darr and Jacobsen[25] stated that the resin cement is not properly polymerized in the initial stages of cementation and that disruption of a restoration's cementation can occur during immediate finishing procedures. According to the authors, restorations may be vulnerable in the first 24 hours. Moreover, factors such as the curing mechanism, choice of adhesive system, and polymerization device can affect the degree of conversion of the cements and, consequently, influence the mechanical properties and clinical performance.[26] Hence, the probable explanations for the increase in the bond strength values over time in the present work are the late curing of the cement, with better polymerization after 3 months, and consequent maturation of the adhesive area. And especially for the IDS.UF group, due to the flow resin providing a more homogeneous sealing of dentin (occluded dentinal tubules), as could be observed in the SEM, the referred maturation of the adhesive layer probably occurred under more favorable conditions, such as by the possible absence of moisture. A strong point also of the present study is that the treatments of the sealed dentin surface, used before the application of resin cement, involved materials and equipment commonly available in dental offices, which improves the clinical applicability of the research protocols. Furthermore, pumice prophylaxis and applying phosphoric acid to the sealed surface for cleaning purposes are common procedures before cementing indirect restorations. A recent study[4] compared the treatment of sealed surfaces with different IDS strategies before applying resin cement for shear tests. They found that there were no statistical differences in bond strength by comparing pumice-only or pumice plus silica coating and silane. Corroborating the idea that simpler and more common procedures may be sufficient for the treatment of sealed dentin before adhesive cementation.

The analysis by SEM revealed that the IDS.U group presented exposure of dentinal tubules after the treatment of the sealed surface, while the IDS.UF group, certainly due to the flow resin layer, kept the dentin protected with the tubules properly sealed. The exposure at IDS.U can be explained by the low film thickness of the unfilled universal adhesive, which creates a surface that is more vulnerable to mechanical stress resulting from the pumice application.[33] This fact confirms the tested hypothesis that the flow resin layer improves the quality of the dentin sealing, as it acts as additional protection to the substrate and the adhesive area. According to Hironaka and others,[19] the resin coating technique really protects the adhesive layer of the IDS, which is subject to degradation, and also enables a better diffusion of the resin cement. Therefore, applying a layer of flow resin can indeed be considered advantageous in the universal adhesive IDS technique evaluated here.

In the present study, we opted for the use of bovine teeth due to the advantages such as: ease of obtaining the teeth in adequate condition, that is, without carious lesions and enamel defects; greater uniformity found in bovine teeth when compared to human teeth; better standardization of samples; and greater area of flat surface, which makes it possible to make a larger number of samples per tooth and improve the performance of the micro-shear bond strength test.[27] Furthermore, comparative studies between human and bovine teeth, which evaluated the bond strength in enamel and dentin, did not indicate significant differences between them.[28] In this way, bovine teeth are seen as a reliable alternative for carrying out the tests proposed in the present study.

Another important factor regarding the tests performed here consists of the characteristics of the smear layer that can also influence the bond strength, which is why it must be standardized in all specimens. In accordance with what is described in the literature, all samples received a final preparation with 600-grit sandpaper, which simulated a dentin preparation performed by fine-grained diamond-tip wear instruments. The standard of laboratory research was followed, with the samples stored in distilled water at 37°C for 24 hours.[29]

The micro-shear tests, initially addressed in the work of Shimada and others,[30] are designed for the evaluation of the bond strength of materials to the dental structure, being used mainly for friable materials, as is the case of cements, which would be damaged if they were submitted to the protocols required to perform the microtensile test. In a comparative analysis, micro-shear is more reliable and advantageous than the traditional shear test, since it evaluates adhesive areas smaller than 3 mm^2. This makes it possible to place several specimens on a single tooth, in addition to requiring a smaller amount of material, which reduces the likelihood of bubbles and irregularities, which could compromise the test. Another important factor is the concentration of stresses in the adhesive area, which promotes a considerable reduction in the percentage of cohesive failures in material and substrate. In fact, in the present study, there was a predominance of adhesive-type fracture patterns in all groups, which was expected, therefore, by the type of test used.

As the main limitations of the present study, one must consider the great variability of the data, and also the difficulty of external validity of the results inherent to *in vitro* studies. Besides that, the micro-shear bond strength test requires a critical execution technique, due to the fact that the positioning of double-sided guides, starch tubes, and samples in the universal testing machine can compromise data collection. Nevertheless, the findings of this laboratory research are important precursors for the development of other studies, especially controlled clinical trials, in order to obtain better scientific evidence for the definition of a safe and reliable protocol for the cementation of indirect restorations on dentin surfaces immediately sealed with universal adhesive and flow resin.

CONCLUSIONS

According to the present work, it can be concluded that the immediate dentin sealing techniques evaluated in the present study did not promote values of bond strength different from the control group (DDS), either in the 24-hour period or in the 3-month period. In the group that used flow resin after sealing with Single Bond Universal (IDS.UF) there was a significant increase in the bond strength values after 3 months, when compared to the time of 24 hours. The qualitative analysis of the fracture pattern showed a predominance of adhesive-type fractures. In the IDS.UF group, the flow resin promoted a more regular surface and the dentinal tubules were effectively sealed.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of the institution in which it was carried out.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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