

Bond Strength to Microtraction and Nanofiltration Using Ethanol Wet Bonding Technique in Fresh Extracted Teeth: An *Ex Vivo* Study

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ABSTRACT

Objective: The objective of this study was to evaluate the bond strength to microtraction and nanofiltration using ethanol wet bonding technique in fresh extracted teeth. **Materials and Methods:** This quasi-experimental *ex vivo* study evaluated 48 teeth that had an indication of premolar extraction due to orthodontic reasons. The protocol of dental preparation and restorative procedure was carried out to evaluate the adhesion resistance by means of the universal testing machine at a loading speed of 0.5 mm/min and 500 MPa. To evaluate the nanofiltration, matches were made that were immersed in ammoniac silver nitrate for 24 h, and then the specimens impregnated with silver were washed thoroughly in distilled water and placed in a photo-developer solution for 8 h under a fluorescent light. All statistical analyses were statistically evaluated with a level of significance $P < 0.05$. **Results:** The ethanol technique without premature failure (PF) group had an average of 31.26 ± 10.26 MPa, whereas the lowest value was found in the water technique group with PF, which had 22.59 ± 12.27 MPa. When performing inferential statistics, it was evidenced that there were statistically significant differences between both techniques with a value of $P < 0.05$. **Conclusion:** According to the results in both cases, the adhesive strength showed superiority in the ethanol wash group. It determines that this technique presents greater tolerance to the residual presence of water. Finally, in relation to nanofiltration we found that there were no significant differences between the groups evaluated.

KEYWORDS: Bond strength, ethanol wet bonding, *ex vivo* study, microtraction, Nanofiltration

INTRODUCTION

The main problems associated with adhesive systems could be partially attributed to the poor infiltration and encapsulation of moistened dentine, by adhesive monomers. The resulting hybrid layer would be suboptimal and more prone to hydrolytic degradation.^[1] Some previous studies have correlated the instability of the resin–dentin bond with the high content of hydrophilic resin monomers in adhesive systems.^[2-4] The vast majority of dental adhesives currently on the market are combinations of hydrophobic and hydrophilic monomers. Hydrophilic groups increase

the wettability of hard dental tissues; hydrophobic groups interact and co-polymerize with the restorative material. Because vital dentin is intrinsically wet, it is virtually impossible to completely dry dentin in a clinical situation. Consequently, manufacturers have developed dentin adhesives that are compatible with humid environments.^[5]

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On the other hand, it has been determined that the hydrophobic components of the adhesives have good miscibility with ethanol, and the so-called hydrophilic monomers also show better solubility in ethanol than in water.^[5] This is because ethanol is an ideal solvent for most hydrophobic monomers; the wet ethanol adhesive technique has been proposed to be applied with hydrophobic adhesives to improve its infiltration and reduce the degradation of the adhesive interface.^[1] The reasoning behind this adhesion technique is that dehydration with ethanol converts etched hydrophilic dentin, allowing the use of relatively hydrophobic monomers to infiltrate the collagen matrix.^[6]

Most works related to the topic of washing with ethanol have already evaluated the results of their use with hydrophobic monomers, obtaining favorable results. Usually, saturation with ethanol is achieved after the application of a series of ascending concentrations of ethanol, taking approximately 3–4 min, which defies the principles of ease of use and simplification of the technique.^[7] Therefore, the use of the wet ethanol adhesive technique and some contemporary hydrophilic adhesive systems, compared to the traditional use of these adhesives on a demineralized matrix saturated in water, can create an adhesive force similar to or greater than 24 h *in vitro*.^[8]

The clinical field adds another important factor to consider when performing adhesive restorations. The pulp pressure existing in each tooth allows the constant movement of fluids to all levels of the dentinal tissue through its tubules. This reason explains why the clinical application of a hydrophobic adhesive system together with the wet ethanol adhesive technique, under a complete dehydration protocol, does not generate the expected results, and it is because in a vital tooth the existing pulp pressure does not allow definitive dehydration of the dentin bed after washing with ethanol, as the flow of liquids is constant from the pulp chamber.^[9]

Finally, the objective of this study was to evaluate the bond strength to microtraction and nanofiltration using ethanol wet bonding technique in fresh extracted teeth. The hypothesis of the study was to show that the ethanol wet bonding technique increases the adhesion of fresh extracted teeth.

MATERIALS AND METHODS

STUDY DESIGN

This study was a quasi-experimental *ex vivo* study and was carried out during the semester of 2018 in the Academic Department of Rehabilitative Stomatology of the Faculty of Dentistry of the Universidad Nacional

Mayor de San Marcos and was reviewed by the Steering Committee of the Research Unit (FO-UNMSM 130818).

SAMPLING METHOD AND SELECTION CRITERIA

The sample size was determined using the means comparison formula through the Stata software, version 12.0 (College Station, TX), determining 48 teeth randomly divided into four groups with each group containing 12 teeth. Patients were selected using the simple random sampling technique. The selection criteria were the following:

Inclusion criteria comprised the following:

- patients 18–35 years old
- patients of both sexes
- patients who agree to sign the informed consent
- patients with indication of premolar extraction due to orthodontic reasons

Exclusion criteria comprised the following:

- patients who do not wish to sign the informed consent
- patients with an altered occlusal scheme
- patients with absence of premolars

Group distribution

- Group 1: Water technique with PF (premature failure)
- Group 2: Ethanol technique with PF
- Group 3: Water technique without PF
- Group 4: Ethanol technique without PF

SAMPLE PREPARATION

For each piece, local anesthesia with vasoconstrictor (2% lidocaine—1:80,000) and absolute isolation was placed; a standardized occlusal cavity was prepared using round and cylindrical diamond cutters with abundant irrigation. The cavities were prepared in order to obtain the longest possible dimensions, which averaged 4-mm wide, 5-mm long, and 3-mm deep; the floor of the dentinal cavity completely flat; and the margins of the full enamel surface. For group 1 and 3, the preparations were etched completely with 35% gel phosphoric acid for 15 s, followed by a 15 s water wash. Then, the cavity was dried gently with absorbent paper without drying it, and keeping it visibly moist, two layers of the Single Bond 2 adhesive (3M ESPE) were applied by rubbing the cavity with the micro applicator for 20 s, followed by a slight application of air for 10 s for solvent evaporation and subsequent curing for 30 s.

For groups 2 and 4, the etched surface was washed with water and then with 100% ethanol for 1 min, avoiding

at all times the evaporation and drying of the dentin saturated with ethanol keeping it visibly moist; the excess ethanol was removed with absorbent paper and two layers of the Single Bond 2 adhesive (3M ESPE) were applied by rubbing in the cavity with the micro applicator for 20s, followed by a slight application of air for 10s and the subsequent photo curing by 30s. The preparations of both groups were restored with a micro hybrid composite resin (Solare) in three increments. Each increase cured for 30s. The LED curing lamp used has a power of 800 mW/cm². Within 20 min after the restorative procedure, the teeth were extracted, stored in distilled water (pH = 7), and kept in a humid environment for 24h at 37°C before being prepared for the microtraction test and nanofiltration analysis.^[9]

Then, teeth were coronal-apically sectioned in the “x” and “y” directions on 1-mm thick sheets with the cutting machine Isomet™ 1000 (Illinois, USA) after the storage period. Each sheet was then sectioned to produce matches with an approximate cross-sectional area of 1 mm². Matches of the most peripheral area that do not contain the restorative material were excluded. Then, the specimens were fixed with cyanoacrylate glue (Multipurpose Adhesive “Triz” 3g; Industrial Beta, Lima, Peru), which is an extraordinary supereffective glue based on cyanoacrylate, easy to use and transparent on a specimen plate for universal testing machine. The microtraction exercise was carried out with a loading speed of 0.5mm/min and 500N to determine the adhesive strength. The cross-sectional area of each specimen tested was measured with a digital caliper (Mitutoyo—Digital, 0–6"/0–150mm, resolution 0.01 mm) (Tokyo, Japan) before specimen fracture.

Then, two matches of each tooth, randomly selected and not subjected to traction, were prepared for the nanofiltration evaluation. These matches were submerged in ammoniacal silver nitrate for 24h, and then the specimens impregnated with silver were washed thoroughly in distilled water and placed in a photo-developing solution for 8h under a fluorescent light.^[4] Then the matches were polished with grain abrasives papers (1000, 1200, 1500, 2000, and 2500) and subsequently washed with plenty of

distilled water to remove any residue. For observation, the specimens were placed in a sample holder to be analyzed by Leica M80 stereomicroscope with wide 8:1 zoom (Heerbrugg, Switzerland).

STATISTICAL ANALYSIS

For inferential statistics, the normality of the data was first assessed with the Shapiro–Wilk test and the Levene test to assess the equality of variances in order to test the investigator’s hypothesis. Subsequently, the means and standard deviations were calculated. The adhesive strength values of the matches of each group were averaged for statistical purposes. To compare the groups, Student *t* test was used for independent samples. The nanofiltration values were evaluated using descriptive statistics. The software IBM SPSS Statistics Base (Chicago, USA), version 22.0, was used to analyze the results.

RESULTS

In this investigation, a sample of 48 teeth was evaluated, which were distributed in four groups (n = 12), and there was no specimen lost during the experimental phase. Table 1 shows that when comparing the adhesion strength of the different techniques, it was found that the highest resistance was in the ethanol technique without PF (group 4) with an average of 31.26 ± 10.26MPa, whereas the lowest value was found in the water technique with PF (group 1), which had 22.59 ± 12.27MPa. When performing inferential statistics, it was evidenced that there were statistically significant differences between both techniques with a value of *P* < 0.05 [Graph 1].

On the other hand, Figure 2 shows that when comparing the nanofiltration it was found that the highest percentage was in the 25% - 50% category, which corresponds to 33.3% of the samples in the group, while in group 2, it was presented with the highest frequency in the 50% -75% category with a prevalence of 33.3%.

DISCUSSION

This study wished to take another step in the optimization of dental adhesion using a contemporary adhesive system with the technique of washing with ethanol in vital pieces, particularly initiating an

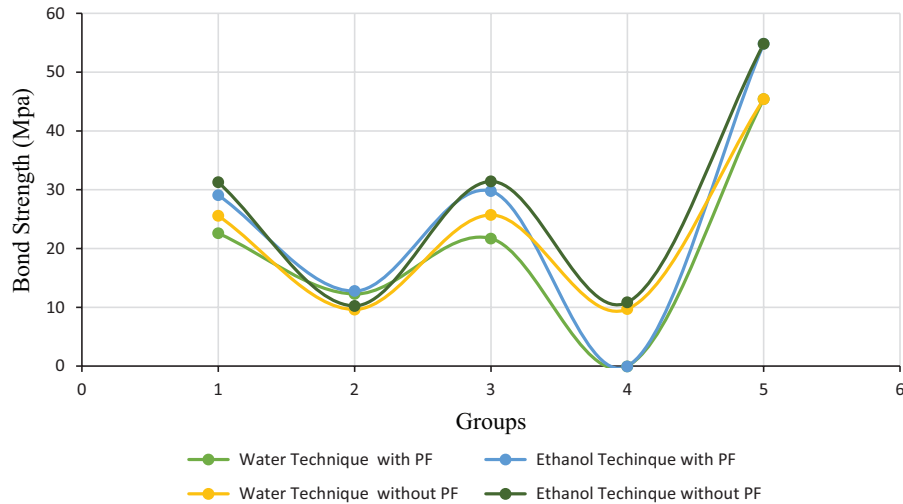
Table 1: Comparison of adhesive resistance of different techniques

Groups	Mean	SD	Median	Min	Max	<i>P</i> *	<i>P</i> **
Water technique with PF (Group 1)	22.59	12.27	21.7	0	45.4	>0.05	0.018
Ethanol technique with PF (Group 2)	29.08	12.76	29.75	0	54.8	>0.05	
Water technique without PF (Group 3)	25.56	9.64	25.7	9.7	45.4	>0.05	0.014
Ethanol technique without PF (Group 4)	31.26	10.26	31.41	10.84	54.8	>0.05	

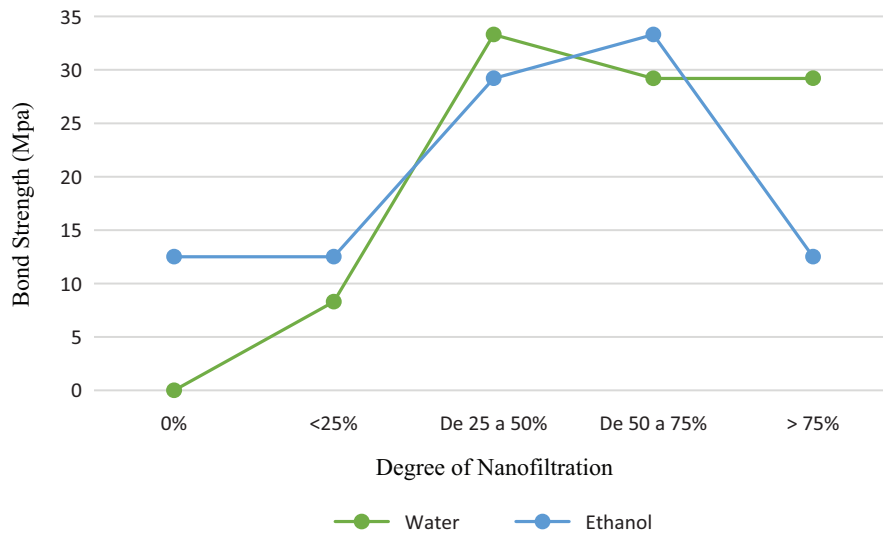
All groups’ bond strengths were measured in MPa. PF = premature failures

*Shapiro–Wilk test *P* > 0.05: all groups presented normality

**Student *t* test *P* < 0.05 statistically significant



Graph 1: Adhesive resistance to microtraction using ethanol wet bonding technique



Graph 2: Nanofiltration using different bonding techniques

investigation with the 3M Single Bond system. Previous research has focused on determining the benefits that result after using the wet ethanol adhesive technique, both on extracted and vital teeth.

Authors such as Kuhn *et al.*^[9] concluded that the technique of complete dehydration with ethanol in vital parts is very sensitive to the residual presence of water when we want to apply a purely hydrophobic (experimental) adhesive system. Their results showed a decrease in adhesive strength in vital parts but not in pieces worked *in vitro*.^[7-9] However, in this study the values obtained in the ethanol wash group are higher (with PF 29.0 MPa [group 2]; without PF 31.26 MPa [group 4]) than those obtained with the conventional washing technique (with PF 22.5 MPa [group 1]; without PF 25.5 MPa [group 3]). This may be because the adhesive used for this investigation has a presence of mixed monomers, which would easily allow the

entry of hydrophobic and hydrophilic monomers into the ethanol wash group. Regarding nanofiltration, our antecedent presented significant differences between the water and ethanol groups, worked in vital pieces, which does not match our results ($P = 0.294$). Later studies with a larger sample quantity could corroborate or not with more certainty the results obtained here. Meanwhile, Li *et al.*^[5] evaluated contemporary adhesive systems (mixture of monomers, hydrophobic, and hydrophilic) under the protocol of wet adhesion with ethanol with simplified dehydration (1 min, waste water from the wash) protocol *in vitro*. Compared to wet adhesion with water, the ethanol wash groups produced similar or higher values of μ -tensile bond strength (μ TBS) at 24 h, because contemporary adhesive systems with monomer mixtures are more tolerant to the residual presence of water when used within an ethanol wash protocol with simplified dehydration. What agrees with this study because the values obtained with

the ethanol wash group are higher (with PF 29.0 MPa; without PF 31.26 MPa) than those obtained with the conventional technique (with PF 22.5 MPa; without PF 25.5 MPa), reaching the same conclusion about the tolerance of wastewater, either due to the presence of pulp pressure (vital parts) or by using the simplified dehydration technique.^[8]

Based on this background, the present study set out to seek response to an adhesive behavior and thus be able to find an improvement in the clinical part. For this reason, we worked on vital pieces using the simplified ethanol wash protocol that allows us to use an adhesive system containing both hydrophilic and hydrophobic monomers. The results of adhesive strength obtained are consistent with what was expected because it was found to be significantly higher in parts restored with the technique of washing with ethanol.^[8,9]

Nagpal *et al.*^[10] also studied the effect of wet ethanol adhesive technique on the immediate and long-term adhesive strength. They used two adhesives (Tetric N Bond and Solobond M, Liechtenstein, Germany) and two washing techniques (water and ethanol). Half of the samples were evaluated immediately in μ TBS and the other half after 6 months of storage in distilled water. In the immediate tests, no significant differences were found regardless of the adhesive used or the washing technique (Tetric N Bond E: 24.57 MPa and A: 21.71 MPa). In the tests carried out at 6 months, the μ TBS decreased dramatically in the wet adhesion group in water, whereas the μ TBS was maintained in the wet adhesion group in ethanol (Tetric N Bond E: 25.31 MPa and A: 12.23 MPa). Unlike this background, in this investigation the adhesive force was significantly different between the water and ethanol groups treated immediately with a two-step etching and washing adhesive (Single Bond 2, E: 29.0 and A: 22.5 MPa).^[10] Guimarães *et al.*^[11] examined the immediate adhesive strength of two contemporary etching and washing adhesive systems (Adper Scotchbond Multipurpose or ASBM, and Single Bond 2 or SB) on demineralized dentin saturated with water and/or with ethanol on extracted teeth using a simplified dehydration protocol (30s). For both adhesives, no significant differences were found; ethanol group (42.9 ± 4.5 MPa) and water wash group (44.9 ± 6.5 MPa) unlike this study where the adhesive strength had significant differences between water techniques and technique of simplified dehydration with ethanol. This results can make a difference in water replacement with the ethanol in the dentin substrate.^[11]

A further step in the optimization of dental adhesion would be the achievement of hydrophobic resinous

monomers completely infiltrated in a demineralized dentin matrix. The ethanol wet adhesive technique brings us closer to achieving this goal; therefore, the replacement of water with ethanol in the demineralized adhesive bed, ready for infiltration of monomers, already allows us to use hydrophobic monomers for this last purpose. *In vitro* tests have been satisfactory in general, but difficulties have arisen regarding its clinical application. Among the difficulties that have arisen are the application time of the complete dehydration protocol, which is not very clinically viable because it needs 3–4 min of washing with different concentrations of ethanol, and the constant contamination of the adhesive bed with water from the pulp chamber, due to the pulp pressure existing in the vital teeth. Due to the difficulties that we still encounter for the clinical extrapolation of the joint use of the wet adhesive technique in ethanol and hydrophobic adhesives, contemporary adhesive systems that contain a mixture of hydrophilic and hydrophobic monomers still clinically prevail.^[12-15]

On the other hand, the results of some studies confirmed that the technique of wet bonding with ethanol improved the quality of the interface. And that the simplified protocol of wet dehydration of ethanol with adhesion strength and stability over time was similar to those obtained with the staged ethanol technique and may have an alternative strategy to achieve the union of the resin to the cement of the root. This challenges the current paradigm of the wet bond requirement for etching and rinsing, creating new alternatives to improve the clinical longevity of the resin–dentin interfaces.^[16-20]

The main limitation of this study was that it used adhesive systems containing mixtures of hydrophilic and hydrophobic monomers (etching and rinsing in two steps), and this could affect the stability of the adhesive over time. Due to the better compatibility of hydrophilic monomers with ethanol, it is sought to determine whether the adhesive stability of contemporary systems can be improved if used with an ethanol wash protocol.^[21-24] However, this study is important because the results can further optimize the performance of dental restorations with the adhesive systems currently used by dental professionals.

This study has a very important clinical impact because an improvement was evidenced when applied under the wet ethanol adhesive technique on vital teeth, particularly initiating an investigation with the adhesive system used. Another importance of this research is that when evaluating the results of the application of this procedure in newly extracted vital teeth, where

the critical factor of intrapulp pressure is added, it is a factor that has not been possible to accurately represent in laboratory tests. The intrapulp pressure is responsible for a continuous movement of the dentinal fluid from the pulp chamber to the different layers of the dentin, through the dentinal tubules. According to our results, this fact will be responsible that the moistened dentine is always present in interacting with the monomers of the adhesive.

CONCLUSIONS

According to the results of the study it is concluded:

1. It is concluded that the adhesive force for the water wash technique was 22.5 and 25.5 MPa with and without PFs, respectively. Meanwhile, the adhesive force for the water wash technique was 29.08 and 31.2 MPa with and without PFs, respectively.
2. In both cases, the adhesive strength showed superiority in the ethanol wash group, which determines that this technique presents greater tolerance to the residual presence of water.
3. Finally, in relation to nanofiltration we found that there were no significant differences between the groups.

ACKNOWLEDGEMENT

Not applicable.

FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICTS OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

Study conception (SC, GA), data collection (SC, GA, DS, RW), data acquisition and analysis (FMT, DAT, RW), data interpretation (DS, GA, RW, FMT), manuscript writing (FMT, DAT, RW, GA).

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

This project is exempted from ethical approval due to it was an experimental *ex vivo* study.

PATIENT DECLARATION OF CONSENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data that support the study results are available from the author (Dr. Frank Mayta-Tovalino, e-mail: fmaytat@ucientifica.edu.pe) on request.

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