

IN VITRO EVALUATION OF DENTINE ADHESIVE RESISTANCE AFTER APPLYING TWO IRRIGATION SEQUENCES

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ABSTRACT

Objective: To evaluate the bond-strength (MPa) of an adhesive system to intracameral dentin after 2 different endodontic irrigation sequences.

Material and Methods: An in vitro double-blinded experimental cross-over study was conducted. 23 teeth were extracted, and sectioned, exposing the pulp chamber. Samples were separated into three groups: the first group (control) used a 0.9% saline solution; in the second group, the conventional sequence of irrigation of the University of Valparaíso was employed (5% NaOCl - 18% EDTA-0.9% saline solution); and finally the third group, the experimental sequence was employed (5% NaOCl, 18% EDTA and 15% saline solution). The sealing technique was then conducted using universal 3M ESPE Single Bond adhesive and Filtek Z350 resin. Subsequently, the bond-strength test was performed in a microtensile, until fracture.

Results: The control group had the highest values of frequency of adhesive and cohesive failure compared to the conventional and experimental group but presented a statistically non-significant association. As for the irrigation protocols, they did not show a major difference in their adhesive bond-strength when compared.

Conclusion: The results of this study conclude that the adhesive resistance is not significantly modified by different irrigation protocols.

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INTRODUCTION

Cleaning and shaping of the Root Canal System (RCS) is one of the main predictors of endodontic success¹, however, it also may alter the proper coronal sealing. Immediate coronal sealing after endodontic treatment is a powerful tool to avoid microleakage and to enhance prognosis. Nowadays, coronal sealing is based on different adhesive systems allowing more dental tissue's conservation, increment the fracture resistance of the remaining dental structure, optimize the coronal restoration's retention and a significant reduction of microleakage through micromechanical bonding of restorative materials to previously conditioned dentin. Nonetheless, dentinal adhesion might be jeopardized by a numerous factors, being the effect of irrigating solutions on teeth during the endodontic treatment one of the steps which may alter the chemical composition of the intracameral and radicular dentinal surface, affecting its interaction with the adhesive materials and their sealing². Adhesive failures can also be found in different levels. Cohesive failures are the most important type of them, because they can occur at the composite-adhesive level⁵, on the other hand, some adhesive failures may occur among two different structures (dentin-adhesive interface or dentin-resin interface)⁶. Sodium Hypochlorite (NaOCl) is a well-known endodontic irrigant which can cause degeneration of the dentin due to dissolution of collagen, carbonic bonds' breaking and protein's structure disorganization. Decrease of bonding force between dentin and adhesive systems is also described, this might be explained because of the collagen's removal from dentin surface.

Ethylenediaminetetraacetic acid (EDTA), is another well-known endodontic irrigant, which has a chelating effect, enhancing dentin demineralization. This compound is used mainly during the final irrigation of endodontic treatment to permeabilize canals and to remove the smear layer once the biomechanical preparation is concluded⁷.

Coronal sealing and bonding among the final restoration and remaining dental tissues are still problem to practitioners^{2,3,4}. There is lack of information of dentin surface and adhesive system reaction after different endodontic irrigation protocols, so it is possible to think, different protocol sequences will have diverse effects and therefore, various bond strength outcomes. This study aims to evaluate the adhesive bond strength (MPa) of an adhesive system to intracameral dentin after the application of 2 different endodontic irrigation protocols.

MATERIAL AND METHODS

An in vitro double-blind experimental cross-over study was conducted to estimate the adhesion resistance of the adhesive bond after the application of two irrigation protocols and a control group. (Figure 1)

23 teeth were extracted for therapeutic reasons at the surgery clinics of the Faculty of Dentistry of the University of Valparaíso, informed consent was obtained from all participants before the extractions, to donate the teeth to this study.

All the extractions were effectuated within a period of no more than 6 months, during 2017, they did not present vertical fractures, previous endodontic treatment, or large coronary destruction that compromised the cervical third. They were placed in distilled water posterior the extraction. Each tooth was cut into thirds in mesiodistal and crown-apical directions, taking the middle third of each tooth as a reference, to expose the roof and floor of the pulp chamber. Two more additional cuts were performed, the first one 0.5mm apical to the pulp chamber ceiling mesio-distally and horizontally, the second one 0.5mm coronal to the floor of the pulp chamber. The teeth were sectioned with a low speed diamond disc saw to expose the walls of the pulp chamber. (Figure 2)

With the pulp chamber exposed, all pulp was eliminated using a sharp end manual instrument. Next, samples were divided into three groups of 23 cuts each. Two of the groups were operated on

with a different irrigation sequence and the remaining group (control) received only irrigation with 0.9% saline solution (Figure 1). Following this, the adhesive system and composite resin were applied.

Walls of the pulp chamber were washed with 10mL of distilled water at room temperature and blown dried for 5 seconds. Later, they were irrigated with water for 30 seconds and dried with sterile gauze on the surface, a layer of 3M ESPE Universal Single Bond Adhesive (self-etching) was applied with a microbrush, eliminating excesses with a jet of air, later then each layer was photopolymerized for 20 seconds with an Ivoclar Vivadent LED curing lamp model Bluephase NMC with a light intensity of 800 MW / cm² calibrated between 430-490 nm. The insertion of the composite resin, Filtek Z350 (3M) was carried out with a manual instrument for resin (Hu-Friedy), placing layers of 2 mm thickness to seal the pulp chamber and each layer was light-cured for 40 seconds with the same lamp of light curing. Then a cut with a low-speed diamond saw was performed, pieces of 1mm by 1mm at the level of the walls of the pulp chamber. The cut was made in a vestibular side-lingual side direction (horizontal), including dentin and composite resin (Figure 2 and 3). Each specimen was labeled according to an endodontic irrigating group; then the bond-strength test took place in a microtensile microtensiometer OM 100, which is a tool manufactured by BISCO Inc, used to test the resistance of adhesives.

Samples were obtained in an environment with controlled temperature and humidity. The specimens were fixed at their ends to a test device, using cyanoacrylate. Each one of these were attached to the fixtures of the universal testing machine; tension was performed, and adhesive failure was calculated until the specimen fractured. Each failure was expressed in megapascals (MPa), being able to evaluate the adhesive bond strength. After the experimental phase, the teeth were destroyed according to the biosecurity protocol.

Figure 1: Study groups distribution

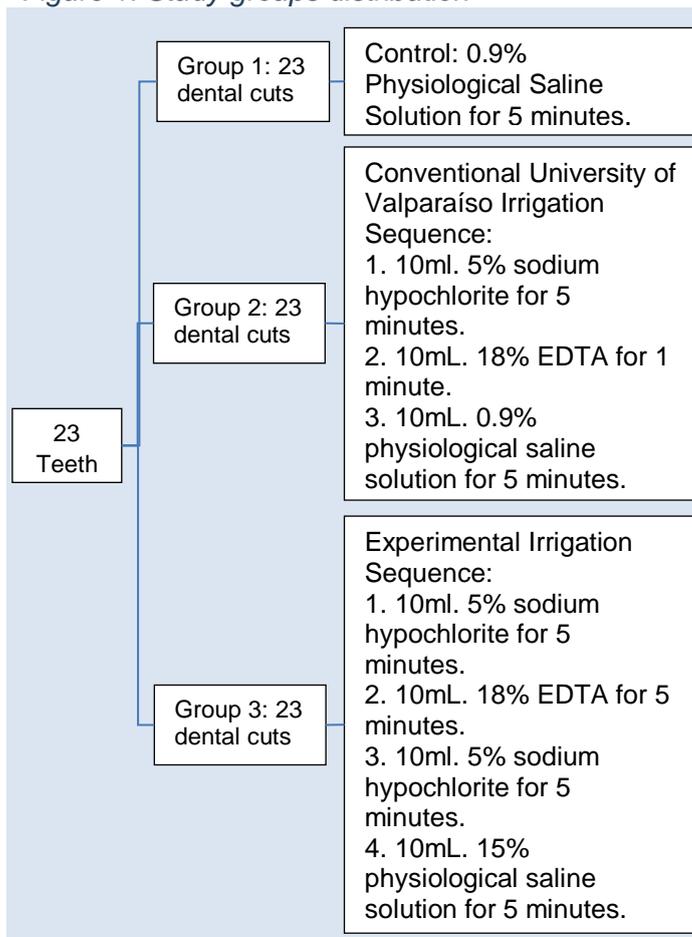
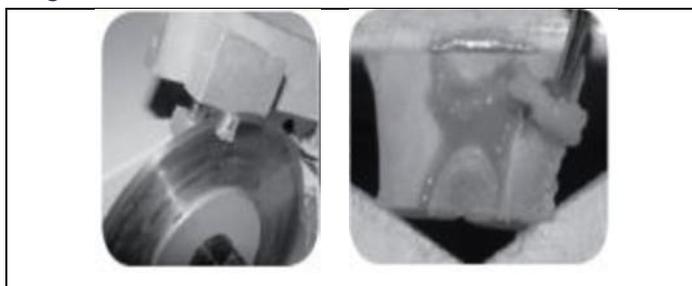
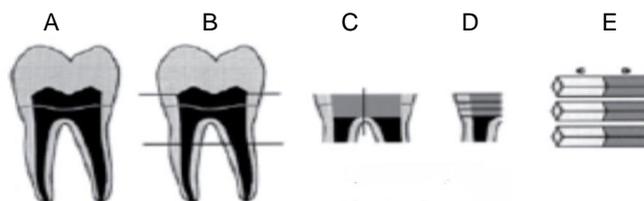


Figure 2: Sectioned teeth



Left: low speed diamond disc; Right: exposure of the walls of the pulp chamber.

Figure 3: Schematic Representation of the sample collection



(A) Mesiodistal cut of the teeth. (B) Pulp chamber cut. (C) Placement of intracameral composite. (D) Cutting of specimens. (E) Finished sample, 1mm area.

Table IV: Chi-square value for association of variables.

Failure	Control	Conventional Sequence	Experimental Sequence	Total
Adhesive	0.0552	0.0464	0.0936	0.1953
Cohesive	0.0170	0.0091	0.0233	0.0494
Mixed	0.0398	0.0416	0.0755	0.1569
Total	0.0112	0.0971	0.1924	0.4016

The variables to be measured in this study were irrigants, adhesive failure, and adhesive bond strength (MPa). Data were registered into an excel database, and the statistical analysis was carried out later with the Stata 13.0 software. The Bartlett and Shapiro-Wilks test were used to determine homogeneity and normality and then a test of equality of medians to determine if there were significant differences between groups. Hypothesis testing for two means was employed. A significance level of 95% was used with a statistical power of 90%. The standard deviation was 1.27 MPa and a minimum difference of 0.315 MPa.

The evaluators were calibrated on what should be considered an adhesive failure, based on operational definitions. Two were the evaluators of the failure, for this reason, the Kappa concordance test was performed, and a minimum of 0.81 was considered to be acceptable.

For the bonding strength test, there was not required the calibration due to the use of microtensile.

RESULTS

During the experimental phase occurred losses both in the conventional irrigation sequence and control group, attributed to fractures of the

test bodies at the time of their installation in the microtensile. The data obtained from the measurements with the microtensile, posterior to the bond-strength test in Mpa. (Table I) The collected data was grouped according to the type of failure they presented, as shown in Table II. (Table II)

Table II: Frequency of failures associated to the irrigation sequence.

Failure	Control	Conventional Sequence	Experimental Sequence
Adhesive	67	76	85
Cohesive	15	2	6
Mixed	18	22	9

The chi-square test was performed to determine the association between the failures and the irrigation protocol and to obtain the observed and expected frequencies (Table III).

With these data, it was possible to arrive at the calculated chi-square value, which was calculated with a significance level of 5% to compare it with the critical chi-square.

The data obtained (Table IV) indicate that the calculated chi-square value is less than the critical chi-square value, for that reason there is not a statistically significant association between variables.

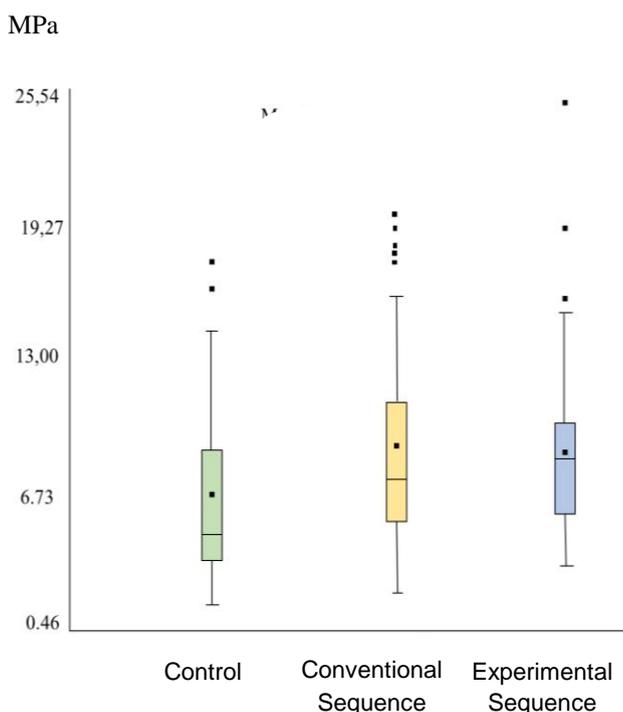
Table III: Observed and expected frequencies for association between variables.

Failure	Control		Conventional Sequence		Experimental Sequence		Total	
	F.E	F.O	F.E	F.O	F.E	F.O	F.E	F.O
Adhesive	0.50	0.67	0.59	0.76	1.18	0.85	2.28	2.28
Cohesive	0.20	0.15	0.24	0.20	0.49	0.60	0.95	0.95
Mixed	0.28	0.18	0.33	0.22	0.67	0.90	1.30	1.30
Total	1.00	0.22	1.18	1.18	2.35	2.35	4.53	4.53

F.O = Observed frequencies; F.E = Expected frequencies

Figure 4 shows the data of the three groups did not follow a normal distribution. Control group and the conventional group are asymmetric upwards and the experimental group asymmetric downwards, however, there is no statistically significant difference among bond-strength. (Figure 4)

Figure 4: Distribution of the sample data



DISCUSSION

The main finding of this study is that the implementation of different sequences of irrigations during endodontic treatment has no clinical relevance on bond-strength (Mpa).

The design of this study had the benefit of using the same body of experimentation subjected to the two irrigation sequences used, plus a control, therefore selection biases were avoided. Furthermore, investigators were calibrated on means of bond-strength obtaining a concordance of 0.81, also it was used a OM 100 microtensile, which is considered a low cost, safe, easy to handle, and widely used in dentistry investigation tool⁸.

The results of this study indicated that the control group had the highest values in the

frequency of adhesive and cohesive failure compared to the conventional and experimental group, but even when it has a statistically non-significant association, its clinical relevance must not be disregarded, due to this irrigation protocol may be altering negatively dentin by the use of less NaOCl, avoiding a coronal sealing, diminishing prognosis, furthermore, independent of the use of NaOCl, adhesion force tends to decrease after an endodontic treatment, reporting a decrease of up to 23% in the bond-strength⁴.

The results of this study revealed that the control group showed significantly lower adhesive bond strength. As for the irrigation protocols, they did not show a major difference in their adhesive bond-strength when compared, this is consistent with what was expressed by Nagpal et al, 2014, where the EDTA-Hypochlorite combination showed a higher adhesive bond strength than controls with physiological saline solution in the use of self-etching adhesive, this may be due to the effect on the hybrid layer formed on the dentin surface, which would help improve adhesion⁹. However, these results are discordant with Carvalho et al. 2017, who stated that adhesive systems were not influenced by any endodontic irrigating protocol; Only the "adhesive system" factor was statistically significant. Compared to our results, where there is a difference between experimental groups, not being able to affirm that endodontic irrigation protocols affect the bond strength¹⁰. This could have happened because only the self-etching adhesive system was used. In different studies, conventional adhesives report high levels of bond strength compared to sixth and seventh-generation self-etching adhesives, due to the formation of water vesicles at the adhesive interface¹¹.

Cecchin et al., 2010, proposed that higher adhesion values are obtained using adhesive systems on dentin that has not previously been treated with NaOCl and EDTA irrigation solutions; the results on said study revealed the irrigation with 1% NaOCl during 1 Hrs (applied every 5 minutes) decreased the bond-strength 13. One possible explanation for this is due to the superficial morphology of NaOCl treated dentin,

where the smear layer is not eliminated, and thus, dentinal tubules are not exposed, added to the formation of free radicals and oxidation produced by NaOCl on the tooth surface, where the presence of oxygen limits the properties and polymerization of adhesive systems.

Coinciding with what was described by Barutcigil et al., 2012 on the fact that if there is a relationship between the application of EDTA in different concentrations with a reduction in the adhesive bond strength, considering that any alteration of calcium (Ca^{+2}) concentration may cause a modification of the dentin composition and therefore in its mechanical characteristics and bond-strength to biomaterials¹⁴.

Adhesion to dentin depends on the presence of residual Ca^{+2} in the bonding area, as evidenced by a significant reduction in bond-strength of some adhesive materials caused by partial depletion of superficial Ca^{+2} .¹⁵ This is why the adhesive bond strength values are expected to be lower after endodontic irrigation protocols have been performed, this could happen due to differences in the methodology implemented¹⁶.

Because this is an in-vitro study, results obtained do not represent conditions present in the oral environment, although dehydrated dentin shows a lower resistance to fracture¹⁴, when conducting the study in a clinical context, multiple factors could also increase the possibility of adhesive failures, such as the filling materials used, or the application of absolute isolation.

It is suggested to expand the sample size and their characteristics to allow better evidence of coronal sealing after endodontic treatments. Also, it is recommended to consider the storage time in distilled water, because it could influence the results of bond strength and adhesive failure. It is advised to use different adhesive systems since their various compositions would alter dentin differently.

CONCLUSION

It is concluded that bond strength is not modified by different irrigation protocols, even

when between groups there are significant differences. Factors that could explain these results were not using different adhesives systems that could have acted differently based on their different properties and interactions with dentin previously treated by the irrigation protocols, hence altering the function of the hybrid layer during postendodontic treatment restoration.

CONFLICT OF INTEREST

The authors declare not having any conflict of interest.

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