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INFLUENCE OF OZONATED WATER APPLICATION ON RESIN COMPOSITE ADHESIVE RESISTANCE

***[1]Renata Takashiba BORBA, [2]Poliana Maria de Faveri CARDOSO, [3]Júlio Katuhide UEDA, [3]Marcio José MENDONÇA, [3]Paula BERNARDON, [4]Rafael Andrade MENOLLI and [3]Veridiana CAMILOTTI**

[1]MS of Dental School, Western State University of Paraná, UNIOESTE, Cascavel, PR, Brazil
[2]Student of Dental School, Western State University of Paraná, UNIOESTE, Cascavel, PR, Brazil
[3]Professor of Dental School, Western State University of Paraná, UNIOESTE, Cascavel, PR, Brazil
[4]Adjunct Professor, Professor of Pharmaceutical School, Western State University of Paraná, UNIOESTE, Cascavel, PR, Brazil

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***Corresponding author: Renata Takashiba BORBA**

ABSTRACT

Objective: To evaluate the influence of different cleaning solutions on the bond strength of an adhesive system and a composite resin. **Method:** 80 bovine dental crowns were selected, divided into four groups (n = 10): SS = saline solution; CHX = 2% chlorhexidine; EDTA = EDTA; OW = ozonated water (4ppm). Each group was subdivided into 2 storage periods (24h and 30 days). Then, three composite bulk fill flow resin cylinders were made using a Tygon matrix with 1 mm internal. The photoactivation was performed by LED light apparatus for 40 seconds. The micro-shear bond strength test was performed in a universal test machine (EMIC). The data were submitted to ANOVA 2 criteria statistical analysis, followed by the Tukey test, $p < 0.05$. **Results:** There were no statistical differences between the solutions studied. **Conclusion:** Different solutions previously used for acid etching do not interfere in the bond strength.

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INTRODUCTION

The aesthetic composite resins proprieties promote different therapeutic indications such as restorations in anterior and posterior teeth, direct facets and fissures sealing (Ferracane, 2011). For the longevity of any of these indications it is fundamental to obtain an adequate union between the restorative materials and the dental structure (Martins, 2008).

To increase the adhesive system bond strength with the restorative material and with the dental surface, it is important to perform proper cleaning of the dental cavity after its preparation. For even with the total removal of the carious tissue, there are bacteria that remain in the prepared cavities and these can penetrate through the material-tooth interface. Thus, the use of an antimicrobial agent in the disinfection of contaminated tooth walls or restorative materials capable of inactivating these bacteria has been recommended to avoid recurrence of caries and to improve the retention of restorative materials to dental structures, reducing microleakage in restorations (Silva and Gill, 2013). Commonly used cleaning agents are 2% chlorhexidine (CHX), EDTA and saline solution. CHX is the widely used oral antiseptic, with low toxicity and broad-spectrum antibacterial activity, which may reduce or eliminate postoperative sensitivity. It has action on Gram positive and Gram negative, aerobic and anaerobic bacteria (Hajizadeh, at al., 2013). EDTA is a chelating agent that forms a stable and soluble complex with dentin calcium and is useful in smear layer removal (Montagner, *et al*., 2015).

In the search for new substances with antimicrobial action, the development of research using ozone, in the gas form and also as a solution, has been growing in recent times. Ozone is a colorless gas with characteristic odor and antimicrobial capacity. This gas forms oxidative radicals in the water presence, which acts on the cell membrane, affecting the osmotic equilibrium, causing microorganisms death (Tiwari, *et al*., 2017; Savab, *et al*., 2018).

It is possible to find in the market devices that produce ozone by means of a generator that associates an oxygen atom with an O^2 molecule, expressed by $O + O^2$, forming the ozone (O^3). This unstable molecule is generated by the passage of an electric discharge through pure oxygen (Azarpazhooh and Limeback, 2008). In this way, the objective of this research was to evaluate the influence of different cleaning solutions on the composite bond strength stored for 24h and 30 days in distilled water.

MATERIALS AND METHODS

Study Design: According to the sample calculation, using a completely randomized design with the aid of bioestat program 5.3, a minimum of 6 (n) teeth per group was defined, with power analysis equal to 90% and a standard error of 0.01. For the sake of safety, 80 teeth were divided into four groups according to the cleaning solution (n = 10), the description of the materials used are in table 1 and 2 and the distribution of the groups is shown in Figure 1.

Three Bulk Fill Flow cylinders (Opus Bulk Fill Flow, FGM, Joinville, SC, Brazil) in a single photoactivated increment for 40 seconds with 1200 mW / cm² LED light apparatus (Bluephase EDTA, irradiance: 1200 mW / cm², IvoclarVivadent , Barueri, SP, Brazil) were fabricated using a Tygontubing (Tygontubing, TYG-030, Saint-Gobain Performance Plastic, MaimeLakes, FL, USA) matrix with 1 mm internal diameter and 2 mm height. Then stored for 24 h and 30 days at relative humidity 37 ° C.

Ozonated water production: 200mL of ozonated water with 4ppm was produced with the aid of an ozone generator (O & L1.5 RM, Ozone & life, São José dos Campos, SP, Brazil). The ozone in this system was generated by the action of electric current on the molecules of high purity oxygen, supplied by a cylinder containing reducing valve with manometer. For the production of ozonated water, 200 mL of autoclaved distilled water was placed in a glass tube system coupled to the ozone generator, in which the gas was bubbled into the water at 4ppm.

Table 1. Description of technical groups

Groups (24h and 30 days)	Application technique
SS – Saline Solution CHX- Chlorhexidine 2% EDTA- EDTA OW- Ozonized water solution	1. Preconditioning with saline solution; chlorhexidine 2%, EDTA, and ozonated water 2. Conditioning with 37% phosphoric acid for 15 seconds; 3. Washing for 30 seconds and air-dry for 10 seconds; 4. Application of 2 layers of the Universal Single Bond adhesive (3M ESPE) with the aid of a disposable brush for 10 seconds in each application; 5. Air jet for 10 seconds; 6. Photoactivation for 20 seconds.

Table 2. Materials used composition

Saline Solution	0,354g de Na+; 0,546g de Cl-; pH=6,0	Eurofarma
Chlorhexidine digluconate 2%	2% Gluconate; Water; Glycerin; Ethanol; Polysorbate 20; Sodium saccharate.	Rioquímica, São José do Rio preto, SP, Brazil
Trisodium EDTA	Disodium ethylenediaminetetraacetic acid; Sodium hydroxide; Distilled water.	Biodinâmica, Ibiporã, PR, Brazil.
Ozonated water (4 ppm)	Ozonated water solution in the 4ppm concentration.	Fabrication Unioeste, Cascavel, PR, Brazil
Condac 37%	37% orthophosphoric acid	FGM Joinville, SC, Brazil
Single Bond Universal	Alcohol, water, silica treated silica, 10-MDP, Bis-GMA, 2-hydroxyethyl methacrylate, decamethylene dimethacrylate, acrylic and itaconic acid copolymer, camphorquinone, N, N-dimethylamonoethyl, methyl ketone, silane	3M ESPE, St. Paul, MN, USA.
Filtek Bulk Fill Flow Resin	Silane treated silica, silane treated silane, diurethane dimethacrylate (UDMA), bisphenol A polyethylene glycol diether dimethacrylate, bisphenol A diglycidyl ether dimethacrylate (BisGMA), treated silanized zirconia, polyethylene glycol dimethacrylate, triethylene glycol dimethacrylate (TEGDMA), 2,6-Di -tert-butyl-p-cresol	3M ESPE, St. Paul, MN, USA.

Test specimens preparation: A total of 80 bovine incisor teeth, recently extracted and free from periodontal tissue, were stored in 1% thymol solution under refrigeration at 7ºC. The teeth were sectioned, in high rotation on abundant irrigation with diamond tip nº 4138 (KG Sorensen, Cotia, SP, Brazil), to separate the crown from the root. Then, the crowns were included in PVC tubes with acrylic resin keeping the vestibular surface free. After inclusion, each crown was taken to the politriz for wear, polishing and obtaining a flat dentin face. The samples sanded with silicon carbide silica sand 240, 400 and 600, then the elements were stored in relative humidity until the moment of their use. Prior to the restorative treatment, the teeth were randomly divided according to the cavity cleaning material (n = 10): SS = physiological saline; CHX = 2% chlorhexidine; EDTA = EDTA; OW = ozonated water. The groups were subdivided according to the storage time in distilled water (24h and 30 days). After dentin cleaning, the groups were submitted to adhesive treatment.

To ensure sterilization and cleaning of the system, the autoclaved distilled water contained in the glass tube was ozonated for twenty minutes. After this time, this water was discarded, and the same volume was replaced in the glass tube coupled to the ozone generator, to produce the water to be used in the experiment. Then, the titration test was performed to prove the concentration obtained, then the ozonated water manufacturing procedure was performed again.

Micro-shear test: After the period of 24h and 30 days, the composite resin cylinders were tested in a universal testing machine (EMIC) with a load cell of 20 kg. The shear load using a 0.2 mm orthodontic wire in the form of a loop was applied to the base of the cylinders at a speed of 0.5 mm / min until the union get broken. The microcrystalline bond strength was calculated and expressed in MPa.

Failure Mode: The fractured surfaces resulting from the μSBS test were cleaned in an ultrasonic bath and gold-sputtered for

Table 3. Bond strength values (mean ± standard deviation, MPa) according to the experimental groups

Time	Mean ± Standard Deviation							
	SS		CHX		EDTA		OW	
24 hours	3.3750 ±	1.3246 [A]	3.8937 ±	1.8390 [A]	3.7933 ±	1.6209 [A]	2.8843 ±	1.4060 [A]
30 days	3.2380 ±	1.3266 [A]	3.8697 ±	1.8459 [A]	3.6517 ±	1.4494 [A]	2.8670 ±	1.4082 [A]

* In each row and column, equal letters mean there is no statistically significant difference ($p < 0.05$).

Table 4. Frequency distribution of failure modes (%): AD, adhesive failure; M, mixed CR, cohesive failure in composite resin; CD, cohesive failure dentin

24h	G0	G1	G2	G3
CD	13,3	0	0	10
AD	56,7	86,7	96,7	60
M	40	33,3	20	46,7
CR	23,3	13,3	13,3	23,3
30days				
CD	0	0	0	0
AF	3,33	36,66	30	0
MF	96,66	63,33	70	100
CR	0	0	0	0

examination by scanning electron microscopy (EVO MA 10, Carl Zeiss). Failure modes were divided into five types: 1) adhesive failure; 2) mixed failure; 3) cohesive failure in composite resin; and 4) cohesive failure in dentin.

Statistical test: The mean values obtained from the bond strength for each test specimen of the experimental groups were initially submitted to the test of adherence to the D'AGOSTINO normality curve, with a positive result. Subsequently, the values obtained were submitted to the ANOVA 2 criteria test, followed by the Tukey test ($p < 0.05$).

RESULTS

Using the BioEstat 5.3 statistical program, the D'AGOSTINO Normality test was performed with a confidence level of 95% (significance level (α) of 5%). The deviation obtained in all groups and thirds is contained within the limits of the critical values tabled at the level of significance (α) of 5%, translating a $p<0.05$. The variables in question had a normal distribution. Subsequently, variance analysis (ANOVA) showed a statistically significant difference between the groups ($p<0.05$), but the Tukey test did not detect this difference. A tabela 4 mostra os resultados do tipo de fratura. É possível observar maior prevalência de fratura adesiva para todos os grupos, seguida de fratura mista. A fratura coesiva de dentina (CD) só ocorreu para o grupo do soro fisiológico (G0) for the 24-hour period. For the period of 30 days there was a higher prevalence of mixed failure followed by adhesive for all groups. Cohesive failures were not observed.

DISCUSSION

The results presented in this study showed that there were no statistically significant differences between saline solutions, chlorhexidine 2% and EDTA. This is corroborated by a study by Camilotti, *et al*. (2013) and Pelegrine, *et al*. (2010). In a study by Carrilho (2007) shows that the use of chlorhexidine did not change the values of union strength, which coincides with the present study. Different results were found by Breschi, *et al*. (2010), where 2% chlorhexidine decreased the values of union strength in contrast to the other groups, this difference can be explained by these authors having used chlorhexidine after acid conditioning without rinsing and the union resistance test was the In this sense and study by Suma, *et al*. (2017), it was shown that the use of chlorhexidine without its rinsing significantly reduces the bond strength. The fact that the cleaning solutions do not interfere with adhesive resistance results is of great value, since the microorganisms presence can lead in the long term to the adhesive restorations failure. Takahashi and Nyvad (2016), reported the presence of bacteria inside dentinal tubules after cavity preparation. In the study by Krunić J, *et al*. (2018) observed that antibacterial effect of OW on residual bacteria after incomplete removal of caries was similar to that of 2% CHX with OW being more biocompatible with pulp tissue. Among the solutions studied the OW has been proposed as an antiseptic alternative, thanks to the powerful antimicrobial action and high biocomputability. OW has great applicability with truly promising results as shown by the work of Anumula *et al*. (2017) who evaluated the effects of mouthwashes on the *Streptococcus mutans* reduction from the dental biofilm. The results indicated that the effects of mouthwashes with ozonated water reduced *Streptococcus mutans* from the dental biofilm, and the longer the exposure time, the greater the bacterial reduction. In the restorative dentistry, Baysan *et al*. (2000) evaluated the ozonated water antimicrobial effects on primary lesions of root caries, against *Streptococcus mutans* and *Streptococcus sobrinus*, for ten and twenty seconds; and in the second study evaluated the antimicrobial effects of ozone on saliva, against the same microorganisms for only ten seconds. Significant reduction of *Streptococcus mutans* and *Streptococcus sobrinus* was observed in the samples of the primary lesions of root caries, in ten and twenty seconds of treatment by ozonated water, in comparison to the samples from the control group.

In the present study the ozonated water solution obtained similar results to the others, which agrees with the research carried out by Pithon and Santos (2010) and Cossellu *et al*. (2015). This finding is quite interesting since the larger amount of O^2 present in the ozonated water could interfere with the composite resin polymerization process and affect bond strength values (Johansson, *et al*., 2007). Perhaps the conditioning with phosphoric acid after the application of OW together with the storage of 24 hours is enough to eliminate the free O^2 promoting the good results found. In this way the antimicrobial advantages of the ozonated water can be united without interfering in the union resistance of the adhesive

system and composite resin. EDTA solution has dentin collagen fibers chelating effect, which can increase the bond strength with the adhesive system, especially in the autoconditions, where the demineralization pattern is lower than the conventional ones (Lima, *et al*., 2018) showed that EDTA improves the bond strength when applied previously to the self-etching adhesive system. As in the present study a conventional adhesive system was used, the chelating capacity of the collagen fibers of the EDTA was without obvious action, since the conditioning with phosphoric acid completely removes the Smear Layer. This finding is in agreement with Sauro, *et al*. (2010) work, that this solution acts like the others when it is used with conventional adhesive system. Carvalho, *et al*. (2017) in their studies also concluded that this cleaning solution does not affect the bond strength. Differently from the results found by Halaur *et al*., 2017, for these authors cleaning with EDTA decreases bond strength. However, these authors used root dentin that has a different tubular configuration of the coronary dentin selected in this study.

Regarding the storage period, 60 days aging is able to provide an aged surface of the composite resin that contains reduced radical activity of monomers functional groups (Padipatvuthikul and Mair, 2007). This phenomenon was found in the work of Kasraei, *et al*. (2016), where 90 days storage in distilled water significantly reduced bond strength results by the micro-shear test. However, in the present study the storage did not influence the values found, perhaps the time of 30 days is insufficient to generate damages in the adhesive interface. The distribution of failure modes showed the prevalence of adhesive and mixed failures, with a prevalence of mixed failures after 30 days storage, probably due to strong bonding at the adhesive interface. However, variations in the failure mode can be reflected by several factors, including the storage media, the test used for bond strength analysis, and the different contents of the restorative materials tested. Other laboratory tests and clinical trials are therefore needed to confirm the data obtained in this in vitro study. In view of the limitations of this *in vitro* study, it can be concluded that ozonotherapy may be a new therapeutic strategy in dentistry due to its antimicrobial activity, biocompatibility and good immune response of the individuals to this solution, clinical work is fundamental to confirm these results.

Conclusion

Within the limitations of this *in vitro* study, we can conclude:

Both variables, cavity cleaning solutions and storage time, do not interfere in the bond strength results.

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