Influence of different phosphoric acids in enamel adhesion

Christopher Cadete de Figueiredo[1]
Diego Alves Cunha[1]
Igor Figueiredo Pereira[1]
Julio Cesar Campos Ferreira Filho[1]
Bianca Marques Santiago[2]
Ana Maria Gondim Valença[3]

ABSTRACT

The aim of this study was to evaluate *in vitro* the shear bond strength of a composite resin after acid etching with phosphoric acid 37% with and without chlorhexidine. Thirty bovine incisors were divided into two groups (n = 15), according to the type of acid etching applied in enamel – G1 (phosphoric acid 37%) and G2 (phosphoric acid 37% with chlorhexidine 2%). The conditioning of the surface followed the manufacturers' recommendations; after this step, there was the application of adhesive and the insertion of composite resin in a rubber matrix with a center hole of 4 mm. The specimens were submitted to analysis of shear strength in a Shimadzu AGS-X Universal Testing Machine with crosshead speed of 1 mm/min and the values measured in MPa. After the test, the Adhesive Remnant Index (ARI) was assessed. Data were analyzed by descriptive and inferential statistics, adopting a significance level of 5%. The mean shear strength reported for the two groups were, respectively, 12.02 MPa and 11.86 MPa (unpaired t-test; p-value > 0.05). As for the ARI, the most frequent scores for G1 and G2 were 3 and 5, with no statistically significant difference between groups (Mann-Whitney test; p-value > 0.05). Under the experimental conditions of the study, we conclude that the addition of chlorhexidine to phosphoric acid 37% did not affect the bond strength to the enamel surface nor the type of failure found.

Keywords: Dental acid etching, chlorhexidine, dental materials, shear strength.

Influência de diferentes ácidos fosfóricos na adesão ao esmalte

RESUMO

O objetivo deste estudo foi avaliar *in vitro* a resistência ao cisalhamento de uma resina composta após condicionamento com ácido fosfórico 37% com e sem clorexidina. Trinta incisivos bovinos foram divididos em dois grupos (n = 15) de acordo com o tipo de ataque ácido aplicado ao esmalte – G1 (ácido fosfórico a 37%) e G2 (ácido fosfórico 37% com clorexidina a 2%). O condicionamento da superfície obedeceu às recomendações dos fabricantes e, em seguida a

1. Undergraduate student, School of Dentistry, Universidade Federal da Paraíba;
2. PhD, Professor of Legal Dentistry, Universidade Federal da Paraíba;
3. PhD, Professor of Pediatric Dentistry, Universidade Federal da Paraíba.

Corresponding Author: Christopher Cadete de Figueiredo. Rua Iolanda Henriques Cavalcante, 19 – Ed. Luciana I Ap. 304. Bairro: Jardim Oceania. Cidade: João Pessoa/PB. CEP: 58037-062. E-mail: christophercadete@yahoo.com.br

esta etapa, realizou-se a aplicação do adesivo e procedeu-se a inserção da resina composta em matriz de borracha com orifício central de 4 mm. Os corpos de prova foram submetidos ao teste de resistência ao cisalhamento em máquina de ensaios mecânicos Shimadzu com velocidade de carga de 1mm/min e os valores registrados em MPa. Após o ensaio, foi observado o Índice de Remanescente Adesivo (IRA). Os dados foram analisados por estatística descritiva e inferencial, adotando-se nível de significância de 5%. A média da resistência ao cisalhamento registrada para os dois grupos foi, respectivamente, 12,02 MPa e 11,86 MPa (teste t não pareado; p-valor> 0,05). Quanto ao IRA, os escores mais frequentes para G1 e G2 foram 3 e 5, não sendo observada diferença estatisticamente significante (Teste Mann-Whitney; p-valor>0,05). Nas condições experimentais do estudo, conclui-se que a adição de clorexidina ao ácido fosfórico 37% não interferiu na resistência de união à superfície do esmalte nem no tipo de fratura encontrado.

Palavras-chaves: Ataque ácido dentário; clorexidina; materiais dentários; resistência ao cisalhamento.

INTRODUCTION

One of the critical stages for the good performance of adhesive systems is the stage of acid etching. The technique of acid etching to prepare the enamel should be rigorously performed so as to avoid diminishing the strength of restorations (1).

Conditioning can influence the quality of adhesion. Therefore, it is necessary to take into account the type of acid to be used, its concentration, its time of exposure on dental surface, and substrate composition (2).

Most adhesives use phosphoric acid at 30 to 40% concentration as a surface conditioner. This substance has been shown to be very efficient in promoting proper adhesion to the dental surface (1) and is widely used in enamel conditioning, because, besides increasing surface roughness, it also decreases the tension of enamel surface, facilitating the penetration of the adhesive (3).

Many candidates for restorative or orthodontic treatments are susceptible to dental caries, gingivitis, and other bacterial diseases. Bacterial contamination may negatively affect the physical qualities and adhesiveness of resinous materials, increasing microleakage at the margins of adhesive restorations (4).

Thus, it has been recommended that enamel conditioning agents should be associated with antimicrobial solutions or those which inhibit bacterial growth (4). Chlorhexidine is an antiseptic and antibacterial agent widely used in dentistry due to its ability to control plaque and gingivitis without forming resistant organisms in the oral flora (5).

Among the uses of chlorhexidine in dentistry, there is its presence in acid conditioners.

A recent study analyzing the antimicrobial activity of phosphoric acid 37%, associated or not with chlorhexidine 2%, against strains of *Streptococcus mutans* (ATCC 25175), *Lactobacillus casei* (ATCC 9595*), S. salivarius* (ATCC 7073), *S. oralis* (ATCC 10557), and *S. mitis* (ATCC 903) found that phosphoric acid 37% showed inhibitory effect on all tested strains. The association of phosphoric acid 37% with chlorhexidine

2% exhibited greater inhibitory effect against *S. oralis*, but not against the other microorganisms (6).

Considering that acid conditioners may show variation in their composition, it is interesting to determine whether the addition of chlorhexidine 2% to phosphoric acid 37% would influence the bond strength of adhesive systems.

Among the tests employed to assess adhesive bond strength of odontological materials to dental surface, one of the most important are the shear bond tests, in which shear bond strength is defined as the maximum tension that a material can withstand before breaking under shear (7).

In shear tests, it has been usually chosen to use bovine substrate, because of its similarity and some advantages compared with human dental tissue, such as: size of crystals, morphology, possibility of obtaining standardized flat surfaces with excellent polishing before bonding, due to the fact that the thickness of the enamel is appropriate for this purpose (8).

Based on the foregoing, the aim of this study was evaluate *in vitro* the shear bond strength of a composite resin to bovine enamel after acid etching with phosphoric acid 37% with and without chlorhexidine, as well as the Adhesive Remnant Index (ARI).

METHODS

Sample

Thirty bovine incisors, which had no cracks and/or fractures, were divided into two groups (n = 15), according to the type of acid etching applied in enamel – G1 (phosphoric acid 37%, Attaque gel, Biodinâmica) and G2 (phosphoric acid 37% with chlorhexidine 2%, Acid gel, Villevie).

Preparation of specimens

In order to define a working area on the medium third of the buccal surface of the tooth, the geometrical center of this surface was marked with a pencil by drawing a longitudinal and a transversal line on the tooth, with their intersection consisting of the center of the buccal surface. With the purpose of flattening the previously demarcated center of the surface, teeth were included in chemically activated colorless acrylic resin, making sure to cover the buccal surface with the resin and pressing the center of the surface against a glass plate.

After being bonded to the resin, the buccal surface of the teeth was worn out with wet and dry sandpaper of decreasing granulation as follows: with sandpaper no. 120, surface was worn out longitudinally, leading to the appearance of an enamel area of approximately 6mm^2. After that, a sandpaper no. 400 was used transversally, then a sandpaper no. 800 was applied longitudinally until reaching an area of approximately

30mm^2. Finally, sandpapers no. 1200 were used to promote a superficial polishing of the enamel. After enamel preparation, the roots and the excess of acrylic resin in the incisal edge of teeth were removed with diamond disc, obtaining then two flat parallel surfaces, which allowed for them to be adjusted in a metallic testing support for shear tests.

Acid etching of enamel and resin bonding

Surface conditioning followed the manufacturers' recommendations. At first, the acid was applied to the dry surface for 30 seconds. Afterwards, two layers of adhesive (Adper Scotchbond 1 XT, 3M® ESPE) were applied and polymerized for 40 seconds and then the composite resin (Z-350 XT, 3M® ESPE) was inserted in a rubber matrix with a center hole of 4 mm. Until the initial polymerization of the material, which lasted for 20 seconds, the washer remained on the bonding site, in order to avoid the displacement of material from this area. After the washer was removed, the cylinder was polymerized for 10 seconds on each of its side surfaces (upper, left side, and right side), and for more 20 seconds on its external surface. Until the moment of debonding, specimens were stored in a humid environment, with relative humidity of 100%.

Shear test

Specimens were submitted to analysis of shear strength in a Shimadzu AGS-X Universal Testing Machine with crosshead speed of 1 mm/min; values were measured in megapascal (MPa), with the rupture strength of the material recorded in Newtons (N) and divided by the bonding area (mm^2).

Evaluation of adhesive remnant index

Subsequently, the Adhesive Remnant Index (ARI) was analyzed in order to evaluate the site of bond failure and the presence of residual adhesive, using the percentage of resin adhered to enamel and attributing ARI scores for each specimen (Bishara, Trulove, 1990), as described in Table 1. Data were analyzed by descriptive and inferential statistics, adopting a significance level of 5%.

TABLE 1 – Adhesive Remnant Index scores.

SCORE	AMOUNT OF ADHESIVE ON THE TOOTH
1	Entire adhesive on the tooth
2	Over 90% of adhesive on the tooth
3	Over 10% and under 90% of adhesive on the tooth
4	Under 10% of adhesive on the tooth
5	No adhesive on the tooth

Statistical treatment of data

Statistical analysis was performed with the non-parametric Mann-Whitney and unpaired t-tests, using the statistical package SPSS (Version 17.0) and adopting a significance level of 95% (p-value<0.05).

RESULTS

The mean shear strength reported was 12.02 MPa for G1 and 11.86 MPa for G2 (unpaired t-test; p-value > 0.05). As for the ARI, the most frequent scores for G1 and G2 were 3 and 5, with no statistically significant difference between groups (Mann-Whitney test; p-value> 0.05). These findings are shown in Table 2.

TABLE 2 – Mean values for shear strength (in MPa) and Adhesive Remnant Index (ARI) scores for the studied phosphoric acids.

Type of acid	Shear strength (MPa)	ARI scores				
		1	2	3	4	5
Phosphoric acid 37% without chlorhexidine	12.02 (±4.08)	0	0	3	5	7
Phosphoric acid 37% with chlorhexidine	11.86 (±3.72)	0	0	7	3	5

DISCUSSION

Chlorhexidine is a synthetic compound derived from bis-biguanide and that, due to its characteristics, shows a high level of activity, which is typical of high standard antimicrobial agents, without having the secondary effects that most agents do. Because of this degree of activity, small concentrations of chlorhexidine salts are usually enough to inhibit the reproductive process or kill most bacterial species (9).

Therefore, the concentration of 2% is enough to produce this effect, and the addition of phosphoric acid in gel form provides an additional prophylactic medium in dental treatment.

However, there were no studies in the literature evaluating the effect of the addition of chlorhexidine to phosphoric acid on bond strength between enamel and adhesive. Notwithstanding, there are *in vitro* studies on the effects of the application of chlorhexidine in its different formulations on the shear bond strength of orthodontic brackets, but these studies were not enlightening.

By means of studies with shear bond tests, it was observed that the application of chlorhexidine digluconate negatively influenced bond strength to dentin, corroborating the hypothesis that this substance affects dentin hybridization by adhesive systems (10). Similar results were obtained by Santos et al. (11) and Ercan et al. (12) in studies in

which shear bond strength decreased if chlorhexidine was used with self-conditioning adhesive systems.

When promoting applications of chlorhexidine, both in gel and varnish forms, in different stages during the bonding of orthodontic brackets in permanent molars, it was found that there were no significant differences in bond strength (13).

However, studies which used the same methodology concluded that the solution does not influence bond strength to dentin (14). Previous studies using tensile tests confirmed that the use of chlorhexidine 2% did not adversely change adhesion to dentin as well (15). The values found with the microtensile test in the research conducted by Bengtson et al. (4) proved that there was no statistically significant difference between groups treated only with chlorhexidine digluconate 2% and untreated groups. These findings are therefore in accordance with studies by Carrilho (16) and De Castro (17), which did not find changes in bond strength, even when the disinfectant agent was used after dentin conditioning. The effect of chlorhexidine on bond strength to dentin is related to the adhesive system used, and current systems did not change their adhesiveness after the application of this substance (18).

Demir et al. reported the higher shear bond strength of an orthodontic resin bonded to enamel previously treated with chlorhexidine 0.2% (19). Cacciafesta et al. reported in their study a decrease in bond strength in bovine enamel treated with chlorhexidine after bracket bonding (20).

The fact that the addition of chlorhexidine to phosphoric acid did not influence bond strength to enamel, as opposed to what occurs in the dentin, may be justified by the low amount of organic matter present in the adamantine structure (2%), while its inorganic portion corresponds to 86% and 12% of water (21). In this line of thought, considering that chlorhexidine would interfere with dentin hybridization by adhesive systems by means of hydrolytic degradation of hydrophilic resin and deterioration of collagen fibrils (22), this mechanism would not occur in a high mineralized tissue such as enamel, in which adhesion establishes differently.

Moreover, it is important to emphasize that the concentration of chlorhexidine present in the phosphoric acid is 2%, suggesting that it does not affect the physical and chemical characteristics of the product, allowing for an effective conditioning of enamel surface. The remaining components of phosphoric acid are thickener and distilled water.

Although laboratory studies have limitations, since they did not fully reproduce clinical conditions (23), *in vitro* research has the benefit of providing isolated data of variables of interest, controlling the influence of other factors. Thus, the present study made it possible to analyze whether the addition of chlorhexidine to phosphoric acid would adversely affect resin adhesion to dental enamel, an aspect little investigated in the literature.

CONCLUSION

Under the experimental conditions of the study, we conclude that the addition of chlorhexidine to phosphoric acid 37% did not affect the bond strength to the enamel surface nor the amount of remaining adhesive on the enamel.

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