

Effect of Dental Erosion and Methods for its Control on the Marginal and Internal Adaptation of Restorations with Different Adhesive Systems

Efeito da Erosão Dental e de Métodos para seu Controle na Adaptação Marginal e Interna de Restaurações Confeccionadas com Diferentes Sistemas Adesivos

Janaina Emanuela Damasceno^a; Felipe Vilasboas Rodrigues^a; Luana Mendonça Dias^a; Patricia Akemi Nishitani Shibasaki^a; Max José Pimenta Lima^a; Roberto Paulo Correia de Araújo^a; Richard Mark Foxton^b; Andrea Nóbrega Cavalcanti^{*ac}

^aDentistry Course, School of Medicine and Public Health of Bahia. BA, Brazil.

^bKings College London Dental Institute, King's College London, SE1 9RT, London, UK.

^cSchool of Dentistry, Federal University of Bahia. BA, Brazil.

*E-mail: andreancavalcanti@yahoo.com.br

Recebido em: 06/03/2019; Aprovado em: 30/07/2019

Abstract

The dentin exposed to erosive challenges is often superficially protected to prevent progression of the lesion. This study investigated the marginal and internal adaptation of composite resin restorations made on surfaces that had first been treated with different methods for controlling erosion. Cavities with margins in dentin were prepared in bovine incisors (n=360) and were divided into three groups according to the method for controlling the erosive challenge: negative control, topical application of fluoride and resin-modified glass ionomer varnish. The specimens were then randomly divided into three sub-groups according to the exposure to simulated gastric acid solution (DES) (5% HCl, pH=2.2) and subsequent remineralization (RE): negative control, 9 and 18 cycles of DES-RE. Finally, teeth were divided into four groups, depending on the bonding agent used for composite resin restoration (n=10): conventional etch-and-rinse adhesive system (2 and 3 steps) and self-etching (1 and 2 steps). Front and internal images of the interface tooth/restoration were recorded in stereoscopic microscope (15x) to quantify the percentage of adhesive failures. Despite the promising results of the resin-modified glass ionomer varnish after 9 cycles; no protective material prevented increased internal defects after 18 erosive cycles. More continuous internal margins were noted with etch-and-rinse acid systems after more intense erosion. The maintenance of internal margins in eroded substrates was positively influenced by the resin-modified glass ionomer varnish and, under the most aggressive challenge, by the use of etch-and-rinse adhesives systems.

Keywords: Tooth Erosion. Dentin. Acid Gastric.

Resumo

A dentina exposta a desafios erosivos é muitas vezes superficialmente protegida para prevenir a progressão da lesão. Este estudo investigou a adaptação marginal e interna de restaurações de resina composta realizadas em superfícies que foram previamente tratadas com diferentes métodos para controlar a erosão. Cavidades com margens em dentina foram preparadas em dentes incisivos bovinos (n=360) e divididas em três grupos de acordo com o método de controle do desafio erosivo: controle negativo, aplicação tópica de flúor e verniz de ionômero de vidro modificado por resina. Os espécimes foram então divididos aleatoriamente em três subgrupos de acordo com a exposição à solução de ácido gástrico simulada (DES) (HCl a 5%, pH = 2,2) e posterior remineralização (RE): controle negativo, 9 e 18 ciclos de DES-RE. Por fim, os dentes foram divididos em quatro grupos, de acordo com o agente de união utilizado para a restauração da resina composta (n=10): sistema adesivo convencional (2 e 3 passos) e autocondicionante (1 e 2 passos). Imagens frontais e internas da interface dente/restauração foram registradas em microscópio estereoscópico (15x) para quantificar a porcentagem de falhas adesivas. Apesar dos resultados promissores do verniz de ionômero de vidro modificado por resina após 9 ciclos; nenhum material de proteção impediu o aumento de defeitos internos após 18 ciclos erosivos. Margens internas mais contínuas foram observadas com sistemas adesivos convencionais após erosão mais intensa. A manutenção das margens internas em substratos erodidos foi influenciada positivamente pelo verniz de ionômero de vidro modificado por resina e, sob o desafio mais agressivo, pelo uso de sistemas de adesivos convencionais.

Palavras-chave: Erosão Dentária. Dentina. Ácido Gástrico.

1 Introduction

Endogenous and exogenous acids are the main causes for the increase in incidence and prevalence of dental erosion.^{1,2} However, the erosive potential from gastric juice is seen as more serious than that coming from exogenous acid because of a countless frequency of the episodes, especially in patients that present disturbances and illnesses known as Gastroesophageal Reflux Disease (GERD).^{1,3,4} Moreover, in comparison with other acids coming from the diet, the gastric juice has a lower pH, and higher titratable acidity that leads to

the development of deeper erosion.^{1,3,4}

The methods for protection of the dental structures against the effect of erosion may include the use of substances responsible for neutralization of the acids and remineralization of the dental tissues.⁵⁻¹¹ Some studies have tested the use of fluoride and glass-ionomer based sealer as efficient superficial protectors against the effect of the erosion.^{5,6,12-15} Authors suggest that they form a protective film on the surface of the tooth, form calcium fluoride^{5,6,8-13} and release of calcium and phosphate.^{14,15} However, the use of surface protectors or

desensitizers in some cases might not be sufficient to control the effects from dental erosion, so restorations for advanced lesions with exposed dentin are recommended.^{16,17}

Among the restorative materials used to rehabilitate the negative effects of dental erosion, composite resins have been recommended due to its adhesion to tooth structure, color varieties and optical effects. An important issue related to the use of composites in patients with history of dental erosion is quality of the adhesion formed in the eroded substrates, especially in dentine.^{13,18-20} Also, it is uncertain if the previous modification of the dentin substrate with surface protectors or desensitizers interferes with the adhesive restoration and if it is necessary to first remove the surface protection remaining on the surface.

The current bonding protocols can be classified into conventional, two and three-step ones, where there is a need for prior phosphoric acid etching; and either one or two-step self-etching adhesives, which do not require prior phosphoric acid-etching. Considering the variety of bonding systems available and their distinct actions on dentin surface,^{9,13,17} it is worthwhile determining better protocols for treating dentin exposed to erosive challenges.

Therefore, the aim of this research was to simulate the effect of erosion by gastric acid on previously protected dentin with various materials and to evaluate the effectiveness of the adhesive restorations using conventional etch-and-rinse and self-etching bonding protocols. The doubts that defined this study were: does the presence of such superficial coverings interfere in the adhesion capacity? What if an adhesive restoration must be performed in a previously protected tooth? The null hypotheses were that there would be no influence from the surface protection method of the dentine, from the intensity of the erosive challenge, or from the type of the adhesive system on the percentage of continuous margins found in composite resin restorations.

2 Material and Methods

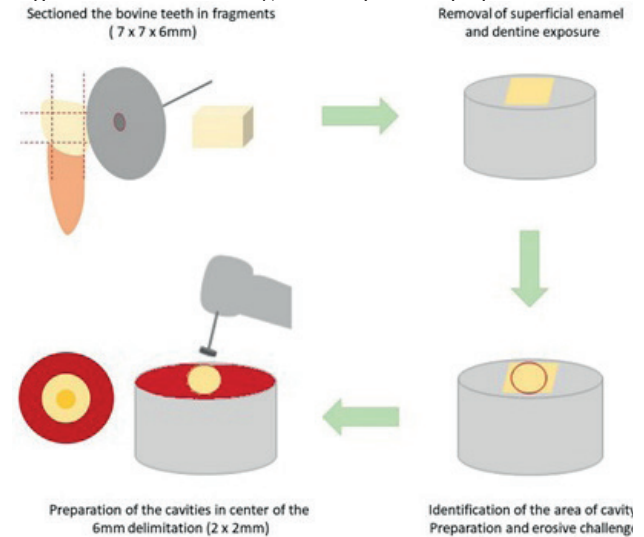
2.1 Specimen preparation

In the present study, bovine incisors were used (n=360) which were cleaned and stored in 0.1% thymol solution. The teeth were measured and sectioned with a flexible diamond disc (KG Sorensen, Cotia - SP) mesio-distal and incisio-occlusal in order to obtain approximately 7x7x6mm fragments.

The vestibular enamel surface was removed using a polishing machine until the dentine was exposed (Arotec S/A Indústria e Comércio, Cotia - SP). Polishing was done under constant water irrigation using 400 and 600-grit polishing disks (Bosch Brasil, São Paulo - SP). Afterwards, the flat dentine surface was partially covered by a circular tape with diameter of 6 mm. In order to identify the area for the preparation of the cavity and the erosive challenge, two layers of enamel varnish were applied around the tape. Cylindrical cavities with dimensions of 2mm deep x 2mm wide were cut

in the center of the 6mm isolated rounded areas using diamond burs (2294, KG Sorensen, Cotia - SP), which were substituted every 10 preparations. Thus, a standardized boundary of 2 mm was outlined for the erosive challenge around each cavity (Figure 1).

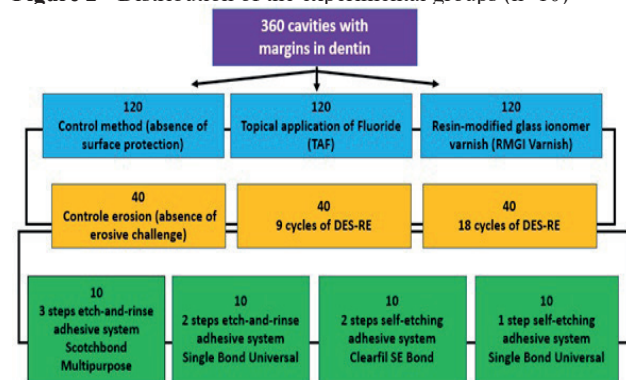
Figure 1 - Schematic design of the specimen preparation



Source: Authors.

The prepared cavities were randomly divided into 36 experimental groups (n=10), according to the method for controlling the erosive challenge, frequency of the simulation of the endogenous erosion and the type of the adhesive system used for restoration (Figure 2).

Figure 2 - Distribution of the experimental groups (n=10)



Source: Authors.

2.2 Methods for controlling the erosive challenge

First, cavities were randomly allocated to one of three methods for controlling the effects of the erosive challenge (n=120), which is described as follows:

- ✓ Control method: the specimens were not submitted to any form of control of erosion and were kept in relative humidity of 37 °C.
- ✓ Topical Application of Fluoride (TAF): 1 ml of neutral 2% NaF (DFL Indústria e Comércio S.A., Jacarepaguá - RJ) was applied according to the recommendations from the manufacturer for 1 minute within the outlined area, followed by a washing with distilled water in an

ultrasonic bath (UNIQUE Industria e Comércio LTDA, São Paulo – SP) for two minutes. After this, they were placed in relative humidity of 37 °C.

- ✓ Resin-modified glass ionomer varnish (RMGI Varnish): product (Clinpro XT Varnish, 3M-ESPE, Sumaré - SP) was applied according to the recommendations from the manufacturer. Equal portions of the two pastes were placed and manipulated for 15 seconds. Straight after, a thin layer was applied on the outlined area of the specimens with a disposable applicator followed by photo-activation for 20 seconds (Ratii Plus, SDI, São Paulo - SP). Finally, they were kept in relative humidity of 37 °C.

2.3 Simulation of erosion by gastric acid

After using the respective method for controlling the erosive challenge, the specimens were subdivided into three groups according to the frequency of the simulation of erosion by gastric acid (n=40).

- ✓ Control erosion: the cavities from this group were immersed in 10 ml of distilled water at 37 °C and had not been subjected to any acidic solution during the cycles of the other subgroups.
- ✓ 9 cycles of DES-RE: every completed cycle consisted of immersing the cavity in 10 ml solution of hydrochloric

acid (5% HCl, pH=2.2) for two minutes in room temperature. After this, the specimens were washed with the help of disposable syringe containing 20ml distilled water and immersed in remineralizing solution.²¹ Its composition included 1.5 mmol/L Ca, 0.9 mmol/L PO₄, 0.15 mol/L KCl, and 20 mmol/L TRIS buffer at pH 7.0 and its use was based on the work of Toda and Featherstone.²² Between the cycles the units were stored in relative humidity of 37 °C.

- ✓ 18 cycles of DES-RE: the specimens in this subgroup were subjected to double the cycle frequency in this way promoting a more aggressive challenge. Every cycle was carried out as described previously.

The methodology of the present study use was based on the work of de Queiroz *et al.*²

2.4 Adhesive restoration

At the end of each phase of controlling the erosive challenge and simulation of endogenous erosion, the specimens were again sub-divided into four experimental groups, according to the type of the adhesive system used for the composite resin restoration. The adhesive systems were applied as recommended by the respective manufacturer (Table 1).

Table 1 - Adhesive system (manufacturer) [category], composition, and recommended application

Adhesive System	Main components	Application
Scotchbond Universal Adhesive (3M-ESPE, Sumaré – SP) [1-step self-etch] [2-steps etch-and-rinse]	MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane	Self-etching strategy: Apply the adhesive for 20 s with vigorous agitation at dry dentin. Gently air thin for 5 s. Light cure for 10 s. Etch-and-rinse strategy: Apply etchant for 15 s. Rinse for 10 s. Air dry to remove excess of water. Keep dentin moist. Apply the adhesive as for the self-etching mode
Clearfil SE Bond (Kuraray South America LTDA, São Paulo – SP) [2-steps self-etch]	Primer: MDP, HEMA, hydrophilic dimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, water Bonding: MDP, bis-GMA, HEMA, hydrophobic dimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica	Apply primer on dry dentin surface and leave undisturbed for 20 s. Dry with air stream for 5 s to evaporate the volatile ingredients. Apply bond and gently air dry. Light cure for 10 s.
Scotchbond MultiPurpose (3M-ESPE, Sumaré – SP) [3-steps etch-and-rinse]	Primer: aqueous solution of HEMA, polyalkenoic acid copolymer; Adhesive: Bis-GMA, HEMA, dimethacrylates and initiators	Apply etchant for 15 s. Rinse for 10 s. Air dry to remove excess of water. Keep dentin moist. Agitate primer for 15 s, gently air blast for 15 s. Apply adhesive, gently air blast. Light cure for 10 s.

MDP: 10-methacryloyloxydecyl-dihydrogen-phosphate; HEMA: 2-hydroxyethyl methacrylate; bis-GMA: bisphenyl-glycidyl methacrylate

Source: Authors.

Composite resin (Filtek Z350, color A3B, 3M-ESPE, Sumaré – SP) was placed in a single increment. After inserting the composite, a standard polyester tape was placed on the surface and a light-activation was carried out for 20s, with light intensity 1500mW/cm². The restorations were polished 24 hours later, using polishing discs in descending sequence of abrasiveness (Sof-Lex Pop-on, 3M-ESPE, Sumaré - SP). The quality of the finishing of the margins was verified an optic microscope at 4x magnification.

2.5 Evaluation of marginal adaptation of the adhesive systems

After the restorations were polished, the external margins were examined in a stereoscopic microscope (Opton, Parque Industrial San José, Cotia – SP), photographed in 15x enlargement and examined using an image-processing program CorelDRAW. The cavities were then sectioned at the center with a diamond-coated disc, adapted to a precision

cutter under constant cooling. This way, an analysis was carried out of the internal surface of the two halves, as described before.

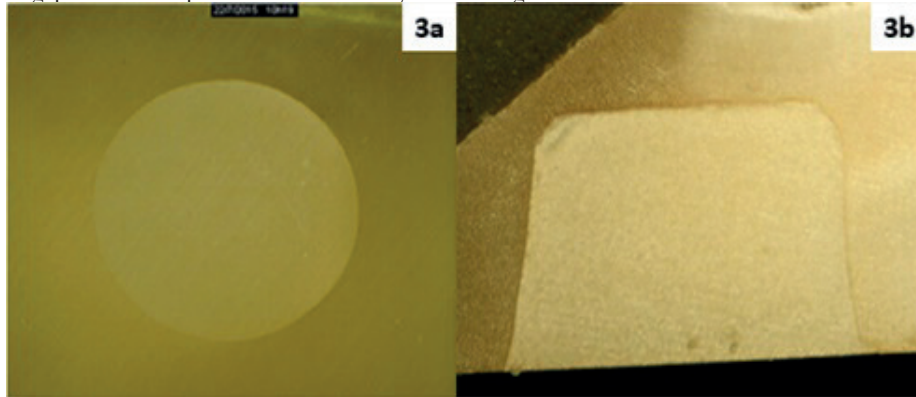
All specimens were subjected to a quantitative exam of the quality of the margins, by a calibrated examiner. For this evaluation, the percentage of continuous margins were measured around the length of the margins over the whole surface.

In order to calculate the length of the external margin the following formula was used: , considering $r=1\text{mm}$. Thus,

the total percentage of the external margin (100%) was equivalent to 6,28mm (Figure 3a). Regarding the internal margin, the length was where $l=2\text{mm}$ was considered (Figure 3b). So, 100% of the internal length is equivalent to 6mm. Using these values, the criteria taken into account in the evaluation were determined according to Bortolotto et al.²³ study where:

- Percentage of the continuous margins: proportion relative to the absence of gaps, interruptions on the continuity of the margin.

Figure 3 - Photographs a stereoscopic microscope of 15x magnification to evaluate the absence of gaps and interruptions in the continuity of the margin.



3a- 100% of the external continuous margin. 3b- 100% of the internal continuous margin.

Source: Authors.

2.6 Statistical analysis

Initially an exploratory analysis was carried out of the data to verify the homogeneity of the variances and to determine if the experimental errors presented a normal distribution (parameters of Analysis of variance). Inferential statistical analysis was performed by 3-Way ANOVA and Tukey’s test for multiple comparisons (method for controlling erosion x erosive challenge x adhesive system). This analysis was done using SAS statistical software, version 9.1, with a significance level of 5 %.

3 Results and Discussion

3.1 External margin

No significant interaction between the study factors “method for controlling erosion”, “erosive challenge” and “adhesive system” was observed ($p=0.49$); and no difference was detected between the levels of each factor: method for controlling erosion ($p=0.45$); erosive challenge ($p=0.26$) and adhesive system ($p=0.12$) (Table 2).

Table 2 - Mean (standard deviation) of% of external continuous margin in experimental groups

Adhesive System	Erosive challenge control method	Erosive challenge		
		Control erosion	9 cycles of DES-RE	18 cycles of DES-RE
1-step self-etch	Control method	97.52 (2.26)	97.68 (2.72)	97.62 (2.33)
	TAF	98.96 (2.13)	98.70 (1.75)	96.52(3.83)
	RMGI Varnish	98.53 (2.33)	97.18 (3.19)	98.82 (1.81)
2-steps etch-and-rinse	Control method	99.15 (1.24)	97.36 (2.39)	98.09 (2.23)
	TAF	99.47 (0.69)	98.83 (2.46)	97.20 (3.51)
	RMGI Varnish	98.87 (1.52)	98.38 (2.34)	97.57 (3.42)
2-steps self-etch	Control method	97.48 (2.14)	99.54 (1.02)	99.00 (1.48)
	TAF	99.20 (1.58)	98.50 (2.12)	98.73 (2.29)
	RMGI Varnish	98.42 (1.62)	98.13 (2.25)	97.20 (3.01)
3-steps etch-and-rinse	Control method	98.92 (2.73)	99.10 (2.39)	99.04 (1.59)
	TAF	99.05 (1.67)	98.90 (1.77)	98.70 (3.02)
	RMGI Varnish	97.88 (3.30)	98.77 (1.79)	98.71 (2.50)

Coefficient of Variation (CV) = 2.4%

Source: Authors.

3.2 Internal margin

The percentages of the continuous internal margins were not significantly different in the triple interaction between the main factors ($p=0.56$) (Table 3). However, double interactions

between these factors were detected: control method x erosive challenge ($p<0.0001$); erosive challenge x bonding protocol ($p=0.0002$) and control method x bonding protocol ($p=0.03$). The Tukey test was used to detect the respective differences among means (Figures 4, 5, 6).

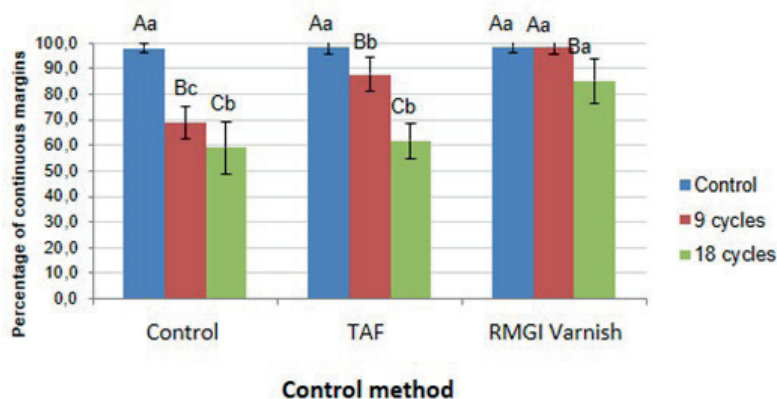
Table 3 - Mean (standard deviation) of% of internal continuous margin in experimental groups

Adhesive System	Erosive challenge control method	Erosive challenge		
		Control erosion	9 cycles of DES-RE	18 cycles of DES-RE
1-step self-etch	Control method	97.73 (2.12)	64.08 (5.89)	50.43 (10.77)
	TAF	98.02 (2.12)	87.57 (6.70)	59.27 (8.21)
	RMGI Varnish	98.41 (2.07)	98.22 (2.02)	83.78 (8.66)
2-steps etch-and-rinse	Control method	98.11 (2.08)	69.62 (4.02)	63.94 (5.17)
	TAF	98.60 (2.30)	88.08 (6.41)	64.86 (5.59)
	RMGI Varnish	98.73 (1.66)	98.75 (1.65)	88.17 (8.69)
2-steps self-etch	Control method	97.93 (1.93)	65.91 (4.25)	57.40 (10.48)
	TAF	97.90 (2.87)	86.93 (7.04)	58.06 (6.26)
	RMGI Varnish	97.72 (2.44)	96.85 (3.48)	80.24 (7.51)
3-steps etch-and-rinse	Control method	98.37 (2.16)	75.17 (5.48)	64.67 (7.22)
	TAF	98.48 (2.12)	88.67 (6.23)	64.89 (5.59)
	RMGI Varnish	98.74 (2.11)	98.80 (1.73)	88.95 (7.71)

Coefficient of Variation CV=6,6%

Source: Authors.

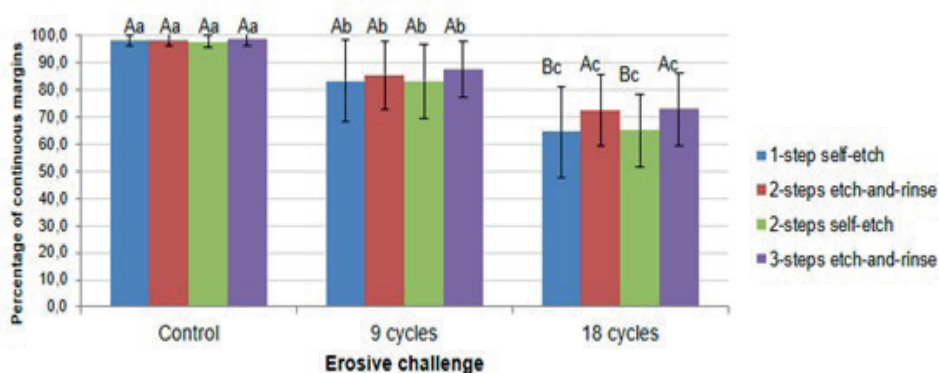
Figure 4 - Statistical interaction between “control method” and “erosive challenge”.



*The data of the bonding protocol was grouped. Capital letters compare the erosive challenges in every control method and lower case letters compare the control methods for every level of the erosive challenge (3-way Anova /Tukey test, $p<0,0001$).

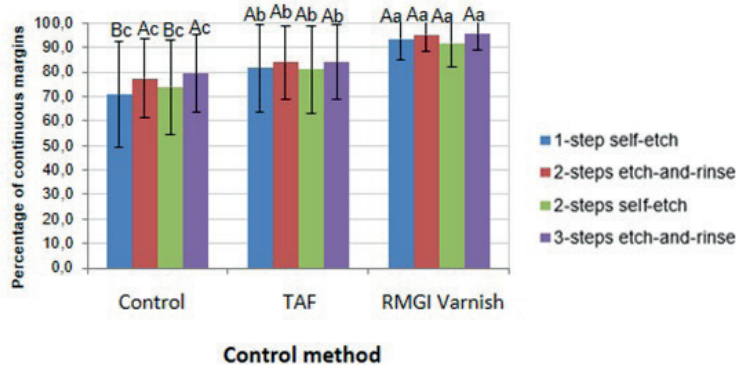
Source: Authors.

Figure 5 - Statistical interaction between “erosive challenge” and “bonding system”.



*The data of the method for controlling erosion was grouped. Capital letters compare the bonding protocols in every erosive challenge and lower case letters compare the erosive challenges for every level of the bonding protocol (3-way Anova /Tukey test, $p<0,0001$).

Source: Authors.

Figure 6 - Statistical interaction between “control method” and “bonding system”.

*The data of the erosive challenge was grouped. Capital letters compare the bonding protocols within every control method and lower case letters compare the control methods for every level of the bonding protocol (3-way Anova / Tukey test, $p=0,03$).

Source: Authors.

Regardless of the type of the bonding protocol used, in the absence of the control method or in the presence of the TAF, there was a significant reduction of the percentage of the continuous margins after the 9 and 18 cycles. However, when the glass-ionomer sealant was used as a barrier, a reduction in the continuity was noticed only after 18 erosive cycles.

Comparing the method of protection against the erosive challenge, in the absence of challenge, there is no significant difference between the forms of protection. After 9 cycles, there was greater percentage of continuous margins when the RMGI Varnish was used, followed by the TAF and the absence of protection. Finally, after 18 cycles, the glass-ionomer sealant exhibited a higher percentage of continuous margins. Nevertheless, TAF showed similar values to the absence of protection (Figure 5).

The statistical interaction between “erosive challenge” and “bonding protocol” (Figure 6) indicates that in the absence erosion and after 9 cycles, the bonding protocols presented a similar percentage of margins with continuity. However after 18 cycles, a higher quantity of continuous margins with the adhesive systems requiring prior acid etching were observed. Regardless of the type of surface protection employed, there was a significant reduction in the percentage of the continuous margins of all the bonding protocols when there was an increase in intensity of the erosive challenge.

Finally, the interaction between “control method” and “bonding protocol” showed that, regardless of the intensity of the challenge, all the bonding protocols present a higher percentage of continuous margins in conjunction with glass-ionomer sealant, followed by the TAF and the absence of surface protection. Additionally, comparing the results of the four systems, it can be seen that the quality of the continuous margins is similar on surfaces exposed to glass-ionomer sealant and to TAF. However, the adhesive systems that required prior acid etching presented higher values in comparison with the others on the surfaces exposed to the negative control of the protection method.

The aim of the methods for controlling the endogenous

erosion is to avoid progression of the lesions by controlling the dissolution of surfaces by acids.²¹ But in certain situations the restoration of previously eroded dentin may be required, either due to the intensity of the erosive challenge or to treatment limitations.^{13,18-20,24,25} However, there are challenges in bonding to eroded dentine,²⁰ and there is limited information related to dentine substrate that have previously been exposed to treatments aimed at controlling the erosion, which then need to be restored.

Evaluation of the restoration margins is an important tool for identifying possible faults in the bonding protocols at the interface of the tooth/restoration.²⁵ However, the methodologies for marginal adaptation verification may also present some limitations, for example, the need to excessively polishing surfaces for microscopic viewing. This procedure can justify the absence of significant findings for external margins in the present study. In other words, even if it was present, a marginal flaw could not be observed, since the margins had been heavily polished for observation purposes. On the other side, internal defects related to loss of adherence were clearly noticeable in this investigation.

The way dental surfaces are exposed to acids varies in different studies.^{1,6-11,20,21,26} Some studies use even periods of time of erosion,^{1,6-11,20,21,26} while in others the use of cycles DES-RE is favored.^{7,20,21} According to Austin et al,²¹ the cyclical use of hydrochloric acid solution at pH 2.2 and titratable acid 86.5mmol OH/L would represent the worst case scenario of endogenous erosive challenge, in which the individual would be repeatedly regurgitating acidic content in oral cavity. The authors carried out a simulation of erosion by gastric acid in enamel, using a progressive quantity of DES-RE cycles (3, 6 and 9), with 2 minutes in demineralizing solution with HCl, pH 2.2 and 60 minutes in remineralizing solution of artificial saliva with pH 7.

In the current study, the option was to use DES-RE cycles because patients who suffer from endogenous erosion normally have various crises during the day and within short periods of time. Every acidic episode takes between 1-2 mins,

and it is always associated to remineralizing action of the saliva, that raises the pH of the oral cavity.^{4,26} Based on this, an adaptation of the acid cycle described by Austin *et al*²¹ was made in this project; increasing the level of intensity of the acidic challenge (18 cycles).

One of the tested hypotheses in the present study was that topical application of fluoride would reduce demineralization of the dentin, generating a substrate more favorable to bonding. According to the findings of the present study, a favorable potential for fluoride ions to reduction the internal defects after 9 erosive cycles was observed as compared with absence of protection. Ganss *et al*⁸ evaluated the mineral loss on enamel and dentine after cycles of DES-RE. The TAF was more efficient on dentine, where the progression of loss of erosive mineral was almost completely interrupted. Nevertheless, after 18 cycles, the favorable effect of the TAF did not continue. Other research has also shown a limited potential for TAF protection after successive demineralizations, because CaF₂ deposits are easily dissolved, exposing the surface to acidic environment.^{5,13} Elkassas *et al.*¹² evaluated the micro-hardness of enamel exposed to fluoride components followed by DES-RE. In the study, the authors state that fluoride acted as a barrier, but its capacity for promoting remineralization was limited by availability of calcium and phosphate ions. It is important to stress that the present work aimed to understand the influence of TAF on the bonding ability of adhesive systems in laboratory conditions, and thus only it involved one application of TAF gel. However, it is well known that clinically, the contact of teeth with fluoride ions can be supplemented using various sources, for example dentifrices, mouthwashes and varnishes. Additionally, TAF can be included as an important routine during clinical visits, increasing the possibility of early detection of the progression of erosive lesions.

Considering the possibility that resin-modified glass ionomer varnish may stay longer on the dentine surfaces, the capacity of Clinpro XT Varnish to protect dentine substrate and to favor the bonding protocols was also investigated in this study. According to the manufacturer, the material is glass-ionomer modified by resin, based on polyalkenoic acid. It contains calcium glycerophosphate that can release calcium and phosphate in the long run. In this work, when the RMGI Varnish was used as surface protection, there was an increase in the continuity of internal margins, even after 18 erosive cycles, showing it to be more efficient than ionic fluoride. In the study of Zhou *et al.*¹⁵ this material showed a better capacity for remineralization of erosive lesions. The authors suggested that this effect is due to the capacity of the sealant to prevent demineralization, and because of its remineralizing potential coming from the release of calcium and phosphate. In spite of the substrate being different from the one investigated in this work, its protection capacity seems to be the same. Apparently, by reducing demineralization on the dentine, which consequently leads to a lower exposure of the demineralized organic matrix (DOM), the presence of

the RMGI Varnish resulted in significant improvement in the continuity of the margins as compared with TAF, even after a more aggressive challenge.

Even with a better bonding, a slight reduction in the continuity of internal margin was observed after 18 cycles. These findings suggest that, with high erosive challenge, not even RMGI Varnish would protect the dentine substrate completely, maintaining its capacity for bonding. According to Zimmerli *et al.*²⁰ adequate hybridization of dentine surfaces that were profoundly eroded can be inhibited by not covering the thin net of collagen fibrils sufficiently. One limitation of present investigation was the inability to determine if RMGI Varnish continued to be present on surfaces or if it was partial or totally removed by the action of the erosive challenge, exposing areas of demineralized organic matrix. Apart from this fact, what is required is a new, more profound investigation. It needs to be stressed that *in vitro* studies often create more aggressive scenarios than the clinical environment, and their results should be carefully considered.

Another factor observed was that after 18 cycles there was a lower quantity of internal continuous margins of the self-etching systems, in relation to those requiring prior etching with phosphoric acid. Self-etching bonding protocols, as tested in the present study, owe their good results to 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) in its composition. Studies has confirmed that 10-MDP allows for a stable chemical bonding formation to the adhesive interface, with formation of ionic links with calcium or covalent with phosphate moieties.^{13,24} However, we need to take into consideration the fact that erosive challenge results in phosphate and calcium loss on the surface of the dentine, and this fact can be the reason for higher presence of adhesive faults on the adhered surfaces with these protocols.

Similarly, the composition of Single Bond Universal included polyalkenoic acid copolymer, whose function is related to the chemical bonding with hydroxyapatite of the tooth. In the present study, the performance of this bonding agent after acid etching of the surfaces was statistically better than its self-etching use. As showed in recent works,^{19,24,27} it can be deduced that the competition of 10-MDP and polyalkenoic acid copolymer by minerals on previously demineralized surfaces, resulted in the micromechanical mechanism showing more faults at the interface. On the other hand, it is important to observe that self-etching systems, especially those that contain 10-MDP form more stable bonding as time passes, with lower reduction of the bonding resistance and low nanoinfiltration.^{20,24} As the present work only evaluated immediate bonding to the eroded dentine, it is fundamental that new investigations are carried out to determine this behavior long-term.

All the null hypotheses tested in the present study were rejected, since the control methods, intensities of erosive challenge and the type of the bonding agents showed a relation to the changes in the continuity of the internal margins. However, above all, it is clinically important for patients

with endogenous erosion that there is immediate protection of the surfaces exposed to erosive challenge. This task can either avoid the progress of erosive lesions or create better substrates, in case adhesive restorations come to be necessary. The immediate results obtained in this study indicate a way that obviously needs to be proved with more investigations, especially in the long-term.

4 Conclusion

Bearing in mind the limitations of the present *in vitro* study, it can be concluded that the continuity of internal margins of composite resin restorations was favored by previous application of resin-modified glass ionomer varnish on erosive challenge. Using a severe erosion protocol, the bonding protocols requiring prior acid etching presented more adequate immediate results.

Acknowledgments

Authors thank FAPESB, grant RED 019/2013 and 3M-ESPE for material support for this investigation.

References

- Bartlett D W and Coward PY. Comparison of the erosive potential of gastric juice and a carbonated drink in vitro. *J Oral Rehab* 2001;28:1045-7.
- Queiroz, MMV; Shibasaki, PAN; Lima, MJP; de Araújo, RPC; Foxton, RM; Cavalcanti, AN. Effect of erosion and methods for its control on the surface roughness of composite resin. *Rev Odonto Cienc* 2017;32(2):88-93
- Farahmand F, Sabbaghian M, Ghodousi S, Seddighorae N, Abbasi M. Gastroesophageal reflux disease and tooth erosion: a cross-sectional observational study. *Gut Liver* 2013;7(3):278-81.
- Ranjitkar S, Kaidonis JA, Smales RJ. Gastroesophageal reflux disease and tooth erosion. *Int J Dent* 2012;1-10.
- Algarni AA, Lippert F, Hara AT. Efficacy of stannous, fluoride and their combination in dentin erosion prevention in vitro. *Braz Oral Res* 2015;29(1):1-5.
- Boteon AP, Kato MT, Buzalaf MAR, Prakki A, Wang L, Rios D, et al. Effect of Proanthocyanidin-enriched extracts on the inhibition of wear and degradation of dentin demineralized organic matrix. *Arch Oral Biol* 2017;84:118-24. doi: 10.1016/j.archoralbio.2017.09.027.
- Comar LP, Cardoso CAB, Charone S, Grizzo LT, Buzalaf MAR, Magalhães AC. TiF4 and NaF varnishes as anti-erosive agents on enamel and dentin erosion progression *in vitro*. *J Appl Oral Sci* 2015;23(1):14-8.
- Ganss C, Klimek J, Schäffer U, Spall T. effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. *Caries Res* 2001;35:325-30.
- Kato MT, Leite AL, Hannas AR, Calabria MP, Magalhães AC, Pereira JC et al. Impact of protease inhibitors on dentin matrix degradation by collagenase. *J Dent Res* 2012;91(12):1119-23.
- Kleter GA, Damen JJM, Everts G, Niehof J, and Ten Cate JM. The influence of the organic matrix on demineralization of bovine root dentin in vitro. *J Dent Res* 1994;73(9):1523-9.
- Tjäderhane L, Buzalaf MAR, Carrilho M, Chaussain C. Matrix Metalloproteinases and other matrix proteinases in relation to cariology: the era of 'dentin degradomics'. *Caries Res* 2015;49:193-208. doi: 10.1159/000363582.
- Elkassas D and Arafa A. Remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. *J Dent* 2014;42:466-74.
- Flury S, Koch T, Peutzfeldt A, Lussi A, Ganss C. The effect of a tin-containing fluoride mouth rinse on the bond between resin composite and erosively demineralised dentin. *Clin Oral Invest* 2013;17:217-22.
- Sohn S, Yi K, Son HH, Chang J. Caries-preventive Activity of Fluoride-containing Resin-based Desensitizers. *Oper Dent* 2012;37(3):306-15.
- Zhou SL, Zhou J, Watanabe S, Watanabe K, Wen LY, Xuan K. *In vitro* study of the effects of fluoride-releasing dental materials on remineralization in an enamel erosion model. *J Dent* 2012;40:255-63.
- Aranha AC, Siqueira Junior CAS, Cavalcante LMA, Pimenta LAF, Marchi GM. Microtensile bond strengths of composite to dentin treated with desensitizer products. *J Adhes Dent* 2006;8(3):85-90.
- Flury S, Lussi A, Peutzfeldt A. Long-term bond strength of two benzalkonium chloride-modified adhesive systems to eroded dentin. *Bio Med Res Inter* 2017:1-8.
- Ding M, Shin SW, Kim MS, Ryu JJ, Lee JY. The effect of a desensitizer and CO2 laser irradiation on bond performance between eroded dentin and resin composite. *J Adv Prosthodont* 2014;6:165-70.
- Lenzi TL, Raggio DP, Soares FZM, Rocha RO. Bonding performance of a multimode adhesive to artificially-induced caries-affected primary dentin. *J Adhes Dent* 2015;17(2):125-31.
- Zimmerli B, Munck J, Lussi A, Lambrechts P, Meerbeek BV. Long-term bonding to eroded dentin requires superficial bur preparation. *Clin Oral Invest* 2012;16:1451-61.
- Austin RS, Stenhagen KS, Hove LH, Dunne S, Moazzez R, Bartelett DW, et al. A qualitative and quantitative investigation into the effect of fluoride formulations on enamel erosion and erosion-abrasion in vitro. *J Dent* 2011;39:648-55.
- Toda S and Featherstone JD. Effects of fluoride dentifrices on enamel lesion formation. *J Dent Res* 2008;87(3):224-7.
- Bortolotto T, Doudou W, Kunzelmann KH, Krejci I. The competition between enamel and dentin adhesion within a cavity: an in vitro evaluation of class V restorations. *Clin Oral Invest* 2012;16:1125-35.
- Muñoz MA, Luque I, Malaquias P, Hass V, Reis A, Bombarda NHC et al. In vitro longevity of bonding properties of universal adhesives to dentin. *Oper Dent* 2015;40(3):282-92.
- Soares CG, Carracho HG, Braun AP, Borges GA, Hirakata LM, Spohr AM. Evaluation of bond strength and internal adaptation between the dental cavity and adhesives applied in one and two layers. *Oper Dent* 2010;35(1):69-76.
- Saunders JGC and McIntyre JM. The ability of 1.23% acidulated phosphate fluoride gel to inhibit simulated endogenous erosion in tooth roots. *Aust Dent J* 2005;50(4):263-66.
- Muñoz MA, Luque I, Hass V, Reis A, Loguercio AD, Bombarda NHC. Immediate bonding properties of universal adhesives to dentine. *J Dent* 2013;41:404-11.