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Can surface protection prevent the loss of hardness on dentin and composite resin surfaces exposed to erosive challenges?

¿Puede la protección de la superficie prevenir la pérdida de dureza en las superficies de dentina y resina compuesta expuestas a erosión?

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Abstract: Objective: This study investigated the effect of endogenous erosion on the microhardness of dentine and a nanofilled composite resin. Procedures for preventing erosion were also studied. **Materials and Methods:** 90 bovine dentine specimens were divided into three groups in accordance with the method for preventing: negative control, topical application of fluoride and resin-modified glass ionomer varnish. 120 composite resin specimens were distributed into four groups, which also included a resin sealant, among the preventive procedures. Specimens were then randomly divided into three sub-groups according to the exposure to simulate gastric acid solution and subsequent remineralization: negative control, 9 and 18 cycles. Surface analysis was carried out by measuring the Knoop hardness. The data obtained were statistically analyzed using 2-way ANOVA and Tukey test. **Result:** The mean hardness of dentine and of the composite specimens resin exhibited lower hardness after 18 cycles. However, the resin-modified glass ionomer varnish resulted in greater values compared to the other preventive procedures. **Conclusion:** A resin-modified glass ionomer varnish seems to be a promising method for minimizing the damage caused by endogenous acid, but its protection can be reduced depending on the intensity of the erosive challenge.

Keywords: Tooth erosion; hardness; dentin; glass ionomer cements; composite resins; hydrochloric acid.

Resumen: Objetivo: Este estudio investigó el efecto de la erosión endógena sobre la microdureza de la dentina y una resina compuesta de nanorrelleno. También se estudiaron los procedimientos para prevenir la erosión. **Materiales and Métodos:** 90 muestras de dentina bovina se dividieron en tres grupos de acuerdo con el método para prevenir: control negativo, aplicación tópica de fluoruro y barniz de ionómero de vidrio modificado con resina. Se distribuyeron 120 muestras de resina compuesta en cuatro grupos, que también incluían un sellador de resina, entre los procedimientos preventivos. Las muestras se dividieron al azar en tres subgrupos de acuerdo con la exposición para simular la solución de ácido gástrico y la remineralización posterior: control negativo, 9 y 18 ciclos. El análisis de la superficie se realizó midiendo la dureza Knoop. Los datos obtenidos se analizaron estadísticamente mediante ANOVA de 2 vías y prueba de Tukey. **Resultados:** La dureza media de la dentina y de la resina de muestras compuestas exhibió una dureza más baja después de 18 ciclos. Sin embargo, el barniz de ionómero de vidrio modificado con resina resultó en valores mayores en comparación con los otros procedimientos preventivos.**Conclusión:** Un barniz de ionómero de vidrio modificado con resina parece ser un método prometedor para minimizar el daño causado por el ácido endógeno, pero su protección puede reducirse dependiendo de la intensidad del desafío erosivo.

Palabra Clave: *Erosión de los dientes; dureza; dentina; cementos de ionómero vítreo; resinas compuestas; ácido clorhídrico.*

INTRODUCTION.

Dental erosion is defined as a chronic loss of the dental hard tissue, which occurs during a chemical process that does not involve microorganisms. It can have an extrinsic origin in the form of frequent consumption of acidic drinks and foods; or an intrinsic origin due to gastric acid reflux and recurring episodes of vomiting.[1-3]

Loss of dental structure is a common sign in patients who suffer from gastro esophageal reflux disease and the progress of erosion can be reduced or stopped after acid suppression therapy.[4] Gastric acid has a low pH (around 2.0) and higher titratable acidity, which explains its potential for causing erosion.[5] When it is found in the oral cavity, it can reduce the pH of the environment and quickly demineralize the surface of the teeth."6" Therefore, in depth studies on the effects of endogenous acid in the oral cavity are important.

Erosive endogenous lesions typically appear on the palatal surfaces of the upper anterior teeth and on the occlusal surfaces of the lower first molars.[7] The wear may be limited to enamel and although it is a reversible process, in more severe cases, it can reach the underlying dentine. This second phase is irreversible.[1-3]

Dental erosion does not only affect the teeth, but it can also reduce the clinical effectiveness and durability of dental restorations in contact with the acid.[8] Erosive agents such as citric acid, natural and artificial sour fruit juices (that have a higher pH than the endogenous acid) can reduce the dental and composite resin structure hardness and lead to the development of rough surfaces. Moreover, the intensity of this effect depends on the duration.[9,10] This is important in the treatment of patients with gastric disturbances, as they can have various episodes of erosive challenge during the day.

Some surface protection methods that have been investigated aim to avoid or reduce the rate of the advancement of erosion on teeth and restorations. Fluoride[11,12] or calcium phosphate[13] based materials show a limited capacity for surface remineralization when faced with erosion.

However, the mineral availability to reverse the demineralization process is always a concern when patients experience frequent erosive challenges. Therefore, some materials such as sealants and varnishes can be more effective as they can be bonded to the surfaces to protect them.[14,15] Nonetheless, there is still limited knowledge on the behavior of these materials when facing continuous episodes of endogenous erosion.

In clinical practice, it is important that practitioners know how the gastric acid interacts with the dental structure and restorative agent in order to obtain successful long-term results. With this in mind, the aim of this study was to investigate the consequences of simulated exposure to gastric acid on the microhardness of dentine and a nanofilled composite resin to determine the efficacy of some surface protection methods against erosive challenge. The experimental hypotheses tested were that the Knoop hardness of the dentine and composite resin would be affected by the preventive method and intensity of the erosive challenge.

MATERIALS AND METHODS.

Specimen preparation

Dentine surfaces

Thirty bovine incisors were used to obtain 90 dental fragments. The following criteria were adopted for selecting the teeth: absence of physical damage such as discoloration, cracks and cavities. The selected teeth were stored in 0.1% thymol solution until the moment they were used.

The teeth were cleaned using a scalpel blade to remove organic debris and polished with pumice stone paste and water using brushes in a low-speed handpiece.

Separation of the roots was carried out using a double-sided diamond disc (n. 7020, KG Sorensen, São Paulo, SP) under constant irrigation. The teeth were divided into quadrants using the diamond disc, which resulted in four specimens. Each specimen was embedded in polystyrene resin, exposing only the vestibular surface.

The surface of the vestibular enamel was removed using 400, 600 and 1200 grit sandpapers (Vonder-ODV, Feira de Santana - BA) until dentine was exposed in a polishing machine (Arotec S.A, Aropol2V, Cotia-SP) under constant irrigation.

Composite resin surfaces

To obtain one hundred and twenty samples in composite resin (Filtek Z350 XT, 3M-ESPE, Sumaré - SP), a 1.5mm thick and 6.0mm diameter cylindrical mold was used. The mold was filled with composite resin and a polyester matrix followed by a 500g weight was placed on top for 30 seconds to level the material. After this period, the top surface was light cured, in direct contact with the strip using a LED light (Radii Plus, SDI, Victoria, Australia), with intensity of 1.500mW/cm^2, for 20 seconds.

The specimens were then stored for 24 hours at 37°C in relative humidity and darkness. After this, they were placed in polystyrene resin leaving the top surface of the samples free (the side polymerized in contact with the polyester matrix). This surface was polished using 1200 grit sandpaper (Vonder-ODV, Feira de Santana-BA) and diamond paste in polishing cloths (granulation 1µm and 3µm) in the polishing machine.

The dentine and composite resin specimens were randomly divided into 9 dentine groups (n=10) and into 12 composite resin groups (n=10), according to the method for preventing the erosive challenge and frequency of the simulation of the endogenous erosion (Figure 1).

Methods for preventing the erosive challenge

The procedures tested to prevent the effects of erosive challenge are described as follows:

-Negative control (preventive method): The specimens were not exposed to any method for preventing erosion. They were kept at at 37°C in relative humidity.

-Topical Application of Fluoride: 1 ml of neutral 2% NaF (DFL Indústria e Comércio S.A., Jacarepaguá-RJ) was applied for 1 minute on the polished surface, followed by washing with distilled water in an ultrasonic bath (UNIQUE Industria e Comércio LTDA, São Paulo,SP) for two minutes. After this, they were placed at 37°C in relative humidity.

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

-Resin Sealant: Application of the product (Fortify, Bisco, Schaumburg, EUA) was in accordance with the manufacturer´s recommendations. Then, conditioning was carried out using 37% phosphoric acid for 15 seconds, followed by washing for 30 seconds and drying.

Immediately after, a thin layer of sealant was applied over the polished surface of the specimen, and then light cured for 10 seconds. Only the composite resin surfaces were covered with this material.

Simulation of erosion by gastric acid

After the application of the respective method for preventing the erosive challenge, the samples were kept at 37°C for 7 days in relative humidity, when they were randomly subjected to conditions of erosion simulation by gastric acid (n=10). The methodology of the present study use was based on the work of de Queiroz *et al.*[16]

-Negative control (simulated erosion): The specimens from this subgroup were immersed in 10ml distilled water at 37°C and were not subjected to any acidic solution during the process of simulating erosion from the other subgroups.

-9 cycles of DES-RE: every completed cycle consisted of immersing the specimen 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 a disposable syringe containing 20 ml distilled water and immersion in a remineralizing solution.[2] Its composition included 1.5 mmol/L Ca, 0.9 mmoll/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.[17] Between cycles, the units were stored in relative humidity at 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.

After the respective challenges, the samples were washed and kept at 37°C in relative humidity for 24 hours.

Knoop microhardness evaluation

The Knoop hardness number (KHN) was determined using an electronic microhardness tester (HMV-G21DT, Shimadzu, Okaya city, Nagano).

For each dentine and composite resin specimen, three indentations were made with a 245.2 mN (HK 0.025) load applied for 15 seconds.

STATISTICAL ANALYSIS

The mean KHN from the three indentations of each specimen was subsequently calculated. Initially, an exploratory analysis of the data was carried out to verify the homogeneity of variances and to determine if the experimental errors showed normal distribution (Analysis of variance parameters). Inferential statistical analysis was carried out by 2-way ANOVA and Tukey's test for multiple comparisons.

A statistical program (SAS version 9.1) was used for this purpose with 5% significance level.

RESULTS.

Tables 1 and 2 show the means and standard deviations of Knoop microhardness found in the dentine and composite resin experimental groups, respectively.

Dentine surfaces

According to the statistical analysis of the data of dentine hardness, a significant correlation between main factors *"preventive method"* and *"erosive challenge"* (p<0.0001) was observed, showing dependence between the levels of one factor with the results of the other. This statistical interaction was developed by Tukey's test.

Firstly, comparing the intensity of DES-RE cycles within each preventive method, it could be noted that dentine surfaces covered with resin-modified glass ionomer varnish presented similar hardness regardless of the degree of erosive challenge. However, the mean hardness of dentine specimens without protection (negative control) and of the ones exposed to topical fluoride significantly decreased after 18 erosion cycles.

When the preventive methods were compared under each level of simulated erosion, no significant differences in hardness were found, both in the negative control condition and after 9 DES-RE cycles. On the other hand, after 18 DES-RE cycles, greater hardness was found in groups that received some method for preventing erosion (resin-modified glass ionomer varnish as well as topical application of fluoride).

Composite resin surfaces

The 2-way ANOVA of composite resin hardness data did not reveal a significant interaction between the main factors (p=0.23). For this reason, the preventive methods and the erosive challenges provided independent results that were detected by the Tukey test. Initially, when comparing the erosive challenges, (p=0.001) it could be seen that regardless of the type of preventive method, higher means of hardness were found in groups not exposed to the acid, yet lower means were observed after 18 DES-RE cycles. Exposure to 9 DES-RE cycles resulted in intermediate results, with no significant differences among the methods.

On the other hand, when comparing the preventive methods (p=0.02), it was found that the hardness values observed in the group exposed to resin-modified glass ionomer varnish were statistically higher than the ones of the negative control. Surfaces exposed to the resin sealant and topical application of fluoride showed intermediary means, which were not significantly different to the other groups.

Figure 1. Distribution of experimental groups in each substrate: dentin (n=10) and composite resin (n=10).

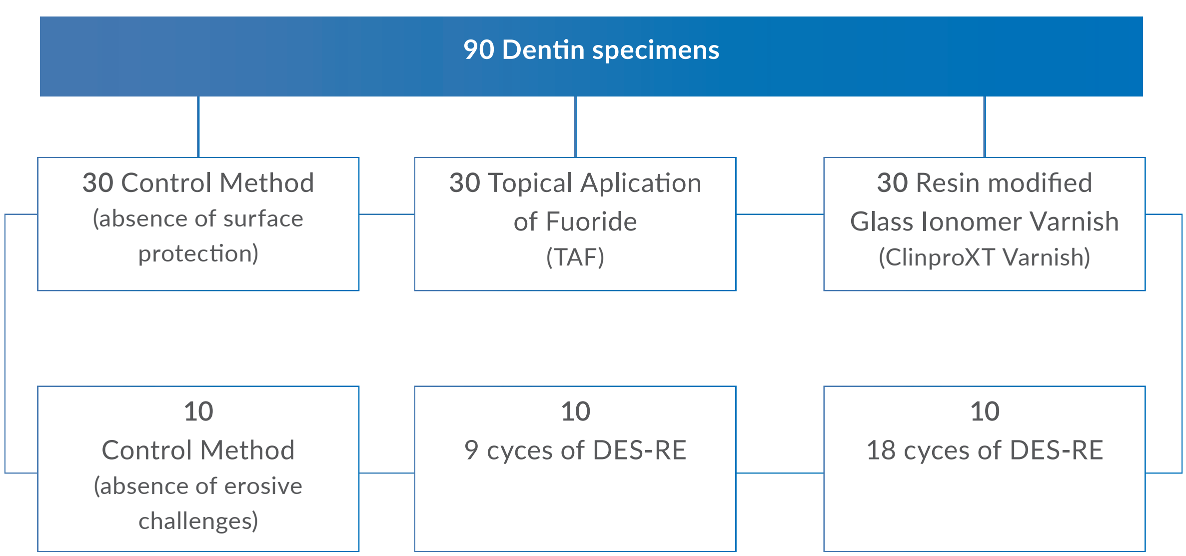

Table 1. Mean (standard deviation) of Knoop microhardness of dentine in the experimental groups.

Methods for preventing the erosive challenge	Simulation of erosion by gastric acid		
	Negative control	9 DES-RE cycles	18 DES-RE cycles
Negative control	31.9 (1.4) Aa	29.7 (3.8) Aa	14.8 (5.0) Bb
Topical application of fluoride	31.1 (4.7) Aa	30.0 (5.2) Aab	23.6 (6.5) Ab
Resin-modified glass ionomer varnish	27.0 (4.9) Aa	26.0 (6.1) Aa	26.0 (2.5) Aa

Mean values followed by different letters show statistically significant difference (2-way ANOVA/Tukey, alfa=5%). Capital letters compare the preventive methods within different levels of simulated erosion and lower-case letters compare erosive challenges within the levels of preventive method.

Table 2. Mean (standard deviation) of Knoop microhardness of composite resin in the experimental groups.

Methods for preventing the erosive challenge	Simulation of erosion by gastric acid			
	Negative control	9 DES-RE cycles	18 DES-RE cycles	
Negative control	64.7 (5.9)	60.4 (8.8)	53.5 (10.9)	B
Topical application of fluoride	68.2 (7.2)	63.8 (5.5)	53.6 (6.4)	AB
Resin-modified glass ionomer varnish	66.5 (13.2)	68.8 (22.0)	68.0 (15.6)	A
Resinous sealant	70.7 (6.1)	61.5 (6.3)	58.9 (4.2)	AB
	a	ab	b	

Different letters show statistically significant difference (2-way ANOVA/Tukey, alfa=5%). Capital letters compare the preventive methods and lower-case letters compare erosive challenges.

DISCUSSION.

Dental surgeons have an important role in early diagnosis of some disorders through detection of structural changes in the hard tissues of the teeth. Moreover, in some situations, in addition to eliminating the cause of the pathology, restorative interventions may be necessary, to protect the remaining structure and health of the dentine-pulp complex. It is important to know how restorative agents behave under erosive conditions.[14] Acids can reduce surface hardness and increase the roughness of composite resin, both effects being dependent on time.

This is due to the degradation of the matrix, a hydraulic rupture of the bond between silane and fillers. Progressive degradation alters the microstructure and leads to formation of pores on its surface.[18]

One of the findings of this investigation was that, regardless of the preventive method used, higher means of composite resin hardness were found in the groups that had not been exposed to acid, and lower means after 18 DES-RE cycles. Thus, one hypothesis was accepted since the erosive challenge affected the composite resin hardness.

The composite resin used in this study is classified as nanofilled and its matrix is UDMA based. The chemical composition and filler content (smaller particle size and higher proportion of fillers) can interfere with the resistance and surface wear of the composite.[19] Monomers such as UDMA are more hydrophilic and susceptible to water absorption. Consequently, they might be more susceptible to acid breakdown.[2,20]

The susceptibility of the resin matrix to degradation by erosive challenges, means that it is important to find ways that may limit or prevent damage and lengthen the durability of restorations. In this study, it was not possible to detect a significant statistical interaction between the preventive methods and the erosive challenges on composite resin, thus the effects were analyzed independently. Understanding this statistical result is important for detecting any differences between the levels of preventive methods.

It should be clarified that all the levels of erosive challenge were grouped for such comparisons and the surfaces protected with resin-modified glass ionomer varnish showed significantly greater hardness compared to the negative control (absence of surface protection).

It is common knowledge that composite resins present higher hardness values compared to glass-ionomer based materials.[8,9,21] The contrary finding observed in the present investigation might be explained by the capacity of the sealant to act as a mechanical barrier for the protection of the restorative material, and by possibly buffering the HCl through the release of minerals from the glass-ionomer based material.[10,13]

The resin-modified glass ionomer varnish tested in this investigation mainly consists of polyalkenoic acid, 2-hydroxy ethyl metacrylate (HEMA), calcium glycerophosphate, Bis-GMA and fluoro-aluminosilicate glass. The material can release fluoride, calcium and phosphate ions during the period of the surface protection. Other methods for surface protection of the composite resin were also tested in this study. The resin sealant and the topical application of fluoride resulted in intermediary hardness values, higher than those with no protection and lower than the resin-modified glass ionomer varnish ones.

The resin sealant appeared able to prolong the longevity of the composite resin restorations.[22] Nonetheless, in the present investigation, it is possible that the UDMA based sealant, with no fillers in its composition might have suffered irreversible damage during the erosive challenges with consequent softening of its organic matrix.[23]

Sources of fluoride have previously been studied in association with composite resin restorations.[11] The expected effect of the fluoride ion on composites is to form a chemical barrier, blocking the contact of acid with the restorative surface, this way offering some superficial protection.

However, it seems that fluoride has limited potential for protection due to its low capacity to remain on surfaces. Also, it is easily degraded, which leads to its protective properties decreasing more and more with every exposure to acid.[24,25] Previous research has shown that the application of acidulated fluorphosphate gels can cause composite resin deterioration (Filtek Z350 and Grandio), increasing their roughness and reducing their microhardness.

These damaging effects are due to the erosion of the resin matrix and the silane interface, as well as the dissolution of inorganic fillers.[3,26] This is why careful consideration should be given to the use of acidulated fluorphosphate gels. Dentine has a lower concentration of calcium by volume because of its greater protein content. When it is exposed to acids, erosion proceeds faster than in enamel.[5]

The experimental hypothesis regarding the erosive effect on dentine samples was also accepted according to the findings of this study, once a reduction in hardness after 18 DES-RE cycles was noted, though only in the absence of surface protection and in groups exposed to topical application of fluoride.

Fluoride seems to be effective in preventing, stabilizating or reversing the demineralizing process. Researchers have observed that after exposure to citric acid (pH=2.6), NaF reduced the dental wear significantly compared with the groups that were not exposed to a surface control treatment.[11,14] Moreover, remineralizing agents containing different calcium phosphate and fluoride ion concentrations increased the potential for remineralization compared with artificial saliva.[13]

Despite this, it is crucial to recognize that in order to promote remineralization, it is fundamental that adequate amounts of calcium and phosphate ions are available.[27] Not all commercial fluoride-containing toothpastes offer protection against the loss of dental enamel caused by acids.[28]

According to Reynolds *et al.*,[27] for every two ions of fluoride, ten calcium ions and six phosphate ions are necessary to form a cell unit of fluorapatite. Some studies have concluded that groups treated with topical fluoride showed a moderate influence on dentine wear, but significantly higher than without it.[15,29]

Algarni *et al.*,[25] stated that in conditions that simulate the frequency of daily acid exposure, routine mouth rinsing using solutions that contain NaF (250ppm F) significantly reduced the amount of the loss of dentine surface as compared with tin (Sn) and the control group. But Sundaram *et al.*,[23] found that fluoride applications did not reduce the amount of wear in comparison with the erosion control group. It could be postulated that the inability of fluoride to protect dentine surfaces against 18 DES-RE cycles is again related to its low capacity to stay on the surface and the fact that it is easily dissolved.

Dentine sealants or desensitizing agents have been suggested to protect and seal exposed areas of the dentine.[23] According to the authors, this noninvasive technique can be beneficial in the initial phases of the loss of hard tissue by delaying the wear and possibly lengthening the longevity of teeth.

An important finding of this study was that the dentine hardness in groups protected with resin-modified glass ionomer varnish remained unaltered, even after 18 DES-RE cycles. A study carried out by Elkassas *et al*.,[13] showed that the protective capacity of this material is related to:

(1) the adhesive potential of the glass-ionomer resin component that infiltrates and seals the surface; and

(2) the remineralizing activity by continual release of fluoride, calcium and phosphate throughout the life of the coating.[13] Nonetheless, this effect might be limited in terms of time and there is a need for maintaining[14] longer protection, improved predictability and longevity of dental treatment.

One limitation of this study is the *in vitro* evaluation of the surface hardness as it is not as reliable as in vivo studies. The oral cavity is complex and other factors, found in every individual, could not be considered. Such conditions could aggravate the effects of erosion such as diet, the effect of saliva, pH of the oral cavity and mechanical abrasion from tooth brushing.

CONCLUSION.

This study found that the microhardness of dentine and composite resin decreases as the frequency of the acid challenge increases.

This is a topic that should be subject to further research since endogenous erosion is becoming a significant contemporary oral health problem. Offering preventive strategies to patients who suffer from dental erosion should be a primary concern.

Among the preventive methods tested against erosion caused by endogenous acids, resin-modified glass ionomer varnish showed a promising result that should be confirmed in future investigations.

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