

Influence of Remineralizing Dentifrice in the Treatment of Erosive Enamel Lesions

Influência de Dentifricio Remineralizante no Tratamento de Lesões Erosivas em Esmalte

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Recebido em: 23/10/2018

Aprovado em: 17/12/2018

Abstract

The objective of this in vitro study was to evaluate the influence of different remineralizing agents in the treatment of enamel erosive lesions. Specimens of 4mmx4mm and 3mm thickness were made from the buccal surface of bovine incisors (n=10) and randomly divided into 4 groups. G1 = application of the remineralizing dentifrice, G2 = application of the remineralizing agent, G3 = remineralizing dentifrice + remineralizing agente, G4 = application of fluoride varnish (positive control), G5 = no treatment. Specimens were immersed in refrigerant solution during a period of 10 days. The surface roughness was analyzed by means of confocal laser scanning microscopy. The data were analyzed for homogeneity (Levene's) and normality (Kolmogorov-Smirnov). Parametric tests with analysis of variance were performed on two criteria: time factor and treatment factor, and Tukey post-test for differentiation of means. All tests were statistically significant at 5% ($\alpha = 0.05$). The results showed statistically significant difference, demonstrating the reduction of surface roughness after the first treatment (G3) and the other groups (G1, G2 and G4) only after the second treatment. It was concluded that the use of dentifrice composed of calcium silicate and sodium phosphate influenced the roughness of the eroded tooth enamel of the bovine tooth.

Keywords: Dentifrices. Tooth Erosion. Tooth Enamel.

Resumo

O objetivo deste trabalho in vitro foi avaliar a influência de diferentes agentes remineralizantes no tratamento de lesões erosivas em esmalte. Foram confeccionados espécimes de 4mmx4mm e 3 mm de espessura a partir da superfície vestibular de incisivos bovinos (n=10) e divididos aleatoriamente em 4 grupos. G1=aplicação do dentifricio remineralizante, G2= aplicação do agente potencializador remineralizante, G3= dentifricio remineralizante + agente potencializador remineralizante, G4=aplicação de verniz fluoretado (controle positivo), G5=nenhum tratamento (controle negativo). Os espécimes foram imersos em refrigerante durante um período de 10 dias. A rugosidade superficial foi analisada por meio de microscopia confocal de varredura a laser. Os dados foram analisados quanto à homogeneidade (Levene's) e normalidade (Kolmogorov- Smirnov). Foram realizados testes paramétricos com análise de variância a dois critérios: fator tempo e fator tratamento, e pós-teste de Tukey para diferenciação das médias. Todos os testes estatísticos tiveram nível de significância de 5% ($\alpha=0,05$). Os resultados obtidos mostraram diferenças estatisticamente significantes, demonstrando a redução da rugosidade da superfície do esmalte logo após o primeiro tratamento (G3) e para os demais grupos (G1, G2 e G4) somente após o segundo tratamento. Concluiu-se que a utilização de dentifricio composto por silicato de cálcio e fosfato de sódio influenciou na rugosidade do esmalte erodido do dente bovino.

Palavras-chave: Dentifricios. Erosão Dentária. Esmalte Dentário.

1 Introduction

The dental erosion is a pathology, chronic disease, which consists of dental hard tissue loss due to chemical phenomena¹. This loss is also defined as a result of the non-bacterial chemical attack, usually involving acidic substances. This leads to a progressive softening of the tooth surface with subsequent irreversible loss of dental hard tissue. Dental erosion affects mainly the enamel but can also cause hypersensitivity if it reaches the dentin, or in severe cases, exposure the pulp or even tooth fracture².

The acids determine the pH of the oral cavity. If below 5.5, which corresponds to the critical limit for the demineralization of the enamel, the erosive process can begin. Thus, the dental

erosion can be caused by intrinsic or extrinsic factors³.

The Intrinsic factors are the result of endogenous acid, usually gastric acid that contact the teeth especially in patients who suffer from anorexia, bulimia and gastrointestinal disorders, regurgitation, salivary deficiencies such as low salivary flow, low pH and buffer capacity and autoimmune diseases^{3,4}.

The extrinsic factors are related to the frequent consumption of acidic foods or drinks and exposure to contaminants acids in the work environment. The consumption of citrus fruits, juices and industrialized drinks, especially sodas, has increased significantly in recent years and has been associated with an increase in the prevalence of dental erosion⁴.

It is believed that the erosive power of acidic drinks

involves several factors, which can lead to dental erosion. Changes in pH, buffering capacity, type of acid, frequency of exposure, duration of each episode of erosive exposure, chelating properties and content of calcium and phosphate, are the most common factors².

Clinical manifestations, such as superficial lesions on smooth surfaces and bulging and flattening of cuspids can develop even in the initial stages, which may lead to exposure of the coronal dentine. The dental hypersensitivity is common in patients with erosion, and on the progressive loss in the long term the tooth substance may become so extreme that the fracture of the same can occur⁵. Clinically the lesions of erosion have appearance of milk glass, the depressions are wide, there is an increase of the incisal translucency and there may be sensitivity³.

Previous studies have observed that between the enamel and the oral environment is a physic-chemical process of demineralization versus remineralisation⁶. The rate of demineralization depends on several factors, including the pH and the duration of the acid challenge. Before the initial loss of tissue, remineralization can occur through the substitution of mineral ions loss of the salivary reservoir of calcium and phosphate ions⁵.

During the erosion process, acid or chelating agents interact with the surface of hydroxyapatite crystals after spreading through the dental biofilm, by acquired film and the layer of lipids and proteins. When the hydrogen ion acts directly on the enamel surface, it combines with carbon and/or phosphate and promotes an acid condition, due to the removal of minerals from the crystal surface. This erosion may cause dentine sensitivity, loss of vertical dimension, pulp exposure and aesthetic impairment when the anterior teeth are involved⁷.

Products containing fluoride are well known to play an important role in the prevention of dental caries, as well as the treatment of cervical non-cariou lesions^{8,9}.

Studies show that fluoride toothpastes have beneficial effect when compared to non-fluoride toothpastes on the abrasion of dentin and enamel subjected to erosive challenges, since they have the potential to reduce the development of tooth wear^{10,11}. They can also be useful in supporting the tooth remineralization, increasing the acid resistance of tooth surfaces after the acid attack⁵.

Recently, the use of a calcium silicate and sodium phosphate-based toothpaste (YAZ[®]) has been indicated for the treatment of erosive lesions¹². The regular use of this toothpaste can promote the regeneration of the enamel recovering its mineral composition and working mainly in the initial and invisible stages of the erosion (REGENERATE Enamel Science[®]).

The objective of this study was to evaluate the *in vitro* effects of this new toothpaste (REGENERATE[®]) before erosive challenges in specimens of bovine enamel, through analysis of surface roughness by confocal laser scanning.

The null hypothesis was statistically significant in the eroded enamel surface roughness after different treatments.

2 Material and Methods

2.1 Teeth Selection

The teeth (bovine incisors) were cleaned with periodontal curettes and pumice stone paste with water applied with Robinson brushes. Then, it was carried out with the aid of an exploratory probe the visual examination in the stereomicroscope for the teeth selection to the study. The teeth sterilization was performed with 10% formalin solution (pH=7.0) prepared with phosphate buffer, in which they were immersed during a week; they were washed thoroughly and then stored in distilled and deionized water at 4°C, changing the water daily until completing a period of 7 days.

2.2 Preparation of specimens

The roots were separated from dental crowns using a diamond disc under refrigeration to water, coupled to a cutting machine. Afterward, the vestibular side of the crown was cut in the mesiodistal direction and incisal-cervical, obtaining blocks of 4.25mm x 4.25mm and 3.00mm thickness, resulting in a surface area of approximately 18.0mm². After due preparation of specimens, these were individualized: each specimen was fixed with sticky wax sculpture at the bottom of a cylindrical container made of plastic, stored individually, so that left only exposed the surface that has been eroded and the other half in isolation is the control region.

2.3 Erosive Challenge

Then they were subjected to erosion challenges in Coca Cola[®] (Cia. de Bebidas Ipiranga, Ribeirão Preto, SP, Brazil). Its erosive potential has been studied and discussed in various scientific studies¹³⁻¹⁵. In addition, it is a widely consumed beverage around the world. The volume was determined in accordance with the exposed area of each specimen and remained immersed for 1 minute. After this time, the erosive solution was discarded, and the specimens were washed with distilled and deionized water for 10 seconds and slightly dried with absorbent paper. This procedure was performed 3 times a day, with intervals of on average 4 hours between the challenges, for a total period of 10 consecutive days. The specimens were stored in distilled water between the challenges.

2.4 Experimental Groups

The specimens were randomly divided and treated as shown in Table 1. The treatments were carried out in two stages. In the first week the products were applied on the exposed surface (experimental region) with micro brush region in the different treatments, being removed the excess with gauze and stored in distilled water. This water was changed after 24 hours and kept in the refrigerator. And after 7 days, the specimens were again subjected to treatments.

Table 1 - Experimental groups.

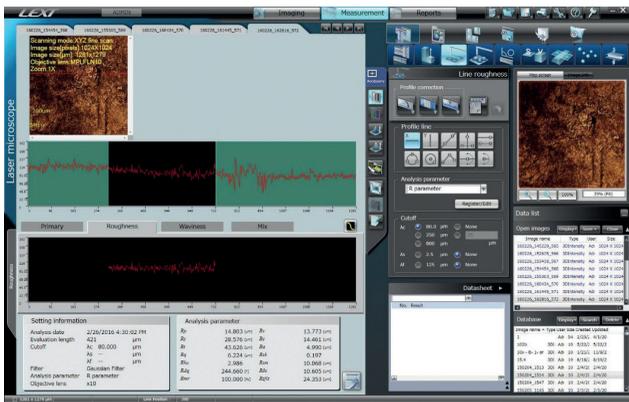
| Groups | Treatment | Application time |
|--------|--|----------------------|
| G1 | Regenerate Advanced Toothpaste | 1 minute |
| G2 | Regenerate Boosting Serum | 3 minutes |
| G3 | Regenerate Advanced Toothpaste + Regenerate Boosting Serum | 1 minute + 3 minutes |
| G4 | Fluoride varnish 5% (positive control) | 4 minutes |
| G5 | No treatment (negative control) | - |

Source: Data from the survey.

Evaluation by confocal laser scanning

After the erosive/acid challenge , followed by the application of the desensitizing agents, the topographic profile 3D was analyzed (wear profile), with the purpose of checking the surface roughness in the different experimental groups (Figure 1).

Figure 1 - Analysis of surface roughness. Image representative of surface roughness analyzed by confocal laser scanning (OLS4000).



Source: The authors.

2.5 Statistical analysis

The data were analyzed regarding the distribution and

normality in the test of homogeneity (Levene’s test) and test of normality (kolmogorov - Smirnov test).

Given this condition, parametric tests were performed with analysis of variance to two criteria (time factor and factor treatment) and Tukey’s post-test for differentiation of the averages. All statistical tests had a significance level of 5% ($\alpha=0.05$).

3 Results and Discussion

Regarding the types of treatments carried out in each group, the results presented in Table 2 showed that there was a statistically significant difference between the groups presented, when comparing the ranges of treatments ($p<0.05$), showing reduction in the enamel surface roughness.

Analyzing the results, the G3 group showed a statistically significant difference immediately after the first treatment (2.407 μ m) when compared to the other groups.

The groups G1, G2 and G4 showed statistically significant differences only after the second treatment.

The analysis of the different groups in relation to their control region and experimental region there was a statistically significant difference in the treatment times, presenting the benefit to eroded dental enamel.

The results of this study showed that the null hypothesis was rejected, because there were statistically significant changes in the eroded enamel surface roughness.

The specimens were divided into control region and experimental region. During the erosive challenge with Coca Cola®, only the vestibular side of the experimental region was eroded, while in the control region there was impermeabilization of specimens through the protective base (nail varnish) and green sticky wax. Thus, it was possible to observe the results, in that there was a statistical standardization in the initial region of all groups in the non-eroded region(control)

Table 2 - Values of surface roughness (μ m) in the different treatments.

| Groups | Initial (Baseline) | | 1 st Treatment | | 2 nd Treatment | |
|--------|--------------------|---------------------|---------------------------|---------------------|---------------------------|---------------------|
| | Region Control | Experimental Region | Region Control | Experimental Region | Region Control | Experimental Region |
| G1 | 0.523 ^a | 3.562 ^c | 0.517 ^a | 3.374 ^c | 0.508 ^a | 2.517 ^b |
| G2 | 0.568 ^a | 3.597 ^c | 0.523 ^a | 3.291 ^c | 0.511 ^a | 2.533 ^b |
| G3 | 0.577 ^a | 3.466 ^c | 0.558 ^a | 2.407 ^b | 0.539 ^a | 2.380 ^b |
| G4 | 0.562 ^a | 3.378 ^c | 0.527 ^a | 3.254 ^c | 0.510 ^a | 2.667 ^b |
| G5 | 0.551 ^a | 3.456 ^c | 0.544 ^a | 3.468 ^c | 0.532 ^a | 3.599 ^c |

*different letters within the same column represent statistically significant difference ($p<0.05$).

Source: Data from the survey.

It was used for the study of bovine teeth, due to the ease of obtaining and its standardization, because these have become models of references for research by presenting characteristics, properties and a morphology similar to that of

humans, in addition to microhardness and mineral composition equivalent to human dental characteristics, with no statistical difference between the human and bovine enamel on the surface roughness^{16,17}.

It was opted for Coca Cola® to produce the erosive challenge, because the other in vitro studies^{18,19}, where there is similarity with the clinical situation and due to the greater part of the world population consuming sodas, which have low pH²⁰. This low pH around 2.5 is capable of dissolution of dental enamel. In this study, the specimens which received erosion showed standardization, i.e., the loss of minerals was uniform for all groups.

In a study analyzing the dental erosion induced by different beverages, Coca Cola® was considered the most erosive agent between the studied hot and cold drinks (37%). After 10 minutes of exposure to Coca Cola®, the exposed tooth surface showed effects such as prisms of enamel clearly visible, cracks, rough surfaces, showing signs of demineralization, the SEM (scanning electron microscopy), showed visible debris and prisms exposed and the hardness tends to decrease²¹. This explains the results found in the present study, it was observed the demineralization of the tooth surface after erosive challenge with Coca Cola®.

A new technology was developed based on the combination of calcium silicate, sodium phosphate salts and fluoride, which proposes to increase the natural mineralization processes of saliva and the formation of minerals of dental enamel. The proposed mechanism can help repair the enamel after acid challenges²². These comprise the REGENERATE® paste. In the present study, the paste REGENERATE® was used in the groups G1, G2 and G3, where in G1 only the Regenerate Advanced Toothpaste was applied, while in G2, the Regenerate Boosting Serum. The results showed statistics changes after the second treatment in groups G1 and G2.

In specimens of G3 the Regenerate Advanced Toothpaste and Regenerate Boosting Serum were applied. The first is composed of calcium silicate, sodium phosphate and fluoride 1459 ppm and has been used to provide reinforcements to the health of the enamel, and the second is a dual gel composed by a part A: calcium silicate, phosphate salts and sodium monofluorophosphate and part B (gel activator): sodium fluoride, being indicated as an adjunct to the daily use of Regenerate Advanced Toothpaste²³. Before the treatment in this group, it was observed that already in the first treatment, it is possible to reduce the roughness of the enamel surface, being maintained after the second treatment, being the last one the only one to show statistical difference in the first treatment. And also, it was observed a study, where the dual phase gel increased the remineralization rate of enamel from the first application, and that the combination of the two Regenerate® was better than the paste alone²³.

This calcium silicate and sodium phosphate-based treatment can provide protection to the enamel by several mechanisms: can release calcium ions to the surrounding oral fluids under acidic conditions, thus increasing the local concentration of calcium, the degree of saturation in relation to enamel hydroxyapatite and inhibiting the dissolution. It also operates as a buffer for the protons absorption, which also

contributes to prevent a drop of local pH and thus damage to the enamel before the acid; has the ability of hydroxyapatite formation, thus shifting the balance for remineralization and resulting in a reduction of mineral loss²³.

The G4, where Fluorniz® was applied, presented statistical result only after the second treatment. But its beneficial potential to dental health is already widely studied (positive control). The fluorine acts as an inhibitor of the demineralization mediated by acid and also as a promoter of remineralization²⁴. In one study, where varnish agents were tested (Duraphat, varnish of xylitol, varnish CPP-ACP) showed the same capacity for protection against changes in surface roughness among them. These positive results can be explained by the formation of a protective layer of CaF₂ (calcium fluoride), in dental tissue by application of fluoride topic²⁵, being that they act as a physical barrier that inhibits the contact of acid with the enamel and also act on the demineralization²⁰.

In the present study, G5 has not received any of the treatments (negative control). Thus, only keeping specimens in distilled water, there was no benefit or treatment, which can compare with the other groups which received treatment and showed benefits to the enamel.

In the present study two treatments were standardized with an interval of seven days between them. Literature studies used different intervals, such as the one that dealt with the enamel surface for three minutes in times 0, 8, 24 and 36 hours²⁶, different from the protocol used in this study in which it was sought a similarity with the frequency at which the pastes and the fluoride varnish are exposed to the tooth surface.

In the literature, many techniques have been used to investigate the effects of erosion challenges in dental hard tissues. Micro-indentation, surface profilometry, micro-radiography, chemical analysis and SEM, were considered the most advanced in the evaluation²⁷. In the present study, the use of confocal laser scanning, allowed to understand qualitatively the processes of demineralization of the enamel surface through the observation of the specific and structural morphology that characterize the enamel itself²⁶, analyzing the loss of substance and the characteristics of the enamel surface. It is interesting that in future studies, these groups be subjected to analysis of longitudinal microhardness.

The study demonstrated that these treatments can bring advantages to the eroded enamel and affirmed the erosive potential of Coca Cola®. However, it is important that further studies and tests be performed to ensure the use of these materials in a longer period of time in therapy and their periodic control to the patient's oral health, in addition to allow the surgeon dentist to recommend and instruct their patients regarding the damages he frequency of Coca Cola® and the possible treatments of dental erosion.

4 Conclusion

The groups subjected to the erosive challenge and treated with new toothpaste composed of calcium silicate/sodium phosphate and also, with the use of sodium fluoride to 5% significantly altered the values of bovine enamel surface roughness.

Acknowledgments

To Fundação de Amparo à Pesquisa de Minas Gerais [Support Foundation to Research of Minas Gerais State] (FAPEMIG PIBIC-2017/6) for granting scientific initiation scholarship and the Programa de Apoio à Pesquisa da Universidade de Uberaba [Program of Support to the research of the University of Uberaba] (PAPE-UNIUBE 2017/6) for supporting the research.

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