Effect of surface protection on the permeability of eroded dentin

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Abstract

Context: Eroded dentin might present the opening of dentinal tubules, increasing permeability, and consequently dentinal hypersensitivity.

Aims: This study evaluated the permeability of dentin surfaces exposed to different levels of erosion and methods of surface protection.

Materials and Methods: Dentine samples (3 mm × 3 mm × 1 mm) were prepared from bovine incisors (n = 90) and divided into three groups according to the method of controlling erosive challenge: Negative control, topical fluoride application, and glass ionomer sealant. Subsequently, they were randomly divided into three subgroups according to the exposure of simulated gastric acid solution (Demineralization-DES) (5% HCl, pH = 2.2), and remineralization (RE); negative control, 9 and 18 cycles DES-RE. The dentin permeability was measured by assessing the hydraulic conductance (µl/min.cmH₂O.cm²). Statistical analysis was performed by two-way ANOVA and Tukey’s test.

Results and Conclusions: Greater permeability was observed after 18 erosive cycles, followed by exposure to 9 cycles and negative control (P < 0.0001). The application of glass ionomer sealant resulted in a major reduction of the hydraulic conductivity, regardless of the erosive challenge. Control groups and topical fluoride application showed similar results. In conclusion, the severity of erosive challenge contributed to the increase of dentin permeability. Besides, the glass ionomer sealant was the only protection agent that promoted significant effects in dentin permeability.

Keywords: Dentine permeability; dentine; tooth erosion

INTRODUCTION

Erosion is a type of tooth wear, defined as hard tissue loss by a chemical process, without bacterial involvement. The prevalence of dental erosion has increased in recent years, mainly due to consumption of acidic foods and eating disorders such as gastroesophageal reflux disease. The tooth enamel is the most commonly affected substrate; however, if the dentin enters into contact with the oral environment, it might also be affected.

The erosive challenge on exposed dentin causes the removal of the smear layer and opening of dentinal tubules, increasing its permeability. The increase of hydraulic conductance through the dentinal surface possibly will promote the occurrence of hypersensitivity. According to the hydrodynamic theory, the movement of fluids through the dentinal tubules leads to stimulation of receptors present in the pulp and dentin, exacerbating the nerve impulse, promoting pain, localized and transient, without odontoblastic involvement.

To minimize the effects of erosion and reduce the teeth mineral loss by acids, surface protection techniques have been used such as topical fluoride application and surface sealing with glass ionomer sealants.

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Fluoride topical application is certainly one of the standard techniques for dental remineralization.\cite{10-12} Fluorides act forming calcium fluoride on the surface, reducing the acid dissolution, and protecting the enamel or dentin against the erosive challenge.\cite{12,13} However, the glass ionomer sealant can promote a more durable contact with the surface, due to its stable dentin union.\cite{7,15} Studies have shown that sealing materials have great potential for remineralization of degraded tooth surface through release of calcium phosphate and fluoride ions.\cite{11,14,16} Nevertheless, there is a lack of information regarding the ability of these materials to resist the erosive challenges, maintaining the obliteration of dentin tubules and surfaces.

The aim of this study was to compare the permeability of dentin surfaces protected with fluoride and glass ionomer sealant and exposed to different degrees of erosion. The hypothesis is that the surface protection will decrease dentin permeability even under more aggressive erosive challenge.

**MATERIALS AND METHODS**

**Study design**

- **Main factors**
  - Method for controlling erosive challenge in three levels: negative control, topical fluoride application, and glass ionomer sealant
  - Erosive challenge in three levels: negative control, 9 DES-RE cycles, and 18 DES-RE cycles.
- Experimental units: Bovine dentin samples.
- Dependent variable: Hydraulic conductivity ($\mu\text{l/min/cmH}_2\text{O/cm}^2$).

**Sample preparation**

In this study, thirty bovine incisors were used. Teeth were obtained with the consent of the Ethics Committee on Animal Use UFBA (45/2015). In the selected units, cleaning was performed with N.15 scalpel blade, Robson brush, and pumice to remove the waste. Cleaned teeth were stored in 0.1% thymol solution (Chromate Chemical Products Ltd., Diadema, SP, Brazil), under refrigeration (4°C).

Each tooth was sectioned in the medium distal, and labial-lingual directions using a water-cooled diamond disc adapted to a precision cutter (Extec Corp®, Enfield, CT, USA). Sections resulted in fragments from the cervical third of the labial surface with dentin on both sides, with 1.0 mm of thickness and 3 mm of width. Each dental unit provided an average of three fragments ($n = 90$).

The specimens were randomly divided into nine experimental groups ($n = 10$), according to the method for controlling the erosive challenge and the exposure to simulated gastric acid solution.

**Methods for controlling the erosive challenge**

The control methods of the erosive challenges were performed as follows ($n = 30$).

**Negative control**

The samples were not subjected to any form of erosion control, only kept in relative humidity at 37°C.

**Topical fluoride application**

On each surface, 1 ml of neutral 2% NaF (DFL Ind. Com., Jacarepaguá, RJ, Brazil) was applied for 1 min, followed by washing with distilled water in a ultrasonic bath for 2 min (UNIQUE, São Paulo, Brazil) during 2 min. Subsequently, they were placed in relative humidity at 37°C.

**Glass ionomer sealant**

Product (Clinpro XT Varnish, 3M-ESPE, Sumaré, SP, Brazil) was applied according to the recommendations from the manufacturer. Initially, conditioning was carried out with 37% phosphoric acid (Biodinâmica Quím. e Farm. Ltda., Ibirapuã, PR, Brazil) for 15 s. After this, equal portions of the two pastes were placed and manipulated for 15 s. Straight after, a thin layer was applied on the dentin surfaces with a disposable applicator followed by photoactivation for 20 s (Radii Plus, SDI Brazil, São Paulo, SP, Brazil). Finally, they were kept in relative humidity at 37°C.

**Simulation of erosion by gastric acid**

After the use of the methods for erosive challenge control, the specimens were divided into three groups according to the simulation of erosion by gastric acid ($n = 10$).

**Negative exposure control**

The samples of this subgroup were kept in relative humidity of 37°C as the cycles were carried out in the remaining subgroups.

**Nine cycles of DES-RE**

Each completed cycle consisted of immersing the sample in 10 ml solution of hydrochloric acid (5% HCl, pH = 2.2) for 2 min in room temperature. After this, the specimens were washed with the help of disposable syringe containing 20 ml distilled water and immersed in remineralizing solution.\cite{13} Its composition included 2.0 mmol/L Ca, 20 mmol/L PO4, and 0.075 mol/L of acetate with pH = 7 and its use was based on the work of Toda and Featherstone.\cite{10} Between the cycles, the units were stored in relative humidity of 37°C.

**Eighteen cycles of DES-RE**

The samples of this subgroup were subjected to a doubled frequency of cycles to promote a more aggressive challenge. Each cycle was carried out as previously described.
Dentin permeability evaluation

To perform the evaluation of dentin permeability, the unprotected surface (inner part of the dentin specimen) was exposed to acid etching with 37% phosphoric acid for 20 s, washed with running water for 30 s, and dried with paper towels. Then, the smear layer was standardized with water-cooled sandpaper grain 600 (Bosch Brazil, São Paulo, SP, Brazil) for 30 s on each side. The specimens were ultrasonically washed with distilled water, dried, and taken to the permeability machine (Odeme, Lucerne, SC, Brazil). Such surface conditioning was used according to the recommendations from the manufacturer of the permeability device.

The hydraulic conductance measurements were performed in the most centralized region of the dentin, simulating the hydraulic conductance from pulpal pressure. Therefore, the shift amount of the air bubble in the capillary tube of the machine was checked. This procedure was performed three times for each sample (5 min each), and the average was taken for obtaining the offset value. With this value it was possible to obtain the filtration rate through the formula:

\[ Q = \frac{SV \times D}{L \times T} \]

Where: \( Q \) = filtration rate (µl/min); \( SV \) = standard volume (µl); \( D \) = bubble displacement in the capillary tube (mm); \( L \) = Capillary Length (mm); \( T \) = time in minutes (min). Having obtained the value of the filtration rate, this value was submitted to another formula to obtaining the hydraulic conductance value of dentin µl/cm²/min/cmH₂O (Lp) of each disc by the formula:

\[ Lp = \frac{Q}{P \times (A)_{sup}} \]

Where: \( Lp \) = dentine hydraulic conductance in µl.cm²/min. cmH₂O; \( Q \) = filtration rate (µl/min); \( P \) = hydrostatic pressure difference over the dentin (cmH₂O); \( (A)_{sup} \) = exposed dentinal surface area (cm²).

Statistical analysis

First, the data analysis was performed to verify the homogeneity of variances and to determine whether the experimental errors had normal distribution or not (analysis of variance parameters). The inferential statistical analysis was achieved by two-way ANOVA Tukey’s test for multiple comparisons. This analysis was done in the SAS statistical software, version 9.1, with 5% significance level.

RESULTS

Table 1 demonstrates the mean and standard deviation of the hydraulic conductance percentage of the experimental groups.

| Method for controlling the erosive challenge | Erosive challenge | Tukey |
|---------------------------------------------|------------------|-------|
| Negative control                            | 2.243 (0.31)     | A     |
| 9 DES‑RE cycles                             | 1.269 (0.19)     | B     |
| 18 DES‑RE cycles                            | 2.543 (0.34)     | A     |
| Topical application of fluoride             | 2.73 (0.54)      |       |
| Glass ionomer sealant                       | 3.313 (0.44)     |       |
| Tukey                                       | 2.967 (0.8)      |       |
| Negative control                            | 3.649 (0.75)     |       |
| 9 DES‑RE cycles                             | 4.564 (1.28)     |       |
| 18 DES‑RE cycles                            | 3.981 (0.89)     |       |

According to the statistical analysis, there was no significant interaction between the main factors studied “control method” and “erosive challenge” (\( P = 0.06 \)), indicating independence between the levels of a factor on the results of other. Therefore, the main factors were analyzed individually.

When erosive challenges were compared together (\( P < 0.0001 \)), it appears that, regardless of the protection form used, higher permeability values were observed after 18 erosion cycles, followed by 9 erosive cycles and negative control group.

In relation to protection methods (\( P < 0.0001 \)), it is noted that, in all erosive challenge levels, the glass ionomer sealant application has resulted in a significant reduction of the hydraulic conductance of dentin. In addition, groups without a control method and subject to topical fluoride application showed similar results between them and significantly higher than those of glass ionomer sealant.

DISCUSSION

The present study evaluated through hydraulic conductance, the dentinal permeability of bovine incisors, protected by fluoride or glass ionomer sealant and exposed to different degrees of erosion. According to the experimental hypothesis, surface protection would decrease dentin permeability even under more aggressive erosive challenge.

There is a great difficulty of obtaining human healthy teeth for dental research. However, bovine incisors are frequently used in studies involving dentin erosion due to morphological similarity to the structure of human tooth.\(^{[17,18]}\) In addition, their large size facilitates obtaining >1 sample per dental unit.

Dentin permeability constitutes the passage of substances through the dentin, which might occur through diffusion or filtration. The measurement of hydraulic conductivity is able to set the dentin permeability by laboratory...
methods. The method of choice for this study was filtering because it is the most used method to obtain permeability and measurement of obliteration capacity of dentinal tubules protected by agents. The fluid used was distilled water, which has a good ability to penetrate the dentin. This method determines the values of the hydraulic conductance by moving the passage of fluids through dentin by a pressure gradient.

The way which erosive challenge is simulated varies between studies. Several authors present the demineralization time on a number of days and others by the number of cycles DES-RE performed. For this study, the erosive challenge determined by DES-RE cycles was selected since this is what best simulates the episodes of gastroesophageal reflux, once that this disease is characterized by multiple crises at various times of the day, 1–2 min, followed by the remineralizing action of saliva.

In this study, regardless of the form of protection used, higher permeability values were found after 18 cycles of erosion, followed by exposure to 9 erosive cycles and the negative control group. This finding can be explained as the way that erosion damage acts in dentin. In dentin, lesion progression is mediated by the presence of demineralized organic matrix (DOM) enzymes, and chemical degradation affects the DOM, increasing tissue loss over time. This fact is directly dependent on the aggressiveness of the acid challenge.

Another finding of this research was the lack of protection resulting from topical application of neutral sodium fluoride. Studies have shown that, during the erosive challenge, the topical fluoride application will lose its effectiveness on dentin, once the protective effect is associated with inter, intra, and peritubular retention of calcium fluoride on the surface. Over the cycles, more fluoride is dissolved, exposing the surface to demineralization. Moreover, during erosion cycles, the smear layer will be removed, consequently increasing dentin permeability due to tubule opening.

One limitation of the present study was that only one topical application of neutral sodium fluoride was performed. Likewise, Austin et al. investigated the effect of a single application of SnF$_2$ and NaF solutions and NaF/CaF$_2$ highly concentrated varnish in an in vitro model of repeated cycles of erosion. The authors found that only the fluoride varnish results in a qualitative and quantitative effect on the enamel surface, with the potential to modify gastric surface erosion. However, this potential was not significant after multiple cycles. Thus, according to the authors, any therapeutic benefit of fluoride products for professional application remains uncertain.

Different results could have been obtained if >1 topical fluoride application was conducted. Castillo et al. concluded that daily application of an experimental mouthwash containing the combination of TiF$_4$ and NaF has the ability to reduce dentin loss in vitro and might be a good alternative treatment for erosion lesions and dentin hypersensitivity.

Besides the common clinical prescription of fluoride sources for patients that suffer from frequent episodes of erosion, the controversy around the true efficiency has brought attention to more permanent methods for surface protection such as varnishes and sealants. In the present study, the glass ionomer sealant showed notorious results in reducing dentin permeability. This result collaborates with the paper of Elkassas and Arafa which claimed that remineralizing agents as calcium phosphate-based sealants and varnishes promote a higher remineralizing effect and greater resistance to acid, compared to the artificial saliva. Therefore, providing superior propensity to remineralization and surface degradation inhibition. Furthermore, the sealant performs a mechanical surface protection as consequence of dentinal tubules obliteration, staying in longer contact with the tooth.

The null hypothesis tested in this investigation was rejected since the glass ionomer sealant was able to significantly reduce dentinal permeability. Despite the good results obtained with this relatively new material, more studies must be conducted, particularly in long-term, to establish its real effectiveness and to define the clinical protocol for patients that suffer from constant episodes of dentin erosion. Finally, it is important to emphasize among clinicians need for not only treating the sequels of tooth erosion but also monitoring patients with a history of gastrointestinal and eating disorders. On this way, it will be possible to control erosive lesions progression, as well as reduce symptomatology, such as tooth sensitivity, and give a better quality of life for these patients.

**CONCLUSIONS**

Within limitations of this in vitro study, it is possible to conclude that the severity of erosive challenge contributed to increase in dentin permeability. The glass ionomer sealant was the only surface protection agent that promoted significant effects on the erosive challenge control.

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Nil.
Conflicts of interest
There are no conflicts of interest.

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