EFFECT OF 4% TITANIUM TETRAFLUORIDE SOLUTION ON THE EROSION OF PERMANENT AND DECIDUOUS HUMAN ENAMEL: AN IN SITU/EX VIVO STUDY

Ana Carolina MAGALHÃES1, Daniela RIOS2, Heitor Marques HONÓRIO3, Alberto Carlos Botazzo DELBEM4, Marília Afonso Rabelo BUZALAF5

ABSTRACT

This in situ/ex vivo study assessed the effect of titanium tetrafluoride (TiF4) solution on erosion of permanent (P) and deciduous (d) human enamel. Ten volunteers wore acrylic palatal appliances containing 4 enamel samples, divided into two rows: TiF4 and no-TiF4 (control). Each row contained one deciduous and one permanent enamel sample. During the 1st day, formation of a salivary pellicle was allowed. At the 2nd day, the 4% TiF4 solution was applied on one row (TiF4), while the other row remained untreated (control). From the 3rd until the 7th day, the samples were subjected to erosion by immersion in a cola drink for 5 min, 4 times/day. Enamel alterations were determined by microhardness testing (%SMHC). Data were analyzed using 2 two-way ANOVA and Tukey’s post hoc test (α=0.05). The mean %SMHC (±SD) amounted to: P (TiF4 - 73.32 ± 5.16 and control - 83.49 ± 4.59) and d (TiF4 - 83.01 ± 7.41 and control - 75.75 ± 2.57). In conclusion, the application of 4% TiF4 solution reduced the softening of permanent enamel but not of deciduous enamel significantly. However, no significant differences were detected between the permanent and deciduous enamel when the factor substrate was considered.

Key words: Dental erosion. Enamel. Fluoride. Titanium tetrafluoride.

INTRODUCTION

While there is a decline in the prevalence of dental caries in most developed countries19, an increasing prevalence of other dental disorders, such as dental erosion, has been observed18. Dental erosion is defined as the loss of tooth substance by chemical processes (acids) not involving bacteria16. The most important sources are dietary acids (acidic foods and drinks)14 and gastric acids (regurgitation and reflux disorders)13. In modern societies, the increased consumption of acidic drinks, such as soft drinks, sport drinks, fruit juices and fruit teas, is becoming the most important factor in the etiology of dental erosion14,16.

Topical application of fluoride on dental hard tissues is considered as a major approach in the prevention of dental erosion. High-concentrated fluoride applications, such as oral rinses, gels or varnishes, have been considered as most effective in reducing the development of enamel erosion7.

The fluoride agents that have been investigated in most in vitro studies about dental erosion are those that have been used over years for caries prevention: sodium fluoride (NaF), acidulated phosphate fluoride (APF), stannous fluoride (SnF2) and amine fluoride (AmF). More recently, the preventive effect of other fluoride agents, such as 4% titanium tetrafluoride (TiF4) solution, have been investigated in erosion tests5,9,10,17-26.

While some studies showed an inhibitory effect of TiF4 on erosion5,9,10,17-26, other studies did not found a protective effect17,25,26. In addition, studies evaluating the effect of this compound on deciduous enamel erosion are missing as yet. As some controversy remains about the susceptibility of deciduous teeth compared to permanent teeth to erosion1,11-13,15, it is assumed that the efficacy of TiF4 on these substrates might also be different. Therefore, the purpose of this study was to assess the effect of TiF4 solution on permanent and deciduous human enamel subjected to erosion in situ/ex vivo.
MATERIAL AND METHODS

Enamel Samples and Palatal Appliance Preparation

The experimental design of the study is illustrated in Figure 1.

Permanent (4x4x3mm) and deciduous (2x2x2mm) human enamel samples were prepared from freshly extracted impacted third molars and deciduous incisors, respectively. The teeth were sterilized by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. The enamel surface of the samples was ground flat with water-cooled 320-, 600- and 1200-grit Al₂O₃ papers (Buehler, Lake Bluff, IL, USA) and polished with felt paper wet by diamond spray (1 µm; Buehler). The initial surface microhardness determination was performed by five indentations in the center of the enamel blocks (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan).

Twenty permanent and twenty deciduous enamel samples with a mean surface microhardness of 368.24 ± 11.57 KHN and 358.51 ± 12.57 KHN, respectively, were randomly assigned to two groups (TiF₄ and control). Two cavities of 5x5x3mm were prepared on the left and right sides of intraoral acrylic palatal appliances. Samples were allocated to treatments by stratified randomization according to the mean surface microhardness. In each row, one sample of permanent and deciduous enamel was fixed with wax.

In Situ Phase

This study was approved by the Research Ethics Committee of the Bauru School of Dentistry, University of São Paulo (Protocol #78/2005). Ten healthy adult volunteers (6 females and 4 males, aged 19-30 years) residing in the same fluoridated area (0.70 mgF/L), who fulfilled the inclusion criteria described below, took part in this study. All participants presented normal salivary parameters, such as adequate stimulated and non-stimulated salivary flows (1.88 ± 1.00mL/min and 0.60 ± 0.33mL/min, respectively) and salivary pH (7.32 ± 0.30). No active erosive lesions or new carious cavities were found.

The volunteers wore acrylic palatal appliances containing 4 human enamel samples, divided into two rows: TiF₄ (TiF₄) and no-TiF₄ (control). Each row contained one deciduous and one permanent enamel sample. In the first 24 h, the samples were not subjected to erosive process in order to allow the formation of a salivary pellicle. At the 2nd day, the application of the fluoride solution (4% TiF₄) was made extraorally by one of the researchers. The 4% (1.29 M F) TiF₄ solution was prepared by dissolution of powdered TiF₄ (Aldrich Chemical Company, Milwaukee, WI, USA) in deionized water. The pH of the fresh solution amounted to 1.2. The TiF₄ solution was applied in drops for 1 min on one row (TiF₄), while the other row remained untreated (control). The drop was left undisturbed until the surface appeared dry. Additional drops were applied in the same
manner until 1 min had elapsed\(^2\). After that, the excess of the solution was removed with a cotton swab.

From the 3rd to the 7th day, erosive challenges were made extraorally 4 times a day and at predetermined times (8:00am, 12:00pm, 4:00pm and 8:00 pm). For erosion of the samples, the volunteers were instructed to remove the palatal appliance and immerse it in a cup containing 150 mL of a freshly opened bottle of regular Coke\(^®\) (pH-2.6, 32.1 mg Ca/L, 18.1 mg P/L, 0.26 ppm of F, 0.1 mL of 0.2 M NaOH to increase one unit pH in 3 mL of drink; Spal, Porto Real, RJ, Brazil). Immersion of the enamel samples was performed for 5 min at room temperature.

The volunteers received instructions to wear the appliances continuously, even at night, and remove them at meals (3 times a day, 1 h/each meal). During this period the appliance was stored in wet gauze. Oral hygiene was performed right after meals using a fluoridated dentifrice (1,500 ppm F, Sorriso\(^®\), Brazil). Subsequently, the appliance was replaced into the mouth.

**Microhardness Measurement**

Five indentations were made in the center of the permanent and deciduous enamel samples each 100 µm apart from the other (Knoop diamond, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan). Microhardness was assessed prior to the treatments (baseline: SMH) and after the in situ phase (final: SMH1). The percentage of surface microhardness change was calculated as follows: % SMHC = \[100\% \times (\text{SMH1} – \text{SMH}/\text{SMH})\].

**Statistical Analysis**

The software GraphPad for Windows (San Diego, CA, USA) was used. The assumptions of equality of variances and normal distribution of errors were checked for all tested variables. Since the assumptions were satisfied, two-way repeated-measures ANOVA (factors dental substrate and fluoride) and Tukey’s post hoc tests were carried out for statistical comparisons. For comparison between the initial surface microhardness of permanent and deciduous enamel, Student’s t test was performed. The significance limit was set at 5%.

**RESULTS**

Table 1 shows the mean SMH and SMH1 as well as the %SMHC (±SD) of permanent and deciduous enamel. The initial surface microhardness of deciduous enamel was significantly lower than the permanent enamel (student’s t test, p=0.01). With regard to the %SMHC (softening), no significant differences were found for the factors “type of dental substrate” (F=0.367, p=0.54) and “fluoride treatment” (F=0.719, p=0.40). However, a significant interaction between the two factors was found (two-way ANOVA, F=27.157, p<0.0001). Analysis by Tukey’s test showed that the application of the 4% TiF\(_4\) solution reduced the softening of permanent enamel significantly (p<0.05). In contrast, softening of deciduous enamel was significantly higher when the TiF\(_4\) solution was applied (p<0.05).

**DISCUSSION**

In order to simulate the daily life situation as closely as possible, an in situ ex vivo model was chosen to test the effect of a TiF\(_4\) solution on erosion of permanent and deciduous human enamel. To the best of our knowledge, this is the first study evaluating the effect of TiF\(_4\) on erosion of deciduous teeth in situ ex vivo. Professionally applied highly fluoridated solutions, gels or varnishes are recommended for individuals who present moderate to severe dental erosion, with the objective to prevent new or
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To simulate the professional application of the TiF₄ agent by a dentist, the solution was applied only once. The excess of the solution was removed before the appliance was replaced into the mouth to avoid any carry-across effect of the treatment. Measurement of the surface microhardness immediately after application of the TiF₄ solution was not performed to allow for remineralization of the enamel surface and stabilization of the titanium glaze layer. However, as the TiF₄ solution presents an acidic pH, further studies should evaluate the surface hardness change immediately after its application. Similar to previous studies, surface microhardness (%SMHC) was used as response variable since it is a suitable tool for determination of early stages of enamel dissolution.

Regarding to the dental substrates, deciduous enamel and dentin are thinner than permanent. Therefore, the erosive process reaches the dentin earlier and leads to an advanced lesion after a shorter exposure period to acids, when compared to permanent teeth. However, the analysis of the susceptibility to erosive softening of permanent and deciduous dental hard tissues revealed conflicting results. Deciduous enamel is assumed to be more susceptible than permanent enamel when the initial softening passes into a progressive mineral dissolution and surface loss. The different susceptibility to erosion of permanent and deciduous enamel might increase rather over time and/or with increasing erosive capacity of the acid. In the present study, deciduous enamel presented significant lower initial surface microhardness than permanent enamel. However, neither of the substrates differed significantly in the degree of acid softening, when only the factor “substrate” was considered in the statistical analysis.

The results of present study showed that the TiF₄ solution was able to reduce the softening of permanent enamel. The protective action of TiF₄ has been attributed not only to the action of fluoride, but also to the action of titanium. The low pH of the TiF₄ solution (around 1.2), favors the linking leading to an increased fluoride uptake by enamel. Moreover, it is suggested that titanium interacts with the enamel surface because of the low pH of the agent, thus giving a protective action of TiF₄ has been attributed not only to the action of fluoride, but also to the action of titanium. The low pH of the TiF₄ solution (around 1.2) could cause greater enamel loss on application because the deciduous enamel is less mineralized and thinner than the permanent enamel. Thus, deciduous enamel might be more susceptible to alterations caused by the TiF₄ solution, which has a lower pH when compared to the most commonly consumed erosive drinks (pH 2.6). This result confirms previous findings that the erosion susceptibility of deciduous enamel increased with a higher erosivity of the acid.

CONCLUSION

The TiF₄ solution was able to reduce permanent enamel softening caused by the erosive challenge in situ. In contrast, softening of deciduous enamel was increased by the treatment compared to control. Since both substrates exhibited a relatively high surface softening after the erosive challenge, the protective capacity of TiF₄ has to be evaluated in further studies.

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