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Original Article

Impact of Saliva and Intraoral Appliance on Erosion Lesions Rehardening Ability - A Pilot Study

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Abstract

Objective: To evaluate the ability of different periods of salivary exposure and two different removable appliances to rehardening initial erosive lesions. **Material and Methods:** This randomized, single blind *in situ* study was conducted with 2 crossover phases. The factors under study were: period of salivary exposure (15 minutes, 30 minutes, 1 hour and 2 hours) and type of oral appliance (maxillary or mandibular). Two hundred enamel blocks were selected by initial surface hardness (SHi). Enamel blocks were demineralized *in vitro* (0.05M citric acid; pH2.5 for 15 seconds), surface hardness (SHd) was remeasured and 160 blocks were selected and randomized among groups. Thus, there were 2 blocks per period of salivary exposure in each type of oral appliance for each one of the 10 volunteers. In each phase, one of the removable appliances was tested. The response variable was percentage of surface hardness recovery (%SHR=[(SHf-SHd)/SHi)]x100). Two-way ANOVA and Tukey's post hoc test were applied adopting 5% of significance. **Results:** No difference was found among oral appliances on enamel rehardening ($p>0.01$). Salivary exposure of 2 hours promoted similar enamel rehardening when compared to 1 hour ($p>0.05$), which showed similar rehardening to 30 min. All mentioned period of salivary exposure promoted superior rehardening than 15 min ($p>0.01$). **Conclusion:** The salivary time exposure between erosive attacks might be 2 hours to achieve a feasible maximum rehardening. In addition, both maxillary and the mandibular appliance have presented a similar rehardening ability.

Keywords: Tooth erosion, Tooth Remineralization, Saliva.

Introduction

Dental erosion is defined as a chemical process that involves gradual loss of dental hard tissue by intrinsic or extrinsic acids of non-bacterial origin [1]. There is evidence that the presence of erosion is growing [2]. A recent systematic review and meta-regression analysis showed that the estimated prevalence of erosive wear in permanent teeth of children and adolescents is 30.4% [3]. The erosive lesion presents two distinguished aspects. In initial erosive lesions, the acid promotes loss of structural integrity and mechanical strength, resulting in surface enamel softening [4,5]. This phase is termed *dental erosion* [6]. The subsequent wear process induced by prolonged erosive challenge with repeated softening events or abrasive forces corresponds to *erosive tooth wear* [4,5]. Considered as a multifactorial condition, the knowledge of chemical, biological and behavioral etiological factors is essential for erosion understanding [6,7]. Due to its protective potential, saliva has been described as an important factor that influences the prevention and development of dental erosion [7-10]. Therefore, studies designed to evaluate preventive methods for erosion should be ideally conducted *in vivo* [11,12]. However, the pattern of lesion progression on *in vivo* studies is uncertainty, since erosion cannot be assessed alone [12]. In addition, in vivo studies are renowned for their difficulty to promote accurate measurements of tooth tissue loss. Under these circumstances, *in situ* models were developed to overcome the challenges posed by *in vivo* studies. *In situ* models have the advantage of controlling erosive challenge while exposing the samples to the oral environment [12].

In situ models usually present two approaches: removable appliances for continuous or intermittent use and fixed appliances for continuous use [12]. In principle, the exposition of the eroded surface and near-surface enamel layer to a supersaturate solution - such as saliva - could replace the mineral lost [13]. However complete remineralization cannot occur, since salivary proteins inhibit calcium phosphate precipitation [13]. Therefore, numerous studies have shown that only some degree of remineralization is possible [8,14,15]. Nevertheless, the period need for maximum rehardening between erosive challenges by saliva is not fully understood [14].

It is known that the calcium, phosphate and fluoride present in saliva may contribute to the repair of initial erosion lesion [10]. However, on remineralized enamel, the mineral deposit occurs in form of crystals on the surface, instead of regrowth of partially dissolved crystals [14]. Depending on the gland by which it is secreted, saliva may provide different levels of protection in several sites at the oral cavity [16]. Thus, the use of maxillary or mandibular oral appliances might also influence on the degree of rehardening on *in situ* studies. It is important to bear in mind that there is no standardization of *in situ* experimental models. The studies usually differ on the time of remineralization and the type of intraoral appliance. These variables may influence the intensity of enamel alteration resulted from erosive challenge, which may also influence on the effect of the preventive measure under study.

Taking the aspects addressed above into consideration, the aim of this study was to evaluate the ability of different periods of salivary exposure and two different removable appliances to rehardening initial erosive lesions.

Material and Methods

Experimental Design

This randomized, single blind in situ study was conducted with 2 crossover phases. The factors under study were period of salivary exposure (15 minutes, 30 minutes, 1 hour and 2 hours) and the type of oral appliance (maxillary or mandibular). Two hundred enamel blocks with hardness values between 312 and 378 Kp/mm² were selected by the initial surface hardness (SHi) and submitted to in vitro demineralization (0.05M citric acid; pH 2.5) during 15 seconds, for initial erosion lesion development. Surface hardness after erosion (SHd) was measured and blocks with hardness values between 108 and 221 Kp/mm² were selected and randomized among groups. Thus, there were 2 blocks per period of salivary exposure in each type of oral appliance for each one of the 10 volunteers. In each phase, one of the removable appliances was tested. The response variable was percentage of surface hardness recovery.

Enamel Blocks Preparation

Enamel blocks (4X4X3 mm, n=220) were prepared from the labial surfaces of bovine incisors crowns. The blocks were cut using ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4-mm thickness spacer. The blocks' surfaces were ground flat with water-cooled silicon carbide discs (600, and 1200 grade papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wet by 1 μm diamond spray (Buehler, Ltd., Lake Bluff, IL, USA). The blocks were cleaned using an ultrasonic device for 10 min and firstly selected according to absence of white spots and cracks using a microscope (x40). The samples were sterilized using ethylene oxide.

Surface hardness (SHi) was determined through the average values of five indentations performed at distances of 100 μm from each other (Knoop diamond, 25 g, 10s, Hardness tester from Buehler, US). Two hundred blocks of enamel were selected according to the surface hardness values to be demineralized in vitro (initial erosion lesion). More than the number of blocks required was demineralized allowing the discharge of nonstandard demineralized blocks.

Initial Erosion Lesion

Bovine enamel blocks (n=200) were subjected to short-term acid exposure by immersion in citric acid (0.05M; pH 2.5) for 15 seconds under agitation (Flatbed oscillator, 60 rpm), resulting in surface softening without tissue loss. The surface hardness after demineralization was measured (SHd) at distances of 100 μm from the baseline surface hardness, to obtain the degree of softening. Next, the surface hardness was measured again (SHd = mean of 5 indentations taken 100 μm below

the initial) and 160 enamel blocks were selected for randomization to the 10 volunteers, to period of salivary exposure and to the type of removable appliance.

In Situ Phase

This study was approved by the Research Ethics Committee of the Bauru School of Dentistry, University of São Paulo (Protocol 141.316/2012) and conducted in full accordance with the Declaration of Helsinki. Informed consent was obtained from each volunteer at the beginning of the study, prior to confirmation of their eligibility for the study. The participants had the right to withdraw from the study at any time and for any reason without prejudice.

Ten healthy adult volunteers (seven female and three males, aged 19–30 years) residing in the same fluoridated area (0.70 mg F/l) participated in the study, [17] after satisfying the following inclusion criteria: physiological stimulated salivary flow rate (>1ml/min), adequate oral health, with no caries or erosion lesions. The exclusion criteria were systemic illness, pregnancy or breastfeeding, under orthodontic intervention and use of fluoride compounds in the last two months.

The intraoral palatal appliance was made of acrylic resin on the plaster model of the upper arch for each volunteer. Each appliance had two vertical rows on the palatine, in the right and left side, each row presented two cavities with 8 x 8 x 3 mm for 2 blocks fixation. The blocks were fixed with wax and were carefully adapted to the level of the resin surface of the appliance. The intraoral mandibular appliance of each volunteer was made over a plaster model of lower arch, by positioning a soft silicon plate with 1 mm thickness over the model subjected to laminator. The excess beyond the limits of the cervical of teeth was removed by cutting the plate. Eight blocks were fixed with wax on the buccal surfaces, being 4 in the right and another 4 in the left side of posterior teeth.

Seven days prior to and during the experiment period, the volunteers brushed their teeth with standardized fluoride toothpaste (Total 12, 1,100 ppm F, Colgate, Brazil). The volunteers were also warned to not use any other fluoride product. Toothbrushing with fluoride toothpaste was performed by the volunteers one hour prior to the insertion of intraoral appliances and the initial of the experiment.

The study was conducted in two phases in which each phase a different appliance (maxilla or mandible) was used. The volunteers have used the intraoral appliances during 2 hours. After each salivary exposure period under study (15 minutes, 30 minutes, 1 hour and 2 hours) the appliance was removed from the oral cavity to remove the respective block for immediate evaluation of surface hardness. Then, the device was reinserted into the mouth and used until the next time. The volunteers were instructed to avoid food and drink consumption during the intraoral phase of the study.

Final Surface Hardness

The final surface hardness (SHf) was measured at distances of 100 μm from the surface hardness after erosion (SHd), as described above. The mean values of the five measurements were

used to calculate the percentage of surface hardness recovery (%SHR = [(SHf-SHd) / SHi)] x100) for each block, then the mean value of the %SHR of 2 blocks for each volunteer (n = 10) in each arch was calculated.

Statistical Analysis

Statistical analysis was performed with SigmaPlot version 12.3 (2011 Systat Software, Germany). The assumptions of equality of variances and normal distribution of errors were checked. Since the assumptions were satisfied, two-way ANOVA and Tukey's post hoc test were applied. The significance level was set at 5%.

Results

There was no difference between type of intraoral appliance (maxillary or mandibular) regarding the rehadening of enamel blocks (p >0.01). The results showed that the 2 hours of salivary exposure promoted similar rehardening effect when compared to 1 hour (p>0.05). There was no significant difference between 30 minutes and 1 hour (p>0.01). Thirty minutes, 1 hour and 2 hours resulted in higher mineral deposition in relation to 15 minutes (p>0.01). The means of hardness recovery of the groups under study are displayed in table 1.

Table 1. Mean and standard deviation of the percentage of surface hardness recovery (%) for the studied groups (n=10).

Times of Remineralization	Surface hardness recovery/ %SHR (±DP)	
	Maxillary [A]	Mandibular[A]
15 minutes[a]	9.30 (±7,77)	7.40 (±6,70)
30 minutes[b]	15.14 (±11,46)	16.92 (±11,66)
1 hour[c,b]	20.76 (±9,93)	18.74 (±8,32)
2 hours[c]	25.28 (±13,83)	23.75 (±12,41)

Groups whose means are followed by distinct letters differ significantly (Two way ANOVA/Tukey's Test, p < 0.05).

Discussion

Owing to the high prevalence of dental erosion, [18] a rising number of studies have been conducted to better understand the processes involved in this condition and to search for preventive therapies [12,19,20]. Fluoride and alternatives to fluoride for the prevention and treatment of dental erosion has been suggested based on scientific data originated from in vitro and in situ studies [15,21-23]. However there is no standardized protocol for in situ studies, additionally the influence of the area of the mouth in which the removable appliance is located and the ideal period between erosive challenges on the rehardening of eroded enamel is unknown.

Saliva is a fluid constituted by inorganic and organic components and it is secreted by three pair of major salivary glands (parotid, submandibular and sublingual), which are localized in different areas of oral cavity [24]. Since each type of major glands produces saliva with variety in quantity and quality [25,26], it was believed that the remineralizating potential of saliva could be different at various locations in mouth. The mechanical and/or gustatory salivary stimulation is able to increase

saliva production, especially of the parotid gland, which might result in higher amount of calcium and phosphate ions available for precipitation onto enamel [27,28]. On the other hand, saliva secreted by the sublingual and submandibular gland contains a high concentration of lysozyme and mucin [29]. Thus, it was expected higher mineral deposition potential at the maxillary appliance than at the mandibular. In the present study, no difference on the degree of enamel hardness recovery was found between maxillary and mandibular appliances. One hypothesis is that despite the localization in the lower jaw, the blocks were also close to the parotid gland, since they were fixed on the region of first molar tooth. There was no apparatus to inhibit blocks exposure to mechanical forces [11]. Therefore another hypothesis for the hardness recovery is the remove of the softened enamel by the tongue abrasion (maxillary appliance) and soft tissue (mandibular appliance), reaching a harder surface when compared to the eroded one [30,31]. It is important to report that the volunteers complained about the mandibular device, reporting discomfort and interference in occlusion.

Considering the above facts, additional studies are needed with a more comfortable type of mandibular appliance with methods to avoid the influence of abrasion on the samples. Ideally, the specimens should be positioned in the lingual region, since this region is constantly bathed by saliva produced by sublingual / submandibular glands [32].

In order to standardize the protocol for future in situ studies, this study evaluated the effect of different times of salivary exposure (15 min, 30 min, 1 and 2 hours). The longest period established was two hours, which was designed to reflect a more realistic condition, where after an erosive challenge the dental tissue would be exposed to the remineralizing effect of saliva for this period, until the occurrence of a new acid attack. After 15 minutes, enamel blocks did not presented a significant hardness recovery. There was a progressive hardness gain in the course of time. This result suggests that between erosive attacks the waiting time might be for about 2 hours to achieve a feasible maximum rehardening. However, there is still need for further understanding regarding the salivary rehardening effect overnight.

Conclusion

Considering the present methodology**,** it could be concluded that salivary time exposure between erosive attacks might be 2 hours to achieve a feasible maximum rehardening effect on enamel erosion. In addition, either maxillary or mandibular intraoral appliances have presented similar rehardening ability on initial erosion lesions.

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