PROTECTIVE ROLE OF ACQUIRED PELLICLE ON ENAMEL EROSION

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Abstract

Materials and method: The aim of the study was to evaluate, by several methods, dental erosion in 0.1 and 1.0% citric acid in vitro and to assess the protective potential of the experimentally formed salivary pellicle (24 h in vitro). Enamel slabs were embedded in epoxy resin and polished. Erosion was performed in citric acid for 1, 5 or 10 min, and recorded as calcium release.

Results: Significant microhardness loss on non-pellicle-covered specimens was measured after 1 min exposure to 0.1% citric acid. Microhardness loss was time- and concentration-dependent. Salivary pellicle significantly inhibited both microhardness loss, except for the 10 min immersion in 1.0% citric acid.

Discussions and conclusions: The results obtained support the general conclusion that salivary pellicle effectively protects enamel surface against short-term erosion in organic acids.

Keywords: acquired salivary pellicle, enamel erosion, in vitro study, microhardness, microscopic scanning.

INTRODUCTION

The frequent consumption of fruits, natural or carbugoseous juices containing citric acid may cause an irreversible pathology, defined as dental erosion. Apart from the acids from extrinsic sources, erosion may be also caused by acids of intrinsic origin, such as the gastric acid, especially in patients suffering from gastro-intestinal disfunctions.

The acid attack in the diet or as a consequence of regurgitations does not generate an immediate enamel dissolution, because the dental surface is covered by the acquired salivary pellicle which protects it. This pellicle, with a selective adsorption of the salivary proteins and of other macromolecules, prevents acid diffusion and influences the ionic transport, behaving as a membrane with selective permeability on the enamel surface [1]. The investigations developed along time demonstrated the protective properties of the acquired pellicule against enamel erosion. As a conclusion, the scope of the present study was to quantify the protection level induced on the in vitro – formed pellicle against the attack of citric acid.

MATERIALS AND METHOD

Preparation of enamel samples

The study was developed on freshly extracted teeth, extraction having orthodontic or periodontal causes. 10 teeth (premolars and molars), cleaned of bacterial plaque and periodontal tissue, have been selected.

The enamel-dentin blocks (approx. 5x5x3 mm) were prepared by means of a fine diamond-like disk, active on the edge, and cooling with water. Longitudinal sections were made in meio-distal and vestibulo-oral direction, followed by their processing through polishing, according to the protocol. The samples were inserted in self-polymerizable epoxicid resin (Epofix Risin, Struers, Denmark) with the enamel surface exposed on one of block’s surfaces, then polished with abrasive paper, on using a Buehler (Rathenow, Germany), Minimet model finishing device.

Obtaining of the salivary pellicle

The whole amount of mixed saliva was collected from 5 volunteers with no odontal
pathology or salivary disfunctions, subjected to no drug treatment.

Taking over of saliva was performed at 12.00 a.m. (on considering that, at this hour, its mineral composition is the most stable). Stimulated saliva was used for the study as, in the case of acid food and beverages consumption, reflex stimulation of the salivary flow occurs. Salivary secretion was stimulated by chewing of a paraffin block.

Saliva was expectorated directly in ice-cooled vials and centrifuged for 10 min at 4°C and 2.000 g (Biofug 22R, Heraeus Sepatech GmbH, Germany). The supernatant was separated and used for pellicle formation, according to Hannig [2]. Half (30) of the enamel samples was soaked in saliva at 37°C for 24 h, while the other – non-treated, without pellicle – was used as a control. 3 ml of saliva were employed for each sample.

**Experimental**

On the whole, 70 samples were prepared, 60 of them being selected for the investigation. Surface microhardness (SMH) of the 60 enamel samples was measured and described in 8 places for each sample, as a basic value. The samples – both the covered and the non-covered ones – (30 in each group) were immersed in 5ml 0.1 or 1% citric acid, for 1, 5 sau 10 min, so that 12 subgroups, each one containing 5 samples, were obtained. After their taking out from citric acid, the enamel samples were washed with distilled water and mounted on stubs with silicone-based impression material, for SMH measurement and for studying the indentations of Vicker hardness by means of an optical metallographic Neophot 21 microscope.

The SMH of Vickers hardness was measured with a device testing microhardness with a square diamond-like head at 136°, under 50 g weight indentations and a loading time of 10 s (VEB Zeiss Jena, Germany). The diagonal lines of the indentations were analyzed with an integrated measuring system, while the SMH value was calculated in Vickers units (HV). For both types of samples – covered and uncovered – the SMH value was measured immediately after the acid treatment. The differences between the two measurements represented the losses.

**STATISTICAL ANALYSIS**

The mean values of surface hardness losses were calculated for each subgroup. The tests referring to the differences between groups, on the existence of the pellicle and exposure to acid, were performed in two ANOVA ways, using the SPSS N variant 9.0 for Windows, separately for two different concentrations of citric acid. The comparisons were considered statistically important at a p < 0.05.

**RESULTS**

The SMH value of the unpolished enamel samples was of 305.9 ±14.2HV. A 24.2HV loss of SMH was observed 1 min after the exposure to 0.1% citric acid, the value increasing with increasing both the exposure duration and the concentration of citric acid. The samples soaked in 1.0% citric acid for 10 min showed a considerable SMH loss (252.1HV), while exposure to a concentration of 0.1%, for 10 min, determined a loss of 125.6 HV.

![Fig. 1. Variation of microhardness in samples with (blue) and without (pink) pellicle, after a 0.1% acid attack for 1 min](image)

The enamel samples covered with the acquired pellicle have a much lower SMH loss in all cases under analysis, with the exception of the 1.0% concentration for 10 min.

Significant losses were registered on specimens uncovered by a salivary pellicle, about 1 min after the exposure to 0.1% citric acid, comparatively with the experiental group (fig. 1).
DISCUSSION

The enamel samples were soaked in human saliva for 24 h, to form a pellicle layer, in the cases in which no major differences were observed between the samples soaked for 24 h and, respectively, 7 days [3].

0.1 and 1.0% concentrations of citric acid, used at its own pH values (2.81 and 2.34) occur in most of the acid beverages [4,5].

The exposure time, from 1 to 10 min, reflects a normal duration of consumption, while a prolonged exposure is far from the nutritional conditions [6]. In the present study, even a very short exposure generated a visible loss of SMH. The treatment for 10 min at a concentration of 1.0% generated an 50% higher increase of SMH, along with important structural modifications.

The loss of SMH due to erosive destruction was inhibited/reduced by the experimentally-formed salivary pellicle [7,8]. The protecting role of the salivary pellicle was lost after a 10 min exposure, at a 1.0% concentration of citric acid. Nevertheless, in the other subgroups, the protection assured by the pellicle was evidenced, the loss of SMH being more reduced than in the samples uncovered with the pellicle.

An important part is represented by pellicle formation, performed in vitro. The literature of the field evidences differences between the in vivo and the in vitro pellicles. It has been demonstrated that maximum in vitro protection is attained 60 min after the formation of the pellicle layer [8,9]. Nevertheless, other studies showed that the time necessary for obtaining an efficient protection is of 7 days [10], whereas other authors identified major differences among the 18 hours pellicles in vivo and in vitro [11]. Laboratory experiments showed that the protecting role may be attributed to the mucus. However, a recent study evidenced no major difference in the protection assured by the saliva obtained from different glands [9]. The basal, electrodense layer of the pellicle is characterized by a higher resistance to the action of the acid, possibly containing proteins with high affinity towards calcium. Other studies demonstrated that the in vitro formed pellicle contains proteins in native form, while the in vivo pellicle is characterized by considerable enzymatic degradation [12,13].

CONCLUSIONS

The data provided by the present investigation and their statistical analysis showed that, in the experimental group, the average values of microhardness (VHN) are higher than those of the control.

The differences are more significant for the sub-groups treated with 0.1% citric acid, for 1 min, with/without a pellicle. For the sub-groups treated with 1% citric acid for 10 min, with/without pellicle, the microhardness differences are negligible.

The obtained results suggest the efficiency of the protection induced by the salivary pellicle, which depends on both exposure time and acid’s concentration.

A comparison between the modifications induced by the citric acid upon the enamel protected by the pellicle, as well as upon the non-protected one, indicates that the presence of a pellicle for 24 h protects the surfaces against severe erosive destruction.

References


