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In situ enamel morphology evaluation after acidic soft drink consumption: protection factor of contemporary toothpaste

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Abstract: *Objective:* The enamel erosion induced by acidic soft drinks is an increasingly important problem. The aim of this study was to investigate the effect of soft drinks on enamel erosion and the protection offered by representative modern toothpastes using a new '*in situ scanning electron microscopy (SEM)-replica technique*'. *Methods:* Six patients were selected to receive *in vivo* enamel replicas, fabricated with a polyvinyl-siloxane/polyether impression material. Scanning electron microscopy was used to evaluate the morphology of enamel surface before and after exposure to lemon juice and SPRITE®. The protective effectiveness of toothpaste was further evaluated with the same method. Furthermore, to validate the effectiveness of the *in situ* SEM-replica technique, we compared it to a direct *in vitro* SEM investigation on extracted teeth. *Results:* Scanning electron microscopy investigation of the *in situ* replicas showed severe enamel morphology alterations after acidic soft drink exposure. On the other hand, it was also showed the protective effectiveness of toothpaste in preventing enamel erosion induced by acidic soft drinks. The direct *in vitro* SEM investigation provided similar enamel erosion results and proved the effectiveness of the *in situ SEM-replica technique.* *Conclusion:* Acidic soft drinks induce enamel erosion but regular use of toothpaste might reduce the risk for enamel erosion. The *in situ* SEM-replica technique provides an accurate method for tracing enamel morphology alterations and erosion induced by acidic soft drinks.

Key words: dental hygiene; enamel protection; erosion; *in situ* scanning electron microscopy-replica; prevention; toothpaste

Introduction

The erosion of dental hard tissues induced by acidic dietary components is an increasingly important problem, especially among children (1–3). The erosion process occurs as a result of the non-bacterial chemical reaction of the hydrogen ions in the acid with dental hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, resulting in a release of mineral ions (Ca^{2+}, OH^- and PO_4^{3-}) (4, 5). The main acid involved in these processes is citric acid, a constituent of many fruit juices and acidic soft drinks (6). The typical concentration present in many acidic soft drinks is 0.2–0.004 M for the fruits juices and 0.015–0.05 M for the acidic soft drinks (7). The three carboxyl groups confer the high chelating properties on citric acid, which forms soluble complexes with calcium ions, enhancing the enamel dissolution to achieve saturation levels of the calcium-acid complex (4, 8).

Most studies have focused on the chemical aspects of the erosive phenomenon, and it has been established that the erosive potential of acidic soft drinks is influenced by many factors, including pH, titratable acidity, and calcium, phosphate and fluoride contents (9, 10). On the other hand, further *in vitro* studies have emphasized the dissolution and demineralization effects or the simultaneously protective effectiveness of frequently applied of highly fluoride-concentration gel (11). Unfortunately, information about the *in vivo* protective role of the most recent home hygiene products for the prevention of dental erosion require further investigation. Therefore, the quantity and quality of the enamel protection provided by available products should be identified.

Recently, a new accurate replica technique has been developed which uses a double polyvinyl siloxane/polyether impression for scanning electron microscopy (SEM) examination to provide *in vitro* (12) and *in vivo* (13) evidence of the morphological modification of dental tissues. Thus, the aim of this study was to evaluate whether contemporary toothpaste has a protective effect in preventing enamel demineralization induced by acid soft drinks.

The study aimed to investigate *in situ* the effect of citric acid solution on enamel morphology with and without the application of toothpaste after acid soft drink challenge using a SEM-replica technique. The *in situ* technique was also compared to an *in vitro* control experiment using a direct SEM investigation in order to evaluate the accuracy of the replica technique.

Materials and methods

In situ morphology evaluation by SEM-replica technique

Six dental hygienist students were selected for this study and informed consent obtained under a protocol approved by the Review Board of the Department of Dental Sciences, University of Bologna, Italy. The participants were selected according to the age (19–30) in order to have a representative group of young people who usually assume acidic soft drinks. The six upper anterior teeth in each patient were used in the experiment. The experimental teeth of each patient were softly brushed for 1 min with water to remove any bacterial biofilm or toothpaste remaining. A first control impression replica (polyvinyl siloxane impression material; President light, Coltene, Switzerland) was obtained from each enamel surface.

Subsequently, enamel surfaces of the three right teeth were treated with a representative contemporary toothpaste containing 0.32%, sodium fluoride (1500 ppm) (AZ Complete; Procter & Gamble, Strombeek-Bever, Belgium) to evaluate the protective effectiveness in preventing enamel erosion. A small quantity (~0.2 ml) of toothpaste was used to cover the entire surface of the teeth to be then softly brushed for 1 min. using a electric toothbrush, Oral-B Professional-Care 5000 at 8.800 oscillations min^{-1} (Procter & Gamble).

The enamel surfaces of the three left teeth were untreated to evaluate the effect of the acid beverage on the enamel. A beverage containing citric acid was tested: a fresh-squeezed lemon juice and Sprite® solution (25:75 w/v) with pH (~2.5), measured using a pH-meter Jenway 3030 with Phoenix KO201B electrode, was used to simulate a typical scenario of the daily assumption of different soft drinks containing citric acid.

Each patient maintained 50 ml of beverage in the oral cavity for 4 min. Every 1 min the beverage was removed and replaced with fresh beverage to simulate the average daily consumption. The interval during solution replacement was 30 s. The total quantity of beverage used for each patient was 200 ml. A final tap water rinse for 2 min completed the experiment. At this point the experimental protocol involved a second impression-replica to obtain a record of any enamel modification induced by the acidic beverage or toothpaste protective effect. The obtained impression replicas were developed as a positive of the replica using a high precision polyether impression material (Permadyne Garant; 3M/ESPE, St Paul, MN, USA). The polyether replicas were coated with gold and examined using an SEM (Model 5200; JEOL, Tokyo, Japan) at 5–10 kV.

In vitro direct SEM evaluation

In order to validate the effectiveness of the *in situ* SEM-Replica, we compared it to a direct *in vitro* SEM investigation. Thus, six extracted upper human incisors and canines (age: 18–30) were used in a further *in vitro/in situ*-analogous experimental protocol to determine whether the SEM-replica test is a suitable method for the study of morphological enamel modifications. The teeth were randomly divided in three groups, with two teeth in each based on the three treatments investigated in the *in vivo* study: (i) control-brushed enamel, (ii) acid attack and (iii) toothpaste versus acid attack. Subsequently, the samples from each group were treated following the same experimental directives as the *in vivo* protocol. Finally, the samples were dehydrated through an ascending series of alcohols, coated with gold, and directly examined using an SEM (Joel 5200, JEOL, Tokyo, Japan) at 5–10 kV.

Results

In situ SEM-replica test

The SEM observation of *in situ* replicas of the sample surface only softly brushed showed homogeneous enamel morphology. No enamel prisms could be detected on the enamel surface (Fig. 1). Subsequent to the acidic soft drink challenge, the SEM observation of the *in situ* replicas of the left upper teeth surfaces untreated with toothpaste showed porosities and structural alterations of the core of the prisms (Fig. 2a and b). The right upper teeth surfaces treated with toothpaste showed only low porosity and no exposure of prisms (Fig. 3).

In vitro direct SEM evaluation

Direct SEM investigation showed that the experimental teeth in the acid attack group untreated with AZ Complete toothpaste and following SPRITE and lemon juice immersion for 5 min were affected by demineralization and erosions. It was interesting to detect severe erosions characterized by demineralization localized in the core of the enamel prisms (Fig. 4b). The experimental samples of the toothpaste versus acid attack group treated with AZ Complete toothpaste and following lemon juice immersion for 5 min showed a smooth surface with no evidences of erosion. The picture suggested the presence of what seemed to be a protective pellicle on the enamel surface that prevented enamel erosion (Fig. 4a).

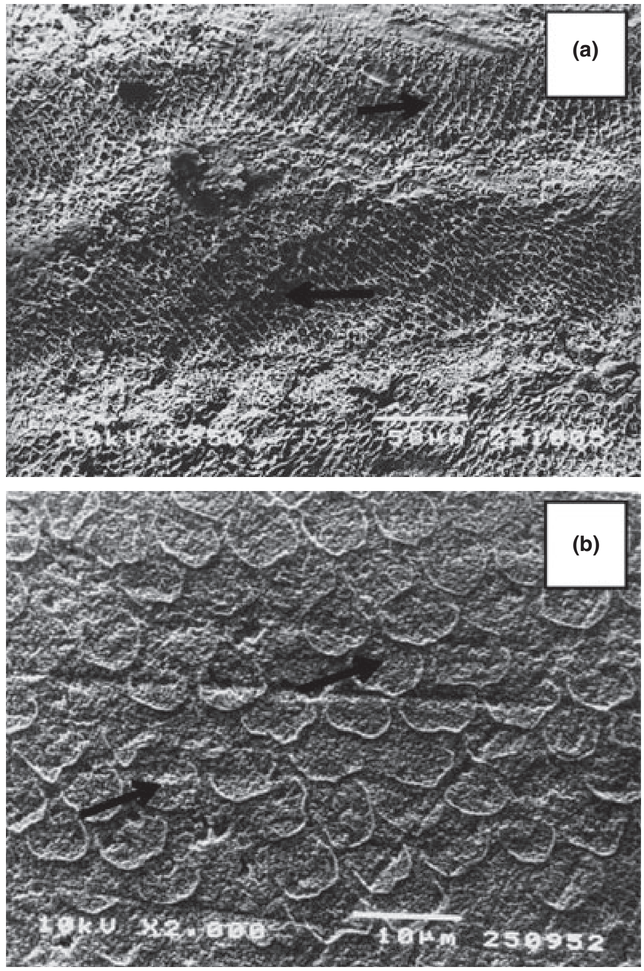

Fig. 2. (a) SEM-replica micrograph showing the surface of the experimental teeth group untreated with AZ complete toothpaste and following lemon juice assumption. It is possible to detect a surface with several porosities and enamel demineralization; (b) higher magnification of previous SEM-replica micrograph showing several porosities and erosions characterized by demineralization of the prisms core.

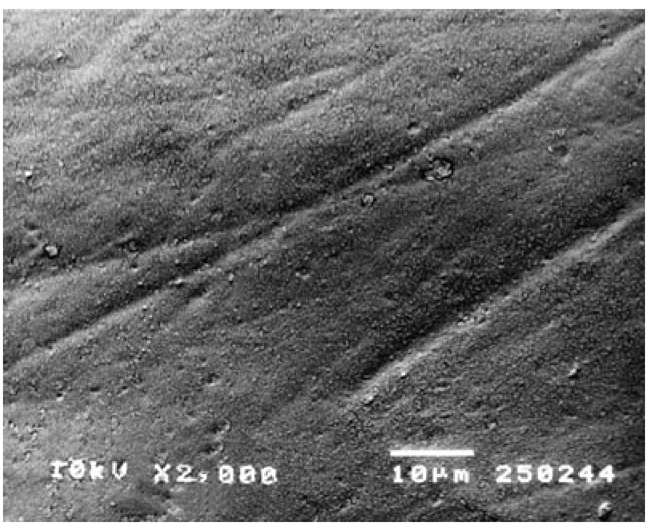

Fig. 1. SEM-replica micrograph showing the enamel surface (General Control) before acid citric attacks, a smooth surface with no porosities or demineralization is shown. It is possible to observe a smooth surface with no porosities or demineralization presence, although some grooves are probably due to excessive home tooth brushing.

Discussion

Citric acid is the main constituent of acidic beverages with an erosive potential which can induce demineralization of dental

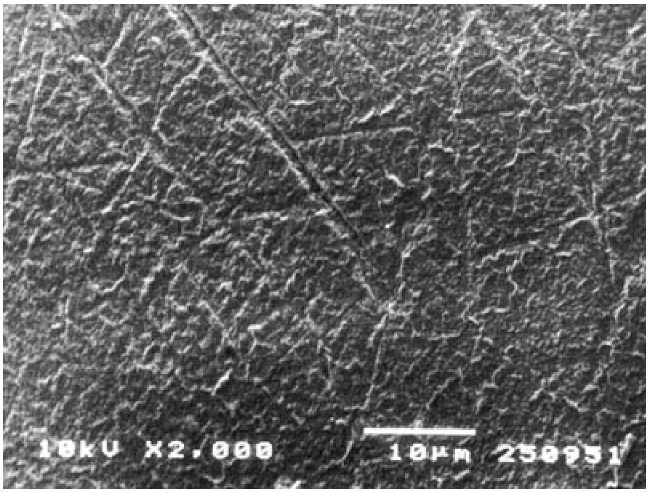

Fig. 3. SEM-replica micrograph showing a portion of the experimental teeth group after treatment with AZ complete toothpaste and following acid drink assumption. A smooth surface with no porosities or erosions presence was detected.

hard tissue (4, 6). The pH (~2.5) of the citric acid solution used in the study is representative for the pH of soft drinks and acidic beverages (14–26). The erosive potential of citric acid is pronounced due to the fact that citric acid acts as a chelator, binding minerals such as calcium. However, it is not relevant at low pH, especially not at pH 2.5 as used in the present study (10).

The aim of this study was to determine *in situ* whether recent commercial toothpaste would reduce enamel erosion by citric acidic drinks and offer a protection factor for enamel erosion. The data also provided additional information on enamel erosion induced by acid soft drinks. Furthermore, the experimental *in situ* model was designed to simulate a possible scenario, in which the application of oral home hygiene products is commonly of 3 min duration and acidic soft drinks are sipped for 4 min.

The *in situ* SEM-replica technique accurately traced the enamel morphology alterations and erosion induced by acidic drinks at less than 0.2 μm (17, 18). Actually, it was interesting to observe that as a substantial consumption of acidic soft drink may affect the morphological structure of enamel resulting in an exposure of enamel prisms. However, erosion *in vivo* may be generally influenced by several factors including re-mineralization periods and salivary film formation (20, 27).

In point of fact, the comparisons of the *in situ* and *in vitro* microscopy results have provided different types of evidence of erosion. The results of the *in vitro* experimental project showed some differences between the enamel alterations due to acid drink exposure in the two different environments.

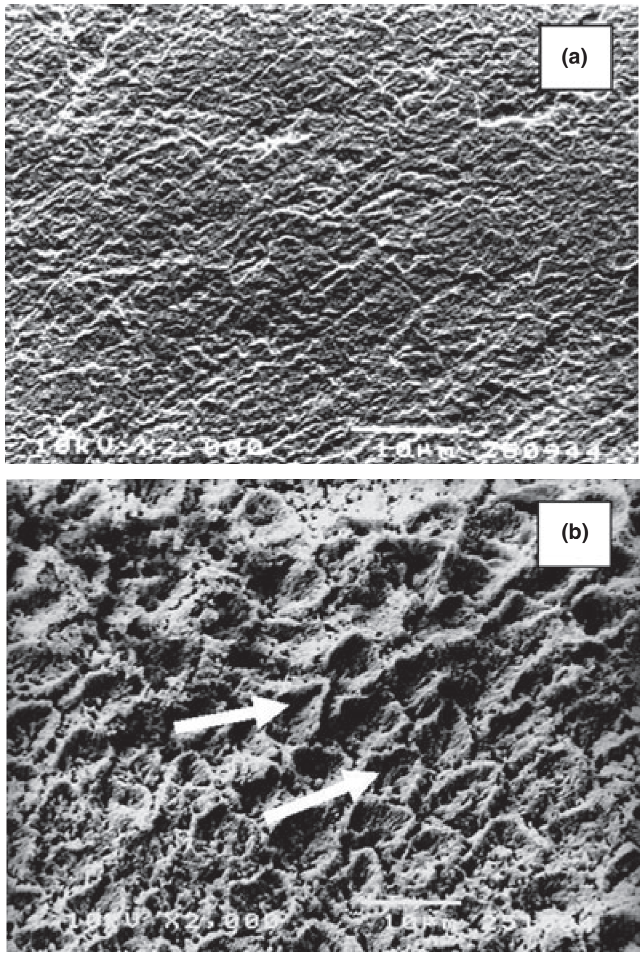

Fig. 4. Micrographs obtained by direct SEM investigation. (a) picture shows the experimental teeth group treated with AZ complete toothpaste and following lemon juice immersion for 5 min. It is possible to detect a surface with several porosities and erosions. A smooth surface with no porosities or erosions presence was detected; (b) picture shows the surface of the experimental samples of the *toothpaste vs acid attack* group treated with AZ complete toothpaste and following lemon juice immersion for 5 min. It is possible to observe the presence of a severe erosion and prisms exposure.

Because of the 4 min. time limit, this can be explained in part since there was no protection for the enamel *in vitro*, whereas the *in situ* environment would afford some protection such as the buffering capacity of saliva (20, 27).

On the other hand, the *in situ* results also showed that the application of toothpaste as an enamel pretreatment offered protection against alterations by erosion. It can be speculated that the protective effect of the toothpaste may have been due to the formation of a protective layer probably induced by its chemical constituents. Moreover, pretreatment of enamel with high (i.e. 1500 ppm) concentration of fluoride may have a protective effect due to fluoride uptake that reduces acid solubility of enamel (11).

In conclusion, within the limitations of the methodology of the study, it can be concluded that an increased consumption of acid soft drink may cause enamel demineralization and that the use of toothpaste plays a protective role in reducing enamel erosion. Moreover, the *in situ* SEM-replica technique provides an accurate method for tracing enamel morphology alterations and erosion induced by acidic drinks. In effect, it is useful to observe the substantial morphological modifications of the structure of enamel and the exposure of the enamel prisms. However, further research is needed to identify the quantity and quality of the enamel protection afforded by the modern toothpastes, and to confirm the validity of the protective action. Such research should be conducted as an inter-disciplinary cross-correlated study to evaluate the effective validity of the 'factor protection' which will allow in future the mathematical determination of the algorithm capable of developing an index of toothpaste protectiveness.

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