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EVALUATION OF CALCIUM CONCENTRATION IN SOLUTION - IN VITRO STUDY OF FLUORIDE ACTION ON DENTAL ENAMEL

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Abstract. In the present study the mineral loss/gain (ΔCa) in dental enamel during de-/remineralization process, depending on used fluoride concentration, were measured *in vitro* in pH cycling conditions. 20 sound premolars extracted for orthodontic reasons were divided in 4 subgroups. Artificial lesions were created on each of them. They were all subject to a pH cycling regimen for 2 weeks. During the pH cycling regimen each of the 4 subgroups received different treatment: Placebo paste, fluoride tooth paste (1440 ppm F), fluoride gel (12,000 ppm F), and rinsing solution (227 ppm F). Calcium concentration in de-/remineralization solution at the beginning and at the end of the study was determined using the spectrometer with atomic absorption. The relative succession of the fluoride containing products in terms of efficiency was established to be: fluoride tooth paste ≥ fluoride rinse > fluoride gel.
Key words: fluoride, calcium, dental enamel, prevention

Rezumat. În acest studiu s-a măsurat *in vitro* pierderea/câştigul de calciu (ΔCa) din smalţul dentar uman în cursul proceselor de de-/remineralizare, în funcţie de concentraţia în fluor a produsului utilizat, în condiţii simulate de pH ciclic. S-au utilizat 20 de premolari umani extraşi în scop ortodontic. Aceştia au fost divizaţi în 4 subgrupe şi supuşi unui regim de pH ciclic timp de 14 zile. În cursul procesului de pH ciclic cele 4 subgrupe au fost tratate diferenţiat, după cum urmează: I. Pastă Placebo; II. Pastă de dinţi (1440 ppm F); III. Gel fluorizat (12.000 ppm F); IV. Soluţie de clătire (227 ppm F). Concentraţia iniţială şi finală a calciului în soluţiile de de-/remineralizare a fost determinată cu ajutorul spectrometrului de absorbţie atomică. Succesiunea relativă a produşilor utilizaţi, din punct de vedere al eficienţei în prevenirea leziunilor de smalţ, a fost stabilită ca: pasta de dinţi ≥ soluţia de clătire ≥ gel fluorurat.
Cuvinte-cheie: fluor, calciu, smalţ dentar, prevenţie

INTRODUCTION

In a previous study we obtained qualitative data scanning electron microscopy (SEM) regarding the potential of fluoride containing products (tooth paste with 1.440 ppm F, fluoride gel with 12.000 ppm F, rinsing solution with 227 ppm F) to induce remineralization in artificial enamel lesions (1).

We managed to determine a "hierarchy" in terms of remineralization potential of fluoride containing compounds, as follows: fluoride tooth paste> rinsing solution> fluoride gel by SEM. The same succession was obtained in other studies (2).

OBJECTIVE

The aim of this study was to evaluate fluoride action on dental enamel in de-/remineralization processes by measuring in solution the average mineral loss and mineral gain (as ΔCa) in a pH cycling model. Treatments with the same fluoride products (tooth paste, fluoride gel, rinsing solution) were used as in the previous study (1). Calcium content after 2 weeks of pH cycling model was determined by spectroscopy with atomic absorption.

MATERIAL AND METHOD

20 human premolars extracted for orthodontic reasons were used.

Teeth preparing and artificial lesion producing

The teeth were selected after visual examination, using the following criteria: healthy enamel, without apparent caries, macroscopic fissures, abrasions or pigmentations.

As pre-treating: there was used washing with inert detergent followed by rinsing with double deionized water.

Conservation: here was used a saline solution, 4-5˚C, for 48 hours at the most.

Preparing for the experiment were slight polishing of the enamel surfaces, covering with acid–resistant varnish and leaving a 5x5 mm window on the vestibular surfaces. The premolars were cut in two through the window, and half of each was used as a control and the other half was treated accordingly. We obtained 20 control and 20 test samples, corresponding to: I. Placebo paste (no F added); II. Fluoride toothpaste; III. Fluoride gel; IV. Rinsing solution (5 control samples and 5 test samples for each testing subgroup).

In preparing artificial lesions was used the same method as in the previous article (1).

MATERIAL USED

a. *Fluoride containing products with topical application.* Data concerning fluoride compounds used can be found in table 1.

Table 1. Type, characteristics, and using recommendations of fluoride compounds

Product	*Method*	*Fluoride concentration*	*Product source*	*Application frequency*
1. Placebo tooth paste (no fluoride)	Brushing, 3 minutes	-	OTC product	2 brushing/day
2. Fluoride tooth paste (NaF)	Brushing, 3 minutes	0.32% NaF 1.440 ppm	OTC product	2 brushing/day
3. Fluoride gel (MFP)	Topical application, 4 minutes	1.2% MFP 12.000 ppm	Professional product	1 application/2 weeks
4. Rinsing solution (NaF)	Immersion in solution, 1 min.	0.05% NaF 227 ppm F	OTC product	1 application/ day

b. *Demineralization solution (D).* It simulates the composition of human saliva when the pH drops under the critical value and it contains the following substances: 2.0 mmol/l $CaHPO_4$, 74 mmol/l CH_3COOH, pH 4.3 adjusted with NaOH (2).

c. Remineralization solution (R). It is a supersaturated solution with about the same degree of saturation of apatite mineral in saliva with respect to the dental enamel. The R solution simulates the repairing action of saliva after the acid attack and its composition is similar with the one used by Featherstone and Ten Cate: 1.5 mmol/l $Ca(NO)_3 \cdot 4H_2O$, 0.9 mmol/l KH_2PO_4, 150 mmol/l KCl, 20 mmol/l buffer solution, pH 7 adjusted with HCl (3,4).

d. In a supplementary experiment it was used a remineralization solution (R_1) with no phosphate contain, hence, under saturated with respect to the apatite minerals from dental enamel.

The solutions D, R and R_1 were prepared in the laboratory using chemically pure reactive.

EXPERIMENTAL PLAN

1. *Managing the pH cycling regimen.* All 40 samples were included in a pH cycling regimen for 14 days which simulates the daily succession of de-/ remineralization processes in the oral cavity. The interventions schedule corresponds to the previous study (1).

2. *Determining the variations of D and R solutions following the pH cycling regimen.* ΔCa parameter was considered as an indicator of mineral loss/ gain, following the pH variations after two weeks. The initial and final calcium amount in D, R and R_1 solutions ware determined by spectrometry of atomic absorption.

3. *Data management.* The results regarding calcium amount variations in D and R solutions, the differences between samples treated with fluoride containing compounds and the differences between experimental and control groups were processed using SPSS 10.0 statistical program for Windows.

RESULTS AND DISCUTIONS

a. *Calcium amount variations in demineralization solutions (D).*

As figure 1 shows, it can be noticed that the variations in calcium content are positive ($\Delta Ca>0$), meaning that the difference between calcium content in solution D before and after the pH cycling regimen represents the increase of calcium in solution, hence, the mineral loss from dental enamel during acid attack. There are no statistical significant differences between test and control samples. At the same time, all fluoride containing products seem to have comparable potential in reducing enamel demineralization.

b. Calcium amount variations in remineralization solutions (R).

Two types of experiments were performed: (A) experiment which uses the R solution, with composition saliva-like, and (B) experiment which uses the R_1 solution (the remineralization solution with no phosphate content). The experimental findings are presented in figure 2 and figure 3.

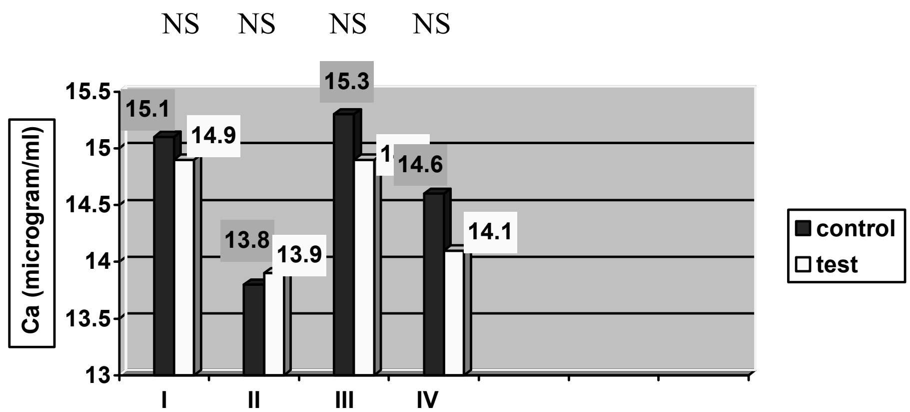

Fig. 1 Mean increase in calcium content (μg/ml) in demineralization (D) solution after 14 days of pH cycling regimen. I – Placebo paste; II – fluoride toothpaste; III – fluoridated gel; IV –fluoride rinse. NS = not statistically significant.

According to the findings from the (A) experiment, ΔCa parameter presents a negative variation during remineralization process ($\Delta Ca<0$) meaning that calcium deposes from solution on samples. The differences between test and control samples in the groups II-IV are statistically significant ($p<0.05$). The tooth paste without fluoride (I) has approximately the same values as the control. The most effective product seems to be the tooth paste (II).

A possible explanation for the poor results of fluoride gel is the fact that the fluoride ion is covalent bound in the molecule (5). At the same time this finding suggests the existence in the oral cavity of a more complex mechanism for delivering the fluoride ion from the fluoridated gel (5, 6). However, the differences between the three fluoride containing products are not very sharp.

In the additional experiment (B), it was used the remineralization solution R_1, without phosphate (KH_2PO_4), under saturated with respect to the apatite minerals from dental enamel. In this case, a positive ΔCa variation can be observed, meaning that calcium concentration in solution is increasing and in enamel is decreasing, respectively. Due to the presence of under saturated solution in phosphate the calcium will continue to come out of the dental enamel, corresponding to the dissolving phase in the process of caries progression. This *in vitro* finding suggests that if the mineral content of the saliva is insufficient (xerostomia, thick dental plaque), the demineralization process will continue, even after the acid attack has stopped (7,8).

A.

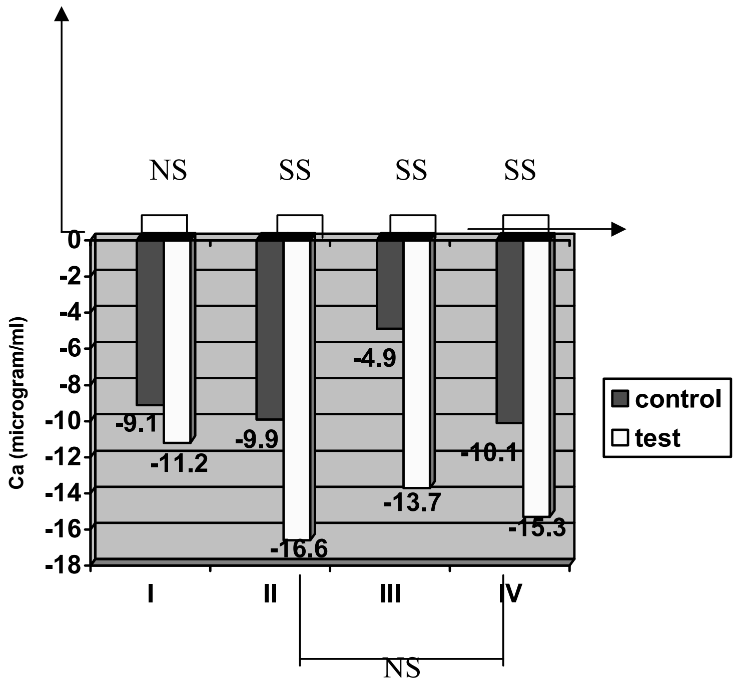

Fig. 2. The mean decrease in calcium amount (µg/ml) in remineralization solution (R) after 14 days of pH cycling. I – Placebo paste; II – fluoride toothpaste; III – fluoridated gel; IV – fluoride rinse. SS – Statistically significant; NS – not statistically significant

B.

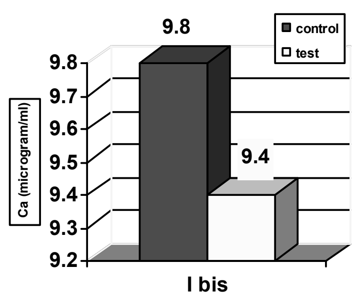

Figure 3. The mean calcium content increase (µg/ml) in remineralization solution (R_1) after 14 days of pH cycling

CONCLUSIONS

1. The *in vitro* determination of ΔCa variation in oral demineralized and remineralized solutions intended to establish to what extend the chemical changes in solution depend on fluoride concentration in dental products.
2. In the remineralization phase the efficiency of fluoride ion is more obvious in terms of enhancing the repairing process. In the demineralization phase, the effect of fluoride ion is almost negligible.
3. Although we used markedly different fluoride concentrations in dental products (226 ppm, 1.440 ppm, 12,000 ppm), the effects on the enamel de- and remineralization (observed by SEM and chemical analysis) are not essentially different.
4. The relative succession in terms of efficiency is: tooth paste > rinsing solution > fluoride gel, confirming previous results obtained by SEM and other experiments from the literature in the field.
5. As far as fluoride gel is concerned, although viscosity and high concentration should determine a higher remineralization potential comparing to the other products, yet the poor results obtained are the consequence of its chemical structure, where fluoride ion is covalent bound in the molecule. The low protection to mineral loss from dental enamel and the low repairing action of MFP comparing to NaF suggests the fact that delivering the fluoride ion from the fluoride gel implies more complex mechanisms, as mentioned before.

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