

Comparative Study Regarding the Effect of Different Remineralizing Products on Primary and Permanent Teeth Enamel Caries Lesions

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The aims of this study were to investigate the surface topography and to compare the remineralization potential of four commercial remineralizing products on deciduous and permanent teeth enamel. After artificial caries lesion formation, the enamel samples were divided into five experimental groups for permanent teeth, respectively deciduous teeth. In group 1 the samples have been stored in distilled water (control group). In group 2 each enamel sample was brushed two times a day for fourteen days with a commercial fluoride gel (Colgate® 6+), in group 3 each enamel sample was brushed two times a day for fourteen days with a commercial fluoride gel (CarrefourKids® +6). In group 4 a water-based cream with fluoride and hydroxyapatite (Remin Pro®, Voco) was applied for 5 minutes two times a day for fourteen days. In group 5 the enamel samples were rinsed with 20 mL antibacterial mouthwash with alcohol free sodium fluoride (Colgate® Plax) for 30 s two times a day for fourteen days. Between the remineralizing cycles, the samples have been stored in artificial saliva. The samples were analyzed using a scanning electron microscope and an EDX detector. For primary and permanent teeth samples, lower values of calcium and phosphorus ion concentrations were recorded in groups 2-5 when compared to group 1. For groups 2 and 3 the values of calcium and phosphorus ion concentrations were nearly the same. In group 4 the concentration of both ions recorded the highest level from all study groups (groups 2-5). The lowest values of calcium and phosphorus ion concentrations were observed in group 5. All the products tested in this study had the capacity to remineralize dental enamel of primary and permanent teeth, but the remineralization was not complete. The products containing fluoride and hydroxyapatite showed a higher remineralization potential when compared to fluoride products.

Keywords: primary teeth, permanent teeth, enamel, remineralization, EDX, SEM

Dental decay is a bacterial disease [1] that involves a dynamic process of cyclical demineralization and remineralization [2, 3]. It is considered a health condition when net mineral losses (etching) and net mineral gain (remineralization) remain in balance [4].

Remineralization of the incipient caries lesions could be supported by fluoride therapy [5, 6]. Fluoride plays a key role in the remineralization of the tooth by accelerating the absorption of calcium and phosphate ions, and by incorporating the molecular structure of apatite [7], making the remineralized surface more resistant to subsequent acidic attack [8]. Fluoride takes the place of a hydroxide in the apatite structure resulting fluorapatite, which is stronger and more acid-resistant than the natural hydroxyapatite. Previous studies demonstrated that the major cariostatic effect of topical fluoride application is not due to the incorporation of fluoride in the hydroxyapatite crystal lattice [9]. After topical treatment, globular complexes of CaF_2 are coating the enamel surface as a major reaction product [10-14]. Calcium fluoride-like deposits acts as a fluoride reservoir due to their solubility and to their release of fluoride during dissolution [15]. Also, they act as a diffusion barrier at the tooth surface, more efficient than fluoridated apatite [16].

The benefits of remineralization when using fluorides are primarily achieved by applying topical fluoride [17]. The agents that could be used include sodium fluoride, stannous fluoride and acidulated phosphate fluoride. They might be applied as professional products (in-office gels,

foams, rinses and varnishes) or at home products (pastes, gels, and rinses) having different concentrations of fluoride ion, ranged between 230 and 22.600 ppm. The effectiveness of the treatment depends on the concentration of fluoride, on the frequency and the duration of application [18].

The purposes of this research were to investigate the surface topography and to compare the potential of four commercial remineralizing products containing fluoride and hydroxyapatite to remineralize the enamel of deciduous and permanent teeth.

Experimental part

Specimen preparation

Fifteen primary molars and eight premolars were extracted in the Clinic of Pediatric Dentistry, Faculty of Dental Medicine, University of Medicine and Pharmacy, Iași, Romania. The primary teeth were extracted from 13 children of about 10 years old (7 female and 6 male) and the permanent premolars were extracted by orthodontic reason from 8 children of about 12 years old (5 female and 3 male). The patients were informed about the study and consented to donate their teeth after extraction. The teeth selected in this study presented no dental caries, erosive or wear lesions on their buccal or lingual surfaces. After the extractions, the teeth were rinsed with water, cleaned of debris and stored in distilled water until the start of the study. This study had the approval of Institutional Ethical Committee of University of Medicine and Pharmacy.

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Group	Remineralizing Material	Composition	Commercial products
2	Commercial fluoride gel	1450 pp, F	Colgate® 6+
3	Commercial fluoride gel	1000 ppm F	CarrefourKids® +6
4	Water-based cream with fluoride and hydroxyl apatite	Hidroxi apatite, sodium fluoride (1450 ppm F) and xylitol	Remin Pro®, Voco
5	Antibacterial mouthwash with natriumfluorid, alcohol free	Natriumfluorid 0.05% (225 ppm F)	Colgate® Plax

Table 1
COMMERCIAL
REMINERALIZING
PRODUCTS USED IN
STUDY

Artificial caries lesions formation and enamel surface remineralization

The teeth were stored in 0.1 M lactic acid solution adjusted to a pH of 4.0, for 14 days. The solution was renewed every five days. After artificial caries lesion formation, the permanent teeth enamel samples were obtained by cutting the buccal and lingual surfaces of premolars using low speed diamond discs (Komet Dental, Brasseler GmbH&Co, Germany), under watercooling. For temporary teeth the enamel sample was represented by the whole temporary crown. All the enamel samples of permanent and primary teeth were divided into five experimental groups.

In group 1, the samples have been stored in distilled water (control group). In groups 2 and 3 the enamel samples was brushed two times a day for fourteen days with a commercial fluoride gel (Colgate® 6+, CarrefourKids® +6). The interval between brushing sessions was of 8 h. Every brushing session has been performed for 30 seconds using an electric toothbrush with a constant pressure and using a bean sized toothpaste aliquot wetted with tap water, closely resembling the in vivo usual tooth brushing procedure. After every treatment session, every enamel sample was washed with tap water using a cleaned toothbrush in order to remove residual toothpaste. In group 4, a water-based cream with fluoride and hydroxyapatite (Remin Pro®, Voco) was applied for 5 min two times a day for fourteen days. In group 5 the enamel samples were rinsed with 20mL antibacterial mouthwash with alcohol free natrium fluoride (Colgate® Plax) for 30 s two times a day for fourteen days. The composition of the commercial remineralizing products used in this study are presented in table 1. Between the remineralizing cycles, the samples have been stored in artificial saliva (AFNOR NF S90-701). All the samples were then washed and kept in distilled water.

Surface topography and chemical analysis

The surface topography has been analyzed using a scanning electron microscope VEGA II LSH (TESCAN, Czech Republic) and the quantitative and qualitative chemical composition has been evaluated using an EDX detector (QUANTAX QX2, BRUKER/ROENTEC, Germany). The detector has an active area of 10 mm² and it can analyze all items heavier than carbon with resolution below 1.33 eV (MnK, 1,000 cps). Quantax QX2 uses a third generation detector which does not require liquid nitrogen cooling and is faster than the traditional detectors. VEGA II LSH microscope, operated entirely by computer, contains an electron gun with tungsten filament that can achieve a 3 nm resolution at 30 kV, with a magnifying power between 30 and 1,000,000 X in the resolution mode and a scanning speed between 200 ns and 10 ms per pixel.

Results and discussions

The surfaces topography of some primary enamel samples from control and after using remineralizing products are presented in figure 1. In group 1 aspects of distinct dissolution of enamel surface were observed (fig. 1 – 1.1 and 1.2). Exposure of enamel prisms and the loss of interprismatic and prismatic material were showed by SEM evaluation. For the samples in groups 2 and 3, rare areas of dissolution were surrounded by large areas of remineralization (fig. 1 – 2.1, 2.2, 3.1, 3.2). The remineralizing pattern in these groups was very similar. In group 4 large areas of remineralization were clearly observed (fig. 1 – 1.4).

Chemical analysis of the enamel showed that the highest concentration of enamel ions was represented by calcium and phosphorus ions. For that reason, only calcium and phosphorus ions were reported as a result of enamel samples quantitative chemical analysis. The mean values of calcium and phosphorus ions in enamel, expressed as weight percents (wt%), are presented in table 2.

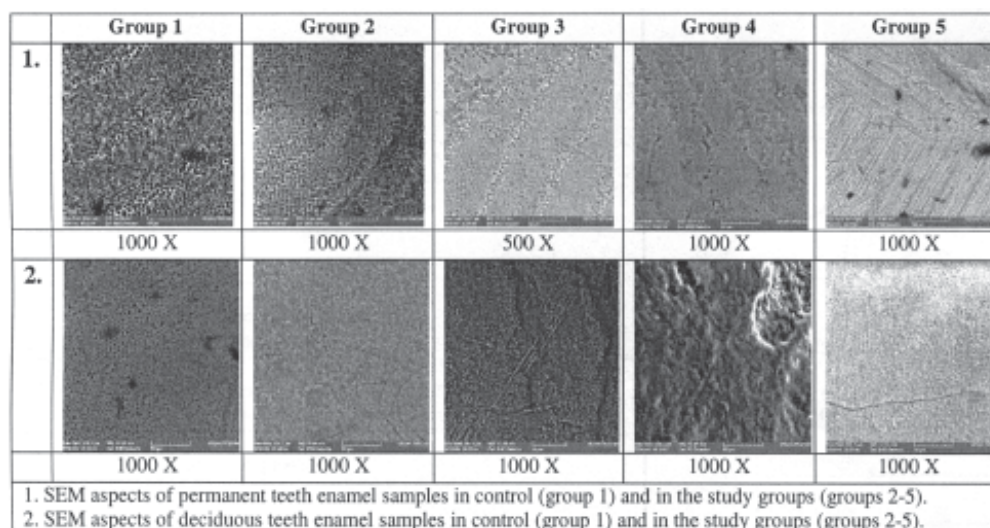


Fig. 1. Enamel SEM aspects in control and in the study groups

Ions concentration (wt%)	Permanent teeth enamel				
	Group 1	Group 2	Group 3	Group 4	Group 5
Calcium	48.86±0.11	44.47±3.05	42.51±0.14	45.27±0.39	38.87±0.32
Phosphorous	25.37±3.28	22.97±1.14	22.56±2.98	24.22±2.19	22.24±4.57
	Deciduous teeth enamel				
	Group 1	Group 2	Group 3	Group 4	Group 5
Calcium	45.68±5.74	43.98±4.04	43.88±1.02	44.13±3.70	37.27±2.64
Phosphorous	22.88±4.52	21.99±2.49	21.73±1.58	22.63±0.44	20.79±2.61

Table 2
MEAN VALUES OF ENAMEL
CALCIUM AND PHOSPHORUS ION
CONCENTRATIONS (WT%) ± SD
FOR THE CONTROL AND STUDY
GROUPS

For primary teeth samples, lower values of calcium and phosphorus ion concentrations were recorded in groups 2-5 when compared to group 1. For groups 2 and 3 the values of calcium and phosphorus ion concentrations were nearly the same. In group 4 the concentration of both ions recorded the highest level from all study groups (groups 2-5). The lowest values of calcium and phosphorus ion concentrations were observed in group 5 (table 1). In all groups the calcium and phosphorus ion concentrations had the same tendency of variation.

For permanent teeth samples, the values of calcium and phosphorus ion concentrations were very close to those from control in groups 2 and 3. In group 4 the value of both ions concentration was higher than in groups 2,3 and 5, but lower then that recorded in control group. In group 5 was recorded the lowest values of calcium and phosphorus ion concentrations from all the study groups (table 2). The values of both ions were lower in enamel samples of primary teeth when compared to permanent enamel samples.

Usually acidic buffers were used to create caries-like lesions in vitro (acetic acid or lactic acid) [19, 20], which not necessarily produce a subsurface lesion, but the surface of the enamel is etched away. There was a constant dissolution from the enamel surface towards the bulk of the enamel [21]. In this study, 0.1 M lactic acid solution was used to create the artificial caries lesions.

In our study the best results regarding the remineralization of dental enamel were found when water-based cream with fluoride and hydroxyapatite (Remin Pro®, Voco) was used. Reconstruction of demineralized tissue with a material having the same inorganic composition seems to be a very good method to increase the remineralization [22]. Using fluoride and hydroxyapatite can increase enamel remineralization due to the formation of surface apatite coating the enamel [23]. Previous studies showed that synthetic hydroxyapatite biomimetic coat is less crystalline than natural apatite in enamel, but it does represent a repair process corresponding to deposition of apatite inside the enamel demineralized area [23].

When fluoride products were used in our study, the remineralisation potential was lower than that obtained using the product with hydroxyapatite. Other studies showed that fluoride is far from a complete remineralization substance of dental enamel [24]. It was demonstrated that only the application of high concentrations of fluoride had the capacity to increase significantly the effect against caries and to promote remineralization. Both commercial fluoride gels used in the present study contain more than 1000 ppm fluoride. They did not have the possibility to repair the enamel structure, as it also have been demonstrated by previous studies [25].

Conclusions

All the products tested in this study had the capacity to remineralize dental enamel of primary and permanent teeth, but the remineralization was not complete. The products containing fluoride and hydroxyapatite showed a higher remineralization potential when compared to fluoride products.

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