Comparative Study Regarding the Reduction of Dental Hard Tissues Demineralization Using Different Commercial Remineralizing Products

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The aims of this study were to investigate the surface topography and to compare the calcium and phosphorous ion concentration in enamel, dentine and cement when three different commercial remineralizing products are used after demineralization of the dental hard tissues. Twenty caries-free extracted teeth were split in two halves. A free window of enamel (E), dentine (D) and cement (C) in the cervical area of the slices was preserved. The slices were randomly divided in four groups (A-D). In control group (group A) the slices were subjected to a demineralization period of 1 hour, three times a day, 14 days. Between demineralization cycles, the samples were stored in artificial saliva. In groups B, C and D the same demineralization cycles were applied, but the first and the last demineralization period were followed by the application of Colgate Total® toothpaste (Colgate Company) for 3 min (group B), MI Paste Plus (GC Corporation) for 3 min (group C) and Home Care Fluoride gel (Alpha-Dent Company) for 1 minute (group D). The samples were analyzed using a scanning electron microscope coupled with an EDX detector. The increase of calcium and phosphorous ion concentrations was significantly higher in groups C and D when comparing to groups A and B (ANOVA and post hoc Bonferroni tests, $p < 0.05$). MI Paste Plus and Home Care Fluoride gel showed a significantly higher remineralization capacity when comparing to Colgate Total® toothpaste.

Keywords: enamel, dentine, cement, remineralization, EDX, SEM

The most common method to inhibit tooth demineralization and to increase remineralization is represented by topical fluoride application. The specific mechanism by which fluoride is capable to protect enamel from demineralization is the ion diffusion in the subsurface caries lesion and its absorption to the crystal surface of carbonated hydroxyapatite [1]. Now it is very well established that low concentrations of fluoride are needed during acid attack to inhibit enamel demineralization. Fluoride can be released in oral cavity from dentifrice, foams, mouth rinses, gels, varnishes or cements. Previous clinical studies showed reduction in the incidence of incipient caries lesions [2] and a decrease in the depth of demineralization [3] when different fluoride products were used. The efficiency of these products depend on patient cooperation, some of them being used at home and some in dental office.

Using Recaldent™ technology, which contains casein phosphopeptides and amorphous calcium phosphate (CPP-ACP), good results were obtained in stopping the progression of incipient caries lesions and even in reversing their evolution. Casein phosphopeptides (CPP) have the capability to increase the concentration of calcium and phosphate in oral biofilm, to inhibit the demineralization of dental hard tissues and to increase the remineralization process [4].

The aims of this study were to investigate the surface topography and to compare the calcium and phosphorous ion concentration in enamel, dentine and cement when three different commercial remineralizing products are used after the demineralization of dental hard tissues.

Experimental part

For this study were used 20 extracted teeth having no dental caries, erosive or wear lesions on their mesial or distal cervical areas. The selected teeth also presented a free dentine area between enamel and cement in the proximal cervical area. The teeth selection according to these criteria was made by evaluation using an optical microscope (Nikon Eclipse E 600, Nikon, Japan) at a 10 X magnification. The teeth were split bucco-lingually in two halves using diamond discs (Komet Dental, Brasseler GmbH&Co, Germany), under watercooling. The extern part of the slices was covered with acid resistant varnish (Resist and shine, L'Oreal, Paris). A free window of enamel (E), dentine (D) and cement (C) (4X4 mm) in the cervical area of the slices was preserved. The slices were randomly divided in four groups (A-D). In control group (group A) the slices were subjected to a demineralization period of 1 hour, three times a day, 14 days. The chemical composition of the demineralization solution, having a *p*H of 5, was: 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 150 mM KCl, 0.1 mM sodium acetate. Between demineralization cycles, the samples were stored in artificial saliva (AFNOR NF S90-701). In groups B, C and D the same demineralization cycles were applied, but the first and the last demineralization period were followed by the application of Colgate Total® toothpaste (Colgate Company) for 3 minutes (group B), MI Paste Plus (GC Corporation) for 3 minutes (group C) and Home Care Fluoride gel (Alpha-Dent Company) for 1 min (group D).. The samples were analyzed using a scanning electron microscope (VEGA II LSH, Tescan, Czech Republic) and an EDX detector (QUANTAX QX2, Bruker/ Roentec, Germany).

Results and discussions

SEM aspects showed a reduction of demineralization areas after using all the remineralizing solutions. In enamel an obvious reduction of pore dimension was observed in groups B, C, and D when compared to group A (fig. 1).

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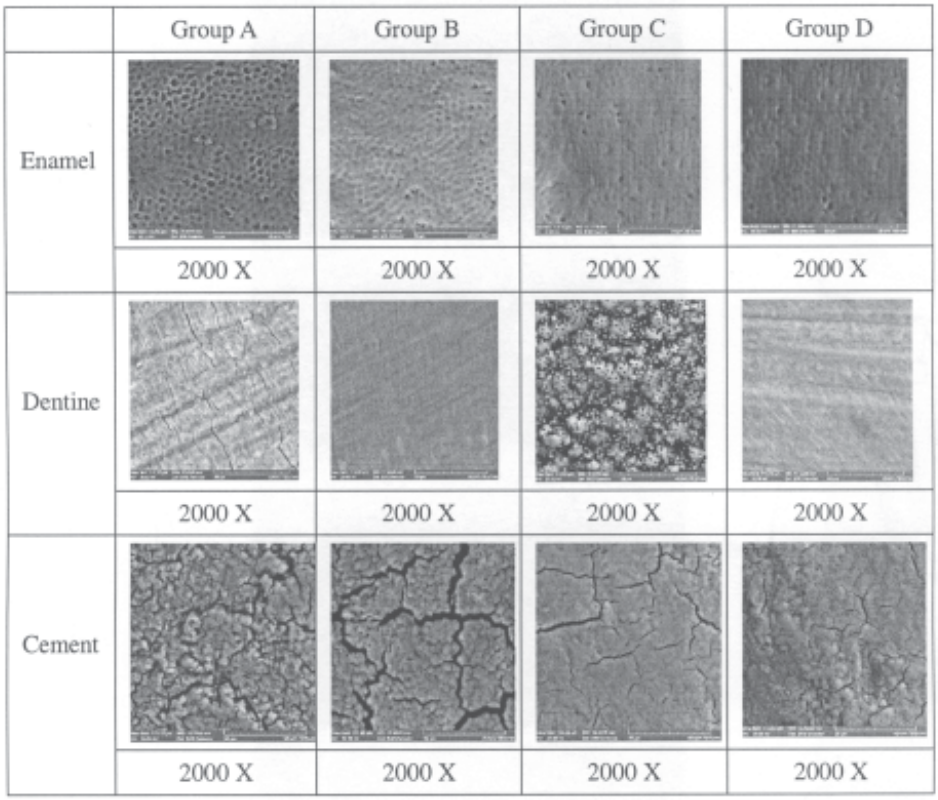

Fig. 1. SEM aspects of enamel, dentine and cement in control and study groups

Mean calcium and phosphorus ion concentrations (wt%) ± standard deviation

	Enamel		Dentine		Cement	
	Calcium	Phosphorus	Calcium	Phosphorus	Calcium	Phosphorus
Group A	30.57±0.21	20.12±0.16	15.46±0.44	9.99±0.36	13.12±0.22	7.10±0.23
Group B	30.90±0.36	20.37±0.21	15.91±0.27	12.00±0.27	13.77±0.32	10.15±0.41
Group C	32.25±0.44*	22.76±0.43*	20.41±0.11*	16.00±0.31*	14.92±0.25*	11.66±0.33*
Group D	38.63±0.33*	23.27±0.18*	24.89±0.14*	17.79±0.17*	16.55±0.61*	16.20±0.15*

*Significant statistical difference

Table 1
MEAN CALCIUM AND PHOSPHOROUS ION CONCENTRATIONS (WT%) IN CONTROL AND STUDY GROUPS

The mean calcium and phosphorous ion concentrations in E, D, C are presented in table 1. An increase of both calcium and phosphorus ion concentrations was recorded in groups B, C, and D when compared to group A. In enamel, dentine and cement, the highest values of calcium and phosphorus ion concentrations were in group D.

The increase of calcium and phosphorous ion concentrations was significantly higher in groups C and D when comparing to groups A and B (ANOVA and post hoc Bonferroni tests, $p < 0.05$). In groups C and D the increase of calcium and phosphorous ion concentration was significantly higher in dentine when comparing to enamel and cement (Mann-Whitney test, $p < 0.05$).

The anticariogenic properties of dairy products due to the casein, calcium and phosphate were demonstrated in a lot of previous *in vitro* and *in vivo* studies [5-10]. CPP from MI Paste product have the capacity to stabilize calcium phosphate in a CPP-amorphous calcium phosphate (ACP) complex [11]. These complexes have the capacity to prevent enamel demineralization by buffering free calcium and phosphate ion activity during acid challenge [12]. |They also keep calcium and phosphate ions supersaturated on the enamel surface [13]. Some studies demonstrated that CPP can be incorporated into salivary pellicle and in this way to be a reservoir for additional release of calcium and phosphate ions. Both ions are responsible for rebuilding enamel structure [12]. Remineralized enamel using CPP-ACP seems to be more resistant to acid challenge when compared to normal enamel [14].

In our study the commercial product that contains CPP-ACP (MI Paste Plus™) showed a greater potential in enamel, dentine and cement remineralization when compared to fluoride gel or fluoride toothpaste. Similar results were obtained in other studies, when it was registered a significantly less change in hardness reduction compared to a placebo paste and to a fluoridated paste [15]. Previous studies showed a good remineralization potential of the product, similar to that obtained by using fluoride products [16]. Significant protective effect against acid demineralization of enamel was also demonstrated

by Sakaguchi et al. (2005, 2006) [17, 18]. The synergistic effect of CPP-ACP and fluoride leaded in our study to best results in remineralizing dental hard tissues. Other studies demonstrated that the association of CPP-ACP with fluoride was more efficient then dentifrice containing only fluoride in the attempt to remineralize the enamel subsurface lesions in situ [13].

The results of this study confirm the findings from other studies that reported greater demineralization resistance of enamel after coating the surface with MI Paste Plus™ [19] (Guilio, 2009). In a clinical study regression of incipient caries lesions was recorded after using MI Paste™ [20]. In contradiction with present results, some studies reported no significant difference in the attempt to remineralize subsurface enamel lesions using MI Paste™, MI Paste™ with Crest® toothpaste, Crest® toothpaste alone, and PreviDent 5000 Plus™ or to prevent the progression of artificial carious lesions [21]. Tooth mousse increases the resistance of demineralized enamel surface and is more effective in remineralization when compared with fluoride products [22].

Conclusions

In the conditions of the present study, MI Paste Plus™, Home Care Fluoride gel and Colgate Total® toothpaste increased the calcium and phosphorous ion concentration in enamel, dentine and cement. MI Paste Plus and Home Care Fluoride gel showed a significantly higher remineralization capacity when comparing to Colgate Total® toothpaste. For MI Paste Plus™ and Home Care Fluoride gel the remineralization of dentine was higher than that of enamel and cement.

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