In vitro Study Regarding the Effect of Various Commercial Remineralizing Products on Primary and Permanent Teeth Dentine Caries Lesions

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The aims of this study research were to investigate the surface topography and to compare the remineralization potential of various commercial remineralizing products on primary and permanent teeth dentine. After artificial caries lesion formation, all the dentine samples of permanent and primary teeth were divided into five experimental groups. In group 1 (control group) the samples have been stored in distilled water. In group 2 and group 3 the dentine samples was brushed two times a day for fourteen days, using an electric toothbrush with a constant pressure and using a bean sized toothpaste for 30 s on brushing session; in group 4 on the dentine samples a water-based cream with fluoride and hydroxyapatite was applied for 5 min two times a day for fourteen days; in group 5 the dentine samples were rinsed with 20 mL antibacterial mouthwash with alcohol free natrium fluoride 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. In conclusion, the dentin remineralization, both in the temporary teeth and young permanent teeth, was increased, very best quality has been achieved in the study group four. The chemical analysis showed that the highest concentration of ions in dentin was represented by ions of calcium and phosphorus; the values of both ions were lower in samples dentin of temporary teeth compared to dentin of young permanent teeth samples.

Keywords: primary teeth, young permanent teeth, dentine, remineralization, EDX, SEM

Dentin is a complex tissue that contains apatite, collagen, other proteins and water [1, 2]. Initial caries affects the mineral component of dentin and exposes collagen fibers, creating conditions for rapid destruction of the entire network dentin [2]. An important requirement in operative dentistry and preventive restorations is developing *smart* materials capable of inducing the remineralization in carious dentin (demineralized).

The remineralization of demineralized dentin (bioremineralization) is the process of mineral restoring by forming inorganic mineral material [3].

Fluoride mainly has the effect of slowing down the process of demineralization, the enamel and dentin loses calcium and phosphate when exposed to an acidic environment following ingestion of foods and drinks containing sugars. It also helps the remineralization areas showing early signs of loss of calcium or phosphate, in other words *a cure for* opaque appearance. The greatest benefit is achieved if a low level of fluoride in the oral cavity is kept constant throughout the day [4-6].

Fluoride delivered directly (topically) on tooth surfaces by toothpastes and mouthwash help to maintain the level of fluoride in the oral cavity [7, 8] and offers its additional benefit of fluoride administered systemically by water fluoridation [9-11].

Brushing with fluoride toothpaste [12] is considered to be the most important factor in the observed decline in tooth decay in many countries [13]. Tooth brushing with fluoridated paste [14] and flossing helps to eliminate bacteria and reduce the risk of both caries and periodontal disease in oral cavity [15].

The aims of this research were to investigate the dentine surface topography of temporary and young permanent teeth before and after remineralization using various commercial products and to compare the potential of various commercial remineralizing products containing fluoride and hydroxyapatite to remineralize the dentine of primary and permanent teeth.

Experimental part

For this study eight premolars were used that were extracted by orthodontic reason and eight primary molars were extracted in the Clinic of Pediatric Dentistry, Faculty of Dental Medicine, University of Medicine and Pharmacy, Ia^{o}i, Romania.

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.

The dentine samples were obtained by cutting the buccal and lingual surfaces of premolars and primary molars using low speed diamond discs (Komet Dental, Brasseler GmbH&Co, Germany), under watercooling.

The dentine samples were stored in 0.1 M lactic acid solution adjusted to a *p*H of 4, for 14 days. The solution was renewed every five days.

After artificial caries lesion formation, all the dentine samples of permanent and primary teeth were divided into five experimental groups:

-group 1 (control group): the samples have been stored in distilled water;

-group 2 (Colgate® 6+): the dentine samples was brushed two times a day for fourteen days, using an electric toothbrush with a constant pressure and using a bean sized toothpaste for 30 seconds on brushing session

-group 3 (CarrefourKids® +6): the dentine samples was brushed two times a day for fourteen days, using an electric

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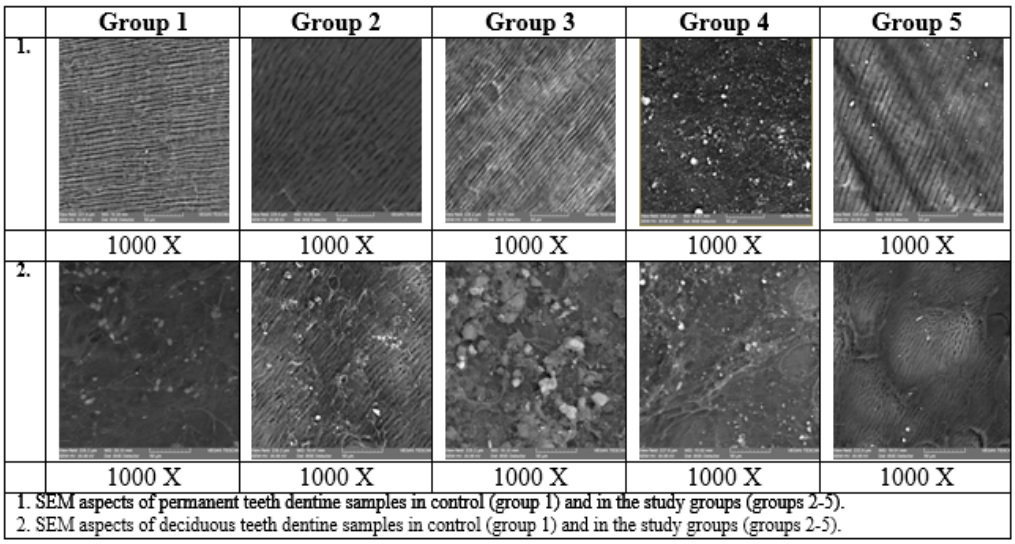

1. SEM aspects of permanent teeth dentine samples in control (group 1) and in the study groups (groups 2-5).
2. SEM aspects of deciduous teeth dentine samples in control (group 1) and in the study groups (groups 2-5).

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

toothbrush with a constant pressure and using a bean sized toothpaste for 30 s on brushing session

-group 4 (Remin Pro®, Voco): on the dentine samples a water-based cream with fluoride and hydroxyapatite was applied for 5 min two times a day for fourteen days;

-group 5 (Colgate® Plax): the dentine samples were rinsed with 20 mL antibacterial mouthwash with alcohol free natrium fluoride for 30 s two times a day for fourteen days.

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.

The dentine 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

In the control group for young permanent teeth and deciduous teeth is observed widening of dentinal tubules (fig.1).

SEM aspects of dentin in the groups 2 and 3 for youth permanent teeth and deciduous teeth shows dentiari partially filled tubules (fig.1).

The remineralization of groups 2 and 3 were very similar where the remineralization agent appears to be linked collagen.

A SEM aspect of dentin in the group 4 for youth permanent teeth and deciduous teeth clearly shows dentiari tubules are almost completely obliterated. Additionally mineral crystals precipitated during remineralization appear better treatment associated with the network of collagen (fig.1).

The remineralization model in this group was observed this process to a greater extent than previous remineralization agents used in the previous study groups.

SEM aspects of dentin in group 5 shows partially obliterated dentinal tubules. Although not observed any precipitation the agent remineralization seems to be related to the collagen (fig.1).

Chemical analysis showed that dentin highest concentration of ions in dentin was represented by ions of calcium and phosphorus. For this reason, only ions of calcium and phosphorus values were reported as a result of the quantitative chemical analysis of samples dentin.

The mean values of calcium and phosphorus ions in dentin, expressed as weight percents (wt%), are presented in table 1.

In permanent teeth samples, the concentration levels of calcium and phosphorus ions were very close to those of the control group in the study groups 2 and 3. In group 4 ion concentration value was higher than in the study groups 2, 3 and 5, lesser but less than those in the control group. In group 5 they were recorded the lowest values of calcium and phosphorus ion concentration toward all study groups.

For temporary teeth samples, lower values of ion concentration of calcium and phosphorus were recorded in 2-5 groups compared with group 1. For groups 2 and 3 values of calcium and phosphorus ion concentration were almost identical. Lot 4 ion concentration in both înregistreazÎ highest levels in all study groups (groups 2-5). The lowest concentration of calcium ions and phosphorus were observed in group 5.

In all groups ion concentration of calcium and phosphorus they had the same variation trend; the values

Table 1

MEAN VALUES OF DENTINE CALCIUM AND PHOSPHORUS IONS CONCENTRATIONS (WT%) ± SD IN CONTROL AND STUDY GROUPS

	Permanent teeth dentine				
Ions concentration (wt%)	Group 1	Group 2	Group 3	Group 4	Group 5
Calcium	31,35±0.12	20.74±2.08	20.51±0.34	26.03±0.93	19.05±0.23
Phosphorous	14.28±2.25	10.69±1.05	10.48±1.89	11.14±1.91	8.24±2.75
	Deciduous teeth dentine				
Calcium	11.86±3.37	6.89±3.03	6.47±1.20	9.31±2.07	4.03±0.85
Phosphorous	6.76±3.25	4.51±1.94	3.73±1.85	5.79±0.56	2.89±1.16

of both ions were lower in samples dentin of temporary teeth compared to dentin of permanent teeth samples.

In studies in vitro test models are frequently used in dental research. The major advantage of in vitro models is the ability to conduct experiments with variable alone in a highly controlled environment [16]. The cyclic *p*H (chemical model caries) [17], like the one used in this study, has become the model of choice to evaluate the caries, tooth decay main preventive measures. Although it is not possible to completely simulate complex biological aspects of decay, laboratory models are still of great importance for research caries [18]. The substrates used for in vitro include the cavities in the enamel and/or dentin. To reduce variability and achieve more reliable results it is recommended to use unique sections of caries [19]. In this study, 0.1 M lactic acid solution was used to create the artificial caries lesions.

The ability of a material to induce the formation of apatite demineralized dentin (the ability remineralization) is strictly tied to the biointeractivitate and bioactivity, the ability to evoke a positive response from the biological environment [20, 21]. Various methods were used to assess the effect of remineralization process in the dental tissues [22, 23]. The assessment methods can provide quantitative and qualitative information [24]. In our study analysis demineralized dentin remineralization process was used artificial scanning electron microscope (SEM) for surface topography dentin and EDX analysis of quantitative and qualitative chemical composition of dentin.

Using SEM it was noted that the remineralization of artificially demineralized dentin was more evident in case it is used the remineralization commercial product containing both fluorine and hydroxyapatite seen in the almost total closure of dentinal tubules. The re-mineralization of artificial demineralized dentin been likened to commercial products in the form of toothpaste containing fluoride only.

Analysis of the chemical composition of quantitative and qualitative dentine in this study able to show that the concentration levels of calcium and phosphorus were very close to those of the control group in the study groups that were used commercial products in the form of toothpaste containing only fluorine. In the group that was used for remineralization commercial product containing both fluorine ions and hydroxyapatite both values were the highest compared with other study groups, but smaller than in the control group.

Conclusions

Remineralization type of study groups 2 and 3 showed the same pattern in the two groups using as a commercial product remineralization toothpaste with fluoride gel form from different two companies slightly different concentrations of fluoride (Colgate® 6+, 1450ppm F^-; CarrefourKids® +6, 1000ppm F^-).

Dentin remineralization, both in the temporary teeth and permanent teeth at young people, was more very best quality in the study group four, that the study group has been used Remin Pro® (Voco), a commercial product containing besides fluoride and hydroxyapatite.

In the study group 5 (in which the use fluoridated mouthwash Colgate Plax no alcohol) was present also remineralization, but meager Toward the other three study groups.

So all products tested in this study had the ability to remineralize tooth dentin temporary and permanent youth, but remineralization was not complete.

The values of both ions were lower in those dentinÎ of temporary teeth in young permanent teeth compared with samples.

Products containing fluorine and hydroxyapatite have demonstrated a greater remineralization of teeth dentin in both temporary and permanent young teeth when compared to products which contain only fluorine.

References

1.DUCKWORTH, R. M., Int. Dent. J., **43**, 1993, p.529.
2.STOLERIU, S., IOVAN, G., GEORGESCU, A., SANDU, A.V., ROSCA, M., ANDRIAN, S., Rev. Chim. (Bucharest), **63**, no. 1, 2012, p. 68.
3.ELLIOTT, J.C., Studies in Inorganic Chemistry, Elsevier, **18**, 1994, p. 243.
4.GAVRILA, L., MAXIM, A., BALAN, A., STOLERIU, S., SANDU, A.V., SERBAN, V., SAVIN, C., Rev. Chim. (Bucharest), **66,** no. 8, 2015, p. 1159.
5.MIHALAS, E., MAXIM, A., BALAN, A., MATRICALA, L., MAXIM, D.C., TOMA, V., PETCU, A., Rev. Chim. (Bucharest), **66,** no. 6, 2015, p. 843.
6.BALAN, A., ANDRIAN, S., SAVIN, C., SANDU, A.V., PETCU, A., STOLERIU, S., Rev. Chim. (Bucharest), **66,** no. 4, 2015, p. 562.
7.ISSA, A.I., TOUMBA, K.J., Caries Res, **38**, no.1, 2004, p. 15
8.DOMENICK, T. Z., BMC Oral Health, **6**, no.1, 2006, p.9.
9.VANHERLE, G., DECLERCK, D., Verh K Acad Geneeskd Belg, **65**, no. 4, 2003, p.233.
10.BÁNÓCZY, J., MARTHALER, T.M., Fogorv Sz, 97, no.1, 2004, p.3.
11.CAREY, C.M., J Evid Based Dent Pract., **14**, 2014, p.95.
12.ELKASSAS, D., ARAFA, A., Journal of dentistry, **42**, 2014, p.466.
13.LUCA, F.A., IOAN, C.A.M., SASU, C., LUCA, A.C., Revista de Cercetare 'i Interventie Socialã, **49**, 2015, p. 80.
14.STOVELL, A.G., NEWTON,B.M., LYNCH, R.J., Int Dent J., **63**, no.2, 2013, p. 57.https://www.ncbi.nlm.nih.gov/pubmed/24283285
15.HIROSE, M., MURATA, Y., FUKUDA, A., FUJITA, Y., OTOMO, M., YAHATA, S., SAITOH, M., Pediatric Dental Journal, **25**, no.2, 2015, p.45.
16.SKUCHA-WAK, M., GIBAS, M., TANASIEWICZ, M., TWARDAWA, H., SZKLARSKI, T., Adv Clin Exp Med., **24**, no.5, 2015, p. 891.
17.LENZI, T.L., CALVO, A.F., TEDESCO, T.K., RICCI, H.A., HEBLING, J., RAGGIO, D.P., BMC Oral Health, **11**, no.15, p.79.
18.TEN CATE, J.M., Caries Res., **49**, no.1, 2015, p.3.
19.CHEN, Z., CAO, S., WANG, H., LI, Y., KISHEN, A., DENG, X., YANG, X., WANG, Y., CONG, C., WANG, H., ZHANG, X., PLoS One., 10, no.1, 2015, p.e0116553.
20.RYOU, H., NIU, L.-N., DAI, L., PUCCI, C.R., AROLA, D.D., PASHLEY, D.H., TAY, F.R., J Dent Res., **90**, no.9, 2011, p.1122.
21.KOIKE, T. , POLAN, M.A.A., IZUMIKAWA, M., SAITO, T., Biomed Res Int., **2014**, 2014, p.745139.
22.MANESH, S.K., DARLING, C.L., FRIED, D., Proc SPIE Int Soc Opt Eng., **1**, 2009, p. 71620.
23.SHETTY, S., HEGDE, M.N., BOPANNA, T.P., J Conserv Dent., **17**, no.1, 2014, p.49.
24.LI, Q.L., NING, T.Y., CAO, Y., ZHANG, W., MEI, M.L., CHU, C.H. BMC Biotechnology, **14**, 2014, p. 32.

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