Study Regarding the Influence of Different Fluoride Compounds on Dental Hard Tissues Resistance to Acid Challenge

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The aim of the study is to assess the effect of different fluorine compounds on dental hard tissues resistance against acid attacks by enamel hardness evaluation. Seventy five enamel slabs were split in five groups (I-IV) according to the five toothpaste included in this study. All the groups were divided in three subgroups (a-c). The samples of subgroup a were immersed in artificial saliva during the study (control), the samples of subgroup b were submitted to acid attack for 8 min, twice a day, 7 days and the samples of subgroup c were immersed in a toothpaste suspension for 3 min, 1 h before the exposure to acid attacks. The mean enamel Vickers hardness number(VHN) was recorded for each sample using the digital device CV 400 DAT (Namicon). The protective effect of each toothpaste (PR%) was evaluated as following PR% = (VHNc-VHNb)/(VHNq-VHNb) x 100. This study demonstrated a high protection against erosive attacks provided by all types of toothpastes tested. The higher protective effect was recorded by the toothpaste Blend-a-med Pro-Expert, followed by Splat Professional Active, Lacalut Alpin and Sensodyne Repair. The toothpaste having no fluoride in composition demonstrated the lowest protective effect. The association of fluoride with other active ingredients increased the protective effect of toothpastes.

Keywords: remineralisation, hard dental tissues, erosive attacks, fluorine

Despite the modern therapeutic solutions provided by the technological progress and the international dental research, dental caries and non-caries lesions remain unsolved issues. The dental erosion is a pathological process associated with localized chronic loss of hard dental tissues produced by acid action, electrolytic or chelation processes, no bacteria being involved. One of the most important factors implied in this process is represented by the extrinsic food acids and intrinsic acids from stomach [1-4]. The nutritional imbalance due to the highly intake of cariogenic products, poor oral hygiene, the lack of efficient preventive programs, bad habits, stress, and toxic professional environment, represent big challenges in the maintenance of a satisfactory oral health.

Numerous researchers [1, 5-7] highlighted the role of fluorine in the protection of hard dental tissues. Its qualities and some adverse effects still remain controversial issues [8]. The fluorine is playing the principal role in the remineralisation processes by accelerating the growth of the inorganic crystals [1, 9-12]. Also, the fluorine blocks the microorganisms metabolism in the biofilm and hinder the synthesis of dextran and levan implicated in the adhesion of bacterial biofilm to the hard dental tissues. The fluorine changes the electric potential to the enamel surface and hinders the deposition of bacterial compounds to this level.

Previous studiess proved that the local fluoridation method have effectiveness in remineralisation of dental hard tissues and has minimal adverse effects [9, 13, 14]. The add of fluorine in toothpastes has the aim to maintain permanent low levels of fluorine in the oral cavity, which might favour the remineralisation of the incipient carious lesions and the increase of the resistance of dental hard tissues to the further acid attacks. The fluorine concentration in the toothpastes is correlated with the reduction of the dental caries incidence. For each 500 ppm fluorine$^-$ concentration that exceed 1000 ppm, the incidence of dental caries decreases with 6% [9]. The optimal level of fluorine in toothpastes must not be higher than 1500 ppm for adults. It was demonstrated thet the daily use of fluorides toothpastes determines a decrease of the dental caries incidence with 15-30% [10, 15, 16].

In most countries, the toothpastes are sold without any medical prescription. Usually, to avoid the adverse effects of fluorine, the recommendation is not to overpass 10 mg fluorine /weigth. Dental fluorosis is the undesired effect of fluorine overdose [1, 17, 18].

Though the preventive mechanisms of the local fluoridation are well known [1, 19-21], still incomplete elucidated issues are the following: which is the optimal level of fluorine to prevent the demineralization processes, what particularities are implied in the mechanism of stopping the dental caries evolution process, and the way how the enamel's hardness is modified during demineralization-remineralisation cycles under the action of different fluorides compounds with different concentrations.

Despite the effectiveness of toothpastes with fluorine, there is a trend from parents to avoid the use of fluoride toothpastes by their children due to the erroneous or partially true mass-media informations. In this context, this research aims to clarify some aspects related to the use of toothpastes, with or without fluorine in composition, in relation to fluorine concentration and their active agents.

The aim of the study is to assess the effect of different fluorine compounds on dental hard tissues resistance against acid attacks.

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Table 1
TOOTHPASTES USED IN STUDY

Nr.	Market product:	Fluorine concentration:	Active ingredients:	Manufacturer:
I	**Splat Professional Biocalcium**	No fluorine	Calcium Lactate, Hydroxyapatite.	Splat-Cosmetica, LTD, Moscow, Russia.
II	**Splat Professional Active**	1000 ppm F	Sodium Monofluorophosphate, Calcium Glycerophosphate.	Splat-Cosmetica, LTD, Moscow, Russia.
III	**Blend-a-med Pro-Expert**	1450 ppm F	Stannous Fluoride, Natrium Fluoride.	Procter & Gamble UK, Germany.
IV	**Lacalut Alpin**	1476 ppm F	Natrium Fluoride, aluminium fluoride (Olaflur).	Homburg, Germany.
V	**Sensodyne Repair**	1450 ppm F	Natrium Fluoride, Calcium Sodium Phosphosilicate (Novamin)	Smith Kline Beecham, Ltd, EUCH CQ, UK.

Table 2
DAILY EXPERIMENTAL PROTOCOL

First cycle	9.00 10.00	immersion in suspension toothpaste (3 min)→ washing in deionised water→ immersion in saliva immersion in acid (8 min) →washing in deionised water →immersion in saliva
Second cycle	18.00 19.00	immersion in suspension toothpaste (3 min)→ washing in deionised water→ immersion in saliva immersion in acid (8 min) →washing in deionised water→immersion in saliva

Experimental part

In this study fifty five permanent healthy bicuspids extracted for orthodontic reason were used. Immediately after the extraction, the teeth were washed to remove the soft tissue, blood and bacterial biofilm. The teeth with visible dental caries or hypoplasia were excluded. The teeth were stored in sodium cloride at room temperature. The teeth were sectioned using diamond discs (Extec Corp, Enfield, CT, USA) at slow speed under water cooling,in order to obtain 4mm x 4mm enamel slabs. All enamel surfaces were examined using an optical microscope (Siemens, Germany) and any sample with surface defects was excluded. The slabs were fixed in epoxy resin in metal cylinders to assess the Vickers enamel microhardness. Seventy five slabs having Vickers hardness number (VHN) between 310 and 330 were selected for study.

The selected slabs were divided in five study groups (I-V), according to the five toothpastes that will be tested. The toothpastes used in this research are presented in table 1.

Each group was divided into three subgroups (a-c). The samples of subgroup a were immersed in artificial saliva during the study and were used as a reference to assess the changes induced during the experiment. The samples of subgroup b were submitted to acid attack for 8 minutes, twice a day, 7 days. Twenty mL of citric acid 1% having a *p*H of 2.3 were used to simulate the acid attack. Between acid attacks, the samples were maintained in 20 mL of artificial saliva (Fusayama - Mayer, *p*H = 7). Artificial saliva was changed two times daily.The samples of subgroup c were immersed in a toothpaste suspension for 3 min , 1 hour before the exposure to acid attacks. The aqueous suspension consisted in fresh 1/3 dilution from toothpaste (5 g) mixed with 15 g distilled water, prepared with 5 min before the experiment. After each immersion, the samples were washed 10 s in deionised water. The experiment protocol is presented in tabel 2.

For all the samples, enamel hardness was measured using the digital device CV 400 DAT (Namicon). The indentations were performed with a 50 g pressure force. For each sample the minimum distance between indentations was 40μm and the number of indentations was five. In order to be accepted, each indentation should had sharp margins, an uniform diagonal aspect and a smooth surface in the tested area. The mean Vicker hardness number (VHN) was recorded for each sample as a result of five hardness determinations.

To express the protective effect of each toothpaste, next formula was used PR% = (VHNc-VHNb)/ (VHNq-VHNb) x 100, where PR% is the remineralization rate, VHa is the initial microhardness (subgroup a), VHb is the micro-hardness after acid attack (subgroup b), VHc is the micro-hardness after the use of toothpaste followed by the erosive attack (subgroup c).

SPSS software, version 11.5 was used for statistical analysis. The mean, standard deviation, maximum, and minimum values in subgroups and groups were described. ANOVA, Tukey's tests and paired t-test (where á < 0.05 was considered significant) were used to compare the results.

Results and discussions

The distribution of the maximum and minimum VHN values for group I varied as follow: in subgroup Ia, from 329 to 319, in subgroup Ib from 241 to 227, subgroup Ic from 261 to 249. For group II, the distribution of maximum and minimum VHN values was as follows: in subgroup IIa from 320 to 330, in subgroup IIb from 239 to 225, in subgroup IIc from 267 to 250. For group III, the distribution of maximum and minimum VHN values was as follows: in subgroup IIIa from 328 to 318, in subgroup IIIb from 240 to 225, in subgroup IIIc from 283 to 264. For group IV (LA) the distribution of maximum VHN values was as follow: subgroup IVa from 329 to 318, subgroup IVb from 238 to 226, subgroup IVc from 272 to 260. For group V the values varied as follow: subgroup Va from 330 to 319, subgroup Vb from 239 to 226, subgroup IVc from 279 to 263.

The mean hardness values are presented in table 3. The mean values for the group I (SPB) are as follow: subgroup Ia - 323.2, subgroup Ib - 232.0, subgroup Ic - 253.2. The mean values for group II (SPA) are as follow: subgroup IIa - 324.6, subgroup IIb- 231.8, subgroup IIc - 260.0. The mean values for group III (BPE) are as follow: subgroup IIIa - 322.2, subgroup IIIb-234.2, subgroup IIIc - 275.6. The mean values for group IV(LA) are as follow: subgroup IVa -324.0, subgroup IVb - 232.8, subgroup IVc - 268.6. The mean values for group V (SR) are as follow: subgroup Va - 324.6, subgroup Vb - 232.0, subgroup Vc - 274.0.

Table 3
THE VICKERS HARDNESS MEAN VALUES AND STANDARD DEVIATIONS FOR STUDY GROUPS

Group/	Subgroup	Mean	Standard Deviation
I (SPB)	I a I b I c	323.2 232.0 253.2	3.7682 7.4833 4.6043
II (SPA)	II a II b II c	324.6 231.8 260.0	3.7148 5.2630 6.3245
III (BPE)	III a III b III c	322.2 234.2 275.6	5.6302 5.8051 7.0064
IV (LA)	IV a IV b IV c	324.0 232.8 268.6	5.2915 5.4497 5.0793
V (SR)	V a V b V c	324.6 232.0 274.0	5.6833 5.4772 5.4420

The highest values of mean hardness were recorded in subgroup a of each group, followed by subgroup c and subgroup b.

Different enamel remineralization rates were obtained in groups (tabel 4). The lowest remineralisation rate was provided by the toothpaste from study group I, followed by group II, IV, V and III.

In this study, the protective effect against erosive attacks was assessed by measuring the enamel microhardness. Many researchers used microhardness method to quantify the degree of demineralization and remineralization of dental hard tissues [17, 22-26].

The importance of fluoride in dental remineralization is well known. Our results showed that the toothpaste having fluoride as active ingredient leaded to a significantly better protection when compared to the product having no fluoride in composition. These findings are in agreement with the results of previous studies [17, 27]. The optimal fluorine concentration in the products for topical application is a controversial issue. Our study proved that lower fluorine concentrations provided lower protective effect against acid attack. In the present study the hardness values obtained when a toothpaste having 1000 ppm fluorine was evaluated were lower when compared to the groups where toothpastes having 1450 ppm or 1476 ppm were assessed. These results confirm the findings of other studies regarding the protective effect of the products in relation to fluoride concentration [15]. Difference in protective effect of toothpastes having 1450ppm and 1476 ppm was also recorded in this study. This is probably related to the different active agents, others then fluoride, included in each toothpaste. The highest protective effect was detected for toothpaste that contains stannous fluoride and natrium fluoride as active agents. Similar results were obtained by R.V. Faller et al., which tested various toothpastes with different active agents and confirm the fact that toothpastes with stabilized stannous fluoride provide superior protection against acid attacks [25]. Also, the authors proved the importance of both fluorine concentration and active agents in obtaining the protective effect. They suggested that the toothpastes that contain

Tabel 4
REMINERALISATION RATE PROVIDED BY THE TOOTHPASTES

	VHa	VHb	VHc	PR%
I	323.2	232.0	253.2	22.99
II	324.6	231.8	260.0	30.38
III	322.2	232.2	253.2	47.04
IV	324.0	232.8	268.6	39.25
V	324.6	232.0	274.0	45.35

the same active agent have different abilities to release the product to the enamel surface.

Previous studies analyze the effect of a toothpaste with low fluorine concentration (450 mgF/g, natrium fluoride) in combination with calcium citrate and trimetha-phosphate sodium. Positive results were obtained due to the effective association between these active agents [26].

Conclusions

This study demonstrated a high protection against erosive attacks provided by all types of toothpastes tested. The higher protective effect was recorded by the toothpaste Blend-a-med Pro-Expert, followed by Splat Professional Active, Lacalut Alpin and Sensodyne Repair. The toothpaste having no fluoride in composition demonstrated the lowest protective effect. The association of fluoride with other active ingredients increased the protective effect of toothpastes.

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