“OVIDIUS” UNIVERSITY OF CONSTANTA,

ROMANIA

FACULTY OF DENTAL MEDICINE

ABSTRACT

DOCTORATE THESIS

LOCAL EFFECT OF FLUORIDE AND OF THE ROMANIAN PRODUCT FLUOROSTOM ON THE TOOTH ENAMEL

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KEYWORDS: fluoride, enamel, demineralisation, remineralisation, prevention, topical fluoride, SEM/EDX

Introducere

The remarkable improvements in the oral health field acquired in the last half of the century are due to a solid scientific basis regarding the prevention of oro-dental diseases, which were developed and applied at community level, in the clinical practice and in the education for oro-dental health. Oro-dental diseases prevention is defined as "the strategy to reduce disease specific risk factors or strengthening factors that can influence the susceptibility of developing it, as well as activities meant for therapy or reduction of specific manifestations of a already installed disease“. (Cuculescu M. 2010 şi Dănilă I. 2005).

The health programs addressed to the community are in general focused both on primary prevention – elimination or reduction of risks or the provision of protection against diseased before their emergence, or on secondary prevention – screening and early detection and intervention for stopping the evolution of diseases after their begining.

Within the main WHO primary prevention strategies of oro-dental diseases, an important chapter is represented by the fluoridation applied at various levels: community (fluoridation of drinking water, educational programs of fluoridation with fluoride supplements – tablets or oral), professional (fluoride supplements –tablets or oral rinse, local application of gels and solutions with high concentration of fluoride, use of restorative materials that release fluoride) and individual (use of toothpastes and mouthwashes that contain fluoride).

La baza conceperii studiilor efectuate în cadrul acestei teze de doctorat au stat tendințele practicii medicale actuale de reorientare spre conceptul preventiv şi spre cel de terapie minim invazivă, cu implicarea produselor pe bază de fluor, bazate pe resultsle numeroaselor studii de cercetare din domeniu, cât şi direcțiile prioritare ale OMS privind sănătatea orodentară.

Due to this context and under the conditions in which in literature, the issues related to the action of fluoride over the dental hard tissues are either disputed or minimized, the intention to systemize as many information as possible regarding this topic and to attempt possible correlations between these and certain personal observations represents a necessary aspect.

The aspects that I proposed to follow in the paper hereof are the following: selecting and systemizing a documentary material that should include the data in the specialized literature regarding the sources of fluoride, the metabolism thereof, the effects of fluoride on the human body and dental tissues, the etiopathogeny of dental caries and of fluorosis as well as of the phenomena associated to these, as well as the importance of fluoride upon the processes of demineralization and remineralization, as well as the ultrastructural changes determined by the action of fluoride on the surface of the enamel.

The general part of this thesis presents the present state of knowledge through a systematic selection and a documentary material which reflect the literature information. It is structured into five chapters:

Chapter I GENERAL CONTEXT OF FLUORIDE USES IN THE DENTAL MEDICINE The first chapter is structured in three subchapters. The first one is an overview of the data on Fluoride history, obtainment and use and a short history of fluoride use in dental medicine. The second one is about fluoride distribution in the drinking water from Constanta County and fluoride exposure. The last one presents the fluoride effects on human health: fluoride metabolism and its effects on human body.

Chapter II BASIC NOTIONS REGARDING TOOTH ENAMEL is a review of the basic anatomy and physiology of dental enamel: tooth development process, enamel formation and development, normal enamel structure, physical and physiological aspects of enamel surface.

Chapter III CURRENT CONCEPT REGARDING THE FLUORIDE ACTION MECHANISMS through the four subsections presents the effect of fluoride on the morphology and solubility of dental structures, factors influencing the incorporation of fluoride in enamel after topical application of preparations F, oral rinsing with fluoride and recommendări the use of fluoride in the future.

Chapter IV EXCESS FLUORIDE EFFECTS ON THE DENTAL HARD TISSUES summarizes the principal aspects of dental fluorosis: general aspects and mechanisms of production and enamel fluorosis pathognomonic characteristics, risk factors as well as issues related to endemic fluorosis.

Chapter V MODERN INVESTIGATING TECHNIQUES OF THE DENTAL HARD TISSUES STRUCTURE presents the fundamental aspects of the interaction of light and optical properties of dental hard tissues. The second part of this chapter contains descriptions of the techniques used in the studies surveyed: optical or photonic-stereomicroscope microscopy, electron microscopy, SEM / EDX analysing system, and Vickers microhardness test, salivary microcrytallization index (IMK).

The personal research part encloses seven distinct chapters - presenting the research motivation and two clinical trials and three *in vitro* studies, having the structure imposed by scientific research: objective, materials and methods, results, discussion and conclusions. The last chapter of the personal part presents the final conclusions of the research.

Chapter VI STUDY MOTIVATION, STATISTICAL ASSESSMENT METHODS addressing the topic titled "LOCAL EFFECT OF FLUORIDE AND OF THE ROMANIAN PRODUCT FLUOROSTOM ON THE TOOTH ENAMEL" was determined by the need for a proper understanding and highlighting the action of the product Fluorostom Romanian, used within the National Program for tooth decay Prevention I.5 that was conducted starting with 2001 in the the entire Constanța County.

Any approaches related to research activity were carried out in accordance to the legal provisions as well as research deontological compliance. The clinical examinations, made for the *in vivo* trials were carried out in accordance to the provisions of Law no. 46/21 January 2003, concerning the patient rights, after having achieved the agreement for participation in scientific research. Patient’s privacy was complied. The aim and methods of the research have been explained to each patient participant in the survey and after this the informed consent has been obtained.

The statistical analysis and data management were performed using the SPSS 17 (SSPS Inc., Chicago, USA) and to generate the graphs the specialized module from Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, USA) was used.

Chapter VII CLINICAL ASSESSMENT STUDY OF THE REMINERALIZATION EFFICIENCY OF TOPICAL FLUORIDE COMPOUNDS ON ENAMEL INCIPIENT CARIES

Aim

- ✓ Comparative assessment of the remineralization efficiency of incipient carious lesions associated to the fixed orthodontic treatments, of three types of compounds with topical administration that have different active compounds;
- ✓ Analysis of the anti-plaque effect by assessing the oral hygiene indexes;
- ✓ Monitoring the conduct of remineralization compounds from the point of view of the remineralization capacity of the incipient carious lesions by surface analysis and process extension.

Material and methods The study involved 40 patients aged between 14 and 25 years old, clinically healthy, that presented enamel demineralization processes (of *white-spot* type), to whom local remineralization treatments were administered. The participants in the study were initially assessed and then monitored for a period of 12 months and the evolution of lesions was reassessed after 6 and 12 months. During the research period, the following parameters were assessed:

1. Analysis of the anti-plaque effect by assessing the oral hygiene status by using the bacterial plaque index Quigley-Hein (OH).
2. The depth of the demineralization focal point;
3. Surface demineralization.

The 40 patients presented **58 teeth with demineralization processes** that were randomly divided in 4 lots *(Table 1)*.

Tabel 1. Structure of the patient's lots and demineralization processes

Lot	No. of patients	No. of demineralization processes
I.	10 patients	16 teeth
II.	12 patients	16 teeth
III.	10 patients	14 teeth
IV.	8 patients	12 teeth
Total	40	58

Lots I, II, III, received local remineralization tratment and lot IV was the control lot and a solution without remineralization potential was administered (distilled water). The compounds used in the study, were administered as follows:

Lot I: Fluorostom (sodium fluoride active compound NaF),

Lot II: GC Tooth Mousse (RecaldentTM/ CPP-ACP formula)

Lot III: Reminal Ca/P-F (calcium, phosphate and fluoride active compounds) *(Table 2)*.

Tabel 2. Compounds used in this study..

Lot	*Compound*	*Active compound*	*Origin*	*Modality of use in the study*

I.	Fluorostom	Sodium fluoride	*ICCF*, Bucharest, Romania	Local administration for 25-30 minutes
II.	GC Tooth Mousse	RecaldentTM/ CPP-ACP formula	*GC Europe N.V.*, Leuven, Belgium	
III.	Reminal Ca/P-F	Calcium glycerophosphate, sodium fluoride	*Celit LTD,* Russia	

Results

Percentage distribution according to batches, it appears that the 58 outbreaks of demineralization are divided as follows: group 1 (F) 10 patients - 25%, group 2 (CAP) 12 patients - 30%, group 3 (CaPF) 10 patients - 25%, and group 4 (control) 8 patients - 20% of the initial batch size of 40 patients.

A. Analysis of the anti-plaque effect by assessing the state of oral hygiene by using the bacterial plaque index Quigley-Hein (OH).

The mean value of oral hygiene indexes for lot 1 of 1.75, for lot 2 of 2.12, for lot 3 of 1.73 and for lot 4 of 1.99, with a mean of 1.9 for the entire lot. Detailing the analysis of the oral hygiene index comparatively by lots, the mean values by the four lots were 1.75, 1.12, 1. 72 and 1.98; in the case of the first 3 lots, the field of values is large enough and in the case of the 4th lot, the field of values was narrowed (between 1.02 min. and 2.80 max.).

1. Depth of demineralization focal points (depending on the color intensity)

Table 3. Depth of demineralization points (color intensity) for Lot 1: with fluoride remineralization

Teeth	Color intensity level				
	initial	After the first application	After 2 weeks	After 6 months	After 12 months
Mean	5.94	4.94	4.69	3.63	1.75
	decrease	1	1.25	2.31	4.19
	%	16.83	21.1	38.89	70.53

In Table 3, which presents the descriptive statistical analysisis, a constant decrease of individual color intensity of (CI) from one time to another can be observed.

Following the analysis of the field of values it can be acknowledged that initially after the first application, after 2 weeks and after 6 months, it maintains large – a fact which certifies a high variation of the color intensity from case to case, but it significantly narrows down at the end of the treatment period (val. min. = 1.00, val. max. = 4.00). Differences between means are statistically significant at each moment in time, thus the variations of color intensity are significant, a fact which indicates that the treatment was efficient.

Table 4. Depth of demineralization points (color intensity) for Lot 2: CaP remineralisation

Teeth	Color intensity level				
	initial	After the first application	After 2 weeks	After 6 months	After 12 months
Mean	5.69	2.69	3.13	3.56	1.50
	decrease	3	2.56	2.13	4.19
	%	52.72	45	37.43	73.63

From *(Table 4),* the following are acknowledged: very high decrease of the mean value after 2 weeks; even if an increase is acknowledged subsequently, at the end of the treatment period, the color intensity mean remained however much lower than initially. This fact is reflected also at the level of individual values – a fact demonstrated by the evolution of the field of values, initially the values were min.=2.00 – max.= 9.00, and in the end they were min.=1.00 – max.=3.00. The t-test for dependent samples indicated that the differences between means are statistically significant only upon the first application – initial moment and during the interval 6-12 months

From the descriptive analysis (*Table 5*), the following are acknowledged: accentuated decrease of the mean values after first application, followed by low variations after 2 weeks and respectively 6 months, followed again by a drastic decrease after 12 months.

Table 5. Depth of demineralization points (color intensity) for Lot 3: CaPF remineralisation

Teeth	Color intensity level				
	initial	After the first application	After 2 weeks	After 6 months	After 12 months
Mean	5.93	4.43	1.21	3.00	2.36
	decrease	1.5	4.72	2.93	2.64
	%	25.3	79.6	50.6	39.73

Testing the significance level of variations by applying t-test, as in the previous cases, we also notice a statistically significant decrease as of the initial moment until the first application, then statistically insignificant decreases and in the end another significant decrease from 6 months to 12 months. The differences between the means are statistically significant at each moment in time, so the variations of the color intensities are significant, the treatment is efficient.

Table.6. Depth of demineralization points (color intensity) for Lot 4 control

Teeth	Color intensity level			
	initial	after 2 weeks	after 6 months	after 12 months
Mean	7.08	7.08	7.33	8.17
	increase	0	0.25	1.09
	%	0	3.53	15.39

With respect to previous cases, in the case of witness lot, an increase of the individual values of color intensity, constantly in time and for each patient, are acknowledged.Using the *t*-test as in the previous cases, we can conclude that these increases are significant only from 6 to 12 months (p=.002<0.005).

Table 7. Color intensity evolution in the 4 lots

Color intensity level	Mean values			
	LOT 1 F	LOT 2 CaFP	LOT 3 CaP	LOT 4 control
initial	5.94	5,69	5.93	7.08
after the first application	4,94	2,69	4.43	
	↓16,83%	↓52.72%	↓25.3%	
after 2 weeks	4.69	3.13	1.21	7.08
	↓ 21,1%	↓45%	↓79.6%	↑ 3.53%
after 6 months	3,63	3,56	3	7.33
	↓ 38,89%	↓ 37.43%	↓ 50.6%	↓ 3,92%
after 12 months	1,75	1.5	2.36	8.17
	↓ 70,53%	↓ 73.63%	↓ 39.73%	↑ 15.39%

3. Total demineralization area

Table 8 Total demineralization area for Lot 1 (F)

	Demineralization area (mm^2)		
	initial	**6 months**	**12 months**
Total amount:	**46.5**	**39.5**	**35.4**
Decrease:		**7**	**11.1**
	%	**15.05**	**23.87**

Applying t-test for testing the significance level of the variations of demineralization areas for Lot 1 (F), *statistically significant differences* were revealed both between the values at the initial moment upon the first application ($p=.000<0.005$) and between the values registered after 6 months and 12 months ($p=.000<0.005$). .

Table 9 Total demineralization area for Lot 2 (CaP)

	Demineralization area (mm^2)		
	initial	**6 months**	**12 months**
Total amount:	**48.80**	**46.2**	**41**
Decrease:		**2.6**	**7.8**
	%	**5.33**	**15.98**

Applying the t-test for testing the significance level of the variations of the demineralization areas for Lot 2 (CaP), *statistically significant differences* were revealed both between the values at the initial moment upon the first application ($p=.000<0.005$) and between the values registered after 6 months and 12 months ($p=.000<0.005$).

Table 10 Total demineralization area for Lot 3 (CaPF)

	Demineralization area (mm^2)		
	initial	**6 months**	**12 months**
Total amount:	**45.60**	**39.5**	**35.7**
Decrease:		**6.1**	**9.9**
	%	**13.38**	**21.71**

For Lot 3 (CaPF), statistically significant differences were acknowledged both between the values at the initial moment upon the first application ($p=.000<0.005$) and between the values registered after 6 months and 12 months ($p=.000<0.005$) (Table 10).

Table 11 Total demineralization area for Lot 4 (martor)

Demineralization area (mm^2)		
initial	**6 months**	**12 months**
Total amount:41.3	**42.8**	**44.2**
Increase:	**1.5**	**2.9**
%	**3.63**	**7.02**

For Lot 4 (witness), after the application of the Student *t*-test –for checking the significance level of the variations, no statistically significant differences were acknowledged either between the values at the initial moment upon the first application ($p=0.087>0.005$) or between the values registered after 6 months and 12 months ($p=0.441>0.005$) (Table 11).

Table 12 Evolution of total demineralization areas

Total demineralization area	**Amount of values**			
	LOT 1 – F	**LOT 2 – CaP**	**LOT 3 – CaPF**	**LOT 4 –martor**
initial (mm2)	**46.5**	**48.8**	**45.6**	**41.3**
initial – after 6 months	**39.5** ↓ *15.05%*	**46.2** ↓ *5,33%*	**39.5** ↓ *13,38%*	**42.8** ↑ *3,63%*
initial – after 12 months	**35.4** ↓ *23.87%*	**41** ↓ *15.98%*	**35.7** ↓ *21,71%*	**44.2** ↑ *7.02%*

Chapter VIII STRUCTURAL ASPECTS OF THE FLUORIDE ACTION ON THE ENAMEL MICROHARDNESS AFTER BLEACHING TREATMENT - *IN VITRO* STUDY

Aim

- ✓ *in vitro* assessment of the action of two bleaching products on the enamel microhardness;
- ✓ *in vitro* assessment of the capacity of certain remineralization products based on fluoride to influence the changes that may occur following the action of the bleaching products.

Material and methods

Within this study the evolution of dental enamel microhardness using the *Vickers method,* was assessed:

1. Following the action of bleaching professional whitening products:
   A. Opalescence Xtra Boost (Ultradent Products Inc., South Jordan, Utah, SUA) - 38% hydrogen peroxide
   B. Viva Style (Ivoclar Vivadent AG, Schaan, Liechtenstein) - 30%;carbamide peroxide
2. Following the remineralization action of certain products based on fluoride Flor-Opal Varnish (Ultradent Products Inc., South Jordan, Utah, SUA).

The enamel samples were divided randomly in 3 equal lots (n=10), as it can be noticed from the *Table 13* below.

Tabel 13. Structure of the study lots

Lot	*Nr. samples*	*Samples distribution pattern*
I.	**10**	1a. without bleaching and without remineralization
		1b. without bleaching and with remineralization
II.	**10**	2a. with bleaching and without remineralization
		2b. bleaching and remineralization
III.	**10**	3a. bleaching and without remineralization
		3b. bleaching and remineralization

The surface microhardness (SMH) of the 30 enamel samples was measured with a microhardness testing device with a diamond head in quadrangular pyramid shape with a apical angle of 36 °, with a constant load of 50g and a loading time of 10 seconds. The diagonals of the indentations were analyzed and measured by using optical metallographic microsocpe, Neophot type 21 (ZEISS - Jena, Germany), and the surface microhardness was calculated in Vickers units (HV).

Results

In the case of lots „with remineralization" (1b, 2b, 3b) there is an increase of the microhardness of the enamel. Moreover the notice very high values of the standard deviation (of the degree of spread of the mean values) in case of Lot 3.

In the case of witness lot, the values of hardness of enamel varied between 312 and 328 Mpa for the subgroup where the remineralization system was not used and between 314 and 330 Mpa in the subgroup where the remineralization system was used.

In the case of lot that included the teeth whitened with the system Opalesance Xtra Boost the values of hardness of enamel varied between 278 and 305 Mpa in the subgroup where the remineralization system was not used and between 289 and 307 Mpa in the subgroup where the remineralization system was used.
In the case of teeth that included the teeth whitened with the system Viva Style the values of hardness of the enamel varied between 254 and 301 Mpa in the subgroup where the remineralization system was not used and between 267 and 302 Mpa in the subgroup where the remineralization system was used.

Comparatively with the witness lot, a decrease of the values of microhardness of enamel in the teeth that were subject to whitening treatment was obtained. A greater decrease of the microhardness values was noticed at the lot whitened with the product Viva Style (carbamide peroxide 30%).

Moreover an increase of the microhardness values was acknowledged both at the control lot and at the lots of study on the teeth whitened with both whitening products and subject to the remineralization therapy with Flor-Opal Varnish (Ultradent Products Inc., South Jordan, Utah, SUA).

The data was analyzed by using the non-parametric statistical test Mann-Whitney. No statistically significant results were obtained upon comparing the hardness values of the enamel between the witness lot and the lots including the teeth whitened with both systems and either upon comparing the hardness of the enamel after whitening, but when the remineralization system was used ($p>0.005$).

Chapter IX *IN VITRO* STUDY OF CORRELATIONS BETWEEN MICRO-CRYSTALLIZATION SALIVARY INDEX (IMK) AND DENTAL FLUOROSIS SEVERITY

Aims

- ✓ Assessing the micro-crystallization saliva index in patients that present dental fluorosis with different degrees of severity.
- ✓ Establishing the correlations between the micro-crystallization saliva index and severity of dental fluorosis, in view of establishing certain practical recommendations with respect to therapeutic management of the existing fluorosis.

Material and methods

For this study were clinically examined 114 children aged between 7 and 14 years from Amzacea locality, Constanta county, where the amount of fluoride in drinking water has a concentration of 2.8 mg / l Clinical evaluation was carried out in natural daylight and its purpose was to determine the severity of dental fluorosis in accordance with Thylstrup-Fejerskov index. (Thylstrup A şi Fejerskov O, 1978).

For each patient the unstimulated saliva was collected into a measuring cylinder. Saliva collection was made at 12 am (considering that at this hour the mineral composition of saliva is the most stable). Same amount of saliva (0.5ml) was collected with a pipette and was applied on a glass plaque. The preparation obtained were dried for 30 minutes at thermostat (thermostat model TC-80m2, House Slave Trade Manufacturer, Company, Inc., Moscow, Russia) (Figure IX-1) at a temperature of 37 ° C, then was studied with Nikon Eclipse E 600 microscope (Nikon Instech Co. Manufacturer., Ltd. Kawasaki, Japan). The images were downloaded and stored in the computer.

Results

Analyzing the results of the micro-crystallization changes of saliva in the examined patients, it was concluded that in all cases, the micro-crystallization types fell within one of the following shapes: flake or dandelion, fern aspect – characteristics of an increased micro-crystallization index, respectively of an increased saliva remineralization capacity; ramified aspect; fir tree, acillary aspect, regular, dense ramification; combinations of the aspects mentioned above – characteristic of an average micro-crystallization index, respectively of an average saliva remineralization capacity; Multiple points, oval or cubical formations - characteristic of a low micro-crystallization index, respectively of a low saliva remineralization capacity. In order to establish the correlations between the values of the IMK indexes and the severity of fluorosis, the data were statistically analyzed and that there were no significant correlation between the saliva micro-crystallization index and the degree of dental fluorosis.

Chapter X CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE (CPP-ACP) AND ITS EFFECT ON DENTAL HARD TISSUES: SEM/EDX EVALUATION

Aims

- ✓ Highlighting the SEM morphological changes induced in vitro in the structure of the enamel surface layer under conditions simulating the daily fluctuations of pH in the oral cavity, in the the presence of remineralization preparations with and without fluoride.
- ✓ Assessment results obtained concerning the therapeutic effect of remineralization and demineralization inhibitory capacity through the action of these products, using the EDX system for determining the chemical composition of both qualitative and quantitative.

Material and methods

Thirty samples were prepared from 15 sound human third molars, that were cutted into two half. The formation of artificial carious lesions was induced. Surface examination was performed with scanning electron microscope SEM VEGA II LSH (producer TESCAN Czech company) connected to a EDX detector QUANTAX QX2 (producer company Bruker / Roentec Germany). All samples were submitted for 14 days to a pH-cycling regimen. The pH-cycling model originally described by Featherstone et al. (1986) was modified by several experiments (data not shown) until establishment of a set of conditions preserving the enamel surface and producing early carious lesions. The samples were kept individually in a demineralizing solution for 3 h, and in a remineralizing product for 21 h each day. After each cycle, the blocks were returned to 100 ml distilled water. This cycle was repeated daily for 14 days.

Remineralizing product was assured by the local application of two compounds with topical application, cream based on casein phosphopeptide and amorphous calcium phosphate with or without association of fluoride.
The enamel samples were divided randomly in 3 equal lots (n = 10), as it can be noticed in the table below *(Table 14)*.

Table 14. Structure of the study lots

Lot	*No.of samples*	*Samples distribution*
V.	**10**	Control – pH cycling
VI.	**10**	pH cycling + Recaldent GC Tooth Mousse (CPP-ACP)
VII.	**10**	pH cycling + Recaldent GC MI Paste Plus (CPP-ACP and 900 ppm fluoride)

The processed samples were examined at the electron microscope SEM VEGA II LSH, (TESCAN Cehia). A substantial advantage within the research was represented by the possibility of using the system EDX QUANTAX QX2 (Bruker/Roentec Germany) coupled at SEM, that allowed the mapping of the examined surfaces, establishing the chemical composition from the quality and quantity point of view. The observations carried out had as purpose the analysis of the changes that occur at the level of the dental surfaces included in the study following the treatments applied.

Results

The following figures present images indicating sound enamel surface *(Figura 1 a)* and images of the created artificial lesions before *(Figura 1 b, c şi d)* and after applying the pH cycling regime *(Figura 2)*. As it can be noticed, the sound enamel surfaces are characterized by an ordered, uniform, continuous structure, with equal inter-crystalline spaces and of reduced sizes *(Figura 1 a)*. Moreover, the demineralization of the surface enamel was more evident prior to the simulation of the daily fluctuations of pH in the oral cavity *(Figura 1 b, c şi d)* than after applying the regime of pH cycling *(Figura 2)*.

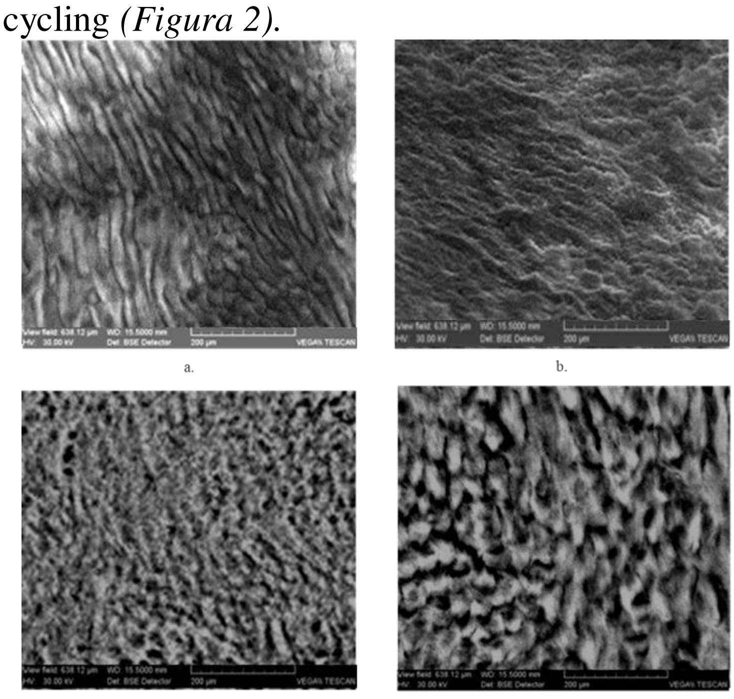


Figure 1 a) Sound enamel surface; b), c) şi d) Artificially created carious lesion, after the demineralization stage. (SEM X 1000)

Following the partial dissolution of crystals by inducing the artificial lesions, the superficial structure of the enamel becomes porous, with larger inter-crystalline spaces, as it can be noticed in the figure below, that exemplifies the sample number 1 *(Figure 2 a,b* and *c).*

In case of *Lot RECALDENT™ GC Tooth Mousse*, on the enamel surface of sample number 1, microscopic irregularities were noticed as adherent granules or globules (Figure 3 a, b and c). These represent the deposited mineral fraction following the mobilizing of the calcium and phosphate from CPP-ACP.

In case of *Lot 3 RECALDENT™ GC MI Paste Plus,* the formation of a reaction layer on the surface of the enamel could be noticed. The morphological changes of the enamel surface after the application of CPP-ACPF, analyzed by SEM, displayed the presence of globular precipitate in all samples.

As it can be noticed (Figure 3 a, b and c), that exemplifies the sample number 1, on the surface of the enamel crystalline, amorphous, globular structures with different sizes and agglomeration degrees were noticed. These globular deposits do not cove entirely the surface of the enamel in any of the samples noticed at the electron microscope (SEM).

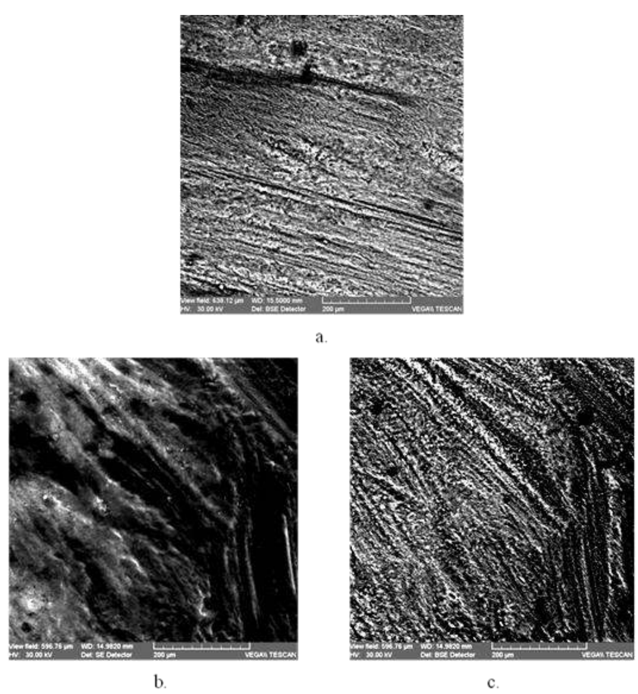
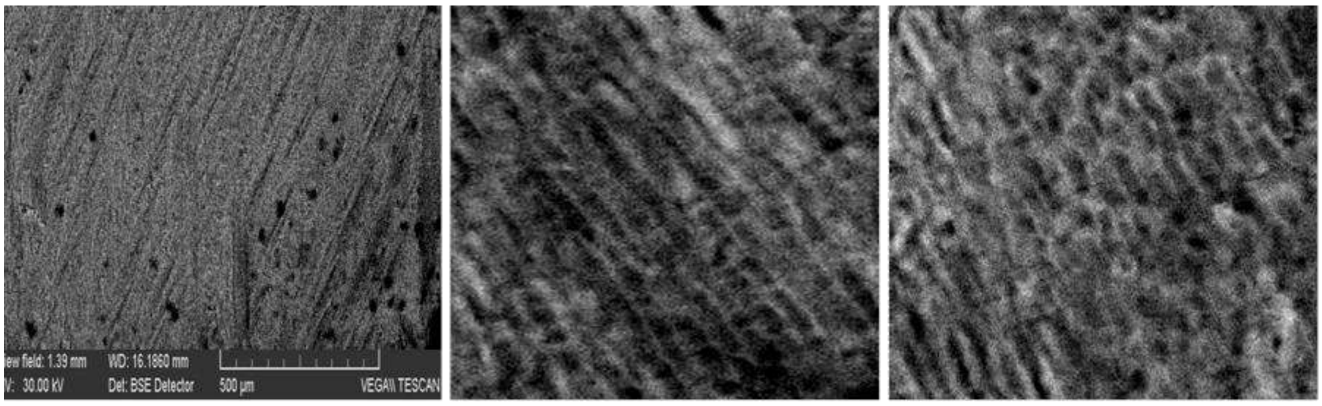
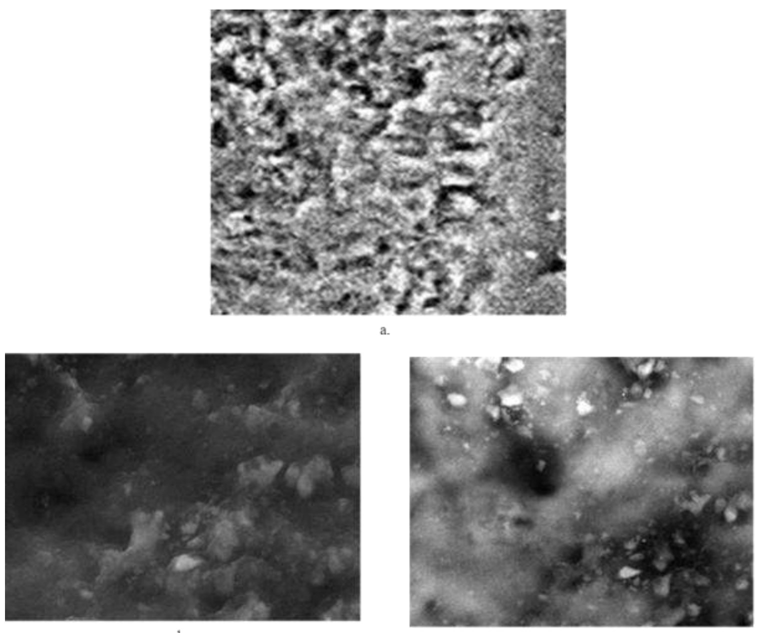


Figure 2 a) Image of enamel Lot 1 control – Sample number 1- after the pH cycling, b) and c) magnification (SEM X 1000)



a. b. c.

Figure 3 a) Image of enamel Lot 2 RECALDENT™ GC Tooth Mousse – Sample number 1- (SEM X 500); b) and c) magnification (SEM X 1000) (represents the redeposited mineral fraction following the mobilizing of calcium and phosphate in CPP-ACP)



b. c.

Figure 4 a) Image of enamel Lot 3 RECALDENT™ GC MI Paste Plus – Sample number 1- (SEM X 500); b) and c) magnification (SEM X 1000) and display of fluoride by the emergence of the precipitate of globular shape

The EDX analysis displayed the presence of calcium and phosphor ions in all the analyzed sections. The fluoride was detected in reduced quantities in the case of Lot 1 control – Samples number 2 and 8, in the case of Lot 2 RECALDENT™ GC Tooth Mousse – Sample number 1, 2, 6 and 9. In the case of Lot 3 RECALDENT™ GC MI Paste Plus – the EDX analysis displayed the presence of the fluoride in in all the analyzed sections.

The Ca^{2+} level of the analyzed enamel areas, indicated a significant decrease in the case of the control lot with an increase for the Tooth Mousse lot and an accentuated increase in the case of the MI Paste lot.The same tendency can be noticed also in the case of P ions.

In order to test the homogeneity of variance of these values, the Levene test was applied. The obtained score for Ca^{2+} was 1.319 at sig = .284 >.05 and for F^- 1.975 at sig = .178 >.05 which indicates an insignificant difference from statistical point of view thereof (valid for Ca^{2+} and F^-) and leads to the conclusion that the dispersions inside the two groups are homogenous. In the case of P ions the score was 5.497 at sig=.010<.05 which indicates a significant difference.

Following the ANOVA analysis, the values of the ***F*** parameter were of 16,279 for Ca^{2+} and 21,686 for F^- for (p = 0.0001), which allows the rejection of the null hypothesis and the acceptance of the research hypothesis according to which the level of calcium (Ca^{2+}) and fluoride (F^-) significantly varies at the enamel level in the case of the 3 lots. For phosphor (P), the research hypothesis is rejected (p = 0.05).

Chapter XI COMPARATIVE STUDY USING SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE X-RAY SPECTROSCOPY TO ASSESS MORPHOLOGICAL MODIFICATIONS TO THE ENAMEL SURFACE PRODUCED BY THREE SODIUM FLUORIDE SOLUTIONS

Aims

- ✓ the comparative assessment of enamel surface morphology and analytical assessment of calcium and fluoride ions after topical application of three sodium fluoride solutions: A, B having the same concentration (0.05% of sodium fluoride) and C with higher concentration (0.1% of sodium fluoride).
- ✓ assessment of the remineralisation capability of solution A which has not previously been studied.

Material and methods

Twelve sound premolars, extracted for orthodontic purposes from patients aged between 12-15 years, were used. Demineralization areas were created by etching with 37% phosphoric acid gel for 60 seconds. Enamel sections were immersed in 100 ml of three solutions, twice daily for 30 days: A) Fluorostom (0.05% Sodium Fluoride) B) Colgate Total Protection 0.05% (Sodium Fluoride). C) Sensodyne Pro Namel 0.1% (Sodium Flouride). Surface examination was performed using a scanning electron microscope (Olympus SZX7). The Calcium and Fluoride concentrations in the enamel surfaces were assessed using energy-dispersive X-ray spectroscopy (Inspect S. FEI Company).

Table 15. Sodium fluoride and control solutions used in the study.

Nr. Crt.	Products	Amount	Fluoride concentrations	Origin	Application frequency
1.	Solution A Fluorostom	100 ml	0.05% sodium fluoride (220 ppm F^-)	2 applications per day for 30 days	immersion in solution
2.	Solution B Colgate Total rotection	100 ml	0.05% sodium fluoride (220 ppm F^-)		
3.	Solution C Sensodyne Pronamel	100 ml	0.1% sodium fluoride (450 ppm F^-)		
4.	Solution M distilled water	100 ml	0%		

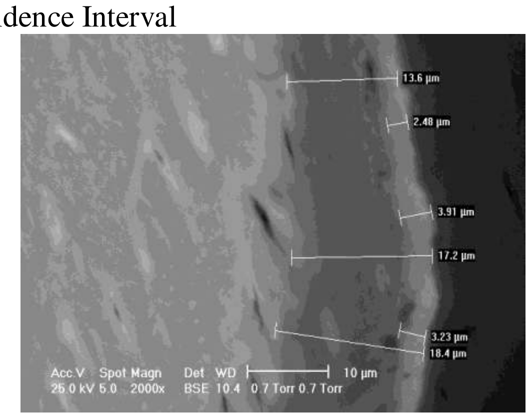
Results

The stereomicroscope highlighted areas of enamel with fluoride remineralization depth (penetration). Remineralization and initial demineralization depth were then analysed by the scanning electron microscopy (SEM) at X2000 magnification operated at 25.0kV (*Figures 5 a, b* and *c*) and energy-dispersive X-ray spectroscopy (EDS). Mean remineralization depths were: A 5.73 µm [95% CI, 2.4-12 µm] B 5.63 µm [95% CI 2.5 - 12.7 µm] and 8.28 µm [95% CI 2.91-15.54 µm] for C *(Table 16)*. There were significant differences between A versus C (p = 0.0006) and B versus C (p = 0.003) and no significant differences between A versus B (p = 0.88).

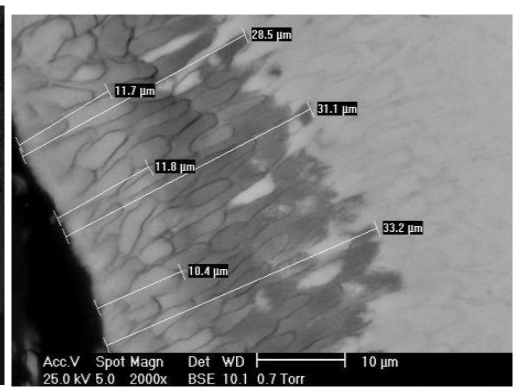
Table 16 Mean initial demineralization, residual demineralization and remineralization:

Group	Initial Demineralization (µm)	Residual Demineralization (µm)	Remineralization (µm)
GROUP A Fluorostom	21.15 [13.6 - 37.30]	15.53 [10.8 - 27.8]	5.73* [2.4.-.12]
GROUP B Colgate total Protection	19.33 [10.4 - 33]	13.79 [4.2 - 29.8]	5.63* [2.5 - 12.7]
GROUP C Sensodyne Pro Namel	31.25 [13.5 – 51.9]	21.87 [8.7 - 38.9]	8.28* [2.91.-.15.54]

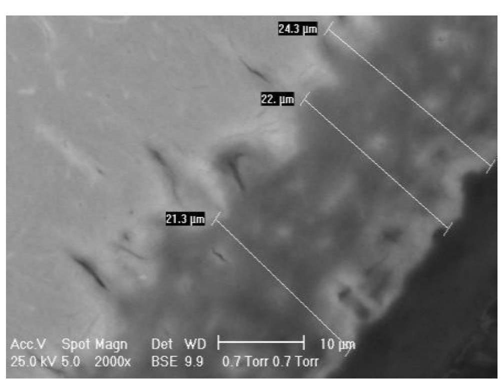
*Paired difference t-test; p<0.05
95% Confidence Interval



a.



b.



c.

Figure 5 a, b, c Measurement of the fluoride penetration depth – tooth no. 2: a) solution A, b) solution B, c) solution C (SEM 25.0kV, magnification X2000)

Morphological appearance of fluoride as globular precipitates was revealed in all treated specimens, regardless of F^- content. The distribution of the deposits was more homogeneous and small in groups treated with higher concentrations and larger in groups treated with lower fluoride concentrations. The energy-dispersive X-ray spectroscopy revealed that the intensity of signals for calcium and fluoride varied significantly across the three groups ($p = 0.0001$).

Because the control group was not subjected to the action of a fluoride solution, SEM enamel surface appearance was not modified, the image showing a similar situation with the demineralization.

The images below illustrate the sound enamel surface *(Figure 6)*, demineralized enamel *(Figure 7)* and measurements of the fluoride penetration depth: a) solution A, b) solution B, c) solution parameters SEM 25.0 C kV, magnification X2000 *(Figure 8 a, b* and *c).*

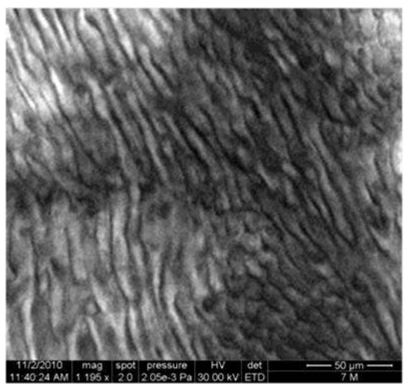
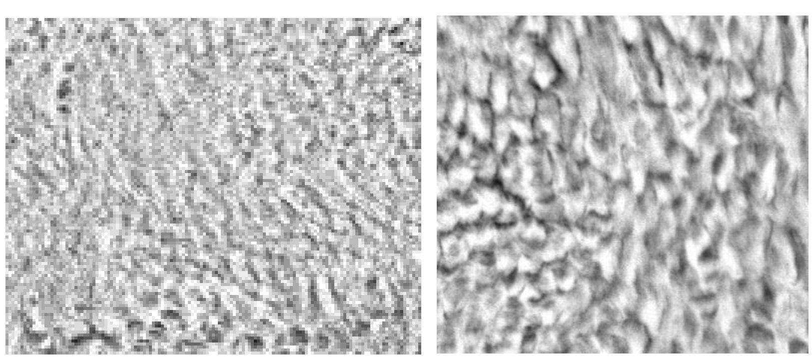


Figure 6. Sound enamel surface – control sample no. 7 (SEM, x1195)

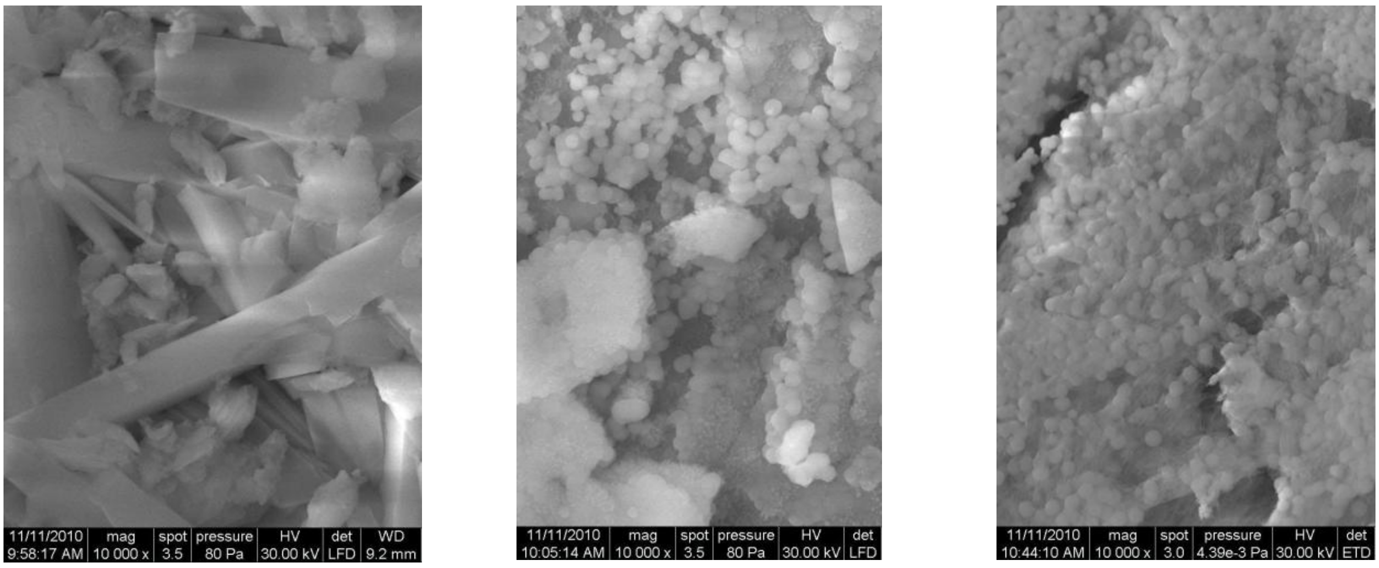
Etching with 37% phosphoric acid for 60 seconds produced a type III etching pattern: irregular pattern of demineralization (Legler et al., 1990), the surface structure of enamel was porous, with larger inter crystalline spaces *(Figure 7 a, b).*



a. b.

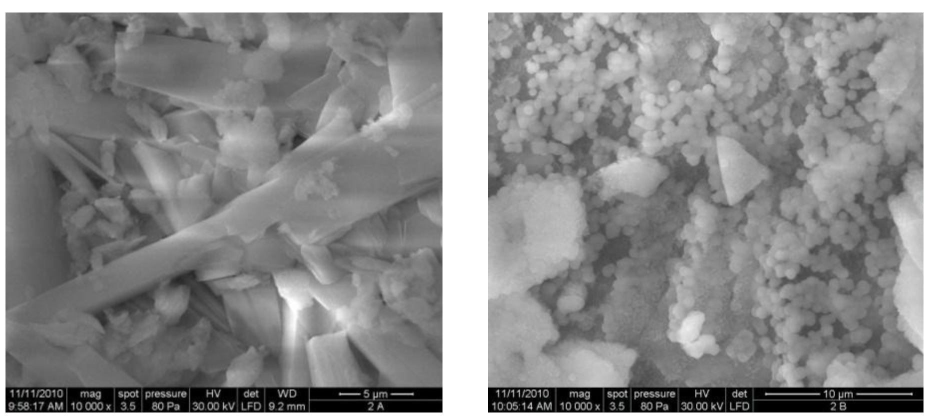
Figure 7 a and b. Demineralised enamel - type III etching pattern - control sample no.7 demineralised (SEM, x1195 and x2000)

Morphological changes on the enamel surface after application of fluoride in SEM revealed the presence of globular precipitate in all samples. The size and degree of agglomeration of the crystalline, amorphous, globular structures varied depending on the concentration of fluoride. These globular deposits did not completely cover the surface enamel in any of the observed samples *(Figure 8 a, b and c).* The density of the deposits on the enamel surface was higher when the fluoride concentration was higher, group C *(Figure 8 c).* Globular deposits observed on enamel samples, treated with lower concentrations of fluoride, were fewer, but larger *(Figure 8 a and b)*. Deposits were spherical, regardless of the concentration of fluoride used *(Figure 9)*.

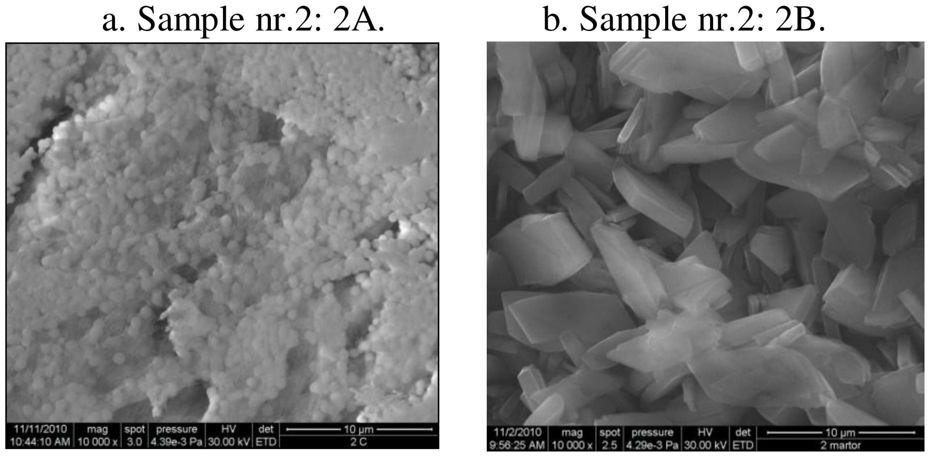


a. b. c.

Figure 8. Density of the CaF_2 deposits on the enamel surface.
a) Group A: Fluorostom sodium fluoride, 220 ppm F^-; b) Group B: 0,05% sodium fluoride, 220 ppm F^-; c) Group C: 0,1% sodium fluoride, 450 ppm F^- x10000



a. Sample nr.2: 2A. b. Sample nr.2: 2B.



c. Sample nr.2: 2C. d. Sample nr.2: 2 control

Figure 9. Globular deposits observed on enamel samples (a, b, c) and control group enamel surface (x10 000)

The EDS analysis highlighted the presence of Ca and F ions in all analyzed sections. After collecting EDS spectrum, an automatic identification of items was carried out, followed by per/ item quantifying, so the letter that appears after each item is the energy level at the time of collection. The collection was made at three energy levels: K. L and M (Wt-mass ratios, at-atomic percentages).

Calcium was the predominant element in all sections studied. The fluoride signals showed an upward trend in the samples treated with solutions with higher concentration (group C) and signals for group A were similar to those in the group B. In the enamel of the control group, fluoride was below the limit of the limit of detection.

The results of the one-way ANOVA test allow the acceptance of the research hypothesis that the Ca and F would vary significantly across the three groups ($p = 0.0001$).

Chapter XII OVERALL CONCLUSIONS, PERSONAL CONTRIBUTIONS AND CONSEQUENCES FOR PRACTICE

➤ Analyzing the results of the first study:

- most effective in a shorter preparation proved to be calcium-based phosphate preparations ierarhizându thus: first calcium-phosphate preparation, the second calcium-phosphate-fluoride preparation and the third fluoride preparation.
- regarding the efficiency over time (6-12 months), better remineralization capacity is observed in decreasing order as follows: the preparation of fluoride, the calcium-phosphate-fluoride preparation and calcium-phosphate preparation.
- preparation of calcium- phosphate - fluoride is situated on an intermediate place between the preparation of calcium phosphate and fluoride preparation. This can be explained by the fact that it meets the other two preparations qualities together.
- Finally, efficacy of fluoride concentration and duration of action in oral environment are correlated with chemical structure and method of administration.

➤ The results of the second study demonstrated an insignificant decrease of the enamel microhardness under the action of both bleaching products included in the study and an improvement of the post-bleaching microhardness values after applying the remineralization agent.

Thus the necessity of including within the clinical bleaching protocol a remineralization stage, in order to counteract the secondary effects that may occur in some patients especially in the case of using increased concentrations of carbamide peroxide, is proven. The products based on fluoride represent an optimum solution that may be used in these cases. They are beneficial both for preventing and counteracting certain dental hypersensitivity phenomena that may occur after the whitening sessions.

The results of the *in vitro* study of correlations between micro-crystallization salivary index (IMK) and dental fluorosis severity no significant correlation could be established between the values of the micro-crystallization saliva index and the degree of affection by fluorosis.
However:

- The performed researches indicated the fact that this index can constitute a useful instrument in assessing the caries susceptibility of patients with fluorosis, contributing to establishing the cariogen and consecutive risk, to selecting the prophylactic and therapeutic personalized protocol of the patient.
- The possibility of testing the remineralization capacity of saliva for each patient represents a real help in predicting the type of individual caries activity of the patient.
- The IMK index, indicator of the remineralization capacity of saliva, can be assessed by a relatively simple, accessible and inexpensive methodology.

➤ Following the comparative assessment of the SEM observations on the samples treated with the remineralization products Tooth Mousse and MI Paste Recaldent under regime of alternative pH-cycling, a significant contribution of the calcium, phosphates and especially fluoride can be noticed in the change of the morphological structure of the enamel lesion.

Following the results obtained regarding the effect of these products and the comparing thereof with the literature data, by using three adequate statistical tests, it may be concluded that the products based on CPP-ACPF (casein phosphopeptideand amorphous calcium phosphate) and especially the ones in combination with fluoride have beneficial effects of remineralization on the incipient carious lesions as well as on the counteraction of the demineralization effects following the simulation of the daily fluctuations of pH in the oral cavity within an interval of 14 days, acknowledged especially at the Control Lot.

➤ In the comparative study using scanning electron microscope and energy dispersive x-ray spectroscopy to assess morphological modifications to the enamel surface produced by three sodium fluoride solutions:

- The remineralization depth, determined by solutions containing sodium fluoride, depends on fluoride concentration and there were no statistically significant differences between samples treated with same concentration of sodium fluoride, and a statistically significant difference when a higher concentration was used.
- After the action of Fluorostom, on the enamel surface produced amorphous globular structures of CaF_2 similar to those observed in samples treated with Colgate Total which contains the same amount of sodium fluoride. Thus both products can be recommended because they produce a CaF_2 globular structure formation.
- EDS qualitative analysis highlighted the presence of the fluoride signals in all samples treated with sodium fluoride solutions.
- The results of this study suggest that the Romanian product Fluorostom is effective for maintaining constant levels of fluoride in the enamel surface and has beneficial effects on reducing the solubility and promoting the remineralization of enamel. Unsurprisingly, as it contains the same concentration of Sodium Fluoride, the remineralisation potential of Fluorostom was the same as that produced by Colgate Total.

CONSEQUENCES FOR PRACTICE

Completion of the thesis, through the conclusions pointed to each of the chapters of part the personal, involve several consequences for practice.

- Some of them support or supplement the results of studies from the literature consulted. Another part, that is the basis of this research:

  - ✓ clearly outlines several aspects of the effects that has fluoride from the romanian product Fluorostom on dental hard tissue: its beneficial effects of remineralization

    and

  - ✓ highlights the pathological effects of increased intake of fluoride from Constanta county as well as morphological-structural features of the teeth in these situations.

- In this thesis the focus is on the importance of understanding the mechanisms that take place within the hard dental structures through the action of fluoride as well as its effects under different conditions.

- Confirming the results obtained from the I.5National Program for Tooth Decay Prevention, program which consisted in weekly rinsing for one minute with the romanian solution "Fluorostom" addressed to the children aged between 6 and 11 years (grades I-IV) of the entire county (Amariei et al., 2005, 2009), consider that these information will be helpful both for dental practitioners and pediatricians from the Constanta county in order to prescribe properly the fluorinated products.

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