Babeș-Bolyai University of Cluj-Napoca
Center for Scientific Research in Physical Chemistry
Faculty of Chemistry and Chemical Engineering

SUMMARY OF THE DOCTORAL THESIS

ADVANCED BIOMATERIALS BASED ON MULTISUBSTITUTED HYDROXYAPATITE WITH MEDICAL APPLICATIONS

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KEYWORDS: nano-hydroxyapatite biomimetic, remineralization, AFM, ion release, multi-substituted hydroxyapatite, toothpaste, forsterite, antibacterial effect.

INTRODUCTION

The aim of this PhD thesis is to investigate the physico-chemical and biological properties of advanced biomaterials with medical applications based on pure and substituted hydroxyapatites, obtained at the Scientific Research Center in Physical Chemistry (CECHIF), namely HAP-5%Zn, HAP-0.23%Mg-3.09%Zn-2%Si-10%Sr, and HAP-2.5%Mg-2.9%Si-1.34%Zn. The scientific advisor is Professor Dr. Maria Tomoaia-Cotisel, Founder (2006) and Director (2006-present) of the CECHIF Center at Babeș-Bolyai University, Cluj-Napoca.

To achieve the proposed objective, multiple aspects were addressed according to a complex multifactorial design, and the results are presented in published or forthcoming articles that will be further discussed.

Thus, Chapters 1 and 2 provide a detailed presentation of theoretical aspects related to the current state of knowledge in oral care technologies and the content of secondary metabolites contained in the Betula genus, which have various uses in oral care products.

Chapter 3 presents aspects related to pure hydroxyapatite (HAP) and three multi-substituted hydroxyapatites containing Mg, Zn, Sr, and Si, synthesized by a wet precipitation method and characterized using different techniques. The presence of the HAP lattice as unique crystalline phase wa n established by XRD and by FTIR spectroscopy. The chemical composition was confirmed by SEM-EDX. The TEM, SEM and AFM imaging showed the morphology of these biomaterials. The elements release in water and in simulated body fluid (SBF) was monitored in time from 1 to 90 days, by using inductively coupled plasma optical emission spectrometry (ICP-OES).

Chapter 4 presents the results of an experiment in which two types of toothpaste were developed, one with nano-HAP and the other with multi-substituted hydroxyapatite (HAP-Mg-Zn-Si) of nano dimensions, which were used to treat artificially demineralized enamel surfaces. The morphology and roughness of the surface of all enamel samples were studied using atomic force microscopy (AFM) before and after the application of the toothpastes. The effect of the toothpastes was highlighted by the average diameter of the ceramic nanoparticles deposited in the smooth surface layer of the enamel, which, at the end of the 10-day treatment, showed a reduced surface roughness comparable to that of natural enamel.

Chapter 5 presents the results of experiments conducted with forsterite (FS, Mg2SiO4), which is a promising candidate for orthopedic and dental applications. This chapter investigates two synthesis methods of forsterite, namely the sol-gel method (FSsg) and precipitation (FSpp), based on a thermodynamic approach. The precursor gel and precipitate were analyzed using thermogravimetric analysis (TG), differential thermogravimetric analysis (DTG), and differential scanning calorimetry (DSC). The FSsg and FSpp powders were characterized by X-ray diffraction

(XRD) and atomic force microscopy (AFM). XRD reveals that the synthesized forsterite has high crystallinity.

Chapter 6 presents the results of experiments in which four new toothpastes based on biomimetic nano-hydroxyapatite with reduced concentration (3.7%) were prepared. Characterizations by X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) revealed crystal sizes between 28 and 33 nm and crystallinity between 28 and 36%. The aim of this study was to compare the effect of these toothpastes on the remineralization of human enamel, which was artificially demineralized by immersion in a phosphoric acid solution. The experiment lasted for ten days. Enamel surface was analyzed by AFM images, and surface roughness before and after treatment was determined. The average size of hydroxyapatite particles ranged from 30 to 40 nm. Surface roughness of enamel slices was investigated using one-way ANOVA and Bonferroni multiple comparison test. Nanosized particles (with an average size of approximately 30 nm for HAP) showed great potential in the process of remineralization by covering regions with enamel lesions.

Chapter 7 presents the results of a study in which four toothpaste formulations were developed, containing four different types of synthetic hydroxyapatite (1 pure HAP and 3 substituted HAPs, namely HAP-5%Zn, HAP-0.23%Mg-3.09%Zn-2%Si-10%Sr, and HAP-2.5%Mg-2.9%Si-1.34%Zn). The experiment lasted for 30 days, and the samples were analyzed at 10-day intervals. Using atomic force microscopy (AFM) techniques, a complex investigation combining morpho-structural and surface quality aspects was accomplished. Topographic images, tridimensional profiles, and the ability of HAP nanoparticles to form self-assembled thin films reveal excellent restorative properties of the tested toothpaste, with nanostructure normalization occurring as soon as 10 days after treatment. At the end of the 30-day treatment period, all four experimental toothpastes led to complete remineralization of the artificially demineralized enamel. This is a key aspect to consider for future studies. AFM has proven to be an efficient technique for investigating the enamel remineralization process, both in terms of morphological and structural changes and surface roughness measurements.

Chapter 8 presents a study aimed at developing toothpaste formulas with combined remineralization and antibacterial effects, using nanohydroxyapatite (nHAP) and birch extract as biomaterials. Eleven types of toothpaste were designed containing various concentrations of birch extract and pure and substituted nHAP (HAP-5% Zn, HAP-0.23% Mg-3.09% Zn-2% Si-10% Sr, and HAP-2.5% Mg-2.9% Si-1.34% Zn). In order to evaluate efficacy, in vitro tests were performed on demineralized enamel slices, analyzing enamel surface repair by atomic force microscopy (AFM) techniques. The antibacterial activity was tested against Enterococcus faecalis, Escherichia coli, Porphyromonas gingivalis, Streptococcus mutans, and Staphylococcus aureus bacterial strains. The results showed improvements in enamel microstructure due to toothpaste treatment and varying antibacterial efficacy of the tested formulas. Promising results were observed with toothpaste P5, containing 5% Zn-HAP and 1.3% birch extract, indicating notable remineralization and antibacterial properties.

Chapter 9 presents a nanocomposite composed of hydroxyapatite (HAP) and silver nanoparticles (AgNPs), referred to as HAP-4.5% AgNPs. HAP was prepared using a wet precipitation technique, while AgNPs were obtained through the reduction of silver nitrate with glucose in a basic medium. HAP, AgNPs, and the HAP-4.5% AgNPs nanocomposite were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM) - energy-dispersive X-ray spectroscopy (EDS), and various imaging methods, including transmission electron microscopy (TEM) and atomic force microscopy (AFM). The antibacterial effect of the HAP-4.5% AgNPs nanocomposite was tested using a nutrient agar diffusion technique against two pathogenic species, one Gram-negative (Salmonella typhimurium) and one Gram-positive (Bacillus cereus), yielding promising results. This hydroxyapatite-silver nanocomposite could be used as a potential antimicrobial component for dental and orthopedic implants or as a bone cement in clinical procedures.

Chapter 10 outlines the General Conclusions of the research in the Doctoral Thesis.

Chapter 11 presents the candidate's accomplishments in the scientific research work included in this Doctoral Thesis.

List of paper original papers

1. Cadar, O., Balint, R., Tomoaia, G., **Florea, D.,** Petean, I., Mocanu, A., Horovitz, O. and Tomoaia-Cotisel, M. Behaviour of multisubstituted hydroxyapatites in water and simulated body fluid. Stud. Univ. Babes-Bolyai, Chem., 62, 269-281, 2017. **I.F. 0.305**
2. **D. A. Florea**, C. Dobrota, R. Carpa, S. Riga, M. Tomoaia-Cotisel. Current status and trends in oral health care technologies. a perspective review. International Journal of Medical Dentistry, 26(1), 38-50, 2022. **I.F. 0.887**
3. A. Avram**, D. Florea**, F. Goga, M. Gorea, A. Mocanu, Gh. Tomoaia, I. Petean, A.-Z. Kun, O. Horovitz, M. Tomoaia-Cotisel. Mechanism in the synthesis of fosterite nanoparticles based on thermodynamic approach. Stud. Univ. Babes-Bolyai, Chem, 68(2), 37-51. 2023. **I.F. 0.3**
4. **D.A. Florea**, A. Mocanu, L.C. Pop, Gh. Tomoaia, C.-T. Dobrota, C. Varhelyi Jr, M. Tomoaia-Cotisel. Remineralization of tooth enamel with hydroxyapatite nanoparticles: an in vitro study. Stud. Univ. Babes-Bolyai, Chem., 68(2), 99-113, 2023 **I.F. 0.3**
5. A. Mocanu, **D. A. Florea**, Gh. Tomoaia, L.-C. Pop, A. Danistean, S. Rapuntean, O. Horovitz, M. Tomoaia-Cotisel. Nanocomposite based on hydroxyapatite and silver with antibacterial activity. Stud. Univ. Babes-Bolyai, Chem. 68 (3), 7-18, 2023, in press **I.F. 0.3**
6. **A.-D. Florea**, L. C. Pop, H.-R.-C. Benea, Gh. Tomoaia, C.-P. Racz, A. Mocanu, C.-T. Dobrota, R. Balint, O. Soritau, M. Tomoaia-Cotisel. Remineralization induced by biomimetic hydroxyapatite toothpastes on human enamel. Biomimetics, Manuscript ID:2590006, in press, **I.F 3.743 Q1**

CHAPTER 1. CURRENT TRENDS IN ORAL CARE TECHNOLOGIES

According to the 2017 Global Burden of Disease study, approximately 3.5 billion people worldwide are affected by dental problems, of which untreated dental caries are the most common problem, Severe periodontal disease caused by poor oral hygiene affects 10% of the world's population. Epidemiological evidence also indicates a link between dental plaque and a number of other diseases of the human body. The oral microbiome has been involved in autoimmune and metabolic diseases such as obesity, diabetes, and cardiovascular diseases.Therefore, research and improvement of oral care products can help improve the overall health of patients.

The purpose of this chapter is to provide a detailed overview of the current state of existing technologies in oral care. A comparative analysis of the benefits and drawbacks of alternative technologies applied in oral care is proposed. Numerous innovative technologies have been introduced in oral hygiene, aimed at personalizing brushing routines and providing feedback on brushing techniques. Smart sensors, smartphone-connected apps that guide users in proper brushing techniques, DNA sequencing technology used to formulate oral care products targeting specific mechanisms of action, are just a few examples of the new technologies applied in oral care. Products are nano-modified, and carriers are used to target specific areas where they are released. Following the analysis conducted, it was concluded that there are several limitations to state-of-the-art oral care technologies, with the main issues in this field being related to the lack of rigorous evidence for many of the newly developed products and the fact that access to such new products is financially prohibitive for a large majority of the population.

CHAPTER 2. THE USE OF *BETULA* SPECIES EXTRACTS IN THERAPEUTIC AND PREVENTIVE ORAL CARE

Chapter 2 provides a detailed presentation of the *Betula* genus, known for its wide variety of secondary metabolites with multiple medical applications. Plant extracts are used in dental practice due to their beneficial activity and minimal side effects. Phytochemical, pharmacological, and toxicological research on Betula species extracts has demonstrated their therapeutic potential. Plants contain hundreds of potentially useful molecules. Birch infusions, decoctions, and isolates are traditionally used in dentistry and otolaryngology for treating gingivitis, glossitis, periodontitis, protecting hard dental tissues, and treating stomatitis. Xylitol extracted from birch tree hemicellulose is used as a sugar substitute in pharmaceuticals, foods, and oral hygiene products such as toothpaste, chewing gum, syrups, and confectionery. Xylitol contributes to reducing the risk of tooth decay through several mechanisms. These include increased salivary flow and pH, reduced xerostomy (dry mouth), gingival inflammation, and dental erosion. Also, xylitol helps to prevent the development of caries and promotes remineralization in the case of primary dentition, can help to reverse the process of advancement of early caries Betulin, a pentacyclic triterpenic alcohol isolated from birch sap, has been incorporated into nanocapsules embedded in toothpaste. *Streptococcus mutans* is considered the main cause of tooth decay. Studies have shown that betuline has significant activity against the biofilm formed by *Streptococcus mutans*. Depending on the dose, betuline can inhibit biofilm formation by up to 93% and the adhesion of bacteria to glass surfaces up to 71%. The biochemical and physiological mechanisms, as well as the detailed preclinical toxicity, bioavailability, pharmacokinetics, and pharmacodynamics of different biologically active molecules, are still under investigation. In addition to their applications in dentistry, compounds isolated from Betula species have demonstrated anti-arthritic, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiviral, gastroprotective, hepatoprotective, and immunomodulatory activities. An integrated, holistic approach is needed to fully understand the potential of the Betula genus.

CHAPTER 3. MULTISUBSTITUTED HYDROXIAPATITES: THE RELIEVING OF IONS IN WATER AND SIMULATED BODY FLUID (SBF)

In order for biomaterials based on synthetic hydroxyapatite (HAP) to be used to replace bone tissue, it is necessary to create hydroxyapatites (HAPs) with structures that resemble the mineral phase of natural bone.

Pure hydroxyapatite (HAP), a complex triple substituted hydroxyapatite,with Mg, Zn and Si (HAPc), and two tetrasubstituted complex hydroxyapatites, containing Mg, Zn, Si and Sr were prepared. The theoretical formula in the table were calculated assuming thatbMg^{2+}, Zn^{2+} and Sr^{2+} ions partially substitute for Ca^{2+} ions in the HAP lattice, while Si, as silicate SiO_4^{4-} ions substitutes some of the phosphate, PO_4^{3-} ions, with a corresponding diminution of the number of OH^- ions, in order tovmaintain the electroneutrality of the lattice, according to the general formula: $Ca_{10-x-y-z}Mg_xZn_ySr_z (PO_4)_{6-u} (SiO_4)_u (OH)_{2-u}$.

The HAP structure, as unique phase present in all samples, was confirmed by XRD investigations. The degree of crystallinity of the obtained hydroxyapatites and the average size of the constituent nanoparticles were determined.

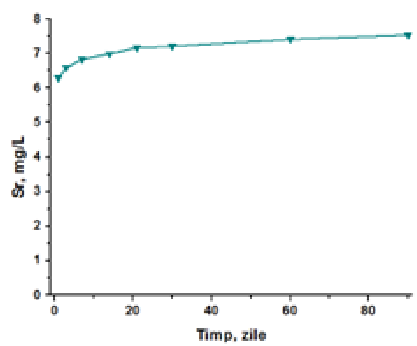

Figure 1. Release of Sr in water after immersion of HAPs samples for 1 – 90 days.

Figure 1 indicates a gradual increase in the release of strontium over time. Studies have also demonstrated that the solubility of strontium-substituted hydroxyapatites continuously increases as the strontium content in the composition grows.

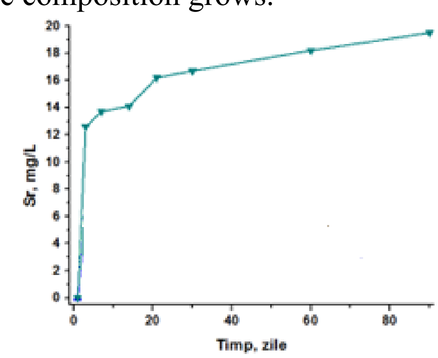

Figure 2. Sr levels in Simulated Body Fluid after immersion of HAPs samples for 1 - 90 days

In simulated body fluid (SBF), the release of strontium (Sr) is higher than in water. There is a greater release of strontium ions from hydroxyapatites in SBF.

The conclusion of this experiment was that a higher concentration of strontium in the structure of HAP affected its release in both water and simulated body fluid (SBF). Multisubstituted hydroxyapatites contain physiologically valuable elements and exhibit prolonged release. This suggests that these biomaterials may have promising biomedical applications.

CHAPTER 4. REMINERALIZATION OF TOOTH ENAMEL WITH HYDROXYAPATITE NANOPARTICLES: AN *IN VITRO* STUDY

Two toothpaste formulations were prepared, one with nano-HAP, referred to as P1, and the other with nano-HAP multisubstituted with Mg, Zn, and Si (ms-HAP, HAP-Mg-Zn-Si), referred to as P2. The ceramic particle content in each toothpaste was 4.0%, and the average size of the nano-hydroxyapatite particles (nano-HAPs) ranged from 30 to 40 nm. The morphology and surface roughness of enamel samples were studied using atomic force microscopy (AFM) before and after the application of the toothpaste treatments. The effect of the toothpastes was highlighted by the average diameter of the ceramic nanoparticles deposited on the smooth surface layer of enamel at the end of the 10-day treatment.

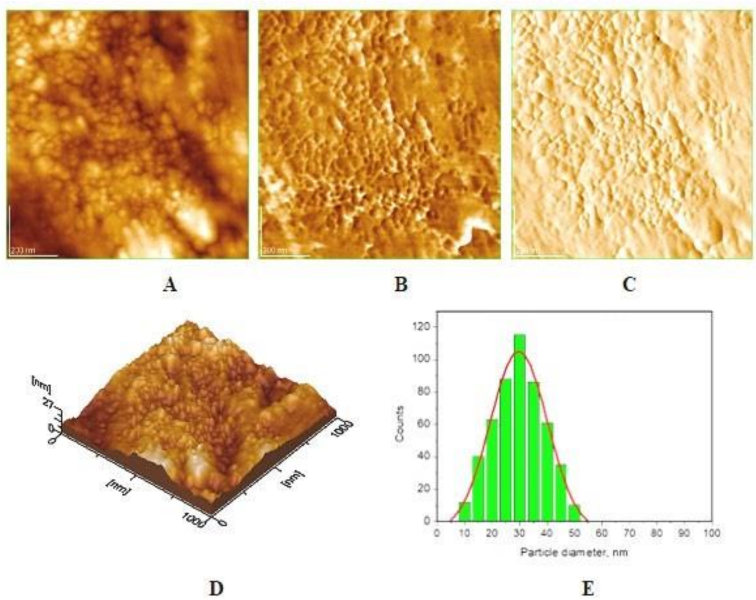

Figure 3. AFM images of remineralized enamel treated for 10 days with P1 toothpaste: 2D topography image (A), phase image (B), amplitude image (C), and 3D topography image (D), for scanned area of 1 μm x 1 μm, and the histogram (E) on image (A): Gaussian distribution (full line) of HAP nanoparticles diameter on remineralized tooth enamel surface (A); average diameter is 30±4 nm.

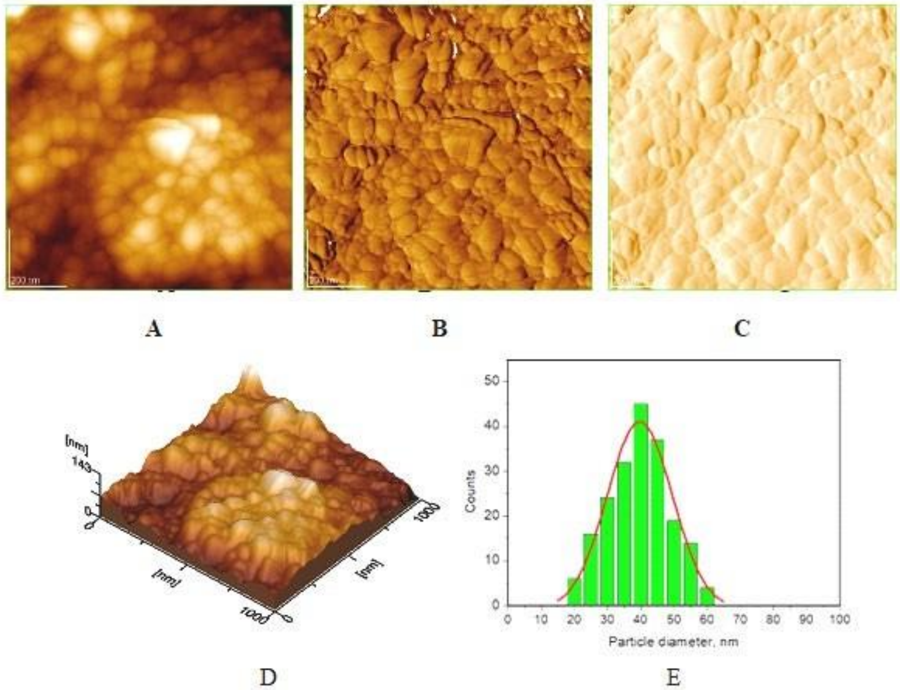

Figure 4. AFM images of remineralized enamel treated for 10 days with P2 toothpaste: 2D topography image (A), phase image (B), amplitude image (C), and 3D topography image (D), for scanned area of 1 μm x 1 μm, and the histogram (E) on image (A): Gaussian distribution (full line) of HAP nanoparticles diameter on remineralized tooth enamel surface (A); average diameter is 40±2 nm.

Another objective of this study was to optimize the tapping mode AFM approach to characterize the ultrastructure of enamel through AFM images, such as 2D and 3D topographies, as well as phase and amplitude images.

This in vitro comparative study demonstrated that both toothpastes can promote enamel surface repair through remineralization and the formation of a protective layer of hydroxyapatite on the treated enamel surface. The P2 toothpaste proved to be the best of the two, leading to stable morphological modifications of the dental enamel surface. In the ten treatment days, the demineralized enamel lesions were remineralized completely as shown by AFM investigations, of structural-morphology and surface roughness. Synthetic ceramic nanoparticles arranged regularly within a smooth protective layer on the enamel surface after treatment, ensuring reduced surface roughness, close to that of natural enamel, thus providing further evidence of the toothpaste's effectiveness.

CHAPTER 5. MECHANISM IN THE SYNTHESIS OF FOSTERITE NANOPARTICLES BASED ON THERMODYNAMIC APPROACH

In this study, various methods for preparing forsterite powder for medical applications based on thermodynamic approaches were investigated, and it was found that the sol-gel method and co-precipitation are suitable procedures for this purpose.

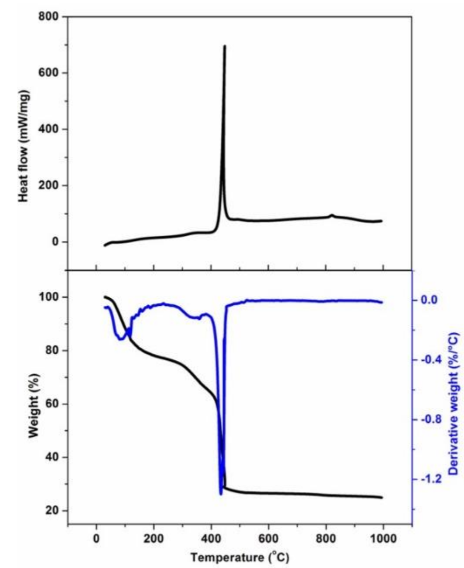

Figure 1. DSC (up) and TG/DTG (down) curves for FSsg dried gel (exo ↑, endo↓)
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The thermal curves in Figure 1 show the processes that occur during the heating of the dried gel obtained after hydrolysis and condensation of precursors, followed by further drying. The removal of water remnants in the dried gel is characterized by an endothermic process up to around 230oC accompanied with a mass loss of around 22%. The following two exothermic processes, one between 233°C and 381°C and the other between 381°C and 465°C, are related to the oxidation of organic components.

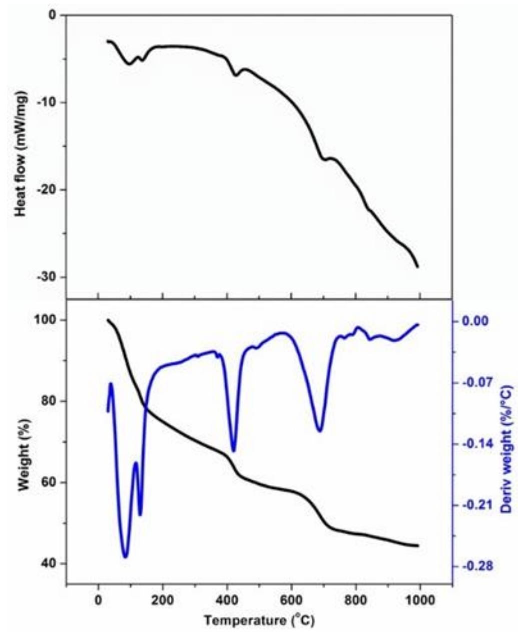


Figure 2. DSC (up) and TG/DTG (down) curves for FSpp dried precipitate (exo ↑, endo↓)

The thermal curves specific to the heating of the dried precipitate are presented in Figure 2. The removal of water remnants in the dried precipitate is characterized by two endothermic processes that occur up to 360°C. The mass loss attributed to these processes is of around 16% for the first and 16.2% for the second. Dehydroxylation of the -SiOH and -MgOH groups can be assigned to the next two endothermic processes, between 366oC and 582°C for –MgOH and at a higher temperature, up to 800°C for –SiOH.

Forsterite crystallizes in the orthorhombic system (space group Pbnm), having the following cell parameters: a = 4.75 Å, b = 10.20 Å and c = 5.98 Å. The diffraction patterns for both forsterite materials, FSsg and FSpp, are presented in Figure 3. Clearly, the obtained powders have a well-defined crystallinity, more so in the case of FSsg.

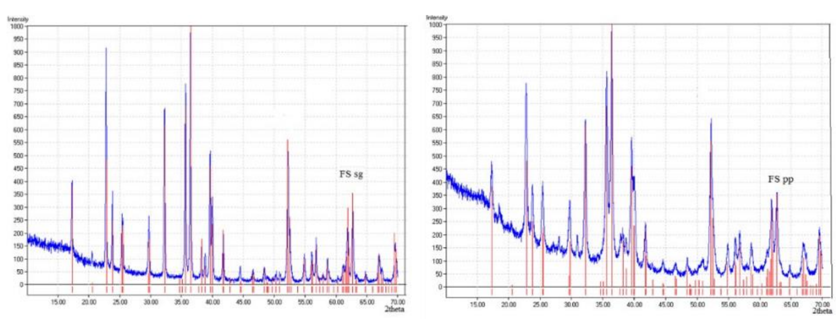


Figure 3. X-ray diffraction patterns for FSsg (a) and FSpp (b)

The choice of synthesis route is quite important as it can affect the characteristics of the final powder – shape and size of nanoparticles, distribution and tendency of agglomeration which in turn affect the microstructural homogeneity of a ceramic and thus limit potential applications. The reaction mechanisms for both sol-gel and precipitation methods were proposed on the thermodynamic approach. Forsterite was successfully synthesized through both sol-gel (FSsg) and precipitation (FSpp) method, leading to smaller nanoparticles (30 nm) through precipitation method when compared to nanoparticles (40 nm) obtained by sol-gel method.

CHAPTER 6. REMINERALIZATION INDUCED BY BIOMIMETIC HYDROXYAPATITE TOOTHPASTES ON HUMAN ENAMEL

Four new toothpastes based on biomimetic, pure, and multi-substituted nano-hydroxyapatite have been prepared, as shown in the following table.

Table 1. Four innovative nanomaterials used to prepare the four toothpastes

Toothpaste Symbol	Type of HAP	Substitution Elements (%, by mass)	Chemical Formula of HAP
P1	HAP-Zn	Zn 5.00	$Ca_{9.22}Zn_{0.78}(PO_4)_6(OH)_2$
P2	HAP	-	$Ca_{10}(PO_4)_6(OH)_2$
P3	HAP-Mg-Zn-Sr-Si	Mg 0.23 Zn 3.09 Sr 10.00 Si 2.00	$Ca_{8.19}Mg_{0.10}Zn_{0.5}Sr_{1.21}(PO_4)_{5.25}(SiO_4)_{0.75}(OH)_{1.25}$
P4	HAP-Mg-Zn-Si	Mg 2.50 Zn 1.34 Si 2.90	$Ca_{8.80}Mg_{1.00}Zn_{0.20}(PO_4)_{5.00}(SiO_4)_{1.00}(OH)_{1.00}$

The XRD patterns for the four HAPs used are given in Fig. 1, along with the pattern for pure HAP from PDF:74-0566 (red vertical lines).

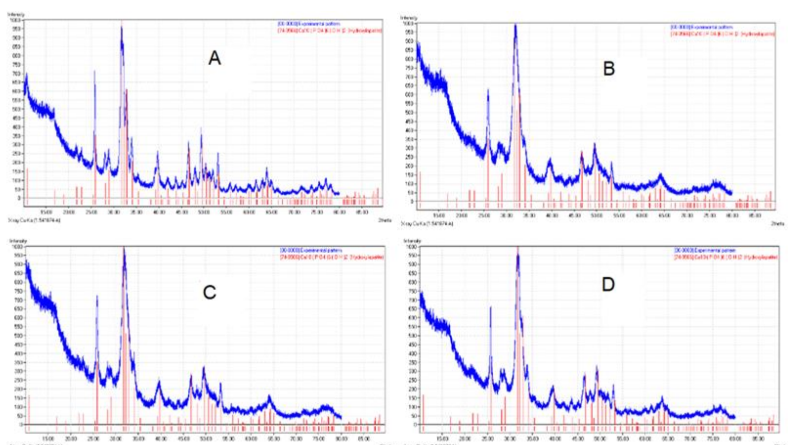


Figure 1. XRD for 4 HAPs: (A) for hydroxyapatite, HAP, $Ca_{10}(PO_4)_6(OH)_2$, (B) for HAP-Zn, $Ca_{9.22}Zn_{0.78}(PO_4)_6(OH)_2$, (C) for HAP-Mg-Zn-Si, $Ca_{8.80}MgZn_{0.20}(PO_4)_{5.00}(SiO_4)_{1.00}(OH)_{1.00}$, and (D) for HAP-Mg-Zn-Sr-Si, $Ca_{8.19}Mg_{0.10}Zn_{0.50}Sr_{1.21}(PO_4)_{5.25}(SiO_4)_{0.75}(OH)_{1.25}$, compared with PDF:74-0566.

The calculated lattice parameters (a and c values) revealed only slight changes with the compositional modification within the HAP structure. The small composition differences lead to a slight distortion of the HAP lattice and, thus, a small drop in its crystallinity (Table 2). It was also discovered that the lattice constants, a and c, diminished slightly with Zn substitution in the HAP structure.

Table 2. XRD estimates of crystallite size, crystallinity degree, and lattice parameters for pure hydroxyapatite, HAP, and substituted hydroxyapatites: HAP-Zn, HAP-Mg-Zn-Si, and HAP-Mg-Zn-Sr-Si

Hydroxyapatites	HAP-Zn	HAP	HAP-Mg-Zn-Sr-Si	HAP-Mg-Zn-Si
Toothpastes	P1	P2	P3	P4
Crystallites size (nm), from XRD data	30.3	33.1	28.2	30.6
Crystallinity (%), from XRD data	30.5	36.6	28.7	30.3
Lattice parameters: a = b (nm) c (nm)	 0.9421 0.6862	 0.9426 0.6881	 0.9466 0.6904	 0.9445 0.6883
Average diameter of NPs (nm), from AFM approach*	40±5	30±3	37±4	38±5

*Average diameter of nanoparticles, NPs, self-assembled as a layer on glass plate, estimated from AFM approach

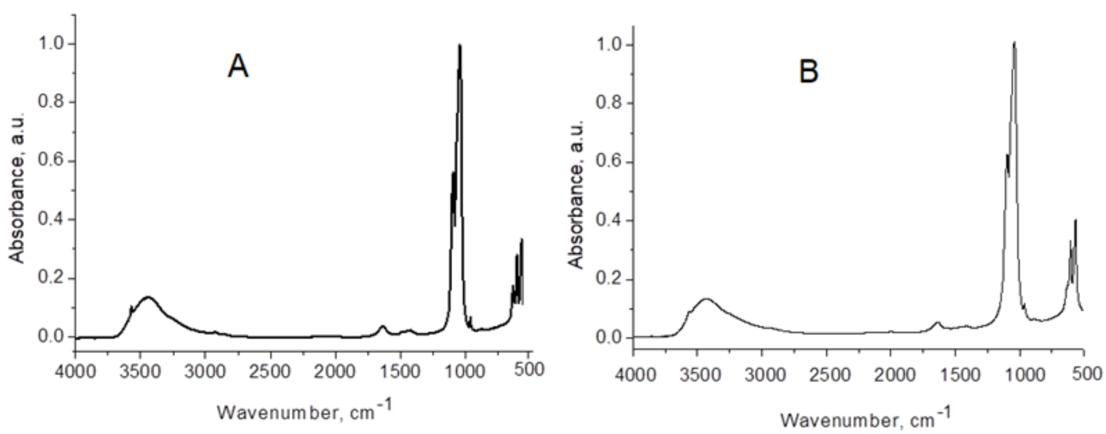


Figure 2. FTIR spectrum of HAP lyophilized, (A), used in P2 toothpaste and of HAP-Mg-Zn-Sr-Si, (B), used in toothpaste P3. Absorbance is normalized to 1.

For SEM-EDX measurement, the powder HAP samples are deposited in slim sheets on SEM grids. The FE-SEM image (Figure 3A) shows individual particles at high magnification. The average diameter of HAP particles was found to be 40.0±7.5 nm.

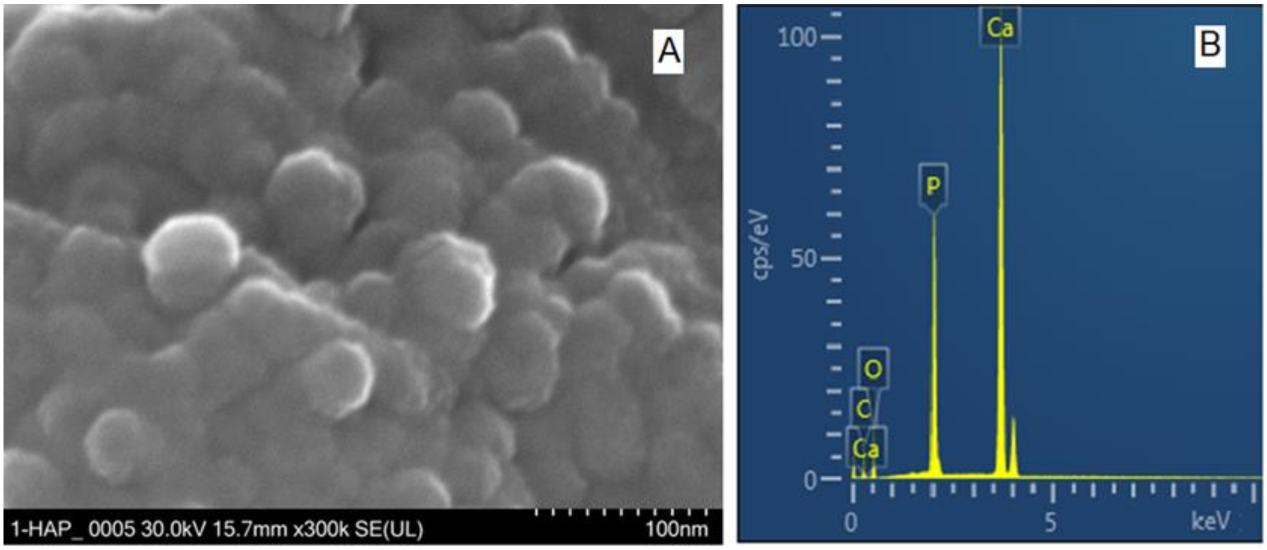


Figure 3. FE-SEM image (A) for HAP; the bar scale is 100 nm; EDX spectrum (B), shows jointly all elements on FE-SEM image (A).

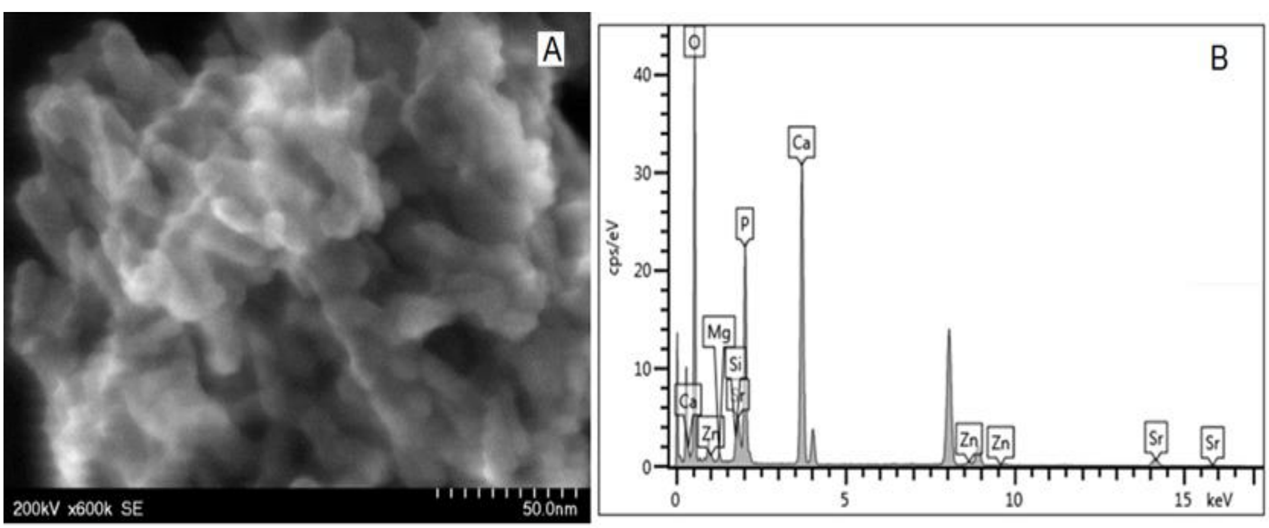


Figure 5. STEM image (A) and EDX spectrum (B) for HAP-Mg-Zn-Sr-Si

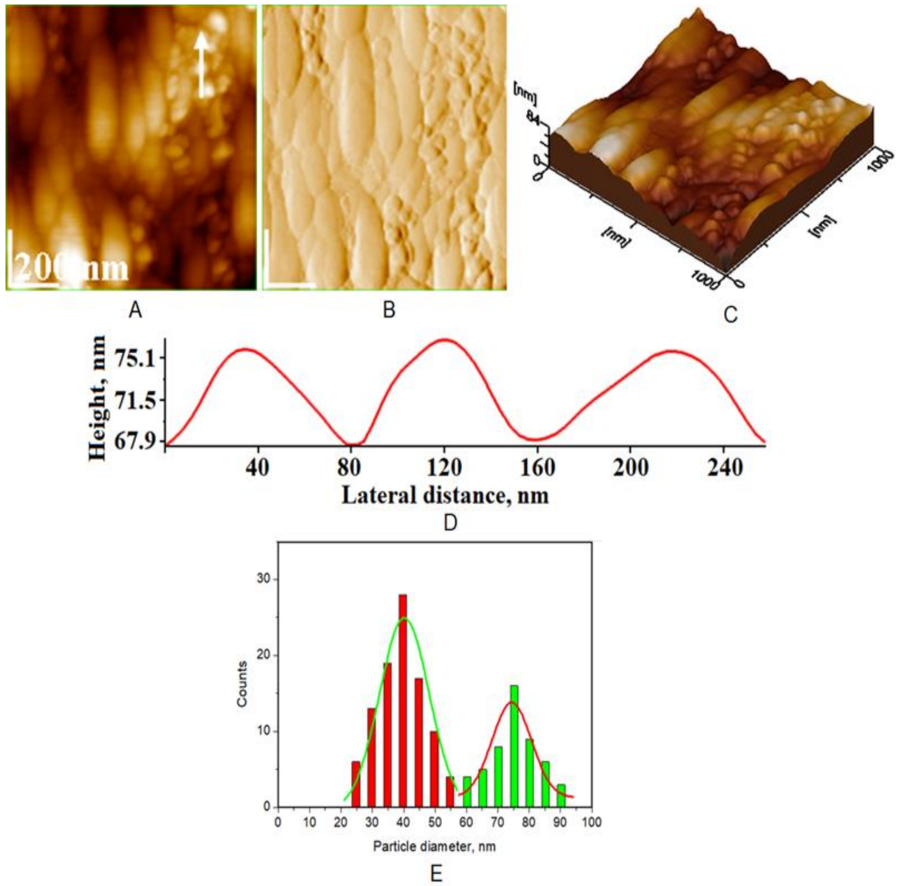


Figure 10. AFM images of two half-slices: one half-slice was untreated (natural enamel for control) marked with white arrow and the other half was demineralized enamel by treatment with phosphoric acid: (A) topography image, (B) amplitude image, (C) 3D image, and (D) cross section profile on white arrow in image (A), representing natural enamel zone, (E) histogram on image (A); average diameter of NPs on natural enamel (42±5 nm) and on demineralized enamel (73±6 nm). Scanned area 1 μm x 1 μm.

The AFM study revealed that P3 toothpaste with tetrasubstituted hydroxyapatite (HAP-Mg-Zn-Sr-Si) performed the best in terms of human enamel remineralization when compared to the other toothpastes (P1 and P4), with approximately the same remineralization efficiency as P2 toothpastes after 10 days of toothpaste treatment. The smallest nano-sized particles (about 30 nm in average size of HAP) showed great potential in the remineralizing process by covering lesion regions of enamel. All of the results of treating human enamel for 10 days with each of the P1–P4 toothpastes lead us to assume that these toothpastes can be used successfully to treat early tooth decay and, more importantly, artificially demineralized enamel.

CHAPTER 7. ENHANCING ENAMEL REMINERALIZATION: NOVEL TOOTHPASTES WITH SUBSTITUTED HYDROXYAPATITE FOR DENTAL HEALTH

In this chapter, the effect of different toothpaste formulations containing hydroxyapatite (with substitutions of network ions such as Sr, Zn, Si, and Mg) on the remineralization of previously acid-treated enamel was studied. The main purpose of this study is to determine whether the addition of these ions would affect the remineralization process when compared to simple hydroxyapatite. Similar research has used commercial toothpastes containing zinc hydroxyapatite [68, 69], zinc carbonate hydroxyapatite [70, 71], nano-carbonate substituted HAP [72], Zn-Mg-hydroxyapatite [73], and Mg-Sr-carbonate hydroxyapatite [71, 74]. However, no toothpaste formulations containing hydroxyapatite with these exact ionic combinations are available. Furthermore, to the best of the authors' knowledge, no studies or commercial products employing toothpaste containing HAP with four ionic substitutions exist, hence this is one of the novelties within this paper.

The four synthesized nanoHAP samples were characterized regarding size distribution using Atomic Force Microscopy before their incorporation into the toothpaste compositions. Thus, all powders were transferred onto glass slides by vertical adsorption from aqueous dispersions.

The adsorption of HAP nanoparticles from an aqueous solution onto a solid substrate demonstrates their ability to form self-assembled thin films. This property of film formation is crucial when considering the desired protective layer formed by certain toothpaste compositions on the enamel surface. Here, HAP-5%Zn (P1) demonstrated well-individualized round nanoparticles with diameters of about 40 nm, positioned in a well-structured film (Figure 1(a-d)), providing a uniform surface free from particle agglomerations. The result is a smooth surface with reduced roughness. Figure 1(e-g) shows that simple hydroxyapatite (HAP) used in sample P2 has round-shaped nanoparticles with smaller diameters, around 30 nm, which are uniformly adsorbed on the glass slide in a thin, smooth, and compact film without generating particle agglomerations.

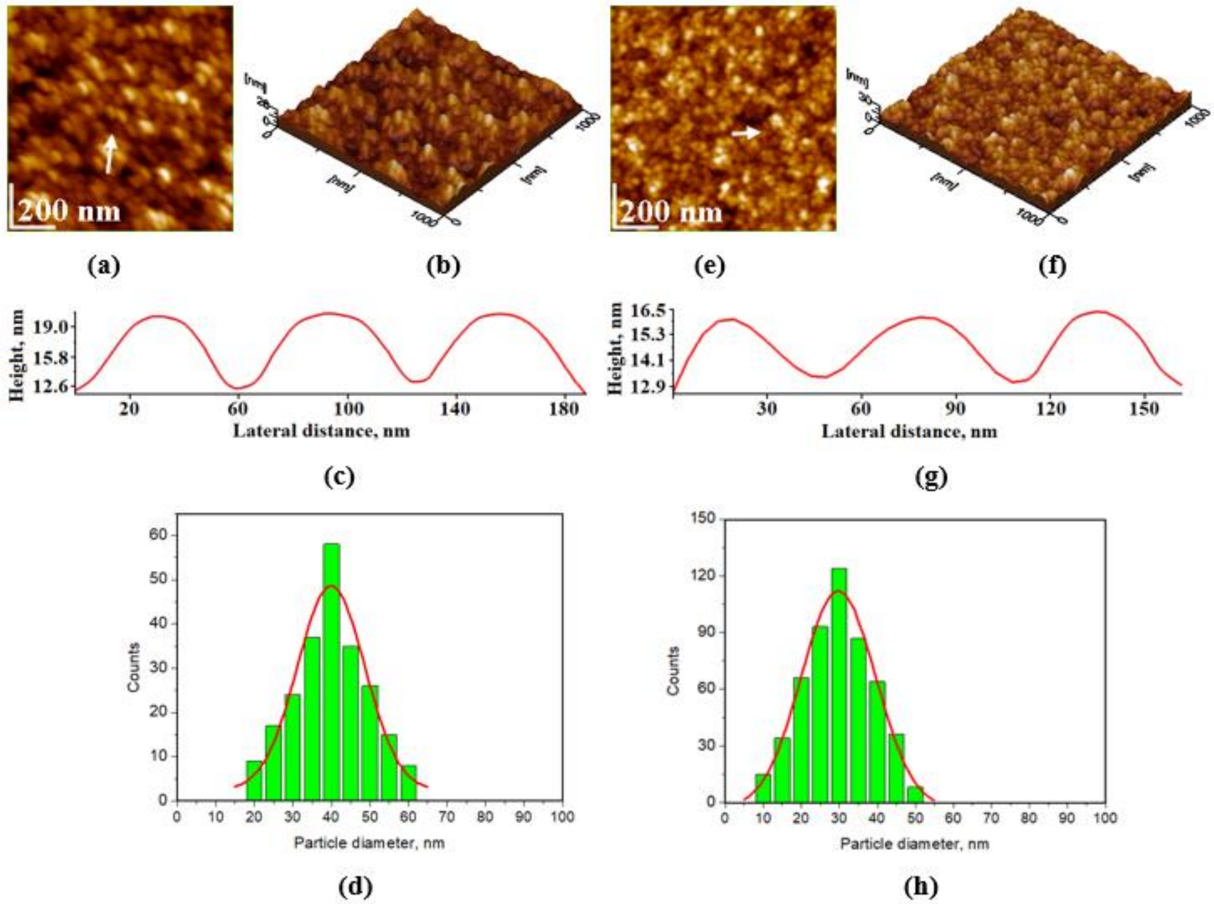


Figure 1. AFM images of the HAP nanoparticles used in the toothpastes: HAP-5%Zn (P1): (a) topographic image, (b) tridimensional image, (c) profile along the arrow in panel (a) and (d) histogram of particle size distribution, RMS=4.0±1.3 nm; simple HAP (P2):(e) topographic image,(f) tridimensional image, (g) profile along the arrow in panel for image (e) and (h) histogram of particle size distribution, RMS=3.4 ± 1.2 nm. Scanned area 1 µm x 1µm.

Figure 2(a-d) exhibits nanoparticles with rounded shapes and diameters of about 40 nm in HAP sample P3 (HAP-0.23%Mg-3.09%Zn-2%Si-10%Sr). Their ability to be adsorbed onto a solid substrate is materialized in a very compact, uniform film. This fact influences thin film roughness, which is slightly higher than in the P2 sample but still lower when compared to P1. Figure 2(e-h) shows a relatively irregular adsorbed thin film of complex hydroxyapatite within sample P4 due to the presence of some larger nanoparticles of about 45 nm that are the central points of a uniform network of hydroxyapatite nanoparticles of about 40 nm that fill up the surroundings. This factor slightly increases the roughness of the thin film.

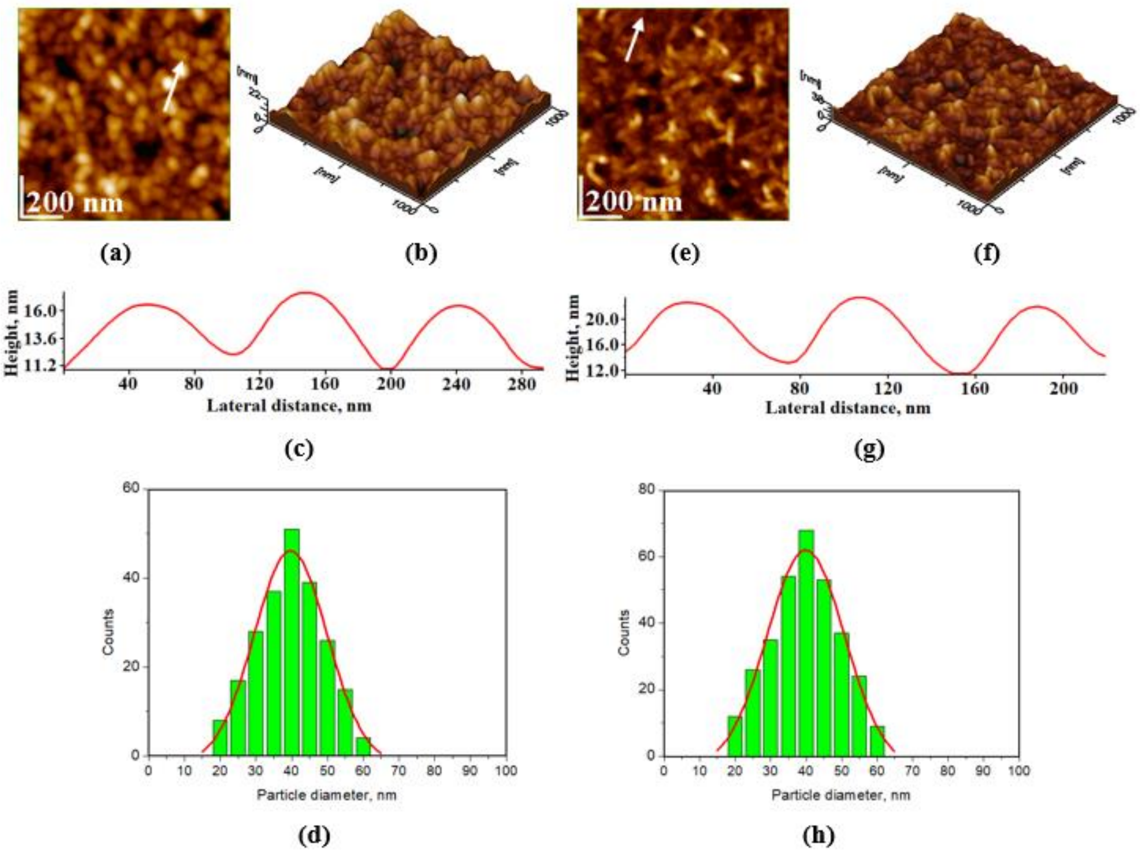


Figure 2. AFM images of the HAP nanoparticles used in the toothpastes: HAP 0.23%Mg-3.09%Zn-2%Si-10%Sr (P3): (a) topographic image, (b) tridimensional image, (c) profile along the arrow in panel (a) and (d) histogram of particle size distribution, RMS=3.4±1.2 nm; HAP-2.5%Mg-2.9%Si-1.34%Zn (P4):(e) topographic image, (f) tridimensional image, (g) profile along the arrow in panel in image (e) and (h) histogram of particle size distribution, RMS=5.2± 2.17 nm. Scanned area 1 µm x 1µm.

Figure 3(a-d) shows the fine microstructure of the healthy enamel (PC - positive control), which is characterized by an excellent cohesion of the mineral material inside as well as between the prisms. The nanoparticles are very-well welded together, leading to a smooth topography of the enamel. Conversely, distinct changes in morphology can be observed with orthophosphoric acid etching. The demineralized enamel (NC - negative control) shows a profound disorganization of the surface after demineralization which can be observed by strongly individualizing the adjacent areas between two consecutive prisms and by eroding the interior of the prisms (Figure 3(g-k)). This comes with increased surface roughness values from a healthy RMS = 70.6 nm to a RMS = 258.3 nm. The histograms in Figure 3f (PC) and Figure 3l (NC) highlight a severe “increase” in HAP size from an average of 40 nm for normal enamel to around 60-80 nm, with an average of 75 nm for etched enamel.

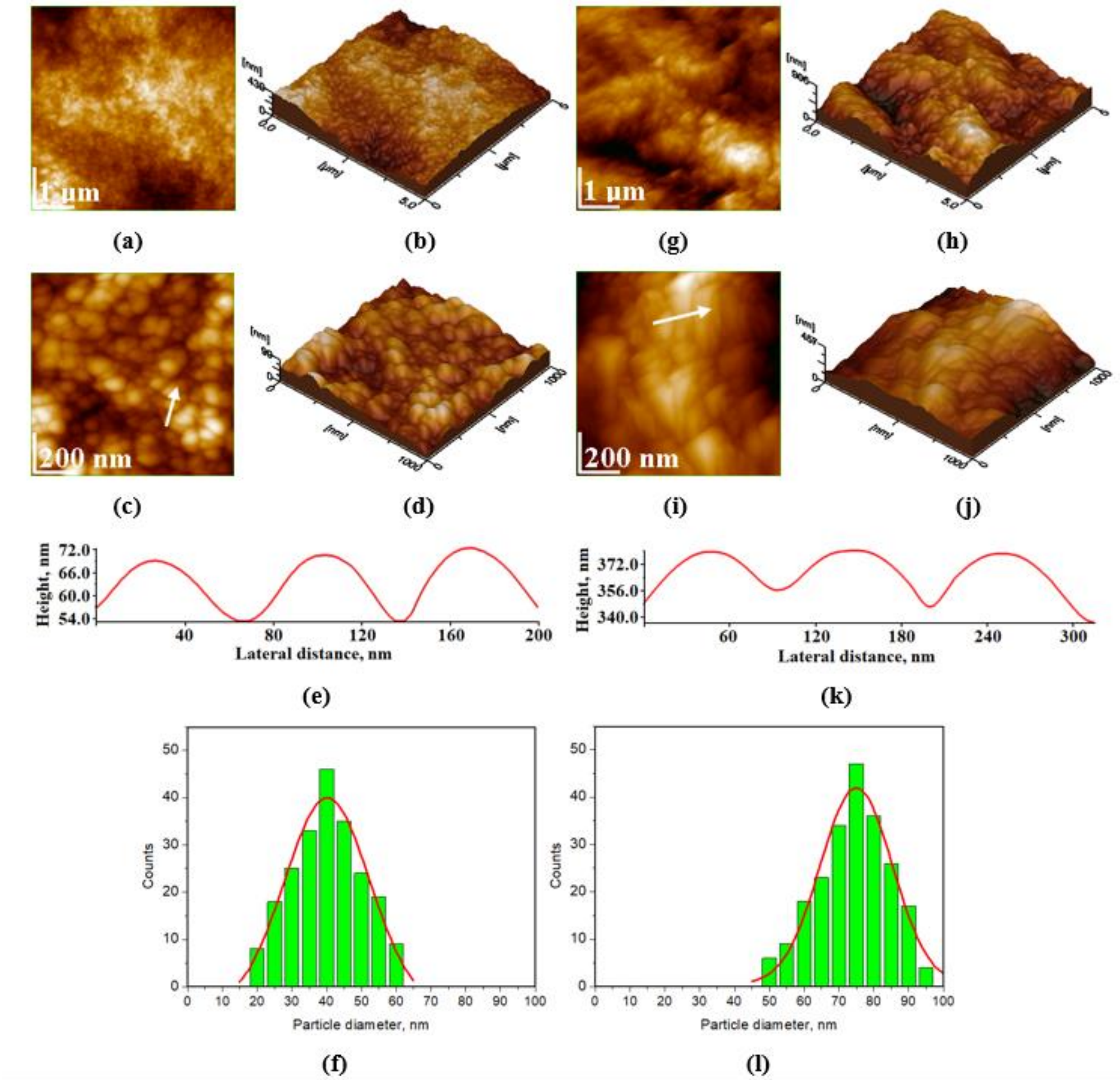


Figure 3. AFM imaging of healthy enamel, positive control (PC): a-f, and, demineralized enamel, negative control (NC): g-l, where a, g are 2D topographical image at scanned area 5 µm x 5 µm; c, i are 2D topographical images at scanned area 1 µm x1 µm; b, h, d, and j are 3D images corresponding to each 2D image; e and k are the profiles along the white arrows in panels c and i; f and l are the histogram of particle size distribution for each sample, PC and NC.

Continuing with the experiment, the remineralization effect of the four toothpaste formulas obtained on artificially demineralized tooth slices was tested. The results showed that all four toothpaste formulas exhibited excellent enamel restoration characteristics. Interestingly, after 10 days of treatment, there was a normalization of the nanostructure of artificially demineralized human enamel, with RMS values almost comparable to those of healthy natural enamel. A better coverage of enamel surfaces can be observed by extending the treatment duration, followed by a consolidation of HAP nanoparticles produced on the enamel surface.

At the end of the 30-day treatment period, all four experimental toothpaste formulas led to complete remineralization of artificially demineralized enamel. This is a key aspect to consider for future studies.

To compare the root mean square (RMS) (nm) data of the remineralization process induced by toothpastes treatments, statistical analysis with One–way ANOVA was applied with an assessment of differences among demineralized enamel (NC), natural enamel (positive control PC) as well as between P1-P4 treated samples. The statistics are pr esented in Figures 6A and 6B. In Figure 6B, where the data were grouped according to time of AFM measurements, it is better observed that the remineralization process induced by the toothpastes is more intense for P2 at all time intervals, P2 reaching the values of the control enamel, followed closely by P3.

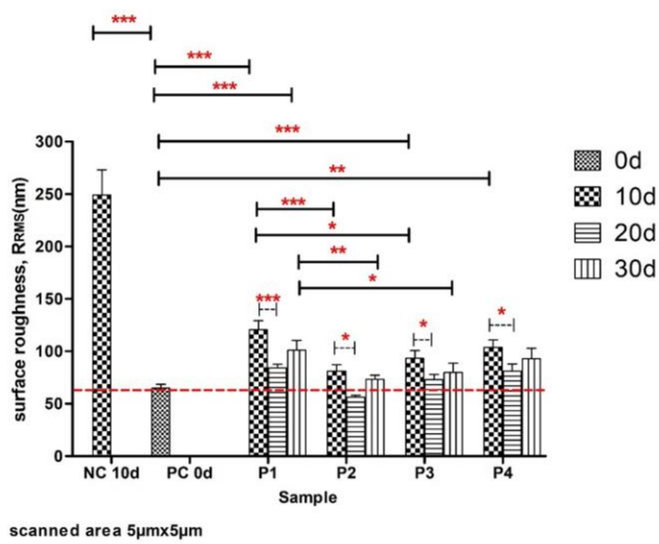


Figure 6A. Graphical representation according to the samples of the RMS values obtained at AFM (scan area 5µmx5µm) after 10, 20 and 30 days of treatments of the demineralized surfaces with the tested toothpastes P1, P2, P3 and P4. The statistical analysis compared the treated samples toothpastes P1, P2, P3 and P4 obtained data with the positive control and respectively the negative control, by using One-Way ANOVA analysis followed by post-test Bonferroni's Multiple Comparison Test. In graphs are illustrated the statistical significance level as follows: p <0.001 *** (extremely significant), p between 0.001-0.01 **(very significant), p between 0.01-0.05 * (significant)

The morphological factors that, together with surface roughness, can describe the efficacy of the enamel remineralization treatment are the shape and size of the HAP nanoparticles that remineralized the enamel surface, the regularity with which they are deposited on the surface, and the compactness of their deposited layers. AFM has proven to be an effective technique for the investigation of remineralization process of human enamel, both in terms of morphological and structural changes as well as of surface roughness measurements.

CHAPTER 8. ADVANCEMENTS IN BIOMATERIALS: NANO-HYDROXYAPATITE AND BIRCH EXTRACTS IN TOOTHPASTE FORMULATION

This study aims to develop functional toothpastes with combined enamel remineralization and antibacterial effects using nano-hydroxyapatites (nHAP) and birch extract. Eleven toothpastes (noted P1-P11) containing different concentrations of birch extract and constant concentrations of pure nHAP or substituted nHAPs (HAP-5%Zn, HAP-0.23%Mg-3.9%Zn-2%Si-10%Sr, and HAP-2.5%Mg-2.9%Si-1.34%Zn) were designed. To assess their in vitro effectiveness, tests on artificially demineralized tooth slices were conducted, analyzing enamel surface repair through AFM investigation.

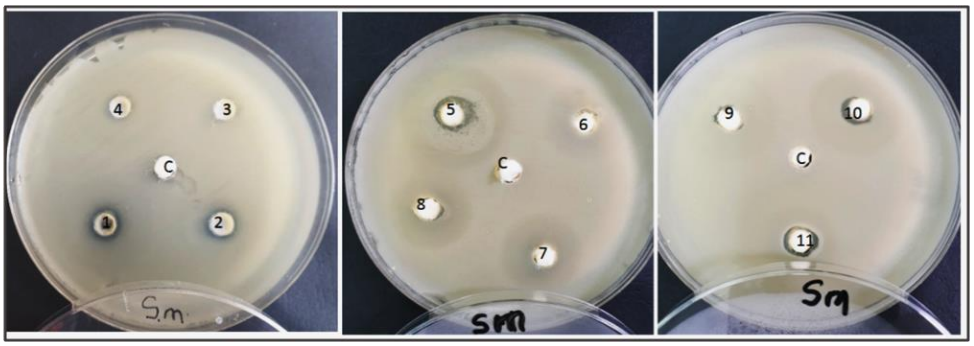


Fig.5. *Streptococcus mutans* inhibition areas after the incubation period

The diameter of the inhibitory area in the Gram-negative bacillus Porphyromonas gingivalis was found to be larger for samples *11,10* and for samples *5* and *6* respectively (Figure 6).

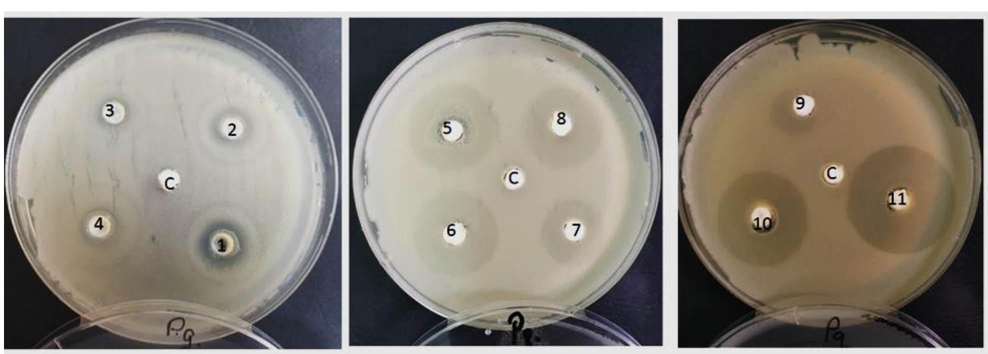


Fig. 6. Inhibition areas of *Porphyromonas gingivalis* after the incubation period

For all samples analyzed, a clear region was detected in the comensal Gram-positive and anaerobic bacteria, Enterococcus faecalis. The diameters varied, with the greatest being recorded in samples 11 and 10 (Figure 7).

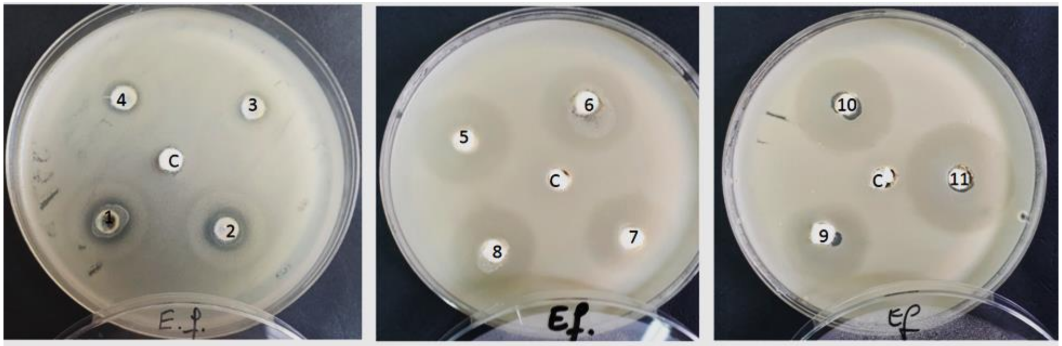


Fig. 7. Inhibition areas of *Enterococcus faecalis* after the incubation period

In the Gram-negative, anaerobic strain, Escherichia coli, inhibition areas were also observed for all tested samples. At this strain the highest inhibition diameter was obtained for sample 11 and 10. The other samples recorded smaller inhibition areas (Figure 8).

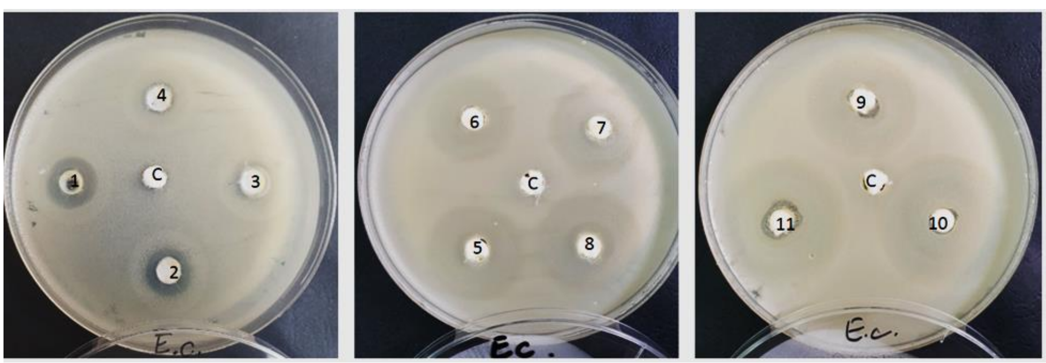


Fig. 8. Inhibition areas for *Escherichia coli* after the incubation period

The existence of sensitivity in the Gram-positive bacterium, Staphylococcus aureus, can be shown for variants P10, P11, P9 and P7. There was no distinct region around the dive in experimental variants P2-P4. (Figure 9).

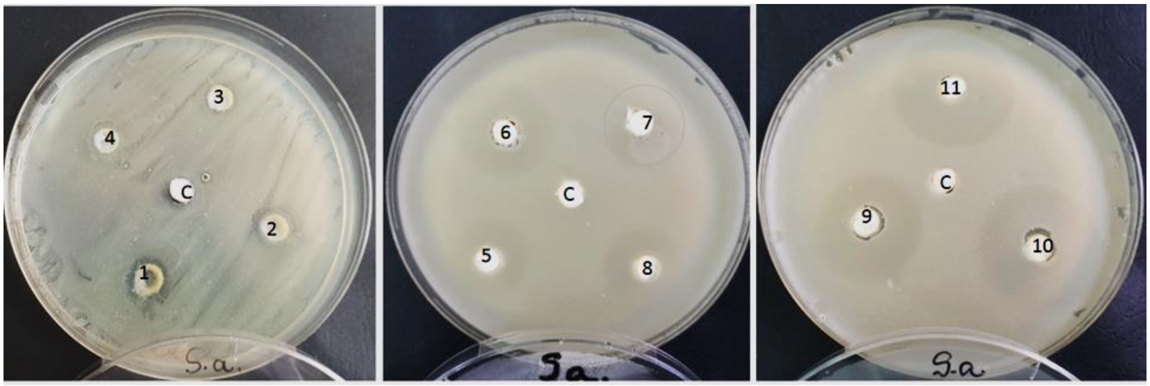


Fig. 9. *Staphylococcus aureus* inhibition areas after the incubation period

Table 3. Inhibition of bacterial growth induced by toothpaste

	Diametrul zonei de inhibiție (mm)				
Eșantion	Streptococcus mutans ATCC 25175	Porphyromonas gingivalis ATCC 33277	Enterococcus faecalis ATCC 29212	Escherichia coli ATCC 25922	Staphylococcus aureus ATCC 25923
P1	8,3±0,6	13,3±1,5	11,3±0,6	15,0±0	9,0±0
P2	8,3±1,2	12,0±0	11,3±1,2	12,3±1,2	-
P3	- *	9,3±0,6	9,3±0,6	10,0±0	-
P4	-	-	9,0±0	8,3±0,57	-
P5	21,6±1,2	27,0±0	24,3±0,6	20,3±0,57	10,3±0,57
P6	16,3±0,6	26,6±1,2	25,0±0	20,3±1,15	20,0±0
P7	16,0±0	17,0±0	15,3±1,2	20,0±0	22,3±1,2
P8	18,3±1,5	25,3±0,6	18,3±1,5	25,3±1,2	16,0±0
P9	22,0±0	15,3±1,2	25,0±0	25,0±0	21,3±0,57
P10	20.3±0,57	28,0±0	29,3±0,6	29,0±0	25,3±1,52
P11	-	32,3±0,6	30,0±0	29,3±0,57	30,0±0

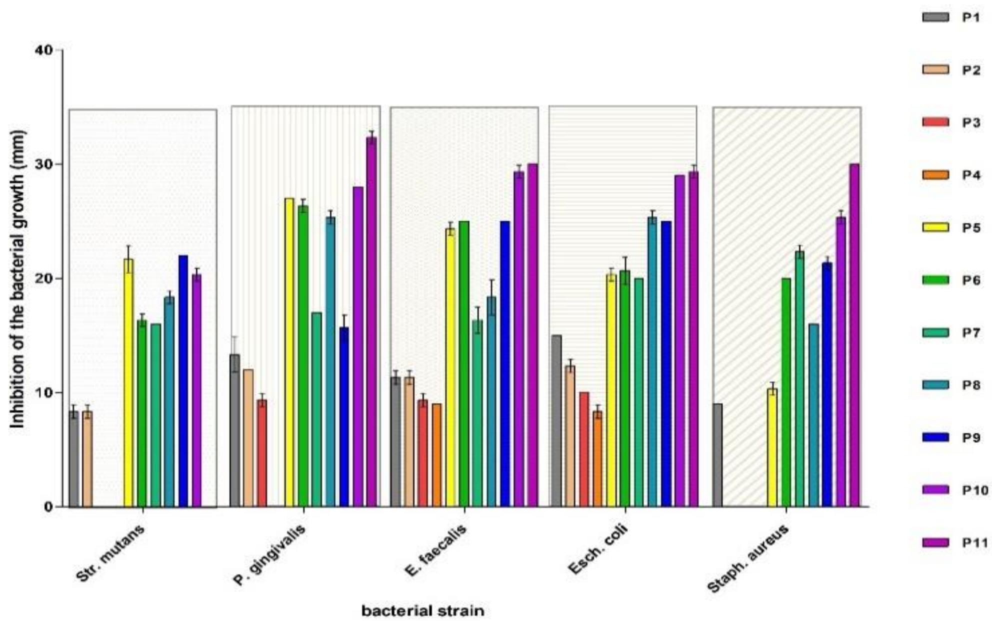


Figura 10. Inhibition of bacterial strain under the action of toothpaste treatment

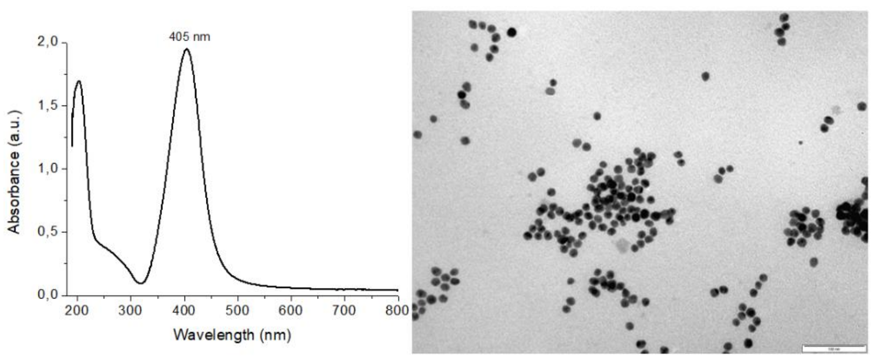
Statistical analysis of surface roughness reveals significant changes between the experimental variations, demonstrating that toothpaste treatments are effective in enamel remineralization. The ranking order of effectiveness in this process is: PC = P2 = P3 > P4 > P1, reflecting the ability of small-sized HAP nanoparticles to penetrate enamel pores and repair microstructural damage. The substituted ions released from the modified HAP structure also contribute to enamel restoration, increasing its strength.

Regarding antimicrobial action, the experiments reveal how different bacterial strains react to the different toothpaste compositions. Even toothpastes containing only nHAP exhibit antibacterial activity, which is enhanced by the addition of zinc as a substitution element in nHAP. Comparing the different groups of toothpaste variants, significant variations in the inhibition of bacterial growth are attributed to the presence of secondary metabolites in birch extract. Among the tested bacterial species, Enterococcus faecalis ATCC 29212 and *Escherichia coli* ATCC 25922 showed the highest sensitivity, followed closely by *Porphyromonas gingivalis* ATCC 33277. *Streptococcus mutans* was ranked in the middle of the tested bacterial species. Among the examined species, *Staphylococcus aureus* ATCC 25923 exhibited the lowest growth inhibition under the applied treatment.

When considering both remineralization and antibacterial efficacy, toothpaste P5 stands out as a compelling option due to its moderate antibacterial activity and good remineralization potential.

CHAPTER 9. NANOCOMPOSITE BASED ON HYDROXYAPATITE AND SILVER WITH ANTIBACTERIAL ACTIVITY

A nanocomposite made from hydroxyapatite (HAP) and silver nanoparticles (AgNPs), namely HAP-4.5 wt% AgNPs, is reported. HAP was prepared using a wet precipitation technique, and AgNPs were made by reducing silver nitrate with glucose in basic medium. HAP, AgNPs and HAP-4.5 wt% AgNPs composite were characterized by X-ray diffraction (XRD), SEM - energy dispersive X-ray spectroscopy (EDS) and different imagistic methods: TEM and AFM. The antibacterial effect of the HAP-4.5 wt% AgNPs nanocomposite was tested using diffusion technique in nutritive agar on two pathogenic species, one Gram-negative (Salmonella typhimurium) and one Gram-positive (Bacillus cereus) and promising results were obtained. This hydroxyapatite-silver nanocomposite can be employed as a potential antimicrobial coating for dental and orthopedic implants, or they can be utilized such as bone cements in clinical procedures.



A B

Fig. 1. UV-Vis spectrum for AgNPs aqueous dispersion (A) and TEM image for AgNPs (B); the scale bar is 100 nm. The average diameter of AgNPs is around 13 nm. The zeta potential value for AgNPs is negative, -39 mV, ensuring a high electrostatic stability of the AgNPs dispersion.

The UV-Vis spectrum of the dispersion containing AgNPs presents the specific SPR absorption band of Ag, with the maximum at 405 nm (Fig. 1A). The spectrum did not change in time for 1 year, thus evidencing the high stability of the AgNPs dispersion. TEM image for AgNPs is depicted in Fig. 1. The mean diameter of the AgNPs was found to be 13.0 ± 2 nm.

The presence of crystalline silver is evident in Figure 2 for HAP-4.5% Ag, where lines corresponding to silver from PDF 89-3722 are observed. Their position is in good agreement with that found for nanosilver particles [56] for the (111), (200), (220), (311) and (222) planes of Ag, corresponding to the peaks at 2θ values of 38.32, 44.50, 64.61, 77.54 and 81.68° respectively. In Figure 2, the most intense diffraction of silver [(111) plane] in HAP-4.5 wt% Ag appears distinctly, while the other Ag diffraction lines are partially superposed on the diffraction peaks of HAP.

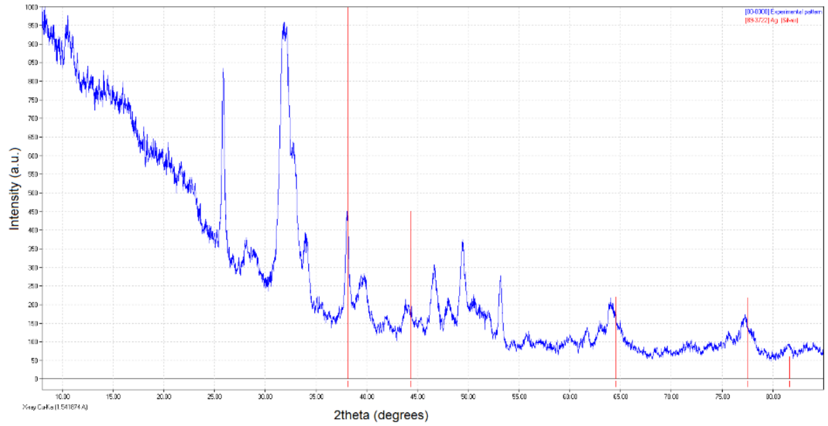


Fig. 2. XRD pattern for HAP-4.5 wt% Ag compared with PDF 89-3722 for Ag (vertical red lines)

The AFM images for the HAP-4.5% Ag nanocomposite on a glass plate obtained by adsorption are presented in Figure 4. As shown in the AFM images (A, B, and C) and the cross-sectional profile (D), the average particle size in this nanocomposite is 54 nm ± 8 nm.

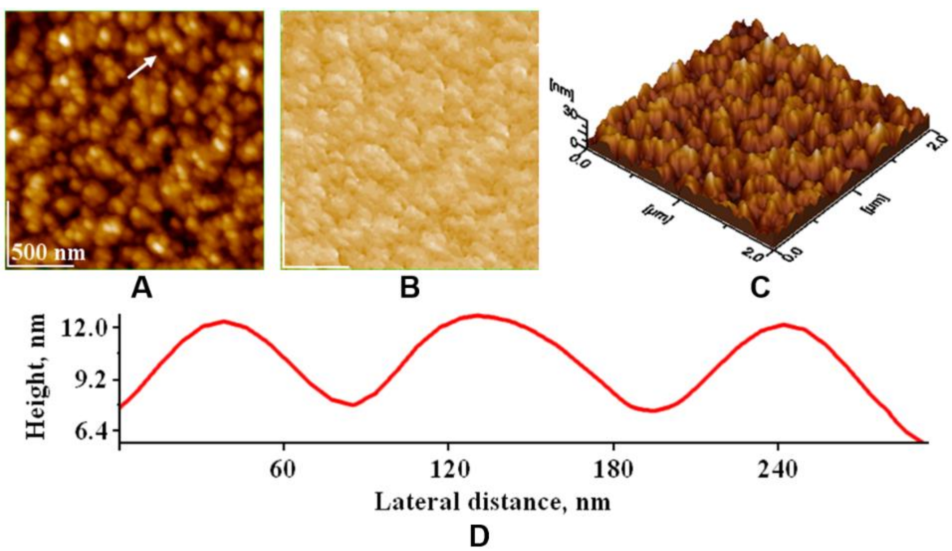


Fig. 4. AFM images of HAP-4.5 wt% AgNPs adsorbed on glass plate: A) topographic image, B) amplitude image, C) 3D image, and D) profile on the white arrow in panel (A). Scanned area 2 µm x 2 µm. Maximum height 30 nm, surface roughness evaluated as root mean square, Rrms = 4.8 nm. Average particle diameter is determined from many profiles (at least 10) as 54 nm ± 8nm.

In conclusion, a straightforward technique for synthesizing HAP-AgNPs with a silver content of 4.5 wt% was effectively developed. Using the XRD, SEM-EDS, TEM, and AFM, the morphological and compositional characteristics of the as-prepared nanocomposite were verified. TEM, SEM, and AFM images showed the dimension and form of HAP particles, both with and without AgNPs, and XRD and EDS measurements confirmed the presence of silver.

Equally Gram-negative and Gram-positive isolates were effectively killed by the HAP-4.5wt%AgNPs that was under investigation. These findings imply that our composite could be regarded as antibacterial materials and that they could be utilized in dentistry and orthopedic implants.

CHAPTER 10. GENERAL CONCLUSIONS

1. The research activity was based on numerous working techniques such as X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) - energy-dispersive X-ray spectroscopy (EDS). The chemical structure was verified through scanning electron microscopy with energy-dispersive X-ray analysis (SEM-EDX). Details regarding the shape of these biocompatible materials were highlighted through imaging techniques, such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM). The release process of elements in water was monitored using inductively coupled plasma optical emission spectrometry (ICP-OES). Antibacterial activity was evaluated using the agar disc diffusion test, a standardized method for antimicrobial susceptibility that involves measuring the diameters of the inhibition zones.

2. Based on the latest information regarding innovative technologies applied in the biomedical field, it can be concluded that oral care products still require in-depth studies to enhance their effectiveness. Research behind these new products are lacking adequate comparisons and are rarely confirmed by controlled clinical studies. Although there is a wide variety of research methods, data sources, and analytical tools available, only a few comparative analyses have been conducted between different products, and many of the results are controversial. The main issues in this field are related to the insufficiency or complete lack of rigorous evidence for many of the newly developed products, as well as the fact that access to such new products is financially prohibitive for a large majority of the population.

3. In the experiments conducted on the release of components into aqueous solutions in the structure of hydroxyapatite (HAP), substitution elements with significant biological effects were introduced, according to physico-chemical research. A higher concentration of strontium in the HAP structure affected its release in both water and simulated body fluid (SBF). Additionally, a tendency to form new biomimetic hydroxyapatite in SBF was observed.

4. In the experiment involving the production of toothpaste containing stoichiometric synthetic hydroxyapatite, it was observed that multi-substituted HAP is the most efficient in toothpaste composition, leading to stable morphological changes on the enamel surface. In the ten days of treatment, demineralized enamel lesions were completely remineralized, as shown by AFM investigations. These toothpastes can be used as potential agents for enamel surface remineralization. Synthetic ceramic nanoparticles were regularly arranged in a smooth protective layer on the enamel surface, ensuring reduced surface roughness, close to that of natural enamel, providing additional evidence of the effectiveness of the toothpaste.

5. In the experiment regarding forsterite synthesis, it can be concluded that the choice of synthesis route can influence the characteristics of the final powder - the shape and size of nanoparticles, distribution, and agglomeration tendency, which, in turn, affect the microstructural homogeneity of a ceramic material and thus limit potential applications. Forsterite was successfully synthesized through the sol-gel method (FSsg) and precipitation method (FSpp),

leading to smaller nanoparticles (30 nm) in the precipitation method compared to nanoparticles (40 nm) obtained through the sol-gel method.

6. In the experiment involving the production and testing of biomimetic toothpaste with a low concentration (3.7%) of hydroxyapatite, it was highlighted that toothpaste P3, containing tetra-substituted hydroxyapatite (HAP-Mg-Zn-Sr-Si), showed the best results in terms of human enamel remineralization compared to the other toothpastes (P1 and P4), with roughly the same remineralization efficiency as toothpaste P2 after 10 days of treatment. Nanoscale particles (with an average size of about 30 nm for HAP) demonstrated significant potential in the enamel remineralization process by covering regions with enamel lesions. All results from treating human enamel for 10 days with each of the toothpastes P1-P4 suggest that these toothpastes can be successfully used to treat incipient dental caries and artificially demineralized enamel.

7. In a subsequent stage of the research, the remineralization effect was tested over a 30-day period, and it was observed that after 10 days of treatment, there was a normalization of the nanostructure of artificially demineralized human enamel, with RMS values almost comparable to those of healthy natural enamel. Better coverage of enamel surfaces can be observed by extending the treatment duration, followed by consolidation of the HAP nanoparticles produced on the enamel surface. The results showed that all four toothpaste formulations had excellent enamel restoration characteristics. Interestingly, at the end of the 30-day treatment period, all four experimental toothpastes led to complete remineralization of artificially demineralized enamel. Morphological factors that, along with surface roughness, can describe the effectiveness of enamel remineralization treatment include the shape and size of HAP nanoparticles that remineralized the enamel surface, the regularity of their deposition on the surface, and the compaction of the deposited layers.

8. AFM has proven to be an efficient technique for investigating the human enamel remineralization process, both in terms of morphological and structural changes and surface roughness measurements.

9. The surface morphology of the studied samples reveals the homogeneous dispersion of globular HAP nanoparticles on enamel surfaces, with varying sizes for different treatments. Statistical analysis of surface roughness indicates significant changes between experimental variations, demonstrating the effectiveness of toothpaste treatments in enamel remineralization. The ranking order of effectiveness in this process is: PC = P2 = P3 > P4 > P1, reflecting the ability of small-sized HAP nanoparticles to penetrate enamel pores and repair microstructural damage. The substituted ions released from the modified HAP structure also contribute to enamel restoration, increasing its resistance.

10. Regarding the antimicrobial action, experiments reveal how different bacterial strains react to different toothpaste compositions. Significant variations in bacterial growth inhibition are noted, attributed to the presence of secondary metabolites in the birch extract. Among the examined bacterial species*, Enterococcus faecalis ATCC 29212 and Escherichia coli ATCC 25922* exhibited the highest sensitivity, followed closely by *Porphyromonas gingivalis* ATCC 33277. *Streptococcus mutans* was positioned in the middle of the tested bacterial species. Among the

examined species, *Staphylococcus aureus* ATCC 25923 showed the lowest growth inhibition under the applied treatment.

11. An optimal toothpaste composition was selected to simultaneously achieve remineralization and antibacterial action, containing zinc-substituted HAP and 1.3% birch extract.

12. In the final stage, an efficient technique was developed to synthesize the HAP-AgNPs nanocomposite with a 4.5% silver content, demonstrating antibacterial effects against Bacillus cereus and Salmonella typhimurium strains. These findings suggest that our composite could be considered as an antibacterial material and could be used in dentistry and orthopedic implants.

CHAPTER 11. SCIENTIFIC RESEARCH ACTIVITY

LIST OF PUBLISHED ARTICLES

Six ISI articles have been published in specialized scientific journals; four works have been published in BDI journals, and one book chapter, with a cumulative impact factor of 5.835.
Total Citations 12, h-index 2; Google Scholar: https://scholar.google.com/citations?user=cx7f380AAAAJ&hl=en&oi=ao

List of Published ISI Articles:

1. Cadar, O., Balint, R., Tomoaia, G., **Florea, D.,** Petean, I., Mocanu, A., Horovitz, O. and Tomoaia-Cotisel, M. Behaviour of multisubstituted hydroxyapatites in water and simulated body fluid. Stud. Univ. Babes-Bolyai, Chem., 62, 269-281, 2017. **I.F. 0.305**

2. **D. A. Florea**, C. Dobrota, R. Carpa, S. Riga, M. Tomoaia-Cotisel. Current status and trends in oral health care technologies. a perspective review. International Journal of Medical Dentistry, 26(1), 38-50, 2022. **I.F. 0.887**

3. A. Avram**, D. Florea**, F. Goga, M. Gorea, A. Mocanu, Gh. Tomoaia, I. Petean, A.-Z. Kun, O. Horovitz, M. Tomoaia-Cotisel. Mechanism in the synthesis of fosterite nanoparticles based on thermodynamic approach. Stud. Univ. Babes-Bolyai, Chem, 68(2), 37-51. 2023. **I.F. 0.3**

4. **D.A. Florea**, A. Mocanu, L.C. Pop, Gh. Tomoaia, C.-T. Dobrota, C. Varhelyi Jr, M. Tomoaia-Cotisel. Remineralization of tooth enamel with hydroxyapatite nanoparticles: an in vitro study. Stud. Univ. Babes-Bolyai, Chem., 68(2), 99-113, 2023 **I.F. 0.3**

5. A. Mocanu, **D. A. Florea**, Gh. Tomoaia, L.-C. Pop, A. Danistean, S. Rapuntean, O. Horovitz, M. Tomoaia-Cotisel. Nanocomposite based on hydroxyapatite and silver with antibacterial activity. Stud. Univ. Babes-Bolyai, Chem. 68(3),7-18, 2023, in press **I.F. 0.3**

6. **A.-D. Florea**, L. C. Pop, H.-R.-C. Benea, Gh. Tomoaia, C.-P. Racz, A. Mocanu, C.-T. Dobrota, R. Balint, O. Soritau, M. Tomoaia-Cotisel. Remineralization induced by biomimetic hydroxyapatite toothpastes on human enamel. Under review Biomimetics, Manuscript ID:2590006, in press **I.F 3.743 Q1**

List of Published Articles in BDI Journals:

7. A. Avram, A. Mocanu, Gh. Tomoaia, C.L. Rosoiu, C.T. Dobrota, D.A. **Florea, M.** Tomoaia-Cotisel, "Unveiling the Complexity of Red Blood Cells: Insights into Structure, Properties and Functions", Academy of Romanian Scientists Annals Series on Biological Sciences, 12(1), 129-154, 2023. https://doi.org/10.56082/annalsarscibio.2023.1.129

8. G.-A. Paltinean, **D. A. Florea**, Gh. Tomoaia, S. Riga, S. Rosoiu, A. Mocanu, M. Tomoaia-Cotisel, 'Perspectives of oral dental care'', Academy of Romanian Scientists Annals Series on Biological Sciences, 11(2), 140-159, 2022.

9. R. Balint, G. A. Paltinean**, D. A. Florea**, Gh. Tomoaia, A. Mocanu, M. Tomoaia-Cotisel. ''Biomimetic and antibacterial composite for orthopedic implants'', Academy of Romanian Scientists Annals Series on Biological Sciences, 11(1), 120-145, 2022. https://doi.org/10.56082/annalsarscibio.2022.1.120

BOOK CHAPTERES

10. D. A. Florea, M. Tomoaia-Cotisel and C. T. Dobrota, "Use of Betula species extracts in therapeutic and preventive oral health care", Chapter 5, in Betula: Ecology and uses, Ed. Carl T. Bertelsen, Nova Science Publishers, Inc., New York, USA, pp. 137-162 (2020) ISBN: 978-1-53617-802-9.

List of Conference Presentations

The dissemination of scientific results from the Doctoral Thesis was achieved through oral presentations at the national conferences organized by the Academy of Scientists of Romania [1-11].

1. D.A. Florea, C.T. Dobrota, R. Carpa, A. Mocanu, M. Tomoaia-Cotisel, RO: '' Tendințe în tehnologiile de îngrijire orală'' / ENG: '' Trends in oral care technologies'', Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Ştiințifică de Toamnă „Tradiții și progrese în știința românească", 18-19 noiembrie 2021, sectiunea Biologie, on-line platforma Zoom, Volum de rezumate, pag. 58 (2021)

2. D.A. Florea, C.T. Dobrota, S. Riga, G. Tomoaia, A. Mocanu, M. Tomoaia-Cotisel, RO: '' Nanomateriale avansate: tehnologie inovatoare utilizată în cercetarea stomatologică'' / ENG:" Advanced Nanomaterials: Innovative Technology Used in Dentistry Research'', Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Ştiințifică de Toamnă „Tradiții și progrese în știința românească", 18-19 noiembrie 2021,sectiunea Medicina, on-line platforma Zoom, Volum de rezumate, pag. 77-78 (2021)

3. C.T. Dobrota, **D.A. Florea**, R. Carpa, Gh. Tomoaia, A. Mocanu, M. Tomoaia-Cotisel, „Therapeutic Formulations containing plant secondary metabolites used to increase the efficiency of antibiotics", Virtual International Scientific Conference on "Applications of Chemistry in Nanosciences and Biomaterials Engineering" NanoBioMat 2022 – Summer Edition, 22-24 June 2022. Oral presentation on Session II, Natural Bioactive Compounds, on line platforma TEAMS (2022).

4. D.A. Florea, C.T. Dobrota, Gh. Tomoaia, A. Mocanu, A. Avram, C.-P. Racz, L.Z. Racz, S. Riga, M. Tomoaia-Cotisel, RO: "Perspective in degradarea smalțului dentar prin microscopie cu forță atomică" / ENG: "Insights into Tooth Enamel Degradation through Atomic Force Microscopy", Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Științifică Națională de Primăvară „ Transformarea digitală în științe", 19-20 mai 2023, sectiunea Biologie, on-line platforma Zoom, Volum de rezumate, pag. 92-93 (2023).

5. D.A Florea, C.T. Dobrota, Gh. Tomoaia, A. Avram, A. Mocanu, A. Mocanu, O. Horovitz, T. Mocan, M. Tomoaia-Cotisel, RO: "Explorarea AFM a legăturilor interfaciale a prismelor de hidroxiapatită și a unităților proteice pe smalțul dentar uman " / ENG: "The Interfacial Bonding of Hidroxyapatite Prisms and Protein Units on Human

6.**D.A Florea**, C.T. Dobrota, Gh. Tomoaia, A. Avram, A. Mocanu, A. Mocanu, O. Horovitz, T. Mocan, M. Tomoaia-Cotisel, RO Teeth Enamel, Explored by AFM", Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Științifică Națională de Primăvară „ Transformarea digitală în științe", 19-20 mai 2023, sectiunea Biologie, on-line platforma Zoom, Volum de rezumate, pag. 107-108 (2023).

7. D.A. Florea, Gh. Tomoaia, C.T. Dobrota, A. Mocanu, A. Avram, O. Horovitz, O. Soritau, M. Tomoaia-Cotisel, RO: "Remineralizarea smalțului dentar uman cu paste de dinți care conțin hidroxiapatite și agenți antimicrobieni naturali " / ENG: "Remineralization of Human Teeth Enamel with Toothpastes Comprising Hydroxyapatites and Antimicrobial Natural Agents", Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Științifică Națională de Primăvară „ Transformarea digitală în științe", 19-20 mai 2023, sectiunea Biologie, on-line platforma Zoom, Volum de rezumate, pag. 108-109(2023).

8. D.A. Florea, Gh. Tomoaia, C.T. Dobrota, L.C. Pop, A. Mocanu, O. Horovitz, T. Mocan, A. Avram, M. Tomoaia-Cotisel, RO: "Restaurări ceramice pentru smalțul dentar uman" / ENG: "Ceramic Restoration for Human Teeth Enamel", Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Științifică Națională de Primăvară „ Transformarea digitală în științe", 19-20 mai 2023, sectiunea Biologie, on-line.

9. D.A. Florea, C.T. Dobrota, A. Avram, Gh. Tomoaia, H.-R.-C. Benea, C.L. Rosoiu, A. Mocanu, M. Tomoaia-Cotisel, RO: “Efectul nanohidroxiapatitelor asupra remineralizării in vitro a leziunilor smaltului uman”, ENG: “The effect of nano-hydroxyapatites on in vitro remineralization of human teeth enamel lesions”, Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Științifică Națională de Toamnă “Știința pentru o societate sănătoasă”, 21-23 septembrie 2023, sectiunea Biologie, on-line, platforma Zoom

10. D.A. Florea, C.T. Dobrota, C.L. Rosoiu, Gh. Tomoaia, A. Avram, A. Mocanu, M. Tomoaia-Cotisel, RO: “Proprietățile antibacteriene ale formulărilor de paste de dinți cu un conținut de hidroxiapatită și extract de mesteacăn”, ENG: “The antibacterial properties of toothpaste formulations comprising hydroxyapatite and birch extract”, Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Științifică Națională de Toamnă “Știința pentru o societate sănătoasă”, 21-23 septembrie 2023, sectiunea Biologie, on-line, platforma Zoom.

11. D.A. Florea, Gh. Tomoaia, S. Riga, H.-R.-C. Benea, A. Avram, C.T. Dobrota, T. Mocan, A. Mocanu, M. Tomoaia-Cotisel, RO: “Eficiența diferitelor paste de dinți pe bază pe hidroxiapatite biomimetice în remineralizarea smalțului uman”, ENG: “Human enamel remineralization efficiency of various toothpastes based on biomimetic hydroxyapatites”, Prezentare Orala, Academia Oamenilor de Stiinta din Romania, Conferința Științifică Națională de Toamnă “Știința pentru o societate sănătoasă”, 21-23 septembrie 2023, sectiunea Științe Medicale, on-line, platforma Zoom.

LIST OF SCIENTIFIC RESEARCH PROJECTS

Ph.D. candidate Florea Alexandra-Diana participated in 1 scientific research project conducted at the Center for Scientific Research in Physical Chemistry, under the supervision of Director Prof. Univ. Dr. Maria Tomoaia-Cotisel, Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University, Cluj-Napoca:

Project PN2 Partnership 241/2017: Development of innovative nanomaterials based on advanced nanotechnology with applications in dental prophylaxis and periodontal diseases, InovaMat
Coordinator: Babeș-Bolyai University
Director: Assoc. Prof. Dr. Aurora Mocanu

MEMBER OF THE CENTER FOR SCIENTIFIC RESEARCH IN PHYSICAL CHEMISTRY

Ph.D. candidate Florea Alexandra-Diana has been a member of the Center for Scientific Research in Physical Chemistry (CECHIF) since 2016. She has benefited from the strategic equipment and facilities in CECHIF laboratories, as well as the highly professional guidance of Prof. Maria Tomoaia-Cotisel, her doctoral supervisor.

Prof. Univ. Dr. M. Tomoaia-Cotişel is the Founder (2006) and Director (2006-present) of the Center for Scientific Research in Physical Chemistry (CECHIF-STAR-UBB) at the Faculty of Chemistry and Chemical Engineering, Babeș-Bolyai University in Cluj-Napoca.

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