NATIONAL UNIVERSITY OF SCIENCE AND TECHNOLOGY POLITEHNICA BUCHAREST

Faculty of Chemical Engineering and Biotechnologies

PhD Domain: Chemical Engineering

- PhD Thesis Summary -

Synthesis and characterization of biomaterials generated by plasma techniques

PhD Student:

MSc. Eng. Maria-Elena ZARIF

PhD Supervisor:

Prof. Dr. Eng. Ecaterina ANDRONESCU

Bucharest

2025

Table of contents

Keywords: calcium phosphates; atmospheric pressure plasma; dentistry; enamel fluoridation; biofilm modulation; radio-frequency magnetron sputtering; matrix-assisted pulsed laser evaporation; chitosan; implant coatings

The PhD thesis was structured in two parts. The **first part**, "*Doctoral theme, methods, and concepts*", describes aspects related to calcium phosphates (CaPs), atmospheric pressure plasma (APP) treatments in dentistry, and two thin film deposition techniques, namely radio-frequency magnetron sputtering (RF-MS) and matrix-assisted pulsed laser evaporation (MAPLE). Therefore, the first part of this thesis aims to offer an outline of the current development in these fields.

Chapter 1, "*Calcium phosphates*"

Chapter 1 begins with the presentation of the physicochemical and biological properties of CaPs. Each CaP was treated separately in terms of Ca/P ratio, density, pH stability, solubility, and crystal system. A more detailed discussion was presented for the hydroxyapatite (HAp) structure (Figure 1) and the substitution of CaPs.

The HAp unit cell (hexagonal structure, space group $P6_3/m$) has [1–3]:

- 2 OH^- ions;
- 6 PO_4^{3-} ions;
- 10 Ca^{2+} ions distributed in 2 sites, from which:
    - 4 Ca^{2+} ions are:
        - Distributed in the Ca(I) sites (also referred to as Ca1 sites);
        - Arranged in columns;
        - Bonded with 9 oxygen atoms from 6 PO_4^{3-} groups (6 strong and 3 weaker bonds);

- 6 Ca^{2+} ions are:
  - Distributed in the Ca(II) sites (also referred to as Ca2 sites);
  - Arranged in a triangular array;
  - Bonded with 7 oxygen atoms form 5 PO_4^{3-} groups and one OH^- group;

To mimic the bone composition, the substitution of CaPs with various ions gained an increased interest. Even if scientific reports have been made on the substitution of several CaPs, the research was focused on the substitution of HAp, which is the main component of bones. The substitution of HAp ions: Ca^{2+}, PO_4^{3-}, and OH^- usually improves its biological properties. They can be divided in cationic, in which calcium is substituted, and anionic substitutions, in which the phosphate or the hydroxyl groups are substituted [1], see Figure 1.

Ions such as Ag^+, Li^+, K^+, Na^+, Mg^{2+}, Sr^{2+}, Zn^{2+}, Fe^{2+}/Fe^{3+}, Cu^{2+}, or Ce^{3+}/Ce^{4+} can replace the Ca^{2+} ions of HAp [2,3]. The hydroxyl groups can be substituted by F^- or Cl^-, while the phosphate group can be substituted by HPO_4^{2-}, SO_4^{2-}, or SeO_3^{2-} [2,3]. The CO_3^{2-} can substitute both PO_4^{3-} and OH^- ions. These substitutions are called B-type and A-type, respectively [2].

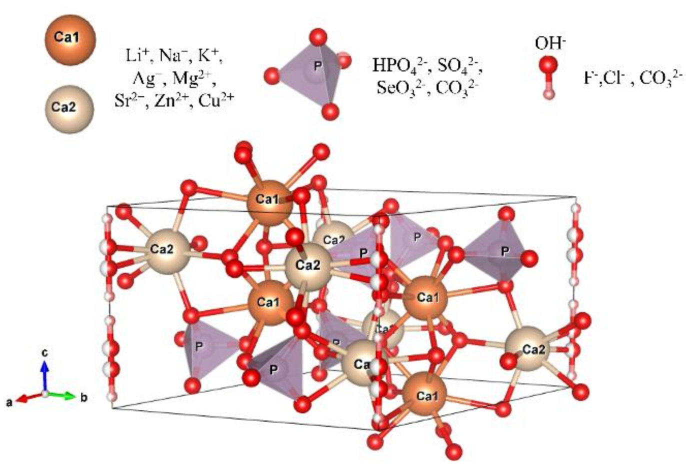

Figure 1. Crystal structure of HAp and ion substitution

The chapter continues with the presentation of the dry (e.g., solid-state and mechanochemical), wet (e.g., chemical precipitation, hydrolysis, hydrothermal, sol-gel, emulsion, and sono-chemical) and high temperature (e.g., combustion and pyrolysis) methods for the synthesis of CaPs. The chapter ends with presenting the characterization techniques selected to investigate the properties of the biomaterials obtained within the PhD thesis (Table 1).

Table 1. Characterization techniques used to investigate the properties of CaPs

Characterization technique	Main investigated properties	Ref.
Electron microscopy		
SEM	• surface morphology; • particle shape and size (e.g., diameter, length); • coating thickness (cross section SEM imaging);	[4]
X-ray Diffraction		
XRD	• crystallinity; • phase identification;	[5]
GIXRD	• crystallinity and phase identification for thin films;	[6,7]
Spectroscopic techniques		
EDX[1]	• elemental composition;	[8]
FTIR	• molecular structure;	[9]
XPS	• chemistry of the surface (elemental composition, chemical and electronic states of the elements);	[10]
Surface topography		
Profilometry	• topographic image of the surface sample; • surface roughness;	[11]
Biological assays		
MTT assay	• cell viability;	[12]
LDH assay	• cell cytotoxicity;	[13]
Antimicrobial assays	• antimicrobial activity;	
Antibiofilm assays	• antibiofilm activity;	

[1]performed using a scanning electron microscope

Chapter 2, "*Atmospheric pressure plasma in dentistry*"

Plasma was discovered in 1879 by the British chemist and physicist Sir William Crookes, while the term "plasma", which derives from the Greek word *plassein*, meaning to form or to mold, was given in 1928 by the American chemist, physicist, and engineer Irving Langmuir. In his studies, he observed that during the passage of an electrical current through a gas, at pressures below the atmospheric pressure, when the discharge was luminescent, it was expanding in the discharge chamber, taking its form [14].

Plasma is considered the fourth state of matter, along with solid, liquid, and gas. If a solid receives enough energy, it will melt into a liquid, which, if it continues to receive energy, will evaporate, reaching the gas state (Figure 2). The energy required for the solid-liquid and liquid-gas transitions is around 10^{-2} eV/particle. However, a higher energy, around 1-30 eV/particle, is necessary for the gas-plasma transition. Therefore, the energy must be higher than the gas ionization energy. Consequently, the gas atoms or molecules are ionized and plasma is formed [14].

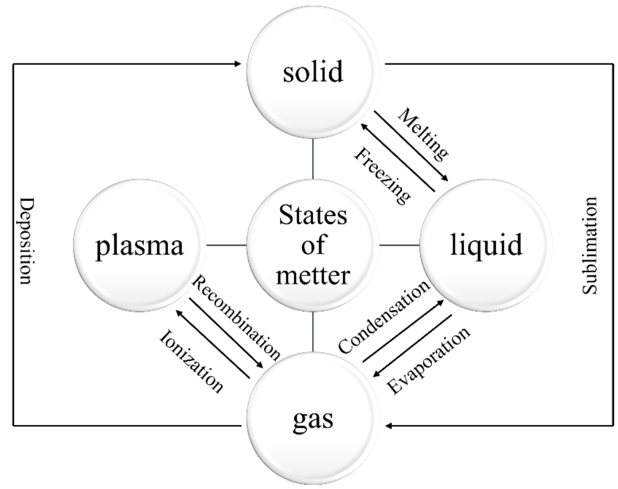

Figure 2. Phase transitions between the four states of matter

Plasma is an ionized gas, consisting of ions (positive or negative), electrons, neutrals (atoms and molecules), and photons, which, at a macroscopic level, is electrically neutral [14].

Plasmas can be divided, based on their temperature, in high temperature and low temperature plasmas. The temperatures of ions (T_i), electrons (T_e), and of the gas molecules (T_g), are similar in the case of high temperature plasmas, meaning that these plasmas are in thermal equilibrium ($T_e \approx T_i \approx T_g$) and their temperature is ranged between 10^6 and 10^8 K. Low temperature plasmas can be divided into thermal, which are quasi-equilibrium plasmas, with $T_e \approx T_i \approx T_g \leq 2 \times 10^4$ K, and non-thermal plasmas, which are non-equilibrium plasmas, with $T_e >> T_i \approx T_g = 300 - 10^3$ K [15,16].

Chapter 2 begins with the classification of the APP discharges into gliding arc, corona, plasma jet, and dielectric barrier discharges, offering a brief description of each one. Among the above mentioned discharges, the last two mentioned are the most studied for the medical field, with clinical and preclinical exploration [17]. The DBD is probably one of the most used methods to generate APPs. It is generated in a gap between two electrodes, from which at least one is covered with a dielectric material, using a high voltage electric current (DC or AC). The dielectric material can be ceramic, glass, quartz, plastic, alumina, or Teflon, and it is used to avoid the formation of an electrical arc, by reducing the current [17–20].

In medicine, a variety of applications for APPs have been proposed. Among them, a few can be reminded, such as wounds treatment, cancer treatment, and dentistry [21]. the applications of APPs in dentistry were divided in two: indirect and direct plasma applications [22,23].

Further, the discussion is focused on presenting results from the scientific literature regarding the direct applications of APP in dentistry, namely dental restorations, endodontic/root canal treatments, tooth whitening, and decontamination – biofilm removal and bacterial sterilization. The chapter ends with a discussion about caries prevention, biofilm modulation, and the role of biofilm in caries development (Figure 3). The few results reported in the scientific literature about the use of APP for this application are described, highlighting the novelty of the first research direction of the thesis.

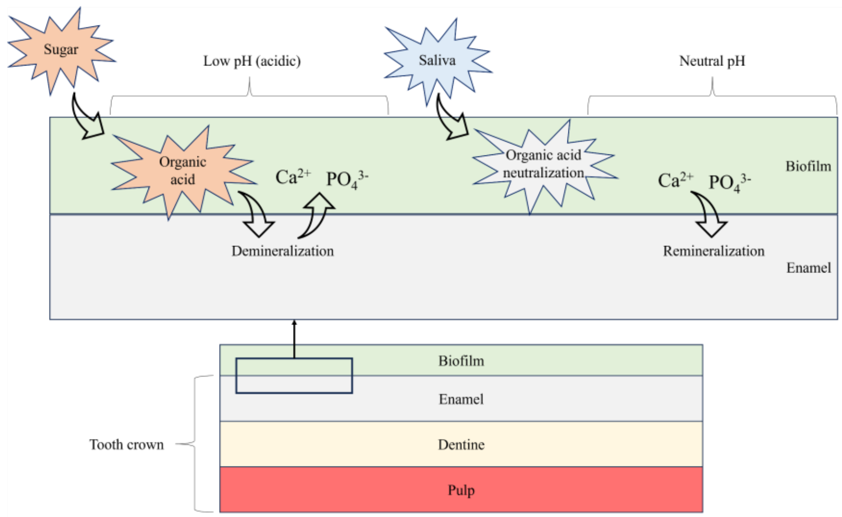

Figure 3. Schematic representation of tooth demineralization and remineralization in the presence or absence of sugars

Chapter 3, "*Thin film deposition techniques*"

Among the thin film deposition techniques, chemical vapor deposition (CVD) and physical vapor deposition (PVD) are the most common. The main difference between CVD and PVD is related to the state of matter of the material to be deposited: gas for CVD and solid for PVD. Therefore, during the deposition, different processes are involved. The CVD deposition involves chemical reactions between the precursor/reactant in the gaseous state and other gases in order to deposit a solid coating. The PVD deposition involves the vaporization or sputtering of a solid material (target), followed by its condensation on the substrate [24–27]. The CVD and the PVD deposition techniques will were briefly described in this chapter, with the main focus on the radio-frequency magnetron sputtering (RF-MS) and matrix-assisted pulsed laser evaporation (MAPLE) techniques.

The second research direction was focused on using two physical vapor deposition techniques for the generation of thin films, RF-MS (Figure 4) and MAPLE (Figure 5). Therefore, this chapter presents the physical principles of these depositions.

Briefly:

- the RF-MS technique:
  - is used for the deposition of dielectric materials to avoid the charge buildup at the surface of the target;
  - involves the sputtering of a target by positive ions, formed during the collisions between electrons and the atoms of the working gas, and the deposition of the sputtered particles on the substrate;

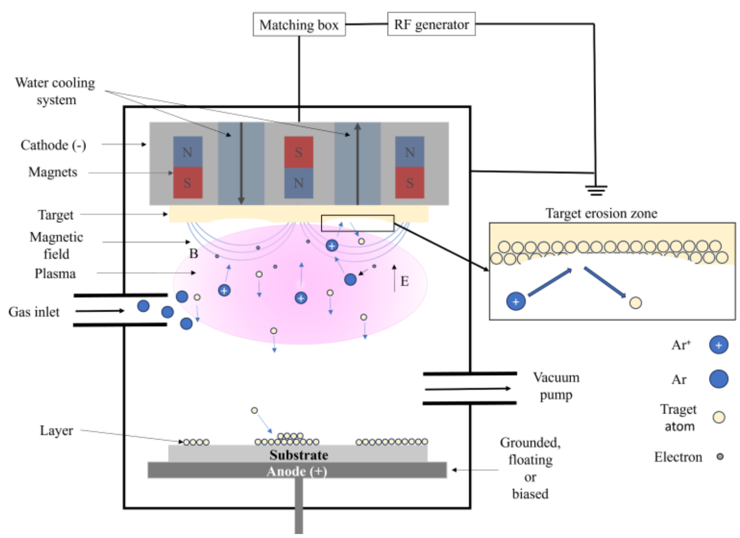

Figure 4. RF-MS set-up and deposition

- the MAPLE technique:
  - is used for the deposition of organic materials to avoid their damage and decomposition;
  - involves the vaporization of a frozen target composed out of a solvent (the matrix) and a solute (the material to be deposited) during a laser interaction and the deposition of the material of interest on the substrate; the solvent is pumped out from the chamber;

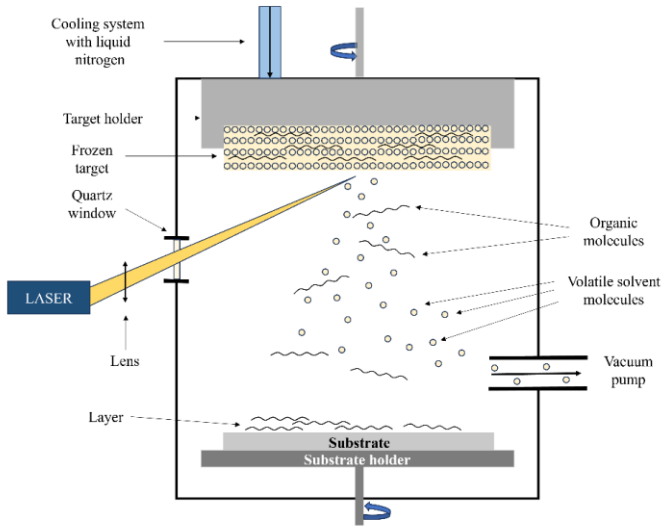

Figure 5. MAPLE set-up and deposition

For a proper selection of the deposition conditions and for the understanding of their influence on the physicochemical and biological properties of the deposited layers, the scientific literature was studied and results about the deposition of CaPs by RF-MS and of polymers by MAPLE were presented within this chapter. The polymer selected for the depositions was chitosan (Figure 6).

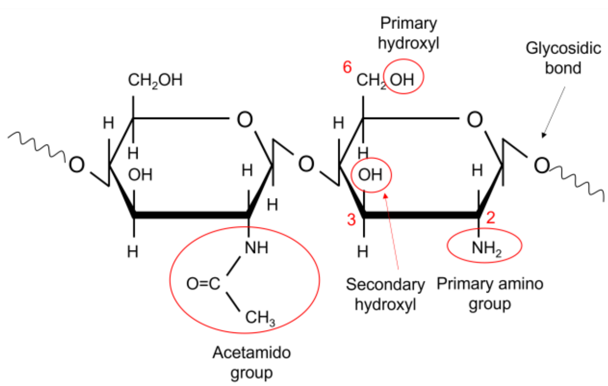

Figure 6. Chitosan chemical structure: N-acetylglucosamine (left) and glucosamine (right) units

CS is a linear cationic polysaccharide derived from chitin through a deacetylation process, chemically or enzymatically. The units of CS are glucosamine and N-acetylglucosamine [28]. The glucosamine units are deacetylated and their proportion defines the deacetylation degree of CS [29]. Considering its molecular mass, CS can be classified into low, medium, and high molecular weight CS, characterized by a mass lower than 100 kDa, between 100 and 1000 kDa, and higher than 1000kDa, respectively [30].

The **second part** of this thesis, "*Research results*", consists in presenting the research aim, goals, and objectives, the scientific results obtained during the PhD studies, the original contributions and conclusions, the future perspectives, and the list of publications.

Two research directions were developed during the PhD studies. The first one refers to the use of APP treatments in dentistry, while the second one to the use of another plasma technique, RF-MS, for bone tissue engineering applications.

Chapter 4, "*Research objectives*"

The aim, goals, and objectives of this thesis are presented in Table 2.

Table 2. Aim, goals, and objectives of the PhD thesis

<table>
<tr><th colspan="2">Aim</th></tr>
<tr><td colspan="2">The use of plasma techniques for biomedical applications, namely for dentistry and bone tissue engineering</td></tr>
<tr><th>Goal 1</th><th>Goal 2</th></tr>
<tr><td>Improvement of fluoride treatments efficiency used in dentistry by atmospheric pressure plasma treatments</td><td>Tailoring the physicochemical and biological properties of calcium phosphate/chitosan composite coatings for bone tissue engineering applications using radio-frequency magnetron sputtering and matrix-assisted pulsed laser evaporation techniques</td></tr>
<tr><th>Objective 1.1.</th><th>Objective 2.1.</th></tr>
<tr><td rowspan="3">Improvement of fluoride retention in an enamel-like model from a commercially available fluoride-containing compound by atmospheric pressure plasma activation</td><td>Identification of the physicochemical modifications of the coatings in relation to the deposition parameters during the RF-MS discharge at high rf power</td></tr>
<tr><th>Objective 2.2.</th></tr>
<tr><td>Development of a laser ablation-based method for the identification of polymers in calcium phosphate films</td></tr>
<tr><th>Objective 1.2.</th><th>Objective 2.3.</th></tr>
<tr><td rowspan="3">Improvement of biofilm inhibition by a combined atmospheric pressure plasma/fluoride treatment</td><td>Identification of the dopant and chitosan role on the biological and physicochemical properties of the coatings</td></tr>
<tr><th>Objective 2.4.</th></tr>
<tr><td>Evaluation of the morphology and molecular structure of calcium phosphate coatings produced in RF-MS discharge at low rf power</td></tr>
</table>

Chapter 5, "*Fluoridation improvement and biofilm modulation by atmospheric pressure plasma*"

The fifth chapter presents the results of the research conducted with the goal of improving the fluoride treatments efficiency used in dentistry by APP treatments [31]. A dielectric barrier discharge (DBD) plasma source was selected to activate the surface of enamel-like HAp samples in the following conditions: working gas – Ar, treatment time – 3 min., gas flow – 2000 sccm, power – 20 W, and a source-to-sample distance of 0.8 mm. After the APP treatment, a fluoride-containing gel was applied for 3 min. For comparison, samples treated only with the fluoride-containing gel or only activated with APP were also obtained. The energy dispersive X-ray spectroscopy (EDX) investigation revealed that the at. % of F was higher for the previously plasma-treated samples, achieving in this manner **Objective 1.1.** (see Table 1). The antibacterial and antibiofilm properties were evaluated on two Gram positive bacteria strains: *S. aureus* and *E. faecalis* and two Gram-negative bacteria strains*: E. coli* and *P. aeruginosa*. The results revealed that the best treatment for reducing the bacterial viability in saline water, the planktonic cell growth, and the biofilm development was the combined one, APP + fluoride-containing gel, achieving therefore **Objective 1.2.** (Table 1).

Chapter 6, "*Calcium phosphates/chitosan composite layers generated by plasma and laser techniques*"

The results presented in the following chapters, from 6 to 9, were obtained during the research conducted with the goal of tailoring the physicochemical and biological properties of calcium phosphate/chitosan (CaP_CS) composite coatings for bone tissue engineering applications using the RF-MS and MAPLE techniques. For a better visualization, the deposition conditions selected for these studies are presented in Figure 7.

The MAPLE deposition was conducted using the same parameters for all the studies to better evidence the influence of the parameters selected for the RF-MS deposition on the physicochemical properties of the layers.

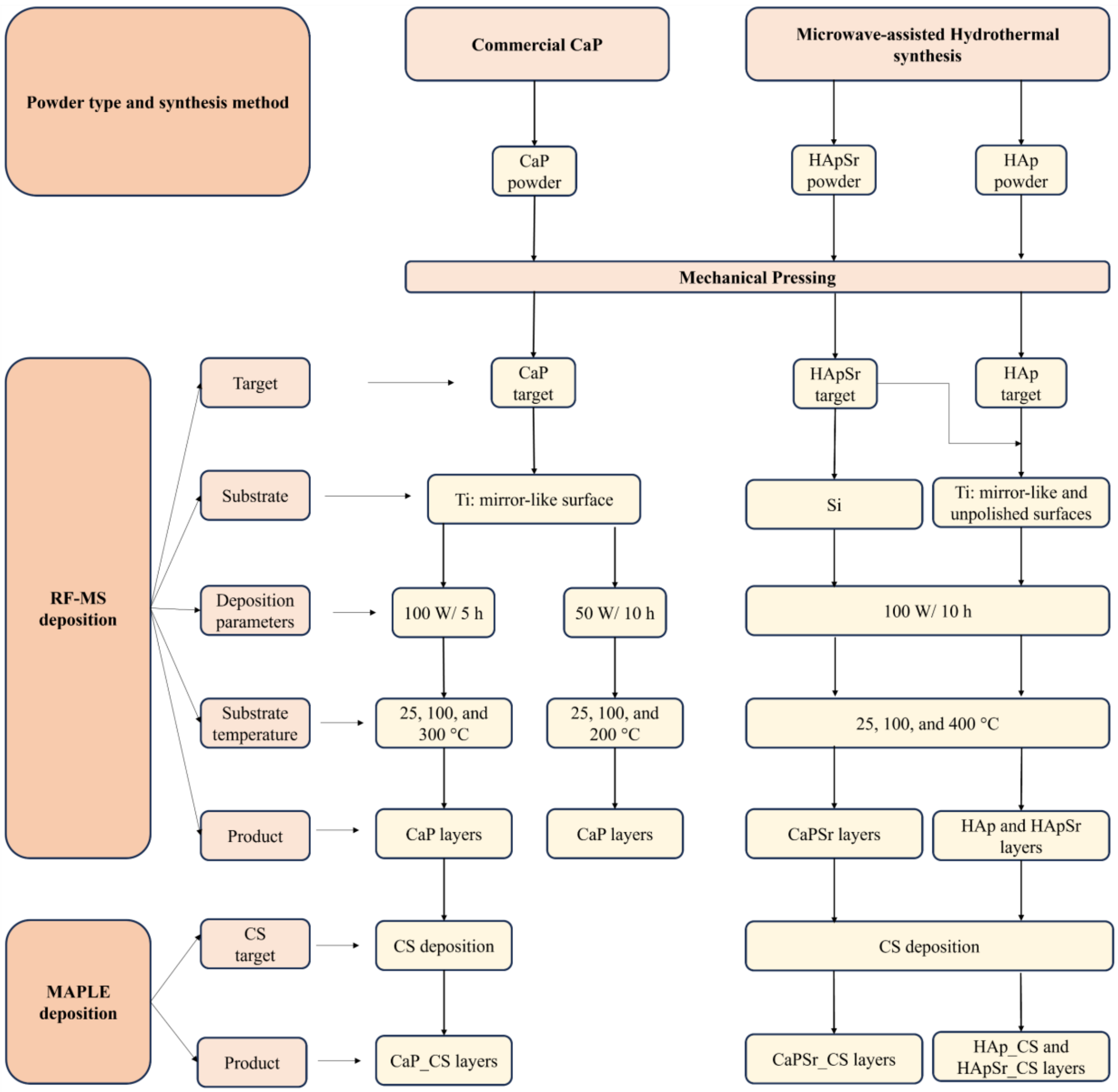

Figure 7. Workflow of the depositions

The sixth chapter highlights the influence of the substrate temperature (25, 100, and 300 °C) during the RF-MS deposition on the physicochemical properties of the CaP layers deposited on Ti substrates at a high working power of 100 W, for 5 h (Figure 7) and of the CaP_CS composite layers [32]. The characterization techniques revealed that as the substrate temperature increases: i) the CaP layers morphology changes from grain-like structures to more compact structures; ii) the Ca/P ratio increases most probably to the due to CaO, which presence was confirmed by X-ray photoelectron spectroscopy (XPS); iii) crystallization appears, as proved by the grazing incidence X-ray diffraction (GIXRD) analysis iv) the embedding of CS into the CaP layers is diminished.

The XPS and the Fourier transformed infrared spectroscopy (FTIR) showed that HAp was formed during the RF-MS deposition and that CS was successfully deposited by MAPLE. Modifications in the molecular structure of the layers were also observed as a function of the substrate temperature. Moreover, the research revealed that the CaP layers were more adherent than the CaP_CS composite layers. By this study, **Objective 2.1.** was attained.

Chapter 7, "*Strontium-doped calcium phosphates/chitosan layers generated by plasma and laser techniques*"

Moving forward, the research was focused on developing a laser-based method for the identification of polymers. In this regard, Sr-doped CaP (CaPSr) layers were deposited on Si substrates at 100 W for 10 h and substrate temperatures of 25, 100, and 400 °C [33]. The composite layers, CaPSr_CS, were obtained after the MAPLE deposition and were investigated by laser induced ablation (LIA) coupled with a quadrupole mass spectrometer (QMS). The LIA-QMS investigation highlighted molecular ions characteristic to CS: i) $C_6H_{11}^+$ (m/q = 83), ii) $C_5H_5O^+$ (m/q = 81), and iii) $NHCOCH_3^+$ (m/q = 58). The last mentioned ascertain the CS acetylation. Therefore, **Objective 2.2.** was achieved. Moreover, the presence of CaP was confirmed by the Ca^+ (m/q = 40), $CaOH^+$ (m/q = 57), P^+ (m/q = 31), and PO_4^+ (m/q = 95) ions currents. The physicochemical properties of the layers were also investigated by scanning electron microscopy (SEM), EDX, and FTIR. As the substrate temperature increased during the RF-MS deposition the morphology of the CaPSr layers changes from grain-like structures to microchannels and the Ca/P ratio is not affected, equal to 2 for all the layers, suggesting that Sr-doped tertacalcium phosphate layers were formed. This was also sustained by the FTIR spectra, in which the absorption bands characteristic to the OH groups were not observed.

Chapter 8, "*The role of Sr and chitosan on the biological properties of composite layers generated by plasma and laser techniques*"

The research was further focused on evaluating the influence of Sr and CS on the biological properties of the composite layers. Chapter 8 presents the results of the research conducted for this purpose. The RF-MS and MAPLE deposition conditions were those reported in the previous chapter. However, for a proper evaluation, the substrate was changed from Si to mirror-like and unpolished Ti samples, as Ti is the most used material for the fabrication of bone implants. Moreover, HAp layers were also deposited to highlight the influence of Sr

(Figure 7). The biological assays (e.g. MTT, LDH, and live/dead cell assays) revealed that all the layers were biocompatible, the most biocompatible being the composite HApSr_CS layers deposited by heating the substrate during the RF-MS deposition [34]. The influences of Sr and CS were better evidenced by the results of the MTT assay. The layers had also antimicrobial properties against *S. aureus*. The layers were also physiochemically investigated. As the substrate temperature increased: i) the grain-like structures were replaced by more compact structures, ii) the differences between the values of the Ca/P and (Ca+Sr)/P ratios decreased, and iii) the absorption bands in the FTIR spectra of the HAp layers characteristic for the ν_3 and ν_4 vibration modes of the PO_4^{3-} narrowed and splitted, respectively, indicating the onset of crystallization. **Objective 2.3.** was achieved after these studies.

Chapter 9, "*Calcium phosphate layers generated by plasma at low rf power – physicochemical properties*"

Considering the outcomes presented above regarding the FTIR investigation, the final step within this thesis was to evaluate the physicochemical modifications of CaP layers generated at a low power of 50 W [35]. A low power can be an optimal choice for heat-sensitive substrates. The deposition time was 10 h and the substrate temperatures were 25, 100, and 200 °C (Figure 7). The SEM investigation revealed that when the substrate was not heated by an oven during the RF-MS deposition, the CaP layer is mainly porous, while increasing the substrate temperature results in more compact layers, with microcavities. The Ca/P ratio was 1.8 for all coatings. The narrowing of the ν_3 band and the splitting of the ν_4 band was observed in the FTIR spectra of the layers deposited by heating the substrate. The peak fitting analysis highlighted the differences in the molecular structure generated by the substrate temperature. The surface chemistry was also changed as a function of the substrate temperature, as shown by the XPS investigation. Therefore, by this study, **Objective 2.4.** was attained.

The PhD thesis ends with three chapters presenting the conclusions - **Chapter 10**, the future perspectives - **Chapter 11**, and the list of publications - **Chapter 12**.

The plasma-based techniques used for the generation of coatings are very versatile considering the number of the deposition parameters which can be varied. As proved by the research results, these can significantly influence the physicochemical and biological properties of the layers.

In this regard, several future perspectives arise. First, based on the results obtained until now, the research should be focused on a more detailed study of the biological properties, both *in vitro* and *in vivo*. Therefore, the most promising composite coatings in terms of cell viability and antimicrobial properties, HApSr_CS layers deposited on Ti by heating the substrate during the RF-MS deposition, should be further tested *in vitro* to evaluate their effects on osteoblasts and osteoclasts, which are the cells involved in bone formation and resorption, respectively. Moreover, other tests, which should simulate the *in vivo* environment, will also be conducted. Finally, to get as close as possible to the approval of such layers for clinical use, the layers will also be evaluated *in vivo*. However, it must be mentioned that, with the aim of clinically use HApSr_CS coatings, the laboratory level RF-MS and MAPLE systems are not enough. Therefore, they should be scaled up. One option could be the deposition of these coatings using industry level set-ups. Of course, this step will also require further research, to ensure that the biological properties of the films deposited with the new systems will be at least preserved, if not better. This future perspective will require a long period of time, and it has the aim of obtaining a final product for industry.

At the laboratory level however, the research on the deposition of CaP/polymer layers will continue by diversifying their composition. For instance, the influence of various dopants and their concentration on the physicochemical and biological properties of CaPs layers generated by RF-MS will be considered for further research. Moreover, considering that depending on the substitutions, the biological properties may be significantly influenced, the future research will also be focused on the deposition of layers from co-substituted CaPs targets, in order to study the synergetic effects. Moving forward, other polymers will be considered for the MAPLE deposition, especially biological polymers.

As a different research direction from the one presented herein, the studies will also be focused on other plasma sources, especially those working at atmospheric pressure, for the generation of CaP/polymer coatings. Among the APP techniques, plasma spraying will be most probably selected due to its advantages.

The studies conducted during the PhD thesis show the potential of plasma techniques for the development of novel materials and treatments for the biomedical field. The novel results were disseminated in 5 scientific papers, with a cumulative impact factor of 20.908:

1. Zarif, M.E.; Yehia, S.A.; Biță, B.; Sătulu, V.; Vizireanu, S.; Dinescu, G.; Holban, A.M.; Marinescu, F.; Andronescu, E.; Grumezescu, A.M.; et al. Atmospheric Pressure Plasma Activation of Hydroxyapatite to Improve Fluoride Incorporation and Modulate Bacterial Biofilm. Int. J. Mol. Sci. 2021, 22, 13103, doi:10.3390/IJMS222313103;

2. Zarif, M.E.; Yehia-Alexe, S.A.; Bita, B.; Negut, I.; Locovei, C.; Groza, A. Calcium Phosphates–Chitosan Composite Layers Obtained by Combining Radio-Frequency Magnetron Sputtering and Matrix-Assisted Pulsed Laser Evaporation Techniques. Polymers (Basel). 2022, 14, 5241, doi:10.3390/polym14235241;

3. Zarif, M.E.; Bita, B.; Yehia-Alexe, S.A.; Negut, I.; Groza, A. Spectral Analysis of Strontium-Doped Calcium Phosphate/Chitosan Composite Films. Polymers (Basel). 2023, 15, 4245, doi:10.3390/polym15214245;

4. Zarif, M.E.; Bita, B.; Yehia-Alexe, S.A.; Negut, I.; Gradisteanu Pircalabioru, G.; Andronescu, E.; Groza, A. Biological and Physicochemical Analysis of Sr-Doped Hydroxyapatite/Chitosan Composite Layers. Polymers (Basel). 2024, 16, 1922, doi:10.3390/polym16131922.

5. Zarif, M.E.; Groza, A.; Yehia-Alexe, S.-A.; Bita, B.; Andronescu, E. The Influence of the Substrate Temperature on the Physicochemical Properties of Calcium Phosphate Layers Deposited by Low Power Radio-Frequency Magnetron Sputtering Discharge. U.P.B. Sci. Bull., Ser. B, 2025, 87, 5–20;

and 9 international conferences:

1. 10th International Conference on Advanced Materials, Bucharest, Romania, (poster presentation): “Sr-doped Hydroxyapatite/ Chitosan composite layers deposited on titanium substrates for bone tissue engineering applications” **Zarif M.E.**; Bita. B.; Yehia-Alexe S.A.; Negut I.; Gradisteanu Pircalabioru G.; Groza A. (**2024**);

2. 2nd International Conference on Laser, Plasma, and Radiation -Science and Technology 2024 (poster presentation): “Physicochemical investigations of strontium-doped calcium phosphate/chitosan composite films deposited by plasma and laser techniques” **Zarif M.E.**; Bita. B.; Yehia-Alexe S.A.; Negut I.; Groza A. (2024);

3. 6th International Conference on Emerging Technologies in Materials Engineering, Bucharest, Romania, (poster presentation): “Calcium phosphate/ chitosan coatings obtained by radiofrequency magnetron sputtering and matrix-assisted pulsed laser evaporation techniques for bone tissue engineering applications” **Zarif M.E.**; Yehia-Alexe S.A.; Bita. B.; Negut I.; Locovei. C.; Groza A. (2023);

4. 22nd Romanian International Conference on Chemistry and Chemical Engineering, Sinaia, Romania (oral presentation): “Atmospheric plasma treatment to modulate biofilm formation and fluoridation of enamel-like hydroxyapatite model” **Holban A.M.**, Vizireanu S., Zarif M., Grumezescu A.M., Birca A., Farcasiu Alexandru Titus, Marinescu Florica, Chifiriuc Mariana Carmen (**2022**);
5. 22nd Romanian International Conference on Chemistry and Chemical Engineering, Sinaia, Romania (poster presentation): “Radiofrequency magnetron sputtering deposition of calcium phosphate coatings – chemical and phase composition”, **Zarif M.E.**, Yehia S.A., Biță B., Groza A., Grumezescu A., Andronescu E. (2022);
6. Virtual International Scientific Conference on “Applications of Chemistry in Nanosciences and Biomaterials Engineering” (oral presentation): “Calcium Phosphate Coatings Obtained by Radiofrequency Magnetron Sputtering For Bone Tissue Engineering Applications”, **Zarif M.E.**, Yehia S.A., Biță B., Groza A., Grumezescu A., Andronescu E. (2022);
7. International Scientific Conference “Applications of chemistry in Nanosciences and Biomaterials Engineering (oral presentation): “Atmospheric plasma treatment to modulate bacterial biofilms” **Zarif M.E.**, Yehia S.A., Satulu V., Dinescu G., Vizireanu S., Holban A., Marinescu F., Grumezescu A., Andronescu E., Farcasiu T. (2021);
8. International Conference on Plasma Physics and Applications, Magurele, Romania (oral presentation): “Atmospheric plasma fluoridation for application in dentistry” **Zarif M.E.**, Yehia S.A., Bita B.I., Satulu V., Vizireanu S., Dinescu G., Grumezescu A.M., Farcasiu A.T., Holban A.M. (2021);
9. International Scientific conference Applications of Chemistry in Nanosciences and Biomaterials Engineering (oral presentation): “Morphological, structural, and chemical characterization of hydroxyapatite obtained through different synthesis routes” **Zarif M.E.**, Holban A.M., Vizireanu S., Dinescu G., Grumezescu A.M., Andronescu E. (2021);

References

1. Cacciotti, I. Cationic and Anionic Substitutions in Hydroxyapatite. In *Handbook of Bioceramics and Biocomposites*; Springer International Publishing: Cham, 2015; pp. 1–68.

2. Filip, D.G.; Surdu, V.-A.; Paduraru, A.V.; Andronescu, E. Current Development in Biomaterials—Hydroxyapatite and Bioglass for Applications in Biomedical Field: A Review. *J. Funct. Biomater.* **2022**, *13*, 248, doi:10.3390/jfb13040248.

3. Ressler, A.; Žužić, A.; Ivanišević, I.; Kamboj, N.; Ivanković, H. Ionic Substituted Hydroxyapatite for Bone Regeneration Applications: A Review. *Open Ceram.* **2021**, *6*, 100122, doi:10.1016/j.oceram.2021.100122.

4. Akhtar, K.; Khan, S.A.; Khan, S.B.; Asiri, A.M. Scanning Electron Microscopy: Principle and Applications in Nanomaterials Characterization. In *Handbook of Materials Characterization*; Springer International Publishing: Cham, 2018; pp. 113–145 ISBN 9783319929552.

5. González, A. 1.5 X-Ray Crystallography: Data Collection Strategies and Resources. In *Comprehensive Biophysics*; Elsevier, 2012; Vol. 1, pp. 64–91 ISBN 9780080957180.

6. López, E.O.; Mello, A.; Farina, M.; Rossi, A.M.; Rossi, A.L. Nanoscale Analysis of Calcium Phosphate Films Obtained by RF Magnetron Sputtering during the Initial Stages of Deposition. *Surf. Coatings Technol.* **2015**, *279*, 16–24, doi:10.1016/j.surfcoat.2015.08.021.

7. Achilli, E.; Annoni, F.; Armani, N.; Patrini, M.; Cornelli, M.; Celada, L.; Micali, M.; Terrasi, A.; Ghigna, P.; Timò, G. Capabilities of Grazing Incidence X-Ray Diffraction in the Investigation of Amorphous Mixed Oxides with Variable Composition. *Materials (Basel).* **2022**, *15*, 2144, doi:10.3390/ma15062144.

8. Gupta, B.D.; Semwal, V.; Pathak, A. Nanotechnology-Based Fiber-Optic Chemical and Biosensors. In *Nano-Optics*; Elsevier, 2020; pp. 163–195 ISBN 978-0-12-818392-2.

9. Thygesen, L.G.; Løkke, M.M.; Micklander, E.; Engelsen, S.B. Vibrational Microspectroscopy of Food. Raman vs. FT-IR. *Trends Food Sci. Technol.* **2003**, *14*, 50–57, doi:10.1016/S0924-2244(02)00243-1.

10. Bekas, D.G.; Tsirka, K.; Baltzis, D.; Paipetis, A.S. Self-Healing Materials: A Review of Advances in Materials, Evaluation, Characterization and Monitoring Techniques. *Compos. Part B Eng.* **2016**, *87*, 92–119, doi:10.1016/j.compositesb.2015.09.057.

11. Mei, L.; Guan, G. Profilometry and Atomic Force Microscopy for Surface Characterization. *Nano TransMed* **2023**, *2*, e9130017, doi:10.26599/NTM.2023.9130017.

12. Mestieri, L.B.; Zaccara, I.M.; Pinheiro, L.S.; Barletta, F.B.; Kopper, P.M.P.; Grecca, F.S. Cytocompatibility and Cell Proliferation Evaluation of Calcium Phosphate-Based Root Canal Sealers. *Restor. Dent. Endod.* **2020**, *45*, 1–7, doi:10.5395/rde.2020.45.e2.

13. Chraniuk, M.; Panasiuk, M.; Hovhannisyan, L.; Żołędowska, S.; Nidzworski, D.; Ciołek, L.; Woźniak, A.; Jaegermann, Z.; Biernat, M.; Gromadzka, B. The Preliminary Assessment of New Biomaterials Necessitates a Comparison of Direct and Indirect Cytotoxicity Methodological Approaches. *Polymers (Basel).* **2022**, *14*, 4522, doi:10.3390/polym14214522.

14. Anghel, S.D. Capitolul I - Plasma Și Parametrii Ei. In *Fizica plasmei și aplicații*; Universitatea Babes-Bolyai, Cluj-Napoca.

15. Lee, J.; Lee, C.W.; Yong, H.I.; Lee, H.J.; Jo, C.; Jung, S. Use of Atmospheric Pressure Cold Plasma for Meat Industry. *Korean J. Food Sci. Anim. Resour.* **2017**, *37*, 477–485, doi:10.5851/kosfa.2017.37.4.477.

16. Xiang, Q.; Liu, X.; Li, J.; Ding, T.; Zhang, H.; Zhang, X.; Bai, Y. Influences of Cold Atmospheric Plasma on Microbial Safety, Physicochemical and Sensorial Qualities of Meat Products. *J. Food Sci. Technol.* **2018**, *55*, 846–857, doi:10.1007/s13197-017-3020-y.

17. Bhattacharjee, B.; Bezbaruah, R.; Rynjah, D.; Newar, A.; Sengupta, S.; Pegu, P.; Dey, N.; Bora, S.; Barman, D. Cold Atmospheric Plasma: A Noteworthy Approach in Medical Science. *Sci. Pharm.* **2023**, *2*, 46–76, doi:10.58920/sciphar02020046.

18. Domonkos, M.; Tichá, P.; Trejbal, J.; Demo, P. Applications of Cold Atmospheric Pressure Plasma Technology in Medicine, Agriculture and Food Industry. *Appl. Sci.* **2021**, *11*, 4809, doi:10.3390/app11114809.

19. Ruchel Khanikar, R.; Bailung, H. Cold Atmospheric Pressure Plasma Technology for Biomedical Application. In *Plasma Science and Technology*; IntechOpen, 2022; Vol. i, p. 13.

20. Palumbo, F.; Lo Porto, C.; Fracassi, F.; Favia, P. Recent Advancements in the Use of Aerosol-Assisted Atmospheric Pressure Plasma Deposition. *Coatings* **2020**, *10*, 440, doi:10.3390/coatings10050440.

21. Braný, D.; Dvorská, D.; Halašová, E.; Škovierová, H. Cold Atmospheric Plasma: A Powerful Tool for Modern Medicine. *Int. J. Mol. Sci.* **2020**, *21*, doi:10.3390/ijms21082932.

22. Cha, S.; Park, Y.-S. Plasma in Dentistry. *Clin. Plasma Med.* **2014**, *2*, 4–10, doi:10.1016/j.cpme.2014.04.002.

23. Li, H.; Zhang, X.; Zhu, X.; Zheng, M.; Liu, S.; Qi, X.; Wang, K.; Chen, J.; Xi, X.; Tan, J.; et al. Translational Plasma Stomatology: Applications of Cold Atmospheric Plasmas in Dentistry and Their Extension. *High Volt.* **2017**, *2*, 188–199, doi:10.1049/hve.2017.0066.

24. Oluwatosin Abegunde, O.; Titilayo Akinlabi, E.; Philip Oladijo, O.; Akinlabi, S.; Uchenna Ude, A. Overview of Thin Film Deposition Techniques. *AIMS Mater. Sci.* **2019**, *6*, 174–199, doi:10.3934/matersci.2019.2.174.

25. Ohring, M. Thin-Film Evaporation Processes. In *Materials Science of Thin Films*; Elsevier, 2002; pp. 95–144.

26. Ohring, M. Chemical Vapor Deposition. In *Materials Science of Thin Films*; Academic Press, 2001; pp. 277–355 ISBN 9780080491783.

27. Martín-Palma, R.J.; Lakhtakia, A. Vapor-Deposition Techniques. In *Engineered Biomimicry*; Elsevier, 2013; pp. 383–398 ISBN 9780124159952.

28. Wang, W.; Xue, C.; Mao, X. Chitosan: Structural Modification, Biological Activity and Application. *Int. J. Biol. Macromol.* **2020**, *164*, 4532–4546, doi:10.1016/j.ijbiomac.2020.09.042.

29. Yan, D.; Li, Y.; Liu, Y.; Li, N.; Zhang, X.; Yan, C. Antimicrobial Properties of Chitosan

and Chitosan Derivatives in the Treatment of Enteric Infections. *Molecules* **2021**, *26*, 7136, doi:10.3390/molecules26237136.

30. Ardean, C.; Davidescu, C.M.; Nemeş, N.S.; Negrea, A.; Ciopec, M.; Duteanu, N.; Negrea, P.; Duda-Seiman, D.; Musta, V. Factors Influencing the Antibacterial Activity of Chitosan and Chitosan Modified by Functionalization. *Int. J. Mol. Sci.* **2021**, *22*, 7449, doi:10.3390/ijms22147449.

31. Zarif, M.E.; Yehia, S.A.; Biță, B.; Sătulu, V.; Vizireanu, S.; Dinescu, G.; Holban, A.M.; Marinescu, F.; Andronescu, E.; Grumezescu, A.M.; et al. Atmospheric Pressure Plasma Activation of Hydroxyapatite to Improve Fluoride Incorporation and Modulate Bacterial Biofilm. *Int. J. Mol. Sci.* **2021**, *22*, 13103, doi:10.3390/ijms222313103.

32. Zarif, M.E.; Yehia-Alexe, S.A.; Bita, B.; Negut, I.; Locovei, C.; Groza, A. Calcium Phosphates–Chitosan Composite Layers Obtained by Combining Radio-Frequency Magnetron Sputtering and Matrix-Assisted Pulsed Laser Evaporation Techniques. *Polymers (Basel).* **2022**, *14*, 5241, doi:10.3390/polym14235241.

33. Zarif, M.E.; Bita, B.; Yehia-Alexe, S.A.; Negut, I.; Groza, A. Spectral Analysis of Strontium-Doped Calcium Phosphate/Chitosan Composite Films. *Polymers (Basel).* **2023**, *15*, 4245, doi:10.3390/polym15214245.

34. Zarif, M.E.; Bita, B.; Yehia-Alexe, S.A.; Negut, I.; Gradisteanu Pircalabioru, G.; Andronescu, E.; Groza, A. Biological and Physicochemical Analysis of Sr-Doped Hydroxyapatite/Chitosan Composite Layers. *Polymers (Basel).* **2024**, *16*, 1922, doi:10.3390/polym16131922.

35. Zarif, M.-E.; Groza, A.; Yehia-Alexe, S.-A.; Bita, B.; Andronescu, E. The Influence of the Substrate Temperature on the Physicochemical Properties of Calcium Phosphate Layers Deposited by Low Power Radio-Frequency Magnetron Sputtering Discharge. *U.P.B. Sci. Bull., Ser. B* **2025**, *87*, 5–20.