

The purpose of this proposal was to develop and characterize biodegradable, multi-element doped hydroxyapatite biopolymer composite coatings onto metallic and ceramic implant materials.

During the first year of the research work, hydroxyapatite, and calcium phosphate (CaP) powders in different phases as well as in amorphous structures were successfully produced by the wet chemical precipitation method. Different calcium and phosphorus precursors were used and bioactive ions such as Mg, Sr, Zn and Ag, were also co-precipitated along with the main CaP particles. The micro and nanostructure of base CaP powders, as well as the ion-doped powders, were thoroughly studied. We optimized the doping parameters and evaluated the distribution characteristics of all the doping elements. We examined how the ionic doping changed the morphology, thus the chemical and biological properties of the powders.

Preparation of HAp and multi-element doped apatite powders and coatings:

The chemicals used to prepare synthetic apatite and ionic doped apatite powders:

Calcium precursors: calcium nitrate, calcium chloride, calcium acetate, calcium gluconate

Phosphorus precursors: sodium dihydrogen phosphate, disodium hydrogen phosphate, diammonium hydrogen phosphate

For ionic substitution: chloride or nitride salts of Mg, Sr, Zn ions in different concentrations and ratios (according to the intensive research work and the optimization process the most optimal doping elements concentration in total was 5 wt%, which most closely resembled the chemical composition of the hard tissues of human bones).

The morphology and elemental structure, and composition of different powders, prepared by wet chemical methods have been thoroughly investigated by Scanning Electron Microscopy, Transmission Electron Microscopy, FT-IR and XRD measurements. The elemental composition and elemental ratio within the coatings were also investigated by X-ray photoelectron spectroscopy.

The XRD measurements revealed that the primarily formed CaP phase in all cases was monetite ( $\text{CaHPO}_4$ ). The CaP phase transformation from monetite to hydroxyapatite was initiated by the treatment of the powders with 1M NaOH or  $\text{Na}_2\text{CO}_3$  solutions (at a pH value of 11). The solubility of the hydroxyapatite phase is the lowest among the other CaP phases at higher pH values thus treating the CaP powders in an alkaline solution will advance the phase transformation through dissolution-precipitation processes. The morphological characterization (by SEM-EDX measurements) and comparison of powders before and after alkaline treatment proved that the phase transformation was completed.

According to the SEM observations, the smallest grain size with an almost amorphous structure was obtained when calcium gluconate was used as a Ca source, while in the case of nitrate and chloride salt of Ca, the crystallinity was higher and well-defined, small, needle-like particles could be obtained.

The post-treatment of powders in alkaline solutions resulted in phase transformation with different Ca/P ratios according to XRD measurement, with larger, agglomerated particles (SEM), and when 1M  $\text{Na}_2\text{CO}_3$  solution was applied, carbonated HAp/apatite phase generation with small, disoriented, needle-like particles in nanometre size was observed. The addition of

different bioactive ions into the starting solution also changed the morphology of the precipitated powders. The substituting ions were also deposited in the form of low-soluble phosphate precipitates along with different CaP phases. The forms and sizes of particles were very diverse, the powders contained large plate-like, rod-like grains in 0.5 – 5  $\mu\text{m}$  sizes and small, needle-like particles as well as agglomerated spherical and flake-like particles. The elemental distribution of substituting elements was homogeneous within the CaP matrix except for silver particles which tended to agglomerate according to the SEM elemental mapping. The morphological characterizations revealed that the different bioactive ions and their concentrations/ratios significantly changed their micro- and nanostructure.

The carbonated apatite content was also proven by FTIR measurements, which presented the characteristic peaks originating from stretching vibrations of  $\text{CO}_3^{2-}$  ions and demonstrated that the  $\text{PO}_4^{3-}$  groups were partially replaced by  $\text{CO}_3^{2-}$  groups, forming B-type carbonated apatite. The FTIR spectra showed that the band intensity related to carbonate vibration mode is higher in the case of ionic doped apatite samples and resulted in a broader peak related to water content. It revealed that the carbonate anionic group substituted in a higher amount in the case of the ion-doped samples. TEM observations also confirmed that the shape of nanoparticles was mostly small, thin, and needle-like that easily form thick aggregates of several 100 nm in diameter. According to TEM-EDS measurements, the Ca/P ratio measured on several aggregates of nanocrystals was approximately 1.8, higher than the value for stoichiometric apatite (1.66). The increased Ca/P ratio can be related to the incorporation of ( $\text{CO}_3^{2-}$ ) anions into the apatite structure. TEM images of ion-doped HAp sample revealed globular particles of diameter  $\sim 30\text{--}70$  nm (which coincides well with the SEM measurements). The individual particles were attached to each other forming 200-500 nm-sized aggregates. According to the TEM-EDS measurements, the Ca/P ratio varied between 1.17 and 1.67, while (Ca+Mg+Zn+Sr)/P ratio ranged from 1.66 to 2.29, indicating a certain degree of inhomogeneity in the amorphous structure and the successful incorporation of doping elements into the calcium phosphate crystals. The Ca:Mg:Sr:Zn ratio of the bioactive ion added apatite sample was 70:11:11:8 (cation %) which also indicated high efficiency of incorporation of additives under the applied synthesis conditions. The XPS measurements revealed the Ca/P ratio to be 1.89 in the pure apatite sample, while for ion-doped apatite, the measured Ca/P ratio was slightly lower, 1.82, and the calculated (Ca+Mg+Zn+Sr)/P ratio was 2.84. The Ca:Mg:Sr:Zn ratio in this case was 64:29:3:4. The addition of doping elements caused a significant decrease in the degree of crystallinity.

Such developed apatite powders were then deposited onto metallic implants' surfaces by spin coating and subsequent heat treatment. According to numerous experiments, the heat treatment also caused phase transformation of CaPs as well as improved the adherence of coatings. This was proven by several measurements since the heat-treated samples contained smaller, densely packed particles and they were well-oriented.

There were also experiments for electrochemical deposition and electrospraying depositions of powder coatings. The electrochemically deposited coatings consisted of mainly large, plate-like grains in the monetite phase. The size, thickness, and orientation of these platelets were largely dependent on the applied deposition parameters. The ion-doped calcium phosphate layer contained particles of different, non-uniform shapes: disoriented small needle-like, larger plate-like, and spheroid grains in various sizes, as well as flake-like agglomerates and blocks in

micrometer size. The size of silver particles within the layers was between 100 and 150 nm, and they tended to agglomerate. For electrospraying deposition, the process was performed at room temperature with Inovenso Ne100 Electrospraying/Electrospinning Machine. An appropriate concentration of CaP powder was dispersed in ethanol with vigorous stirring. The suspension was pumped through a copper nozzle with an automated syringe pump at an adjusted flow rate and high voltage was applied between the two electrodes. The homogeneity and thickness of layers depended greatly on the applied voltage, the flow rate, and the time of deposition. However, the disadvantage of these coatings was their poor adherence to the surface of substrates.

Apatite powder/biopolymer composites were also prepared by the electrospinning technique. Different biopolymers were used as matrices for the apatite (CaP) and trace elements incorporation. The influence of deposition parameters on the chemical and morphological properties of electrosprayed composite samples was studied, evaluated, and discussed. The incorporation rate of apatite particles was dependent on their concentration in the suspensions while their distribution depended on the electrospraying parameters.

Investigation of the structural, morphological, and mechanical properties of previously prepared apatite/biopolymer composites:

For comparison, pure biopolymer fiber mats and apatite-biopolymer composites were prepared by the electrospinning method. The biodegradability of apatite coatings can be modified by incorporating them into adequately chosen biopolymer fibers. It is also important that the apatite-biopolymer composite coatings degrade to non-toxic by-products, being removed by the body completely after ossification. Taking this into account, in the experiments, we have chosen two types of synthetic biopolymers, such as poly N-vinyl pyrrolidone, (PVP) and polycaprolactone, and one natural polymer, cellulose acetate (CA) in order to use them as matrices for apatite and modified apatite powder incorporation.

PVP has unique properties of biodegradability in biological environments and is also an amphiphilic molecule owing to the polar lactam group in pyrrolidone offering hydrophilicity. Polycaprolactone (PCL) is a biocompatible and biodegradable polyester with a low melting point and a glass transition temperature and can act as a perfect adhesive and bonding material between the coating and implant. While cellulose acetate is the acetate ester of cellulose and it has unique properties which make it ideal for many applications, such as filtration, medical coatings, drug delivery, and food packaging. Moreover, it is biodegradable and biocompatible. SEM investigations on samples revealed the fibrous structure of electrospun PVP, PCL and CA, however, their basic microstructure (such as diameters and lengths of fibers, their homogeneity, and their complex structure) was noticeably different. The electrospinning parameters were optimized to get the perfect fibrous structure without any bead formation.

The apatite-biopolymer composites were also prepared by electrospinning method with the same parameters as in the case of pure polymers in order to make them comparable. The morphological studies revealed that the incorporation of all types of apatite into polymers changed the structure of the base polymer matrices. The apatite particles were attached to the polymer fibers as clusters and the surface coverage was discontinuous. There was no significant difference between the incorporation characteristic and powder distribution of pure and ion-modified apatite powders (SEM elemental mapping). According to the SEM observations, long,

entangled, thin fibers could be attained, and the agglomerated apatite particles were entwined by the biopolymers forming a web-like structure.

The adhesion properties of all types of apatite-biopolymer composites were studied by adhesion test kit for coating. It was observed that the electrospun polymer improved the adherence of the coatings compared to the powder coatings.

The reproducibility of the preparation of composite samples was appropriate as well as the distributions of apatite powders within the biopolymer matrix were quite homogeneous owing to the intensive optimization procedures. In addition, their adherence was also sufficient. We also obtained useful information on how the different preparation parameters influenced the grain size, homogeneity of apatite distribution, and therefore the mechanical and chemical properties of different composite coatings.

During the last year of this project the surface roughness, the micro and nanostructure of base apatite, the biominerals added apatite powders as well as the different apatite-biopolymer composites were thoroughly studied and compared. We studied their biological performances and biodegradability rates as well. The results indicated that the optimized composite coatings can be proper candidates for bioresorbable coatings on metallic implants with controlled ion release during middle and long-term implantations.

Investigation of chemical and biological stabilities of apatite coatings as well as apatite-biopolymer composites prepared with different methods:

During our intensive research work on the optimization and achieving the final composition of the biominerals added apatites and according to the thorough review of the structure and composition of natural human bones, we have concluded that the most optimum concentration of the doping elements is when the Ca:Mg:Zn:Sr weight ratio is 97:2.5:0.45:0.05, while the applied Ca:P mole ratio is 5:3. The carbonate content was introduced into the apatite structure by soaking the powder into sodium carbonate solution allowing for ionic exchange. It is reported that all biological apatites contain variable amounts of carbonate and hydrogen phosphate ions. In the bones, carbonate is the predominant substituent, which is typically 2–8% by weight, depending on the source. The carbonate content was clearly proven by FT-IR measurements. The developed biominerals added apatite powders were mainly nanocrystalline or quasi-amorphous, according to the XRD measurements. These types of apatites turned out to be more advantageous as biodegradable coatings since their dissolution rates are noticeably faster compared to the highly crystalline HAp. This fact can be attributed to the smaller particle size and the larger specific surface area. To examine the internal structure of the apatite coating and the biomineralized apatite coating, SEM-FIB cross-sectional analyses were also performed. The cross-section images clearly revealed that the powder coatings were built up from small, needle or thorn-like as well as distorted spherical particles from bottom to top and they formed very porous, sponge-like structures.

The biodegradation rate of both powders and composites was followed over a long time by corrosion measurements. The corrosion rates of the optimally biomineralized apatites were measured by running potentiodynamic polarization measurements in biological solutions. The corrosion characteristics and ionic dissolution rates were compared for powder and composite coatings. In our experimental work, Ti6Al4V alloys were used as substrates which are the most used commercial implant materials. The mechanical properties and surface distribution of

coatings were sufficiently reproducible. The reproducibility was dependent on the appropriate dispersion of the apatite powders within the solvent and the homogeneity of the particle size distribution. For corrosion measurements, we have chosen phosphate buffer solution (PBS) and simulated body fluids since they have the most similarity to the ionic composition of body fluids, hence simulating the biological conditions. The corrosion results showed that the measured corrosion currents ( $i_{\text{corr}}$ ) were the highest in the case of the biomineralized apatite coatings (highest corrosion rate) which can be attributed to the faster dissolution rate of the calcium-magnesium-zinc-strontium apatites. We can assume that these components (Mg, Zn, Sr) will dissolve first from the coatings. The base apatite coatings had slightly lower corrosion rates, proving their slower dissolution mechanisms. The smallest  $i_{\text{corr}}$  values were obtained in the case of uncoated substrate owing to the spontaneously formed  $\text{TiO}_2$  thin layer on the surface which provided high corrosion resistance to the sample. The investigation of the time-dependence of the corrosion of all samples showed that the  $i_{\text{corr}}$  values increased over time in the cases of apatite and biomineralized apatite coatings (around 4-5 times over one month), however, the increasing tendency slowed down considerably after around two weeks of immersion, which can refer to the saturation of the solution or the passivation of the layer. On the other hand, the increasing corrosion current is proof of their biodegradability. Contrary, the  $i_{\text{corr}}$  values hardly changed over time for the titanium alloy proving that the formed  $\text{TiO}_2$  layer was stable and corrosion resistant. The deposition method (spin coating or electrospraying) in this case did not cause any significant change so we can state that the dissolution characteristic of powder coatings was independent of the deposition process.

The ionic dissolution rate was followed and measured over a long time by ICP-OES measurements as well. The samples were immersed in SBF solution and the changes in ionic concentrations were tested at different time intervals (after 1 day, 7 days, 14 days, 21 days, 30 days, and 60 days). The observation was that the dissolution characteristics of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  ions showed a saturation curve, which means relatively fast dissolution during the first 10 to 15 days of immersion then the dissolution rate slowed down and reached a quasi-steady state. On the other hand, for the strontium ion, a steady and very low concentration was measured over the entire investigation period which can be related to its low concentration within the powder coatings.

We can conclude from the corrosion measurements that the ionic doping or biomineralization increased the dissolution rate of the apatite powder since the magnesium-, zinc-, strontium containing apatites had higher solubility product constants ( $K_{\text{sp}}$ ).

Regarding the biomineralized apatite-biopolymer composites, we have tested several polymers, such as PVP, CA, and (PCL) as mentioned above. The results revealed that the PVP was not suitable as a long-term coating on implant materials owing to its very fast dissolution rate, on the other hand, the PCL and CA polymers proved to be suitable since they are properly biocompatible, and their biodegradable rate is sufficiently slow for middle- and long-term applications. During our research work, we tested and compared the electrospinning and the spin coating technique for composite coating preparation. The comprehensive tests revealed that the coatings prepared by spin coating had better mechanical properties with better adhesion compared to the electrospun coatings. This can be attributed to the fact that the fiber structure, obtained from electrospinning provided loosely adherent coatings since the substrate-composite interaction was poor with weaker adhesive attraction sites between the coating and the substrate.

On the other hand, the spin coating provided appropriately adherent, reproducible film layers and their thickness could be easily adjusted to the requirements. The optimized apatite-biopolymer ratio was 1:2 in weight percent. The internal structure and build-up of the biocomposite layers were also checked by SEM-FIB cross-sectional analysis. We have to note that the FIB cut was more difficult to perform for polymers than in the cases of powder coatings since the polymers got strongly charged under the applied ion beams. Despite all the difficulties, the cross-section images of composite coatings revealed a little denser, more compact layer and the elemental mapping also proved that the apatite particles were embedded into the larger polymer particles. It was also visible that the interface between the composite and the substrate seemed to be well-bonded, and more defined with no cracks, which can be evidence of a better adherence. However, due to the embedded small apatite particles, the layers maintained their porous structure from bottom to top very similar to the powder coatings. Overall, the composite coatings also showed a sponge-like structure with a stronger bond in the composite-substrate interface.

The corrosion properties of the developed and optimized composite coatings were also studied and compared. The corrosion measurements were performed with the same parameters and under the same conditions as in the case of powder coatings for better comparison. The results revealed that the measured corrosion currents were higher for the composite coatings than in the cases of powder coatings, approximately 2 and 3 times higher for apatite-PCL composite and for apatite-cellulose acetate, respectively. It is also noteworthy that the corrosion currents of composites increased more significantly with immersion time. After one month of immersion, the corrosion rates were five times higher in the case of apatite-PCL and around 10 times higher for apatite-CA composite coatings. This proves their increased biodegradability rate compared to the powder coatings' and that in these cases there was no observable layer passivation or solution saturation over the investigated period. The dissolution rate increased steadily and gradually over time for both composite coatings.

For composite samples, the ionic dissolution rate was also followed and measured over a long time by ICP-OES measurements in the same way as previously described. The obtained results supported the results of the corrosion study. The concentrations of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ , and even the  $\text{Sr}^{2+}$  ions slightly and steadily increased over time.

The change in morphology of the composite film layers before and after immersion of samples in SBF for two months was examined by SEM. In this case, opposite to the powder coatings, the immersion caused visible changes in the micro- and nanostructure of the layers. The polymer and the apatite particles within the coating could be clearly distinguished. After immersion, the structure of the polymer particles became less defined, and the apatite particles appeared as large agglomerates, incorporated into the polymer matrix.

Finally, we compared the corrosion characteristic and rate of powder coatings and composite coatings and the results clearly revealed that the composite coatings (both CA and PCL based) had faster solubility which proves that they will degrade faster in biological conditions.