UNIVERSITY OF SZEGED
DOCTORAL SCHOOL OF
ENVIRONMENTAL SCIENCES

HUNGARIAN ACADEMY OF SCIENCES
CENTRE FOR ENERGY RESEARCH

# DEVELOPMENT AND CHARACTERIZATION OF BIOCOMPATIBLE AND ANTIBACTERIAL BIOCERAMIC COATINGS

PH.D. THESIS

Prepared by
Monika Furko
Chemist (MSc)

SUPERVISOR:
Dr. Csaba Balázsi
Scientific Advisor (DSc.)

Doctoral School of Environmental Sciences
Department of Applied and Environmental Chemistry
Faculty of Natural Sciences and Informatics
University of Szeged

Szeged

Thin Film Physics Department
Institute for Technical Physics and Materials Science
Centre for Energy Research
Hungarian Academy of Sciences

Budapest

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# 1. INTRODUCTION AND OBJECTIVES

The most commonly used implant materials are the titanium based alloys, such as Ti6Al4V, due to their excellent mechanical strength and chemical stability. Moreover, they have excellent corrosion resistance owing to the spontaneously formed stable oxide layer on their surface. However, bacterial adhesion and biofilm formation on these alloys can easily cause infections after surgery. It is also proven in different literature works that the titanium alloys are bioinert, but not bioactive. Therefore, it is advisable to increase its biocompatibility by applying coatings on their surface. It is well known that hydroxyapatite (HAp) has been widely used in both dentistry and orthopaedic applications due to its excellent osteoconductive properties. Since the HAp has similar chemical composition and crystal structure to the main constituting minerals in human bone tissues, it is considered suitable for bone substitution and reconstruction. However, the excellent bioactivity of hydroxyapatite and the appropriate conditions in the human body are still favourable to the adhesion and reproduction of bacteria on its surface, leading to wound infection and implant failure. It is well known that post-operative infections are still serious problems that need to be solved. Due to post-operative infections, the implants might be rejected by the body and reconstructive surgeries are needed. This is very expensive and it puts financial burden on the healthcare system. Therefore, incorporation of antibacterial particles and other bioactive minerals into the HAp coating during or after the coating preparation process has important clinical value to improve the biocompatibility as well as the antibacterial activity of the medical implants. Applying inorganic antibacterial materials, such as silver, copper, zinc and other metal ions is more effective than any antibiotic treatment because most bacteria can become tolerant to antibiotics. Moreover, silver and in particular the free silver ion is well known for its broad-spectrum antimicrobial activity and its low toxicity to mammalian cells. Biodegradable coatings with good biocompatibility are also desired and tailored for biomedical applications.

With regard to this research area, the aims of my doctoral work were to develop and characterize multi-element doped hydroxyapatite coatings onto implant materials. In my work, the modified hydroxyapatite coatings were prepared by the novel combination of pulse current electrochemical deposition and surface post-treatment with appropriate solutions. The morphology, structure and chemical composition of coatings were thoroughly characterized with different methods, such as scanning electron microscopy (SEM) / transmission electron

microscopy (TEM), energy dispersive X-ray spectroscopy (EDX), X-ray diffractometry (XRD) and Fourier transform infrared spectroscopy (FT-IR). Cross sectional analysing was also performed with focused ion beam (SEM-FIB). The biodegradability of coatings was assessed by complex corrosion measurements (potentiodynamic polarization and Electrochemical Impedance Spectroscopy measurements) in simulated body fluid (SBF). The exact elemental composition of coatings and the concentrations of the dissolved ions after long-term immersion in SBF were studied by inductively coupled plasma optical emission spectrometry (ICP-OES). In addition, biocompatible measurements were also performed to check the bioactivity of coatings using MG-63 osteoblast-like cells as well as antimicrobial tests on one Gram - and one Gram + bacteria.

## 2. EXPERIMENTAL

The coatings were prepared by pulse current electrodeposition using standard two-electrode electrochemical cell, where the cathode was the implant material and the anode was platinum wire under atmospheric condition. Pulse current generator was used to apply square waveforms for electrodeposition with different $t_{on}$ and $t_{off}$ times as well as peak current densities to determine the optimal parameters. Applying $t_{off}$ time in pulse current deposition gives the system time to recover during the zero current periods. Since in naturally occurring biological HAp the Ca/P ratio is 1.67, the electrolyte composition was adjusted accordingly to achieve similar elemental ratio in the layer. In view of this, the electrolyte for pure HAp deposition contained (in g/L) $Ca(NO_3)_2.4H_2O$ – 115.6, $NH_4H_2PO_4$ – 33.30 and 10 mL/L $H_2O_2$ (30%). The role of hydrogen peroxide in the electrolyte was to reduce the $H_2$ evolution process and to generate sufficient amount of hydroxyl ions, since the deposition of hydroxyapatite crystals require high (above 11) pH values at the vicinity of cathode. The electrolyte was vigorously stirred during all the deposition processes and kept at 70 °C and at pH of 4.0 – 4.5. In my research work two different deposition methods were examined to prepare pure hydroxyapatite layers. In the first method, the coatings were deposited with parameters of $t_{on}$: 5 ms, $t_{off}$: 5 ms (high-frequency current impulses), peak current density of 400 mA/cm$^2$ and 10 minutes of deposition time (HAp-I). In this case, the thickness of coatings varied between 25-50 μm. While, in the other method the coatings were prepared with $t_{on}$: 1 ms, $t_{off}$: 10 ms (low-frequency current impulses), peak current density of 5 A/cm$^2$ and with deposition time of 3 seconds (HAp-II). By applying these parameters, much thinner, non-continuous coatings could be obtained, with isolated, 1-2 μm thick calcium phosphate clusters on the surface of implant materials. In all cases, after deposition, the samples were treated in 1M NaOH solution for 2 hours at 70 °C to achieve calcium phosphate phase transformation from monetite to hydroxyapatite.

Three types of multi-element doped hydroxyapatite (adHAp) coatings were prepared with different methods to analyze the effect of electrolyte composition, deposition parameters as well as the preparation methods onto the biocompatible and chemical properties of coatings. In the first one (adHAp-I), the electrolyte used for electrodeposition contained (in g/L) $Ca(NO_3)_2.4H_2O$ – 115.6, $NH_4H_2PO_4$ – 33.30, $AgNO_3$ – 0.85, $Zn(NO_3)_2.6H_2O$ – 1.48 and 10 mL/L $H_2O_2$ (30%). The deposition parameters were $t_{on}$: 5 ms, $t_{off}$: 5 ms, peak current density of 400 mA/cm$^2$ and with deposition time of 10 minutes. These coatings were further investigated without any surface post-treatment.

The second type of adHAp coatings (adHAp-II) were deposited from electrolyte containing (in g/L) $Ca(NO_3)_2.4H_2O$ – 115.6, $NH_4H_2PO_4$ – 33.30, $Mg(NO_3)_2$ – 2.56, $Sr(NO_3)_2$ – 2.10 and 10 mL/L $H_2O_2$ (30%). The pulse electrodeposition parameters were in this case $t_{on}$: 1 ms, $t_{off}$: 10 ms, peak current density of 5 A/cm$^2$ and deposition time of 3 seconds. The surface treatment after deposition was soaking the samples in solution containing 0.01 M $Zn(NO_3)_2$ and 0.0025 M $AgNO_3$ for 24 hours and afterward in 1M NaOH solution at 70 °C for 2 hours with subsequent heat treatment at 150 °C. While, in the third method (adHAp-III), first pure hydroxyapatite coatings with parameters of $t_{on}$: 1 ms, $t_{off}$: 10 ms, peak current density of 5 A/cm$^2$ and 3 second deposition time were deposited and then surface post-treatment was applied by immersing the samples into solution containing 0.01 M $Zn(NO_3)_2$, 0.01 M $Mg(NO_3)_2$, 0.01 M $Sr(NO_3)_2$ and 0.0025 M $AgNO_3$ for 24 hours and afterward in 1M NaOH solution at 70 °C for 2 hours with subsequent heat treatment at 150 °C.

The characteristics of such prepared coatings were analyzed by several different methods. The elemental composition of the samples was determined using EDX method, particle morphologies and structures were observed with SEM and TEM instruments while the cross sectional examinations were performed by FIB measurements. The phases and phase composition of the various nanoparticles in samples were identified with XRD. FT-IR measurements were used to check the quality and the presence of characteristic peaks of different complex anionic groups in calcium phosphates. Complex corrosion measurements were also carried out to assess the biodegradable properties of coatings while the quantity of released ions was measured by inductively coupled plasma atomic emission spectroscopy (ICP-OES) after long-term immersion into SBF solution. The biocompatibility of coatings was investigated by measuring the cell viability by WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) and WST-8 (5-(2,4-disulfophenyl)-3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-tetrazolium, monosodium salt) reagents and with other commercially available biocompatibility test assays: lactate dehydrogenase (LDH) assay, alkaline phosphatase (ALP) assay as well as by staining the living cells with higly fluorescent stains such as Live/Dead cell staining, Calcein/DAPI staining and OsteoImage staining where the specific green fluorescent stain only stain the hidroxyapatite phase deposited by the bone cells during mineralization.

## 3. NOVEL SCIENTIFIC RESULTS

**T1. I successfully deposited nano-sized HAp and multi-element doped HAp (adHAp) coatings onto metallic implant materials by a novel preparation method which consists of the combination of pulse current electrochemical deposition and the appropriate post-treatment of coatings.**

1. I showed by SEM measurements that the doping elements have influence on the size of nanoparticles, on the morphology and crystal structure of coatings deposited by the developed novel preparation method. The doping elements are mainly present in the pores of HAp coatings in the form of insoluble precipitates. I proved the presence of doping elements within the coatings by EDX elemental analysis. [P1, P2, P4, P5]
2. I confirmed by XRD measurements that the coatings were mainly in hydroxyapatite phase. Although the adHAp-I coating contained other calcium phosphate phases, such as monetite ($CaHPO_4$), parascholzite ($CaZn_2(PO_4)_2.2H_2O$) as well as metallic silver. [P1, P2, P4]

**T2. I showed, as a novelty, that non-continuous, bioactive and antibacterial ion-doped hydroxyapatite coatings with sponge-like morphology were obtained using the above described advanced techniques.**

1. I showed that the coatings were deposited onto the implants' surface in the form of isolated clusters by SEM elemental mapping. I proved by cross sectional analysing using focused ion beam that these isolated clusters were 1-2 μm thick and the sizes of these isolated clusters were around several hundred micrometres with irregular shapes. [P5]

**T3. I proved by corrosion measurements that the developed HAp and multi-element modified non-continuous hydroxyapatite coatings could serve well as biodegradable coatings.**

1. I demonstrated that the corrosion rates of non-continuous HAp and adHAp coatings were higher compared to uncoated substrate. This result clearly demonstrates that dissolution processes occur during immersion or during the performed corrosion tests. Since the measured corrosion currents were higher in the case of coated materials, this proves that mainly the dissolution of calcium phosphate phases and the doping elements is dominant over the corrosion of implant substrate. This fact also

demonstrates the biodegradable characteristic of such prepared coatings. [P1, P2, P4, P5]

2. I showed by elemental analysis using ICP-OES that the doping elements dissolved into the electrolyte after long-term immersion in SBF solution in ambient condition.

**T4. I proved that the multi-element modified non-continuous hydroxyapatite coatings possessed better biocompatibility properties compared to uncoated implant materials or even to pure hydroxyapatite coatings.**

1. I showed that the highest biocompatibility belonged to the non-continuous adHAp coatings (adHAp-II and adHAp-III). The cell viability tests, LDH tests and ALP tests all showed steadily and systematically higher values for these samples. Moreover, the cell viability percentage on adHAp-II and adHAp-III samples reached over 90% compared to control group (when only the MG-63 cells were grown in the well plates in DMEM culture media) after two weeks of cell culture. This result means high biocompatibility of this type of coating. [P4, P5]
2. I showed well adherent, dense, living and proliferating MG-63 cells on HAp-II, adHAp-II and adHAp-III samples by Calcein/DAPI staining methods. On the other hand, in the case of adHAp-I coating, large number of dead cells were observed. These findings are in well accordance with the results of cell viability and cytotoxycity measurements. [P4, P5]
3. The antibacterial tests performed on non-continuous, multi-element doped HAp coatings showed sufficient inhibition effect against Gram+ (*S. Aureus*) és Gram- (*E. coli*) bacteria. On the other hand the pure HAp coating did not show any inhibitory effect. Taking the size of the inhibition zones into account, there was no significant difference between the antibacterial activity of adHAp-II and adHAp-III asmples. Moreover, the samples were more effective against Gram+ bacteria than Gram- bacteria.

## 4. PUBLICATIONS RELATED TO THE PRESENT THESIS

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[P5] M. Furko, Z. May, V. Havasi, Z. Kónya, A. Grünewald, R. Detsch, A. R. Boccaccini, C. Balázsi, Pulse electrodeposition and characterization of non-continuous, multi-element doped hydroxyapatite bioceramic coatings, Journal of Solid State Electrochemistry 22(2) (2018) 555-566. IF:2,316

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M. Furko, C. Balázsi, Y. Jiang, T. Wilkins, Pulse current deposition and characterization of silver and zinc loaded calcium phosphate coatings on metallic implantmaterials, Qualitynano Final Conference, 15-17. Jul. 2015, Heraclion, Crete, Greece, poster

M. Furko, C. Balázsi, Development of modified antimicrobial and biocompatible calcium-phosphate/hydroxyapatite layers on metallic implant materials, EUROMAT Conference, 20-24 Sept. 2015, Warsaw, Poland, poster

Furkó M., Balázsi C., Pulzáló árammal implantátum anyagokra leválasztott antimikrobiális és biokompatibilis módosított kalcium-foszfát rétegek elektrokémiai minősítése, X. OATK Conference, 11-13. Oct. 2015, Balatonalmádi, Hungary, poster

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M. Furko, E. D. Bella, M. Fini, C. Balázsi, Biocompatible and cytotoxicity studies on modified calcium phosphate coatings, COST Action MP1301 – NEWGEN, EMRS 19-22. Sept. 2016, Warsaw, Poland, presentation

M. Furko, V. Havasi, Z. Kónya, A. Grünewald, R. Detsch, A. R. Boccaccini, C. Balázsi, Preparation and characterization of multi-element doped hydroxyapatite coatings on metallic implant materials, HSM ANNUAL MEETING, 11-13. May 2017, Siófok, Hungary, presentation

M. Furko, C. Balázsi, Preparation and characterization of electrochemically prepared multi-ion modified calcium phosphate coatings on titanium implants, 15th Conference &Exhibition of the European Ceramic Society (ECerS2017), 9-13. Jul. 2017, Budapest, Hungary, presentation

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## 6. OTHER PUBLICATIONS

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N. Oláh, Z. Fogarassy, M. Furkó, C. Balázsi, K. Balázsi: Sputtered nanocrystalline ceramic TiC/amorphous C thin films as potential materials for medical applications, Ceramics International 41 (4) 5863-5871. (2015) IF: 2.605

M. Furko, M. Lakatos-Varsányi, C. Balazsi: Complex electrochemical studies on silver coated metallic implants for orthopaedic application, J. Solid State Electrochemistry 20 (1) (2016) 263-271. (DOI 10.1007/s10008-015-3026-1) IF: 2.446

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N. Oláh, M. Veres, A. Sulyok, M. Furkó, O. Tapasztó, C. Balázsi, Katalin Balázsi, Biocompatible TiC / amorphous C thinfilmspreparedby DC magnetronsputterin, JVC15, 2014. June 15-20, Vienna, Austria

N. Oláh, L. Illés, A. Sulyok, M. Menyhárd, C. Balázsi, M. Furkó, K. Balázsi, Porlaszott nanokristályos TiC / amorf C vékonyrétegek orvosi alkalmazásokra, Magyar Mikroszkópos Konferencia, Május 28-30, 2014, Siófok, Hungary

## 8. SCIENTOMETRIC DATA

Peer-reviewed papers total: **13;** out of this, related to the topic of thesis: **6.**
Cumulative impact factor: **24.116;** out of this, related to the topic of thesis: **15.233.**
Independent citations total: **20;** out of this, related to the topic of thesis: **11.**