

University of Groningen

Electrochemically deposited antimicrobial hydroxyapatite coatings

Mokabber, Taraneh

DOI:
[10.33612/diss.132596200](https://doi.org/10.33612/diss.132596200)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Mokabber, T. (2020). *Electrochemically deposited antimicrobial hydroxyapatite coatings*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.132596200>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Chapter 7

Summary and outlook

7.1 Summary

Nowadays, in orthopedics, many attempts are focused on providing multifunctional surfaces that are able to simultaneously improve the bioactivity of titanium implants and prevent bacterial infection [1]. Enhanced osseointegration of titanium implants can be achieved by applying bioactive ceramic coatings such as calcium phosphate compounds [2]. Another issue that can restrict the long-term application of the titanium implants is bacterial contamination. To overcome this problem, utilizing silver is highly beneficial because of its strong antimicrobial activity [3,4]. In order to apply multifunctional coatings on metallic substrates, electrochemical deposition has received more attention because of its advantages. This method allows highly irregularly shaped objects, including porous implants, to be coated uniformly [5,6]. Therefore, depositing silver-containing calcium phosphate coatings via electrochemical deposition on titanium is a very promising strategy to enhance the biocompatibility of the implants, also induce the antimicrobial properties. However, still much is unknown about the influence of operating parameters, coating development over time and the mechanical and biological properties of the coatings.

The main aim of this thesis was developing bioactive and antimicrobial silver-containing calcium phosphate coatings via electrochemical deposition. The project focused on the interplay between the effect of different deposition parameters and the chemical and physical properties of the coatings along with fundamental insight into the crystal growth mechanism of electrodeposited calcium phosphate coatings and its influence on mechanical and biological properties of the coatings. In addition, the antimicrobial mechanism and biocompatibility of the silver-containing calcium phosphate coatings were systematically investigated. The main findings obtained from the various research projects that led to the final coating are that the deposition parameters significantly influence the morphology of the coatings, which in turn

affects the cellular behavior and the mechanical properties of the coatings. In addition, the chemical state of the silver in the silver-containing calcium phosphate coatings plays a remarkable role in the antimicrobial activity and the biocompatibility of the coatings.

In **chapter 3**, the influence of deposition parameters (i.e., applied voltage and current density, H_2O_2 concentration in the electrolyte solution, deposition temperature and time) on the chemical composition and the morphology of the deposited Ca-P coatings was investigated. It was found that the type of the applied voltage and current density highly affect the structure of the coatings. Ca-P coatings deposited at pulsed mode are homogeneous with excellent adhesion to the substrate. However, the surface of the coatings deposited at constant voltage is inhomogeneous with delamination, due to the hydrogen evolution and the concentration gradient. Nevertheless, for both pulsed and constant modes, the deposited coatings consist of hydroxyapatite and octa-calcium phosphate, and the mode of the applied voltage does not change the chemical composition of the Ca-P coating. However, considering the quality and homogeneity of the coating structure, the pulsed mode is preferred for depositing Ca-P coatings. At low pulsed voltage (-1.1 V) and with low concentration of H_2O_2 (0.1 wt.%) coatings consist of brushite, octa-calcium phosphate and hydroxyapatite. By increasing the applied voltage and the concentration of H_2O_2 , brushite formation is restricted and the coatings consist of only hydroxyapatite and octa-calcium phosphate phases. At fixed H_2O_2 concentration, altering the applied voltage does not affect the phase composition of coatings, but at higher applied voltages, coatings are non-uniform with defects due to hydrogen evolution; and this effect is exacerbated at higher H_2O_2 concentrations. Therefore, the applied voltage and H_2O_2 concentration are dependent parameters. Considering the quality, morphology, and chemical composition of the coatings, the best condition for Ca-P deposition is -1.4 V and 1.5 wt.% H_2O_2 . The chemical composition of the Ca-P coatings is not affected by neither the deposition temperature nor deposition time. However, at high deposition temperatures (75 and 85 °C), the coatings have a more porous structure with very thin needles. Furthermore, by increasing the deposition time (from 1 to 30 min), the surface morphology of the Ca-P coatings is significantly influenced going from smooth to plate-like and ribbon-like structures.

The nucleation and growth mechanism of electrodeposited Ca-P coatings was the main objective of **chapter 4**. According to our findings, crystal growth of the Ca-P coatings is a time-dependent process. During the course of deposition, the nucleation and growth mechanism of the Ca-P coatings changes and leads to different morphologies at different stages of the deposition. At the first stage of the deposition ($t = 1$ min), the electrolyte is highly supersaturated, which results in a high

nucleation rate. In this stage, the deposited Ca-P coatings are composed randomly oriented and highly branched nanoplates of polycrystalline hydroxyapatite. In the second stage of the deposition ($t = 3$ min), the deposited Ca-P crystals grow along the b and c axes and form micro-sized plates. In the third stage of the deposition ($3 \text{ min} < t < 30 \text{ min}$), due to the increase in the distance between the front of depositing layer and the cathode surface, the degree of the supersaturation decreases. As a result, the deposited Ca-P crystals propagate along the c -axis and become ribbon-like single crystals.

Chapter 5 reported the influence of the morphological changes on the mechanical and biological properties of electrodeposited Ca-P coatings. Micro-scratch tests revealed that the coating with smooth morphology has a strong bonding with the substrate, and also acts as a solid lubricant. It was found that the morphology and thickness of the coatings do not have an influence on the adherence of the Ca-P to the Ti substrate, and the failure mode in all of the coatings is cohesive failure. However, the thicker coating delays plastic deformation of the underlying titanium substrate. Regarding the cellular activities, the differences in surface morphology of Ca-P coatings greatly affect the osteosarcoma cells' behavior. The relatively smooth Ca-P surfaces are beneficial for cellular activities and induce better cell adhesion, viability, and proliferation than the ribbon-like, spiky rough surfaces. The improved cell activity on smooth surfaces can be attributed to the larger contact area. On the other hand, the presence of sharp needles and ribbons on the rough surfaces inflict physical damage onto the cells and restrict proper cell adhesion, which in turn inhibits cell proliferation and viability. Differences in the surface morphology do not induce remarkable changes in the level of osteoblast-relevant gene expression, namely Alkaline phosphatase, Type I collagen, and Osteopontin. In conclusion, osteosarcoma cells are found to be more viable on the smooth Ca-P coatings and therefore, the better cell/tissue compatibility along with desirable mechanical properties make these coatings an optimal candidate for biomedical applications.

The antimicrobial activity and cytotoxicity of the silver-containing calcium phosphate coatings were investigated in **chapter 6**. Two different deposition approaches were explored: one labelled as Ag/Ca-P(1) coating, where micro-sized silver phosphate particles were embedded inside the Ca-P matrix, consequently silver is presented in the form of ions. In the second approach (Ag/Ca-P(2)), silver is presented as metallic nanoparticles on the Ca-P coating. The antimicrobial mechanism of the Ag/Ca-P(1) coating is leaching killing due to the high release rate of silver ions, that causes the bacteria reduction of $76.1 \pm 8.3\%$. The antimicrobial mechanism of the Ag/Ca-P(2) coating is mainly contact killing, and results in a

bacteria reduction of $83.7 \pm 4.5\%$. Moreover, a PBS treatment can improve the bacteria reduction of Ag/Ca-P(1) and Ag/Ca-P(2) coating to $97.6 \pm 2.7\%$ and $99.7 \pm 0.4\%$, respectively. The improvement of antimicrobial activity after PBS treatment can be attributed to the formation of $\text{AgCl}_x^{(x-1)-}$ species, which are very soluble. The formation of the $\text{AgCl}_x^{(x-1)-}$ species on the silver phosphate and the silver nanoparticles results in high silver release rate and leaching killing. The biocompatibility assay reveals that the Ag/Ca-P(1) coating is cytotoxic towards the osteosarcoma cells. In contrast, the Ag/Ca-P(2) coating shows excellent biocompatibility. Finally, it can be concluded that the electrochemically deposited silver-containing calcium phosphate coatings containing silver nanoparticles have excellent antimicrobial activity as well as good biocompatibility, which can be applied on titanium medical implants.

7.2 Outlook

The outcome of this study about electrochemically deposited bioactive and antimicrobial coatings for biomedical application is positive and exciting. We successfully synthesized the coatings on titanium and investigated the effect of different deposition parameters on the properties of the coatings. We identified the optimum deposition parameters according to the structure, morphology, chemical composition, mechanical and biological properties of the coatings. In addition, the role of the chemical state of silver for antimicrobial activity and biocompatibility of the coatings was comprehensively investigated and the antimicrobial mechanism of the coatings was also evaluated. Nevertheless, further research may be considered along some potential lines as follows.

- According to the classical nucleation theories, the nucleation rate is a function of nucleation barrier, which is in turn a function of temperature and supersaturation (Eq. 4-1 and 4-2) [7]. In this thesis, the nucleation and growth mechanism of Ca-P crystals during the electrochemical deposition as a function of electrolyte supersaturation was studied in detail. However, studying the influence of the temperature on the nucleation and growth mechanism of Ca-P crystals is also important for providing a comprehensive guideline for the growth mechanism of Ca-P crystals during the electrochemical deposition.
- From chapter 5, it can be concluded that the Ca-P coatings have a strong adhesion strength to the Ti substrates and after scratch test the failure mode is cohesive failure. One of the most suitable solutions to improve the cohesive adhesion of the coatings is reinforcement of a second phase material such as hard ceramics, polymers and bio-glasses. Carbon nanotubes

are an attractive reinforcement for Ca-P compounds because of their high stiffness and mechanical strength. In recent years a number of studies have explored the efficiency of CNT reinforcement in strengthening HA. The composites of HA/CNT are mainly synthesized via plasma spraying, electrophoretic deposition and spark plasma sintering [8]. Depositing Ca-P/CNT composite coatings via electrochemical deposition to enhance the cohesive adhesion of the coatings is still a challenge that needs more investigation.

- The structure of the bone is composed of natural anisotropic composites that consist of apatite embedded in collagen matrix [9]. Apatite belongs to the family of calcium phosphate compounds, which, indeed, is the main motivation to coat the metallic implants with calcium phosphate compounds. However, in order to mimic the exact structure of the bone, composite coatings of calcium phosphate with collagen are interesting candidates. The deposition of calcium phosphate with collagen can be done simultaneously or a layer of collagen can be deposited on the top of the calcium phosphate coating. These combinations can be highly beneficial to improve the biocompatibility and bioactivity of the coatings. Although, some initial attempts have been made to investigate this possibility [10,11], further analyses and method development are needed to assess the validity of this hypothesis.
- In chapter 6, we evaluated the antimicrobial mechanism and biocompatibility of silver-containing calcium phosphate coatings. There are still some issues that require further analysis. For example, we still do not have a clear idea what main interactions there are between the bacteria and silver ions or silver nanoparticles. Bacteria may be killed through the attachment of the silver nanoparticle to the cell wall, which can penetrate the cytoplasm and eventually cause cell death [12]. It is also possible that silver ions bind and react with electron-donating groups such as amino, carboxyl and thiol groups and weaken the stability of the outer membrane and thereby cause cell death [4,13]. Still another mechanism could be the generation of reactive oxygen-containing species, such as superoxide radicals. A very high level of reactive oxygen species (ROS) cause very high oxidative stress that results in the cellular inactivation [12]. In addition, it would also be interesting to investigate the antimicrobial properties of the of silver-containing calcium phosphate coatings against Gram-negative bacteria such as *E. coli*.

- Antimicrobial biomaterials need to be customized according to the specific clinical application. Although the clinical application of coated titanium implants is growing, the clinical failure has attracted significant attention. The translation of silver-containing calcium phosphate coatings to clinical applications needs not only the development of safe, cost-effective, simple, and eco-friendly synthesis method, but also a comprehensive understanding of interactions between biomaterial and cellular behavior. In other words, because of the complex nature of natural cellular microenvironment, it is necessary to understand and explore the combined effects of biomaterial and the bioenvironment to successfully translate the silver-containing calcium phosphate coatings towards clinical applications such as dental and orthopedic implants.

References

- [1] S. Spriano, S. Yamaguchi, F. Baino, S. Ferraris, A critical review of multifunctional titanium surfaces: New frontiers for improving osseointegration and host response, avoiding bacteria contamination, *Acta Biomater.* 79 (2018) 1–22.
- [2] Y. Zhou, C. Wu, J. Chang, Bioceramics to regulate stem cells and their microenvironment for tissue regeneration, *Mater. Today.* 24 (2018) 41–56.
- [3] C. Marambio-Jones, E.M.V. Hoek, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment, *J. Nanoparticle Res.* 12 (2010) 1531–1551.
- [4] C. Shi, J. Gao, M. Wang, Y. Shao, L. Wang, D. Wang, Y. Zhu, Functional hydroxyapatite bioceramics with excellent osteoconductivity and stern-interface induced antibacterial ability, *Biomater Sci.* 4 (2016) 699–710.
- [5] M. Shirkhazadeh, Direct formation of nanophase hydroxyapatite on cathodically polarized electrodes, *J. Mater. Sci. Mater. Med.* 9 (1998) 67–72.
- [6] N. Eliaz, T.M. Sridhar, Electrocrystallization of hydroxyapatite and its dependence on solution conditions, *Cryst. Growth Des.* 8 (2008) 3965–3977.
- [7] H. Jiang, X.Y. Liu, Principles of mimicking and engineering the self-organized structure of hard tissues, *J. Biol. Chem.* 279 (2004) 41286–41293.
- [8] D. Lahiri, S. Ghosh, A. Agarwal, Carbon nanotube reinforced hydroxyapatite composite for orthopedic application: A review, *Mater. Sci. Eng. C.* 32 (2012) 1727–1758.
- [9] M. Sadat-Shojai, M.-T. Khorasani, E. Dinpanah-Khoshdargi, A. Jamshidi, Synthesis methods for nanosized hydroxyapatite with diverse structures, *Acta Biomater.* 9 (2013) 7591–7621.
- [10] A. Tozar, İ.H. Karahan, A comprehensive study on electrophoretic deposition of a novel type of collagen and hexagonal boron nitride reinforced hydroxyapatite/chitosan biocomposite coating, *Appl. Surf. Sci.* 452 (2018) 322–336.
- [11] G. Ciobanu, M. Harja, Cerium-doped hydroxyapatite/collagen coatings on titanium for bone implants, *Ceram. Int.* 45 (2019) 2852–2857.

- [12] B. Le Ouay, F. Stellacci, Antibacterial activity of silver nanoparticles: A surface science insight, *Nano Today*. 10 (2015) 339–354.
- [13] J. Hasan, R.J. Crawford, E.P. Ivanova, Antibacterial surfaces: The quest for a new generation of biomaterials, *Trends Biotechnol.* 31 (2013) 295–304.

