

BIOLOGICAL AND MECHANICAL RESEARCH OF TITANIUM IMPLANTS COVERED WITH BACTERICIDAL COATING

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Abstract

Materials used in bone implants should not only be non-toxic to the surrounding tissues, but also should promote osseointegration and minimize the risk of infection. Infections are a serious problem contributing to implantation failure. They are associated with pain, immobilization, and the necessity of reoperation. In extreme cases, they can lead to significant inflammatory changes in the bones, which, in turn, can lead to amputation and even death. After implantation, the surrounding tissues are damaged. In addition, implants are susceptible to bacterial colonization due to the lack of microcirculation. Therefore, scientists are working on antibacterial coatings to prevent the adhesion of bacteria before tissue regeneration.

The paper concerns the biological and mechanical properties of titanium implants with an antibacterial coating. The Ti13Zr13Nb alloy samples were coated with hydroxyapatite (HAp) coatings using the electrophoretic deposition technique (EPD). Subsequently, the surface of the samples was modified with silver, copper, and nickel nanoparticles by the immersion method. Different titanium sample types (i.e. HAp-only and nanometals-enriched coatings) were placed in a bacterial solution for a period of one month. Each sample was examined using scanning electron microscopy (SEM), nanoindentation, nanoscratch, and contact angle tests. The significant amount of dead biofilm on the surface proves the effectiveness of antibacterial activity. The wettability assessment showed that the samples were hydrophilic. The conducted tests of mechanical properties indicate the heterogeneity of the coatings.

Keywords: nanometals, scanning electron microscopy, wettability, nanoindentation, antibacterial properties, mechanical properties

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Introduction

Infections are a serious problem that contributes to implant failure. They are associated with pain, immobilization, and also with repeated surgery. In extreme cases, they can lead to significant bone inflammation, which, in turn, may result in amputation or even death. Sources of infectious bacteria include the environment of the operating room, surgical equipment, clothing worn by the medical and paramedical staff, resident bacteria on the patient's skin and bacteria already residing in the patient's body [1].

Implants are particularly susceptible to bacterial colonization due to damage to the microcirculation. Microcirculation plays an important role in the immune system's defence response and antibiotics delivery. Therefore, if bacterial adhesion occurs before tissue regeneration, the host's immune system is often unable to prevent the colonization of microorganisms producing a biofilm matrix. The inhibition of microbial adhesion is essential to prevent postoperative infections [1].

Numerous implant-related infections are caused by staphylococci (roughly four out of five), and two single staphylococcal species, i.e. *Staphylococcus aureus* and *Staphylococcus epidermidis*, account for two out of three infection isolates. They represent, in absolute, the main causative agents in orthopaedics [1].

Biofilms are bacterial communities growing together embedded in an extracellular matrix which is a fundamental structural component of the bacterial community and acts as a protective shield [2]. Microorganisms contained in a biofilm can demonstrate up to 1000 times greater resistance to antibiotics compared to organisms of the same species in the form of plankton. In addition, a biofilm has significantly greater resistance to disinfectants ensured by a complex, cross-linked, hydrophobic structure with reduced gas permeability. The biofilm mode of growth of infecting bacteria on an implant surface protects the organisms from the host immune system and antibiotic therapy [1-3].

Titanium and its alloys belong to the most developed metallic biomaterials used for long-term orthopaedic and dental implants. Among more than 40 alloys investigated so far, the Ti13Zr13Nb alloy has the most suitable mechanical properties, as compared to the most popular technical titanium alloys, Ti-6Al-4V and Ti-6Al-7Nb. The advantages of the Ti13Zr13Nb alloy include also the absence of potentially dangerous elements, such as Al and V, which are presumed to develop Alzheimer's disease and tissue disturbance [4]. The Ti6Al4V and Ti13Nb13Zr alloys have the elasticity modulus (114 GPa and 81.6 GPa, respectively [5]) most similar (from among metal biomaterials) to that of a human bone (18-19 GPa [6]). Moreover, additional modifications of the surface of titanium alloys enable appropriate osseointegration [7].

Bioceramics are the ceramic materials used to replace or regenerate damaged bone and muscular tissues in the human skeletal system. One of the most widely used bioceramics is calcium hydroxyapatite, due to its biocompatibility and mechanical properties close to that of a human bone [8]. Hydroxyapatite materials are of great interest to scientists. Due to their excellent biocompatibility, osteoconductive properties and similarity to the inorganic component of human bone, they are widely used as biomedical materials, e.g. as bone fillers, scaffolds in tissue engineering, bioactive coatings, drug, protein, and gene delivery systems [9].

Being a calcium-based ceramic compound, hydroxyapatite exhibits brittleness, a very high compressive strength (up to 917 MPa), and a very low tensile property. Young's modulus and Poisson's ratio of sintered HAp are directly proportional to its density (porosity). If density increases by 100%, Young's modulus can reach 117 GPa and fracture toughness can reach 1 MPa/m² [8]. Hydroxyapatite coatings can be deposited on titanium substrates using many techniques. The most popular technique is electrophoretic deposition, in which whole HAp particles dispersed in organic alcohol achieve the electric charge, allowing them to deposit on an electrode as a very thin layer [4]. Other methods involve precipitation, the sol-gel method, and the hydrothermal method. Hydroxyapatite has also antibacterial properties, affecting both gram-negative bacteria and gram-positive bacteria thanks to its ability to penetrate the bacterial cell wall through electrostatic action. Hydroxyapatite nanoparticles (nanoHAp) have a wide spectrum of antibacterial activity and can potentially be used in medicine and environmental protection [10]. HAp is used in bone tissue engineering alone or can be doped with various metallic or non-metallic dopants to tailor its properties. Substitution enhances the properties of modified HAp depending on the properties of a dopant [8]. Despite numerous advantages, a noticeable disadvantage of bioceramic coatings (e.g. HAp, nanoHAp) deposited on metallic implants is their tendency to crack and poor adhesion to the substrate. Modifications of the chemical composition of applied coatings can be considered one of the possibilities to alleviate this problem [11].

To ensure the best properties of the material, scientists modify the surface of implants, often using materials in nanometric scale. The use of nanomaterials and nanostructures has revolutionized many fields of medicine and has led to a significant expansion of the list of implantable biomaterials and medical devices. Nanometals are often used due to their unique properties, including antimicrobial and anti-inflammatory ones [12]. Postoperative treatment following implantation is carried out by administering antibiotics. Similar effects can be achieved by the addition of some nanometals, operating as biocidal agents, to the coating.

Silver is usually applied directly to the implant surface as a pure metal or nanosilver [13]. Geissel et al. proved the antibacterial properties of silver nanoparticles using a scanning electron microscope. They showed that *Staphylococcus aureus* bacteria grown on pure SiO₂ had an intact and globally shaped shell, while the samples enriched with silver revealed a damaged cell structure of the surface of the bacteria attached to the particles [14].

The emerging problem of multi-drug resistance and its consequences stimulate researchers to search for effective alternatives to fight biofilm formation. Although the antibacterial properties of materials such as silver, nickel, and copper have been confirmed, it is recommended to create synergistic composite materials as bacterial pathogens can effectively develop resistance against metal nanophases. Therefore, it is very important to improve and maintain their antibacterial potential [15].

Despite the significant advantages of such approach, there is still a danger of negative effects on the human body. In an *in vitro* toxicity study of silver nanoparticles, even low exposure to silver nanoparticles induced oxidative stress and impaired mitochondrial functions. *In vivo* studies in rats on oral toxicity of nanosilver showed that nanosilver accumulated in the liver [16].

Copper is known not only for its high electrical and thermal conductivity but also for its antimicrobial properties. It has long been used as an effective antibacterial, antiviral, and antifungal agent. Copper nanoparticles are of interest for their excellent chemical, physical and optical properties, heat transfer, large surface area to volume ratio, as well as catalytic, magnetic, and biological properties [17-19].

Studies of antimicrobial activity have shown that copper oxide nanoparticles are effective as antimicrobial agents for both gram-positive and gram-negative bacteria. Bacteria have cell membranes with nanometer-sized pores which facilitate the penetration of nanoparticles. This is the cause of cytoplasm degradation, which ultimately leads to cell death. The antimicrobial mechanism is mainly attributed to the strong adsorption of ions to bacterial cells, which confers antimicrobial efficacy in a concentration-dependent manner [20].

Argueta-Figueroa et al. [20] synthesized nanoparticles of copper, nickel, and bimetallic Cu-Ni using a chemical method. Their antibacterial activity has been tested against the commonly used standard human pathogens *Staphylococcus aureus* (gram negative), *Escherichia coli* (gram positive), and additionally *Streptococcus mutans*. *Streptococcus mutans* is physiologically present in the human mouth and contributes to tooth decay. Studies have shown that copper nanoparticles exhibit bactericidal activity against *S. aureus*, *E. coli*, and *S. mutans*, while nickel nanoparticles and bimetallic Cu-Ni nanoparticles only exhibit bacteriostatic activity against the same microorganisms [20].

The paper deals with the analysis of hydroxyapatite (HAp) coatings (without additives and with nanometals) on the titanium alloy. The aim of the study is to compare the biological and mechanical properties of the samples.

Materials and Methods

Preparation of samples

The Ti13Zr13Nb alloy was used as a substrate. The surface was prepared by using abrasive paper SiC up to grit # 2500. The samples were then rinsed with isopropanol and ultrasonically cleaned.

Electrophoretic deposition of HAp coatings

The Ti13Zr13Nb alloy samples were covered with hydroxyapatite coatings by the electrophoretic technique. The EPD was carried out in a suspension prepared by dispersing 0.1143 g of HAp powder (Sigma-Aldrich) in 100 ml of ethanol (anhydrous, 99.8% purity). The EPD was performed at 8 V for 10 min at room temperature. The hydroxyapatite solution was mixed with a magnetic stir bar for 4-5 min prior to each sample procedure. Finally, the deposited coatings were air dried at room temperature for 24 h. Subsequently, the samples were sintered in a vacuum oven for 2 h at 500°C.

Immersion method

The surface of the samples was modified with silver, copper, and nickel nanoparticles using the immersion method. 0.005 g of nanoparticles were dispersed in 50 ml of 99.8% anhydrous alcohol. The size of the nanoparticles is shown in TABLE 1.

TABLE 1. Size of nanoparticles.

Nanoparticles	Size [nm]
Ag	40
Cu	10-30
Ni	10-30

Initiation of bacterial growth

All samples were immersed in a bacterial solution [21] for 30 days. The chemical composition of the solution is presented in TABLE 2, and the list of the added microorganisms in TABLE 3.

TABLE 2. Chemical composition of the bacterial solution [21].

Component	Content [g/dm ³]
Casein peptone	17
Pepton S	3
NaCl	5
Na ₂ HPO ₄	2.5
Glucose	2.5

TABLE 3. List of bacteria added to the solution [21].

Bacteria	Content in liquid [%]
Staphylococcus aureus	20
Staphylococcus epidermidis	20
Enterococcus faecalis	15
Enterobacter cloacae	10
Pseudomonas aeruginosa	35

Surface examination

The morphology of the samples was investigated by a Schottky field emission scanning electron microscope (FEI Quanta FEG 250) with an ET secondary electron detector. The beam accelerating voltage was kept at 10 kV. For the purpose of elemental analysis, energy dispersive X-ray spectroscopy was performed with the EDAX Genesis APEX 2i with the ApolloX SDD spectrometer.

Measurements of contact angle

Eight samples of titanium alloy with a hydroxyapatite coating were tested with the Attension Thete Lite goniometer to assess the wettability of the materials. A drop of water was dropped onto the sample surface. The computer program calculated the contact angle (CA) from the shape of the drop of water immediately after its contact with the surface of the sample, then the measurement was after 5 s and 10 s. The surface of the material is considered hydrophilic if the measured value is below 90° and hydrophobic above 90°.

Mechanical studies - nanoindentation and nanoscratch tests

Nanoindentation tests were performed using the NanoTest™ Vantage device. A Berkovich diamond indenter with an apex angle of 124.4° was used for the analysis. The parameters of the nanoindentation tests are shown in TABLE 4.

TABLE 4. The parameters of the nanoindentation tests.

Parameter	Value
Number of measurements	15
Maximum force [mN]	10
Loading time [s]	10
The dwell period at maximum load [s]	5
Unloading time [s]	10

The modulus of elasticity was calculated after transforming the formula [7]:

$$\frac{1}{E_r} = \frac{1 - \nu_s^2}{E_s} + \frac{1 - \nu_i^2}{E_i} \quad (1)$$

where ν_i is the nanoindenter Poisson's ratio (accepted as 0.07) [7], ν_s is the Poisson's ratio of the tested materials (accepted for HAp as 0.3 [4] and 0.27 for titanium alloy [7]), E_s is the elastic modulus of the tested samples, E_i is the Young's modulus of the nanoindenter (accepted as 1140 GPa) and E_r is the reduced modulus of elasticity [7]. The final equation from which the reduced Young's modulus was determined is:

$$E_s = \frac{E_i E_r (1 - \nu_s^2)}{E_i - E_r (1 - \nu_i^2)} \quad (2)$$

Nano-scratch tests were performed with the NanoTest™ Vantage (Micro Materials). The scratch tests were carried out by increasing the load from 0 mN to 200 mN at a loading rate of 1.3 mN/s at a distance of 500 μm. The adhesion of the coating was assessed based on the observation of an abrupt change in frictional force during the test.

Results and Discussions

Surface examination using a scanning electron microscope

Samples of Ti13Zr13Nb titanium alloy coated with hydroxyapatite with metal nanoparticles were examined using a scanning electron microscope. The received images are shown in FIG. 1.

The scanning electron microscope study showed numerous nanoparticles on the surface. The distribution of the nanoparticles was heterogeneous in the case of Ag and Cu. They also formed numerous agglomerates. The sample with nickel nanoparticles was characterized by the highest homogeneity of the nanoparticles distribution.

The SEM indicated changes in the topography of the implant surface with visible singular bacteria and biofilm colonies. The biofilm showed a tendency to capsulate the nanoparticles, but the cell structure of the bacteria on the surface of the nanoparticles was damaged. This phenomenon was also observed in the studies of the antibacterial properties of silver nanoparticles on SiO₂. Geissel et al. [14] detected that samples enriched with silver showed a damaged cell structure of the *Staphylococcus aureus* bacteria attached to the surface of the particles. The significant dead biofilm proved the antibacterial properties of the coatings. Bartmański et al. [4] presented high magnification SEM images that demonstrate the porous structure of the nanoHAp coatings. For nanoHAp coatings obtained at 0.1 g nanoHAp powder in the solution, the coatings roughness decreased with the increasing voltage, due to the presence of a smaller number of agglomerates on the surface.

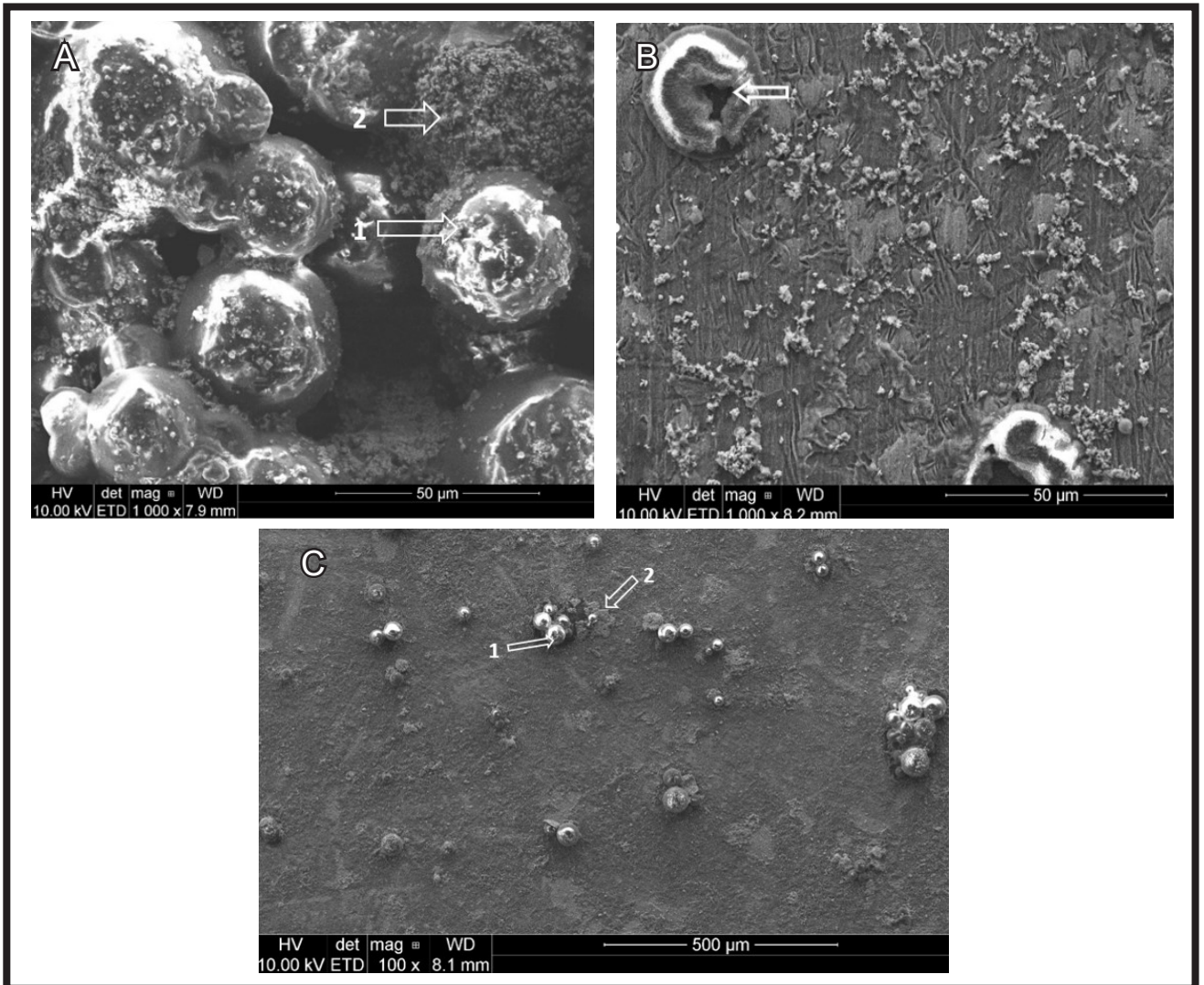


FIG. 1. Surfaces of the HAp coatings with the nanoparticles after 1 month in the bacterial solution. A) Image of the surface of titanium alloy with deposited HAp and silver nanoparticles, visible agglomerates of silver nanoparticles and a living (1) and dead biofilm (2). B) Surface image of titanium alloy with deposited HAp and nickel nanoparticles - visible Ni nanoparticles. C) Surface image of titanium alloy with deposited HAp and copper nanoparticles with visible nanoparticles (1) and dead biofilm (2).

Measurements of contact angle

TABLE 5 presents the results of the contact angles obtained for the tested materials; the results were averaged over 4 measurements. The volume of the drop of water was 4 μ l.

The wettability assessment showed that the samples were hydrophilic, which is consistent with the literature data. The lowest contact angle was observed for the sample with the additive-free hydroxyapatite coating (0°) and the highest contact angle occurred for the copper nanoparticles enriched implant (17.94°). The contact angle values of the hydroxyapatite samples with silver and nickel nanoparticles were 8.88° and 3.33° , respectively.

The nanoparticles significantly increase the value of the contact angle, which is consistent with the literature data [13]. Scientists concluded that the presence of silver nanoparticles on the surface may prevent water droplets from penetrating into the nanoHAp coating, resulting in an increase in the contact angle [13].

The low contact angle values for all specimens may be attributed to their porous surface structure. Contact angles are measures of wettability; their low values correspond to better osseointegration. On the other hand, the best values of contact angle for cell attachment were assessed at 55° and for bone regeneration at 35° to 80° [13]; these values are higher than those obtained in the presented studies.

TABLE 5. Results of the obtained contact angles for the samples as a function of time.

Time [s]	CA mean for the sample without nanoparticles [$^\circ$]	CA mean for the sample with Ag nanoparticles [$^\circ$]	CA mean for the sample with Cu nanoparticles [$^\circ$]	CA mean for the sample with Ni nanoparticles [$^\circ$]
0	67.77 ± 32.98	40.42 ± 17.0	24.35 ± 24.56	38.26 ± 4.6
5	29.57 ± 33.46	12.96 ± 8.0	50.44 ± 24.40	13.62 ± 4.92
10	0 ± 0	8.88 ± 7.3	17.94 ± 22.82	3.33 ± 5.76

Mechanical studies - nanoindentation and nanoscratch tests

FIG. 2 shows one of the nanoindentation load-displacement curve for the examined materials. The nanoindentation tests were performed for all HAp coatings with nanometals and for the titanium samples only after grinding. The mechanical properties - hardness and Young's modulus - are shown in TABLE 6. 15 nanoindentation measurements were pursued.

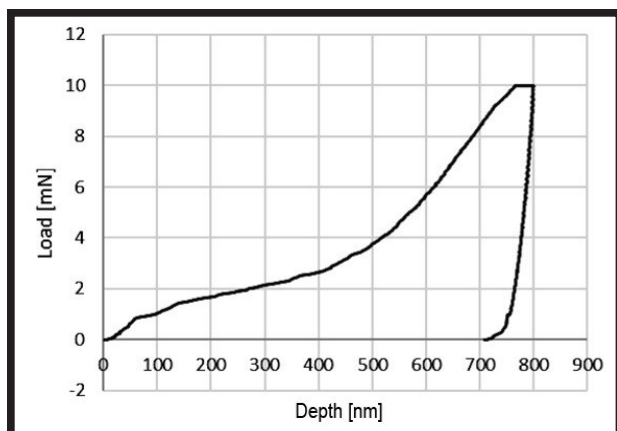


FIG. 2. Nanoindentation load-displacement curve obtained for HAp coating with Ni nanoparticles.

The nanoscratch tests were performed for HAp coating with nanometals. 5 measurements were made for each sample. The obtained results are shown in TABLE 7 based on the diagram of the depth change depending on the distance. First, the distance at which the coating was delaminated was determined, then the appropriate normal force for the distance covered by the tool was determined. The graphs of changes in surface topography before and after the nanoscratch tests are presented in FIG. 3.

TABLE 6. Mechanical properties: hardness and Young's Modulus of samples.

Material	Hardness [GPa]	Young's modulus [GPa]
HAp with Ag nanoparticles	1.95 ± 1.10	74.88 ± 27.15
HAp with Cu nanoparticles	0.82 ± 0.34	55.13 ± 13.88
HAp with Ni nanoparticles	1.30 ± 1.10	80.66 ± 26.54

TABLE 7. Results of nanoscratch tests of the HAp coatings with nanometals.

Measurement	HAp with Ag nanoparticles		HAp with Cu nanoparticles		HAp with Ni nanoparticles	
	Distance [μm]	Load [mN]	Distance [μm]	Load [mN]	Distance [μm]	Load [mN]
1	64	25.6	13	5.2	47	18.8
2	86	34.4	39	15.6	108	43.2
3	82	32.8	68	27.2	100	40
4	80	32	75	30	100	40
5	80	32	68	27.2	119	47.6
average		31.36 ± 3.01		21.04 ± 9.35		37.92 ± 9.96

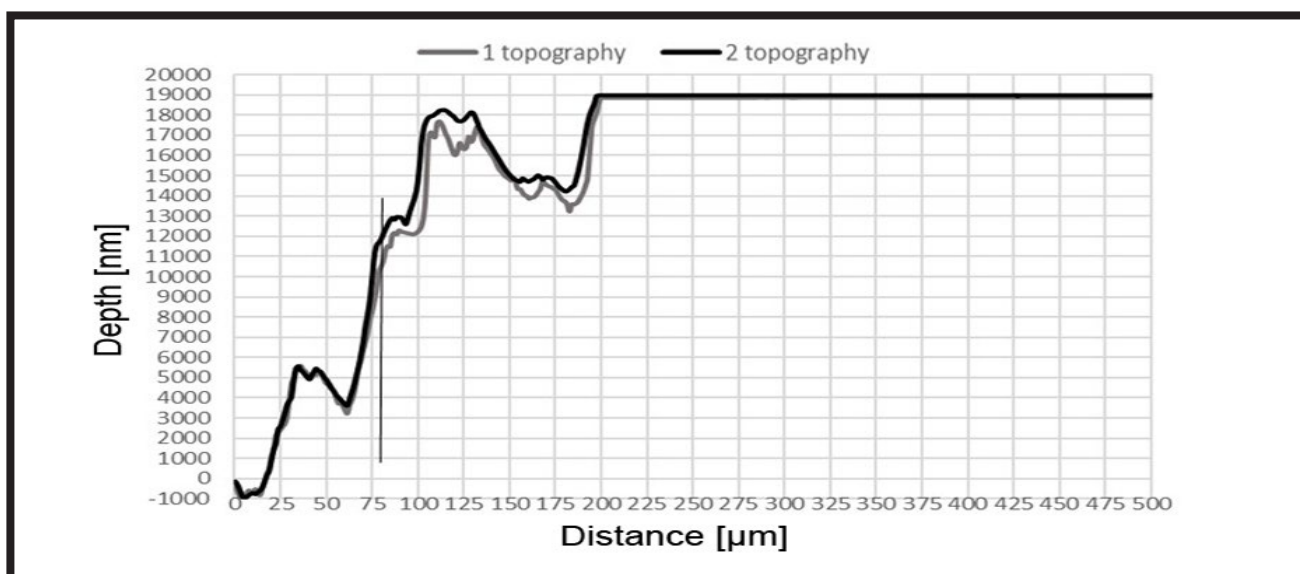


FIG. 3. Nanoscratch test of HAp coating with Ag nanoparticles. The delamination of the coating is marked in the graph (the black line).

The conducted tests of mechanical properties indicate the heterogeneity of the coatings due to the large standard deviation of the obtained hardness results, which is associated with high surface roughness. The greatest heterogeneity was revealed for the hydroxyapatite coating with nickel nanoparticles (1.30 ± 1.10 GPa), and the lowest for the hydroxyapatite coating with copper nanoparticles (0.82 ± 0.34 GPa). The coatings are characterized by low hardness; this value depends on the thickness of the coating (the greater the thickness, the harder the coating). The most advantageous Young's modulus was observed for the hydroxyapatite coating with copper nanoparticles (55.13 ± 13.88 GPa) and its value was the closest to the Young's modulus of bone. Young's modulus result was the highest for HAP with Ni nanoparticles and amounted to 80.66 ± 26.54 GPa. Bartmański et al. [11] observed that Ag nanoparticles addition had negligible influence on the coating's mechanical properties. Still, the presence of Cu nanoparticles, alone or with Ag nanoparticles, resulted in the increased value of hardness and Young's modulus. The positive impact of Cu nanoparticles may be attributed to somewhat decreasing porosity and susceptibility to brittle cracking.

The adhesion of coatings to the metallic substrate is one of the most essential properties determining the quality of the coatings. The loose particles in HAP coatings may even initiate inflammation process and disappearance of bone in its surroundings [4]. The sample with nickel nanoparticles had the best coating adhesion, where the force at which delamination occurred was 37.92 ± 9.96 mN. The smallest force causing delamination of the coating was observed for the sample with copper nanoparticles.

Conclusions



The Ti13Zr13Nb alloy samples were covered with hydroxyapatite coatings through the electrophoretic deposition. The samples surface was modified with silver, copper, and nickel nanoparticles. Eight titanium samples (i.e. an additive-free hydroxyapatite coating, a silver nanoparticles enriched implant, a copper nanoparticles enriched implant and a nickel nanoparticles enriched implant), were placed in a bacterial solution for a period of one month. Each sample was examined using a scanning electron microscope. Wettability and mechanical properties were also assessed.

The significant dead biofilm in their vicinity proves the effectiveness of antibacterial activity. All of the samples had visible high surface roughness, which was confirmed in nanohardness tests using the nanoindentation method. The coatings were characterized by a low hardness (this value depends on the thickness of the coating). The most advantageous Young's modulus was observed in the case of the hydroxyapatite coating with copper nanoparticles, as its value is most similar to the Young's modulus of bone. The sample with nickel nanoparticles had the best coating adhesion. All the samples were hydrophilic, which is associated with possible high osteointegration. In addition, the nanoparticles increased the contact angle value.

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References

- [1] Ribeiro M., Monteiro F. J., Ferraz M. P.: Infection of Orthopedic Implants with Emphasis on Bacterial Adhesion Process and Techniques Used in Studying Bacterial-Material Interactions. *Biomater* 2 (2012) 176-194.
- [2] Cendra M. del M., Torrents E.: Pseudomonas Aeruginosa Biofilms and Their Partners in Crime. *Biotechnol Adv* 49 (2021) 107734.
- [3] Vickery K.: Special Issue: Microbial Biofilms in Healthcare: Formation, Prevention and Treatment. *Materials* 12 (2019) 3-5.
- [4] Bartmański M., Zielinski A., Majkowska-Marzec B., Strugala G.: Effects of Solution Composition and Electrophoretic Deposition Voltage on Various Properties of Nanohydroxyapatite Coatings on the Ti13Zr13Nb Alloy. *Ceramics International* 44 (2018) 19236-19246.
- [5] Surma K., Adach M., Dębowska M., Turlej P.: Projektowanie i analiza obliczeniowa implantu krążka międzykręgowego odcinka szyjnego kręgosłupa przeznaczony do wytwarzania za pomocą technologii przyrostowych. *Aktualne problemy biomechaniki* 17 (2019) 111-122.
- [6] Świeczko-Żurek B.: *Biomateriały*; Wydawnictwo Politechniki Gdańskiej (2009) 72-97.
- [7] Jażdżewska M., Kwidzińska D., Seyda W., Frydrych D., Zieliński A.: Mechanical Properties and Residual Stress Measurements of Grade IV Titanium and Ti-6Al-4V and Ti-13Nb-13Zr Titanium Alloys after Laser Treatment. *Materials* 14 (2021) 1-22.
- [8] Panda S., Biswas C.K., Paul S.: A Comprehensive Review on the Preparation and Application of Calcium Hydroxyapatite: A Special Focus on Atomic Doping Methods for Bone Tissue Engineering. *Ceramics International* 47 (2021) 28122-28144.
- [9] Abdulkareem M.H., Abdalsalam A.H., Bohan A.J.: Influence of Chitosan on the Antibacterial Activity of Composite Coating (PEEK/HAP) Fabricated by Electrophoretic Deposition. *Progress in Organic Coatings* 130 (2019) 251-259.
- [10] Lin K., Chang J.: Structure and Properties of Hydroxyapatite for Biomedical Applications. In *Hydroxyapatite (Hap) for Biomedical Applications*; Woodhead Publishing (2015) 3-19.
- [11] Bartmański M., Pawłowski Ł., Mielewczyk-Gryń A., et al.: The Influence of Nanometals, Dispersed in the Electrophoretic Nanohydroxyapatite Coatings on the Ti13Zr13Nb Alloy, on Their Morphology and Mechanical Properties. *Materials* 14 (2021) 1-16.
- [12] Prasad K., Zhou R., Zhou R., Schuessler D., et al.: Cosmetic Reconstruction in Breast Cancer Patients: Opportunities for Nanocomposite Materials. *Acta Biomaterialia* 86 (2019) 41-65.
- [13] Bartmański M., Cieslik B., Glodowska J., et al.: Electrophoretic Deposition (EPD) of Nanohydroxyapatite - Nanosilver Coatings on Ti13Zr13Nb Alloy. *Ceramics International* 43 (2017) 11820-11829.
- [14] Geissel F. J., Platania V., Gogos A., et al.: Antibiofilm Activity of Nanosilver Coatings against Staphylococcus Aureus. *Journal of Colloid and Interface Science* 608 (2021) 3141-3150.
- [15] Vijayaraghavan P., Rathi M.A., Almaary K.S., et al.: Preparation and Antibacterial Application of Hydroxyapatite Doped Silver Nanoparticles Derived from Chicken Bone. *Journal of King Saud University - Science* 34 (2022) 101749.
- [16] Prabhu S., Poulouse E.K.: Silver Nanoparticles: Mechanism of Antimicrobial. *Int. Nano Lett.* 2 (2012) 32-41.
- [17] Huglot R., Titus W., Agnihotri A.S., Krishnakumar V., et al.: Stable Copper Nanoparticles as Potential Antibacterial Agent against Aquaculture Pathogens and Human Fibroblast Cell Viability. *Biocatalysis and Agricultural Biotechnology* 32 (2021) 101932.
- [18] Ingle A.P., Duran N., Rai M.: Bioactivity, Mechanism of Action, and Cytotoxicity of Copper-Based Nanoparticles: A Review. *Applied Microbiology and Biotechnology* 98 (2014) 1001-1009.
- [19] Ameh T., Sayes C. M.: The Potential Exposure and Hazards of Copper Nanoparticles: A Review. *Environmental Toxicology and Pharmacology* 71 (2019) 103220.
- [20] Argueta-Figueroa L., Morales-Luckie R.A., Scougall-Vilchis R.J., Olea-Mejia O.F.: Synthesis, Characterization and Antibacterial Activity of Copper, Nickel and Bimetallic Cu-Ni Nanoparticles for Potential Use in Dental Materials. *Progress in Natural Science: Materials International* 24 (2014) 321-328.
- [21] Świeczko-Żurek B.: Method of Assessing Biodegradation of Metallic Implants, Method of Obtaining a Bacterial Solution for Assessing Biodegradation of Metallic Implants and a Bacterial Composition for Assessing Biodegradation of Metallic Implants. Patent No. P 409082, 2015.