Ceramics-Silikáty 66 (3), 255-261 (2022)
www.ceramics-silikaty.cz doi: 10.13168/cs.2022.0020

TESTING OF ADHESION AND CYTOTOXICITY OF SOL-GEL COATINGS APPLIED BY DIP-COATING ON POROUS AND NON-POROUS TITANIUM

#DIANA HORKAVCOVÁ*, VERONIKA ZEMANČÍKOVÁ*, KRISTÝNA ŠVAGROVÁ*, EVA JABLONSKÁ**, ADRIANA BERNATIKOVÁ***, PAVEL NOVÁK***

*Department of Glass and Ceramics, Faculty of Chemical Technology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, Czech Republic
**Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 3, 166 28 Prague, Czech Republic
***Department of Metals and Corrosion Engineering, Faculty of Chemical Technology, University of Chemistry and Technology Prague, Technická 5, 166 28 Prague, Czech Republic

#E-mail: diana.horkavcova@vscht.cz

Submitted March 1, 2022; accepted April 5, 2022

Keywords: Porous titanium substrate, Sol-gel, Dip-coating, Adhesion, Tape test, Cytotoxicity test

The properties of existing biocompatible titanium implants can be improved by controlling the porosity of the substrate or surface modification, e.g., by coating. Two types of titanium substrates were used, made by pressing and the subsequent sintering of titanium powder with and without the pore-forming agent NH_4HCO_3, and they were compared with a commercial titanium metal sheet. Furthermore, the substrates were coated with a titanium sol enriched with calcium and phosphates using the dip-coating technology. The adhesion and cytotoxic properties were measured on the coatings with standard methods. The adhesion of the coatings to all types of substrates was measured with a tape test and evaluated as very good (5B). The contact and non-contact cytotoxicity tests confirmed that all the substrates with the coatings and without them were non-toxic to a mice fibroblast cell line (L929).

INTRODUCTION

In dental and surgical implantology of metal materials, research is currently focused on corrosion resistant steel, Co-Cr alloys and titanium substrates that can be applied in exposed places. Although pure titanium has been widely used, alloyed elements provide better results in dental and orthopaedic surgery. Two of the most widely applied titanium alloys are Nitinol, thanks to its elastic memory, and Ti-6Al-4V with excellent corrosion resistance [1-3]. After being implanted, titanium substrates interact with biological liquids and tissues and two reactions may occur. The first one includes the formation of a fibrous capsule around the implant (so-called encapsulation), which leads to its failure; the second reaction involves the direct contact of the implant with the bone without an intermediate layer (so-called osseointegration) [4]. In order to increase the osseointegration of titanium implants, their surfaces may be chemically or mechanically treated [4, 5] or highly porous structures can be developed - so-called scaffolds - with a porosity of 50 – 70 %, with controlled interconnected micro- and macro-pores [6, 7]. In order to create porous metal substrates with good qualities, the effect of sintering temperatures [8] or the addition of pore-forming additives and their concentrations have been tested [9, 10]. Another possibility to treat the surfaces of implants is the formation of coatings which may improve or change the properties of the existing substrates [11-14]. For porous implants with complicated shapes, it is convenient to use technically simple coating methods using, e.g., electrodeposition from solutions [15-17] or sol-gel methods [18-22] which guarantee the perfect coverage of the surface. In order to monitor the ability to precipitate apatite on the surface of a tested material, the so-called bioactivity is tested *in vivo* (in a living organism) and in *vitro* (in model liquids) [23-26]. In order to prevent bacterial infections; in the case of medicinal applications, Ag, Cu and Zn elements may be incorporated into the substrates or coatings that have a toxic effect on microorganisms. Surfaces treated in this way may either kill the bacteria or inhibit their growth [27, 28]. Silver is definitely the most frequently used element, mostly in the form of $AgNO_3$, even though the mechanisms of the antimicrobial action of silver ions are not properly understood. It is suggested that silver ions penetrate inside the bacterial cell, inactivate the membrane proteins, bind with the bacterial DNA

and disrupt DNA replication, impairing the ability of ribosomes to transcribe messenger RNA into the vital proteins required by the cell to function, inactivate cytochrome and consequently cause bactericidal activity [29-32]. The antibacterial effects of silver nanoparticles and ions have been tested against a wide range of gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis*) and gram-negative bacteria (*Escherichia coli*) [33-36]. The concentration of the released silver from the biomaterials into a living organism should not be toxic for cells and, therefore, cytotoxicity tests are performed most frequently on mice fibroblasts (L929) or on human osteosarcoma (HOS). In contact tests, the viability of cells is evaluated primarily by visual measurement (Scanning Electron Microscopy - SEM) on the surface of the tested material with the applied cells, while non-contact tests (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide - MTT method) monitor the viability of the cells in leachates of a culture medium in which the tested material was submerged [37-39]. The coating`s adhesion to substrates [40] or the shear strength [41] are equally important properties. If permitted by the shape and morphology of the biomaterial, the coating adhesion can be measured by standardised tests: the tape test [42], scratch test [43] or pull-out test [44]. The evaluation not only includes the numerical result of the test, but also includes the visual characteristic of the coating after the adhesion test is completed.

The objective of the work was to characterise and test the selected properties of two types of sol-gel titanium coatings applied by the dip-coating technology on newly developed titanium substrates of low and high porosity in comparison with a commercial titanium sheet. The porous structure and surface treatment of the materials not only increases their reaction surface, but may also support the better utilisation of metal substrates in a wide range of applications in orthopaedic and dental implantology.

EXPERIMENTAL

Three types of titanium substrates were used for the experiments. In order to prepare high porous (HP, high-porous) samples, a commercial titanium powder α-Ti 325 mesh (Strem Chemicals, Newburyport, MA, USA) was mixed with a 20 % pore-forming agent NH_4HCO_3, pressed at 400 MPa, annealed at 100 °C/4 hours and sintered at 800 °C/1 hour and at 1300 °C/4 hours. The low porous (LP, low-porous) samples were prepared from a pure titanium powder, pressed and sintered under the same conditions [45]. The third tested substrate (NP, non-porous) was a commercially produced ASTM Grade 2 titanium sheet. All the substrates were mechanically treated with SiC abrasive paper, with a grit size of P400, P600 and P800, in order to achieve a homogenous roughening of the surface. All the substrates were degreased and cleaned in acetone and ethanol in an ultrasonic bath for 10 minutes each.

The basic titanium sol (T) was prepared by mixing tetra-n-butyl orthotitanate, 1 mol·l^{-1} HNO_3, ethanol (denatured with benzine), triton X-100, acetyl acetone. The titanium sol containing calcium and phosphorus (TCP) was prepared from the basic titanium sol with ethanol solutions 2 mol·l^{-1} $Ca(NO_3)_2{\cdot}4H_2O$ and 2 mol·l^{-1} triethyl phosphate. The conditions of the dip-coating, drying and firing are described in [46, 47]. The composition of the sols and the identification of the individual substrates and coatings are provided in Table 1.

Table 1. Type of the titanium substrates, coatings and composition of the sols.

Type of the sample	Titanium substrate	Composition of the sol
NP-T	Non-porous	Basic titania sol
LP-T	Low-porous	
HP-T	High-porous	
NP-TCP	Non-porous	Basic titania sol with 2 mol·l^{-1} $Ca(NO_3)_2.4H_2O$ and 2 mol·l^{-1} triethyl phosphate
LP-TCP	Low-porous	
HP-TCP	High-porous	

The adhesion of the coatings to the substrates was tested with a tape test under the standard ASTM D 3359 02 [42]. A scalpel was used to make a lattice in the coating (parallel cuts 6 × 6, 1 mm apart). A special Permacel-99 tape was applied on the lattice which was peeled off after 60 seconds, approximately at an angle of 180°. After removal of the tape, the coating surface was inspected in the lattice area with an optical microscope (OM, Olympus BX 51, Japan, software Quick photo camera 3.1.). According to a classification table provided in the standard, the level of adhesion was evaluated based on the percentage of the peel-off area of the coating.

The extracts of the samples were tested for cytotoxicity according to the standard ISO 10993-5 (resazurin test for metabolic activity) [48]. The samples were sterilised in 70 % ethanol for 2 hours and then were incubated on an orbital shaker (120 RPM) for 24 hours in a MEM (Minimum Essential Medium) + 5 % Foetal Bovine Serum (FBS) and antibiotics at 37 °C. The surface-to-volume ratio was 1 ml of the medium per 1 - 1.2 cm^2 of the sample surface. L929 murine fibroblasts (ATCC® CCL-1™) were cultured under standard conditions in the MEM (Minimal Essential Medium, Sigma M0446) + 10 % FBS. The cells were seeded in 96-well plates at a density of 104 cells per well. After 24 hours, the medium was replaced by the extracts of the samples. The MEM with the 5 % FBS solution was used as a negative control and the medium without cells was used as a blank. The extracts were prepared from three samples and were performed in six replicates for each

extract. After 24 hours of incubation with the extracts, the cell metabolic activity was determined using the resazurin assay. The resazurin (final concentration of 25 $\mu g \cdot ml^{-1}$) in the MEM without phenol red and with 10 % FBS was added to each well. The fluorescence was measured (FLUOROSCSAN ASCENT™) after 2 hours of incubation (the excitation/emission wavelength being 560/590 nm). The percentage of the metabolic activity of the cells exposed to the extracts relative to the negative (untreated) control was evaluated. The extracts causing a decrease below 70 % of the activity of the control were considered cytotoxic, as described in the ISO standard.

Contact *in vitro* cytocompatibility tests were also performed. The sterilised samples were placed into 6- and 12-well cultivation plates and seeded with the L929 cells. The volume of the cell suspension in the medium (MEM + 10 % FBS + antibiotics) was 2.5 - 3.3 ml and the seeding density was 24,000 cells per cm^2. After 24 hours, the cells growing on the samples were rinsed with Phosphate buffered saline (PBS) (twice) and fixed with Karnovsky's fixative (2 % formaldehyde, 2.5 % glutaraldehyde and 2.5 % sucrose in 0.2 M cacodylate buffer) for 1.5 hours. The samples were then rinsed with a 0.1 M cacodylate buffer and dehydrated in a series of ethanol (50 %, 70 %, 90 % and 100 %, 10 min). Finally, the samples were incubated in hexamethyldisilazane (HMDS) and dried overnight at 28 °C. Before the SEM analysis, the samples were sputter-coated with gold (15 nm thick layer) using a Leica EM ACE600.

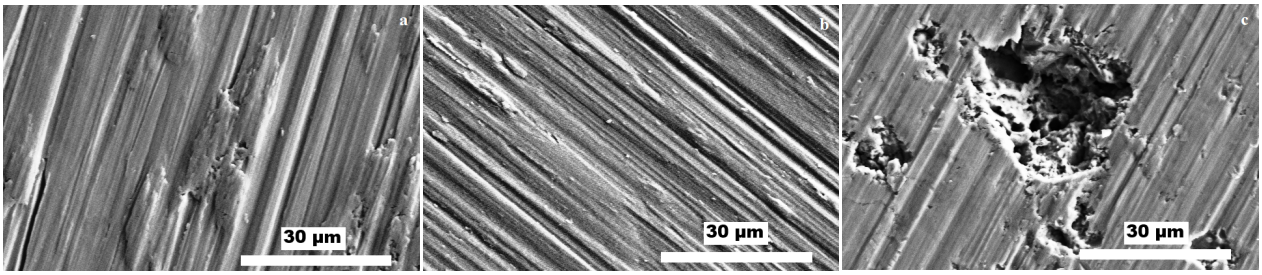
Scanning electron microscopy (SEM, Hitachi S-4700, Japan) was used to characterise the substrate surface after grinding and the coating after firing and after the cytotoxicity contact tests. The measurement was performed at an accelerating voltage of 15 kV and a current of 20 µA with an energy-dispersive spectroscopy analyser (EDS, D-6823, United States).

RESULTS AND DISCUSSION

The substrates were ground with SiC papers of different grit sizes, which made the surfaces of all the substrate types uniform (Figure 1). The mechanical treatment removed any unevenness after the substrates were cut the and the ultrasonic cleaning in acetone and ethanol removed any minor impurities and grease which might have adversely affected the properties of the coatings.

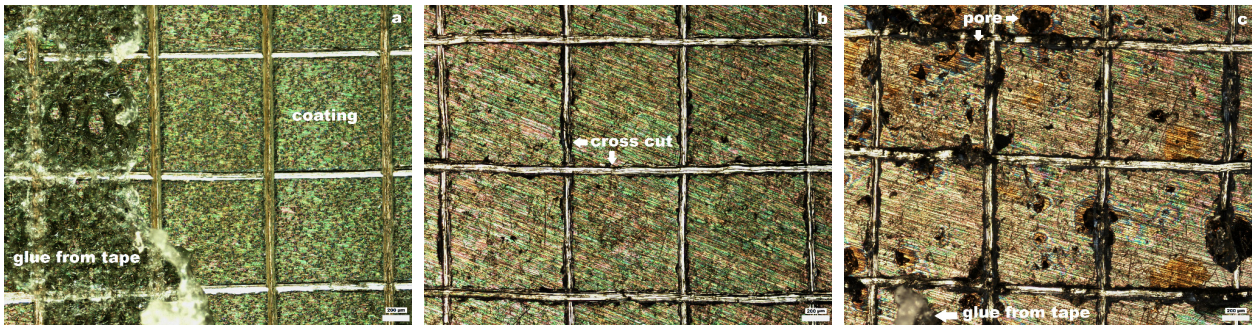
The distribution and depth of the pores in the porous substrates were uneven, both on the surface and in the bulk material. The size of the pores in the low-porous substrate was approximately 10 µm, while it ranged between 10 µm and 200 µm in the high-porous substrates. In the high-porous substrates, the presence of bigger pores supported the partial detachment of the metal substrate top layers.

After the coated substrates were fired, we measured the adhesion of both types of coatings to the substrates; we created the lattice on the surface and applied a special tape, shortly pressed it down and peeled it off. Figure 2 shows the surfaces of the basic titanium coatings applied on three types of titanium substrate after the tape test. The coatings perfectly copied both the surface of the titanium sheet, as well as the surface of the low- and high-porous titanium. The individual cuts of the lattice engraved in the coatings applied on the non-porous and low-porous



a) Non-porous b) Low-porous c) High-porous

Figure 1. (SEM) Surfaces of the titanium substrates after mechanical treatment.



a) Non-porous (NP) b) Low-porous (LP) c) High-porous (HP)

Figure 2. (OM) Surfaces of the basic titanium coating (T) after the tape test on substrates.

substrates were straight (Figure 2a, b), while the lines were slightly curved on the high-porous substrate (Figure 2c). A high number of diversely deep pores made the execution of the tape test more complicated.

The adhesion of the titanium coatings (T) to the substrate was, in many cases, so high that even the optical microscopy made it possible to observe glue leftovers from the adhesive tape on the coating surface (Figure 2a, c). The pores in the substrates and cracks in the coatings did not have a negative effect on the adhesion. The resulting adhesion of all types of coatings (T, TCP) to all three types of substrates (non-porous, low-porous and high-porous) was, under the classification scale in the standard ASTM D 3359-02, evaluated as 5B, i.e., very good, because the adhesive tape tore off 0 % of the coating.

The non-contact cytotoxicity testing (ISO 10993 5) was performed with a test using 100 % extracts from the substrates after grinding and from the substrates with the coatings, using the mice fibroblast cell line L929. A negative control test was performed with a pure medium and the metabolic activity of the samples was compared with the negative control sample. Three samples of each substrate and coating were tested. The relative values of the metabolic activities of the L929 cells after interactions with the individual leachates are shown in Figure 3.

The error bars indicate the sample standard deviations of three replicates from each leachate for the given sample. The toxicity was not confirmed and the metabolic activity of the cells ranged between 97 – 105 %. A value above 100 % means that the cells, after the interaction with leachates from the samples, continued to propagate.

The widely variable porous structures and surfaces of the substrates and various compositions of the coatings did not have a negative effect on the results of the measurements because the values of the relative metabolic activities were nearly identical.

After 24 hours of contact interaction of mice fibroblasts cell line L929 with the substrates that were mechanically treated and with the substrates with two types of coatings, the surfaces were examined with electron microscopy (Figure 4a-i).

On all types of the ground substrates, there was a high share of active cells (non-spherical morphology) trying to spread all over the surface (Figure 4a-c). This suggests that the environment was suitable for cell propagation.

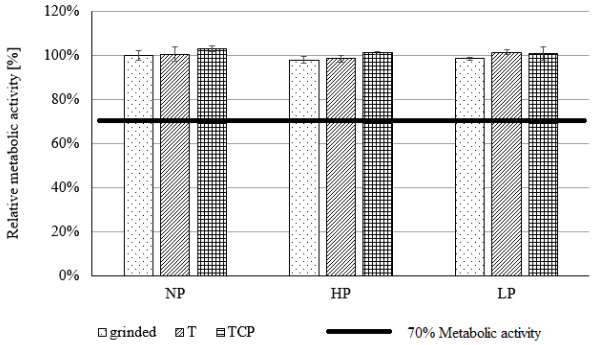
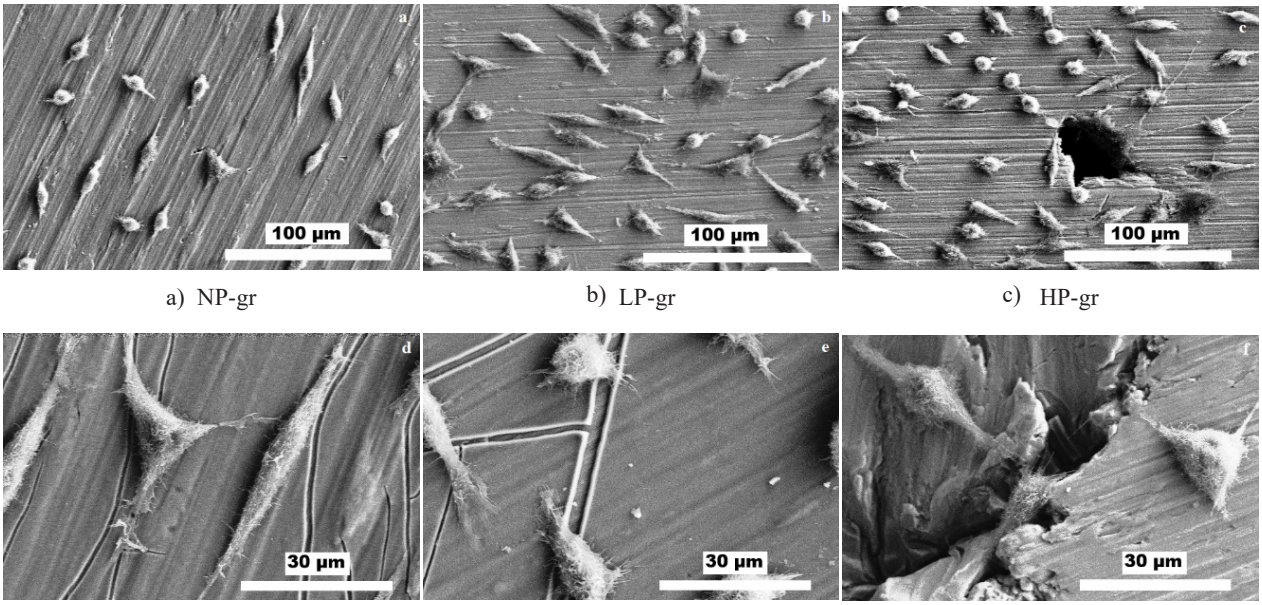


Figure 3. Metabolic activity of L929 after interaction with extract of the ground Non-porous, High-porous, Low-porous (NP, HP, LP) substrates and with substrates coated by the sol T and TCP..

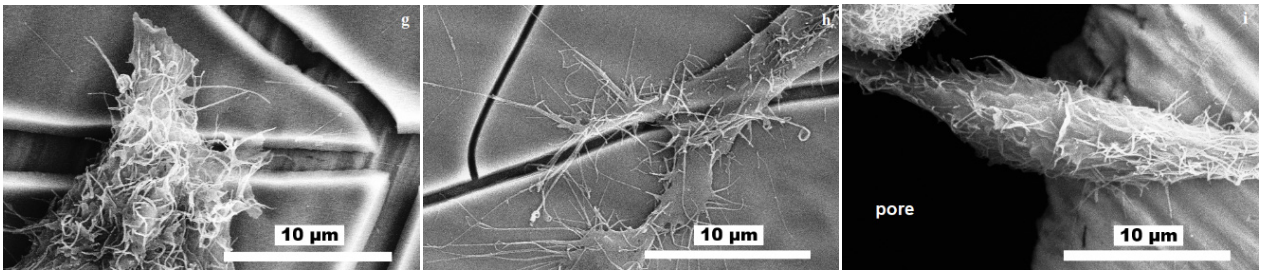


a) NP-gr b) LP-gr c) HP-gr

d) NP-T e) LP-T f) HP-T

Figure 4. (SEM) Surfaces of the substrates after the contact cytotoxicity test: ground: a) NP-gr, b) LP-gr, c) HP-gr; with coating T: d) NP-T e) LP-T, f) HP-T; with coating TCP: g) NP-TCP, h) LP-TCP, i) HP-TCP

continue on the next page ...



g) NP-TCP h) LP-TCP, i) HP-TCP

Figure 4. (SEM) Surfaces of the substrates after the contact cytotoxicity test: ground: a) NP-gr, b) LP-gr, c) HP-gr; with coating T: d) NP-T e) LP-T, f) HP-T; with coating TCP: g) NP-TCP, h) LP-TCP, i) HP-TCP

The diverse porosity and roughness of the surface did not have a significant effect on their behaviour. Also, on the coated substrates, we could see that the cells extended with their filopodia and attempted to take up the largest possible area on the coating, regardless of the substrate type (Figure 4 d-f). The images in Figure 4g, h suggest that the division of the L929 cells is not prevented by cracks in the coatings and that they even get inside the pores (Figure 4i).

CONCLUSIONS

The experimental work focused on a comparison of properties of two titanium substrates prepared by sintering a titanium powder, with and without a pore-forming agent, in comparison with a commercially available titanium made by melting and rolling into a metal sheet. The substrates with different bulk and surface structures were dip-coated with two types of sols - with and without active elements. The microscopy measurements showed that all the coatings perfectly copied the rough surface of all types of the substrates and, despite the cracks, they adhered to the surface very well when measured with the tape test. The non-contact cytotoxicity tests of the coatings on all the substrates confirmed that all the samples were non-toxic because the relative metabolic activity of the cells was over 90 %. The visual observation of the number and shape of the L929 cells showed that all the samples were non-toxic, even after the contact tests. Another step in the experimental work will be static and static-dynamic *in vitro* tests of the bioactivity of the examined types of substrates and coatings.

REFERENCES

1. Bhat S. V. (2021). *Biomaterials*. Alpha Science International Ltd. UK.
2. Park J., Lakes R. S. (2007): *Biomaterials*, An Introduction. 3rd ed., Springer Science.
3. Hansen D. C. (2008): Metal corrosion in the human body: The ultimate bio-corrosion scenario, *Electrochemical Society Interface, 17* (2) 31-34. Doi: 10.1149/2.F04082IF
4. Le Guéhennec L., Soueidan A., Layrolle P., Amouriq Y.: Surface treatments of titanium dental implants for rapid osseointegration. *Dental Materials, 23* (7), (2007) 844-854. Doi: 10.1016/j.dental.2006.06.025
5. Fujibayashi S., Neo M., Kim H.-M., Kokubo T., Nakamura T. (2004): Osteoinduction of porous bioactive titanium metal. *Biomaterials, 25*, 443-450. Doi: 10.1016/S0142-9612(03)00551-9
6. Khodaei M., Valanezhad A., Watanabe I., Yousefi R. (2017): Surface and mechanical properties of modified porous titanium scaffold. *Surface & Coatings Technology, 315*, 61-66. Doi: 10.1016/j.surfcoat.2017.02.032
7. Ryan G., Pandit A., Apatsidis D. P. (2008): Porous titanium scaffolds fabricated using a rapid prototyping and powder metallurgy technique. *Biomaterials, 29*, 3625-3635. Doi: 10.1016/j.biomaterials.2008.05.032
8. Knaislová A., Novák P. (2018): Preparation of porous biomaterial based on Ti-Si alloys. *Manufacturing Technology, 18*, 411-418. Doi: 10.21062/ujep/114.2018/a/1213-2489/MT/18/3/411
9. Yook S.-W., Yoon B.-H., Kim H.-E., Koh Y.-H., Kim Y.-S. (2008): Porous titanium (Ti) scaffolds by freezing TiH_2/camphene slurries. *Materials Letters, 62*, 4506-4508. Doi: 10.1016/j.matlet.2008.08.010
10. Bernatiková A., Novák P. (2016): Optimizing the production of porous alloys based on Ti-Si. *Manufacturing Technology, 16,* 871-874. Doi: 10.21062/ujep/x.2016/a/1213-2489/MT/16/5/871
11. Chouirfa H., Bouloussa H., Migonney V., Falentin-Daudré C. (2019): Review of titanium surface modification techniques and coatings for antibacterial applications. *Acta Biomaterialia, 83*, 37-54. Doi: 10.1016/j.actbio.2018.10.036
12. Ivanova T., Harizanova A., Koutzarova T., Vertruyen B. (2013): Optical and structural characterization of TiO_2 films doped with silver nanoparticles obtained by sol-gel method. *Optical materials, 36*, 207-213. Doi: 10.1016/j.optmat.2013.08.030
13. Hijón N., Cabañas M.V., Izquierdo-Barba I., García M.A., Vallet-Regí M. (2006): Nanocrystalline bioactive apatite coatings. *Solid State Sciences, 8*, 685-691. Doi: 10.1016/j.solidstatesciences.2006.01.008
14. Mavis B., Taş A. C. (2000): Dip coating of calcium hydroxyapatite on Ti-6Al-4V substrates. *Journal of the American ceramic Society, 83* (4), 989-991. Doi: 10.1111/j.1151-2916.2000.tb01314.x
15. Lopez-Heredia M. A., Sohier J., Gaillard C., Quillard S.,

Dorget M., Layrolle P. (2008): Rapid prototyped porous titanium coated with calcium phosphate as a scaffold for bone tissue engineering. *Biomaterials, 29*, 2608-2615. Doi: 10.1016/j.biomaterials.2008.02.021

16. Zhao J., Lu X., Weng J. (2008): Macroporous Ti-based composite scaffold prepared by polymer impregnating method with calcium phosphate coating. *Materials Letters, 62*, 2921-2924. Doi: 10.1016/j.matlet.2008.01.075
17. Lee J.-H., Kim H.-E., Koh Y.-H. (2009): Highly porous titanium (Ti) scaffolds with bioactive microporous hydroxyapatite/TiO_2 hybrid coating layer. *Materials Letters, 63*, 1995-1998. Doi: 10.1016/j.matlet.2009.06.023
18. Manso M., Langlet M., Fernández M., Vázquez L., Martínez-Duart J.M. (2003): Surface and interface analysis of hydroxyapatite/TiO_2 biocompatible structures. *Materials Science Engineering C, 23*, 451-454. Doi: 10.1016/S0928-4931(02)00320-X
19. Guo L., Li H. (2004): Fabrication and characterization of thin nano-hydroxyapatite coatings on titanium. *Surface & Coatings Technology, 185*, 268-274. Doi: 10.1016/j.surfcoat.2004.01.013
20. Xu W., Hu W., Li M., Wen C. (2006): Sol-gel derived hydroxyapatite/titania biocoatings on titanium substrate. *Materials Letters, 60*, 1575-1578. Doi: 10.1016/j.matlet.2005.11.072
21. Rodriguez Paez L., Matoušek J. (2006): Preparation and characterization of TiO_2-MnO sol-gel layers on glass. *Ceramic – Silikáty, 50* (1), 39-43.
22. Novotná P., Matoušek J. (2006): Preparation and characterization of photocatalytical TiO_2-SiO_2-PDMS layers on glass. *Thin Solid Films, 502*, 143-146. Doi: 10.1016/j.tsf.2005.07.259
23. Durán A., Conde A., Coedo A.G., Dorado T., García C., Ceré S. (2004): Sol-gel coatings for protection and bioactivation of metal used in orthopaedic devices. *Journal of Materials Chemistry, 14*, 2282-2290. Doi: 10.1039/b401370k
24. ISO 23317 (2014). *Implants for surgery-In vitro evaluation for apatite-forming ability of implant materials*. 3rd ed, Switzerland.
25. Jalota S., Bhaduri S.B., Tas A.C. (2007): Osteoblast proliferation on neat and apatite-like calcium phosphate-coated titanium foam scaffolds. *Materials Science and Engineering C, 27*, 432-440. Doi: 10.1016/j.msec.2006.05.052
26. Tang H., Guo Y., Jia D., Zhou Y. (2011): Preparation and in vitro characterization of crack-free mesoporous titania films. *Surface & Coatings Technology, 206*, 8-15. Doi: 10.1016/j.surfcoat.2011.06.028
27. Jaiswal S., McHale P., Duffy B. (2012): Preparation and rapid analysis of antibacterial silver, copper and zinc doped sol-gel surfaces. *Colloids and Surfaces B: Biointerfaces, 94*, 170-176. Doi: 10.1016/j.colsurfb.2012.01.035
28. Šlamborová I., Zajícová V., Karpíšková J., Exnar P., Stibor I. (2012): New type of protective hybrid and nanocomposite hybrid coatings containing silver and copper with an excellent antibacterial effect especially against MRSA. *Materials Science Engineering, C 33*, 265-273. Doi: 10.1016/j.msec.2012.08.039
29. Shrivastava S., Bera T., Roy A., Singh G., Ramachandrarao P., Dash D. (2007): Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology, 18*, 225103. Doi: 10.1088/0957-4484/18/22/225103
30. Rai M., Yadav A., Gade A. (2009): Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances, 27*, 76-83. Doi: 10.1016/j.biotechadv.2008.09.002
31. Dallas P., Sharma V.K., Zboril R. (2011): Silver polymeric nanocomposites as advanced antimicrobial agents: Classification, synthetic paths, applications, and perspectives. *Advances in Colloid and Interface Science, 166*, 119-135. Doi: 10.1016/j.cis.2011.05.008
32. Simchi A., Tamjid E., Pishbin F., Boccaccini A.R. (2011): Recent progress in inorganic and composite coatings with bactericidal capability for orthopaedic applications. *Nanomedicine: Nanotechnology, Biology and Medicine, 7*, 22-39. Doi: 10.1016/j.nano.2010.10.005
33. Li W.-R., Xie X.-B., Shi Q.-S., Zeng H.-Y., OU-Yang Y.-S., Chen Y.-B. (2010): Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. *Applied Microbiology and Biotechnology, 85*, 1115-1122. Doi: 10.1007/s00253-009-2159-5
34. Li W.-R., Xie X.-B., Shi Q.-S., Duan S.-S., Ouyang Y.-S., Chen Y.-B. (2011): Antibacterial effect of silver nanoparticles on Staphylococcus aureus. *Biometals, 24*, 135-141. Doi: 10.1007/s10534-010-9381-6
35. Naik K., Kowshik M. (2014): Anti-biofilm efficacy of low temperature processed AgCl-TiO_2 nanocomposite coating. *Materials Science Engineering C, 34*, 62-68. Doi: 10.1016/j.msec.2013.10.00
36. Braem A., Van Mellaert L., Mattheys T., Hofmans D., De Waelheyns E., Geris L., Anné J., Schrooten J., Vleugels J. (2014): Staphylococcal biofilm growth on smooth and porous titanium coatings for biomedical applications. *Journal of Biomedical Materials Research Part A, 102A,* 215-224. Doi: 10.1002/jbm.a.34688
37. Kim H.-W., Koh Y.-H., Li L.-H., Lee S., Kim H.-E. (2004): Hydroxyapatite coating on titanium substrate with titania buffer layer processed by sol-gel method. *Biomaterials, 25,* 2533-2538. Doi: 10.1016/j.biomaterials.2003.09.041
38. Cotolan N., Rak M., Bele M., Cör A., Muresan L.M., Milošev I. (2016): Sol-gel synthesis, characterization and properties of TiO_2 and Ag- TiO_2 coatings on titanium substrate. *Surface & Coatings Technology, 307*, 790-799. Doi: 10.1016/j.surfcoat.2016.09.082
39. He X., Zhang G., Wang X., Hang R., Huang X., Qin L., Tang B., Zhang X. (2017): Biocompatibility, corrosion resistance and antibacterial activity of TiO_2/CuO coating on titanium. *Ceramics International, 43*, 16185-16195. Doi: 10.1016/j.ceramint.2017.08.196
40. Schrooten J., Helsen J.A. (2000): Adhesion of bioactive coating to Ti_6Al_4V oral implant. *Biomaterials, 21*, 1461-1469. Doi: 10.1016/s0142-9612(00)00027-2
41. Gan L., Wang J., Pilliar R.M. (2005): Evaluating interface strength of calcium phosphate sol-gel derived thin films to Ti_6Al_4V substrate. *Biomaterials, 26*, 189-196. Doi: 10.1016/j.biomaterials.2004.02.022
42. ASTM D 3359 – 02 (2006). *Standard Test Methods for Measuring Adhesion by Tape Test.* ASTM International.
43. Zhang S., Xianting Z., Yongsheng W., Kui Ch., Wenjian W. (2006): Adhesion strength of sol-gel derived fluoridated hydroxyapatite coatings. *Surface & Coatings Technology, 200*, 6350-6354. Doi: 10.1016/j.surfcoat.205.11.033
44. Kim H.-W., Kim H.-E., Salih V., Knowles J.C. (2005): Hydroxyapatite and titania sol-gel composite coatings on titanium for hard tissue implants; Mechanical and in vitro biological performance. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 72B*, 1-8. Doi:

10.1002/jbm.b.30073

45. Školáková A., Körberová J., Málek J., Rohanová D., Jablonská E., Pinc J., Salvetr P., Gregorová E., Novák P. (2020): Microstructural, Mechanical, Corrosion and Cytotoxicity Characterization of Porous Ti-Si Alloys with Pore-Forming Agent. *Materials. 13*, 5607, Doi: 10.3390/ma13245607
46. Švagrová K., Horkavcová D., Helebrant A. (2009): Optimization of composition and formation of titania sol-gel coatings on titanium substrate using dip-coating technique. *Ceramics-Silikáty, 63* (3), 255-260. Doi: 10.13168/cs.2019.0018
47. Švagrová K., Horkavcová D., Jablonská E., Helebrant A. (2021): Titania based sol-gel coatings with Ag, Ca-P applied on titanium substrate developed for implantation. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 110*(1), 115-124. Doi: 10.1002/jbm.b.34895.
48. ČSN EN ISO 10993-5 (2010). *Biologické hodnocení zdravotnických prostředků* - Část 5: Zkoušky na cytotoxicitu in vitro, Geneva.