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Titania sol-gel coatings with silver on non-porous titanium and titanium alloys

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Abstract. The objective of the work was to prepare and characterize titania sol-gel coatings on non-porous titanium and newly developed titanium alloys. Basic titania sol contained two forms of silver. Titania sol without silver was used as a reference sample. Coatings were prepared by dip-coating technique during stirring and fired. Coatings after firing were characterized by scanning electron microscopy. All titania coatings were measured to determine their adhesive and bactericidal properties. Adhesion of the coatings to the substrate was measured by tape test. Gram-negative bacteria *E. coli* was used for the bactericidal test. Coated substrates were immersed into suspension of *E. coli* in physiological solution for 24 hours. The *in vitro* cytotoxicity test was performed after one day. The bactericidal effect without toxicity was confirmed for selected coatings.

1. Introduction

Titania coatings containing various ions prepared by a solo-gel method on metallic, glass or other substrates have been frequently used in photocatalysis, solar cells, sensors and biomaterials [1, 2]. The sol-gel method which is based on a controlled mixing process of organic compounds in alcohols and water with an added catalyst is suitable for formation of thin homogenous coatings using techniques like dip-coating [3], spin-coating [4] or spray-coating [5]. One advantage of dip-coating is the possibility to coat substrates of complicated shapes under constant conditions. By using suitable reagents and appropriate coating conditions it is possible to prepare coatings of various thickness [1] with bioactive [4] and adhesive [6] properties. Many authors have investigated effects of silver in titania sol-gel coatings on antibacterial effects against various microorganisms, most frequently against *Escherichia coli* and *Staphylococcus aureus* while in most cases silver was added in form of silver nitrate [7]. There are also other forms of silver suitable for the purpose, e.g. silver acetate or silver phosphate [8,9]. The effect of silver ions on microorganisms is well known, however, the mechanism of the effect has not been fully explained yet. It is assumed that silver ions interact with three components of the bacterial cell to produce the bactericidal effect: with the peptidoglycan cell wall and plasma membrane, with bacterial (cytoplasmic) DNA and with bacterial proteins [10]. Due to the

effect of silver ion, DNA may have lost its replication ability and cellular proteins became inactive [11].

The objective of our work was to prepare thin titania sol-gel coatings containing silver on titanium substrate by dip-coating technique. Antibacterial coatings prepared in this manner on implants might be in the future used for orthopedic applications in order to reduce the risk of post-surgery infection.

2. Experimental

2.1. Materials and methods

Substrates made of pure titanium (Grade 2, ASTM B265) and newly developed titanium alloys TiSi5, TiSi10 sized 30x10x1mm were ground with SiC paper No. 80, 160, 400, 800 and washed with acetone and then in ethanol.

Titania sol was prepared by gradual mixing of tetra-n-butyl-orthotitanate, acetylacetonate, triton X-100, ethanol and 1 mol.l^{-1} HNO_3. The basic titania sol was divided into three parts and various forms of silver were added into two of them. The concentration of silver in the sols samples was always the same (c_{Ag} = 0.015 gml^{-1}). Silver was added into the sols in form of AgNO3 and Ag3PO4 immediately before the coating process. The substrates were dipped once into the individual sols at the rate of 20 cmmin^{-1} and remained in the sols for 30 s. Then the substrates were pulled out from the sols at the rate of 6 cmmin^{-1}. Substrates coating was performed by continual mixing on a magnetic stirrer to ensure homogenous distribution of silver in the sols and subsequently in the coatings. All the coated substrates were fired at 400 °C for 2 hours. Cooling was performed in an oven. The substrates with basic titania coating were identified as Ti-T, TiSi5-T, TiSi10-T, substrates with $AgNO_3$ as Ti-TAN, TiSi5-TAN, TiSi10-TAN and substrates with Ag3PO4 as Ti-TAP, TiSi5-TAP, TiSi10-TAP.

Surfaces of individual types of coatings after firing were investigated with the electron microscope (SEM) Hitachi S4700 with an SDD detector.

2.2. Conditions of the bactericidal, adhesion and cytotoxicity test

The test of bactericidal properties was performed with gram-negative bacteria *Escherichia coli (E. coli*, strain DBM 3138). Bacterial culture was incubated in liquid LB medium and then diluted in physiological solution to the bacteria concentration 10^4 CFUml^{-1}. The test was performed by dipping of the coated substrate into suspension of *E. coli* in physiological solution. Then the substrate was removed and amount of the suspension was spread on a Petri dish with LB agar. Sole suspension without any substrate served as a reference. The dish was placed into a biological thermostat set up at 36.5°C for 24 hours. After the incubation period the dish was photographed and the quantity of surviving *E. coli* was counted.

Adhesion of the coating to the substrate was measured with a cross-cut tape test under ASTM D 3359-2 [12]. Cuts were made into the coatings arranged into a grid shape and a tape (Permacel 99) was applied on the area with cuts. The tape was peel off and the area with the cuts was evaluated visually by comparison with a standard scale. The percentage of the area removed was determined and the classification grade was assigned.

Test for *in vitro* cytotoxicity was performed according to ISO 10993-5 standard [13]. The samples were submersed into MEM + 5% FBS (fetal bovine serum) with antibiotics at 37 ° C with shaking for 24 hours. L929 cells (mouse fibroblasts, ATCC® CCL-1™) were seeded at a concentration of $1\,1\,0^5$ cells ml^{-1} into a 96-well plate and cultured in MEM (Minimal Essential Medium) with 10% FBS at standard conditions (37 °C, 5% CO_2). After 24 hours, the medium was removed and the extracts were applied onto the subconfluent cell monolayer. Sole medium served as a control. Cytotoxic effect of the extracts on the cells was measured after 24 hours of incubation using WST-1 (water soluble tetrazolium salt 1) assay. Cells were incubated with 5% WST-1 in MEM (with 10% FBS) for 4 hours and the absorbance of the yellow formazane formed by metabolic reduction of the reagent was measured at 450 nm. Reduced metabolic activity of cells by over 30 % compared to the control was considered as a cytotoxic effect.

3. Results and discussion

Figures 1d-i show surfaces of the titanium substrates with fired titania coatings with silver in two forms. For comparison figure 1a-c shows the fired substrates with the basic titania coatings without silver.

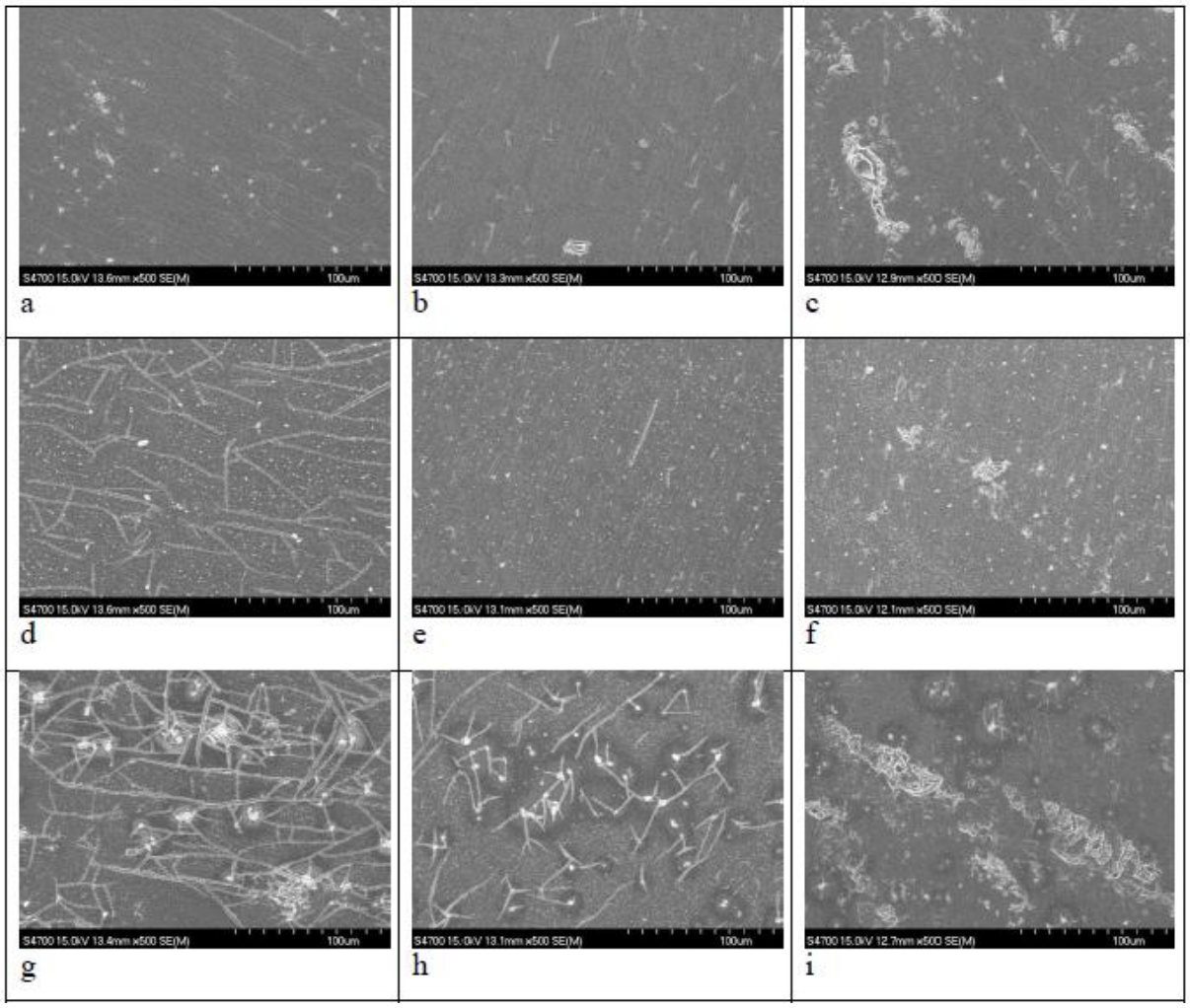

Figure 1. Titania coatings on titanium substrates after firing: a) Ti-T, b) TiSi5-T, c) TiSi10-T, d) Ti-TAN, e) TiSi5-TAN f) TiSi10-TAN, g) Ti-TAP, h) TiSi5-TAP and i) TiSi10-TAP

The figure 1a-c indicates that the basic titania coatings after firing were partly cracked only on substrate TiSi10. Spherical silver particles in all the other types of coatings (Fig. 1d-i) were nearly evenly distributed all over the surface. The size of silver particles added into the sol as $AgNO_3$ (Fig. 1d-f) was in units of nanometers and for particles added as Ag_3PO_4 (Fig. 1g-i) in units of micrometers. The coating TAN on TiSi5 (Fig. 1e) was homogeneous nearly without cracks. In case of the titania coatings with added Ag_3PO_4 (Fig. 1 g-i) the cracks propagated always from particles of the mentioned compound.

Adhesion of the coatings to the substrates measured by tape test was very good, grade 5B as compared to the classification scale. The cracks in the coatings Ti-TAN, Ti-Tap and TiSi5-TAP had no negative effect on the adhesion.

After 24-hours TAN and TAP coatings showed very good bactericidal effect (the number of surviving colonies was 0 - 25) whereas in case of the basic coating T no bactericidal effect was demonstrated (the number of surviving colonies was 800 - 1200 which is comparable with the

reference sample). Figure 2 shows the cytotoxicity of the coatings extracts (T, TAN, TAP) on cell line L- 929 (mouse fibroblasts).

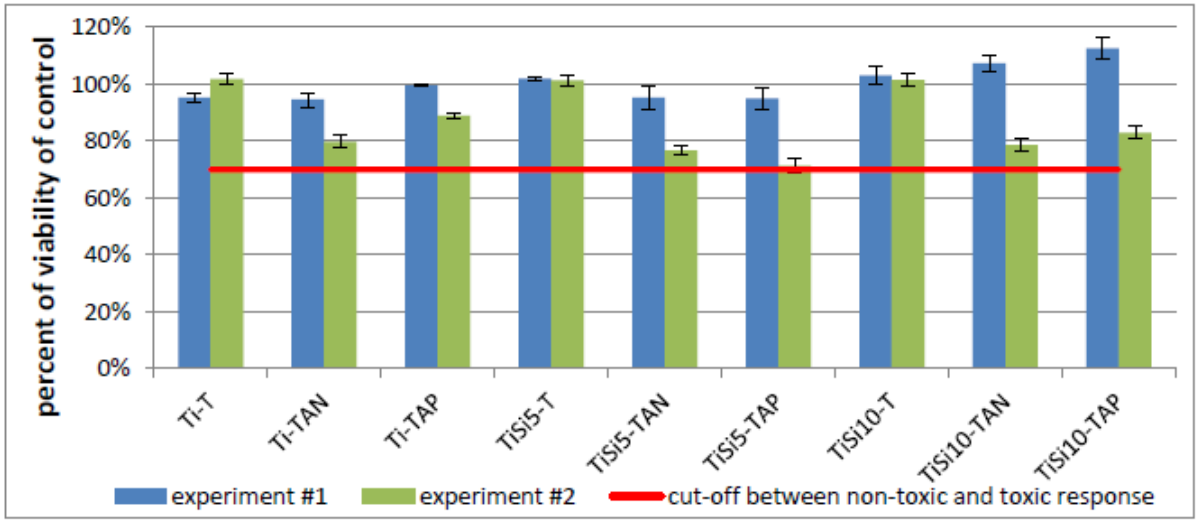

Figure 2. Cytotoxicity of the extracts (T, TAN, TAP) on cell line L-929 (mouse fibroblasts) after 24-hours incubation

After the 24-hours cytotoxicity test, neither of the coatings T, TAN or TAP showed cytotoxic effect, irrespective of the substrate (titanium or titanium alloy TiSi5, TiSi10) used.

4. Conclusion

Titania sol-gel coatings contained silver in form of dissolved AgNO3 and Ag3PO4 particles were successfully prepared by dip-coating technique on three types of titanium substrates. Adhesion of all types titania coatings to the substrates was very good (grade 5B). The 24-hours bactericidal test confirmed significant bactericidal effect of titania coatings containing $AgNO_3$ and Ag_3PO_4 particles. No cytotoxic effect of the coatings was observed.

Acknowledgement

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