COMPARISON OF SOL-GEL SILICATE COATINGS ON Ti SUBSTRATE

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The objective of the submitted work was to prepare and to characterize two types of silicate coatings prepared by the sol-gel method using the dip-coating technique on a titanium substrate. Efforts have been made to use mechanical properties of bio-inert titanium and bioactive properties of a silicate layer enriched with an admixture of compounds identified below. The first group consisted of silicate coatings containing silver, brushite and monetite. The other group of silicate coatings contained calcium nitrate and triethyl phosphate. Mechanically and chemically treated titanium substrates were dipped into sols and dried and fired. Silicate coatings from the first group were also chemically treated in 10 mol.l^{-1} solution of sodium hydroxide. All coatings were measured to determine their adhesive and bioactive properties and furthermore the antibacterial properties were tested in the case of first group. Surfaces of the coated substrates were investigated after the firing and after the individual tests with optical and electron microscopy and X-ray microdiffraction. A tape test demonstrated excellent adhesive property of all coatings to the substrate, classified with degree 5. A static in vitro test demonstrated bioactivity of nearly all the coatings. The basic silicate coating from the first group and one type of coating from the second group were identified as inert. Antibacterial properties of silicate coatings containing silver showed to be different when tested against Escherichia coli bacteria. A complete inhibition of the growth of bacteria under our experimental conditions was observed for the coating containing silver and monetite and a partial inhibition of the growth of bacteria for coatings containing silver and silver in combination with brushite.

INTRODUCTION

With the extending lifespan, congenital defects and increasing number of injuries the demand increases for development and improvement of materials used mainly in surgical procedures. The biomaterials may be metallic, plastic, ceramic, glass-ceramic or polymeric in form of plates, scaffolds, granules or powders.

Many coating methods have been developed to take advantage of mechanical properties of metals or ceramics and of bioactive properties of the coatings. The most frequently used methods include plasma spraying [1], electrophoretic [2], magnetron and pulsed laser deposition [3, 4]. Another sol-gel method, specifically the dip-coating technique, is convenient due to its simplicity, low costs and formation of homogenous thin coatings on substrates of various shapes. This technique ensures overall coverage of the substrate, followed by drying and firing. Its major advantage is the possibility to form coatings of various thicknesses [5-7]. In order to improve bioactivity of the coatings mixed silicate-phosphate-calcium sol-gel layers have been developed on a titanium alloy [8]. Major attention has been paid to sol-gel coatings containing bio-glass, known for its high bioactivity [9-13]. Composite hydroxyapatite-forsterite bioactive glass coatings on stainless steel have been prepared by authors [14].

Bioactivity of biomaterials and coatings is monitored with *in vivo* tests (on live organisms) or *in vitro* tests (interaction with simulated body fluid) [15], while formation of bone-like hydroxyapatite (HA) on the surface of materials/coatings is considered a proof of bioactive behavior. By its composition hydroxyapatite is close to the mineral part of bones and therefore implanted bioactive materials form a direct chemical bond with the bone tissue.

Apart from bioactivity, antibacterial effect is also desirable and therefore silver (Ag) in various forms is incorporated into sol-gel coatings as well [16, 17]. The effect has been tested with various microorganisms, e.g. *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* [18, 19]. The antibacterial effect may be influenced by concentration of silver, by the size, shape and surface of Ag nanoparticles or by a combination of HA powders with silver [20, 21]. Most frequently, the tests monitor the number of colonies of surviving bacteria or the turbidity of bacterial suspension. Another important property of the coatings is their adhesion to substrates, which may be measured with a pull-out test, scratch test or tape test [22-27]. The adhesion of the coatings may be evaluated rather visually than numerically.

The objective of this work was to prepare thin silicate coatings based on TEOS containing Ca-P on a titanium substrate using the sol-gel method and

dip-coating technique. In the first group the coatings contained silver, brushite and monetite (its well-known transformation to HA [28]) and in the second group the coatings contained dissolved calcium nitrate and triethyl phosphate. Another step was to measure their adhesive capacity by means of a tape test, their bioactive behavior by means of an *in vitro* test (simulated body fluid, SBF) and their antibacterial properties against *E. coli*.

EXPERIMENTAL

Titanium substrates (Grade 2, ASTM B 265), sized 30×10×1 mm, were mechanically treated with SiC sand paper No. 500, 600 and washed in acetone in a ultrasonic bath for 10 minutes at laboratory temperature and subsequently in demineralized water. After drying the substrates were chemically treated by leaching in concentrated hydrochloric acid (35 %) for 2 hours at laboratory temperature and subsequently washed several times in demineralized water. After the mechanical and chemical treatment they were dried at laboratory temperature. The mechanical and chemical treatments of the titanium substrates were selected to clean the surfaces from impurities and to improve adhesion of coatings by increasing roughness of the surface. Surfaces of the substrates after the individual treatments were measured with the HOMMEL TESTER T1000 device. The measured length was always 1500 μm and each measurement was repeated 3 times on one sample [25, 26].

The first group contained four types of silicate sols based on TEOS (Tetraethyl orthosilicate, $Si(OC_2H_5)_4$) marked SN, SAN, SANB, SANM, prepared by gradual mixing of reagents listed in Table 1. The sols were mixed with a magnetic stirrer for 120 minutes and left to age for 7 days at laboratory temperature. The aging time is important for long-term stability of sols. After the aging was completed brushite ($CaHPO_4.2H_2O$, Sigma-Aldrich) with the average size of particles ∅ 7.9 μm, and monetite ($CaHPO_4$, Merck) with the average size of particles ∅ 10.4 μm [25, 26] were added into SANB and SANM sols. The second group included two types of silicate sols based on TEOS, marked SCP-I and SCP-II, prepared by gradual mixing of reagents listed in Table 1. Mixing with a magnetic stirrer lasted 24 hours at laboratory temperature.

Triton X-100 (Roth) was added in order to increase sol viscosity. In order to improve bioactivity of the coatings, calcium phosphates were introduced into the sols in the first group in form of brushite and monetite and into the sols in the second group in form of calcium nitrate and triethyl phosphate. Silver nitrate was added into the sols in the first group as a carrier of Ag^+ which has a proven antibacterial effect.

In order to ensure homogeneity of the sols in the first group the coating of titanium substrates was performed under conditions of continual stirring in a dip-coater at laboratory temperature. The dipping rate was 20 cm/min, the withdrawing rate 6 cm/min and the dwell time of the substrates in the sols was 30 s. After the deposition the coatings in the first group were left to dry for 24 hours at laboratory temperature, then 30 minutes at 60°C and fired at the temperature of 500°C for 1 hour, at the heating rate 2°C/min up to 250°C and 5°C/min up to 500°C. The coatings in the second group were fired at the temperature of 600°C for 1 hour, at the heating rate 2°C/min. In both cases the cooling occurred in the oven until the following day. The identification of the coatings was identical with that of the sols used for their development.

Coatings in the first group were subsequently chemically treated by leaching in 10 $mol.l^{-1}$ solution of NaOH for 4 hours statically at laboratory temperature. The ratio of the substrate surface to the volume of NaOH solution was S/V = 0.1 cm^{-1}. The coated surfaces were

Table 1. Composition and identification of silicate sols (coatings) in the first and second groups.

	Composition and identification of sols (coatings)					
	First group				Second group	
Reagents	SN	SAN	SANB	SANM	SCP-I	SCP-II
TEOS	10 ml	10 ml	10 ml	10 ml	13.3 ml	30 ml
1 $mol.l^{-1}$ HNO_3	2 ml	2 ml	2 ml	2 ml	–	1 ml
0.1 $mol.l^{-1}$ HNO_3	–	–	–	–	30 ml	–
Ethanol	30 ml	30 ml	30 ml	30 ml	–	5 ml
$AgNO_3$	–	0.5 g	0.5 g	0.5 g	–	–
Triton - X100	–	–	–	–	2 ml	–
$Ca(NO_3)_2.4H2O$	–	–	–	–	7.32 g	1.9 g
TEP: $(C_2H_5O)_3PO$	–	–	–	–	0.9 ml	0.5 ml
H_2O	2 ml	2 ml	2 ml	2 ml	–	2 ml
Brushite	–	–	4 g	–	–	–
Monetite	–	–	–	4 g	–	–

rinsed 5 times in demineralized water and left to dry in the air until the following day. The purpose of the chemical treatment was diffusion of Na^+ ions into the coating as the ions are expected to support bioactive behavior and intentional erosion of the surface to improve the interaction with SBF.

Adhesion of the coating to the substrate was measured with a cross-cut tape test under ASTM D 3359-2. Cuts were made into the coatings arranged into a lattice pattern and a tape (Permacel 99) was applied on the area with cuts. The tape was peel off (under the angle of 180°) and the area with the cuts was evaluated visually by comparison with a standard scale. The percentage of the peel-off area was determined and the classification grade was assigned to it: 0 % = 5, less than 5 % = 4, 5-15 % = 3, 15-35 % = 2, 35-65 % = 1, over 65 % = 0 [22, 25, 26].

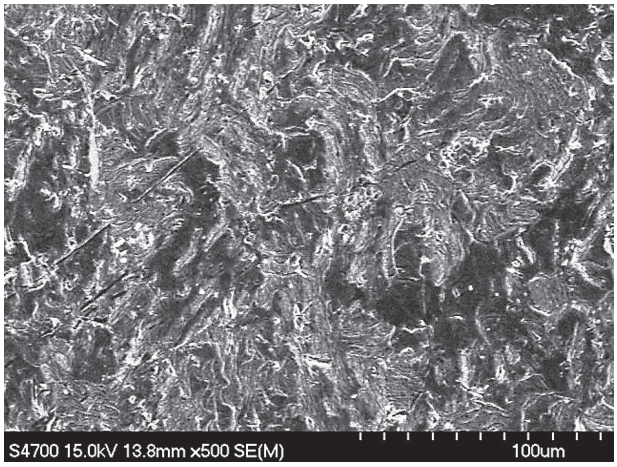
A test of bioactivity was performed *in vitro*, by static exposure of all coated substrates to simulated body fluid (SBF) which simulates the inorganic part of blood plasma. The SBF solution was prepared from the following reagents: KCl, NaCl, $NaHCO_3$, $MgSO_4.7H_2O$, $CaCl_2$, KH_2PO_4. The Tris buffer ((hydroxy methyl) aminomethane, $NH_2C(CH_2OH)_3$) was added to adjust pH = 7.5 and azide (NaN_3) was added to prevent bacteria growth [26]. The ratio of the substrate surface to the volume of SBF solution was S/V = 0.1 cm^{-1}. Coated substrates were placed into plastic bottles filled with SBF and left in a biological thermostat at 37 ± 0.5°C for a period of ca. 20 days. Formation of hydroxyapatite on surfaces of the coated substrates was monitored.

Gram-negative bacteria *E. coli* (strain DBM 3138) was used for the bactericidal test. The bacterial culture was incubated in a liquid LB medium (Luria Bertani, Sigma Aldrich) at 37°C and 230 rpm for a period of 18 hours and then it was diluted to the concentration of bacteria 10^4 cfu.ml^{-1} (colony forming units per ml) in physiological solution (9 g l^{-1} NaCl). The test was performed by immersion of coated substrates from the first group into 2.5 ml suspension of *E. coli* in physiological solution for a period of 24 hours at laboratory temperature. Subsequently, the substrates were taken out from the suspension and 100 µl of each suspension was spread onto LB agar Petri dish. The agar dishes were placed into a biological thermostat set at 37 ± 0.5°C for a period of 24 hours. After the incuba-tion period the dishes were photographed and the numbers of colonies of surviving *E. coli* were counted with NIS Elements AR.3 software. The test was repeated 4 times.

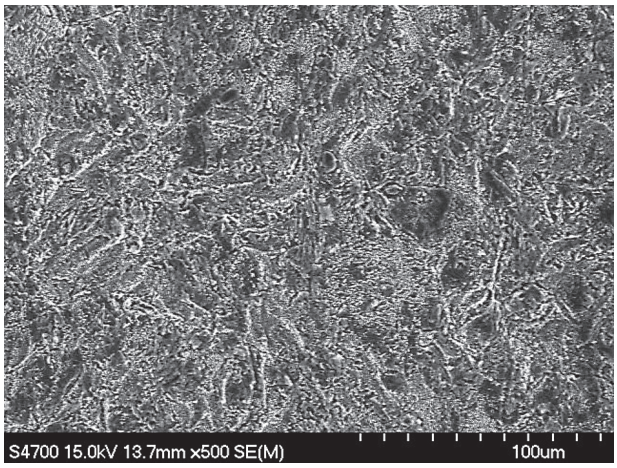
Coated substrate surfaces after the firing, chemical treatment in NaOH solution, tape test and *in vitro* test were inspected with an optical microscope (OM, Jenapol) with lateral illumination and with an electron microscope (SEM, Hitachi S4700 with SDD detector). The XRD analysis (PANalytical X´Pert PRO+High Score plus) was used to determine the phase composition of selected coatings after the *in vitro* test.

RESULTS AND DISCUSSION

Figures 1a and 1b show a visible difference in the structures of titanium surfaces after the mechanical and chemical treatments. A markedly rough surface appeared after leaching in concentrated hydrochloric acid, i.e. the surface roughness increased (and thus also the surface area).



a)



b)

Figure 1. (SEM) Surface of titanium substrate after: a) grinding, b) leaching in HCl.

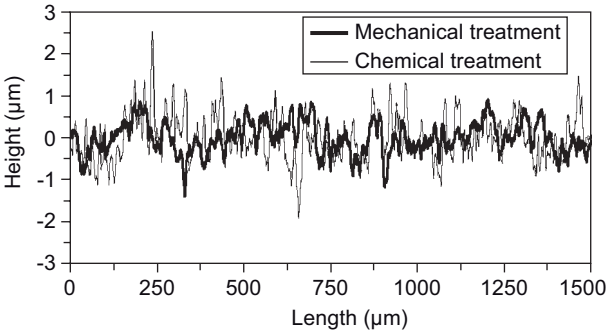
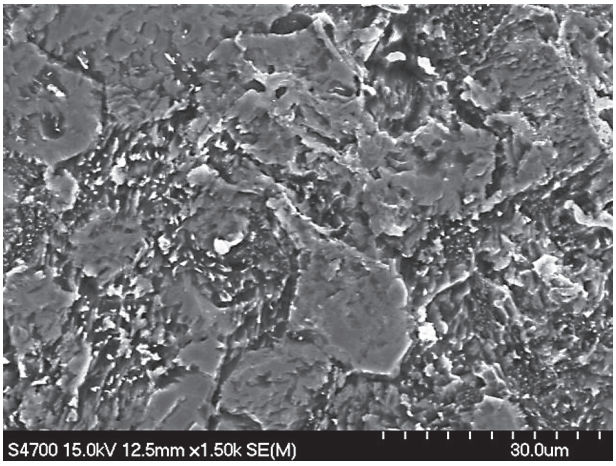


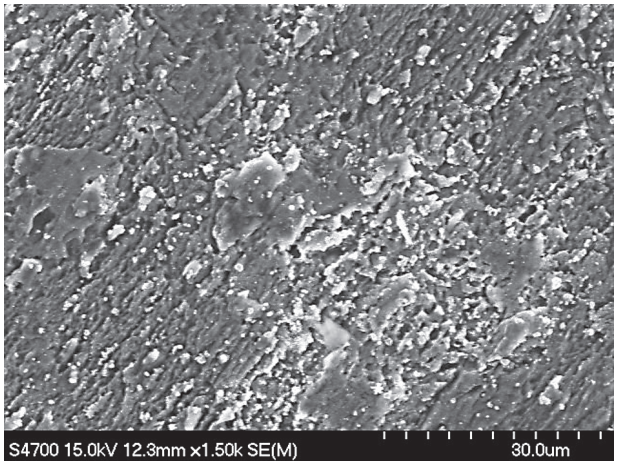
Figure 2. Profile comparison of Ti substrates after mechanical and chemical treatment.

Records from roughness measurements (Figure 2) confirmed accentuation of the highest and lowest points after the chemical treatment.

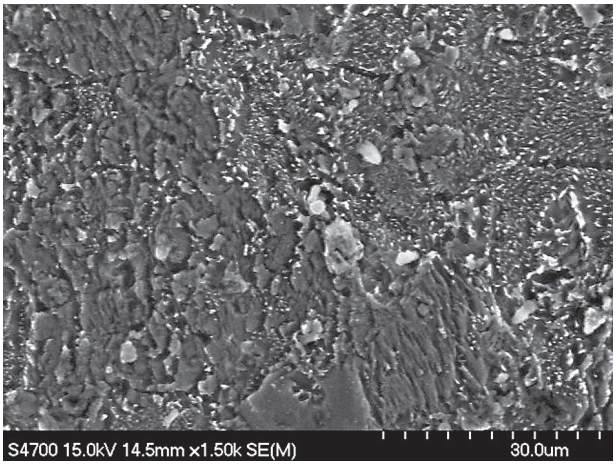
A sol-gel dip-coating technique was used to create silicate coatings on mechanically and chemically treated titanium substrates in the first group, based on TEOS (SN), containing silver (SAN), silver + brushite (SANB) and silver + monetite (SANM); the coatings were dried, fired and statically exposed to 10 mol.l^{-1} solution of NaOH and they are shown in Figures 3a-d. Silica coa-



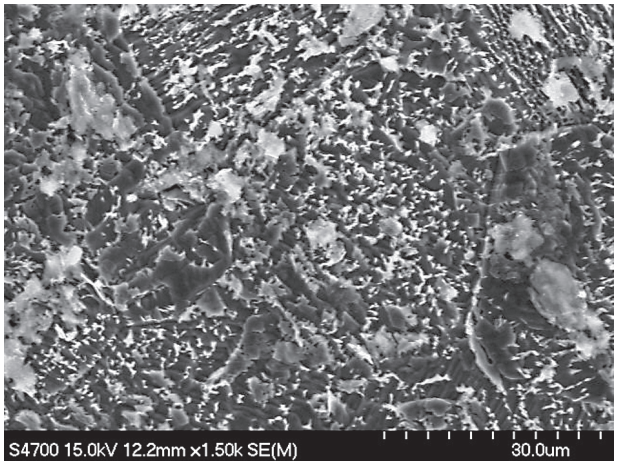
a) SN



b) SAN

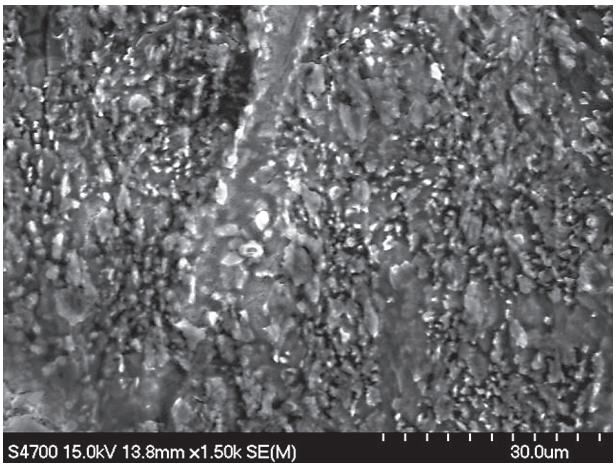


c) SANB

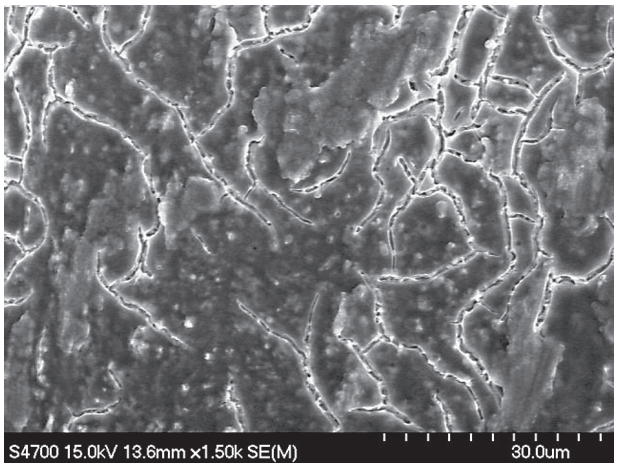


d) SANM

Figure 3. (SEM) Surface of coatings after treatment in NaOH: a) SN b) SAN, c) SANB, d) SANM.



a) SCP-I

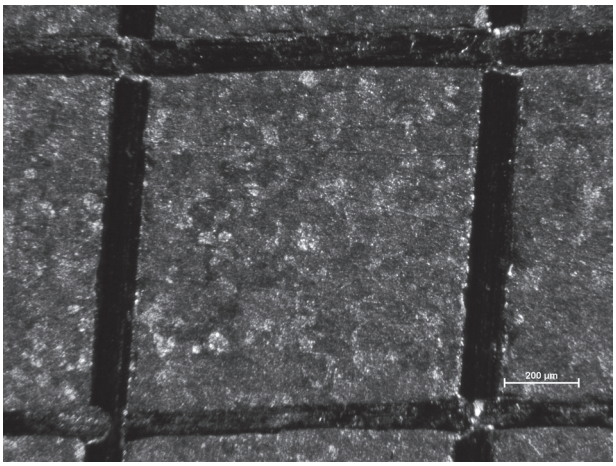


b) SCP-II

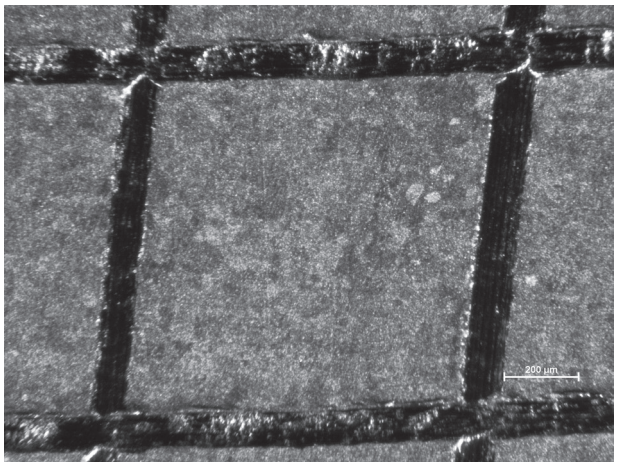
Figure 4. (SEM) Surface of coatings after firing: a) SCP-I, b) SCP-II.

ting (Figure 3a) seems to be dense. Small spherical particles in Figures 3b, c and d are nano-particles of silver while bigger white micro-particles of irregular shape in Figures 3c and 3d are particles of brushite and monetite. Coatings were very thin and they copied the rough surface of the substrates. Particles of silver and powders were evenly distributed all over the coating surface.

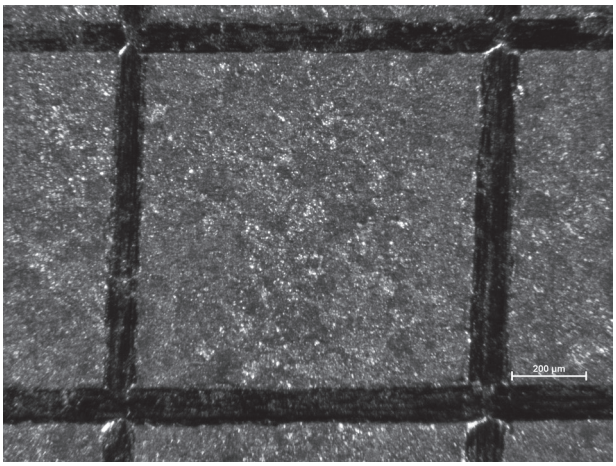
Figures 4a and 4b show surfaces of silicate coatings from the second group, whose sols contained dissolved tetrahydrate of calcium nitrate and triethyl phosphate (TEP) in different ratios. The coating SCP-I (Figure 4a), which also contained Triton, was more compact, it copied the rough surface of the substrate and was without cracks. On the contrary, the coating SCP-II (Figure 4b) was



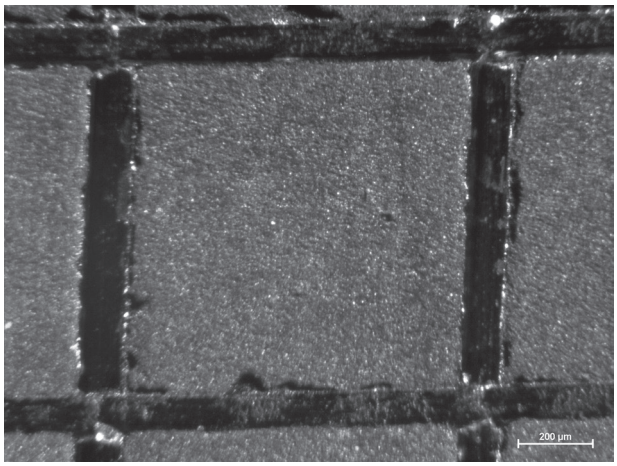
a) SN



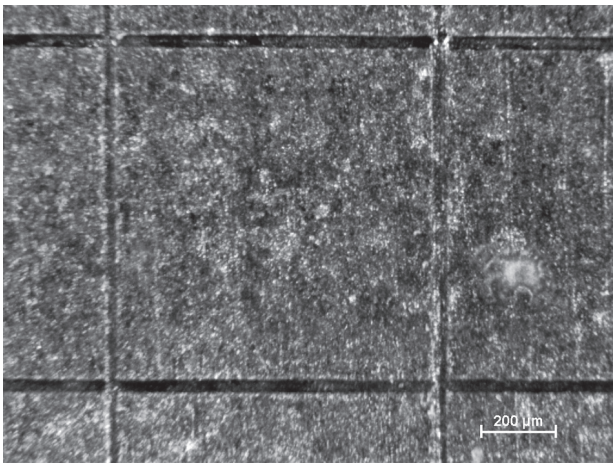
b) SAN



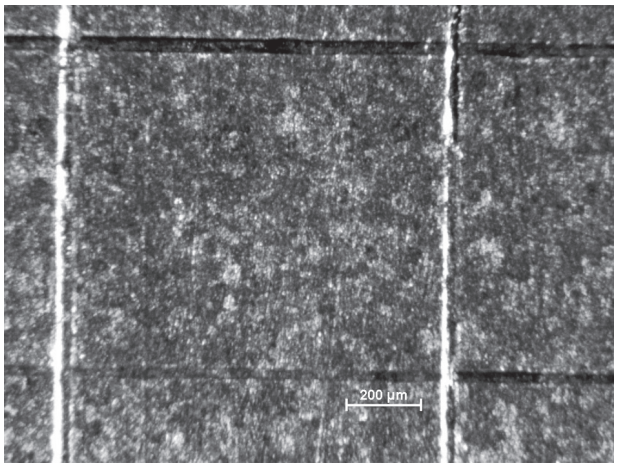
c) SANB



d) SANM



e) SCP-I



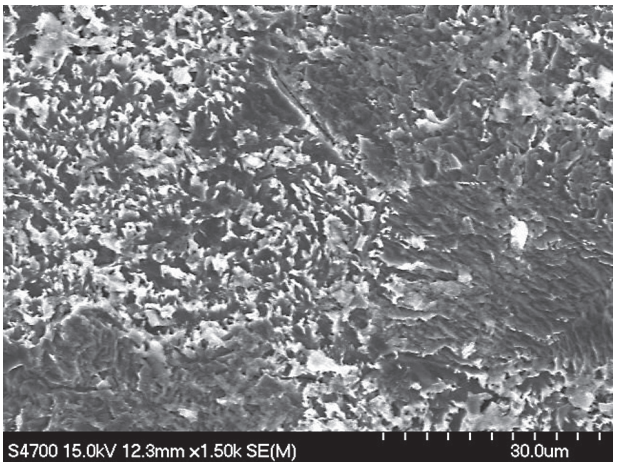
f) SCP-II

Figure 5. (OM) Coatings after tape test: a) SN, b) SAN, c) SANB, d) SANM, e) SCP-I, f) SCP-II.

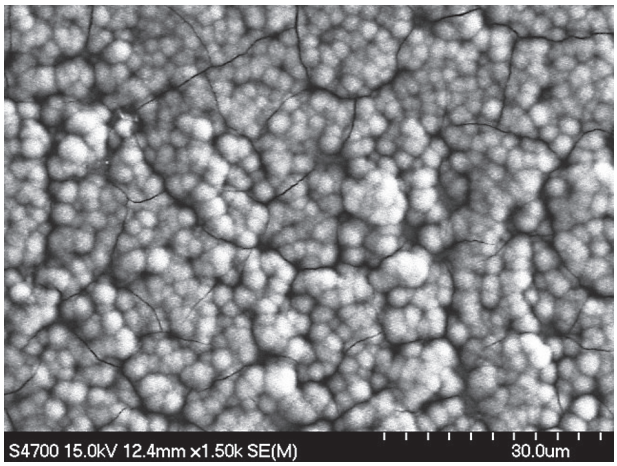
crackled all over the surface of the substrate. The cracks were longer than 30 μm and mutually interconnected.

The adhesion capacity of all the coatings was measured with the cross-cut tape test (ASTM D 3359-2) and evaluated visually (OM) by comparison with an established scale. The monitored indicator was the percentage of a scaling surface. Figures 5a through 5f show the coatings from both the groups after the tape test. After the tape was pulled off the coatings remained intact also inside the grid and along the cuts. The adhesion capacity of all the silicate coatings was excellent and ranked as grade 5.

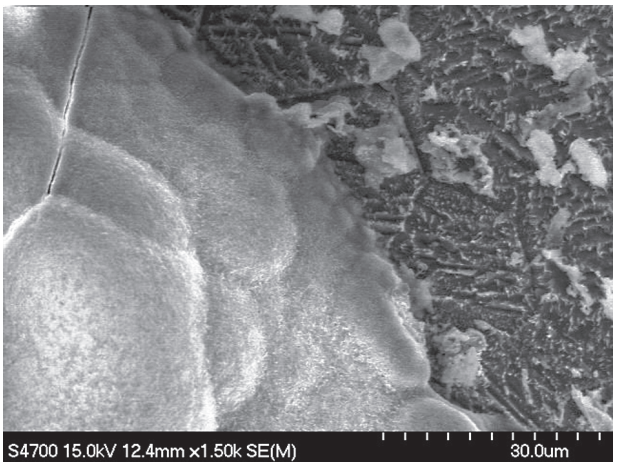
Coatings from both the groups were exposed to a static *in vitro* bioactivity test, where we monitored



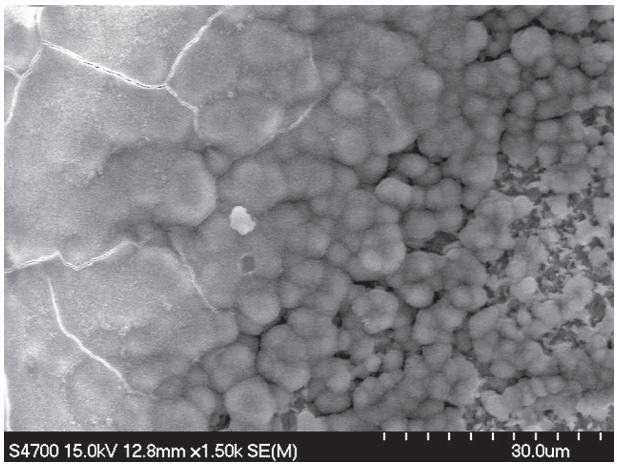
a) SN



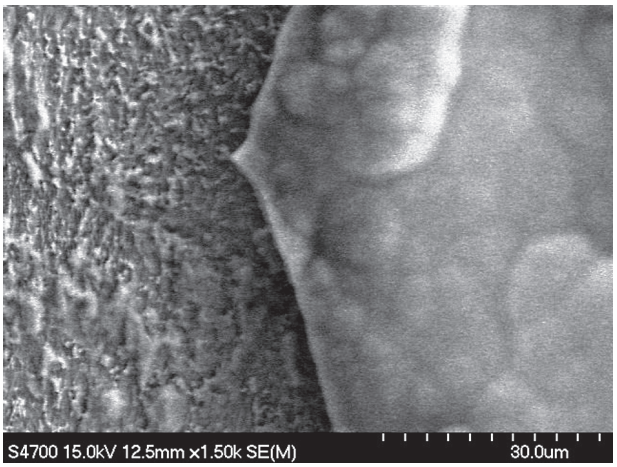
b) SAN



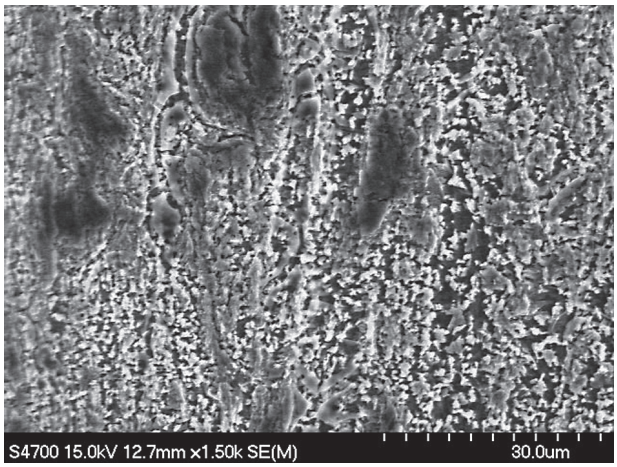
c) SANB



d) SANM



e) SCP-I



f) SCP-II

Figure 6. (SEM) Coatings after in vitro test: a) SN, b) SAN, c) SANB, d) SANM, e) SCP-I, f) SCP-II.

development of bone apatite after the exposure to simulated body fluid. The coatings were investigated with electron microscope after 20 days of exposure and the results are shown in Figures 6a through 6f. From among silicate coatings in the first group, the excellent bioactivity was demonstrated by the SAN coating (Figure 6b), which was completely and evenly covered with a new, probably hydroxyapatite, phase. Quite surprisingly, the coatings containing brushite SANB (Figure 6c) and monetite SANM (Figure 6d) with multiple huge globular clumps of hydroxyapatite nanocrystals on the surface demonstrated only partial bioactivity. The exposed basic silicate coating SN (Figure 6a) demonstrated inhomogeneity caused probably by its dissolution in SBF. In the second group the partial bioactivity was found only for the coating SCP-I (Figure 6e). It demonstrated the same behavior as the SANB and SANM coatings because a large agglomeration of globules typical for hydroxyapatite developed on its surface. The last coating, SCP-II (Figure 6f), demonstrated the same behavior as the SN coating. The surface was visibly disrupted, probably due to a large number of fissures which appeared after the firing and contributed to its partial disruption in SBF.

An XRD analysis of the SAN coating performed after the *in vitro* test (Figure 7) detected a newly developed hydroxyapatite phase (Ref. Code 04-009-8846) that was partly amorphous. On the surfaces of the SANB, SANM and SCP-I coatings there were only islands of the new phase that was visually identical with the phase on the SAN coating. We therefore anticipate that it was probably the same hydroxyapatite as in the case of the SAN coating but, due to its small quantity on the surface of coatings and due to the low crystallinity, it was not detected by XRD analysis.

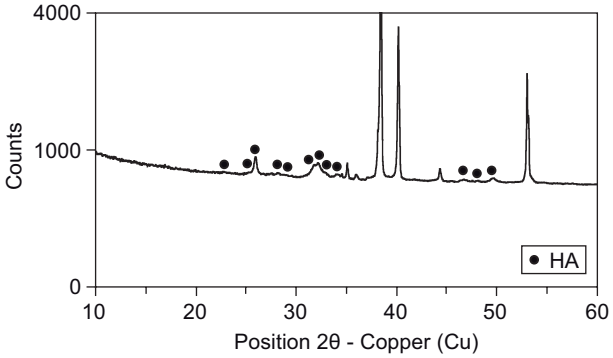
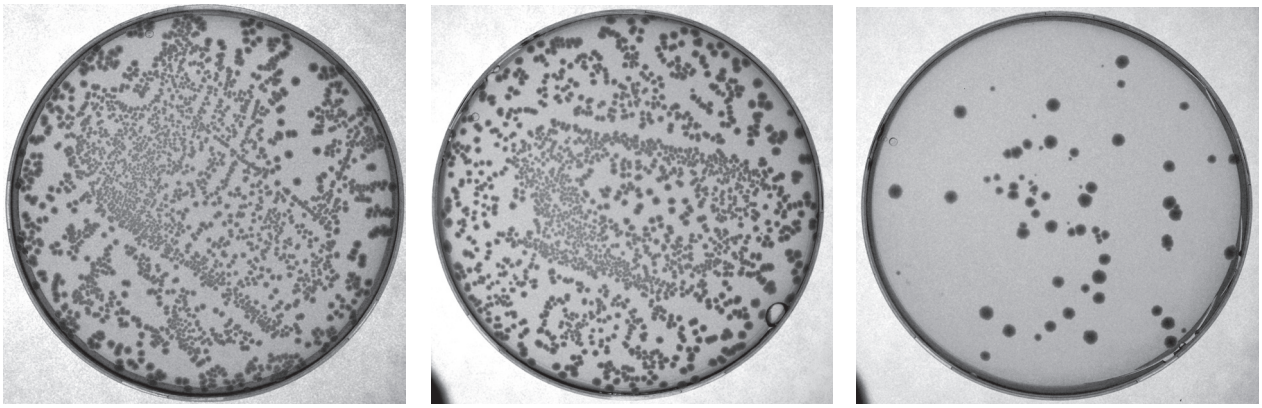
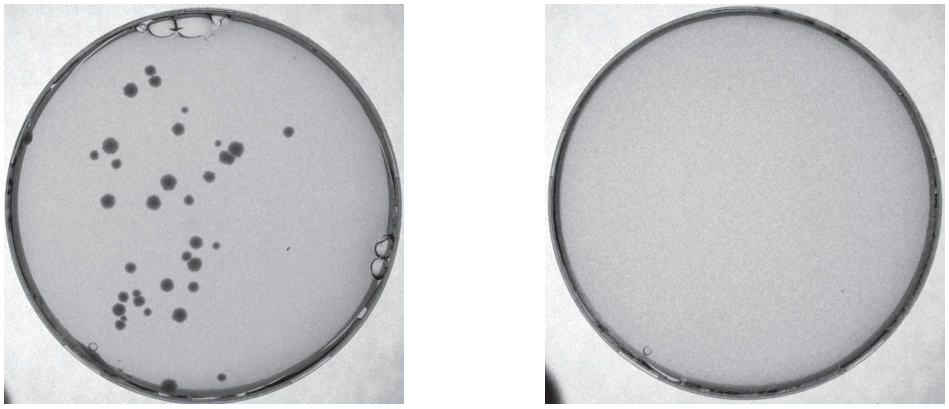


Figure 7. XRD difraction pattern for the coating SAN after *in vitro* test.



a) referent b) SN c) SAN



d) SANB e) SANM

Figure 8. Dishes with LB agar after bactericidal test and numbers of colonies of survived E. coli bacteria: a) referent, b) SN, c) SAN, d) SANB, e) SANM.

The bactericidal test was performed by dipping of all the coated samples from the first group into suspension of *E. coli* in physiological solution with the concentration of bacteria 10^4 cfu.ml^{-1} for 24 hours. Figures 8a through 8e show the representative examples of dishes containing LB agar with colonies of surviving bacteria. The images were compared with a reference sample (Figure 8a, number of colonies of surviving bacteria = 1.9×10^3), which was the same bacterial suspension but without contact with the coated substrate. The images positively show that the basic silicate coating SN (Figure 8b, number of colonies of surviving bacteria = 1.8×10^3) has no antibacterial properties because the number of colonies of surviving bacteria is comparable with the reference sample. The silicate coating containing silver - SAN (Figure 8c) - and the coating containing silver and brushite - SANB (Figure 8d) - have similar antibacterial properties because the order of colonies of surviving bacteria were 10^1 for both coatings. The silicate coating containing silver and monetite - SANM (Figure 8e) - caused 100 % death of bacteria under our experimental conditions.

The role of calcium phosphates, especially monetite, in antimicrobial properties of the prepared coatings has not been completely clarified. One of the options is that calcium phosphates in the sol may react with $AgNO_3$ to produce Ag_3PO_4 [21] (monetite and brushite turned yellow during the development of SANB and SANM sols) and the product may, e.g. in contact with the suspension of *E. coli,* partly dissolve the coatings containing brushite and monetite and this may further support release of Ag^+ ions into the solution and thus improve the antimicrobial properties of silver-containing coatings as the antibacterial effects are most frequently assigned to the release of Ag^+ ions. Another option is that the SANB and SANM coatings containing brushite and monetite have larger surface areas than the SAN and SN coatings. With the same concentration of $AgNO_3$ in the sols, the availability of silver on the surfaces of the prepared coatings with crystals of monetite and brushite is higher, the potential of its release as Ag^+ is also higher and, consequently, the antibacterial effects might be higher than in case of coatings containing no calcium phosphates. However, a question remains about the substantial difference between antibacterial effects of brushite and monetite because repeated tests have always proven 100 % death of bacteria only in contact with the coating containing silver and monetite (SANM). The difference between monetite and brushite consists in chemically bound water in brushite and slightly different surfaces and shapes of their crystals.

CONCLUSION

The sol-gel method using the dip-coating technique was successfully used to prepare two groups of silicate coatings on titanium substrates. The adhesive capacity of all the coatings was very good and on the classification scale it was ranked as grade 5. Nearly all the coatings demonstrated bioactivity in static *in vitro* tests, except the basic silicate coating (SN) from the first group and the silicate-calcium-phosphate coating (SCP-II) from the second group. The silicate coating containing silver (SAN) was after the exposure to SBF covered completely with a new layer, probably of hydroxyapatite, which was confirmed also by XRD analysis. Quite surprisingly, the coatings with silver, brushite and monetite from the first group and the silicate-calcium-phosphate coating (SCP-I) from the second group demonstrated only partial bioactivity and hydroxyapatite developed only in form of islands of agglomerated spherulites. More *in vitro* tests will be performed under static-dynamic and dynamic conditions, where coatings will be in contact with always fresh SBF solution. Antibacterial properties of coatings from the first group were tested by immersion of the coated samples into suspension of *E. coli* in physiological solution. The coating containing silver + monetite (SANM) demonstrated excellent antibacterial effects with 100 % death of bacteria. Coatings containing silver (SAN) and silver + brushite (SANB) demonstrated lower antibacterial effects. The basic silicate sol (SN) did not have any antibacterial properties. More bactericidal tests will be conducted on coatings without silver but containing brushite and, particularly, monetite, in order to determine the possible effect of powders themselves on bacteria. The tests will also investigate coatings with various silver contents.

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