

Hard implant coatings with antimicrobial properties

Claus Moseke · Uwe Gbureck · Patrick Elter ·
Peter Drechsler · Andreas Zoll · Roger Thull ·
Andrea Ewald

Received: 30 March 2011 / Accepted: 2 October 2011 / Published online: 16 October 2011
© Springer Science+Business Media, LLC 2011

Abstract Infection of orthopaedic implants often leads to inflammation immediately after surgery and increases patient morbidity due to repetitive operations. Silver ions have been shown to combine good biocompatibility with a low risk of inducing bacterial resistance. In this study a physical vapour deposition system using both arc deposition and magnetron sputtering has been utilized to produce silver ion doped TiN coatings on Ti substrates. This biphasic system combines the advantages of silver induced bactericidity with the good mechanical properties of TiN. Crystallographic analysis by X-ray diffraction showed that silver was deposited as well in its elementary form as it was incorporated into the crystal lattice of TiN, which resulted in increasing hardness of the TiN-coatings. Elution experiments revealed a continuous release of Ag ions in phosphate buffered saline. The coatings showed significant inhibitory effects on the growth of *Staphylococcus epidermidis* and *Staphylococcus aureus* and practically no cell-toxicity in cytocompatibility tests.

1 Introduction

Besides stainless steel titanium and its alloys are the most commonly used materials for the manufacturing of load bearing implants like knee joint replacements or hip prostheses [1]. The well known biocompatibility of titanium is primarily caused by the spontaneously forming passivation

layer leading to a low level of electronic conductivity and therefore high corrosion resistance [2, 3]. Due to the covalent/ionic bonding character of the oxide layer titanium is a good substrate for the adhesion of proteins and cells [4], a basic requirement for the formation and growth of new bone tissue in the vicinity to an orthopaedic implant. However, the same properties also promote the adhesion of microorganisms like bacteria, which may colonize the implant surface and thus cause inflammation at the implantation site [5]. These periimplantary infections occur in a frequency of up to 4.3% [6, 7], in the case of revision surgery even up to 17%. It is assumed that primarily the surgical intervention itself leads to the contamination of the implant surface [8]. However, also hematogenous or lymphogenous colonization of the implant is possible even though less frequent in transitory bacteraemia, arising from more distant sources of infection [8–10].

In times of increasing problems with multiresistant microorganisms, especially in hospital environments, there are efforts to modify implant surfaces with antimicrobial agents, with alternatives to antibiotic treatment being of growing importance. In the field of implantology several antimicrobial surface coatings have been developed. One established system is the covalent binding of antibiotic molecules to surfaces [11]; another one takes advantage of the anti-adhesiveness of hydrophobic coatings like polyvinylpyrrolidone (PVP) [12]. Further approaches are the introduction of noble metals into the surfaces or the development of coatings releasing antibiotics over long periods [13–16]. Biological tests with these surfaces show partially promising results, even though the latest state of research still does not allow their practical application. Coating of implant surfaces with antibiotics may lead to antibiotic concentrations below the minimal inhibitory

C. Moseke (✉) · U. Gbureck · P. Elter · P. Drechsler ·
A. Zoll · R. Thull · A. Ewald
Department for Functional Materials in Medicine and Dentistry,
University of Würzburg, Pleicherwall 2, 97070 Würzburg,
Germany
e-mail: claus.moseke@fmz.uni-wuerzburg.de

concentration which would result in the development of resistant microorganisms [17, 18]. A promising alternative to antibiotics is the functionalization of biomaterial surfaces with silver ions as they have low toxicity against human cells [19]. Furthermore, only few bacterial species could be proven to show resistance mechanisms against silver [20]. The antimicrobial effect of silver coated medical instruments has been documented in several studies [21–23] and is also being utilized for e.g. artificial heart valves or venous as well as urinary tract catheters [24–26].

Another crucial aspect of implant surface modification for load-bearing applications is the mechanical behaviour of the coating, which has to endure mechanical stress induced by the surgical procedure, especially during insertion into the bone by press-fit methods. Titanium nitride (TiN) is a very well established hard coating, which is commonly used on surfaces exposed to high mechanical loads and stresses, like those of cutting and shaping tools as well as machine parts. TiN as a coating for orthopaedic implants will meld the advantages of increased mechanical stability and improved corrosion resistance [27, 28] and well-proven biocompatibility [29, 30]. The aim of this study was to combine the mechanical qualities of a TiN hard coating with antimicrobial properties of silver. Coatings were produced by the simultaneous application of two methods of physical vapour deposition (arc deposition and magnetron sputtering) and were tested with regard to their phase composition and micro-hardness. The cytocompatibility was proven in osteoblast cell culture experiments and the bacteriostatic qualities were analyzed against various bacterial strains.

2 Materials and methods

2.1 Surface coating

Commercially pure titanium discs (\varnothing 15.5 mm, 1 mm height, ASTM-Nr. B 265-95, Grade 2, Zapp, Düsseldorf, Germany) were sonicated in pure water, alkaline Extran[®]-solution (Merck, Darmstadt, Germany) and finally in pure water at 40°C, with every cleaning step taking 10 min. The cleaned Ti discs were then placed into the recipient of a PVD-system type PLS 570 (Pfeiffer Vacuum, Germany). The coating set up consisted of an arc vaporization source for the titanium target and a radio-frequency magnetron for sputtering the silver target, thus providing the possibility to deposit both metals simultaneously. The deposition of TiN was achieved by introducing nitrogen 5.0 as reaction gas into the vacuum chamber during the arc vaporization of titanium. To allow for continuous TiN and silver deposition the titanium discs have been attached to a sample carousel rotating with a frequency of 0.33 s^{-1} (Fig. 1), thus

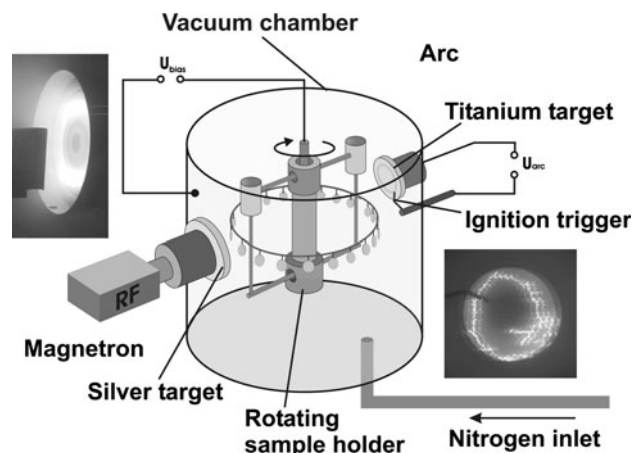


Fig. 1 Setup for physical vapour deposition. The inserted photographs show the burning plasma ring on the magnetron silver target (left) and the electric arc on the titanium target (right)

exposing the substrates alternately to the Ti vaporizer and the magnetron sputtered silver target. Furthermore, a bias voltage U_{bias} was applied to the substrates in order to improve the adhesion and the microscopic properties of the deposited layers [31]. The process parameters for different silver concentrations (arc deposition current, forward power of the applied radio frequency in magnetron sputtering, deposition time) in the surface coating are given in Table 1.

2.2 Coating characterization

The crystal structure of the coatings was determined by X-ray diffraction (XRD) in grazing angle geometry with a Siemens D5005 X-ray diffractometer (Bruker AXS, Karlsruhe, Germany) using Cu-K_α radiation with a voltage of 40 kV and a tube current of 50 mA. Micrographs of the surface were generated using a Helios NanoLab DualBeam System (FEI, Eindhoven, Netherlands). Elemental analysis by energy dispersive X-ray spectroscopy (EDXS) was

Table 1 Combinations of deposition parameters and resulting silver contents as calculated from EDX measurements

Batch No.	Coating time in min	I_{arc} in A	P_F in W	P_R in W	Resulting Ag content in %
1	120	80	0	0	0
2	120	80	308	86	1.4
3	120	80	414	121	3.12
4	150	70	414	76	3.47
5	120	80	414	84	3.74
6	240	60	414	58	4.62

I_{arc} current of the arc vaporizer, P_F the forward power, P_R the reflected power of the applied radio frequency

performed using a Si(Li) detector (Eumex, Heidenrod, Germany). Additional elemental analysis was carried out with a time-of-flight secondary ion mass spectrometer TOF SIMS V (IONTOF, Münster, Germany). Hardness testing was carried out using an atomic force microscope Autoprobe CP Research (ThermoMicroscopes, Sunnyvale, CA, USA) with a nano indenter module for probing hard coatings (Hysitron TriboScope, Minneapolis, USA). The indenter tip was a 90 ° pyramid that could be loaded with a maximum force of 5000 µN. The hardness of the sample surfaces was determined by ten measurements at different locations for every tested sample. Evaluation occurred by the TriboScope Software after preceding calibration of the area function on fused silica using the unload curve according to Oliver and Pharr [32].

2.3 Elution measurements

Titanium samples coated with AgN containing 1.4% Ag and 4.62% Ag, respectively, were immersed in 0.9% NaCl solution over 12 weeks at 50°C in order to determine the elution of Ag⁺ ions from the coatings under quasi-physiological conditions. The elevated temperature was chosen to accelerate the elution process and to simulate a prolonged residence time in physiological environment. For each silver content eight samples were placed in wells containing 1.5 ml of solution. The surrounding wells in the well plate were also filled with solution to compensate eventual water evaporation caused by the relatively high incubation temperature. The elution medium was replaced after 1 day, 2 days, 3 weeks, 6 weeks and 12 weeks and analyzed regarding Ag⁺ concentration with inductively coupled plasma mass spectrometry (ICP-MS, Varian, Darmstadt, Germany) against standard solutions of 50 and 100 µg/l (Merck, Darmstadt, Germany).

2.4 Anti microbiological activity of coated surfaces

The antimicrobial properties of the deposited TiAgN surfaces were examined as described previously [13]. Briefly, the samples were incubated with *Staphylococcus epidermidis*, strain RP62A (ATCC 35984), and *Staphylococcus aureus*, strain RN 4220, gram positive (kind gift from K. Ohlsen, Würzburg, Germany [33]) each for 24 h, washed in phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 7 mM Na₂HPO₂·2H₂O, 1.5 mM KH₂PO₄) and finally fixed in glutaraldehyde. After dehydration in ascending ethanol series the bacterial DNA of both strains was stained with SYBR[®]-green. By measurement of the fluorescence intensity on the sample surfaces the amount of adhered microorganisms was determined in comparison to the control samples consisting of cp-Ti.

2.5 Cell culture and biocompatibility tests

The human osteoblastic cell line MG-63 (ATCC no. CRL-1427, Rockville, MD, USA) was cultivated in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin (all from Invitrogen Life Technologies, Karlsruhe, Germany). The cells were incubated in humidified atmosphere at 37°C and a CO₂ content of 5%. For the cytocompatibility testing the samples and the titanium control were placed in triplicate into the wells of 24-well-plates (Nunc, Wiesbaden, Germany) and covered with 50,000 cells per ml of DMEM. Cell number and cell activity were determined as described earlier [13] after 3, 5, 7 and 10 days of culture.

2.6 Statistical analysis

Three specimens of each coated titanium specification as well as uncoated control surfaces were analyzed. For statistical analysis mean values as well as standard deviations of the obtained data were calculated and the Anova *t* test of Microsoft Excel was performed. Differences between measured values were judged significant, when *P* < 0.05.

3 Results

3.1 Surface characterization

Figure 2a shows the surface of a TiAgN coating that was deposited with an arc current of 60 A and a forward power of the applied radio frequency of 414 W. The coating showed a homogenous and closed structure, however, small and isolated droplets could be observed on the surface. A lateral cut into the sample using the focused ion beam revealed the profile of the coating and a layer thickness of ~1.27 µm (Fig. 2b). Integrated EDX spectra over a wide surface range showed the presence of titanium, nitrogen and silver as dominating compounds of the coating. The averaged silver contents of coatings deposited with different parameters are listed in Table 1. When the electron beam of the SEM was focused on the isolated droplets, the EDX spectra showed a significantly higher silver content, while smaller silver to titanium ratios were detected in areas without droplets. In addition Fig. 3 shows elemental maps obtained by SIMS of surfaces with silver contents of 1.4 and 4.62%. Figure 4 compares the topographic images (Fig. 4a, c) of two different areas on a surface containing 4.62% of silver with their respective backscattered electron images (Fig. 4b, d) and shows that the larger droplets on the surface have the same elemental

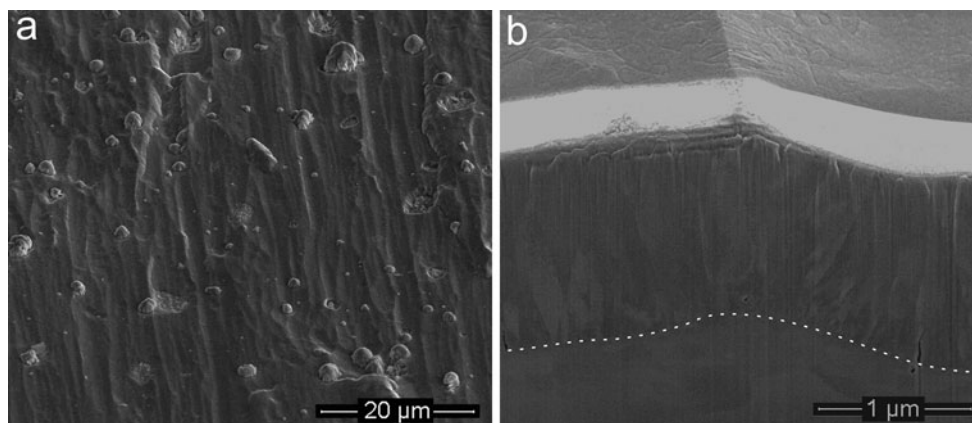


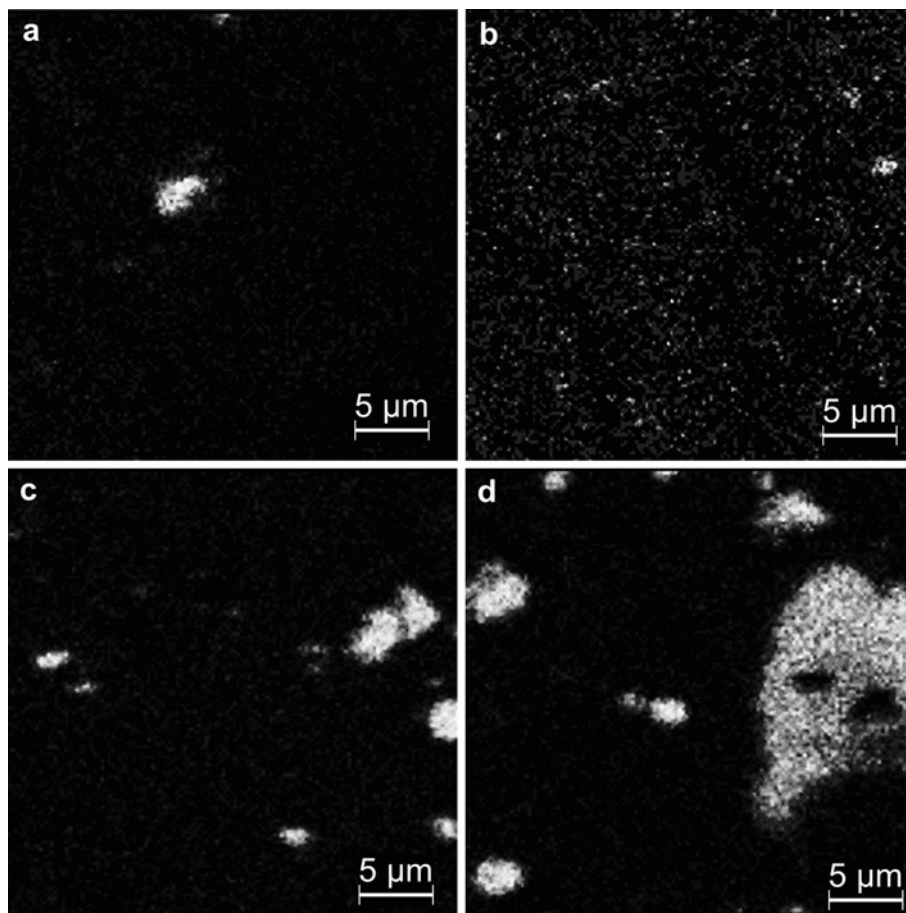
Fig. 2 Scanning electron micrograph of a TiAgN layer containing 4.62% Ag (a) and a lateral cut (obtained by the focused ion beam treatment) of the same coating (b)

composition as the rest of the surface (Fig. 4a, b), while the smaller droplets mainly consist of silver (Fig. 4c, d).

The results from the crystallographic analysis by XRD measurements revealed the basic crystal structure of the deposited coatings (Fig. 5). The basic structure of TiN could be identified in the silver-doped layers, however, their diffraction patterns were shifted to higher lattice constants, as is shown in Fig. 5a by comparison with the

peak positions for the lattice planes (111), (200), and (220) for a silver-free TiN deposit. Regarding the relative intensities of the three peaks also a well pronounced (111) in-plane texture could be observed. Furthermore, in comparison to the peaks of pure TiN the TiAgN peaks were significantly broadened and the integral intensity of the scan area was reduced. As Fig. 5b shows, the peak shift to higher lattice parameters was directly correlated with the

Fig. 3 Elemental SIMS maps of different areas of TiAgN surfaces containing 1.4% (a, b) and 4.6% (c, d) of silver



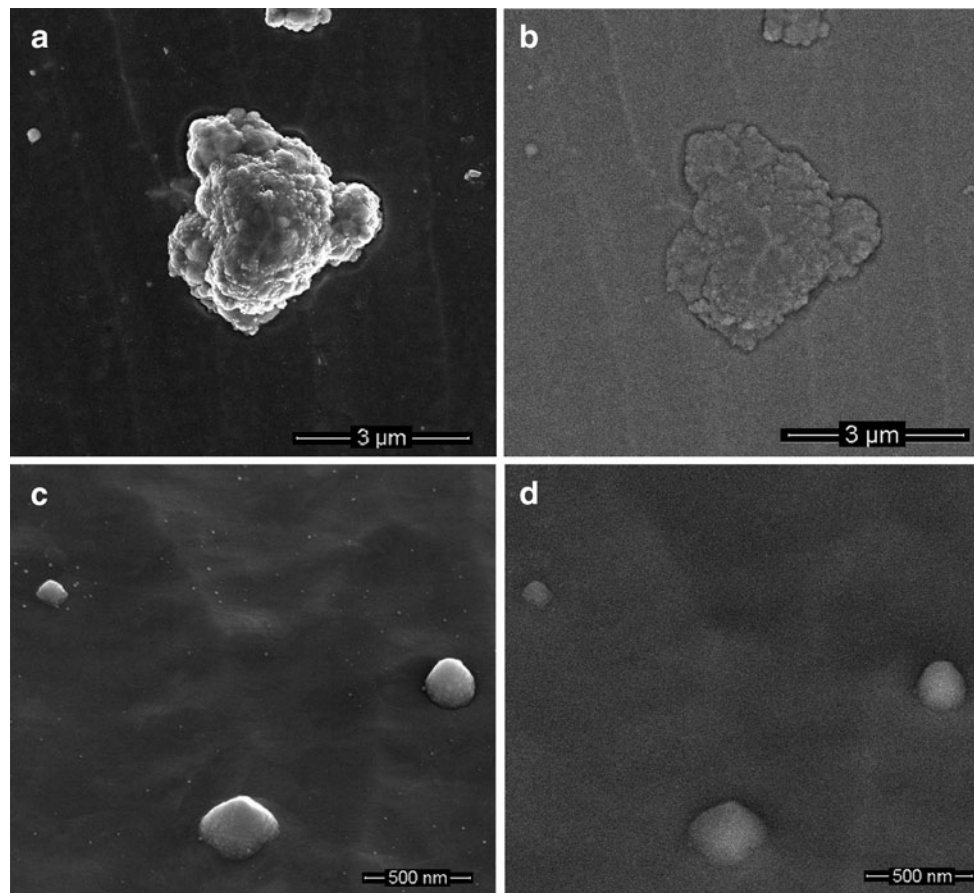


Fig. 4 Scanning electron micrographs of a TiAgN coating on Ti substrate with an average Ag content of 4.62%. **a, c** topographic images of two different areas; **b, d** backscattered electron images of the same areas

silver content in the respective TiAgN coating. Distinctive peaks indicating the presence of elementary silver could not be definitely identified, however in the diffraction pattern of the coating with the highest Ag concentration of 4.62% (shown in Fig. 5a) smeared respectively, overlapping peaks at 2Θ positions of 38.2 and 44.3 degrees could be observed.

The hardness test measurements using nano-indentation showed a hardness of about 21 GPa (Fig. 6) for the pure TiN coatings, while the silver-containing TiN coatings turned out to reveal significantly higher hardness values than the silver free deposits. The highest hardness was achieved for a silver concentration of 1.4% (34 GPa), but also the samples with the highest silver content of 4.62% exhibited a pronounced increase in hardness (29 GPa).

3.2 Ag-release from the surfaces

The release of Ag ions from TiAgN samples with 1.4 and 4.62% silver content was monitored over 12 weeks of immersion in 0.9% NaCl at 50°C. The mass spectrometric analysis of the elution liquid showed that silver was

released from all surfaces almost continuously (Fig. 7). For all eluates measured but the last the determined Ag^+ concentration in the medium of the 4.62% surface was significantly higher than in the medium taken from the 1.4% samples. Obviously the Ag^+ release rate was directly correlated with the silver content in the coating. Furthermore the surfaces of the TiAgN samples containing 4.62% Ag showed a remarkably high increase of the Ag^+ concentration in the analyzed medium after 6 weeks, while the same occurred only after 12 weeks for the samples containing 1.4% silver. At this time point the Ag release from both surfaces tested was at a similarly high level.

3.3 Antimicrobial activity

Coated surfaces containing 1.4 and 4.62% silver, respectively, were chosen to test the antimicrobial properties of TiAgN coatings. Therefore, three types of samples, namely as-deposited coatings, surfaces after 3 day immersion in 0.9% NaCl at 50°C, and surfaces eluted for 9 weeks in 0.9% NaCl at 50°C, were incubated with a bacteria suspension in logarithmic growth phase. All surfaces tested

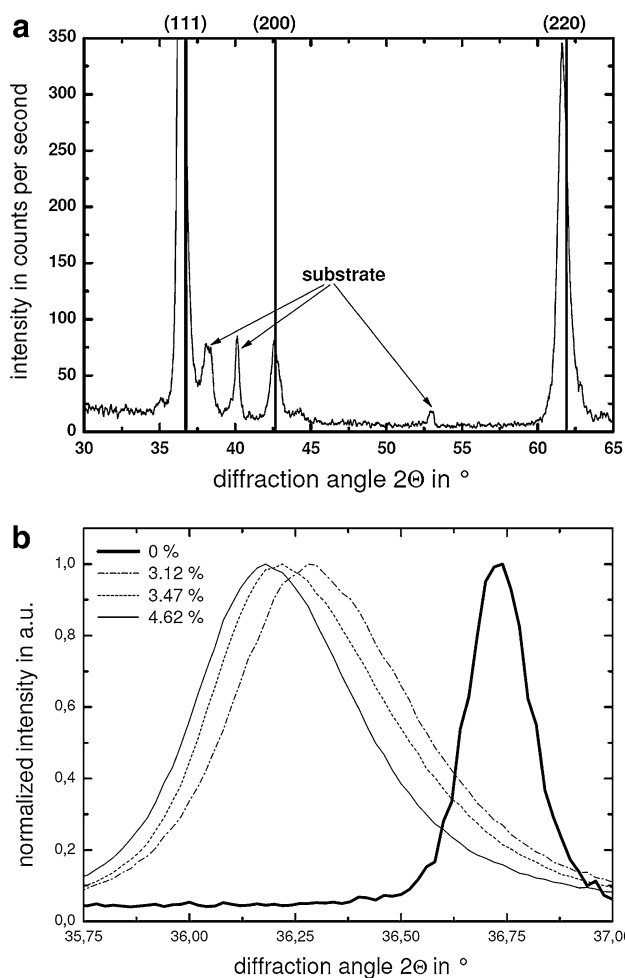


Fig. 5 **a** Diffraction pattern of a TiAgN coating with 4.62% silver on Ti substrate. The peak positions of three characteristic TiN lattice planes are marked with the corresponding Miller indices, **b** Comparison of the diffraction patterns around the peak of the (111) lattice plane of TiAgN coatings containing different amounts of Ag with the pattern of pure TiN. For reasons of clarity the peak intensities have been normalized to comparable values

showed antibacterial activity. In Fig. 8a the effect of the as-deposited surface on bacterial growth is shown. *Staphylococcus epidermidis* was reduced significantly ($P < 0.01$) on both of the tested surfaces (about 80% on 4.62% Ag and about 90% on 1.4% Ag containing TiAgN samples compared to a non-coated titanium control). *Staphylococcus aureus* was reduced only tendentially ($P > 0.05$) by about 20% on both of the surfaces. After 3 days of immersion in NaCl solution before incubation with microorganisms the bactericidal effect on *S. aureus* was significant ($P < 0.01$). On both surfaces the bacterial contamination was significantly reduced by about 75%. The reduction of *S. epidermidis* was less pronounced (25% on 4.62% Ag surfaces, $P = 0.05$; 40% on 1.4% Ag surfaces, $P < 0.05$). After 9 weeks of immersion *S. aureus* was still reduced by 50 and 60%, respectively, ($P < 0.01$). *Staphylococcus*

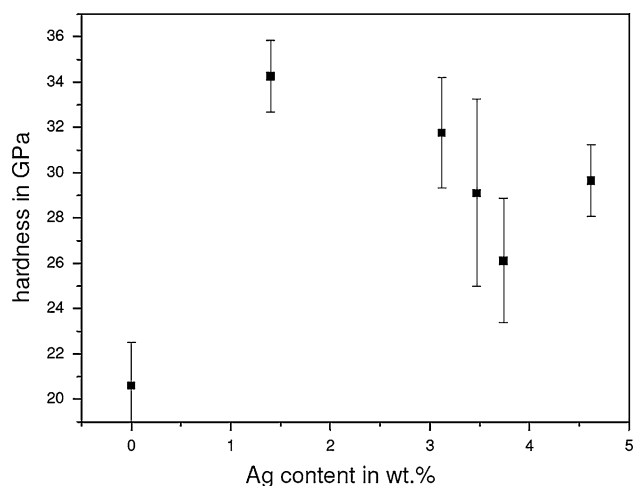


Fig. 6 Nanohardness of TiN–Ag coatings on titanium measured by micro-indentation

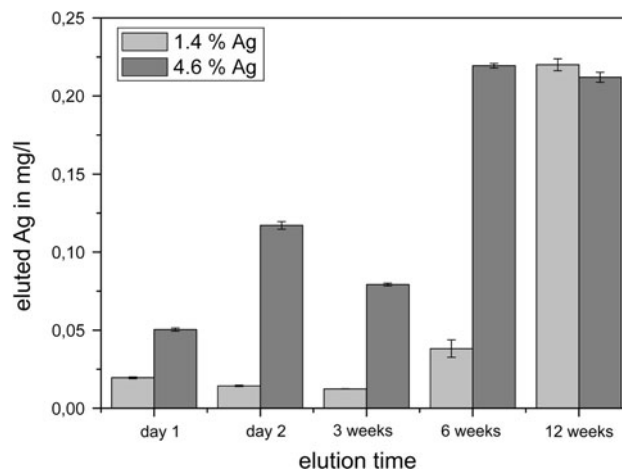


Fig. 7 Silver release into PBS buffer at 50°C of TiN–Ag coatings measured by ICP-MS

epidermidis was still significantly ($P < 0.01$) reduced on the 4.62% Ag containing surfaces but not significantly ($P > 0.05$) on the 1.4% samples. The experiments show a bacteriostatic effect even after 9 weeks of immersion in 0.9% NaCl at 50°C.

3.4 Cytocompatibility tests

The cytocompatibility of TiAgN coatings containing 1.4 and 4.62% silver, respectively, was analyzed by means of cell growth determination and measurement of cell activity using the WST reagent on the basis of DIN EN ISO 10993-5. Compared to commercially pure titanium the cell growth on surfaces containing 1.4% Ag was moderately reduced on day 10 of the culture time (Fig. 9a). At all other time points the cell growth was comparable to the titanium control, after 3 days of culture the growth was even higher

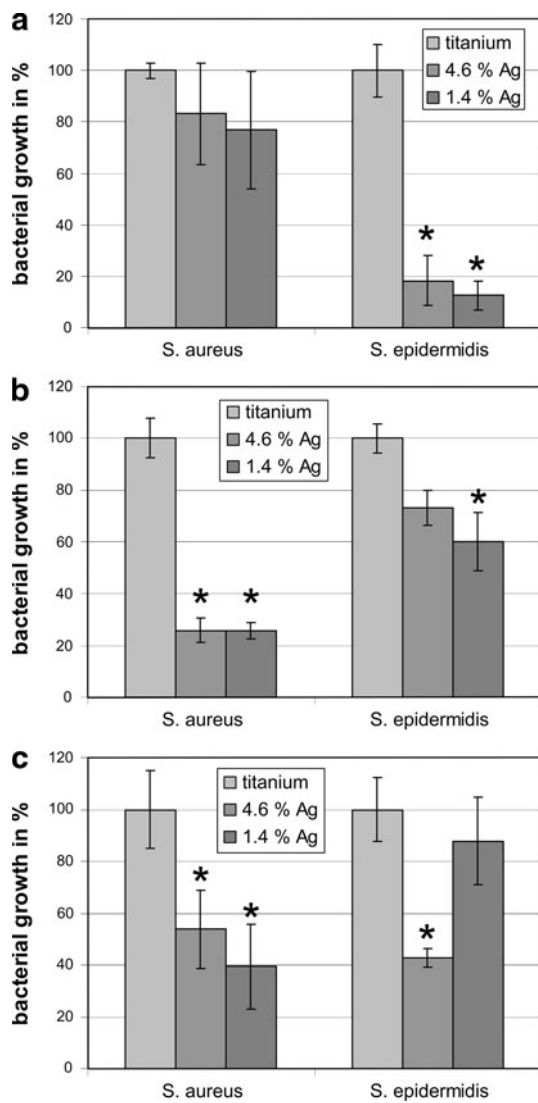


Fig. 8 Antimicrobial activity of TiN–Ag coatings containing 1.4 and 4.62% of silver against *S. aureus* and *S. epidermidis* compared with cp-Ti surfaces. **a** as-deposited, **b** after 3 days of immersion in NaCl solution, **c** after 9 weeks of immersion in NaCl solution. *Significant differences ($P < 0.05$)

($P < 0.05$). Cells grown on samples containing 4.62% Ag even showed increased growth in comparison to the control surfaces on days 3–7 ($P < 0.05$, Fig. 9a). A similar pattern was obtained after measurement of the cell activity (Fig. 9b). The initial activity of cells cultured on surfaces containing 1.4% Ag was higher than the one on titanium control samples ($P < 0.05$). On day 7 and 10 the cell activity decreased whereas the cell number was similar as on the control surfaces ($P > 0.05$). Cells grown on the samples containing 4.62% Ag showed similar activity as cells grown on Ti control surfaces. Therefore, the argenteriferous surfaces showed no cytotoxicity according to DIN EN ISO 10993-5 and to ISO 7405:1997.

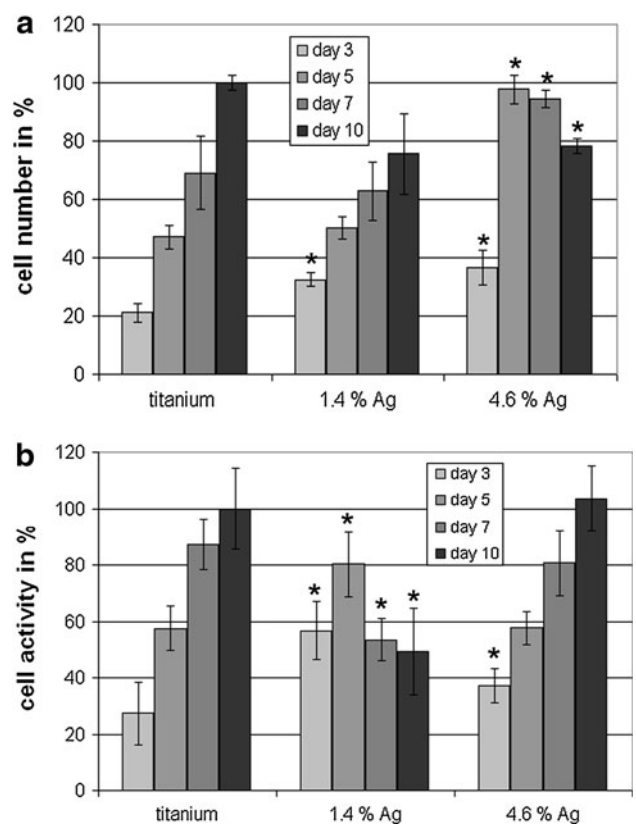


Fig. 9 **a** Proliferation of human osteoblast cell line MG63 on TiAgN coatings, **b** cell activity according to WST test. *Significant differences ($P < 0.05$)

4 Discussion

In this study the combination of two PVD techniques, namely magnetron sputtering and arc evaporation, was utilized to unify the high mechanical stability of TiN coatings with the well-known bacteriostatic effects of silver ions. The silver concentration in the coatings was adjusted by variation of the coating parameters and determined by integrated as well as location-specific EDX measurements and additional SIMS analysis, while the mechanical properties were evaluated by nano-indentation. Furthermore, XRD was used to determine the phase composition and the crystallographic properties of the silver-doped coatings.

The integrated EDX measurements as well as the elemental surface mapping by SIMS showed inhomogeneously distributed silver concentrations in the deposited coatings. EDX spectra obtained with the electron beam focused on apparent droplets revealed significantly higher silver contents than for the droplet-free areas. This location-dependent presence of silver indicates that it was deposited in two different formations during the coating process. Apparently silver was incorporated into the crystal lattice of the TiN phase on one hand and deposited in the form of

droplets containing elementary silver on the other. This was supported by the results from the crystallographic analysis by XRD measurements (Fig. 5). Pure TiN crystallizes in face centred cubic unit cells with a lattice parameter of 4.235 Å in NaCl structure. Basically this structure was maintained for TiN deposited in conjunction with the sputtering of the silver target. However, the diffraction patterns were shifted to higher lattice constants, as was shown by comparison with the peak positions for the lattice planes for a silver-free TiN deposit. The significant broadening as well as the reduced integral intensity of the TiAgN peaks as compared with the peaks of pure TiN support the assumption that silver was partially incorporated into the TiN lattice, particularly because the effect increased with rising silver content in the respective TiAgN coatings. As no characteristic peaks were annihilated, the incorporation occurred most probably by partial substitution of Ti by Ag ions. This resulted in an expansion of the lattice and a distortion of the unit cell due to the high deformability of the atomic shell of silver [34], which may lead to mechanical strain in the host lattice [35].

As diffraction peaks of elementary silver could only be observed in the coating with the highest Ag concentration (and even there not well pronounced), the presence of a well defined elementary silver phase could not be strictly approved by the XRD measurements. Therefore, it can be assumed that the second silver phase dominating the droplets most probably consisted of nanocrystalline respectively, amorphous silver.

The hardness obtained from the test measurements using nano-indentation for the pure TiN coatings was a little bit lower than the published value of (24.7 ± 2.0) GPa [36]. However, the silver containing TiN-coatings turned out to reveal significantly higher hardness values than the silver free coatings, whereby the highest hardness was achieved for a relatively low silver concentration of 1.4%. Most probably this improvement in mechanical resistivity occurred due to the assumed partial substitution of Ti by Ag in the TiN phase and the resulting distortion of the crystal lattice, which leads to the development of mechanical strain and therefore an increase of the elastic modulus. Obviously this effect had a maximum pronunciation for the lowest of the tested silver contents in the TiAgN coating. A similar effect was observed by Hsieh et al. [37] in co-sputtered TaN-Ag nanocomposite films. Their study also showed that the hardness decreases even below the value of undoped TaN, when a certain silver content is exceeded.

Besides the mechanical and crystallographic features of the deposited coatings, particularly the concentration-dependent silver ion release in liquid media and the resulting bactericidal properties were of interest in this study. The monitoring of the Ag⁺ ion release from TiAgN

samples immersed in NaCl solution over 12 weeks was carried out at elevated temperature to mimic a long time usage of the material. As was shown by the mass spectrometric analysis of the elution liquids, silver was released from all surfaces almost continuously. From the comparison of the silver concentrations in all eluates it became obvious that the Ag⁺ release rate was directly correlated with the silver content in the coating. For both applied silver concentrations the samples showed a vast increase of the Ag⁺ elution after certain immersion times (4.62% after 6 weeks, 1.4% after 12 weeks), which may have occurred due to a damage of the crystal lattice caused by corrosion processes. This effect would also explain the steady increase of Ag release with time. It is tempting to speculate that the 4.62% surface is more susceptible to corrosion processes as its metal lattice has incorporated more foreign ions. For antimicrobial activity a prolonged release of at least 0.1 ppb silver ions is required [38], this is assured for the surfaces tested in this study.

The microbiology experiments with *S. aureus* and *S. epidermidis* revealed a bacteriostatic effect of the silver doped surfaces even after 9 weeks of immersion in 0.9% NaCl at 50°C. According to this procedure it can be assumed that implants coated with TiAgN show bactericidal effects over an extended time minimizing the risk of infection. As shown by elution experiments the silver concentration will be still high after 12 weeks indicating a long time bacteriostatic effect. This bacteriostatic effect of Ti coatings was shown in previous research [13, 39, 40]. Here we could prove the activity of Ag⁺ ions in TiAgN coatings.

Relevant for bacteriostatic action of silver is the Ag⁺ ion due to its binding to electron donor groups in sulphur, oxygen or nitrogen containing biomolecules. Thereby low concentrations <35 ppb are bacteriostatic, whereas higher concentrations may be toxic to eukaryotic cells ranging from low and local toxicity up to systemic effects like argyria for very high doses >4–6 g of total silver content in the body [41]. These doses will not be reached with the concentrations released by the coating developed in this study.

However, further studies are necessary to understand the elution process more in detail. In particular, a careful structural examination following the elution process could provide information about which localization of silver in the deposited coatings is most appropriate for the desired release kinetics.

A comparative inspection of recently published literature concerning coatings for medical implants with antimicrobial properties reveals a variety of different methods as well for the coating itself as for the bactericidal functionalization [42]. However, materials intended for the use in load-bearing applications have to meet certain

mechanical requirements, e.g. high resistance against abrasive wear and anti-corrosive properties. The mechanical features of TiN based coatings have been well proven [27–29], yet many approaches have been made to enhance the mechanical properties as well as the bactericidal properties by doping with additional ions [43–46], the most promising being silver and copper. While the dose-dependent and clinically relevant contact bactericidity of surfaces equipped with these ions have been proven for magnetron co-sputtering as well as for ion implantation methods, only little is known about the long-term release kinetics of the antimicrobial species. The combined use of magnetron sputtering and arc evaporation apparently led to the formation of two different silver phases. This was also the case for TiN/Ag nanocomposite coatings obtained by co-sputtering with two magnetron targets, as was recently reported by Iordanova et al. [47], who detected the formation of a second silver phase only in coatings containing more than 10% silver. As this was in contrast to the findings from our studies, where a biphasic silver deposition was found for much lower Ag contents, it appears most probable that the deposition method has a significant influence on the incorporation of ionic additives to the functional coating and hence on the release kinetics. Regarding the latter point the combined utilization of magnetron sputtering and arc evaporation may be a promising technique to achieve coatings with prolonged silver ion release at clinically relevant but subtoxic doses.

5 Conclusion

Recent studies concerning the combination of hard coatings (TiN, TaN, TaON) with silver ions concentrated on co-sputtering methods for the deposition as well of bi-layer systems [48] as of nanocomposite thin films [37, 49, 50]. The coating procedure presented in this paper combined two entirely different methods of physical vapour deposition, namely reactive arc-deposition of TiN and magnetron sputtering of silver. This method was successful in the deposition of TiAgN coatings with reproducible Ag contents that were adjusted using different combinations of arc-deposition currents, forward powers of the applied radio frequency in magnetron sputtering and deposition times. XRD revealed that the resulting coatings consisted of a silver-rich phase and a TiN phase with partial substitution of Ti by Ag in the crystal lattice. This so-called TiAgN-phase showed pronounced lattice distortion in the diffraction patterns. Hardness measurements showed that the TiAgN coatings were harder than silver free TiN. Elution experiments showed a continuing release of Ag⁺ ions from the doped surfaces. The microbiological studies proved the TiAgN coatings to be bactericidal against

S. epidermidis and *S. aureus*, while their cytocompatibility regarding cell growth and cell activity was approximately the same as on uncoated Ti substrates when tested with osteoblast cells. In summary it can be stated that the TiAgN coatings produced by the described PVD method show very promising properties. They combine mechanical resistivity with highly desirable properties concerning their performance in physiological medium. When used as medical implant coatings the continuous release of Ag⁺ ions over several weeks may be very effective in avoiding infection during the healing period subsequent to surgery without reducing the biocompatibility of the implant.

Acknowledgments The authors wish to thank the Deutsche Forschungsgemeinschaft for their financial support (DFG Gb1/13-1) as well as the Fraunhofer-Institut für Silicatforschung (ISC, Würzburg, Germany) and Dr. Martin Kamp (Lehrstuhl für Technische Physik, Würzburg, Germany) for their experimental support.

References

- Dewidar MM, Yoon HC, Lim JK. Mechanical properties of metals for biomedical applications using powder metallurgy process: a review. *Met Mater Int*. 2006;12(3):193–206.
- Zitter H, Plenk HJ. The electrochemical behaviour of metallic implant materials as indicator of their biocompatibility. *J Biomed Mater Res*. 1987;21:881–96.
- Solar RJ, Pollack SR, Korostoff E. In vitro corrosion testing of titanium surgical implant alloys: an approach to understanding titanium release from implants. *J Biomed Mater Res*. 1979;13:217–50.
- Diebold U. The surface science of titanium dioxide. *Surf Sci Rep*. 2003;48:53–229.
- Gristina AG. Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science*. 1987;237:1588–95.
- Otto M. Klassifikation bei protheseninsuffizienz und partikelbestimmung. *Der Pathologe*. 2008;29(2):232–9.
- Darouiche RO. Treatment of infections associated with surgical implants. *N Engl J Med*. 2004;350:1422–9.
- Knobben BA, Engelsma Y, Neut D, van der Mei HC, Busscher HJ, van Horn JR. Intraoperative contamination influences wound discharge and periprosthetic infection. *Clin Orthop Relat Res*. 2006;452:236–41.
- Wodtke J, Lohr JF. The infected implant. *Der Orthopäde*. 2008;37:257–67.
- Ruchholtz S, Träger G, Nast-Kolb D. Die infizierte hüftgelenksprothese. *Der Unfallchirurg*. 2004;107(4):307–19.
- Kennedy JF, Humphreys JD. Active immobilized antibiotics based on metal hydroxides. *Antimicrob Agents Chemother*. 1976;9:766–70.
- Legeay G, Poncin-Epaillard F, Arciola CR. New surfaces with hydrophilic/hydrophobic characteristics in relation to (no)bioadhesion. *Int J Artif Organs*. 2006;29:453–61.
- Ewald A, Glückermann SK, Thull R, Gbureck U. Antimicrobial titanium/silver PVD coatings on titanium. *Biomed Eng Online*. 2006;5:22–31.
- Colon G, Ward BC, Webster TJ. Increased osteoblast and decreased *Staphylococcus epidermidis* functions on nanophase ZnO and TiO₂. *J Biomed Mater Res A*. 2006;78:595–604.
- Engelsman AF, Krom BP, Busscher HJ, van Dam GM, Ploeg RJ, van der Mei HC. Antimicrobial effects of an NO-releasing

- poly(ethylene vinylacetate) coating on soft-tissue implants in vitro and in a murine model. *Acta Biomater.* 2009;5(6):1905–10.
16. Norowski PA, Bumgardner JD. Biomaterial and antibiotic strategies for peri-implantitis. *Biomater.* 2009;88B:530–43.
 17. Gold HS, Moellering RC Jr. Antimicrobial-drug resistance. *N Engl J Med.* 1996;335(19):1445–53.
 18. Gransden WR. Antibiotic resistance. Nosocomial gram-negative infection. *J Med Microbiol.* 1997;46(6):436–9.
 19. Williams RL, Doherty PJ, Vince DG, Grashoff GJ, Williams DF. The biocompatibility of silver. *Crit Rev Biocompat.* 1989;5: 221–3.
 20. Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. *FEMS Microbiol Rev.* 2003; 27(2–3):341–53.
 21. Schierholz JM, Lucas LJ, Rump A, Pulverer G. Efficacy of silver-coated medical devices. *J Hosp Infect.* 1998;40:257–62.
 22. Roy M, Bandyopadhyay A, Bose S. In vitro antimicrobial and biological properties of laser assisted tricalcium phosphate coating on titanium for load bearing implant. *Mat Sci Eng.* 2009; C29:1965–8.
 23. Das K, Bose S, Bandyopadhyay A, Karandikar B, Gibbins BL. Surface coatings for improvement of bone cell materials and antimicrobial activities of Ti implants. *J Biomed Mater Res.* 2008;87B:455–60.
 24. Cook G, Costerton JW, Darouiche RO. Direct confocal microscopy studies of the bacterial colonization in vitro of a silver-coated heart valve sewing cuff. *Int J Antimicro Ag.* 2000;13: 169–73.
 25. Yorganci K, Krepel C, Weigelt JA, Edmiston CE. Activity of antibacterial impregnated central venous catheters against *Klebsiella pneumoniae*. *Int Care Med.* 2002;28:438–42.
 26. Bechert T, Bösward M, Lugauer S, Regenfus A, Greil J, Guggenbichler JP. The Erlanger silver catheter: in vitro results for antimicrobial activity. *Infection.* 1999;27(Suppl. 1):24–9.
 27. Jehn HA, Baumgärtner ME. Corrosion studies with hard coating-substrate systems. *Surf Coat Tech.* 1992;54(55):108–14.
 28. Griepentrog M, Mackrodt B, Mark G, Linz T. Properties of TiN hard coatings prepared by unbalanced magnetron sputtering and cathodic arc deposition using a uni- and bipolar pulsed bias voltage. *Surf Coat Tech.* 1994;74(75):326–32.
 29. Paschoal AL, Vanâncio EC, de Campos Franceschini Canale L, da Silva OL, Huerta-Vilca D, de Jesus Motheo A. Metallic biomaterials TiN-coated: corrosion analysis and biocompatibility. *Artif Org.* 2003;27(5):461–4.
 30. Zhao J, Li L, Li D, Gu H. A study on biocompatibility of TiN thin films deposited by dual-energy ion beam assisted deposition. *J Adh Sci Tech.* 2004;18(9):1003–10.
 31. Olbricha W, Kampschulte G. Superimposed pulse bias voltage used in arc and sputter technology. *Surf Coat Tech.* 1993; 59(1–3):274–80.
 32. Oliver WC, Pharr GM. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *J Mat Res.* 1992;7:1564–83.
 33. Saha D, Bal M. Transformation and expression of a staphylococcal plasmid in *Escherichia coli*. *FEMSLE.* 1993;109:279–82.
 34. Bliz H, Gliss B, Hanke W. Theory of Phonons in Ionic Crystals, Review Article, In: Horton GK, Maradudin AA (eds) *Dynamical Properties of Solids*, vol 1. North-Holland; 1974. p. 343.
 35. Fischer K, Bilz H, Haberkorn R, Weber W. Covalency and deformability of Ag⁺-ions in the lattice dynamics of silver halides. *Phys Stat Sol.* 1972;54:285–94.
 36. Travitzky NA, Zhitomirsky VN. Hardness of titanium nitride coatings fabricated by vacuum arc deposition. *J Mat Sci Let.* 1996;15:1818–20.
 37. Hsieh JH, Tseng CC, Chang YK, Chang SY, Wu W. Antibacterial behavior of TaN–Ag nanocomposite thin films with and without annealing. *Surf Coat Tech.* 2008;202:5586–9.
 38. Kumar R, Munstedt H. Silver ion release from antimicrobial polyamide/silver composites. *Biomaterials.* 2005;26:2081–8.
 39. Zhang Q, Sun C, Zhao Y, Zhou S, Hu X, Chen P. Low Ag-doped titanium dioxide nanosheet films with outstanding antimicrobial property. *Environ Sci Technol.* 2010;44(21):8270–5.
 40. Zhao J, Cai XM, Tang HQ, Liu T, Gu HQ, Cui RZ. Bactericidal and biocompatible properties of TiN/Ag multilayered films by ion beam assisted deposition. *J Mater Sci Mater Med.* 2009;20: 101–5.
 41. Gosheger G, Harges J, Ahrens H, Streitburger A, Buerger H, Erren M, Gonsel A, Kemper FH, Winkelmann W, von Eiff C. Silver-coated megaendoprostheses in a rabbit model—an analysis of the infection rate and toxicological side effects. *Biomaterials.* 2004;26:5547–56.
 42. Zhao L, Chu PK, Zhang Y, Wu Z. Review—antibacterial coatings on titanium implants. *J Biomed Mater Res.* 2009;91B: 470–80.
 43. Subramanian B, Ananthakumar R, Jayachandran M. Microstructural, mechanical and electrochemical corrosion properties of sputtered titanium-aluminum-nitride films for bioimplants. *Vacuum* 85:601–609. 7th international symposium on applied plasma science, Hamburg, 2009.
 44. Zhao J, Feng HJ, Tang HQ, Zheng JH. Bactericidal and corrosive properties of silver implanted TiN thin films coated on AISI317 stainless steel. *Surf Coat Technol.* 2007;201:5676–9.
 45. Oliveira C, Galindo RE, Palacio C, Calderon S, Almeida BG, Henriques M, Espinosa A, Carvalho S. Surface characterization of Ti–Si–C–ON coatings for orthopedic devices: XPS and raman spectroscopy. *Solid State Sci.* 2011;13:95–100.
 46. Kelly PJ, Li H, Whitehead KA, Verran J, Arnell RD, Iordanova I. A study of the antimicrobial and tribological properties of TiN/Ag nanocomposite coatings. *Surf Coat Technol.* 2009;204: 1137–40.
 47. Iordanova I, Kelly PJ, Antonov V, Li H. Influence of concentration of Ag on electron and crystallographic structure of reactively co-sputtered CFUBMS TiN/Ag nanocomposite coatings. *Mater Technol.* 2011;26:40–5.
 48. Köstenbauer H, Fontalvo GA, Keckes J, Mitterer C. Intrinsic stresses and stress relaxation in TiN/Ag multilayer coatings during thermal cycling. *Thin Solid Films.* 2007;516:1920–4.
 49. De los Arcos T, Oelhafen P, Aebi U, Hefti A, Düggelin M, Mathys D, Guggenheim R. Preparation and characterization of TiN–Ag nanocomposite films. *Vacuum.* 2002;67:463–70.
 50. Hsieh JH, Chang CC, Chang YK, Cherng JS. Photocatalytic and antibacterial properties of TaON–Ag nanocomposite thin films. *Thin Solid Films.* 2010;518:7263–6.