Silver doped metal layers for medical applications

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Abstract. Biological, physical and mechanical properties of silver-doped layers of titanium alloy Ti6Al4V and 316L steel prepared by pulsed laser deposition were studied. Metallic silver-doped coatings could be a new route for antibacterial protection in medicine. Thin films of silver and silver-doped materials were synthesized using KrF excimer laser deposition. The materials were ablated from two targets, which were composed either from titanium alloy with silver segments or from steel with silver segments. The concentration of silver ranged from 1.54 at% to 4.32 at% for steel and from 3.04 at% to 13.05 at% for titanium alloy. The layer properties such as silver content, structure, adhesion, surface wettability, and antibacterial efficacy (evaluated by *Escherichia coli* and *Bacillus subtilis* bacteria) were measured. Film adhesion was studied using scratch test. The antibacterial efficacy changed with silver doping up to 99.9%. Our investigation was focused on minimum Ag concentration needed to reach high antibacterial efficiency, high film adhesion, and hardness.

1. Introduction

Some of the most serious complications of surgical treatment of fractures are infections [1-3]. The infectious complications prolong healing, prevent fractures healing, and deplete the body's immune system [4-7]. The solution usually requires repeated surgeries and multiplies healing costs and inconvenience for patients [4, 8, 9]. The aim of this work is to find suitable antibacterial materials that will reduce the possibility of an infection or severity of infectious complications in patients after surgical treatment of fractures. The use of fixation screws for temporary immobilization of broken bones entails considerable risk of infection due to the possibility of bacteria spreading along the outer surface of the fixing screws into the body, see Fig. 1. Since silver is known for its excellent antibacterial properties, its use as a suitable dopant seemed a viable road to take [10-17].
In this contribution the silver doped metallic materials (titanium alloy Ti6Al4V and 316L steel) of various concentrations were created by pulsed laser deposition (PLD) method. Silver concentration for various deposition conditions, films crystalline structure, adhesion, hardness, surface morphology, antibacterial properties (evaluated by Gram-positive strain *Bacillus subtilis* and Gram-negative strain *Escherichia coli*) and wettability were also studied.

Our primary goal was to prepare layers with sufficient Ag content for antibacterial effect with maximum adhesion and hardness as close as possible to the substrate.

2. Experimental

**Deposition** - Silver-doped layers of titanium alloy Ti6Al4V and 316L steel were prepared by PLD using a KrF excimer laser ($\lambda = 248$ nm, $\tau = 20$ ns, rep. rate of 10 Hz). The laser beam was focused on a silver target with energy density of 2 J cm$^{-2}$, silver with Ti6Al4V or silver with 316L steel targets with energy density of 5 J cm$^{-2}$. The material was ablated from one target composed from silver and titanium alloy or steel segments. The substrate (Ti6Al4V, 316L steel or Si (100)) was placed 35 mm away from the target and held at room temperature. Films were grown in argon atmosphere at 0.25 Pa. The substrates were cleaned by RF discharge before the deposition process.

**Thickness and roughness** was measured by Alpha-step IQ mechanical profilometer (KLA TENCOR Co.).

**Structure** of layers was determined by XRD in parallel beam geometry and detector scan with stationary sample and grazing angle of incidence (GAOI) were used.

**Concentration of silver** was determined using WDX measurement (WDX – wavelength dependence X-ray analysis) was analysed with EDAX Jeol Supersprobe 733.

**Adhesion** - For determination of adhesion we used macroscratch tester REVETEST (CSM Instruments co.). The REVETEST system is a standard tester which is compatible with DIN EN 1071-3 and ASTM C1624. We used acoustic emission to estimate the delamination point and we visually confirmed it from the photography of the scratch. Rockwell diamond with tip radius of 200 µm was used as indenter.

**Micro adhesion and micro hardness** - For determination of micro adhesion and micro hardness the nanosclerometric head was used. The head is an extension of AFM microscope Solver Next (NT-MDT co.). This system provided us with graphic information of the surfaces by SPM technique with high resolution. The probe with diamond tip was of Berkovich type (radius about eighty nanometres). Calibration measurement on fused silica was performed and the calibration curve was obtained before measurement.

**Antibacterial properties** – The antibacterial properties of Ag-doped films were studied using the gram-negative strain *Escherichia coli* K12 C 600 [18] and the gram-positive strain *Bacillus subtilis* 168, commonly found in soil [19]. Bacteria were grown in Lysogeny Broth (LB) [20] medium and plated on LA plates (LA; LB with addition of 1.5 % of agar). The tested layers were placed into sterile glass chambers and covered with 0.5 ml of overnight cultures diluted in the sterile physiological saline solution at a concentration of cells $10^6$ CFU/ml (Colony Forming Units). Glass chambers were cultivated at 37°C and after 24 hours aliquots of 100 µl were withdrawn and serially diluted up to $10^6$.
in physiological saline solution. 100 µl of each diluted sample were spread over an agar plate. The plates were incubated overnight and the number of growing colonies was counted. The bactericidal efficacy expressing the antibacterial properties of different silver doped films was estimated according the formula [10, 11, 21]:

\[
\text{ABE} (\%) = \frac{\text{AN}_{\text{REF}} - \text{AN}_{\text{EXP}}}{\text{AN}_{\text{REF}}} \times 100\%,
\]

where ABE is the antibacterial efficiency, AN_{REF} is the alive number in reference group, and AN_{EXP} is the alive number in experiment group.

**Wettability** - The wettability of the films was determined by means of contact angle method measured by DSA100 (Krüss co.). The liquid used was distilled water.

3. **Results and discussion**

**Thickness** of created silver layers was 100 nm and 350 nm while that of created silver-doped layers was from 94 nm to 398 nm, depending on target, see Table 1. **Roughness** of silver-doped 316L steel films was from 12 nm to 29 nm and the roughness of silver-doped titanium alloy Ti6Al4V films was from 5 nm to 28 nm, similarly to silver layer, see Table 1. **Composition** - WDX measurement confirmed the increasing concentration of silver with increasing segment of the silver piece on the target - during deposition process. The concentrations of silver ranged from 1.54 at% to 4.32 at% for steel and from 3.04 at% to 13.05 at% for titanium alloy, see Table 1.

| Sample | Substrate | Roughness Ra [nm] | Thickness [nm] | Target (Area) | Ag [at%] |
|--------|-----------|-------------------|----------------|---------------|---------|
| Ag-1   | Ti6Al4V   | 10                | 100            | Ag            | 100     |
| Ag-2   | Ti6Al4V   | 11                | 350            | Ag            | 100     |
| S-1    | 316L steel| 13                | 161            | 316L steel : Ag (40:1) | 1.54    |
| S-2    | 316L steel| 29                | 260            | 316L steel : Ag (20:1) | 3.53    |
| S-3    | 316L steel| 12                | 94             | 316L steel : Ag (10:1) | 4.32    |
| T-1    | Ti6Al4V   | 5                 | 398            | Ti6Al4V : Ag (40:1) | 3.04    |
| T-2    | Ti6Al4V   | 28                | 294            | Ti6Al4V : Ag (20:1) | 5.05    |
| T-3    | Ti6Al4V   | 25                | 198            | Ti6Al4V : Ag (10:1) | 13.05   |

**Crystallinity** - In the XRD spectrum of silver-doped 316L steel films the peak of intermetallic compounds with Ag (compound Ag with Fe, Cr, Ni, and Mo) was identified. In the XRD spectrum of the titanium alloy doped with silver there are no new peaks, see Fig. 2.
Surface morphology was studied using scanning electron microscopy (SEM). The surface of the steel layers was smooth and covered with droplets with diameter up to several micrometres. No changes were observed with increasing amount of silver in the layers, see Fig. 3a. Titanium alloy layers were also smooth and their surfaces were also covered by droplets of similar size. Nevertheless, in contrast with steel layers there was an increase in number of droplets on the layers with increased silver amount, see Fig. 3b.

Fig. 3a. Surface morphology measured by SEM (mag = 400x) of layers of steel 316L with Ag. From left: S-1, S-2, S-3.

Fig. 3b. Surface morphology measured by SEM (mag = 400x) of layers of Ti6Al4V with Ag. From left: T-1, T-2, T-3.
Adhesion – A macro scratch tester was used for adhesion measurements. For samples Ag-1 and Ag-2 of pure silver layers we used linear progressive scratch with initial load 1 N and the end load 5 N. Loading rate was 4 N/min. The lengths of the scratches were 8 mm. Two scratches were performed on each sample. The layers were very soft and the tip penetrated at the start by initial load. For Ag-1 sample there was no delamination observed. In case of Ag-2 sample we observed delamination for critical force approximately 1.75 N, see Fig. 4. Pure silver layers exhibit low adhesion.

Fig. 4. Example of delamination of a 350 nm thick silver layer (Ag-2) load 1.4 N (a) and load 1.75 N (b). Comparison of adhesion for 100 nm (Ag-1) (c) and 350 nm (Ag-2) (d) thick silver layers for the force of 4 N.

For samples S-1, S-2, S-3, T-1, T-2, and T-3 we used linear progressive scratch with initial load 1 N and the end load 30 N. Loading rate was 15 N/min. The length of the scratches was 5 mm. Two scratches were performed on each sample. Samples were tested for two different substrate roughnesses, polished and lathed. Both substrates had similar behaviour. We did not observe any penetration through layer or delamination. The behaviour of the samples was similar to bulk material, see Fig. 5a., 5b.

Fig. 5a. Example of macro scratch on the layers of Ag doped steel 316L. From left to right: sample S-1, S-2 and S-3 (load for all samples 24 N).
Fig. 5b. Example of macro scratch on the layers of Ag doped Ti6Al4V alloy. From left to right: sample T-1, T-2 and T-3 (load for all samples 24 N).

**Micro adhesion and micro hardness** - The adhesion behaviour was verified by micro adhesion measurement, too. Two scratches with different loads were made on each sample. Lengths of the scratches were approximately 10 µm and loads were (0.5 ÷ 3) mN and (2.5 ÷ 5) mN. Scratching speed was 100 nm/s. For the micro scratch test we observed no adhesion failure. The hardness was evaluated from the scratch test too, see Table 2. Tabular values of hardness are approximately 0.25 GPa for pure silver, 5.0 GPa for 316L steel, and 3.4 GPa for titanium alloy Ti6Al4V. For the steel 316L there is obvious decrease in hardness with increasing silver amount in the layers. There is no such decrease in hardness for the titanium layers and the hardness values lie in a narrow band of 0.1 GPa between 4.12 and 4.22 GPa.

**Table 2.** The micro hardness of silver-doped 316L steel layers on 316L steel substrate and silver-doped titanium alloy Ti6Al4V layers on titanium alloy Ti6Al4V substrate.

| Sample | Hardness $H$ [GPa] |
|--------|-------------------|
| S-1    | 5.22 ± 0.79       |
| S-2    | 4.02 ± 0.66       |
| S-3    | 3.74 ± 0.54       |
| T-1    | 4.12 ± 0.43       |
| T-2    | 4.22 ± 0.50       |
| T-3    | 4.15 ± 0.15       |

**Antibacterial properties** - Layers of steel and titanium alloy doped with various concentration of silver (1.54 at%, 3.53 at%, and 4.32 at% for steel; and 3.04 at%, 5.05 at%, and 13.05 at% for titanium alloy) were tested for their antibacterial effect against the *Escherichia coli* (gram-negative) and the *Bacillus subtilis* (gram-positive) strains. Antibacterial efficiency increased with increasing silver content for Ti6Al4V layers, and was good for both of bacteria strains (reached 95% for *Bacillus subtilis* and 99.9% for *Escherichia coli*). For 316L steel we did not observed clear dependence on silver content, but the efficiency was high, too (reached 93% for *Bacillus subtilis* and 99.9% for *Escherichia coli*). Antibacterial efficiency significantly increased over time, see Fig. 6. Pure silver has excellent antibacterial properties (exceeds 99.9 %), but is very soft and has a low adhesion, inappropriate for practical use. After 24 h cultivation the silver doped metal layers with more than 4 at% of Ag exhibit good anti-bacterial ability. This is consistent with the results of silver-doped ceramic layers [10, 11].
Fig. 6. Antibacterial efficiency of silver-doped metal layers against the *Bacillus subtilis* (gram-positive) and the *Escherichia coli* (gram-negative) strains, after 2 and 24 hours.

**Wettability** - The wettability of the films was determined by using contact angle method. Distilled water was used as the liquid. The highest values of the contact angle were observed for pure silver layers. For layers of Ti6Al4V doped with silver, increasing contact angle values with increasing content of silver were observed. For layers of steel 316L there was no significant change in contact angle with silver content. Each type of layer was prepared on substrates with two different roughnesses, polished (P) and lathe turned (T). Contact angles on lathe turned substrates were slightly higher, see Fig. 7.

Fig. 7. The wettability of silver-doped 316L steel layers on 316L steel substrate (P – polished surface and T – turning surface) and silver-doped titanium alloy Ti6Al4V layers on titanium alloy Ti6Al4V substrate (liquid was distilled water).

4. Conclusions

The metallic (titanium alloy Ti6Al4V and 316L steel) layers with various concentration of silver were prepared by PLD. Composition was determined by WDX. The amount of silver in Ti6Al4V layers was from 3.04 to 13.05 at%. The amount of silver in 316L steel layers was from 1.54 to 4.32 at%. Minor changes were observed in crystalline structure of the doped steel, which can be assigned to intermetallic compounds with silver. We did not observe new peaks in XRD spectrum of the titanium
alloy doped with silver. No silver crystalline phase was found. All surfaces of the layers were covered with droplets. In case of the titanium layers the number of droplets increases with the increasing silver concentration. The adhesion of the silver-doped 316L steel and Ti6Al4V was outstanding. We did not observe any delamination of layers. The transition between the layer and substrate was not observed. The hardness of 316L steel layers decreased with increasing silver content. There is no such decrease in hardness for the titanium layers. Antibacterial efficiency increased with increasing silver content for Ti6Al4V layers, and was good for both bacteria strains. For 316L steel we did not observe any clear dependence on silver content, but the efficiency was high, too. Antibacterial efficiency significantly increased over time. The silver doped metal layers with over 4 at% of Ag exhibit good anti-bacterial ability. Wettability measurement showed that the contact angle of Ti6Al4V layers doped with silver increases with increasing silver content. For layers of 316L steel there was no significant change in the contact angle with varying silver content. The prepared layers were verified as a new possibility to prepare effective protection against incidence and propagation of infections during treatment of complicated fractures.

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References

[1] Džupa V, Ryantová V, Skála-Rosenbaum J, Vyhálek F, Fríc M, Grill R, Horák L and Pavelka T 2008 Acta chirurgiae orthopaedicae et traumatologiae Cechoslovaca 75 293
[2] Weber G B 2004 Asepsis and the risk of infection In: ed. Weber G B Minimax fracture fixation (Davos Platz, AO Publishing) 1–18
[3] Wininger D A and Fass R F 2003 Bone and joint infections In: ed. Finch R G, Greenwood D, Norrby S R and Whitley R J Antibiotic and chemotherapy (8th ed. Edinburgh, Churchill Livingstone) 733–739
[4] Hanssen A D and Rand J A 1998 J. Bone Jt Surg. 80-A 910
[5] Jahoda D, Nyč O, Imša J, Kučera E, Hanek P, Chrz P, Pokorný D, Tawa N, Landor I and Nosan A 2007 Acta Chir. orthop., Traum. čech., 74 397
[6] Stuhlbreiter G, Gaebel G, Kramer W and Neugebauer W 1989 Akt. Traumatol. 19 28
[7] Šťastník M 2004 Klín. Mikrobiol. Inf. Lek. 10 73
[8] Gallo J, Landor I and Vavřík P 2006 Acta Chir. orthop., Traum. čech. 73 229
[9] Hanssen A D 2005 Clin. Orthop. 437 91
[10] Jelinek M, Weiserová M, Kocourek T, Jurek K and Strnad J 2010 Laser Physics 20 562
[11] Jelinek M, Weiserová M, Kocourek T, Zezulová M and Strnad J 2011 Laser Physics 21 1265
[12] Chen W, Liu Y, Courtney H S, Bettenga M, Agrawal C M, Bumgardner J D and Ong J L 2006 Biomaterials 27 5512
[13] Chung R J, Hsieh M R, Huang K C, Perng L H, Chou F I and Chin T S 2005 Journal of Sol-Gel Science and Technology 33 229
[14] Lansdown A B G, Samson B, Laupattarakasem P and Vuttiviorjan A 1997 Br. J. Dermatos 137 728
[15] Gosheger G, Hardes J, Ahrens H, Streitburger A, Buerger H, Erren M, Gunsel A, Kemper F H, Winkemann W and Eiff C 2004 Biomaterials 25 5547
[16] Chen Y, Zheng X, Xie Y, Ding C, Ruan H and Fan C 2008 J. Mater Sci: Mater Med 19 3603
[17] Choi J W, Cho H M, Kwak E K, Kwon T G, Ryoo H M, Jeong Y K, Oh K S and Shin H I 2004 Key Engineering Materials 254-256 47
[18] Appleyard R K 1954 Genetics 39 440
[19] Madigan M, Martinko J 2005 *Brock Biology of Microorganisms (11th edition), Prentice Hall*

[20] Luria S E 1953 *Cold Spring Harb Symp Quant Biol* 18 237

[21] Huang L, Li D Q, Lin Y J, Wei M, Evans D G and Duan X 2005 *J Inorg Biochem* 99 986