PARTICULATE SILVER COATING ON 316L STAINLESS STEEL FOR BIOMEDICAL IMPLANTS

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ABSTRACT

In this study, the particulate silver coatings were prepared on a 316L stainless steel by electroplating method. The effect of 3 main influencing factors, namely current density, electroplating time and silver solution composition, on the structure and properties of the coating was investigated. The SEM data indicated that the average size of silver grains was about 20 µm. The optimal parameters for electrodeposition were $AgNO_3$ concentration of 0.05 M, current density of 0.5 A/dm^2 and plating time of 10 minutes. The optimal coating had lower silver ion release in simulated body fluid although the silver weight of this coating was higher than that of other coatings. Antibacterial efficiency of this coating was over 92 % in 60 min (against *Escherichia Coli* bacteria). After immersion for 168 h in simulated body fluid, concentration of silver released is not toxic to human body.

Keywords: Silver coating, particulate silver, electroplating, biomedical implants.

1. INTRODUCTION

For centuries, silver has been used for the treatment of burns, wounds and several bacterial infections [1]. Nowadays, silver has been incorporated into a variety of products and parts, especially products that are used in biomedical field such as artificial teeth, bone... to increase the antibacterial ability and to protect human health. According to the published scientific works, silver has been studied in a variety forms such as silver ion, silver zeolite, silver nanoparticles, silver metallic films or particulate silver coatings [1, 2]. However, the particulate silver coatings have not been studied much.

The 316L stainless steel (316L SS) has a compatibility with the human body; the mechanical properties of the 316L SS are suitable for bone structure; ease of fabrication and low cost. Therefore, it has been widely used in the field of temporary implants since 1960s and 1970s [3]. However, after implantation for 10-13 years in the human body, the accumulation of Fe, Cr, Ni ions that are released from 316L SS is significantly higher than that of non-implanted fluid [4]. In addition, corrosive products may cause local or systemic infections, terminate bone formation/growth, conjunctivitis, loosen of artificial joint implants, or cause neoplasms [5].

Various methods have been used to deposit the silver coating on the surface of metal substrate such as chemical vapor deposition (CVD), physical vapor deposition (PVD), plasma spraying, chemistry, electroplating, ... Among these methods, the electroplating method has been widely applied due to its low cost and ease of fabrication, that could be applied in many production processes.

Thus, the purpose of this study was to manufacture the particulate silver coating on 316L SS by electroplating method, for use in the surgery and bone implant to limit the risk of infection. The particulate silver coating made by electroplating method has the advantage that it does not completely cover the surface of steel substrate, so it has both the antibacterial ability and retains the biological activity of 316L stainless steel.

2. MATERIAL AND METHOD

2.1. Sample Preparation

The chemical composition of 316L stainless steel samples was presented in Table 1. These steel plates have been machined in the dimensions of 20 × 20 × 1 mm, then cleaned (by sonication using surfactants, distilled water, acetone) prior to the electrodeposition [2]. The electroplating process was performed at room temperature using the 316L SS steel cathode and a silver metal rod anode. The $AgNO_3$ plating solution had the concentration between 0.05 M and 0.5 M; current density was in a range of 0.05÷0.5 A/dm^2; electroplating time varied from 1 to 10 minutes. The purpose of this work was to manufacture the particulate silver coating that did not completely cover the surface of steel substrate so the parameters were selected in the small value range. After preparation, the samples were washed with distilled water for 3 minutes and heat treated in Argon gas atmosphere at 500 ^oC for 15 minutes. This heat treatment mode was optimal mode that was referred to the document 2.

Table 1. The material composition of 316L stainless steel sample.

Element	Al	Mn	Si	Cr	Ni	Mo	P	C	S	Fe
%wt	0.300	0.217	0.556	17.979	9.335	2.148	0.045	0.021	0.002	balance

Table 2. The plating mode by experimental modeling

No.	Concentration of $AgNO_3$, M	Current density, A/dm^2	Electroplating time, minute
1	0.05	0.05	1
2	0.5	0.05	1
3	0.05	0.5	1
4	0.5	0.5	1
5	0.05	0.05	10
6	0.5	0.05	10
7	0.05	0.5	10
8	0.5	0.5	10
9	0.275	0.275	5.5
10	0.275	0.275	5.5
11	0.275	0.275	5.5

Table 3. The technical parameters of the selected plating modes

No.	Concentration of $AgNO_3$, M	Current density, A/dm^2	Electroplating time, minute	Coating area, dm^2
1	0.05	0.500	1	0.02
2	0.05	0.050	10	
3	0.05	0.500	10	
4	0.275	0.275	5.5	

The authors used Modde 5.0 software to design experimental plan for investigating the influence of technological factors on the structure and properties of the coating. This method minimized the number of experiments and offered a scientifically optimal mode. In this study, 11 samples with different plating modes have been examined, as given in Table 2. For comparative study, we selected 4 modes (samples), such as optimal mode, center mode and two other modes with the same plating concentration, but with different current density or plating time (Table 3).

2.2. Analytical methods

2.2.1. Weight of silver coating

The weight of silver coating was determined by the formula 1. Sample weight was measured on a Precisa XR 205SM-DR (Switzerland), with accuracy of 0.00001 g.

$$m_{SC} = m_{SAP} - m_{SBP} \quad (1)$$

in which: m_{SC}: The weight of silver coating; m_{SAP}: The weight of 316L SS sample after silver plating; m_{SBP}: The weight of 316L SS sample before silver plating.

2.2.2. Microstructural study

The surface of the silver coating was scanned with a scanning electron microscope SEM JSM-6010 LV equipment (Japan). Through it, the level of silver particle distribution on the 316L SS substrate and grain size were evaluated.

2.2.3. Phase composition study

The phase composition of the silver coating surface after heat treatment was determined by X-ray diffraction method, using X-RAY D5005/SIEMENS equipment (Germany) with the measurement conditions as following: Temperature of 25 ^oC, 2θ scanning angle from 10˚ to 70˚, step size of 0.03˚, step time of 1s, using Cu anode.

2.2.4. Concentration of silver ion releasing in the simulated body fluid

The simulated body fluid (SBF) was prepared with the chemical composition shown in Table 4. The pH of the solution was 7.4, adjusted with 0.1 M NaOH or 0.1 M HCl [6, 7]. The samples were immersed in separate cups containing 10 ml of SBF, with maintained temperature of 37 ^oC. After 24 h, the immersion solution was analyzed by atomic absorption spectra (AAS) to determine the concentration of silver ion releasing in the SBF solution, adding into the beaker with 10 ml of a new SBF solution. The immersion process and sample measurement were

continued to 168 h. The AAS analysis was performed using a Shimadzu AA6300 equipment (Japan), silver ions were atomized by GFA graphite furnace, using cathode-ray tube and BCG-D2 lighting.

2.2.5. Antimicrobial activity test

As estimated, over 80 % of infections associated with metallic implants are caused by gram negative bacteria [2]. Thus, the antimicrobial activity against *E. coli* ATCC 14169 bacteria of silver plating samples has been then evaluated. In this test, a solution containing 3.7×10^8 CFU/ml *E. coli* bacteria was placed on the fixed surface area of each samples by pipetman. After 60 minutes of exposure, this solution was taken into a tube containing 9 ml of sterile water. Then 1 ml of this solution was transferred to a petri dish containing the culture medium. The diluting process in decimal series was then taken place (Fig. 1), following by incubation at 37 ^oC for 24 hours. The total coliforms were then counted using magnifying lens.

Table 4. The chemical composition of SBF.

No.	Reagents	Concentration (g/L)
1	NaCl	8.00
2	$NaHCO_3$	0.35
3	KCl	0.40
4	Na_2HPO_4.	0.48
5	$MgCl_2.6H_2$	0.10
6	$CaCl_2$	0.18
7	KH_2PO_4	0.06
8	$MgSO_4$.7H	0.10
9	Glucoza	1.00

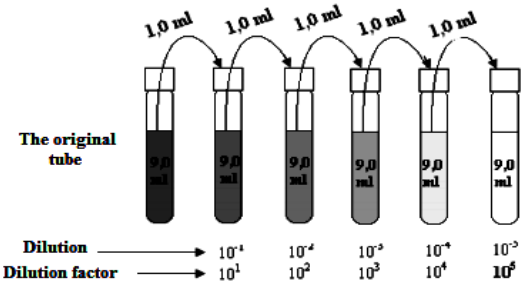

Figure 1. Diluting process in decimal series for antibacterial test.

3. RESULTS AND DISCUSSION

3.1. Weight of silver coating

To simplify presentation, from now on, the parameters of the plating process will be listed briefly and in turn as the concentration of $AgNO_3$ solution/current density/plating time. For example, the sample fabricated with $AgNO_3$ concentration of 0.05M, current density of $0.5A/dm^2$ and plating time of 1 minute will be referred to plating mode of 0.05/0.5/1. The weight of silver coating in the different plating modes was shown in the graph of Figure 2 and Table 5.

Table 5. The weight of silver coating in the different plating modes.

Electroplating mode	Average weight of silver coating, mg/mm^2
0.05/0.5/1	0.00315
0.05/0.05/10	0.02185
0.05/0.5/10	0.03005
0.275/0.275/5.5	0.019201

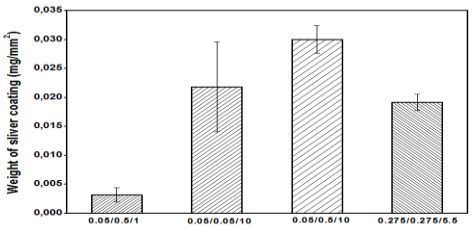

Figure 2. The weight of silver coating in the different plating modes.

The results showed that, the sample made at the plating mode of 0.05/0.5/10 had higher silver coating weight than that of the other samples. It was 9.5 times higher in comparison with the sample in plating mode of 0.05/0.5/1 and 1.4 times higher in comparison with the sample in plating mode of 0.05/0.05/10. Thus, when the concentration of $AgNO_3$ solution was constant, the weight of silver coating was proportional to the plating time and the current density. This was consistent with Faraday's law. However, the efficiency of the plating processes was different. By increasing the plating time from 1 minute to 10 minutes, the silver coating weight increased by 9.5 times. Whereas with the current density from 0.05 A/dm^2 to 0.5 A/dm^2, the silver coating weight increased by 1.4 times. Due to the increase of the current density, H_2 gas could be released from the reduction of H_2O at the cathode surface, which in turn reduced the efficiency of the plating process.

3.2. Microstructural observation

Figure 3 represents the SEM images of silver coating surface. As can be seen in this, the granular structure was observed in all samples at different plating modes. The average size of silver grains was smaller than 20 μm. Deposited silver grains did not completely cover the steel surface, but they dispersed rather uniformly with similar shape and particle size, which was clearly observed for the sample with the plating mode of 0.05/0.5/10. In case of 0.05M solution, the silver coating had smaller particle size than that of 0.275M solution. It was reported that the cathode polarization increased when the plating concentration reduced, thus decreased the particle size [8]. At the same condition of 0.05M solution and 10 minutes plating, 0.5 A/dm^2 current density provided the higher crystal-growth rate than that of 0.05 A/dm^2 current density. Therefore, the coating prepared at 0.05/0.5/10 mode was thicker than that of 0.05/0.05/10 mode.

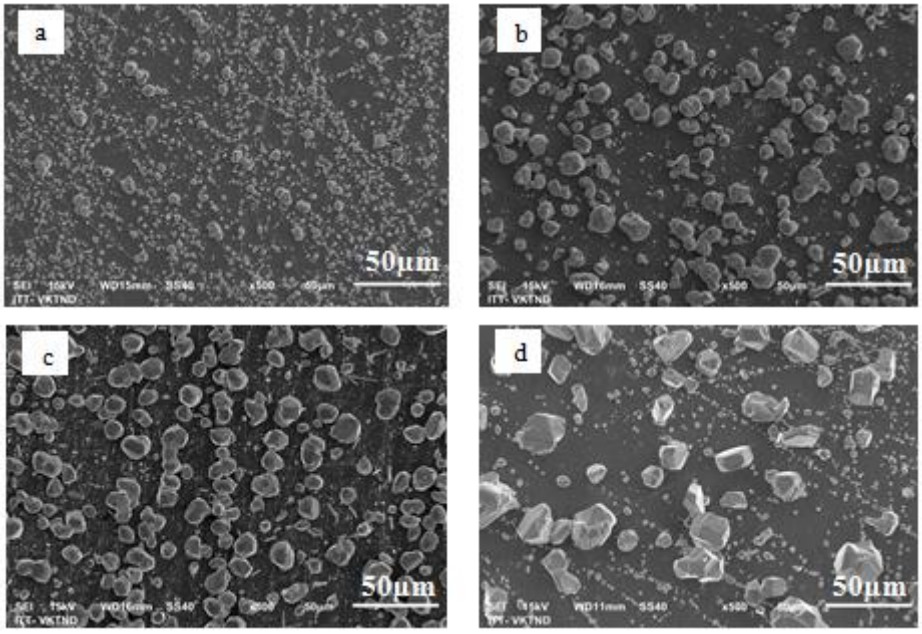

Figure 3. SEM images (×500) of the silver coating at the plating mode of 0.05/0.5/1 (a); 0.05/0.05/10 (b); 0.05/0.5/10 (c); and 0.275/0.275/5.5 (d).

By using low plating concentration and short time deposition, the coating prepared at mode of 0.05/0.5/1 had the smallest particle size (< 8 μm). In case of plating mode at 0.275/0.275/5.5, the surface coverage was smallest.

Thus, in the plating process, the plating concentration was an important factor that influenced on the size and distribution of the silver grains.

3.3. The phase composition study

Figure 4 showed the XRD patterns of coatings after heat treatment. As shown in this figure, after the heat treatment, beside the diffraction peak of Taenite phase (Fe, Ni) representing the 316L SS substrate, there was only one phase that represents silver metal. For silver metal, the 2θ angles of its diffraction peaks were found at 30.82°; 44.41° and 64.60°. This result confirmed that there was no change in the phase composition of the silver coating's surface after heat treatment at 500 °C for 15 minutes.

As seen in the Table 1, chromium content was about 18 % wt. However, no diffraction peak of chromium has been detected in XRD patterns. This result indicated the high coverage of thick silver coating on the steel substrate.

3.4. Concentration of silver releasing in the simulated body fluid

The particulate silver coating has been applied in the field of bone implants. Therefore, when applying to the human body, the concentration of silver ion releasing should be controlled at certain level that is not toxic to patients but still ensures the antibacterial role. To achieve that, according to researches by scientists around the world, silver ion concentrations should not be higher than 10 μg/ml [2]. Therefore, heat treatment was necessary to increase the adhesion not only between silver particles, but also between silver coating and steel substrate, thus might decrease silver release in human body fluids.

In addition, since the steel surface might not locally covered by silver coating, there would have the galvanic couples between silvers and the metals (iron, nickel, chromium), thus reduced the concentration of silver releasing in the SBF solution.

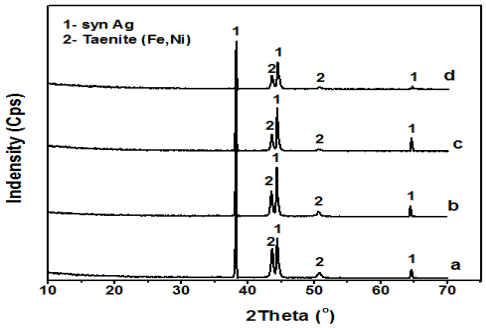

Figure 4. The phase composition on the silver coating's surface at the plating mode of 0.05/0.5/1 (a); 0.05/0.05/10 (b); 0.05/0.5/10 (c); 0.275/0.275/5.5 (d) after heat treatment.

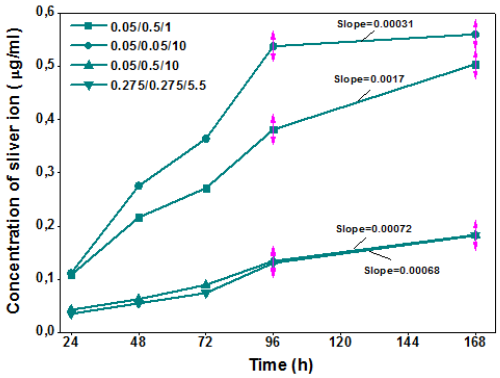

Figure 5. Silver release concentration in the SBF.

The chemical composition of SBF solution was similar to human serum [9]. The acceptable concentration of iron, nickel and chromium releasing in human serum was in range of 750 ÷ 1500 μg/l; 2.6 ÷ 7.5 μg/l; and 0.12 ÷ 2.1 μg/l, respectively [10]. These critical concentrations can

be reached only after long-time exposure (e.g. 10-13 years [11]) . Since the immersion time of sample is only 168 hrs, we did not measure the concentration of iron/nickel/chromium ions releasing in SBF.

Figure 5 represents the release test of silver ions in the SBF. It was reported that if concentration of silver ion releasing in SBF was lower than 10 μg/ml, it was safe for human body. The highest value of 0.0599 μg/ml was seen at the plating mode of 0.05/0.05/10. The lowest value of 0.1822 μg/ml was seen at the plating mode of 0.275/0.275/5.5. Whereas, the releasing concentration for other two samples, at the plating modes of 0.05/0.5/1 and 0.05/0.5/10 were 0.0538 μg/ml and 0.183 μg/ml, respectively. The rate of silver release in SBF rapidly increased in the range of 24 ÷ 96h immersion and insignificant changed after 96 h. From 96 h to 168 h, the evolution of silver ion concentration became slower.

3.5. Antimicrobial efficiency

Table 6. Antimicrobial efficacy of samples after 60 minutes exposure**.**

Sample	CFU/cm^2	Antimicrobial efficacy, %
316L SS	1,23 x 10^5	0
0.05/0.5/1	4,51 x 10^4	63,39
0.05/0.05/10	1,47 x 10^4	88,10
0.05/0.5/10	9,64 x 10^3	92,17
0.275/0.275/5.5	3,59 x 10^2	99,71

As shown in Table 6, at the plating mode of 0.05/0.5/1, the samples have lowest antimicrobial efficiency, due to its lowest weight. It was reported that silver ions could bind DNA to condense DNA molecules, lose its replication ability; interact with thiol group in protein to reduce the activity of protein [12]. In addition, silver ions could also attach to the bacteria membrane, interact with ribosomes and thereby suppresse the expression of enzymes and proteins necessary for ATP production-the main source of energy [13]. As seen in Table 5, the coating prepared at the 0.275/0.275/5.5 plating mode had highest antimicrobial efficiency, due to of its larger particle size, as compared with other modes.

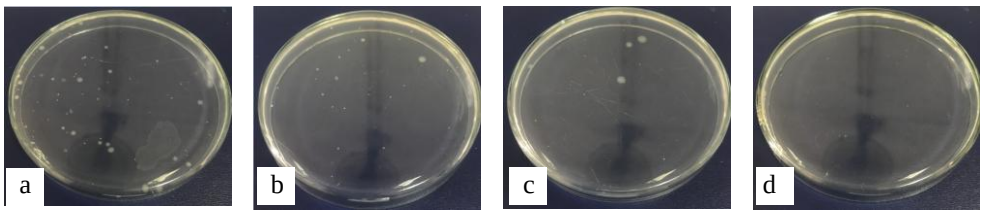

Figure 6. E.Coli bacterials grow in petri dish with dilution factor of 10^2 for the samples at the plating mode of 0.05/0.5/1 (a); 0.05/0.05/10 (b); 0.05/0.5/10 (c); 0.275/0.275/5.5 (d).

4. CONCLUSIONS

This article studied on the fabrication of the silver plating with the technical parameters as follows: $AgNO_3$ solution had the concentration of 0.05 M and 0.275 M; current density was in the range of 0.05 ÷ 0.5 A/dm^2, plating time varied from 1 to 10 minutes, heat treatment was at 500 ^{o}C for 15 minutes.

The SEM images showed that the silver coating had particulate form with silver particles size smaller than 20 μm. The silver ion release test in human body fluids showed that the rate of releasing silver in SBF rapidly increased in the range of 24 ÷ 96 h and insignificantly changed after 96 h.

Experiment data indicated the optimal parameters for electrodeposition as 0.05 M $AgNO_3$; at current density of 0.5 A/dm^2, during a plating time of 10 minutes. At this mode, the silver coating had the best particle distribution, the most homogeneous silver particle size; the highest silver weight, the concentration of silver ion releasing after immersion 168 h in SBF was 0.1833 μg/ml which was not toxic for human body. Antibacterial efficiency of this coating was over 92 % in 60 min (against *E. coli* bacteria).

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