Surface Characterization and Cell Response of Binary Ti–Ag Alloys with CP Ti as Material Control

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In this study, the surface passive films, dissolution behavior and biocompatibility of Ti–Ag alloys (with 5%, 10% and 20% Ag) were evaluated by X-ray diffraction (XRD) tests, electrochemical corrosion tests, X-ray photoelectron spectroscopy (XPS) tests, dissolution tests and *in-vitro* cytotoxicity tests. The surface films on the Ti–20Ag alloy are rich in Ti and much deficient in Ag with respect to alloy composition, as identified by XPS. Compared to CP Ti, Ti–5Ag and Ti–20Ag alloys show larger impedances and lower capacitances, which can be associated with an increase of the passive layer thickness. Moreover, all Ti–Ag alloys exhibit negligible or low metal release in the test solutions. The *in-vitro* cytotoxicity results show Ti–Ag alloys seem to be as cytocompatible as CP Ti. From the viewpoint of surface passive film and cytotoxicity, Ti–5Ag and Ti–20Ag are considered to be more suitable for dental applications.

KEY WORDS: Ti–Ag alloy; Surfaces; Electrochemical corrosion; Cytotoxicity

1. Introduction

Commercially pure titanium (CP Ti) has long been used as dental materials because of its good corrosion resistance and excellent biocompatibility^[1,2]. However, the application of CP Ti has raised some concerns due to the insufficient strength and poor wear resistance. Recently, Ti–Ag dental alloys were developed and a systematic study on the mechanical behavior^[3,4] and corrosion resistance^[5,6] was carried out. Based on the results reported, Ti–Ag alloys are believed to be an excellent candidate for dental materials.

As easy to be understood, dental materials to be used as permanent implants in the human body must be corrosion resistant and tissue compatible. In our previous study^[6,7], addition of Ag to Ti is found to be effective in reducing the corrosion current density and increasing the open circuit potential of Ti in artificial saliva environment. Moreover, tita-

nium/silver coatings consisting of both titanium and silver phases show low ion release rate and little cytotoxic effects^[8].

In order to understand the nature and growth pattern of the films formed on Ti–Ag alloys, X-ray photoelectron spectroscopy (XPS) analysis, open circuit potential tests and electrochemical impedance spectroscopy (EIS) tests were carried out. Furthermore, dissolution behavior and cytotoxicity of Ti–Ag alloys were characterized, thereby to develop new Ti–Ag alloys with good corrosion resistance and biocompatibility.

2. Materials and Method

Ti–*x*Ag binary alloys (*x*=5, 10 and 20, in wt%) were prepared from grade zero titanium, silver wire (99.9% in purity) by arc melting in argon atmosphere. The button ingots were re-melted four times for homogeneity. For XPS tests, dissolution tests and cytotoxicity tests, specimens (10 mm×10 mm×1 mm) were polished step by step till using 2000# silicon carbide paper, and then ultrasonically cleaned with acetone for 10 min. XPS analysis was performed with a PHI

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S5700 XPS system using mono $AlK\alpha$ (1486.6 eV) radiation at vacuum pressure of 10^{-4} Pa. Multiplex (narrow-scan) spectra were obtained with the pass energy of 29.35 eV for O1s, Ag3d and Ti2p peaks.

In the electrochemical corrosion tests, the cell used was conventional three-electroded configuration with a Pt electrode and a saturated calomel electrode (SCE) as the counter electrode and reference electrode, respectively. All the measurements were carried out on an electro-chemical workstation (CHI660C, China). The test solution was Fuayama Mayer artificial saliva (NaCl 0.4 g/L; KCl 0.4 g/L; CaCl₂ 0.6004 g/L; NaH₂PO₄·2H₂O 0.78 g/L; KSCN 0.300 g/L; Na₂S·9H₂O 0.005 g/L; urea 1.000 g/L).

In the corrosion potential tests, the specimens were submerged into the electrolyte without any imposed potential for 7200 s. The variations of the corrosion behavior during the formation of the passive film on the surface of the samples were studied by electrochemical impedance spectroscopy (EIS) technique. The impedance spectra were acquired in the frequency range of 10^4 – 10^{-2} Hz with a 10 mV amplitude sine wave at open circuit potential.

Three solutions were prepared for the dissolution tests. One was Fuayama Mayer artificial saliva as mentioned above. One was 1% lactic acid solution (the pH is about 2.1). The other was 0.1 mol/L H₂O₂+0.9% NaCl solution (pH 4.0 adjusted by lactic acid to accelerate the corrosion rate). Lactic acid could be formed in dental plaques, and H₂O₂ is likely to be generated in the body in the case of inflammation in the surrounding of implant or present in mouth washes. Specimens with the dimension of 10 mm×10 mm×1 mm were immersed in a 7-mL test solution at 37 °C for 168 h. The inductively coupled plasma atomic emission spectrometry (Leeman, Profile ICP-AES) was employed to measure the concentrations of titanium and silver ions, which had dissolved from the experimental alloy plates. An average of three measurements was taken for each group. The composition of surfaces before and after immersion was identified by scanning electron microscopy (SEM, Quanta 200 FEI) with energy dispersive X-ray detector.

Indirect cytotoxicity test was carried out according to a standard procedure described in our previous work^[9] using murine fibroblast cells (L929 and NIH3T3 cells). L929 and NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), which was supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µl/ml streptomycin. Extracts of the Ti–Ag alloys were prepared using serum free DMEM medium as the extraction medium with the surface area/extraction medium ratio of 3 cm²/ml in a humidified atmosphere with 5% CO₂ at 37 °C for 72 h. The control groups involved the use of DMEM medium as negative control, 10% dimethylsulfoxide (DMSO) medium as positive control and CP Ti as material control.

Fig. 1 XRD patterns of various Ti–Ag alloys, with CP Ti as control

Cells were incubated in 96 well cell culture plates at 5×10^3 cells/100 µl medium in each well and incubated for 24 h to allow attachment. The medium was then replaced with 100 µl of extracts. After 2, 4 and 7 days culture in a humidified atmosphere with 5% CO₂ at 37 °C in 96 well plates, 10 µl 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, America) was added to each well. The samples were incubated with MTT at 37 °C for 4 h in darkness, and then 100 µl formazan solubilization solution (10% SDS in 0.01 mol/L HCl) was added in each well overnight. The spectrophotometric absorbance of the samples was measured by microplate reader (Bio-RAD680) at 570 nm with a reference wavelength of 630 nm.

3. Results and Discussion

3.1 Phase identification

Fig. 1 shows the X-ray diffraction (XRD) patterns of different Ti–Ag alloys. The XRD patterns of Ti–Ag alloys are similar to those of CP Ti. It seems that the crystal structure of Ti–Ag alloys is not sensitive to Ag content. No peaks of intermetallic compounds are detected, which is possibly attributed to the high cooling rate during the preparation.

3.2 XPS surface analysis

In practice, a very thin oxide film on the Ti surface plays a decisive role in determining the biocompatibility and corrosion behavior of the Ti implant^[10]. In addition, surface modification is often used on titanium alloys to obtain enhanced wear resistance or high osseointegration^[11]. The knowledge of the material's surface composition is absolutely necessary.

The survey spectra obtained from CP Ti and Ti–20Ag alloy with XPS are shown in Fig. 2. The oxide layers mainly consist of TiO₂. The [Ag]/([Ti] + [Ag]) ratio in the surface oxide film is about 0.019, which is

Fig. 2 XPS surface chemical analyses of CP Ti and Ti-20Ag alloy

significantly lower than that in the substrate (about 0.2). The inset in Fig. 2 shows enlarged images of the sections of the spectra for Ti at about 460 eV. Metallic Ti could be detected for CP Ti. This indicates that the surface oxide film is very thin because the metallic state of elements could be detected through the surface oxide film. In comparison, the metallic Ti peak for Ti-20Ag alloy is not obvious.

3.3 Electrochemical behavior

Fig. 3 presents the evolutions of the open-circuit potential of CP Ti and Ti-Ag alloys as a function of immersion time in the artificial saliva solution. For CP Ti, Ti-5Ag and Ti-20Ag alloys, corrosion potentials increased moderately during initial immersion and stabilized after ~1000 s. This phenomenon indicates the thickening of the passive oxide film. After 7200 s immersion, the corrosion potentials of Ti-Ag alloys are about 150 mV higher than that of CP Ti, and Ti-5Ag alloy shows the highest open circuit potential values compared to other alloys. The change in the corrosion potentials is considered to be caused by the addition of Ag. Similar results can be observed in Ti-Ag binary alloys^[5,6] and Ti-Ag-Cu ternary alloys^[12]. It is assumed thicker or more stable oxide films formed on Ti-Ag based alloys.

The impedance spectra are present as Nyquist plots and Bode plots, as shown in Fig. 4. The nature of the Nyquist plots is similar for all the alloys, consisting of an arc that can be extrapolated to a semi-circle, which is associated with the corrosion process. Ti-20Ag alloy exhibits the largest semicircle radius, suggesting the highest impedance.

In the Bode plots, a highly capacitive behavior, typical of passive materials, is indicated from medium to low frequencies by phase angles approaching -90° , suggesting that a highly stable film is formed on tested alloy in the electrolyte used^[13]. Moreover, the intermediate frequency region (10^3 to 10 Hz) has the maximum phase angle and the

Fig. 3 Open circuit potential of CP Ti and Ti-Ag alloys in artificial saliva solution

Fig. 4 Electrochemical impedance spectra of CP Ti and Ti-Ag alloys immersed in artificial saliva solution: (a) Nyquist plots, (b) Bode plots

$\log|Z|$ vs $\log f$ slope approaching -1 . Those impedance responses correspond to the capacitive behavior of the electrode and describe the dielectric properties of the electronically conducting surface film.

According to the above EIS results obtained from CP Ti and Ti-Ag alloys, a $R_s(Q_1R_1)$ model was used as the equivalent circuit model to fit the EIS data in the case of single passive film presented on the metal surface, as shown in Fig. 5(a). R_s is the solution resistance; R_1 is the passive film resistance and Q_1 is the constant-phase element (CPE) for the passive film.

Table 1 Electrochemical impedance parameters of CP Ti and Ti–Ag alloys in artificial saliva solution

Materials	$R_s/(\Omega\cdot\text{cm}^2)$	$Q_1/(\text{F}/\text{cm}^2)$	n	$R_1/(\Omega\cdot\text{cm}^2)$	χ^2
CP Ti	155.8	3.30×10^{-5}	0.92	1.65×10^6	8.5×10^{-4}
Ti–5Ag	146.0	2.37×10^{-5}	0.93	2.10×10^6	7.3×10^{-4}
Ti–10Ag	175.6	3.19×10^{-5}	0.91	0.99×10^6	6.7×10^{-4}
Ti–20Ag	186.3	2.37×10^{-5}	0.93	4.82×10^6	5.0×10^{-4}

Fig. 5 Equivalent circuit fitted for Ti–Ag alloys (a) and fitted Bode impedance spectra of Ti–20Ag alloy immersed artificial saliva solution (b)

This model has been widely discussed for the single passive layer formed on the surface of titanium alloys^[14,15].

Good agreement between the experimental data and fitted data was obtained, as shown in Fig. 5(b) and the fitted parameters are shown in Table 1. Q_1 and n are the magnitude and the exponent of the constant phase element, respectively. The good consistence between the experimental data and fitted data was obtained with the χ^2 of about 10^{-4} . Large values of R_1 (of the order of $1\times 10^6 \Omega\cdot\text{cm}^2$) are obtained, confirming the formation of a passive film of CP Ti and Ti–Ag alloys. It is also observed that the n values are high, more than 0.9, which indicates that the passive film exhibits a near capacitive behavior. It is interesting that Ti–5Ag alloy and Ti–20Ag alloy give larger impedances and lower capacitances than CP Ti. Large impedances and low capacitances can be associated with an increase of the passive layer thickness^[16]. However, Ti–10Ag alloy exhibits the lowest impedance.

Moreover, it can be seen that the values of n were very close to 1 ($n>0.9$) for all the alloys. This indicates a near capacitive behavior of the passive films formed on CP Ti and Ti–Ag alloys^[17].

Fig. 6 Ion released behavior of Ti–Ag alloys in different solutions: (a) lactic acid solution, (b) H_2O_2 -containing solution, with CP Ti as material control

Compared to R_1 values, the R_s values obtained are relatively low and do not vary notably for different alloys.

3.4 Dissolution behavior and surface analysis

Fig. 6 shows the comparison of the amount of Ti and Ag ions released from Ti–Ag alloys after 7 d immersion in lactic acid solution (Fig. 6(a)) and H_2O_2 -containing solution (Fig. 6(b)). In artificial saliva solution, the Ti and Ag quantities released are very low and below the detection limit of ICP-AES, so the data are not shown.

In lactic acid solution and H_2O_2 -containing solution, all alloys exhibited very low ion released amount, suggesting that Ti–Ag alloys are corrosion-resistant metal in the test solutions^[18]. The quantity of Ti ions released from CP Ti is the highest in both solutions. In lactic acid solution, the Ag concentration is under the detection limit for

Fig. 7 XPS surface chemical analyses of CP Ti and Ti-20Ag alloy before and after immersed in H_2O_2 solution: (a) XPS survey spectrum, (b) the changes in atomic fractions in the alloy surface. (1), (2), (3), (4), (5) and (6) represent non-treated CP Ti, CP Ti immersed in lactic acid solution, CP Ti immersed in H_2O_2 -containing solution, non-treated Ti-20Ag alloy, Ti-20Ag alloy immersed in lactic acid solution and Ti-20Ag alloy immersed in H_2O_2 -containing solution, respectively

all Ti-Ag alloys. CP Ti shows remarkable dissolution, whereas Ti-10Ag alloy shows the least dissolution. Ti-5Ag and Ti-20Ag alloys showed slightly higher dissolution than Ti-10Ag alloy. In H_2O_2 -containing solution, the amount of Ag ions dissolved from Ti-Ag alloys increase with increasing Ag content. Ti-10Ag alloy and Ti-20Ag alloy exhibit similar total ion released amount.

According to the results obtained, Ti-Ag alloys do not release elements in proportion to its composition. The different quantities of the alloying element released depend on the type of solution. The released amount of Ag ions in lactic acid solution was mostly not detectable, namely under the minimal detection limit of ICP-AES, for all Ti-Ag alloys tested in lactic acid solution. In comparison, a significant Ag released amount is observed with the H_2O_2 -containing solution.

In order to elucidate the surface-related chemical characteristics of the alloy, XPS analysis was performed for the CP Ti and Ti-20Ag alloy after immersion in lactic acid solution and H_2O_2 -containing solution, and the spectra are shown in Fig. 7(a). After 7 d immersion, according to the fractions, TiO_2 is pre-

Fig. 8 Cell viability expressed as a percentage of the viability of cells in the negative control after 2, 4 and 7 d of culture in CP Ti and experimental Ti-Ag alloys extraction mediums: (a) L929 cell line, (b) NIH3T3 cell line

dominant state in the surface oxide. Little amounts of suboxide components could be found either coexisting with the TiO_2 phase or lying directly beneath this phase. For Ti-20Ag alloy after immersion in H_2O_2 -containing solution, no peak corresponding to Ag is detected.

Fig. 7(b) shows the atomic fraction of elements in the surface for CP Ti and Ti-20Ag alloy before and after immersion in various solutions. The air-formed oxide film on the Ti-20Ag alloy is rich in Ti and much deficient in Ag with respect to alloy composition. When the alloy is immersed in H_2O_2 -containing solution, the fractions of Ag cations in the surface film decrease as compared with the air-formed surface composition. According to the ion release results shown in Fig. 6(b), it could be deduced that most of Ag cations released into the solution. EDX analyses confirm the releasing of Ag. For Ti-20Ag alloy, when compared to the mean nominal chemical composition (19.62 ± 0.12 wt%), a significant decrease in Ag content (9.50 ± 0.18 wt%) could be observed after immersed in H_2O_2 -containing solution. On the contrary, Ag is moderately enriched in the alloy surface after immersion in lactic acid solution because of the release of Ti.

3.5 In-vitro cytotoxicity

Fig. 8 shows the viability of murine fibroblast cells

Fig. 9 Optical morphologies of L929 and NIH 3T3 cells cultured in the control, CP Ti and Ti–Ag alloys extraction medium for 7 d

L929 (Fig. 8(a)) and NIH3T3 (Fig. 8(b)) cocultured with the extract of the experimental alloys and expressed as a percentage of the viability of cells cultured in negative control. For L929 fibroblasts, there are few differences in cell viability among negative control, CP Ti and Ti–Ag materials at any point in time. In the case of NIH3T3 fibroblast cells, the viability of cells cultured in Ti–Ag alloy extracts slightly decreases compared to that cultured in CP Ti for 2 d, the cell viability decrease with increasing Ag content. After long time (4 and 7 d) culture, Ti–Ag alloy groups exhibit almost the same cell viability as CP Ti group.

The morphologies of L929 and NIH3T3 cells cultured in the extraction media for 7 d are shown in Fig. 9. Most of the cells cultured in the extracts of Ti–Ag alloys grow well and the cell proliferation has not been inhibited. The cells cultured in the extracts of Ti–Ag alloys show a similar attachment and proliferation to the negative control. All in all, the viability and morphologies results suggest excellent cytocompatibility for the Ti–Ag alloys.

4. Conclusion

In this study, Ti–Ag alloys have been prepared and their surface films, dissolution behavior and cytotoxicity have been tested. In comparison with CP Ti, Ti–5Ag and Ti–20Ag alloys show higher corrosion potentials, larger impedances and lower capacitances, which can be associated with nobler electrochemical behavior. The total amounts of dissolved titanium from Ti–Ag alloys are much less than that from CP Ti. The excellent corrosion resistance and the low level of dissolution are mainly attributed to the TiO₂ film on the surface. The cytotoxicity tests show that Ti–Ag alloys indicate similar cell viability to CP Ti. Because of their excellent corrosion resistance, low ion release amount and good biocompatibility, Ti–5Ag alloy and Ti–20Ag alloy are probably to be good candidate as dental materials and can be used for different dental devices.

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REFERENCES

- [1] D.M. Brunette, P. Tengvall, M. Textor and P. Thomsen: *Titanium in Medicine*, Springer, Berlin, 2001.
- [2] M. Niinomi: *Mater. Sci. Eng. A*, 1998, **243**, 231.
- [3] M. Takahashi, M. Kikuchi and Y. Takada: *Dent. Mater. J.*, 2002, **21**, 270.
- [4] K.T. Oh, H.M. Shim and K.N. Kim: *J. Biomed. Mater. Res. Part B*, 2005, **74**, 649.
- [5] S.B. Hong, N. Eliaz, E.M. Sachs, S.M. Allen and R.M. Latanision: *Corros. Sci.*, 2001, **43**, 1781.
- [6] B.B. Zhang, Y.F. Zheng and Y. Liu: *Dent. Mater.*, 2008, **25**, 672.
- [7] B.B. Zhang, B.L. Wang, L. Li and Y.F. Zheng: *Dent. Mater.*, 2011, **27**, 214.
- [8] A. Ewald, S.K. Glückermann, R. Thull and U. Gbureck: *Biomed. Eng. Online*, 2006, **5**, 22.
- [9] X.N. Gu, Y. F. Zheng, Y. Cheng and S.P. Zhong: *Biomaterials*, 2009, **30**, 484.
- [10] A.P.R. Alves, F.A. Santana, L.A.A. Rosa, S.A. Cursino and E.N. Codaro: *Mater. Sci. Eng. C*, 2004, **24**, 693.
- [11] M. Geetha, A.K. Singh, R. Asokamani and A.K. Gogia: *Prog. Mater. Sci.*, 2009, **54**, 397.
- [12] D.K. Kang, S.K. Moon, K.T. Oh, G.S. Choi and K.N. Kim: *J. Biomed. Mater. Res. Part B*, 2009, **90**, 446.
- [13] S.L. de Assis, S. Wolyneć and I. Costa: *Electrochim. Acta*, 2006, **51**, 1815.
- [14] B.L. Wang, Y.F. Zheng and L.C. Zhao: *Mater. Corros.*, 2009, **60**, 788.
- [15] J.E.G. González and J.C. Mirza-Rosca: *J. Electroanal. Chem.*, 1999, **471**, 109.
- [16] W.R. Osorio, A. Cremasco, P.N. Andrade, A. Garcia and R. Caram: *Electrochim. Acta*, 2010, **55**, 759.
- [17] A.K. Shukla and R. Balasubramaniam: *Corros. Sci.*, 2006, **48**, 1696.
- [18] Y. Okazaki and E. Gotoh: *Biomaterials*, 2005, **26**, 11.