Corrosion resistance of Ti-Si-N coatings in blood and cytocompatibility with vascular endothelial cells

Ming Zhang a, b, Ang Gao b, Shengli Ma a, Kewei Xu a, Paul K. Chu b, *

a State Key Laboratory for Mechanical Behavior of Materials, Xi’an Jiaotong University, Xi’an 710049, China
b Department of Physics and Materials Science, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong, China

ARTICLE INFO
Article history:
Received 22 September 2015
Received in revised form 8 March 2016
Accepted 9 March 2016
Available online 10 March 2016

Keywords:
Ti-Si-N coatings
Corrosion resistance
Cell proliferation
Hemocompatibility

ABSTRACT
Ti-Si-N coatings are deposited on titanium alloy by arc-enhanced magnetron sputtering at different negative bias voltages and their corrosion resistance in blood, effects on platelet activation, endothelial cell functions are studied. The Ti-Si-N coatings are made up of crystalline TiN and amorphous Si3N4 phases and show better corrosion resistance compared with titanium alloy. Reduced platelet receptor activation is observed on the Ti-Si-N coatings and that deposited at \( V = 100 \) V shows the longest clotting time and smallest platelet activation. Ti-Si-N coatings also enhance the endothelial cell proliferation and spreading as well as the endothelial nitric oxide synthase (eNOS) expression.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction
Corrosion resistance, suitable mechanical properties, and good hemocompatibility are essential properties of cardiovascular biomaterials [1,2]. Although Ti and its alloys have been widely used in biomedical implants, their wear resistance and hemocompatibility need to be further improved [3]. As the surface of biomaterials plays an important role in interaction with bodies [4], surface modification is often employed to meet the special clinical needs [5,6]. Titanium nitride (TiN) coatings can be deposited to improve the wear resistance [7–9]. However, inherent defects of these coatings, such as pinholes and micro-cracks, degrade their corrosion resistance [10]. The addition of Si in these bio-functional coatings has been reported not only can increase the corrosion resistance, but also enhance the biocompatibility [11,12].

Cardiovascular implants may suffer severe corrosion in blood. After long-term implantation in body, Ti alloy may also cause chronic inflammation, leading to the pH value of the blood to be acidic. Ti alloy thus suffer corrosion due to acidic erosion. On the other hand, scarcity of the oxygen levels in blood also retard the formation of oxide protective layer on Ti alloy. Once the oxide layer is broken, it is vulnerable to corrosion in human blood.

Ternary Ti-Si-N coatings have drawn much attention for potential biomedical applications owing to their excellent mechanical performance, cytocompatibility and corrosion resistance. For example, the corrosion resistance of Ti-Si-N coating was reported to be superior to stainless steel in a Ringer solution system [13]. These properties of Ti-Si-N coatings can be attributed to the microstructure of nano-crystalline TiN and amorphous Si3N4 matrix [14–17]. While TiN is well tolerated by the human body [18–21], Si3N4 will offer acceptable hemocompatibility [22–24].

Endothelialization is an ideal technology to enhance hemocompatibility of cardiovascular implants [25]. Currently endothelialization of Ti-Si-N coating is still focused on the cellular level. Understanding of the molecular biology mechanism in which the coatings interact with platelet and endothelial cells is desirable for further promote endothelialization for Ti-Si-N coatings.

In this work, Ti-Si-N coatings are deposited on Ti alloy at different substrate bias voltages to investigate their effects on corrosion resistance of coatings in human blood plasma, platelet activation marks and endothelial nitric oxide synthase (eNOS) gene expression using human umbilical vein cell line EA.hy926. Real blood is chosen in this study instead of commonly used artificial media such as simulated body fluid (SBF) and phosphate buffered saline (PBS) to better simulate the physiological conditions for cardiovascular devices.
2. Experimental procedures

2.1. Deposition of Ti-Si-N coatings

Arc-enhanced magnetron sputtering (AEMS) was used to deposit Ti-Si-N coatings on polished Ti alloy (Ti6Al4V) samples (Φ 20 mm × 2 mm). Fig. 1 shows a schematic diagram of the AEMS system. A columnar ultra-pure Ti (99.99%) target with the diameter of 60 mm was used to produce arc discharge. Two ultra-pure silicon (99.99%) targets with dimensions of 435 mm × 94 mm × 8 mm were used as silicon source [26]. A rotatable substrate holder was placed in the center. Magnetron sputtering can produce some thermal effects. The thermal effect on substrates during coating deposition can be minimized through proper cooling, and the temperature of the substrate is usually kept below 130 °C. Parameters used during deposition process are listed in Table 1.

2.2. Surface characterization

The coatings were characterized by X-ray diffraction (XRD, XRD-7000, Shimadzu Limited), atomic force microscopy (AFM, SPI3800-SPA-400, Seiko Instruments Inc.), and X-ray photoelectron spectroscopy (XPS, AXIS ULTRA, KRATOS ANALYTICAL Ltd.). Hardness of the coatings was determined by an HV-100 microhardness tester and 10 probe points were selected randomly for each sample. Wear resistance of the coatings was evaluated in human blood plasma at 37 °C by a ball-on-disk tribometer. Alumina balls with the diameter of 3 mm were used as the counterparts with the normal load and sliding distance to be 1 N and 150 m, respectively. The wear loss was calculated by wear track.

2.3. Corrosion behavior

The corrosion resistance of different samples was detected by potentiodynamic polarization tests conducted on Zennium electrochemical workstation (ZAHNER, Germany) with the conventional three-electrode configuration. Samples with exposed surface area of 75 mm² served as working electrode. A Pt foil and saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively. The electrochemical experiments were performed at 37 °C in human blood plasma which was separated from whole blood by gradient centrifugation. The scanning rate was 1 mV s⁻¹. Corrosion potential (Ecorr) was derived from the curves and the corrosion current density (Icorr) was determined from the polarization curves by Tafel extrapolation [27].

2.4. Contact angle measurements

The static (sessile drop) water contact angles were determined on the Rame-Hart imaging system (USA). For each sample, at least three individual measurements were taken at different locations [28].

2.5. Hemolysis assay

The potential of samples that induce hemolysis was assessed using a hemolysis assay. Blood samples were obtained from healthy human donors. Distilled water was used as positive control which caused complete hemolysis, and NaCl (0.9% (w/v)) used as negative control. Absorbance was measured by microplate reader (Powerwave XS MQX200R). The hemolysis ratios were calculated according to the following formula: 

\[
R = \left( \frac{A_{eC1}}{C2_{eC1}} \right) \times 100% 
\]

where R is the hemolysis ratio (%), and A, C1, and C2 are the absorbance of samples, negative control, and positive control, respectively [29].

2.6. Clotting time

Thrombin time (TT) reflects the time of fibrinogen activation by material. Normal values for TT are 12–14 s. The TT assays were performed using coagulation instrument according to the standard test procedures [30]. Briefly, platelet poor plasma (PPP) was obtained by centrifuging fresh human blood at 3, 000 rpm for 10 min at 4 °C. 500 μl of PPP was added to the samples and incubated at 37 °C for 15 min. Then 100 μl of incubated PPP was mixed with 100 μl of TT clotting reagent and incubated at 37 °C for 3 min.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working pressure (Pa)</td>
<td>0.4</td>
</tr>
<tr>
<td>N₂ flow rate (sccm)</td>
<td>12</td>
</tr>
<tr>
<td>Ar₂ flow rate (sccm)</td>
<td>4</td>
</tr>
<tr>
<td>Columnar Ti target current (A)</td>
<td>60</td>
</tr>
<tr>
<td>Si targets current (A)</td>
<td>4</td>
</tr>
<tr>
<td>Substrate temperature (°C)</td>
<td>120</td>
</tr>
<tr>
<td>Negative bias voltage (V)</td>
<td>50/100/150/200</td>
</tr>
<tr>
<td>Deposition time (min)</td>
<td>60</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic diagram of the AEMS system. (a) Lateral view, (b) Top view, black areas represent permanent magnet.
The TT was determined using the coagulation instrument.

2.7. Detection of activated platelets

To determine the P-selectin (CD62p) and PAC-1 expression on platelet, platelet-rich plasma (PRP) was prepared by centrifuging whole blood. Samples were immersed in PRP at 37 °C for 10 min. The adherent platelets were removed by trypsin. The post-incubation PRP was mixed with phycoerythrin (PE) conjugated CD62p and fluorescein isothiocyanate (FITC) conjugated PAC-1 monoclonal antibody (BD Biosciences, San Jose, California, USA). After staining with antibodies, samples were incubated for 30 min in dark and diluted with 1 ml sheath fluid. The level of platelet activation was then analyzed by flow cytometry (FACS Calibur; BD Biosciences).

2.8. Cell proliferation, morphology and spreading

Human umbilical vein cell line, EA.hy926 (Shanghai cell bank, catalog number GNHu39), was used in the biological assays. Cells were cultured according to the standard American Type Culture Collection (ATCC) method and culture reagents were purchased from Life Technologies (Gibco, USA).

Cells were seeded on the sterilized samples at a density of $2.5 \times 10^4$ cells/cm$^2$. After incubation for 24 h, cell morphology and spreading were examined by fluorescence staining using CFDA SE kit (Beyotime, China) according to the manufacturer’s instructions. After culturing for 1 and 6 days, cell proliferation were evaluated quantitatively by CCK-8 kit (Biotime, China) following manufacturer’s instruction. To assess the proliferation.

2.9. Nitric oxide production

The nitric oxide (NO) secreted level was evaluated by nitric oxide fluorescence probe (Beyotime, China) according to the manufacturer’s instruction after cells were seeded at a density of $2.5 \times 10^4$ cells/cm$^2$ and cultured for 6 days.

2.10. Determination of eNOS mRNA expression

Total RNA was extracted from cells after 72 h of incubation on samples using Trizol kit (Beyotime, China). RNA samples were reverse transcribed into complementary DNA by using cDNA kit (Beyotime, China) according to the manufacturer’s instructions. Real-time PCR reactions were performed in triplicate on a Chromo Real-time PCR system (BIO-RAD, USA) with PCR kit (Beyotime, China). The eNOS mRNA expression was normalized to GAPDH mRNA expression levels [31].

2.11. Western blotting analysis of eNOS expression

After cultured on the samples, cells were lysed with protein extraction kit (Beyotime, China). Total protein extracted from cells was then transferred and incubated with the corresponding antibody. Gel was imaged with a Gel Doc XR System (BIO-RAD, USA). All protocols are performed following standard laboratory procedures [31].

2.12. Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine the statistical significance of the data. Three samples were used in each group and the results were reported as means ± standard deviations. Difference at $p < 0.05$ was considered to be significant [4].

3. Results and discussion

3.1. Microstructure and surface characterization

Fig. 2 shows the dependence of Si contents in Ti-Si-N coatings on different negative bias voltages. The atomic percentage of Si was determined by XPS. It can be seen that Si concentration increases with the negative voltage up to 100 V. Since Si are lighter than Ti, Si ions can be accelerated more easily under the influence of negative bias voltage. Therefore, more Si will be deposited in Ti-Si-N coating with the increase of applied voltage. However, with the negative voltage further increases above 100 V–200 V, Si content in Ti-Si-N coating decreases gradually. In this high-voltage regime (100–200 V), Ti ions can be accelerated efficiently to the substrate, leading to the increase of Ti content in coatings. Furthermore, these energetic Ti ions will displace surface atoms. So Si content continuously decreases due to this re-sputtering phenomenon [32].

The XRD patterns of the Ti-Si-N coatings deposited at various bias voltages are shown in Fig. 3. Diffraction peaks of crystalline TiN are observed. The absence of characteristic peaks corresponding to Si$_3$N$_4$ or Ti-silicide phases may be ascribed to their amorphous state. When the applied negative bias voltage exceeds 100 V, Ti-Si-N coatings exhibit a strong preferred orientation of TiN (1 1 1), indicating that TiN form larger crystalline grains, which can be attributed to less Si content in Ti-Si-N coatings. At a smaller negative bias voltage, the broadening of TiN (1 1 1) peak and its decreased intensity indicate the decrease of TiN grain size. This is result from the fact that amorphous Si$_3$N$_4$ will hinder the crystalline growth of TiN.

Fig. 4 shows the N 1s spectra of the Ti-Si-N coatings deposited at various negative bias voltages. After deconvolution [33,34], two peaks corresponding to TiN and Si$_3$N$_4$ can be observed. Combined with XRD results, it is concluded that Si$_3$N$_4$ exists in amorphous state in deposited coatings. It has been reported that the Si-N bonds can increase the hydrophilicity of the coating, which is beneficial for the inhibition of platelet activation [35]. In addition, the intensity of the peak corresponding to Si$_3$N$_4$ increases and reaches to maximum when the negative bias voltage is over –100 V.

The surface morphologies of Ti-Si-N coatings deposited at different negative bias voltages are assessed by AFM as shown in Fig. 5. At small negative bias voltages, amorphous matrix Si$_3$N$_4$ inhibits the growth of TiN crystal. Hence the coatings are smooth and homogeneous, particularly that deposited at –100 V, due to the
densification by ion bombardment. At higher negative bias voltages above 100 V, the roughness increases perhaps due to re-sputtering effect.

From the biological perspective, the surface morphologies of the biomaterials have great influences on the biological processes, like protein adsorption [36,37]. Conformation of the adsorbed proteins is influenced by the surface morphology [38]. Cell behaviors, such as adhesion, proliferation, and differentiation, are also greatly affected by the surface morphologies [39]. Cell adhesion and spreading on the material surface is necessary for cell proliferation. Rougher surface provides adequate number of attachment sites for focal adhesion complexes. Biomaterials with nano-morphologies have been reported to be able to supports cell adhesion and proliferation [40–43]. The probable cause is that the nanostructure of a material equivalent to the nano-architecture of the extracellular matrix and cell membrane receptors.

Fig. 6 shows the hardness of Ti-Si-N coatings deposited at different of negative bias voltages. As detected by SEM, the Ti-Si-N coatings are about 1 μm in thickness. The maximum value of hardness are achieved when the applied negative bias voltage is 50 V. In Ti-Si-N coatings, smaller grains give rise to more grain boundaries. When the grain size is below a critical value, grain boundary sliding, rather than dislocation slip, becomes the main mechanism of plastic deformation. Amorphous Si3N4 between grains will acts as “glue” and prevent movement of grain boundary, thus resulting in increased hardness of the coating [44,45]. At higher applied negative bias voltage of 100 V, the increase of Si content (as shown in Fig. 2) in coatings may discrete TiN grains, thus leading to the decrease of bonding strength between TiN and Si3N4 and consequent decrease of the coating hardness [44]. On the other hand, some studies have suggested that high hardness of the Ti-Si-N coating results from the residual stress caused by ion bombardment during the deposition process [46]. Based on the above XRD analysis, crystalline grains of TiN in coatings will grow larger when deposited at the negative bias voltages above 100 V. Larger TiN grains may induce residual stress decreased, which is harmful to the coating hardness [35]. Therefore, higher bias voltage leads to the further drop in hardness of Ti-Si-N coatings [47]. However, the hardness of Ti-Si-N coatings is still very high after the elimination of residual stress by annealing [48]. Then it can be inferred that residual stress is not the main cause of the hardness drop. The hardness decreases with the increased bias voltage can be ascribed to the microstructural change in the Ti-Si-N coatings due to the variation of Si concentrations. Therefore, maximum hardness
of Ti-Si-N coating is only achieved from the best combination of ultra-fine TiN grains and amorphous Si₃N₄ [44].

One of the drawbacks of Ti and its alloys is their low antifriction properties, which can be improved by deposition of Ti-Si-N hard coatings. Table 2 shows the wear rate of uncoated and Ti-Si-N coated Ti alloy against alumina balls. When alumina balls are used, the wear mechanism of the samples belongs to the abrasive wear. In this case, the hardness of the material has a greater impact on the wear rate [49]. Therefore, in blood plasma friction medium, coated Ti alloy with higher hardness possesses superior wear resistance over uncoated ones.

### 3.2. Corrosion behavior

The Corrosion behavior of Ti-Si-N coatings are determined in blood plasma at 37 °C. As shown in Fig. 7, compared with the pristine Ti alloy, Ti-Si-N coated samples show more positive corrosion potentials and decreased corrosion currents. The better corrosion resistance of Ti-Si-N coatings can be attributed to their non-columnar and dense microstructure, as well as poor electrical conductivity of Si₃N₄ [50,51]. The presence of amorphous Si₃N₄ in TiN matrix eliminates defects such as cracks and pinholes. Thus corrosive media are blocked from reaching to the inner coating and intergranular corrosion are prevented. In this viewpoint, Ti-Si-N coating with higher Si₃N₄ content will show better corrosion resistance for the coated sample. This may explain why the Ti-Si-N coating deposited at the negative bias voltage of 100 V exhibits the best corrosion resistance. Moreover, blood plasma is a complex fluid, which not only contains blood cells, but also is rich in plasma proteins and sodium chlorides. These blood components have great influence on the corrosion process of cardiovascular implants. After contacting the blood, proteins and amino acids adsorb to the implant surface. Owing to the insulating Si₃N₄ phase in Ti-Si-N coatings, these adsorbed macromolecules will serve as a diffusion barrier.

### Table 2

| Wear resistance of Ti alloy and the Ti-Si-N coatings under blood condition. |
|---|---|---|---|---|
| Ti alloy | Ti-Si-N coatings | | | |
| | | −50 V | −100 V | −150 V | −200 V |
| Wear rate, mm³/Nm | 1.75 × 10⁻⁶ | 3.67 × 10⁻⁶ | 4.13 × 10⁻⁶ | 4.67 × 10⁻⁶ | 5.26 × 10⁻⁶ |
| Friction coefficient | 0.49 | 0.53 | 0.51 | 0.57 | 0.63 |
barrier thus resulting in the enhanced corrosion resistance. All in all, our experiments corroborate that Ti-Si-N coatings have better corrosion resistance than uncoated Ti alloy in a real blood environment.

3.3. Water contact angle

The water contact angles of the pristine Ti alloy and the deposited coatings are presented in Fig. 8. Small increase of the contact angles on the coated samples indicate their more hydrophobic properties. Wettability (hydrophilic or hydrophobic) of the material surface greatly affect the protein adsorption and the following cell adhesion [52].

3.4. Hemolysis rate

The hemolytic activity is an important parameter for cardiovascular devices. The hemolysis ratios obtained from the Ti-Si-N coatings are showed in Fig. 9. All of them are below 5%, indicating acceptable hemolysis levels of the coating as hemolysis value within 5% is generally considered nonhemolytic according to ISO 10993-4. Because relatively smooth surface reduces damages to the blood cells and hemolysis, minimum hemolysis ratio is observed on the coating deposited at negative bias voltage of 100 V.

3.5. Clotting time measurements

The TT results are presented in Fig. 10. The coating prepared at –100 V shows the longest TT value than Ti alloy control and the other coatings. After the cardiovascular devices contact with blood, their surface will rapidly adsorb plasma proteins [53]. Si-N bonds play an important role in protein adsorption here because Si-N has a strong affinity to albumin which promote anticoagulation. The TT results indicate that Ti-Si-N coatings diminish platelet activation and fibrinogen binding compared with that of Ti alloy.

3.6. Platelet activation marker expression

Fig. 11 shows the plot of PAC-1 vs CD62p expression in platelet activation among the platelet contacting with the different samples. The platelet-rich plasma in contact with the Ti-Si-N coatings...
show reduced degrees of PAC-1 and CD62p co-positive (up right quadrant of bivariate plot) platelet activation than that of Ti alloy control. Only the CD62p positive platelets increase significantly indicating that Ti-Si-N coatings reduce fibrinogen adsorption and platelet activation. Hemocompatibility of the Ti-Si-N coatings is also affected by the deposited bias voltage, which is in agreement with the TT test results.

It is well known that the first event that occurs when biomaterials contact with blood is the adsorption of plasma proteins followed by platelet adhesion [54]. In our test, the anti-CD62p antibody and PAC-1 antibody specifically bind to the P-selectin receptor and activated form of GP IIb/IIIa receptor, respectively. P-selectin receptor is involved activation in early step, while the GP IIb/IIIa receptor has the highest density on platelets. Under normal circumstances, GP IIb/IIIa does not adsorb fibrinogen. Upon platelet is activated, high-affinity binding site for soluble fibrinogen will be exposed. This situation will leads to platelet aggregation [54]. It is generally believed that rough surfaces will increase the adhesion area, resulting in more platelet adhesion. However, if the surface roughness is smaller than the pseudopods of platelets, surface topography will no longer be a major factor for platelet adhesion [55]. The Ti-Si-N coating deposited at −100 V shows the lowest percentages of dual label (co-positive), which can be ascribed to its surface properties. XPS shows that the Si–N bond reaches a maximum at −100 V. The polarity of Si–N bond has negative charge, which is able to suppress adsorption of fibrinogen.

3.7. Cell proliferation and spreading

The proliferation of endothelial cells on the Ti alloy control and Ti-Si-N coatings is qualitatively assayed using CCK-8 kit after culturing for 1 and 6 days and the results are presented in Fig. 12. In the first day, the proliferation of cells cultured on Ti-Si-N coatings are relatively high. After 6 days, endothelial cells show significantly enhanced proliferation on the Ti-Si-N coatings (−50 and −100 V) compared with the Ti alloy control. Hence, the Ti-Si-N coatings deposited at the negative bias voltage of 50 V and 100 V enhance the proliferation of endothelial cells.

Fig. 13 shows the fluorescence images of the stained cells on deposited coatings after culturing for 24 h. After exposure to the fluorescent stain, cytoskeleton fibers are green and the nuclei marked by DAPI are blue. The endothelial cells seeded on all samples are elliptical having a rounded or polygon morphology, indicating good adhesion and spreading on all surfaces. However, differences of the cytoskeleton organization and spreading can be observed between the Ti alloys and the Ti-Si-N coatings. Cells on
the Ti-Si-N coatings deposited at $-50$ V and $-100$ V are denser and their spreading area are larger. The endothelial cells fully covering the surface and remain viable in long term culture on Ti-Si-N coatings (>1 week).

The existence of both Si$_3$N$_4$ and TiN in Ti-Si-N coatings are of great importance in biomedical application [18,56,57]. It has been reported that Si$_3$N$_4$ and Si can promote endothelial cell proliferation and up-regulate the expression of vascular endothelial growth factor (VEGF), which can promote NO synthesis and also is essential to mediating the cardiovascular function [58]. TiN is the dominant phase in Ti-Si-N coatings. TiN coatings have been shown can improve endothelial cell functions [59]. For example, the actin cytoskeleton pathway and expression of vimentin are up-regulated on the TiN coating. Vimentin is the main member in intermediate filament protein family and increase of the vimentin expression would result in higher cell motility. Therefore, Ti-Si-N coatings, particularly those deposited with the negative bias voltages of 50 V and 100 V, can promote the endothelial cell proliferation and spreading.

**Fig. 12.** Endothelial cell proliferation on the titanium alloy and Ti-Si-N coatings deposited at various bias voltages using the CCK-8 assay. * indicate $p < 0.05$ vs. Ti alloy.

**Fig. 13.** Fluorescence microscopy images of the endothelial cells cultured on different samples for 24 h: (a) Titanium alloy, (b) Ti-Si-N coating deposited at $-50$ V, (c) Ti-Si-N coating deposited at $-100$ V, (d) Ti-Si-N coating deposited at $-150$ V, (e) Ti-Si-N coating deposited at $-200$ V. The actin cytoskeleton and cell nucleus in endothelial cells are visualized by fluorescent staining of the actin filaments (green) and nucleus (blue), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).
3.8. Regulation of eNOS levels in endothelial cells

Endothelial nitric oxide synthase (eNOS) is an enzyme that synthesizes nitric oxide (NO), which plays an important role in cardiovascular homeostasis and also inhibits platelet adhesion in vivo [31,60,61]. In order to investigate the effects of the Ti-Si-N coatings on endothelial cell functions, the relative NO levels from the vascular endothelial cells seeded on the Ti-Si-N coatings and Ti alloy are determined. The relative NO secretion levels from endothelial cells cultured on these surfaces for 6 days are shown in Fig. 14. Six days are adequate to ensure a good coverage of endothelial cells on the sample surface. It can be seen from Fig. 14 that NO released are significantly enhanced from the cells on Ti-Si-N coatings deposited at the negative bias potential of 50 V and 100 V. It can be ascribed to the capacity of Si₃N₄ existing in the Ti-Si-N coatings to promote NO secretion of endothelial cell.

The effects of Ti-Si-N coatings on eNOS mRNA are shown in Fig. 15. The expression of eNOS mRNA is found to be up-regulated by 1.4 and 1.3 times for Ti-Si-N coatings deposited at bias voltages of −50 V and −100 V, respectively. The expression of eNOS protein in EA.hy926 cells is also investigated by western blot analysis (Fig. 16). The Ti-Si-N coatings deposited at −50 V and −100 V significantly enhance eNOS protein expression by 1.7 times and 2.0 times, respectively. They also significantly up-regulate the eNOS protein levels than the other Ti-Si-N coatings and Ti alloy.

Herein, we evaluate the effects of Ti-Si-N coatings on the eNOS gene expression of endothelial cells. Ti-Si-N coatings may increase the activity of eNOS by their unique structure, in which the TiN crystals are embedded in the amorphous Si₃N₄ [62]. Si₃N₄ has been applied for the adsorption of phosphorylated proteins [63]. Phosphorylation is an important post-translational modification of proteins [64] and intracellular signal transduction regulates the synthesis and secretion of NO from endothelial cells. The phosphoinositide 3-kinase and adenylate cyclase pathways promote the eNOS activity. The eNOS suffers phosphorylation of serine residues. In addition, Si promotes endothelial cell proliferation and up-regulates the vascular endothelial growth factor (VEGF) expression in endothelial cells.

4. Conclusion

Ti-Si-N coatings are deposited on Ti alloy by arc-enhanced magnetron sputtering at different negative bias voltages. The hardness increases with Si concentration and the best hardness is achieved at bias voltages of −50 and −100 V. At a bias over −100 V, the Si concentration decreases due to sputtering. A smooth surface morphology is also observed from the coating deposited at −100 V. Corrosion behavior tests indicated a better resistance for corrosion of Ti-Si-N in comparison to Ti alloy in blood plasma. Reduced platelet receptor activation is observed from the coatings and that deposited at −100 V shows the longest clotting time and smallest platelet activation. The eNOS expression is enhanced. The Ti-Si-N coating deposited at −100 V possesses the desirable corrosion resistance and hemocompatibility suitable for cardiovascular implants.

Acknowledgements

The work was supported by City University of Hong Kong Strategic Research Grant (SRG) No. 7004188 and Hong Kong Research Grants Council (RGC) General Research Funds (GRF) No. CityU


[59] Dayun Yang, Xiaoying Lü, Ying Hong, Tingfei Xi, Deyuan Zhang, The molecular mechanism for effects of TiN coating on NiTi alloy on endothelial cell function, Biomaterials 35 (2014) 6195–6205.


