Vascular endothelial cell compatibility of superhard ternary Ti–Si–N coatings with different Si contents

Ming Zhang, Shengli Ma, Keweix, Paul K. Chu

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

Abstract

Superhard ternary Ti–Si–N coatings with different Si atomic concentrations are deposited on titanium alloy substrates by arc-enhanced magnetron sputtering (AEMS) and the vascular endothelial cell compatibility is studied. In vitro studies show that the surface hydrophilicity and hemolysis are about the same as those of the titanium alloy control and platelet adhesion and protein adsorption tests suggest a close relationship between the surface energy and blood compatibility. Endothelial cells cultures reveal better proliferation and anti-platelet adhesion on the coatings compared to the titanium alloy control and the Ti–Si–N coating with 20 at% Si exhibits excellent endothelialization.

1. Introduction

In spite of continuous improvements, the blood compatibility of biomedical implants such as cardiovascular stents and artificial heart valves remains one of the critical problems that trigger failure [1,2]. The blood compatibility of biomaterials is closely related to surface properties such as roughness, composition, and elemental chemical states [3], and surface modification is an effective way to enhance the surface blood compatibility without affecting the favorable bulk properties such as materials strength and sturdiness. Coatings such as gold, titanium nitride, silicon nitride, silicon carbide, iridium oxide, and diamond-like carbon (DLC) have been prepared on cardiovascular stents in order to prevent or alleviate formation of wear debris and inhibit platelet adhesion and activation [4–6].

Endothelium can maintain vascular homeostasis, regulate vascular tone and thrombosis, and foster muscle cell proliferation and migration. Damaged vascular endothelial cells and sub-endothelial matrix exposure may cause thrombosis and neointimal hyperplasia [7,8]. This is very important to re-endothelialization of injured parts to repair vascular functions. Studies have shown that cultivation of endothelial cells on the material surface in contact with blood is a good choice and clinical trials have been conducted.

In arteries and heart valves, there are high impact and force imposed by the blood flow and hence, it is important to improve the adsorption capacity of endothelial cells on biomaterials in order to maintain endothelial cell functions.

Recently, ternary Ti–Si–N coatings have drawn much attention because of their excellent mechanical performance such as superior hardness attributable to its phase and microstructure of the nanocrystalline TiN and amorphous Si3N4 matrix [9]. They are thus suitable for bio-tribological applications such as artificial heart valves and hip joints. Although much is known about their attractive mechanical properties, the biological properties of Ti–Si–N coatings, especially the blood compatibility [10], are relatively not well known. Ternary Ti–Si–N coatings can be prepared by chemical vapor deposition (CVD) and physical vapor deposition (PVD) [11–13] and their mechanical properties depend on the microstructure and processing parameters. In biological applications, the blood compatibility is related to the surface properties of the biomaterials. In particular, surface endothelialization can produce anti-thrombogenic coatings that mimic natural blood vessels [14] and so it is of both scientific and technical significance to investigate the interactions between Ti–Si–N coatings and vascular endothelial cells. In this work, Ti–Si–N coatings with different Si atomic concentrations are deposited on medical titanium alloy by arc-enhanced magnetron sputtering (AEMS) and the relationship between the surface properties and blood compatibility as well as vascular endothelial cell compatibility is systematically investigated.
2. Experimental details

2.1. Deposition of Ti–Si–N coatings

Arc-enhanced magnetron sputtering (AEMS, Beijing Powertech Co. Ltd.) was used to deposit the Ti–Si–N coatings on polished titanium alloy (Ti6Al4V) samples with a diameter of 20 mm and thickness of 2 mm. A columnar titanium target with a diameter of 60 mm and length of 450 mm produced the arc discharge in the presence of a hollow and permanent magnet placed in the center of the hole. The two ultra-pure Ti (99.99%) and two ultra-pure Si (99.99%) targets with dimensions of 435 mm \( \times \) 94 mm \( \times \) 8 mm were used to prepare the Ti–Si–N coatings. A Ti interlayer was first deposited using the columnar Ti target (operated at 20 V and 60 A) at 0.3 Pa in the arc discharge mode for 10 min to improve adhesion between the substrate and coating. The important deposition parameters are listed in Table 1.

2.2. Microstructure and surface characterization

The structure of the coatings was characterized by X-ray diffraction (XRD, XRD-7000, SHIMADZU LIMITED) using Cu K\( \alpha \) radiation. The surface morphology was examined by atomic force microscopy (AFM, SPI3800-SPA-400, Seiko Instruments Inc.) and the chemical states were determined by a X-ray photoelectron spectroscopy (XPS, AXIS ULTRA, KRATOS ANALYTICAL Ltd.) using Mg\( (1253.6 \text{ eV}) \) radiation.

2.3. Contact angle measurements

The static (sessile drop) water contact angles were determined on the Rame-Hart imaging system (USA) [15]. The mean contact angle was calculated from at least three individual measurements taken at different locations on each sample.

2.4. Hemolysis ratios

In order to obtain the hemolysis ratios of the samples, blood samples were obtained from healthy human donors. 4 ml of blood was diluted with 5 ml of 0.9\% (w/v) sodium chloride solution and then 10 ml of 0.9\% (w/v) sodium chloride was added. Additionally, 10 ml of the 0.9\% (w/v) sodium chloride solution (negative control, \( n = 4 \)) and 10 ml of double distilled water (positive control, \( n = 4 \)) were prepared for antitheses. All the samples were kept at 37°C for 30 min and immediately incubated in 0.2 ml of the whole blood at the same temperature. After 60 min, the samples were centrifuged for 5 min and the supernatant was analyzed at 540 nm to determine the absorbance of cells undergoing hemolysis using a microplate reader (Powerwave XS M0X200R). The hemolysis ratios were calculated by the following relationship [16]:

\[
R = \frac{(A - C1)}{(C2 - C1)} \times 100\% \quad \text{with} \quad R \text{ being the hemolysis ratio (\%)},
\]

\( A \) the absorbance (%), \( C1 \) the absorbance of the negative controls (%), and \( C2 \) the absorbance of the positive control (%).

2.5. Adsorbed human plasma proteins and cell culture medium proteins

Fresh whole blood from a volunteer was centrifuged at 3000 rpm to obtain the platelet-poor plasma (PPP). The samples without PBS immersion were put on a 12-well culture plate and 2 ml of PPP was added and incubated for 2 h at 37°C. Afterward, each sample was rinsed ten times with PBS to detach the loose proteins. The plates were soaked in 1 wt\% aqueous sodium dodecyl sulfonate (SDS) solution for 30 min to desorb the protein and a protein analysis kit (Micro BCA, Pierce, Rockford) was utilized to determine the concentration of the PPP in the SDS solution based on the BCA method [17]. The amount of PPP adsorbed on the polymer surface was calculated from the concentration in the SDS solution. We also do a protein adsorbed assay of cell culture medium, method as described above. The data were expressed as means ± standard deviation (\( n = 4 \)) based on the analysis of variance and Student t-test.

2.6. Platelet adhesion

Platelet adhesion on different samples was examined by fluorescence staining. The membrane probe Dil was purchased from Beyotime (China) and the fresh human platelet-rich plasma (PRP) incorporated with the Dil solution was incubated at 37°C for 10 min. The platelets were separated from the red dye by high speed centrifuging and then the samples were immersed in PRP (1 ml per sample), incubated at 37°C for 1 h, and rinsed with PBS three times. The stained samples were observed under an inverted fluorescence microscope.

2.7. Cell culture

The endothelial cell line (EAhy926) was provided by Shanghai cell bank (catalog number GNHu39) of The Chinese Academy of Sciences. They served differentiated endothelial cell functions, namely angiogenesis, homeostasis, thrombosis, blood pressure, and inflammation. Furthermore, they could be cultured to high passages without appreciable changes in the growth rate and phenotype, thus avoiding the diversity of primary isolated endothelial cells from different individuals, limitation of replication potential, and senescent tendency in cultures. The cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin in culture dish and incubated in a humidified atmosphere of 5% CO\(_2\) at 37°C [18].

2.8. Cell proliferation

After sterilization with 75% alcohol, the samples were placed on a 12-well culture plate and 1 ml of the DMEM medium was added. The concentration of endothelial cells was 5 \( \times \) 10\(^4\) cells/ml. Endothelial cell proliferation was investigated by the CCK-8 kit (Biotime, China) after incubation for 1 and 5 days. The medium was removed and the samples were washed twice with PBS. The fresh medium containing CCK-8 reagent was added to each sample and incubated at 37°C for 3 h under standard culturing conditions. Afterward, 100 \( \mu \)l of the blue solution were transferred to a 96-well plate. The absorbance was measured at 570 nm on a microplate reader and all the proliferation experiments were performed in triplicates [19].

---

**Table 1**

Typical instrumental parameters adopted in the fabrication of the Ti–Si–N coatings arc-enhanced magnetron sputtering.

<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>( N_2 ) flow rate (sccm)</td>
<td>12</td>
</tr>
<tr>
<td>Ar flow rate (sccm)</td>
<td>4</td>
</tr>
<tr>
<td>Ti targets current (A)</td>
<td>10</td>
</tr>
<tr>
<td>Si targets current (A)</td>
<td>1, 3, 5, 7</td>
</tr>
<tr>
<td>Substrate temperature (°C)</td>
<td>120</td>
</tr>
<tr>
<td>Negative bias voltage (V)</td>
<td>100</td>
</tr>
<tr>
<td>Deposition time (min)</td>
<td>120</td>
</tr>
</tbody>
</table>
2.9. Cell morphology and spreading

5 × 10⁴ endothelial cells were seeded on the surface of the samples and cultured for 5 days in an incubator to study cell spreading. The CFDA SE kit (Beyotime China) was used for endothelial cells staining and the staining method with based on the manufacturer’s instructions. After staining was completed, each sample was added to 1 ml of fresh medium and cultured for 24 h to ensure all cells were stained. A fluorescence microscope was used to examine the samples afterward.

2.10. Relative nitrite assay

5 × 10⁴ endothelial cells were seeding on the samples for 5 day. The supernatant was collected from the cell culture plate and 1 ml of the supernatant analyzed. The endothelial cells secreted nitric oxide and the nitric oxide assay kit (Beyotime, China) were used according to the manufacturer’s instructions. A microplate reader was employed to measure the absorbance of the supernatant of each sample to determine the relative nitric oxide secretion absorbance value.

2.11. Inhibition of platelet adhesion

Blood donated by volunteers was added with heparin for anticoagulant and centrifuged for 10 min at 1500 rpm at 4 °C. The supernatant was the platelet-rich plasma (PRP) [20]. Using the Dil dye kit for platelet dyeing, centrifugation was performed to remove the free dye molecules. The operation was carried out in ice water. The samples covered with endothelial cells were transferred to a new 12-well culture plate and cultured for 5 days. The endothelial cells underwent CFDA SE staining with freshly prepared PRP 500 and were incubated for 1 h. After absorbing the PRP, the samples were washed 3 times with PBS to remove loose platelets and then a fresh medium was added. The platelets adhered to the samples were examined by fluorescence microscopy.

3. Results and discussion

3.1. Microstructure

The Si atomic concentrations of samples 1A (360 W Si target power), 3A (1 kW), 5A (1.7 kW), and 7A (2.5 kW) determined by XPS are 20, 32, 39, and 46 at%. It should be noted that the Si atomic concentrations are those of the whole coatings, not only of the TiN/Si₃N₄. The XRD results are shown in Fig. 1. As the Si atomic concentration increases, the Ti–Si–N coatings exhibit orientations of [2 0 0], [2 2 0], and [3 1 1]. No diffraction peaks associated with Si₃N₄ is observed suggesting that Si exists in an amorphous Si₃N₄ phase. The coatings exhibit strong preferred orientation in the crystal planes of [2 2 0] and [3 1 1], indicating that TiN in the coatings grow continuously forming big crystalline grains. The intensity of the [2 2 0] and [3 1 1] peaks increases gradually and the peaks become narrower with increasing Si atomic concentrations. This peak narrowing behavior originates from a larger grain size and generally, the size of the TiN crystal grains diminishes as the Si concentration increases. However, it has been observed that the TiN grain size can increase if the Si concentration is very high [21].

As the Si atomic concentration increases, the TiN crystal size decreases possibly due to the rapid growth of amorphous Si₃N₄. The crystal mixed with amorphous materials is better suited for the application. The Ti–Si–N surface topography is generally island-like and the size of these islands in the crystal depends on the volume entrapped. The changes in the island dimensions may also affect the functions of the vascular endothelial cells.

3.2. Chemical bonding and states

XPS is performed to acquire bonding information from the Ti–Si–N coatings deposited under typical conditions and the results are shown in Fig. 2. As shown in Fig. 2(A1)–(A4), the intensity of the Si₂N₄ peak increases gradually with Si concentration while that of the TiN peak diminishes. The Ti 2p spectra in Fig. 2(C) shows that in addition to Si₂N₄, free Si can be found possibly because the Si target current is too high so that some Si atoms do not bind with nitrogen. In particular, as shown in Fig. 2(C1), the intensity of the Si 2p peak of the coating with 46 at% Si is quite large. The Ti peak consists of subpeaks from Ti, TiO, and TiN. The N 1s peak suggests existence of both TiN and Si₂N₄ and it can be concluded from the XRD and XPS data that Si₂N₄ is amorphous and TiN is crystalline in the coatings.

It has been reported that a larger Si concentration in the Ti–Si–N coating increases the mechanical strength. Coatings used in cardiovascular applications require good mechanical strength because if the coatings are ruptured in blood, dangerous debris can be produced and lead to thrombosis or clotting. Hence, good mechanical strength is required from the Ti–Si–N coatings to meet the safety standards in blood-related applications.

3.3. Surface topography

AFM is employed to investigate the influence of the Si concentration on the surface roughness of the Ti–Si–N coatings. As shown in Fig. 3, the surface becomes rougher with increasing Si target current and the root-mean-square (RMS) roughness rises from 0.28 nm to 0.53 nm possibly due to the large TiN grain size. In general, the surface roughness of the Ti–Si–N coatings is still less than that of the polished titanium alloy control (RMS = 0.61 nm). A smoother surface may induce less damage to blood cells in flowing blood. The blood components are blood cells and plasma. Rapid movement of blood cells in a blood vessel does not rupture endothelial tissues and a smoother surface can also reduce the mechanical friction loss.

3.4. Water contact angle

Several studies have demonstrated that increased hydrophilicity can reduce protein adsorption and cell adhesion, and so considerable efforts have been made to introduce hydrophilic characteristics to a hydrophobic surface by coatings and grafting [22].
Fig. 2. XPS spectra of the Ti–Si–N coatings with different Si atomic concentrations: (A) N 1s, (A1) 20 at% Si of N 1s, (A2) 32 at% Si of N 1s, (A3) 39 at% Si of N 1s, (A4) 46 at% Si of N 1s, (B) Ti 2p, and (C) Si 2p, (C1) 46 at% Si of Si 2p.
However, polymeric coatings may have poor adhesion on metals compared to sputtered metallic and ceramic coatings. Fig. 4 shows that the water contact angles do not change significantly on coatings with different Si atomic concentrations and the Ti–Si–N coatings and titanium alloy control also have almost the same hydrophilicity.

The surface energy and surface chemistry affect the wettability and the thrombogenicity of a surface increases with higher surface energy [23]. In the TiN coatings, the [2 0 0] plane has the lowest surface energy and hence, the Ti–Si–N coatings with a predominantly TiN [2 2 0] orientation should have high surface energy [6]. This may be the reason why the water contact angle is not apparently reduced as the Si concentration increases. It has been reported that Si–N bonds enhance the surface hydrophilicity and combined with good critical surface tension, activation of platelets is mitigated [24]. If attachment of platelets and proteins is reduced, the probability of thrombus formation can be lowered. In addition, the interaction between proteins and cells can promote the formation of tissues induced by cell aggregation and facilitate the attachment and proliferation of cells on the surface. Hence,

![Fig. 3. Three-dimensional AFM surface morphology of Ti–Si–N coatings with different Si atomic concentrations as well as titanium alloy control: (a) Ti alloy, (b) 20 at%, (c) 32 at%, (d) 39 at%, and (e) 46 at%.](image-url)
endothelialization on the Ti–Si–N coatings may improve the antithrombotic effects.

3.5. Hemolysis rate

The hemolysis rate is an important factor to evaluate blood compatibility. The lower the hemolysis rate, the better is the blood compatibility. The hemolysis ratios obtained from all the Ti–Si–N coatings are within 5% and similar to that of the titanium alloy control. Fig. 5 suggests that the Ti–Si–N coatings do not exhibit the hemolysis phenomenon. In the Ti–Si–N coatings, the TiN crystal size is small due to Si3N4 formation. The Ti–Si–N coatings also have a relatively smooth surface which can reduce damage of the blood cells. A smaller concentration of Si in the Ti–Si–N coatings is more suitable for blood-related applications.

3.6. Total protein adsorption

Blood plasma is a complex medium containing sugars, fats, amino acids, urea, and different proteins. Protein adsorption from blood plasma onto biomaterials is considered to be a non-selective and competitive process since many different proteins are involved [25]. On tissue scaffolds, protein adsorption is the key factor for proper vascularization whereas on artificial implants that are in contact with blood, protein adsorption can lead to thrombosis. Furthermore, protein adsorption can trigger adhesion of particles, bacteria, and cells possibly promoting inflammation cascades and fouling processes [26]. As shown in Fig. 6, we examine the relative adsorption capacity of plasma protein on different samples and all the samples show low levels of blood protein adsorption after 2 h. Here, the performance of the Ti–Si–N coatings with 20 at% Si is similar to that of the titanium alloy and the adsorption level on the coating with 32 at% Si is slightly below that of other coatings. The total protein adsorption experiments are conducted in the cell culture medium (Fig. 7). The relative amount of adsorption is not
very different, but the titanium alloy and the 20 at% Si Ti–Si–N coatings show a higher adsorption levels. Similar results are observed with respect to plasma protein adsorption.

Protein adsorption on a solid surface may be affected by factors such as the morphology, surface energy, and hydrophilicity [27]. AFM discloses that the Ti–Si–N coatings are smoother than the titanium alloy control, but the surface energy may have a greater influence on protein adsorption than surface roughness. It has been shown that more proteins adsorb on a surface with higher surface energy [28]. In our Ti–Si–N coatings, the primary [2 2 0] plane with a higher surface energy adsorbs more plasma protein. Our results suggest that a Si concentration higher than 20 at% favors protein adsorption.

3.7. Platelet adhesion

Platelet adhesion and activation are the main indicators of hemocompatibility of blood-contacting materials [29]. A small degree of platelet adhesion denotes good blood compatibility and a higher degree of platelet adhesion and activation results in the formation of thrombus. In our experiments, platelet adhesion is examined by fluorescently labeled platelets and the qualitative results are presented in Fig. 8. Many platelets adhere on the surface of the titanium alloy and Ti–Si–N coatings with 20 at% Si, but the number of platelets on the coatings with more than 32 at% Si is smaller. On most samples, the platelets aggregate into bigger clusters and retain the spherical morphology. The results indicate that platelet adhesion is less on the coatings possibly due to the surface morphology. The titanium alloy is rougher than the Ti–Si–N coatings and these extra surface structures may trap platelets. It is believed that a small grain size may also reduce platelet adhesion. All in all, the platelet adhesion results are consistent with those of protein adsorption and water contact angles.

3.8. Cell proliferation

Endothelial cells are seeded onto the samples without PBS immersion and endothelial cell proliferation is investigated by CC#8 viability assay after incubation for 1 and 5 days. Fig. 9 shows the proliferation of endothelial cells on the titanium alloy control and Ti–Si–N coatings. The absorbance is proportional to the amount of cells. In the first day, the absorbance value of the Ti–Si–N coatings with 20 at% and 46 at% are relatively high. After 5 days, the endothelial cells show significantly increased attachment and proliferation on the Ti–Si–N coatings. Hence, the Ti–Si–N coatings enhance proliferation and growth of endothelial cells without cytotoxic effects due to the surface chemistry and favorable absorption of proteins on the Ti–Si–N coatings. Poor hydrophilicity has been linked to absorption of serum extracellular matrix (ECM) proteins from the culture medium [30,31]. Another possible reason is the surface morphology of Ti–Si–N coatings as described previously. The in-walls of vessels consist of endothelial cells and if the coating surface is endothelialized, it mimics the native lining of blood vessels consequently lowering the probability of thrombus formation.

3.9. Cell spreading and morphology

One of the important requirements for endothelialization on materials surface is the formation of a dense single cell layer on the surface [32]. The morphology of vascular endothelial cells on the Ti–Si–N coatings and titanium alloy surface is examined after immunofluorescence staining. Fig. 10 depicts the morphology of the endothelial cells after incubation for 5 days and the results are similar to those of cell proliferation. The endothelial cells seeded on all the samples are elliptical having a rounded or polygon morphology. The endothelial cells also show good spreading and proliferation on the Ti–Si–N coatings. Almost confluent coverage of endothelial cells with elliptic and cobblestone morphology is observed after culturing for 5 days. There is a very dense monolayer of endothelial cells on 20 at% Si Ti–Si–N coating. The endothelial cells completely cover the surface and can simulate the structure of the vascular wall to avoid the occurrence of thrombosis and immune rejection. The results indicate that the distribution and proliferation of endothelial cells on the Ti–Si–N coatings are enhanced resulting in better endothelialization compared to the titanium alloy control.

3.10. Relative nitric oxide

Nitric oxide released by the endothelium plays an important role in the inhibition of thrombosis. Multiple effects including vasodilatation also inhibit platelet aggregation and leukocyte adhesion and control smooth muscle cell proliferation [33]. The relative nitric oxide levels from the vascular endothelial cells seeded on the Ti–Si–N coatings and titanium control alloy surface are determined. The relative nitric oxide secretion levels after the fifth day are shown in Fig. 11. Five days are adequate to ensure good coverage of endothelial cells on the sample surface. The absorbance values observed from the endothelial cells seeded on the Ti–Si–N coatings with 20 at% and 46 at% Si are relatively large. The results are similar to those of the aforementioned cell spreading and coverage.

Nitric oxide produced in the endothelial cells can regulate nitric oxide synthesis. In the Ti–Si–N coatings, the TiN crystals are embedded in the amorphous Si$_3$N$_4$ and the Ti–Si–N coatings constitute a unique structure that may increase the activity of nitric oxide synthesis. In particular, the Si–N bond may interact with the nitric oxide synthesis center so that the tertiary structure is changed.

3.11. Endothelial cells inhibiting platelet adhesion

Platelet adsorption on materials is undesirable and endothelialization can inhibit platelet adsorption as reported in the literature [34]. Platelet adsorption on samples covered by vascular endothelial cells is monitored and compared to that on the titanium control. The vascular endothelial cells on the Ti–Si–N coatings and
titanium alloy are soaked in platelet-rich plasma by immunofluorescence stain for 10 min in a cell incubator. Fig. 12 shows the endothelial cells on the Ti–Si–N coatings and titanium alloy surface. A monolayer of cells is formed but only a few platelets adsorb onto the monolayer of endothelial cells. In comparison, there are more adsorbed platelets on the surface of the titanium control. Comparing the coatings, few platelets adsorb on the 46 at% and 20 at% Si Ti–Si–N coatings. The platelets assay results are consistent with those obtained from the nitric oxide test revealing that platelet adsorption on the Ti–Si–N coatings is inhibited.

Many conventional metal and ceramic biomaterials may cause undesirable reactions in blood. Endothelial cells lining the blood vessel wall form a natural protective layer. If the vascular endothelial cells can exist for a long time on the biomaterials to maintain normal functions, the recovery process of patients after surgery can be shortened. Our results show that Ti–Si–N coatings can induce the presence of a large number of vascular endothelial cells and vascular endothelial cells to secrete nitric oxide. With regard to the Ti–Si–N coatings, Si–N bond and small TiN crystals are key to promoting the growth of normal endothelial cells.

Fig. 8. Fluorescent images of platelets on Ti–Si–N coatings with different Si atomic concentrations and titanium alloy control: (a) Ti alloy, (b) 20 at% Si, (c) 32 at% Si, (d) 39 at% Si, and (e) 46 at% Si.

Fig. 9. Endothelial cell proliferation on the titanium alloy and Ti–Si–N coatings with different Si atomic concentrations using the CCK-8 assay.
Fig. 10. Endothelial cell spreading on the titanium alloy and Ti–Si–N coatings after 1 day and 5 days.
4. Conclusion

Superhard ternary Ti–Si–N coatings with different Si contents are prepared on titanium alloy by arc-enhanced magnetron sputtering and the hemocompatibility is investigated. The surface hydrophilicity and hemolysis of the coatings are about the same as those of the titanium alloy control, and platelet adhesion and protein adsorption reveal a close relationship between the surface energy and blood compatibility. Endothelial cells cultures reveal more cell attachment and proliferation on the coatings compared to the titanium alloy control. Effective inhibition of platelet adsorption is observed from the Ti–Si–N coatings cultured with endothelial cells. Successful endothelialization promotes the blood compatibility of Ti–Si–N coatings which are projected to have large potential in cardiovascular applications especially those requiring tribological performance.

Acknowledgments

The work was jointly supported by City University of Hong Kong Applied Research Grant (ARG) No. 9667066 and Hong Kong Research Grants Council (RGC) General Research Funds (GRC) No. CityU 112212.

References

Ma Dayan, Ma Shengli, Xu Kewei. The tribological and structural character-


Mattart Laurine, Calay Damien, Simon Dorothy. The peroxynitrite donor 3-
morpholinosydnonimine activates Nrf2 and the UPR leading to a cytopro-


[2] Yang Zhihao, Wang Jin, Luo Rong, Ma Shengli, Xu Kewei. The covalent immobilization of heparin to pulsed-plasma polymeric allylamine films on 316L stainless steel and the resulting effects on hemo-


[9] Rabe Michael, Verdes Dorinel, Seeger Stefan. Understanding protein adsorp-


