Bio-tribological properties and cytocompatibility of Ti–Si–N coatings

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Ti–Si–N coatings are synthesized on titanium alloy (Ti6Al4V) by arc-enhanced magnetron sputtering and the microstructure and tribological properties are determined. The friction coefficient of the Ti–Si–N coatings, which is smaller in human serum than ambient air, decreases gradually with Si contents. Protein gel electrophoresis shows that the small friction coefficient is due to adsorbed proteins from the human serum under sliding conditions. The cytocompatibility of the coatings is assessed in vitro by a relative nitrite assay. The Ti–Si–N coatings have a positive effect on nitric oxide synthesis on the endothelial cells. The cell morphology and spreading on the coatings are examined by fluorescence staining. The Ti–Si–N coating with 12 at% Si exhibits the best effects in promoting actin cytoskeleton formation and cell spreading compared to coatings with different Si contents and titanium alloy.

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1. Introduction

Titanium alloys (Ti6Al4V) are widely used in biomedical industry due to the excellent mechanical properties, corrosion resistance, and cytocompatibility. Unfortunately, their poor tribological properties have hampered more extensive clinical applications such as ventricular pumps as well as artificial heart bearings, valves, and valve rings [1–3]. The bio-tribological properties such as friction of biomedical components are crucial to artificial valve operation in cardiovascular tissues and poor friction performance not only compromises the cytocompatibility, but also leads to the formation of thrombus and catastrophic failure [4–8]. Surface modification by chemical vapor deposition (CVD), physical vapor deposition (PVD), and so on can improve the tribological properties of cardiovascular materials and in particular, PVD is environmentally friendly, convenient, and easily controlled [9–13]. PVD describes a variety of vacuum deposition methods used to deposit coating by the condensation of a vaporized form of the desired coating material onto surfaces of specimens. The coating method involves purely physical processes such as high-temperature vacuum evaporation with subsequent condensation, or plasma sputter bombardment rather than involving a chemical reaction at the surface to be coated as in CVD. Transition metal nitride coatings composed of CrN and TiN have been used to improve the wear resistance but inadequate cell adhesion is a drawback. Addition of Si increases the biocompatibility of the biomaterials [14] and ternary Ti–Si–N coatings have drawn much attention because of their excellent mechanical performance such as superior hardness attributable to the phase and microstructure of the nano-crystalline TiN and amorphous Si3N4 matrix [15–18]. Our previous studies have shown that Ti–Si–N coatings are compatible with endothelial cells and endothelialization significantly reduces platelet adhesion [19]. In addition, since Ti–Si–N coatings can prevent propagation of microorganisms and exhibit good osteoblast differentiation of bone marrow mesenchymal stem cells, they have large potential in cardiovascular implants [20].

Protein adsorption is one of the first events in blood-materials interactions and some proteins in blood, especially serum albumin and fibrinogen, play an important role in materials-related lubrication [21]. Hence, study the tribological properties of Ti–Si–N coatings in blood are very important. The composition of human blood is complicated including blood cells and serum. Serum, which constitutes about 55% of blood, is mostly water (92% by volume), and contains dissipated proteins, glucose, mineral ions, hormones, and carbon dioxide. Albumin, the main protein in blood plasma, regulates the colloidal osmotic pressure of blood [22]. So far, the influence of serum proteins on the tribological properties of Ti–Si–N coatings such as friction unclear, but these properties are...
very important and it is also essential to understand the wear mechanism of Ti–Si–N coatings in the presence of human serum.

In contact with blood, human serum albumin first adsorbs onto the biomaterials and affect the friction properties of the Ti–Si–N coatings. Our objective is to investigate the tribological properties of Ti–Si–N coatings in human blood serum and the properties of blood serum proteins after mechanical tests. For comparison, the tribological properties of the Ti–Si–N coatings in ambient air are determined. The relative endothelial nitric oxide synthase (eNOS) activity of the endothelial cells seeded on the Ti–Si–N coatings and vascular endothelial cell morphology are also assessed in vitro.

2. Experimental details

2.1. Deposition of Ti–Si–N coatings

Arc-enhanced magnetron sputtering (AEMS) 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 ultra-pure titanium (99.995%) target with a diameter of 60 mm and length of 450 mm produced the arc discharge in the presence of a hollow and two ultra-pure silicon (99.995%) targets with dimensions of 435 mm, 94 mm, and 8 mm was used to prepare the Ti–Si–N coatings. 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). The surface morphology was examined by atomic force microscopy (AFM, SPI3800-SPA-400, Seiko Instruments Inc.) and chemical states were determined by an X-ray photoelectron spectroscopy (XPS, AXIS ULTRA, KRATOS ANALYTICAL Ltd.). The coating-substrate adhesive strength was measured on a multifunction materials surface tester. The critical load was determined by scanning electron microscopy (SEM) and sound emission signal because of occurrence of cracks and coating-substrate delamination.

2.3. Tribological tests

The tribological tests were performed on a ball-on-disk tribometer under dry conditions and with fresh human serum (provided by the Second Affiliated Hospital of Xi’an Jiaotong University). The dry friction medium was ambient air (relative humidity 30–40%). Alumina balls with a diameter of 3 mm were used as the counter materials and the applied load of 1 N was kept constant at a sliding distance of 120 m. The temperature was controlled by the heater out of the test-bed and kept constant at 37 °C. The coefficient of friction was digitally recorded by a load transducer and after the wear test, the wear tracks were examined by SEM.

2.4. Human serum proteins gel electrophoresis

After the tribological test, the samples were moved to new petri dishes to avoid serum proteins adsorbing onto the petri dishes. After rinsing with the phosphate buffer solution (PBS) to remove loosely bound proteins, the samples were immersed in 1.5 mL of 1% sodium dodecyl sulfate (SDS) solution and placed in a sonicator for 30 min to elute the adsorbed proteins. Afterwards, the eluent containing the adsorbed proteins was collected. To determine the properties of the proteins adsorbed onto the samples and the synthetic analogs during friction tests, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used. The phoresis rel (Sebia, France) software was used to warp the gels automatically to match specific spots on the reference gel to all the other gels. The software was used to generate images of the spots and calculate the relative volume.

2.5. Cell culture

The EAhy926 immortalized human aortic endothelial cell line 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 on culture dishes and incubated.

2.6. Nitric oxide production

5 × 10⁴ endothelial cells were seeded on the samples for 3 day. In order to determine the secreted nitric oxide level, the nitric oxide fluorescence probe (Beyotime, China) was used according to the manufacturer’s instruction. A microplate reader was employed to measure the absorbance of the suspended digest cell of each sample to determine the relative nitric oxide secretion absorbance value.

2.7. Cell cytoskeleton and spreading

The endothelial cells were seeded on the samples at a content of 5 × 10⁴ cell/ml and incubated for 24 h. To study the extent of cell spreading and the morphology of the endothelial cell, fluorescence staining of the cytoskeleton kit (Millipore, USA) and nucleus using DAPI (Beyotime, China) was performed and the staining method was in accordance with the manufacturer’s instruction.

3. Results and discussion

3.1. Microstructure and surface characterization

The Si content is determined by X-ray photoelectron spectroscopy (XPS) method. The Si contents of the samples with the 2, 3, and 4 A silicon target currents are 3, 8, and 12 at%, respectively. The XRD spectra of Ti–Si–N coatings with different Si contents are depicted in Fig. 1. Three TiN peaks are observed and assigned to the (1 1 1), (2 0 0) and (2 2 0) peaks for a Si content of 12 at%. As the Si content increases, the intensity of (1 1 1) peaks decreases but that of the (2 0 0) peaks increases slightly due to decreased size of the TiN crystals. When the Si content is increased to that the amorphous matrix embedding TiN crystals, the amorphous matrix Si3N4...
hinders the growth of TiN forming fine TiN grains. On the other hand, increasing the interfacial energy between the amorphous matrix and TiN increases the (2 0 0) orientation gradually. In the TiN crystal, the (200) peak has the lowest surface energy thus bonding well for cardiovascular devices [23]. Meanwhile, no signals corresponding to crystalline Si₃N₄ are observed suggesting that Si is present in an amorphous phase of Si₃N₄. It is in agreement with previous reports on Ti–Si–N coatings prepared by PVD [29].

It is well accepted that small grains typically have lower dislocation density, and the decrease in grain size leads to an increase in the grain boundary, which acts as obstacles to dislocation flow across grains. The combined effects of low dislocation density and larger grain boundary area contribute to the decrease in the hardness with increasing Si content in the coatings. For the coatings with high Si content, the grain size decreased. In this case, the dislocations are absent in the grains and the plastic deformation mechanism changes from dislocation sliding to grain boundary sliding. However, the high bonding energy of Si–N bonds in the grain boundary resisted the grain sliding, resulting in the super high hardness of the Ti–Si–N coatings.

Fig. 2 shows the XPS spectra for Ti–Si–N coatings with different Si contents. The intensity of the Si₃N₄ peak increases gradually with Si contents but that of the TiN peak diminishes slightly. The N 1s peak suggests existence of both TiN and Si₃N₄ and it can be concluded from the XRD and XPS data that Si₃N₄ is amorphous and TiN is crystalline in the coatings. The combined effects of the low dislocation density and larger grain boundary area are observed for increasing Si contents in the coatings. 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]. The high bonding energy of Si–N bonds in the grain boundary resist grain sliding resulting in the super high hardness of the Ti–Si–N coatings. The structure and tribological properties of the coatings depend on the depositing conditions and Si content [25].

The surface morphology of Ti–Si–N coatings with different Si contents is assessed by AFM and the results are presented in Fig. 3. The Ti–Si–N coatings become rough and homogeneous compared to the titanium alloy. Nevertheless, the surface roughness (root-mean-square, RMS) of the Ti–Si–N coatings decreased gradually with increasing Si content. The AFM images show a columnar morphology which becomes denser with increasing Si content due to the amorphous matrix Si₃N₄ inhibiting TiN crystal growth. A smooth surface reduces the wear rate and prolongs the working life of the Ti–Si–N coatings. The 3D AFM results corroborate the XRD data in that a larger Si content decreases the crystal/particle size resulting in smaller surface roughness. The Ti–Si–N coating shows islands and the size of these islands depends on the volume entrapped. The changes in the island dimensions may also affect the functions of the endothelial cells.

Friction force can generate additional stress that may become important in the contacting body and biomaterials surface. Poor coating-substrate adhesion strength can cause coating delamination and the debris are linked to chronic inflammation. In order to investigate coating-substrate adhesive strength, scratch tests are performed on Ti–Si–N coatings with different Si contents and the results are presented in Fig. 4. There is severe delamination on the Ti–Si–N coatings with 3 at% Si. As the silicon content is increased, the adhesive strength improves and less delamination is observed. When the Si content is increased to 12 at%, the critical load is larger than 40 N. The scratch test shows that more Si improves the adhesive strength because the amorphous Si₃N₄ enhances the hardness of the Ti–Si–N coatings and reduces the probability of coatings rupture. Our results reveal that the coating with 12 at% silicon has the best properties.

Critical loads can be used directly to quantify the “scratch toughness” of coatings [26]. Toughness, which is the ability of the material to absorb energy during deformation up to fracture, is proportional to the difference between lower critical load and upper critical loads. For high Si contents, the critical load is increased. Similar results are found in the literature for Ti–Si–N coatings

Fig. 1. XRD patterns of the Ti–Si–N coatings with different Si contents.

Fig. 2. XPS spectra of the Ti–Si–N coatings with different Si contents with the corresponding Si content in (a), (b), and (c) being 3, 8, and 12 at%, respectively.
The toughness of the coatings is increased by increasing the Si content. Thus, the propagation of crack is inhibited and the wear resistant is promoted.

3.2. Tribological tests

Fig. 5 shows the variation in the friction coefficients under different conditions. In air, all the curves are rough and constant and the friction coefficients decrease slightly with increasing Si contents, as shown in Fig. 5a. The results can be explained by a possible tribochemical reaction which often takes place in Si₃N₄ ceramics as Si₃N₄ reacts with ambient H₂O to produce SiO₂ or Si(OH)₂ tribo-layers [29]. Fig. 5b shows that the friction coefficients decrease greatly and the friction curves are relatively flat for human serum. Owing to the lubricating role of proteins and water in human serum, the samples exhibit much smaller and more stable friction coefficients under bio-lubrication than dry sliding conditions [30,31]. The friction coefficients are generally smaller in biological systems under normal conditions, but can become large under abnormal and diseased conditions. The smaller friction coefficients with human serum illustrate that serum proteins has also improved wear resistance. Fig. 6 shows the morphology of wear tracks of the Ti–Si–N coatings in ambient air and human serum. A wide wear track with many ploughed lines (except Fig. 6d which shows merely machine polishing scratches) and flakes is shown in Fig. 6a–c due to direct contact between the tribo-pair in ambient air. On the contrary, the sample tested in human serum has a narrow and shallow wear track. Almost no wear occurs during sliding in human serum due to lubrication rendered by the serum proteins and water as shown in Fig. 6d–f [32]. In addition, the wear debris disappear from wear track after cleaning with acetone. This indicates that the black debris is not chemically but physically adherent on the Ti–Si–N coatings. Milder wear is observed in human serum and the worn surface is intact without any ploughing and detachment. It can be concluded that the Ti–Si–N coatings prevent the soft titanium substrate from directly contacting the biological medium and improve the friction behavior.

Friction is the resistance to motion when two bodies in contact are forced to move relative to each other. Generally, the force of friction consists of two components: force to shear adhesion and force to plough the asperities on one surface through the other [33]. As most of the surfaces are rough on a micro or nano scale, only the
tips of their asperities will contact when two surfaces are placed in contact. For clean surfaces, the atoms on one surface will attract those on the other and produce strong adhesion, which prevents the sliding of one surface over the other and contributes to the first cause of friction. The asperities on the harder surfaces will plough out grooves in the softer surface, which constitutes the second cause of friction.

During the friction of uncoated Ti6Al4V under dry condition, the Ti element would react with the oxygen and form TiO2 film in the air due to frictional heat accumulation [34]. Due to the brittleness of TiO2 film, the combination of the matrix is not tough and reliable. In this case, the wear particles were removed easily from the surface of uncoated Ti6Al4V. This corresponds to the high friction coefficient of the uncoated Ti6Al4V. When Ti6Al4V were coated with Ti–Si–N coating, with increasing Si content, the change in the surface roughness of the Ti–Si–N coatings plays an important role to the friction. On the one hand, increasing the Si content produces grain refinement which will lead to Hall–Petch hardening effects [35], and an increase of the intergranular amorphous phase in the nanocomposite leads to a certain improvement of the mechanical properties.

Classical theories of wear [36] relate the hardness to the wear resistance of a surface. It is often the case that a hard material has also high wear resistance. Under human serum lubrication, proteins in the human serum were deposited on the surface of specimens, which formed a sticky film on the surface of specimens. The proteins film was supposed to prevent worn surface exposing to air and separate the counter ball and the specimens, which reduced the friction coefficient and prevented propagation of wear track.

3.3. Human serum proteins gel electrophoresis

The bio-tribological properties are crucial to biomedical components with moving parts. The physiological environment can affect the bio-tribological properties of the biomaterials [37]. In practice, cardiovascular materials contact blood which contains

![Fig. 5. Typical friction coefficient vs. sliding distance curves of the titanium alloy and Ti–Si–N coatings: (a) Dry sliding condition, (b) Human serum sliding condition.](image)

![Fig. 6. Wear tracks of the Ti–Si–N coatings with different Si contents: Dry sliding (a) 3 at% Si, (b) 8 at% Si, (c) 12 at% Si and human serum sliding (d) 3 at% Si, (e) 8 at% Si, and (f) 12 at% Si.](image)
blood cells and serum and it has been shown that proteins adsorbed from serum plays an important role. Human serum is a complex mixture of proteins. In order to investigate the properties of human serum proteins adsorption to the Ti–Si–N coatings, SDS-PAGE is used. Fig. 7 shows a typical coomassie blue stained electrophoresis gel image of the human serum proteins eluted from the Ti–Si–N coatings and titanium alloy after bio-tribological tests. The left lane 1 in the gel image is the fresh human serum protein ladder with the corresponding abbreviated name and the human serum proteins collected are separated by their molecular weight in the vertical direction. The software shows five spots in left lane 1. The most abundant proteins are serum albumin (HSA, 45 mg/mL) and four various globulin (α1, α2, β1, β2, 10–20 mg/mL). The reference human serum lane on the other samples also has a large amount of albumin and fewer globulin. There is variation between the adsorbed protein relative volumes in different samples. The relative volume of albumin adsorbed on the samples are 6%, 11%, 13%, and 18% corresponding to titanium alloy, 3 at%, 8 at% and 12 at% Si Ti–Si–N coatings, respectively. The other spots imply hydrolysis or denaturation of proteins perhaps due to the physical effects of friction tests. A small amount of protein hydrolysis or denaturation normally does not cause adverse biological effects [38].

Proteins play a role in lubrication in addition to water in the serum. Proteins contribute to the lubrication process and the tribological protection comes from the deposited proteins. Human serum proteins have certain patches and domains that are soluble in water and others that are not as soluble in water. The portions of the proteins that are relatively insoluble tend to be expelled from water. Hence, amphility (a molecule having a hydrophilic group attached to a hydrophobic hydrocarbon chain) is ultimately responsible for adsorption of proteins from the solution on the biomaterials immersed in serum [39]. According to gel electrophoresis, the Ti–Si–N coatings favor serum protein adsorption leading to the formation of a protective layer and lubricant to enhance the tribological properties by reducing friction.

3.4. Effects of Ti–Si–N coatings on endothelial cells

To investigate the effects of the Ti–Si–N coatings on endothelial cells, the relative nitric oxide levels from the vascular endothelial cells seeded on the Ti–Si–N coatings and titanium alloy are determined. The relative nitric oxide secretion levels after the third day are shown in Fig. 8. The absorbance values observed from the endothelial cells seeded on the Ti–Si–N coatings with 12 at% Si are relatively large. Compared to the titanium alloy, similar absorbance is observed from the other two samples after culturing for 3 days. The Ti–Si–N coatings have a favorable effect on endothelial cells.

Fig. 8. Relative nitric oxide released from the endothelial cells cultured on the titanium alloy and Ti–Si–N coatings after 3 days.

3.5. Cell cytoskeleton and spreading

The organizational structure of cytoskeleton plays an important role in not only endothelial cell spreading and proliferation, but also gene expression [46]. Fig. 9 shows the cytoskeleton and spreading in endothelial cells cultured on the different samples for 24 h. Distinct differences in the cytoskeleton organization and spreading are observed between the titanium alloys and the Ti–Si–N coatings. Well-organized cytoskeleton fibers (green color) and obvious nuclei marked by DAPI (blue color) are detected from the endothelial cells cultured on the Ti–Si–N coatings. The endothelial cells also show good spreading and adhesion on the coatings.

In contrast, endothelial cells on the titanium alloys are sparse and the cytoskeleton fibers and nuclei are vague. In comparison, apparent cytoskeleton fibers and nuclei are observed from the endothelial cells cultured on the Ti–Si–N coatings. The cells spread on the Ti–Si–N coatings to form a confluent monolayer, especially on the 12 at% Si coating. Therefore, the Ti–Si–N coatings promote cytoskeleton organization and cell adhesion compared to the titanium alloy. Cell adhesion benefits serum protein adsorption on the materials [47] and serum albumin and globulin protein adsorption is detected by gel electrophoresis after the bio-tribological tests.

Surface properties including wettability and surface chemistry play important roles in proteins adsorption on biomaterials. A
hydrophobic surface is usually believed to adsorb more proteins than a hydrophilic one. Human serum contains many kinds of proteins, and different proteins may have different adsorption trends. It has been shown that fibronectin has greater adsorption on hydrophilic surfaces, whereas albumin predominantly adsorbs on hydrophobic surfaces. Albumin is most abundant in human serum [48]. The protein gel electrophoresis results show very clearly albumin spots in lanes of Ti–Si–N coatings with less than 12 at% Si having implications for the poor surface hydrophilicity. The relative volume of albumin adsorbed on the Ti–Si–N coatings is the larger than that of titanium alloy and so activation of adsorbed proteins to regulate cytoskeleton organization and cell spreading is greater on the Ti–Si–N coatings than titanium alloy. On the other hand, Si–N bonds may play a more important role in protein adsorption here. Si–N has a strong affinity to albumin to promote cell adhesion [49,50]. With increasing Si contents in the Ti–Si–N coatings, the cytoskeleton fibers are dense and cell spreading is significantly greater than that on titanium alloy, as corroborated by the fluorescence staining results.

4. Conclusion

The tribological properties of Ti–Si–N coatings are evaluated under dry conditions and in the presence of human serum. The coatings have large friction coefficients in ambient air because of the absence of lubricants and the friction coefficient decreases with increasing Si content because TiN crystals are embedded in the amorphous Si3N4. In the sliding tests conducted with human serum, the friction coefficients are quite small due to lubrication by serum proteins. The Ti–Si–N coating with 12 at% Si shows the best coating-substrate adhesive strength which helps to reduce inflammation response arising from wear debris particles. Since Si–N has strong affinity to serum albumin, the Ti–Si–N coatings show enhanced adhesion of endothelial cells. The Si content affects the endothelial cells nitric oxide synthesis as well as cytoskeleton formation and spreading. Our results demonstrate that Ti–Si–N coatings possess the desirable bio-tribiological properties and have large potential as cardiovascular materials.

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