MRI-compatible Nb–60Ta–2Zr alloy used for vascular stents: Haemocompatibility and its correlation with protein adsorption

Xiu-Mei Li a, Hui-Zhe Li b, Shao-Ping Wang b, Hsun-Miao Huang c, Her-Hsiung Huang c, Hong-Jun Ai a,⁎⁎, Jian Xu b,⁎⁎

a School of Stomatology, China Medical University, 117 Nanjing North Street, Shenyang 110002, China
b Shenyang National Laboratory for Materials Science, Institute of Metal Research, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang 110016, China
c Biomaterials & Electrochemistry Lab, Department of Dentistry, National Yang-Ming University, Taipei City 112, Taiwan

A R T I C L E   I N F O
Article history:
Received 15 January 2014
Received in revised form 29 April 2014
Accepted 23 May 2014
Available online 2 June 2014

Keywords:
Haemocompatibility
Platelet adhesion
Protein adsorption
Niobium
Stent

A B S T R A C T
Nb–60Ta–2Zr is a newly developed MRI-compatible alloy used for vascular stents. In this work, its haemocompatibility was investigated, including platelet adhesion (lactate dehydrogenase activity), platelet activation (P-selectin expression), coagulation and haemolysis. For comparison, parallel assessments for these factors were performed for the niobium, tantalum, 316L stainless steel (316L SS) and L605 Co–Cr alloy (L605). In addition, albumin and fibrinogen were selected to examine the correlation of protein adsorption with platelet adhesion and metal surface properties. The propensity for platelet adhesion and activation on the Nb–60Ta–2Zr alloy was at nearly the same level as that for Nb and Ta but was slightly less than those of 316L SS and L605. The mitigated platelet adhesion and activation of the Nb–60Ta–2Zr alloy is associated with its decreased adsorption of fibrinogen. The Nb–60Ta–2Zr alloy has a longer clotting time and exhibits significantly superior thromboresistance than 316L SS and L605. Moreover, the haemolysis rate of the Nb–60Ta–2Zr alloy satisfies the bio-safety requirement of the ISO 10993–4 standard. The favourable haemocompatibility of the Nb–60Ta–2Zr alloy provides evidence of its good biocompatibility and of its suitability as a candidate stent material.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The percutaneous coronary intervention (PCI) approach is increasingly used for patients recovering from blockage in the coronary arteries. Currently, balloon-expandable vascular stents are manufactured primarily from austenitic stainless steel (e.g., AISI 316L) or cobalt–chromium alloys (e.g., L605) [1,2]. When considering stent materials, apart from the mechanical properties, corrosion resistance and biocompatibility, radiography visibility is also a critical issue during the rigorous deployment process for the stent. To reduce X-ray radiation-induced damage to the surgery operator, stent deployment navigated by magnetic resonance imaging (MRI) is a highly promising approach. Furthermore, cardiac MRI examination is also an important tool for the diagnosis of cardiovascular disease, such as the assessment of cardiac vessel morphology and plaque characterisation [3]. Consequently, compatibility of stent materials with MRI becomes a considerably important property. However, paramagnetic metals, such as the 316L SS and the Co–Cr alloy, exhibit high volume magnetic susceptibility (χv), owing to their constituent ferromagnetic elements, such as iron, cobalt and nickel. Under a magnetic field with intense magnetic strengths, artefacts in the images are generated as a result of a distortion in the magnetic field [4]. In order to reduce image artefacts, the χv of stent metals should be as comparable to the surrounding tissue as possible, which is in a range of (−11.0)−(−7.0) × 10−8 [5]. Recently, niobium-based alloys, such as the Nb–28Ta–3.8W–1.3Zr [6,7] and Nb–60Ta–2Zr alloys [8], have been considered as an MRI-compatible metal for stent fabrication due to their remarkably lower χv values. As such, the χv of the Nb–60Ta–2Zr alloy is only approximately 3% of the value of 316L SS, providing a significant advantage in MRI compatibility. Meanwhile, the Nb–60Ta–2Zr alloy has a Young’s modulus of 142 GPa, a yield strength of ~330 MPa (comparable to 316L SS), an elongation of ~24%.

As the stent materials contacted with blood, understanding the blood compatibility of Nb-based alloys is essential. Blood–material interactions trigger a complex series of events including protein adsorption, platelet and leukocyte activation/adhesion, and complement and coagulation activation, which are highly interlinked [9]. Interaction between an implanted material and blood starts with the adsorption of plasma proteins onto the material surfaces within a few seconds, leading to the formation of a protein adsorption layer of 10–20 nm. The type and quantity of the proteins that are first adsorbed onto the surface will influence the subsequent coagulation process. Fibrinogen (Fb) is a key structural glycoprotein involved in blood clotting by assembling to form a fibrin clot following thrombin activation. Additionally, Fb is largely responsible for mediating platelet–surface interactions by serving as a ligand for the
Moreover, albumin (Ab) is the most abundant protein in the circulatory system. Ab adsorption would lead to passivation of a surface, thereby slowing down thrombus generation. In other words, adsorption of Ab helps to mitigate coagulation by reducing adhesion and activation of platelets. Therefore, understanding the adsorption behaviour of Fb and Ab on a material is a fundamental aspect for the characterisation of haemocompatibility.

Surface properties, such as wettability (generally referred to as hydrophilicity/hydrophobicity), are key material parameters that affect the biological response to an implanted material. However, observations regarding the effects of surface wettability on protein adhesion have not always been consistent [11–13]. Hydrophobic surfaces are believed to be more protein adsorbent than hydrophilic surfaces because of the strong hydrophobic interactions occurring at these surfaces, in direct contrast to the repulsive solvation forces arising from strongly bound water at the hydrophilic surface [12,14,15].

In our work, the newly developed MRI-compatible Nb–60Ta–22Zr alloy was selected to assess its haemocompatibility, including platelet adhesion (lactate dehydrogenase activity), platelet activation (P-selectin expression), coagulation and haemolysis. For comparison, parallel assessments for these factors were also performed for pure niobium and tantalum, 316L stainless steel (316L SS) and L605 Co–Cr alloy. In addition, relationships between protein adsorption, platelet adhesion and the surface properties of these metals are discussed.

2. Materials and methods

2.1. Material preparation

Cuboidal metal specimens with a dimension of 10 mm × 10 mm × 2 mm were taken from bulk materials of the Nb–60Ta–22Zr alloy, Nb, Ta, 316L SS and L605 Co–Cr alloy. The chemical compositions (wt.%) of 316L SS and the L605 Co–Cr alloy are listed in Table 1. The specimen surface was successively ground using SiC abrasive paper up to 2000 grit. Subsequently, the specimens were mechanically polished with diamond paste up to 1 μm grade and were then ultrasonically washed successively in acetone, dehydrated alcohol, 75% alcohol, and ultrapure water for 10 min in each solution.

2.2. Characterisation of surface roughness and wettability

Surface roughness of the as-polished metallic specimens was determined with a LEXT OLS4000 laser scanning confocal microscope (Olympus, USA). Arithmetical mean deviation of the profile (Rd) was obtained in terms of the surface roughness measurement with an evaluation length of 4 mm. At least three independent samples for each alloy were measured.

Wettability of the metal surfaces was characterised by the water contact angle. Measurements of the contact angle were conducted using the sessile drop technique performed on a contact angle goniometer (JY-82, China) at 25 °C. Doubly distilled water and diiodomethane were used as wetting agents. A 5 μl droplet of the liquid was dropped on the surface of the sample, and an image of the droplet was captured immediately after stabilisation and every 5 s. The profile of the droplet was automatically fitted and analysed with the software supplied by the manufacturer using the Young–Laplace approach. At least three samples were measured for each metal species, and three different sites were chosen on each sample. The final values are given as the mean ± standard deviation (SD).

Based on the contact angle measured with the two different liquids, the surface free energy (SFE), or surface tension, of each contacted surface can be determined using the Owens and Wendt approach [16]. In the Owens and Wendt approach, it was assumed that dispersive and polar intermolecular forces operate across the interface; therefore, the value of the total SFE, γ, is the sum of the two components,

\[ \gamma = \gamma_d + \gamma_p \]  

where \( \gamma_d \) and \( \gamma_p \) are the dispersive and polar components of the SFE, respectively. The solid–liquid interfacial tension can then be written as

\[ \gamma_{SL} = \gamma_S + \gamma_L - 2\sqrt{\gamma_S \gamma_p} \]  

where \( \gamma_{SL} \) represents the interfacial tension between the solid and the liquid and \( \gamma_S \) and \( \gamma_L \) are related to the solid surface energy and the liquid surface energy, respectively.

At the state of the three-phase equilibrium, the relation between the \( \gamma_S \), \( \gamma_L \) and \( \gamma_{SL} \) and the contact angle \( \theta \) is expressed as Young’s Equation:

\[ \gamma_L \cos \theta = \gamma_S - \gamma_{SL} \]  

Combining Eqs. (2) and (3) leads to

\[ \gamma_L (1 + \cos \theta) = 2\sqrt{\gamma_S \gamma_p} + 2\sqrt{\gamma_S \gamma_p} \]  

4. According to Eq. (4), both polar \( \gamma_p^f \) and dispersive \( \gamma_d^f \) components of the SFE of each material can be obtained from the values of the \( \theta \) measured with two testing liquids, doubly distilled water and diiodomethane, with known surface tension components, \( \gamma_S^f, \gamma_L^f, \gamma_d^f, \) as listed in Table 2.

2.3. Protein adsorption

The amounts of plasma Fb and Ab adsorbed to metal specimens were determined using the method described in Ref. [17]. The metal specimens were immersed in phosphate-buffered saline (PBS) containing bovine serum fibrinogen (BSF, Sigma, USA, F8630) or albumin (BSA, Sigma, USA, A1933). The BSA was selected because it presents 76% sequence identity with the human serum albumin (HSA) [18]. The concentrations of Fb and Ab in PBS (pH = 7.4) selected were 0.3 mg/ml and 3 mg/ml, respectively, which represented a 10% dilution of the plasma level to mimic human blood, which has a Fb concentration of 2–4 mg/ml and an Ab concentration of 35–55 mg/ml. After incubation in the protein-containing solution at 37 °C for 1 h, the specimens were washed in distilled water to remove the unadsorbed proteins, treated with 5% sodium dodecyl sulphate (SDS) at 37 °C overnight, and ultrasonicated for 10 min to desorb the proteins. The amount of proteins adsorbed on the metals was calculated in terms of their concentration in the SDS solution. The micro Bichinchoninic Acid (BCA) protein assay kit (Pierce Biotechnology, USA, #23235) was used to determine the concentration of proteins in the SDS solution.

Table 1

<table>
<thead>
<tr>
<th>Chemical composition (wt.%)</th>
<th>Cr</th>
<th>Ni</th>
<th>Mo</th>
<th>W</th>
<th>Mn</th>
<th>Si</th>
<th>C</th>
<th>Fe</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>316L SS</td>
<td>18.4</td>
<td>14.3</td>
<td>3.0</td>
<td>15.1</td>
<td>1.53</td>
<td>0.68</td>
<td>0.02</td>
<td>Bal.</td>
<td></td>
</tr>
<tr>
<td>L605</td>
<td>19.3</td>
<td>8.7</td>
<td>15.1</td>
<td>1.55</td>
<td>0.12</td>
<td>Bal.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4. X-ray photoelectron spectrometer measurement

A specimen of Nb–60Ta–2Zr was incubated in whole blood for 1 h at 37 ± 0.5 °C and rinsed with PBS solution and deionised water. Together with the as-polished sample, the chemical compositions of the sample surfaces were analysed using an X-ray photoelectron spectrometer (XPS). The XPS measurements were performed in an ESCALAB250 surface analysis system (Thermo VG, USA) using a monochromatic Al Kα X-ray source of 1486.6 eV at a take-off angle of 90°. The beam spot size was 500 μm × 500 μm. The pass energy was 50 eV with an energy step size of 0.1 eV. The measured binding energies were calibrated referring to the C 1s peak with the binding energy value of 284.8 eV. The background was subtracted from the measured spectrum according to Shirley’s method [19]. The curve fitting of the XPS spectra was performed by determining the peak position, height, width and Gaussian/Lorentzian ratio, using a commercial software XPSPEAK4.1 for analysis.

2.5. Platelet adhesion and activation

Using the methods described in Ref. [20], fresh human blood was collected from a healthy donor who had not taken any medication, such as aspirin or other drugs, for at least 10 days. To prepare the platelet-rich plasma (PRP), blood was anticoagulated with sodium citrate (3.2 wt.%) at a ratio of 9:1 and centrifuged at 75 g for 5 min. Platelet density of the PRP was adjusted to 3.0 × 10¹¹/l by dilution with platelet poor-plasma (PPP). The investigated metal specimens were incubated with 0.8 ml PRP at 37 °C for 2 h in a 24-well plate. After incubation, the specimens were washed with PBS to remove non-adherent platelets.

For scanning electron microscopy (SEM) observation, adhered platelets on the specimens were fixed with 2.5% glutaraldehyde solution for 12 h at room temperature, dehydrated in a series of graded ethanol at 50%, 75%, 90%, and 100% for 15 min at each concentration, dealcoholised with isooamyl acetate with 50%, 75%, 90% and 100% for 15 min at each concentration, and then dried. The specimen surfaces were deposited with a gold coating with a thickness of 10 nm and measured with a spectrophotometer (UV752N, China) at 545 nm. Conditions. The blood was transferred to a 1.5 ml tube, ethylene diamine tetraacetic acid (EDTA) was added to a final concentration of 10 mM, and the mixture was centrifuged at 2000 g for 10 min to obtain the PPP. Concentration of P-selectin in the plasma was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Enzyme-linked Immunosorbent Assay Kit for Selectin, Platelet, USCN, Wuhan China, E90569Hu) [22].

2.6. Coagulation assays

To prepare the PPP, fresh human blood from a healthy donor that contained sodium citrate (3.2 wt.%) at a ratio of 9:1 was centrifuged at 2000 g for 10 min. The metal specimens were placed in a 24-well plate and incubated with 1 ml of PPP at 37 °C for 1 h. Measurements of prothrombin time (PT), activated partial thrombin time (APTT) and thrombin time (TT) were performed with an automated blood coagulation analyser (CA-500, Sysmex, Japan). As a negative control, the clotting characteristics of the PPP were taken as the blank values of parameters PT, APTT and TT, and were compared with the plasma in contact with the samples.

2.7. Haemolysis assessment

Haemolysis testing was performed according to ASTM F 756-00 [23]. Fresh human blood from a healthy donor that contained sodium citrate (3.2 wt.%) at a ratio of 9:1 was diluted with 0.9% saline (8 ml of blood was diluted with 10 ml of 0.9% saline). The metal specimens were incubated at 37 °C for 0.5 h in the tubes with 10 ml of 0.9% saline. Then, 0.2 ml of diluted blood was added to the tubes. The incubation period was extended for an additional 1 h at 37 °C. Ten milliliters of 0.9% saline and distilled water were chosen as the negative control and positive control, respectively. Then, all tubes were centrifuged at 750 g for 5 min. The supernatant liquid was transferred into a cuvette and measured with a spectrophotometer (UV752N, China) at 545 nm. The haemolysis was calculated based on the optical density (OD) using the following formula:

\[
\text{Haemolysis} = \frac{OD_{\text{test}} - OD_{\text{negative control}}}{OD_{\text{positive control}} - OD_{\text{negative control}}} \times 100.
\]  

2.8. Statistical analysis

The presented data are given as the mean ± standard deviation (SD). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 17.0. Statistical comparisons were made using one-way ANOVA. With a conservative post-hoc analysis, the Student–Newman–Keuls (SNK) method was used to determine statistical significances between materials [24]. Differences were considered to be statistically significant when \( p < 0.05 \).

---

Table 2
Surface properties of tested liquids and investigated metals at as-polished state.

<table>
<thead>
<tr>
<th>Material</th>
<th>Surface roughness Ra (nm)</th>
<th>Water contact angle (°)</th>
<th>Surface free energy γ (mJ/m²)</th>
<th>Dispersive component γd (mJ/m²)</th>
<th>Polar component γp (mJ/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doubly-distilled water</td>
<td>−</td>
<td>−</td>
<td>72.8</td>
<td>21.8</td>
<td>51</td>
</tr>
<tr>
<td>Diiodomethane</td>
<td>−</td>
<td>−</td>
<td>50.8</td>
<td>50.8</td>
<td>0</td>
</tr>
<tr>
<td>Nb–60Ta–2Zr alloy</td>
<td>132 ± 5</td>
<td>66.6 ± 9.2</td>
<td>44.9</td>
<td>33.9</td>
<td>11.0</td>
</tr>
<tr>
<td>Pure Nb</td>
<td>162 ± 2</td>
<td>64.0 ± 6.8</td>
<td>48.0</td>
<td>36.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Pure Ta</td>
<td>136 ± 1</td>
<td>63.2 ± 5.9</td>
<td>47.6</td>
<td>35.6</td>
<td>12.0</td>
</tr>
<tr>
<td>316L SS</td>
<td>31 ± 2</td>
<td>80.5 ± 2.2</td>
<td>37.9</td>
<td>33.1</td>
<td>4.8</td>
</tr>
<tr>
<td>L605 Co–Cr alloy</td>
<td>35 ± 1</td>
<td>72.9 ± 1.5</td>
<td>47.7</td>
<td>42.2</td>
<td>5.5</td>
</tr>
</tbody>
</table>
3. Results

3.1. Characterisation of surface properties

The surface properties of the five investigated metals, including surface roughness, water contact angle and surface energy, are listed in Table 2. For the as-polished samples, the surface roughness of the Nb–60Ta–2Zr alloy is comparable to that of Ta, but slightly less than that of Nb. In contrast, the roughness of 316L SS and L605 is approximately 30 nm, which is markedly lower than that of the three Nb/Ta-based metals. As noted, the surface roughness of L605 is similar to that of 316L SS, which agrees with the results presented by Fritsche et al. [25]. These findings indicate that the surface roughness of all investigated metals is much less than the size of platelets (3–4 μm) [9] and comparable to the size of proteins (BSA: 14 nm × 4 nm × 4 nm; BSF: 47 nm × 5 nm × 5 nm [26]). As a consequence, the effect of specimen surface roughness on platelet activity is expected to be negligible, but the influence on protein adsorption will most likely be relevant.

As indicated in Table 2, there is no detectable difference in the water contact angle between the Nb–60Ta–2Zr alloy and Nb and Ta. Because the native film on the surface of Nb and Ta exists in the form of niobium and tantalum oxides [27,28], respectively, it is not surprising that the current water contact angle data for Nb and Ta are very similar to those of Nb2O5 (72° ± 1.8°) and Ta2O5 (60° ± 1.8°), respectively [29]. The water contact angle of 316L SS is slightly larger than that of L605 and the three Nb/Ta metals. In fact, our results indicate that the contact angle of 316L SS is larger than that of Co, and the three Nb/Ta metals. In fact, our results indicate that the contact angle of the Nb–60Ta–2Zr alloy is subtly enhanced.

3.2. Protein adsorption

Fig. 1 shows a diagram of the adsorption amount of BSA and BSF to the investigated metals and the ratio of BSA/BSF. Compared to Nb and Ta, the BSA adsorption to the Nb–60Ta–2Zr alloy is slightly enhanced. The difference in adsorption amounts between Nb and Ta is insignificant. Also, the differences between 316L SS and the other metals were not significant (p > 0.05). However, adsorption amount of protein to the L605 was significantly higher than to the Nb, Ta and Nb–60Ta–2Zr alloy (p < 0.05). With respect to L605, the adsorption amount of BSA to the Nb–60Ta–2Zr is reduced by approximately 17%.

For BSF adsorption, the Nb–60Ta–2Zr alloy, Nb and Ta also exhibit a lower adsorption amount, with no significant differences between them. 316L SS and L605 manifest a higher adsorption amount compared to the Nb, Ta and Nb–60Ta–2Zr alloy (p < 0.05), but no significant differences exists between them (p > 0.05). Compared with 316L SS and L605, the Nb–60Ta–2Zr alloy exhibits a reduced BSF adsorption by approximately 16% and 24%, respectively.

As noted in prior studies [31,32], the ratio of adsorbed Ab/Fb can be used as an indicator for material haemocompatibility. As displayed in Fig. 1, the ratio of adsorbed BSA/BSF increases according to the following sequence: Ta (0.79) > Nb–60Ta–2Zr (0.78) > Nb and 316L SS (0.73) > L605 (0.71). In this sense, Nb–60Ta–2Zr and Ta with a value somewhat higher than the others are expected to have better haemocompatibility. The variation of the amount of the protein adsorption for all metals is in a range of 5.7%–15.4%. Thus, the difference in the BSA/BSF ratio between each metal seems insignificant.

3.3. XPS analysis

Fig. 2 shows the XPS survey spectrum of the outer-most surface of the Nb–60Ta–2Zr alloy in two states, as-polished and immersed in whole blood for 1 h. For the as-polished state, Nb, Ta, Zr, O and C peaks were detected, as seen in Fig. 2. The presence of the C 1s peak is attributed to air contamination during sample transfer into the XPS chamber. The outer-most layer of the as-polished sample is mainly composed of Nb, Ta, Zr and O, with the relative atomic concentrations of 20.2%, 15.2%, 1.5% and 63.0%, respectively. This suggests that niobium and tantalum oxides are formed on the surface of the Nb–60Ta–2Zr alloy and are major components of the surface layer. For the sample immersed in whole blood, the elements detected on the surface were mainly C, O and N, with relative atomic concentrations of 67.8%, 14.0% and 16.9%, respectively. Small amounts of S, Cl, P, Ca and Mg were also found, at concentrations of 0.50%, 0.26%, 0.23% and 0.13%, respectively, which were determined by an additional examination using high-resolution spectra.

For the Nb–60Ta–2Zr sample immersed in whole blood for 1 h, the high-resolution XPS deconvoluted spectra were measured for the O 1s, N 1s, C 1s and S 2p peaks, as shown in Fig. 3(a)–(d), as the representative, while the spectra for Cl, P, Ca and Mg elements were omitted. The O 1s peaks in the high-resolution XPS spectra appear at 530.9, 531.7 and 532.6 eV, which represent O2−, OH− and H2O, respectively. An intense N 1s signal is located at the binding energy value of 399.8 eV with a
width of 1.6 eV (FWHM). The presence of N is attributed to amino and amide groups from the organic layer adsorbed on metal surfaces. As one of the major elements in proteins, the S 2p peak appears at the binding energy of 163.6 eV. The C 1s peak from the immersed sample includes the prominent hydrocarbon (C–H), the hydroxyl carbon (C–O), and the carbon bound to nitrogen (C–N) and amide carbon (N–C=O). These peaks are identified by their characteristic binding energies at approximately 284.8, 286.2 and 288.0 eV, respectively [33]. The low binding energy signal for C–H bond results from hydrocarbon contamination [34], whereas the carbon contributes high binding energy signals to the C–O, C–N and N–C=O groups of protein molecules. Thus, the presence of these characteristic protein functional groups provides further evidence that the Nb–60Ta–2Zr alloy surface, once in contact with whole blood, is covered by an adsorbed protein layer that cannot be removed by rinsing in PBS solution and deionised water.

3.4. Platelet adhesion and activation

Fig. 4(a)–(e) presents a group of SEM images of adhered platelets on the five metal substrates after being subjected to immersion in PRP for 2 h. In parallel, high-magnification images corresponding to each species are shown in Fig. 5(a)–(e). In all cases, as shown in Fig. 4, a number of adherent and clustered platelets adhered and spread on the metal substrate surfaces. The adherent platelets on the surface of the Nb–60Ta–2Zr alloy are uniformly distributed, with a covered area of approximately 30%, as seen in Fig. 4(a). Spot-shaped platelets are partially aggregated together. Areas free of adherent platelets remained unchanged, even if the material was exposed to the PRP. The area covered by adherent platelets on the surfaces of Nb (~29%) and Ta (~28%) is slightly less than that of the Nb–60Ta–2Zr alloy, as seen in Fig. 4(b) and (c). However, this is not statistically significant. The adherent platelets distributed on Nb and Ta are mainly in the isolated form, with a lack of significant aggregation. In contrast, more extensive coverage of adherent platelets appears on the surface of 316L SS and L605 and reaches a level of approximately 44%, as displayed in Fig. 4(d) and (e), but the difference between the two metals was not significant. For the area fraction of adherent platelets, a significant difference was found between the Nb–60Ta–2Zr alloy, Nb and Ta and the 316L SS and L605 alloy. The higher density of adherent platelets on 316L SS and L605 is also accompanied by remarkable aggregation, as seen in Fig. 4(d) and (e). As a result of platelet spreading, 316L SS and L605 surfaces are covered by layers of platelets. Therefore, the Nb–60Ta–2Zr alloy, Nb and Ta have a significantly weaker propensity for platelet adhesion compared to 316L SS and L605. More adhesion on 316L SS and L605 is associated with more severe aggregation. This means that the haemocompatibilities of the Nb/Ta based alloys are superior to those of 316L SS and L605.

As seen in the high-magnification SEM images in Fig. 5(a)–(e), the majority of adherent platelets are 1–2 μm in size and are dendritic and wide-spread in shape. According to the Goodman’s classification [35], the morphology of most of the platelets can be dendritic (D) or spread dendritic (SD). Spreading (S) or fully spreading (FS) shapes indicate platelet activation. Platelets with a round (R) shape are rarely present. Adherent platelets mutually interact by pseudopodia. The degree of spreading seems dependent on the substrate species. On the surface of the Nb–60Ta–2Zr alloy, Nb and Ta, growth of platelet pseudopodia is well established, and the spreading of inter-pseudopodia is not yet prominent. Most of the platelets are in the D or SD shapes, with some R or FS states. In 316L SS and L605, pseudopodia growth and platelet spreading seem to be more developed, as indicated by their SD and S states, as shown in Fig. 5(d) and (e). Some platelets are in the FS stage. Consequently, considering the morphologies of adherent platelets, we can suggest that the Nb–60Ta–2Zr alloy, Nb and Ta have a weaker propensity for platelet

Fig. 3. High-resolution XPS deconvoluted spectra of (a) O 1s, (b) N 1s, (c) C 1s and (d) S 1s.
activation compared to 316L SS and L605. This is consistent with the finding that the area covered by platelets on the surfaces of 316L SS and L605 is more extensive than that on Nb–60Ta–2Zr, Nb and Ta.

Based on the values from the SEM images, the area fraction of platelet adhesion on each metal is displayed in Fig. 6. Nb and Ta have a lower coverage area. There is no significant difference between Nb, Ta and the Nb–60Ta–2Zr alloy (p > 0.05). In contrast, 316L SS and L605 show a higher coverage with respect to the Nb/Ta metals (p < 0.05). Compared to 316L SS and L605, the area fraction of platelet adhesion in the Nb–60Ta–2Zr alloy is reduced by approximately 30%. There was no significant difference between 316L SS and L605 (p > 0.05).

LDH is used as a measure to characterise platelet adhesion on materials [21], while P-selectin is a marker of material-induced platelet activation [22]. Fig. 7 illustrates the LDH and P-selectin assay results for the five investigated metals. There is no significant difference between Nb–60Ta–2Zr, Nb and Ta for the LDH level (p > 0.05). However, platelet adhesion according to LDH in the Nb–60Ta–2Zr alloy is significantly lower compared to 316L SS and L605 (p < 0.05), with a reduction of approximately 19% and 21%, respectively. In contrast to the other metals, platelet adhesion on Ta is approximately 11% lower than on the L605 alloy. There were no significant differences between 316L SS and L605 (p > 0.05).

LDH is used as a measure to characterise platelet adhesion on materials [21], while P-selectin is a marker of material-induced platelet activation [22]. Fig. 7 illustrates the LDH and P-selectin assay results for the five investigated metals. There is no significant difference between Nb–60Ta–2Zr, Nb and Ta for the LDH level (p > 0.05). However, platelet adhesion according to LDH in the Nb–60Ta–2Zr alloy is significantly lower compared to 316L SS and L605 (p < 0.05), with a reduction of approximately 19% and 21%, respectively. In contrast to the other metals, platelet adhesion on Ta is approximately 11% lower than on the L605 alloy. There were no significant differences between 316L SS and L605 (p > 0.05).

As displayed in Fig. 7, after incubation in whole blood for 1 h, sP-selectin expression for all materials significantly increases with respect to the negative control (p < 0.05). This suggests that platelets which adhered to all metal surfaces were already activated during incubation. In contrast to 316L SS and L605, sP-selectin expression levels in Nb–60Ta–2Zr and Ta are significantly lower (p < 0.05), whereas the level for Nb is between the two groups, without a significant difference (p > 0.05). With respect to 316L SS and L605, sP-selectin expression in the Nb–60Ta–2Zr alloy is reduced by approximately 10% and 12%, respectively. Platelet activation on 316L SS and L605 is more easily to occur compared to the case on the Nb–60Ta–2Zr alloy and Ta.

Thrombus formation on the stent material has been a long-term challenge for the development of new stents, and this thrombus formation is due to platelet-mediated reactions and coagulation of blood plasma [36]. Blood coagulation involves a series of enzymatic reactions resulting in the polymerisation of soluble fibrinogen into an insoluble fibrin gel. The blood coagulation pathway is divided into intrinsic and extrinsic branches, and then, the intrinsic and extrinsic branches merge into a common pathway [9].

PT, APTT and TT are essential in the assessment of haemocompatibility of a material that will potentially contact blood in the body [37]. PT and APTT, two markers assessing the anticoagulant properties of surfaces [38], correspond to the extrinsic and intrinsic coagulation systems, respectively. TT is a marker for the common pathway of coagulation, indicating the material-induced formation of thrombin-mediated fibrin [39]. The longer the PT, APTT and TT, the lower the bioactivity of coagulation factors [40,41]. Extension of the PT and APTT suggests the
inhibition of the extrinsic and intrinsic pathways, respectively, whereas a prolonged TT reflects the inhibition of thrombin activity or fibrin polymerisation [42]. The clotting times for all metals after contact with PPP for 1 h, according to the PT, APTT and TT, are displayed in Fig. 8(a)–(c) and listed in Table 3.

In our study, the test times were limited to the ranges of 10–14 s (PT), 22–38 s (APTT) and 14–21 s (TT). As shown in Fig. 8 and Table 3, the PT, APTT and TT values of all metals are in the range of 11.8–12.23, 32.07–37.2 and 16.97–17.5 s, respectively. These values all fall within the normal range, reflecting that these metals are acceptable regarding the coagulation system. In other words, the level of blood coagulation on all investigated metals is tolerable, without any detectable adverse effect.

PT values in all cases are lower than that of the negative control (p < 0.05). There is no significant difference between Nb–60Ta–2Zr and Ta (p > 0.05), while the difference between Nb–60Ta–2Zr and the other metals is significant. The result for Nb–60Ta–2Zr is approximately 2.5%, 3.4% and 3.4% longer than that for Nb, 316L SS and L605, respectively.

Fig. 5. High-magnification SEM micrographs of adherent platelets on the (a) Nb–60Ta–2Zr alloy, (b) Nb, (c) Ta, (d) 316L SS and (e) L605 after contact with PRP for 2 h at 37 °C. The shapes of adherent platelets on the surfaces as indicated by arrows: R (round), D (dendritic), SD (spread dendritic), S (spreading) and FS (fully spreading).

Fig. 6. Area fraction covered by platelet adhesion on the Nb–60Ta–2Zr alloy, Nb, Ta, 316L SS and L605 after incubation in PRP for 1 h at 37 °C. Error bars indicate the standard deviation for n = 5.

Fig. 7. Platelet adhesion and activation expressed with LDH and sP-selectin, respectively, for the Nb–60Ta–2Zr alloy, Nb, Ta, 316L SS and L605 after contact with PRP (LDH) or whole blood (sP-selectin) for 1 h at 37 °C. Error bars indicate the standard deviation for n = 6.
but comparable to that for Ta. However, the PT of Ta is also significantly longer than that of 316L SS and L605 ($p < 0.05$) but comparable to that of Nb. Again, the values for Nb, 316L SS and L605 are significantly different ($p > 0.05$).

As indicated by the APTT assay, the clotting time of the Nb–60Ta–2Zr alloy and Ta is approximately 11.4%, 12.4% and 12.8% longer than that of Nb, 316L SS and L605 ($p < 0.05$), respectively. However, there is no significant difference between the Nb–60Ta–2Zr alloy and Ta, Nb and the negative control. The value for Nb was not significantly different from those for 316L SS and L605 ($p > 0.05$). The times for 316L SS and L605 are shorter than that of the negative control ($p < 0.05$).

In all cases, the TT values are lower than that of the negative control ($p < 0.05$). Nb–60Ta–2Zr and Ta are significantly different from 316L SS and L605 ($p < 0.05$). The value for Nb was not significantly different from those of the other four metals. Compared to 316L SS and L605, the TT of Nb–60Ta–2Zr was ~3% higher.

Differences in PT, APTT and TT between the materials reflect whether the intrinsic and extrinsic pathways are initiated due to contact with the materials and whether the intrinsic pathway is a predominant factor. As suggested by Hong et al. [43], thrombogenic effects are triggered by contact-activated factor XIIa. Consequently, regarding the suppression of coagulation system activation, the current clotting time data suggest that the Nb–60Ta–2Zr alloy is superior to 316L SS and L605. Because a lower tendency for thrombus formation is partially related to the suppression of coagulation protein activation [44], a longer clotting time corresponds to a stronger thromboresistance. Combined with the results of platelet adhesion (see Figs. 6 and 7), the Nb–60Ta–2Zr alloy exhibits better thromboresistance.

### 3.6. Haemolysis

The haemolysis rate of all investigated metals is shown in Table 3. The haemolysis response to a material indicates the destruction of red blood cells, which directly impairs the ability of the circulatory system to transport oxygen to tissues. Haemolysis is influenced by multiple factors, such as material chemistry, mechanical stress to an erythrocyte, and metal ions leached from the implant [45,46]. Haemolysis initiates the loss of membrane integrity of red blood cells and the release of haemoglobin into surrounding fluid. The lower the haemolysis rate, the better the blood compatibility. According to the ISO 10993–4 standard [47], to ensure biological safety, the haemolysis rate of an implanted material is required to be below 5%. As shown in Table 3, the haemolysis rate of all investigated metals is far below the accepted threshold value of 5%, thus satisfying the requirement of haemocompatibility according to the ISO 10993–4 standard. This result also indicates that haemolytic behaviour is insensitive to the variation of material surface properties, such as roughness and wettability, and chemical compositions.

### 4. Discussion

#### 4.1. Correlation of surface wettability with protein adsorption

Instead of bulk material properties, the surface characteristics of an implanted material, including surface chemistry [48,49], surface roughness [50], and surface wettability [25], determine the material–biological interactions such as protein adsorption and cell attachment. Wettability is the tendency for a liquid to spread on a solid substrate and is characterised by the contact angle formed at the interface between solids and liquids. In general, proteins adsorb more to hydrophobic surfaces than hydrophilic surfaces because proteins compete with water for adsorption to surfaces, and because the hydrate shell of proteins interferes with surfaces during a hydrophilic interaction. However, with small changes in wettability, the relationship between the water contact angle and protein adsorption does not simply follow the rule that the higher the water contact angle, the greater the protein adsorption [10].

In this work, the difference in the water contact angle between Nb–60Ta–2Zr and L605 is insignificant, as shown in Table 2, but the difference in protein adsorption is significant. However, the water contact angle of 316L SS is larger than that of L605, whereas the protein adsorption is insignificant. As a result, the water contact angle does not directly correlate with protein adsorption.

Regarding the adsorption of Fb and Ab to Nitinol surfaces, Shabalovskaya et al. [51] showed that Fb adsorption is directly proportional to the polar component of SFE. Contrary to an obvious correlation between Fb adsorption and polar surface energy, the Ab

---

**Table 3**

<table>
<thead>
<tr>
<th>Material</th>
<th>PT (sec)</th>
<th>APTT (sec)</th>
<th>TT (sec)</th>
<th>Haemolysis rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>12.7 ± 0.1</td>
<td>33.7 ± 0.25</td>
<td>18.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Nb–60Ta–2Zr alloy</td>
<td>12.2 ± 0.1</td>
<td>36.2 ± 0.6</td>
<td>17.5 ± 0.2</td>
<td>1.38 ± 0.26</td>
</tr>
<tr>
<td>Pure Nb</td>
<td>11.9 ± 0.1</td>
<td>32.5 ± 0.6</td>
<td>17.2 ± 0.1</td>
<td>1.44 ± 0.12</td>
</tr>
<tr>
<td>Pure Ta</td>
<td>12.1 ± 0.2</td>
<td>37.2 ± 0.7</td>
<td>17.4 ± 0.1</td>
<td>1.38 ± 0.1</td>
</tr>
<tr>
<td>316L SS</td>
<td>11.8 ± 0.2</td>
<td>32.2 ± 0.6</td>
<td>17.0 ± 0.2</td>
<td>1.62 ± 0.13</td>
</tr>
<tr>
<td>L605 Co–Cr alloy</td>
<td>11.8 ± 0.1</td>
<td>32.1 ± 0.6</td>
<td>17.0 ± 0.2</td>
<td>1.62 ± 0.13</td>
</tr>
</tbody>
</table>
adsorption to Nitinol surfaces seems to fluctuate approximately 75 ng/cm². The lack of regularity in Ab adsorption was also noted in a study of Ti surfaces [11]. Michiardi et al. [52] investigated the influence of surface energy on competitive protein adsorption on oxidised NiTi surfaces. The amount of adsorbed Ab linearly correlated with the polar component of the SFE, in a range of 8–14 mJ/m². For fibronectin adsorption, there was not a linear correlation between the amount of adsorbed fibronectin and surface energy.

Similarly, we also plotted the polar component in SFE against adsorption amount of BSA and BSF for the five investigated metals, as shown in Fig. 9(a) and (b), respectively. The polar component and BSA adsorption are distinctly separated into two groups, 316L SS and L605 and Nb–60Ta–2Zr, Nb and Ta, and there is no well-defined correlation, as seen in Fig. 9(a). The lower the polar component, the higher the Ab adsorption. This is contrary to the case of NiTi as reported by Michiardi et al. [52]. In Michiardi’s work [52], changes in the polar component of the SFE occurred in a given material, but we did not concern on this issue in our work. A similar trend in the relation between BSF adsorption and the polar component of the SFE was also found in the present study, as shown in Fig. 9(b).

As shown in Fig. 1, the difference in adsorption amounts between Nb and Ta is insignificant. This is in agreement with results reported by Byrne et al. [53]. In their work, the adsorption amount of two proteins to Nb and Ta was the same, at ~300 ng/cm², as measured by wavelength-dispersive spectroscopy (WDS). Moreover, our finding that the lack of a distinguishable difference between Nb and Ta in terms of BSF adsorption is in accordance with the results in Ref. [53], in which the Nb and Ta adsorption amounts were reported as 635 and 655 ng/cm², respectively, as measured by spectroscopic ellipsometry (SE).

4.2. Platelet response to the materials and its significance

Ab and Fb are in high abundance in the plasma, and they have different responses to the material surfaces. While Ab is considered a passivating protein [54], Fb is an adhesive protein with a central role in coagulation, platelet activation and aggregation [55]. However, the adsorption of proteins to a material from blood and plasma is very complex and cannot be simply predicted from the behaviour in monolithic protein-containing solution.

On the other hand, the LDH assay is a tool to measure adhered platelets [20]. Because LDH exists in cytoplasm in all tissues undergoing glycolysis and gluconeogenesis, LDH could be found in platelets. Furthermore, P-selectin is a marker of platelet activation, which is stored in the α-granules of platelets and in the Weibel–Palade bodies of endothelial cells. After platelet activation, P-selectin is expressed on the surface of platelets and secreted into plasma; thus, P-selectin correlates with biomaterial thrombogenicity [56]. Soluble P-selectin (sP-selectin) in plasma originates from platelets.

Fig. 10(a) and (b) plots protein adsorption amount against the LDH and sP-selectin assay results, respectively. As shown in Fig. 10(a), the increased adsorption of BSF is relevant to enhanced platelet adhesion, while this is not the case for BSA. For sP-selectin, the relationship between platelet activation and the adsorption amount of BSA and BSF exhibits a trend similar to that for LDH versus protein adsorption, as seen in Fig. 10(b).

In addition, it is noticed that, as displayed in Fig. 4(d) and (e), the difference in platelet adhesion behaviour between the 316L SS and L605 was not significant. It is similar to the previous observation reported by Hansi et al. [57], in which there was no difference in the rate of platelet adhesion/µm² stent surface area between these two metals. Based on the

![Fig. 9. Plot of protein adsorption amount against polar components of surface free energy. (a) BSA and (b) BSF.](image)

![Fig. 10. Plot of protein adsorption amount against platelet adhesion and activation. (a) LDH and (b) sP-selectin.](image)
area fraction of adherent platelets on the investigated metals, as shown in Fig. 6, a significant difference was found between the Nb–60Ta–2Zr alloy, Nb and Ta and the 316L SS and L605 alloy. We noticed that similar results were also observed by Sprague et al. [48], in which platelet adhesion on Ta was much lower than on 316L SS and L605.

Platelet adhesion, shape change and secretion of granules upon exposure to material surfaces in flowing blood are the initial and critical reactions that facilitate thrombus formation and growth [58], contribute to blood coagulation and play a crucial role in stent thrombosis [59]. Typically, platelet–material interactions have been assessed by evaluating the material for the presence of adherent platelets and assessing the extent to which they have become activated [22]. Our results indicate that the degree of platelet adhesion and activation on the Nb–60Ta–2Zr alloy is less than that on 316LSS and L605, which indicates that the Nb–60Ta–2Zr alloy has acceptable thromboreactivity as a vascular stent material. However, there is a need for an in vivo assessment for haemocompatibility in future works.

4.3. Haemolytic behaviour

As shown in Section 3.6, haemolytic behaviour for the investigated five metals is insensitive to the variation of material surface properties, such as roughness and wettability. This is also in agreement with previous observations [60], in which the haemolysis behaviour of as-polished 316L SS, Ti and NiTi was similar to the negative control and much lower than the positive control, indicating that NiTi, 316L SS, and commercially pure Ti are comparably non-haemolytic materials. Furthermore, the haemolysis of materials is also insensitive to surface modifications. Niemoeller et al. [45] and Kempe et al. [46] showed that the release of metal ions is a key factor influencing the haemolysis of a material. In our study, we showed that the haemolysis rates of the Nb–60Ta–2Zr alloy, Ta and Nb are lower than those of 316LSS and L605, which is directly related to the high corrosion resistance of the Ta-containing oxide layer [8,61] that helps to reduce the release of metal ions into the red blood cell. The haemolysis rate of the NiTi alloy can be reduced via Ta implantation due to better corrosion resistance [62].

Finally, to our knowledge, very few studies have been carried out on the blood compatibility of pure metals used in various medical devices. Hong et al. [43] noted that metals have varying thrombogenic and complement-activating properties. Similar to titanium and indium, tantalum was found to exhibit pronounced thrombogenic properties, whereas aluminium, nickel and, in particular, iridium were essentially non-thrombogenic. Tin and zirconium were intermediate activators. All metals activated complement to a similar degree, with the exception of aluminium, which had more pronounced activating properties. Our results on the haemocompatibility of pure Nb and Ta provide valuable information for the selection of elements for designing biocompatible alloys.

5. Conclusions

With an air-formed surface oxide film, the Nb–60Ta–2Zr alloy has higher hydrophilic characteristics to Nb and Ta and is less hydrophobic than 316L stainless steel and the L605 Co–Cr alloy. Fairly weak adsorption of albumin and fibrinogen to the Nb–60Ta–2Zr alloy, Nb and Ta is consistent with their less hydrophobic features, in contrast to the 316L stainless steel and the L605 Co–Cr alloy. For the investigated metals, the affinity of protein adsorption is approximately consistent with their propensity for platelet adhesion and activation. The Nb–60Ta–2Zr alloy’s propensity for platelet adhesion and activation remains at nearly the same level as Nb and Ta but is slightly weaker than that of 316L SS and the L605 Co–Cr alloy. The mitigated platelet adhesion and activation of the Nb–60Ta–2Zr alloy is associated with its decreased adsorption of fibrinogen.

Regarding blood coagulation, the Nb–60Ta–2Zr alloy has a longer clotting time and exhibits significantly superior thromboreactivity compared to the 316L SS and the L605 Co–Cr alloy. Furthermore, the haemolysis rate of the Nb–60Ta–2Zr alloy (~1.4%) is comparable to those of pure Nb and Ta but is slightly lower than those of 316L SS and the L605 Co–Cr alloy. The Nb–60Ta–2Zr alloy satisfies the bio-safety requirement as listed by the ISO 10993-4 standard (≤5%). As a consequence, the reasonable haemocompatibility of the Nb–60Ta–2Zr alloy indicates that the alloy has good biocompatibility as a potential material for stents; however, an in vivo assessment of the haemocompatibility is required.

Acknowledgments

The authors gratefully acknowledge Prof. Ke Yang for providing the samples of the 316L stainless steel and Co–Cr (L605) alloys. The authors gratefully acknowledge the assistance with the XPS tests from Dr. Qiang Kang and Ms. Bin Zhang, and with the plasma preparation using whole blood from Dr. Zhen-dong Zhu.

References
