Surface characteristics and biological properties of paclitaxel-embedding PLGA coatings on TiNi alloy

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Abstract

Surface characteristics and biological properties of paclitaxel-embedding poly(lactide-co-glycolide) (PLGA) coatings on TiNi alloy have been studied systematically. Surface topography, chemical construction, paclitaxel cumulative release characteristic and interactions between platelets and paclitaxel-embedding PLGA coatings are studied by atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS), high performance of liquid chromatography (HPLC) and platelet adhesion test. AFM results show that the surface roughness decreases from 350 nm to below 52 nm after completion of PLGA coatings on passivated TiNi alloy samples. XPS results indicate the PLGA coating without paclitaxel has the maximum percentage of C\textsubscript{C} bond, which is about 61\%, and the minimum percentage of C\textsubscript{O}C\textsubscript{O} and O\textsubscript{C}O bonds comparing with PLGA coatings after adding paclitaxel. HPLC results show that paclitaxel releases fast in vitro from the paclitaxel-embedding PLGA coatings at the beginning, and then the cumulative release amount of the paclitaxel increases slowly with increasing of incubation time. The amount of paclitaxel released is not related to the paclitaxel embedded. It has been found that the amount of platelets adhered on the surface of passivated TiNi alloy sample are greater than that on the surface of the PLGA coated on TiNi alloy. With the increase of paclitaxel, the amount of adhered platelets increases and the shape of platelets were activated obviously. When the content of paclitaxel reaches 30\%, the platelets exhibit a phenomenon of significant accumulation.

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1. Introduction

TiNi alloys have been intensively used in biomedical field due to its excellent shape memory effect, superelasticity and corrosion resistance [1,2]. These properties have made them used as new medical devices such as different kinds of diagnostic and therapeutic catheters, stents, needle wire localisers, orthodontic arch wires, implantable drug delivery system, etc [3]. The specific superelasticity of TiNi alloy is 10–20 times higher than stainless steel, which plays an important role on the security and flexibility after implanted [4]. TiNi alloy has been a promising material of stent due to its easy placement, low cost, reduced patients’ discomfort and recovery time, etc. Furthermore comparing with 316L stainless steel, TiNi alloy has outstanding fatigue resistance [5].

The biodegradable polymer poly(lactide-co-glycolide) (PLGA) degrades into lactic and glycolic acids which both can be eliminated from body as carbon dioxide and water, and PLGA has been increasingly used to delivery drugs [6]. Jackson [7] used PLGA blending with other polymers for the formation of paclitaxel loaded films on the surface of Teflon strips in order to applying as perivascular “wraps” to prevent restenosis. In present paper, paclitaxel-embedding PLGA coatings with a variable dosage were prepared. The surface characteristics and biological performance are studied, which can lay a foundation for developing new generation of drug-eluting TiNi alloy stents.

2. Experimental

2.1. Sample preparation

The 10 × 10 mm\textsuperscript{2} square substrates of TiNi alloy were used in this study. The substrates were firstly prepared by wet grinding with silicon carbon paper to a mirror surface, and then immersed into \textit{HNO}\textsubscript{3} for 30 min to get passive surface. Finally they were

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ultrasonically cleaned in acetone, alcohol and distilled water in sequence. The paclitaxel-embedding PLGA coatings were prepared by dissolving the appropriate amount of PLGA and paclitaxel in dichloromethane. The mixed solutions were stirred by a magnetic stirring apparatus for about 1 h until all components had completely dissolved and the solutions were virtually clear. After that the mixed solutions were deposited on TiNi alloy plates by dipping technique.

2.2. Surface characterization

The surfaces of the paclitaxel-embedding PLGA coatings were investigated using a Nano-scope III Digital Instruments Inc. for atomic force microscopy (AFM), surface chemical construction was determined by X-ray photoelectron spectroscopy (XPS) equipped with an ESCA PHI 500 spectrometer, and the X-ray source with a Al Kα.

2.3. In vitro release of paclitaxel from coatings

The in vitro release of paclitaxel from coatings was measured in phosphate buffer saline (pH 7.4) (PBS) by HPLC (Waters 510, USA). The chromatography was performed using a C-18 column with the size of Ø4.5 × 250 mm, the mobile phase consisted of 20% methanol–32% acetonitrile–48% water and a flow rate of 1.0 ml/min with detecting wave length 227 nm. The released paclitaxel was extracted by centrifuging the collected PBS after adding 1 ml dichloromethane at a rotary speed of 2000 r/min for 4 min. The underlayer solution was paclitaxel-rich phase. The concentration of released paclitaxel was determined by comparing with paclitaxel standard curve.

2.4. Platelet adhesion test

Platelet adhesion test was performed to investigate the quantity, morphology, and the degree of activated platelet. The whole blood from a volunteer was treated with the anticoagulant of 3.8% sodium citrate, and then centrifuged at a rate of 3000 r/min to obtain the platelet-rich plasma (PRP). The uncoated and paclitaxel-embedding PLGA coated on TiNi alloy samples were immersed in PRP and incubated at 37 °C for 60 min, then the samples were rinsed with a 0.9% NaCl solution to remove the nonadherent platelets. The adhered platelets were fixed in 4% glutaraldehyde solutions at room temperature, and then the samples were dehydrated, degreased and dried at critical point. Before observed by scanning electron microscopy (SEM), the specimens were coated with a layer of gold by sputtering.

3. Results and discussion

3.1. Surface topography

After the completion of preparation of paclitaxel-embedding PLGA coatings on TiNi alloy plate, the surface of different

![Fig. 1. Three-dimensional AFM images of surface for (a) passivated TiNi alloy, (b) 10% paclitaxel-embedding PLGA coating, (c) 20% paclitaxel-embedding PLGA coating, (d) 30% paclitaxel-embedding PLGA coating on TiNi alloy.](image-url)
paclitaxel-embedding coatings and also the passivated TiNi alloy were inspected by AFM and the three-dimensional images are shown in Fig. 1. Fig. 1(a) presents the passivated surface of TiNi alloy before coating with hump surface feature which has a surface roughness about 350 nm. It was noticeable that the surface of 10% paclitaxel-embedding PLGA coating is improved significantly, and the surface roughness decreases from 350 nm to below 17 nm. AFM results also show that the surface roughness of different paclitaxel-embedding PLGA coatings on TiNi alloy samples decreases, as listed in Table 1. It is easy to notice that the surface roughness of the coatings increases with increasing of paclitaxel dosage.

The XPS techniques are adopted in present work to investigate the surface composition of paclitaxel-embedding PLGA coatings on TiNi alloy. Fig. 2 shows the C1s high-resolution spectra of different paclitaxel-embedding PLGA coatings on TiNi alloy plate. The spectra are least-squares fitted using Gaussian–Lorentzian lines and the corresponding integral background. The fitted results demonstrated that the broad C1s bond consists of three different carbon bonds, including C–C, C=O–C=O and O–C=O appeared with the peaks at 285.0, 286.8 and 289.3 eV.

The surface percentage of carbon bonds in different paclitaxel-embedding PLGA coatings are given in Fig. 3. As the figure shows, the PLGA coating without paclitaxel has maximum percentage of C–C bond, which is about 61%, and the minimum percentage of C=O–C=O and O–C=O bonds which are about 23% and 25%, respectively, comparing with PLGA coatings after adding paclitaxel.

### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>RMS (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passivated TiNi</td>
<td>350.00</td>
</tr>
<tr>
<td>0% paclitaxel-PLGA</td>
<td>5.32</td>
</tr>
<tr>
<td>10% paclitaxel-PLGA</td>
<td>17.00</td>
</tr>
<tr>
<td>20% paclitaxel-PLGA</td>
<td>26.58</td>
</tr>
<tr>
<td>30% paclitaxel-PLGA</td>
<td>51.17</td>
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</tbody>
</table>

3.2. Paclitaxel release from PLGA coatings in vitro

The cumulative release behaviour of paclitaxel from paclitaxel-embedding PLGA coatings is presented in Fig. 4. As the figure shows, the initial release burst is prominent for all three paclitaxel-embedding coatings after incubation in phosphate buffer saline at pH 7.4. The released amount increases slowly with the increasing of incubation time. It is interesting to find that not only in initial burst release period but also in subsequent slowing release period, the amount of released paclitaxel does not have close relationship with the amount of paclitaxel embedded. As can be seen, the amounts of the released paclitaxel are 19.4%, 21.5% and 23%, respectively, after incubation in PBS at pH 7.4 for 3 days. After incubation for 12 days, the amounts of released paclitaxel are 23.4%, 24.5% and 30%. The factor influencing initial burst release is related with “swelling behaviour” of high polymer in aqueous media, in which case small molecule of paclitaxel can release from loose surface. Another factor might be related to the drug near to the outer surface which is covered by a...
thin PLA film releases when the thin film is damaged in PBS solution.

3.3. Platelet adhesion test

The interactions between the platelets and the paclitaxel-embedding PLGA coatings were evaluated by platelets adhesion test shown in Fig. 5. The adhered platelets on passivated TiNi alloy samples shown in Fig. 5(a) seem partially activated, with few pseudopodia spreading on the boundary of platelet. On the surface of PLGA coating without paclitaxel, platelets present one or two short pseudopodia spreading. While on the surface of PLGA-coatings after paclitaxel embedded, platelets exhibits spreading to different extent, as can be seen in Fig. 5(c)–(e). Moreover, the activated degree increases with the increasing amount of paclitaxel. Platelets on the surface with 30% paclitaxel-embedded sample show a phenomenon of significant accumulation.

Fig. 6 shows the amount of platelets adhered on different paclitaxel-embedding PLGA coatings. As the figure shows, the amount of platelets adhered on bare TiNi alloy sample with a rough surface is the largest, while the adhered amount on PLGA coating is the smallest. Comparing with PLGA coating without paclitaxel, the platelets adhered on paclitaxel-embedding PLGA coatings increase.
4. Conclusions

(1) The surface roughness of TiNi alloy treated with mechanical polish and then immersed into HNO₃ was measured to be 350 nm, which decreases significantly after completion of paclitaxel-embedding PLGA coatings on it and the surface roughness increases with increasing of the amount of paclitaxel embedded.

(2) The PLGA coating without paclitaxel has the maximum percentage of C–C bond, which is about 61%, and the minimum percentage of C=O–C=O and O=C=O bonds comparing with PLGA coatings after adding paclitaxel.

(3) The initial release burst was prominent for all paclitaxel-embedding PLGA coatings, then the amount of released paclitaxel increases slowly with increasing of the release time. It has been proved that the release behaviour of paclitaxel is not related close with the amount of embedded paclitaxel.

(4) The amount of platelets adhered on the surface of passivated TiNi alloy sample is the largest, and the PLGA coating without paclitaxel is the lowest. With the increase of paclitaxel embedded the activated degree of platelets increases. When the contents of paclitaxel reach 30%, the platelets exhibit a phenomenon of significant accumulation.

References