Hemocompatibility of lanthanum oxide films fabricated by dual plasma deposition

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Abstract

Lanthanum oxide films were fabricated using dual plasma deposition. X-ray photoelectron spectroscopy (XPS) showed that La existed in the +3 oxidation state. X-ray diffraction (XRD) revealed a (101) oriented hexagonal structure. Blood platelet adhesion tests and endothelial cell cultures were used to evaluate the hemocompatibility of the as-deposited films. Scanning electron microscopy (SEM) and optical microscopy were employed to evaluate the surface morphology of the blood platelets and endothelial cells on the films. The results showed that the number of adhered, aggregated and morphologically changed platelets was reduced compared to that observed on low-temperature isotropic carbon (LTIC). Endothelial cells culture tests indicated good adhesion and proliferation of human umbilical vein endothelial (HUVE) cells in vitro. Our study suggests that lanthanum oxide films are potential blood-contacting biomedical materials.

Keywords: Lanthanum oxide; Dual plasma deposition; Blood platelets; Endothelial cells

1. Introduction

Thrombogenicity of artificial organs can lead to life threatening complications resulting in significant morbidity and mortality. In addition to relatively low toxicity, rare earth elements exhibit anticoagulant, antiemetic, antiseptic, immunomodulatory, antineoplastic properties and have thus aroused considerable interest in medicine due to these and other pharmacological effects [1,2]. Furthermore, rare earth oxides have excellent mechanical, chemical, thermal, optical, and electrical properties [3]. Lanthanum oxide films can be produced by chemical vapor deposition, electron beam evaporation, sputtering, spray pyrolysis, and molecular beam epitaxy [4–7]. However, there have been few reports on the blood compatibility of rare earth oxide films. In the work reported here, lanthanum oxide films were synthesized using dual plasma deposition derived from plasma immersion ion implantation and deposition (PIII&D). The low deposition rate and relatively long time for diffusion and relaxation between metal plasma pulses yield good quality films. Blood platelet adhesion and endothelial cell culture tests were used to study the hemocompatibility in vitro. Our results reveal unique hemocompatibility properties making La2O3 suitable for blood-contacting biomedical materials.

2. Experimental methods

Lanthanum oxide films were produced in a plasma immersion ion implanter equipped with a metal cathodic arc plasma sources [8,9]. The dual plasma consisting of oxygen and La ions was created by bleeding oxygen gas into the vicinity of the metal arc discharge plume when the cathodic arc was triggered. The lanthanum oxide films were deposited on silicon wafers at working pressure of 1.6 × 10−2 Pa. The processing deposition time was 2 h and the substrate temperature was 500 °C. The film thickness was measured with a Taylor–Hobson surface profiler. The structure and chemical properties of the films were determined by X-ray diffraction (XRD) at a glancing angle of 0.7° and λ = 1.5418 Å using the PAN Analytical X’pert PRO
and X-ray photoelectron spectroscopy (XPS) employing the Kα X-ray line of Al using the Physical Electronics PHI 5802 instrument.

In vitro platelet adhesion tests were performed to identify the blood compatibility of the lanthanum oxide films in comparison with low-temperature isotropic carbon (LTIC) [10,11]. LTIC is generally regarded to be an acceptable anticoagulant material. The samples were incubated in human platelet-rich plasma with agitation for 2 h at 37 °C. After rinsing with physiological saline, fixing with glutaraldehyde solution, dehydrating by gradient alcohol, dealcoholizing by gradient isooamyl acetate and critical point drying, the specimens were coated with a 10–20 nm thick gold layer to conduct scanning electron microscopy (SEM) and optical microscopy to evaluate the quantity and morphology of the adherent platelets. Ten fields at a magnification of 1000 were chosen at random to obtain statistical averages of the adherent platelets based on the point counting method.

HUVE (human umbilical vein endothelial) cells were isolated and cultured according to the method of Jaffe et al. [12]. 200 μl of the incubation liquid with approximately 3.5×10⁵ viable cells/ml were added onto the sample surface and incubated at 37 °C in 5% CO₂/air for 1–3 days. After rinsing, fixing, dehydrating and dealcoholizing, critical point drying and coating with gold layer, cells were examined by optical microscopy and scanning electron microscopy.

3. Results and discussion

The thickness of the film determined by surface profilometry was about 136 nm. According to the X-ray diffraction patterns in Fig. 1, the La₂O₃ films deposited at 500 °C were hexagonal La₂O₃ with a preferred (101) orientation. XPS was used to study the chemical states of lanthanum after about 10 nm of the surface had been etched by Ar ion sputtering to remove surface contaminants. The presence of La, O and Ar is shown in the XPS spectrum in Fig. 2. Figs. 3 and 4 depict high-resolution La3d and O1s XPS spectra, respectively. The binding energies were normalized using the argon Ar2p peak. The La3d spectrum shows two doublets and the energy peaks appearing on the high energy side of the 3d5/2 and 3d3/2 peaks are satellite peaks. Not only are the La3d states split into two lines, 3d5/2 and 3d3/2, because of a spin–orbit interaction, but also each line is split due to a transfer of an electron from O2p to the empty 4f shell of La leading to the 3d⁵4f⁵ final state [13]. The most intense peak of La 3d5/2 is at approximately 835.1 eV, and it can be assigned to La₂O₃ [14,15]. The energy difference between the 3d3/2 and 3d5/2 states is approximately 17 eV. The main peak at 530.5 eV
in the O1s spectrum corresponds to $O^{2-}$ of the metal oxide [15,16], whereas the other peaks at about 532.6 eV are derived from water and hydroxide absorbed on the surface [17]. The XPS results show that La is in the $+3$ oxidation state.

The statistical results of platelet adhesion for lanthanum oxide and LTIC are displayed in Fig. 5. After incubation in PRP for 2 h, the number of adherent platelets on the lanthanum oxide film decreased in comparison with LTIC. Fig. 6 depicts the SEM micrographs showing the morphology of the adhered platelets on the $La_2O_3$ and LTIC surfaces after incubation for 120 min. It can be seen that the platelets on lanthanum oxide exhibit fewer aggregation and pseudopodium compared to those on LTIC. Our experiments demonstrate that the scalar and active levels of adhered platelets on lanthanum oxide are lower than those on LTIC. Here, denaturing and aggregation of the platelets are observed to be impeded, and platelet adhesion is also reduced on the lanthanum oxide surface. The actual mechanism may be quite complicated. $La_2O_3$ films have been reported to form carbonate and hydroxide in air [18,19], but in our experiments, very little $La^{3+}$ ions may be released from the lanthanum oxide during incubation as they were not detected by inductively-coupled plasma mass spectrometry (ICPMS). $La^{3+}$ ions may interact with platelet activator molecules such as adenosine diphosphate (ADP) and 5-hydroxytryptamine, and it can influence the adhesion, aggregation of platelet and blood coagulation. It has been reported that millimolar concentrations of $La^{3+}$ ions inhibit platelet aggregation induced by ADP. However, high concentrations of $La^{3+}$ ions cause nonspecific clumping of platelets [1]. Some researchers think that $La^{3+}$ ions can reduce the surface charge of platelets and alter their electrophoretic mobility as a result of binding to the external surface [20]. It has also been reported that $La^{3+}$ ions inhibit certain platelet responses by decreasing the lipid fluidity and it is highly unlikely that $La^{3+}$ penetrates the platelet plasma membrane [21]. Therefore, we believe that the platelet adhesion, denaturing and aggregation of lanthanum oxide film are inhibited based on these possible mechanisms.

The number of endothelial cell increases significantly after 3 days (average of ten fields at a magnification factor of 50 under optical microscopy). Fig. 7 shows the surface morphology of the endothelial cells on the lanthanum oxide after 1 day and 3 days. The cells proliferate on the surfaces exhibiting an elongated and spread morphology after 1 day, and grow to confluence with typical cobblestone morphology after 3 days. Our results reveal

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Fig. 5. Statistical results of platelet number on the surface of LTIC and lanthanum oxide film.

Fig. 6. SEM micrographs of in vitro platelet adhesion tests: (a) and (b) LTIC; (c) and (d) lanthanum oxide.
that HUVE cells exhibit good adhesion and proliferation behavior on the lanthanum oxide film and the materials have good cytocompatibility with HUVE cells. Owing to the similarities between rare earth ions and Ca\(^{2+}\) in some physical and chemical properties, they may influence cell functions. Lanthanides can bind to cell membrane proteins, sialic acid residues of membrane glycoproteins, and phospholipid bilayers. However, results on cardiac muscle cells, liver cells, epithelia cells, muscle cells, and blood cells suggest that lanthanide ions cannot penetrate artificial phospholipid bilayers and probably cannot enter healthy cells\(^\text{[1]}\). La\(^{3+}\) has been shown to accelerate the synthesis of 3T3 and 3T6 cells\(^\text{[22]}\). An appropriate concentration of rare earth elements can expedite the growth and proliferation but a concentration that is too high can lead to some toxicity. La\(^{3+}\) may promote or inhibit the formation and bone-resorbing activity of osteoclast-like cells depending on its concentration\(^\text{[23]}\). Therefore, there appears to be a “dose effect” associated with rare earths\(^\text{[24]}\). It should, however, be mentioned that other effects have also been observed. For instance, a certain concentration of rare earth compounds may inhibit the growth of leukemic cells, induce them to apoptosis, and impose no significant inhibitory effects on normal bone marrow hematopoietic progenitor cells\(^\text{[25]}\). More work is required before the exact enhancement mechanism can be elucidated.

### 4. Conclusion

Lanthanum oxide films synthesized by dual plasma deposition have very good hemocompatibility activity. XPS results showed that La is in the +3 oxidation state. XRD analyses reveal the (101) oriented hexagonal La\(_2\)O\(_3\) structure. The adhered, aggregated, and morphologically changed platelets are reduced on La\(_2\)O\(_3\) in comparison with LTIC. HUVEC cells exhibit good adhesion and proliferation behavior on lanthanum oxide. The combination of these unique properties suggests that lanthanum oxide films are potential blood-contacting biomedical materials.

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### References