Blood Compatibility of Titanium-Based Coatings Prepared by Metal Plasma Immersion Ion Implantation and Deposition

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Abstract – In this work the suitability of some Ti-based coatings as coating for blood-contacting implants is analyzed. Layers of pure Ti, Ti nitride, Ti oxynitrides ($TiN_{1-x}O_x$ with $x = 0.25$, 0.50, and 0.75) and Ti oxides were deposited from a plasma produced by cathodic arc evaporation under addition of N_2 and/or O_2. The oxynitrides are crystalline with the fcc structure of TiN up to $x = 0.25$. For $x = 0.5$ a two-phase system of fcc TiN and fcc TiO has been found. In dependence on the deposition parameters, amorphous and crystalline layers (anatase + brookite, or rutile) of TiO_2 have been obtained. The rutile layers were doped by implantation of P. To study the correlation between coating structure and blood compatibility, the clotting time of blood plasma as well as the adhesion and activation of blood platelets on the surface was investigated. TiN and oxynitrides showed the longest clotting time compared to rutile. Minimum platelet adhesion has been observed for TiO_2. Contrasting tendencies in the dependence of clotting time and platelet adhesion on the microstructure have been stated. However, for P^+-doped rutile both enhanced clotting time and improved platelet adhesion were observed. Platelet adherence and activation always showed similar trends.

1. Introduction

For blood-contacting medical implants, like heart valves or vascular stents, it is important to minimize the tendency of their surface to adsorb blood proteins and to induce blood clotting, hence, to reduce the danger of thrombosis. Ti and Ti-based materials are known to be good biocompatible in general, and a good hemocompatibility for Ti oxide [1] and Ti-nitride-oxide [2] recently have been demonstrated. However, other studies also showed enhanced haemostatic reactions on Ti with its natural oxide film [3]. The release of growth factors from activated blood platelets should be the reason for the good osteoconductive properties of the metal [4]. This indicates that the reaction of the body upon a Ti surface is influenced by more parameters. The preparation mode of the oxide film, its thickness, roughness, crystallinity and carbon contamination have been discussed [5, 6], but a complete picture could not be achieved, and the number of detailed studies is low. This is one subject of this investigation. The other topic is the impact of a possible electron transfer from fibrinogen into the surface on the blood compatibility. The crystal forms anatase and rutile of TiO_2 are semiconductors with band gap energies of approximately 3.0 eV. By ion implantation of n-doping elements it is possible to inhibit an electron acceptance of the surface. An alternative way to inhibit the electron acceptance is the implantation of a strong electron donor in a redox system.

In this study Ti-based layers with different phase composition were prepared by MePIIID (Metal Plasma Immersion Ion Implantation and Deposition). Ion implantation was applied to modify the electronic properties of TiO_2 films. The clotting time of blood plasma and the adhesion and activation of human blood platelets were studied in order to find correlations between the structure of the Ti oxide, oxynitride, as well as nitride and its blood compatibility.

2. Experimental

As substrate thermally oxidized monocrystalline silicon was used. The Ti-based layers were deposited by MePIIID. The schematic diagram of the MePIIID equipment is presented in [7].

The Ti plasma is produced by a cathodic arc discharge operated in direct current mode and filtered by means of a curved magnetic filter. Ions from the plasma are accelerated by applying a negative bias voltage to the substrate. In this way, deposition and ion implantation are combined. The substrate temperature increases strongly with increasing implantation voltage and deposition time. To limit the temperature to a given value, the deposition was interrupted approaching T_{max} and started again after a certain cooling time. Ti oxides and/or nitrides are formed by additional supply of oxygen and/or nitrogen into the vacuum chamber near the substrate. The method of MePIIID allows to obtain Ti-based coatings with different stoichiometry and phase composition in dependence on the deposition parameters. The preparation parameters are collected in Table I.

After deposition selected TiO_2 samples were implanted using an ion implanter with P^+ (30 keV, 10^{15} cm^{-2}) or Cr^+ (30 keV, $5 \cdot 10^{17}$ cm^{-2}). For P-im-

planted samples the effect of post-implantation annealing (900 °C, 1 h in vacuum) was also studied.

Table I. Preparation conditions for the Ti-based layers

Specimen temperature	25–500 °C
Gas flow rate (F)	60–180 sccm
Substrate bias (U)	0–2.5 kV
Coating deposition rate (R_{dep})	0.2–1.1 μm/min
Cathodic arc current (I)	110 A
Basic vacuum	$0.5–1 \cdot 10^{-3}$ Pa
Working pressure	$0.5–1 \cdot 10^{-1}$ Pa
Substrate	SiO_2 on Si (100)

X-ray diffraction (XRD) measurements were carried out with a step scan diffractometer with a thin film attachment in grazing incidence geometry ($\omega = 1°$) using Cu K_α radiation. The roughness of the deposited layers was measured by atomic force microscopy (AFM) in tapping mode on representative 10×10 μm^2 areas.

The blood plasma clotting time was determined by manually stirring 50 μL recalcified standard human plasma (Dade Behring) on the samples at a constant temperature of 37 °C and measuring the time until the formation of a fibrin clot. The percentage of adherent blood platelets from platelet-rich plasma after 45 min contact with the surface was determined by the lactate dehydrogenase method [8]. The expression of the platelet activation marker CD62P was measured by flow cytometry in the platelets of the supernatant of the previous assay. For the biological examinations, the polyethylene Thermanox® was used as a reference.

3. Results and Discussion

Phase composition and microstructure of the formed Ti-based layers can be influenced in a controlled way by selecting the deposition parameters. This offers the possibility for a systematic study of the correlation between blood compatibility and microstructure of the layer. In Table II a review of the obtained structures in dependence on the deposition parameters is presented.

The thickness of all produced layers was about 1 μm with exception of the amorphous and nanocrystalline titanium oxide films (~ 350 nm).

By Ti-oxide layers without ion implantation ($U = 0$ kV) amorphous or nanocrystalline structures are formed. For a high oxygen flow rate F (180 sccm) the XRD patterns are typical for an amorphous layer (Fig. 1(a)). For the low oxygen F (60 sccm) very broad diffraction peaks are observed, which can be interpreted as due to a nanocrystalline phase mixture of anatase and possibly brookite (Fig. 1(b)).

For the highest ion energy ($U = 2.5$ kV) crystalline films composed of two phases are formed. The phases observed are strongly dependent on the oxygen flow. For the high F (180 sccm) a crystalline mixture dominated by rutile and a contamination of anatase is formed (Fig. 1(d)). Anatase is formed in the beginning of the deposition directly at the surface as proved by XRD measurements with different incident angle. The low F (60 sccm) leads under the same conditions to formation of anatase + brookite (Fig. 1(c)) analogous to the deposition without bias.

Table II. Dependence of the layers structure on the deposition parameters

Structure		$F(O_2)$, sccm	$F(N_2)$, sccm	U, kV	Subsequent ion implantation	Annealing, °C
Ti oxides	amorphous TiO_2-layer	180	–	0	Cr^+(30 keV, $5 \cdot 10^{17}$cm^{-2})	–
	nanocrystalline anatase + brookite	60	–	0	–	–
	anatase + brookite	60	–	–2.5	–	–
	rutile	180	–	–2.5	P^+(30 keV, 5×10^{15}cm^{-2})	900
Ti-based layers	$TiO_{2-x}N_x$ (rutile)+TiO	135	45	–2.5	–	–
	TiO+TiN	90	90	–2.5	–	–
	$TiN_{1-x}O_x$	45	135	–2.5	–	–
	TiN	–	180	–2.5	–	–
	α-Ti	–	–	–2.5	–	–

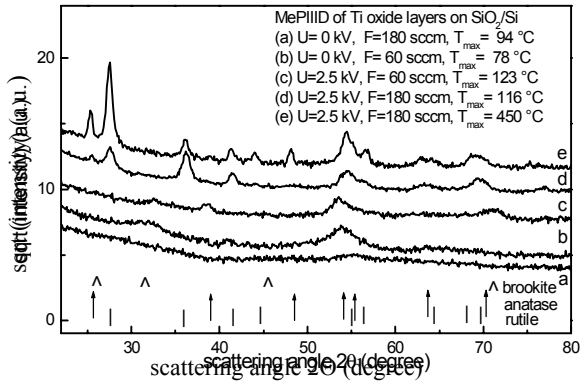

Fig. 1. XRD pattern of Ti-oxide layers deposited with different parameters

Enhanced substrate temperature leads to better crystallization of the oxide layer. This can be seen by the sharper reflections in the XRD patterns comparing depositions at $T_{max} = 450$ °C and $T_{max} = 116$ °C using $U = 2.5$ kV and $F = 180$ sccm (Fig. 1(d), (e)). The phase composition is nearly independent of the temperature. Amorphous and nanocrystalline layers have a lower roughness (~ 5 nm) compared to crystalline Ti-oxide layers (~ 35 nm).

By Ti-based layers without gas flow crystalline film composed of the hexagonal α-Ti phase is formed (Fig. 2(a)). For the nitrogen flow without oxygen the XRD patterns are typical for a titanium nitride TiN with a face centered cubic lattice (Fig. 2(b)). For the lower O_2 flow compared to the N_2 flow with a relation

of the gases partial pressures $p(N_2)/p(O_2) = 3/1$ the TiN peaks shift to higher angles (Fig. 2(c)) indicating the lattice shrinking. For the equal partial pressure of N_2 and O_2 the broad diffraction peaks are observed, which can be interpreted as due to a phase mixture of fcc TiN and fcc TiO (Fig. 2(d)). Enhanced O_2 flow leads to the beginning of the formation of rutile and anatase at the substrate surface (compare Figs. 2 (e) and (*f*).

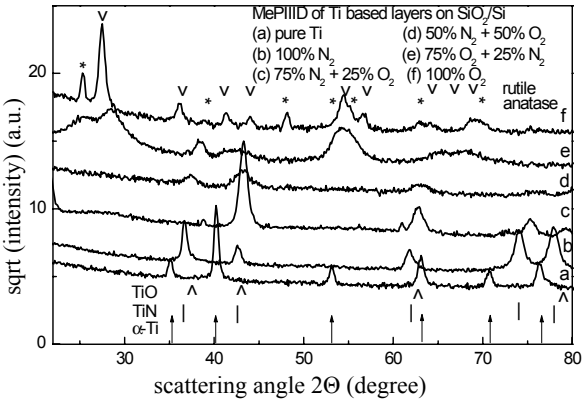

Fig. 2. XRD pattern of Ti-based layers deposited with different partial pressures of N_2 and O_2: (a) without *F*; (b) only N_2; (c) $p(N_2)/p(O_2) = 3/1$; (d) $p(N_2)/p(O_2) = 1/1$; (e) $p(N_2)/p(O_2) = = 1/3$; (f) only O_2, see Fig. 1(e)

The differences in clotting time were statistically not significant. However, as a trend the maximum clotting time, i.e. the best blood compatibility at this aspect, has been found for Ti oxides by the P^+- doped rutile (II-P) and the Cr^+-implanted amorphous TiO_2 layer (II-Cr) (Fig. 3, a). The well crystallized samples dominated by the rutile structure show the lowest clotting time, i.e. highest activation of the clotting cascade. The behavior of the amorphous and nanocrystalline samples is in between.

The clotting time on Ti, TiN and oxynitrides is higher compared to rutile (Fig. 3, b).

The trends of platelet adhesion are contrary to the behavior of the clotting time except for P-doped rutile (Fig. 4, a, b). P-implantation in rutile results in reduced platelet adherence compared with the unimplanted rutile layer. Cr-implantation in TiO_2 increases the platelet adherence significantly over the P-implanted rutile. The same trends with smaller differences were seen for the activation marker CD62P on the platelets (not shown).

The properties of Ti-oxide surfaces can change by various influences after the deposition. Substoichiometric Ti oxides quickly oxidize in air. Organic compounds inevitably adsorb from air on the surface and also cannot be removed by the applied cleaning procedure with acetone, ethanol and water.

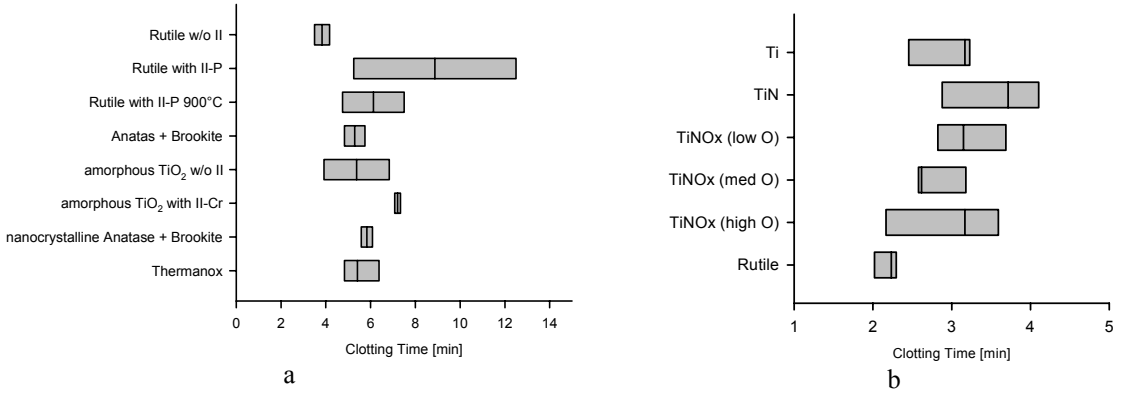

Fig. 3. Clotting time (median and quartils of the blood clotting time on the test surfaces): a) Ti-oxide layers; b) Ti-based layers

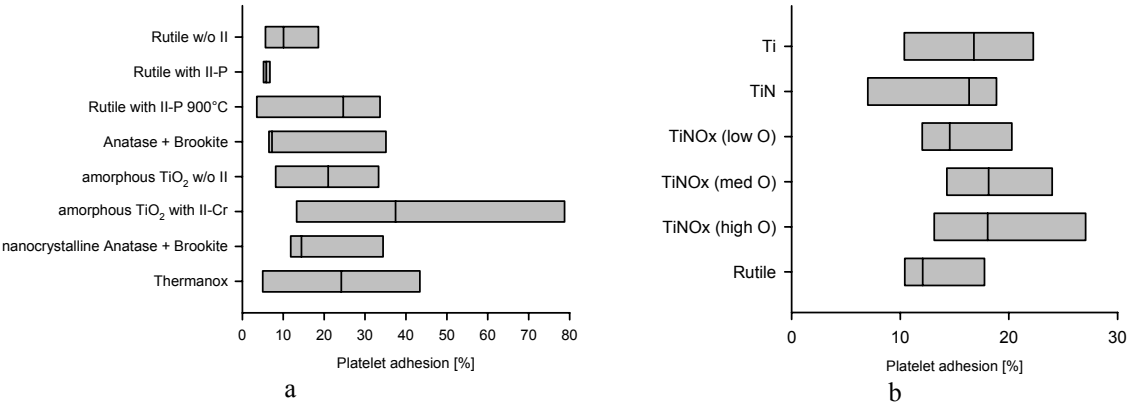

Fig. 4. Platelet adhesion (bars indicate median and quartils of the percent adherent platelets from a sample platelet-rich lasma on the test surfaces): a) Ti-oxide layers; b) Ti-based layers

The biological effects therefore are also influenced by these substances. The biological testing was performed within one month after the deposition to keep these influences low. Compared to the oxidation of a mechanically polished and pre-cleaned sample, the film deposition in this study produces the better-defined surface.

UV light induces long-term changes of the surface, including the formation of hydroxygroups on the surface, increased hydrophilicity and even the formation of free reactive oxygen species. These surface changes go parallel with an enhanced calcium phosphate precipitation from a saturated solution, and calcium adsorption also influences the adsorption of proteins. The samples in this study therefore were stored and handled without exposure to direct sun light.

The different behavior of clotting time and platelet adhesion can be understood taking into account, that these two hemostatic processes also *in vivo* have different initiators, i.e. the clotting cascade is activated mainly on the intrinsic pathway via pre-kallikrein and factor XII activation, whereas thrombocytes mainly are activated on a surface via adsorbed proteins like fibrinogen and von Willebrand factor. Only at a more downstream step, there are various crosstalks between the two systems via the fibrin monomers, which activate blood platelets, and by factors released from the platelets, like the platelet factors 3, 4 and the plasminogen activator inhibitor.

4. Summary and Conclusions

Metal plasma immersion ion implantation and deposition provides a useful technique to control composition and structure of titanium-based films. In dependence on the deposition parameters amorphous and nanocrystalline structures, crystalline layers composed of anatase and brookite, layers dominated by the rutile phase, as well as different titanium oxynitrides, have been produced.

The biological results of this study should not be over-interpreted, but seen as rough trends. The crystal structure and crystallite size of Ti-oxide films appear to influence the activation of the plasmatic clotting system only to a minor degree. Implantation of P or Cr into Ti oxides reduced the clot forming property of the surface.

The microstructure of the Ti-based layers showed an opposite effect on platelet adherence and activation of the clotting cascade. However, P^+-doped rutile showed an improved behavior in both cases. A possible explanation for the action of P may be that P-doping hinders the electron exchange between proteins and the modified surface. However, P and its oxides in the surface my also act as biochemical functional groups with beneficial effects [9].

The roughness of the layers below 50 m appears to be less important, as the layers with low roughness showed no exceptional behavior. However, a very smooth surface was only found for the amorphous and nanocrystalline layers, so that an independent effect of the roughness could not be verified.

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