JOURNAL OF MATERIALS SCIENCE **36** (2001) 2067–2072

In Vivo and *In Vitro* investigation of titanium oxide layers coated on LTI-carbon by IBED

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A layer of titanium oxide layer was coated on low temperature isotropic pyrolytic carbon (LTI-carbon), a prevailing material used for artificial heart valves' fabrication, by ion beam enhanced deposition (IBED). Glancing angle x-ray diffraction (GAXRD), X-ray photoelectron spectroscopy (XPS), Rutherford backscattering spectroscopy (RBS), atomic force microscopy (AFM) and transmission electronic microscopy (TEM) were used to characterize the deposited titanium oxide layer. The results show that the layer is polycrystalline with TiO, Ti_2O_3 and TiO_2 coexisting and the root-mean-square (RMS) roughness of the surface is measured to be 8.7 nm. Platelet adhesion experiments show that the adherent platelet on titanium oxide layer is about four times less than that on LTI-carbon. *In vivo* investigation was performed by implanting LTI-carbon and a titanium oxide layer coated LTI-carbon into the femoral artery of a dog for 4 weeks. By means of scan electron microscopy, coagulation, fibrin, deformed blood red cells and aggregation of adherent platelet were found on the surface of the uncoated LTI-carbon, whereas, nothing but a few normal-shaped blood red cells were found on the titanium oxide coated LTI-carbon. It can be concluded that titanium oxide coated LTI-carbon has a much better blood compatibility than that of the LTI-carbon.

1. Introduction

Low temperature isotropic pyrolytic carbon (LTI-carbon) is a prevailing material used for mechanical heart valves' fabrication because of its good combination of blood compatibility and high resistance to degradation, wear and fatigue [1]. However, so good hemocompatibility as the LTI-carbon is, it is still far from satisfactory. The patients who have mechanical heart valves in their bodies have to take anticoagulant lifetimely to prevent thrombogenicity. Long-term anticoagulant taking may have latent risk to the patients, especially to pregnant women. Therefore, people kept on finding a certain kind of biomaterial that excels LTI-carbon in blood compatibility. In order to reserve the good mechanical property of LTI-carbon, not bulk material but a thin solid film coated on LTI-carbon is considered to be a good way to improve the blood compatibility of mechanical heart valves. Among all kinds of materials, our interests focused on titanium oxide because of its good hemocompatibility and biocompatibility [2, 3].

Ion beam enhanced deposition (IBED) is a good way for surface layer preparation because the chemical composition and structure of the prepared layer can be easily controlled via adjusting the process parameters. In addition, the prepared layer not only dense, but also adhered to the substrate tightly. A series of titanium oxide layers with different composition and structure have been synthesized by IBED in our laboratory [5, 4], and the related behavior in blood compatibility were investigated through *in vitro* experiments. From these experimental results, it is found that the titanium oxide layer with (100)-preferred orientation exhibits a better hemocompatibility than that of LTI-carbon [6–8].

In this paper, titanium oxide layer was deposited on LTI-carbon substrate, the composition and structure of the prepared layer is analyzed and *in vivo* investigation was performed and discussed in detail.

2. Experimental

The titanium oxide layers were deposited onto LTI-carbon substrate, whose surface finish beyond ∇10, by ion beam enhanced deposition (IBED). The electron beam evaporation of Ti was fixed at a rate of 0.4 nm/s in an oxygen atmosphere. A beam of energetic xenon ion was used to bombard the growing titanium oxide layer

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with the energy of 40 keV, simultaneously. The angle of incidence of titanium vapor stream and xenon ion to the substrate were 45°, respectively. The current density of ion beam was 40 mA/cm^2. The base pressure of the target chamber was 9×10^{-5} Pa and 8.2×10^{-3}Pa when backfilled with oxygen to grow the titanium oxide layer. Prior to deposition, the substrates were cleaned by bombard with 20 keV Xe ions for the dose of 2×10^{15} ions/cm^2. The thickness of the prepared layer was about 1.2 mm.

The chemical states of titanium, oxygen and carbon on the surface and near-surface of the synthesized titanium oxide layers were studied by X-ray photoelectron spectroscopy (XPS). The spectra were curve-fitted using a computer assisted Gaussian-Lorentzian peak model. The binding energy of the C 1s line was taken as 284.6 ± 0.4 eV for calibrating the obtained spectra. Rutherford backscattering spectroscopy (RBS) was performed using a 2 MeV He^+ ion beam at a scattering angle of 165° to investigate the ratio of O/Ti of the synthesized titanium oxide layer. The structure of the layer was investigated by Glancing angle X-ray diffraction (GAXRD) with the glancing angle of 2°, as well as TEM. AFM was used to observe the surface morphology of the synthesized titanium oxide layer and LTI-carbon.

In platelet adhesion experiment, the ACD-blood that taken from a healthy volunteer was centrifuged at 100 g for 10 minutes to obtain a platelet-rich plasma (PRP). The samples were merged into the PRP and incubated in 37°C aqueous thermostat for 1 hour. Subsequently, the samples were rinsed in 0.9% NaCl solution. After fixing and critical point drying, the specimens were observed by scan electron microscopy (SEM) to investigate the morphology of the adhered platelet. The quantity of the platelets that appeared in a certain vision field (the magnification was fixed at 500×) was counted. For each sample, 10 such vision fields were randomly selected to obtain a quantity statistic of the adhered platelets.

In vivo investigation was performed by implanting the titanium oxide coated LTI-carbon and the uncoated LTI-carbon into the left and right femoral artery of a same dog. No medicine was provided to these dogs after surgeon. 4 weeks later, these samples were taken out carefully and steeped in 2.5% glutaraldehyde solution for 24 hours. After dehydration, clinic point drying and Au deposition, these samples were observed under scan electron microscope.

3. Results

O/Ti ratio of the prepared titanium oxide layer calculated from RBS data equals to 1.85. It suggests that the layer is lack of oxygen and cannot be formed by pure titanium dioxide phase. Low-valent titanium oxide phases such as titanium monoxide and titanium sesquoxide must coexist with titanium dioxide phase. XPS spectra agree with the RBS result. Fig. 1a is the XPS spectrum of Ti 2p from surface of the titanium oxide layer deposited on LTI-carbon and Fig. 1b shows the high resolution spectrum of Ti 2P of the titanium oxide layer after sputtering for 10 minute. From this figure it can be seen that having exposed to the air for some

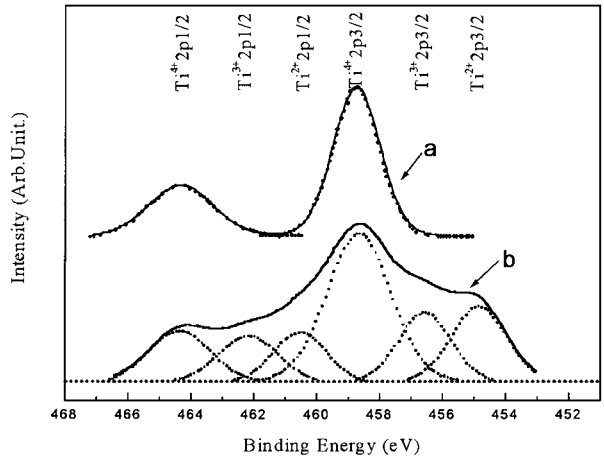


Figure 1 Ti 2p XPS spectrum of titanium oxide layer on LTI-carbon wafer (a) on the surface of the layer (b) after 10 min of argon ion sputtering.

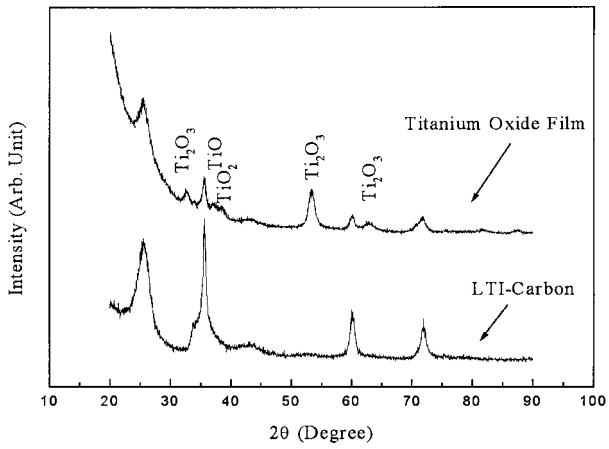
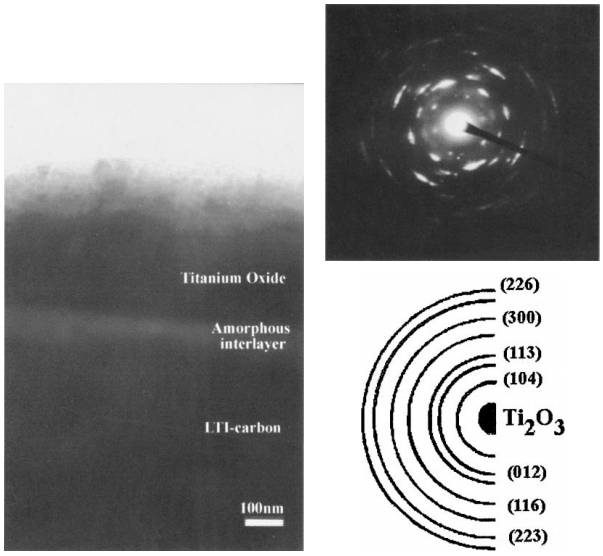


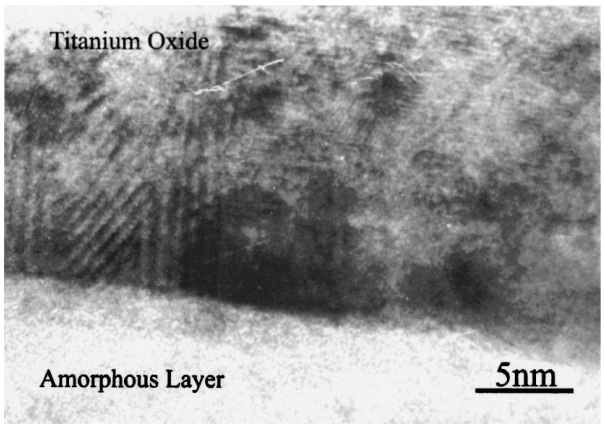
Figure 2 GAXRD patterns of titanium oxide layer.

time, the surface of the prepared titanium oxide layer is naturally oxidized to form a TiO_2 layer. After 10 minutes of argon ion sputtering, this natural oxidized layer was etched, and the spectrum shows to be dominated by a peak at ~458.9 eV which is assigned to the Ti^{4+} in TiO_2. There are also other two peaks located according to the deconvolution procedure at ~456.9 eV and ~455 eV, which is Ti^{3+} state and Ti^{2+} state in the Ti_2O_3 and TiO respectively. The peak separation between Ti 2p1/2 Ti 2p3/2 is about 5.7 ± 0.1 eV. The XPS spectrum indicates that TiO_2, Ti_2O_3 and TiO are coexisted in this titanium oxide layer.

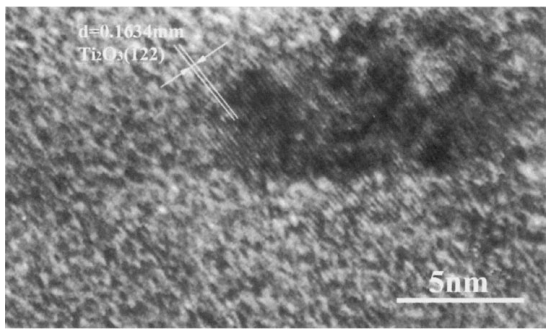
Fig. 2 shows the glancing angle diffraction patterns of titanium oxide layer coated LTI-carbon and uncoated LTI-carbon. From this figure it can be seen that the prepared layer is polycrystalline without obviously preferred orientation. This is because that LTI-carbon is a kind of amorphous structure. In general, the layer grown on amorphous substrate is easily grown to be polycrystalline structure with obscured orientation [9]. This result is good agreement with TEM result. Fig. 3a is the cross-section TEM image of the sample, it shows that a "titanium oxide/amorphous transitional layer\LTI-carbon substrate" layered structure is formed. By electronic spectroscopy analysis, it is found that this amorphous transitional layer which is caused by Xe^+ ion beam bombarding is attributed to titanium oxide, and



(a) (b)



(c)



(d)

Figure 3 TEM images of titanium oxide layer coated on LTI-carbon (a) cross-section TEM image (b) electron diffraction pattern (c) Morié fringe (d) Ti_2O_3 lattice fringe.

the layer is about 40–100 nm in width with rough borders. The selected area electronic diffraction pattern and its schematic diagram that taken from the titanium oxide layer shown in Fig. 3b exhibit a polycrystalline ring. It shows that the layer is dominated by Ti_2O_3 polycrystalline phase. From cross-section high-resolution electron microscopy (CHREM) images shown in Fig. 3c and d, the lattice image of (122) plane of a Ti_2O_3 crystal is clearly seen in addition to the moiré fringes.

Fig. 4 is the three-dimensional AFM images of titanium oxide layer coated on LTI-carbon. The layer is dense and the RMS roughness of the deposited layer is about 8.7 nm.

TABLE I Experimental conditions of titanium oxide layers' synthesis

	O_2 pressure (Pa)	Ion beam energy (keV)	Preheated temperature (°C)	Evaporation rate of Ti (nm/sec)	Current density (μA/cm^2)
Layer 1	8.26×10^{-4}	80	200	0.4	40
Layer 2	1.5×10^{-3}	80	200	0.4	40
Layer 3	8.6×10^{-4}	40	200	0.4	40
Layer 4	1.5×10^{-3}	40	200	0.4	40
Layer 5	8.26×10^{-4}	40	Room temperature	0.4	40

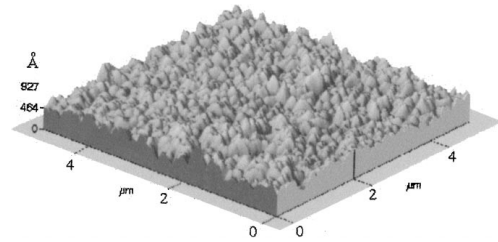


Figure 4 Surface morphology of titanium oxide layer coated on LTI-carbon.

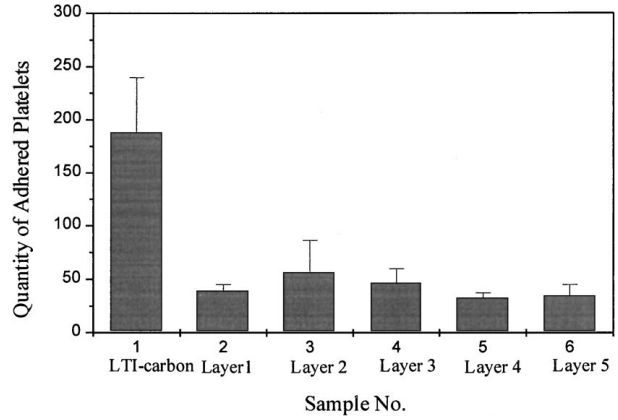
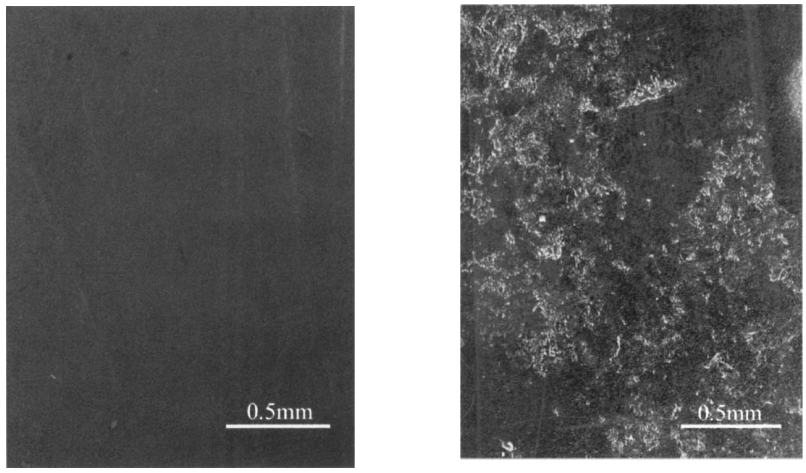


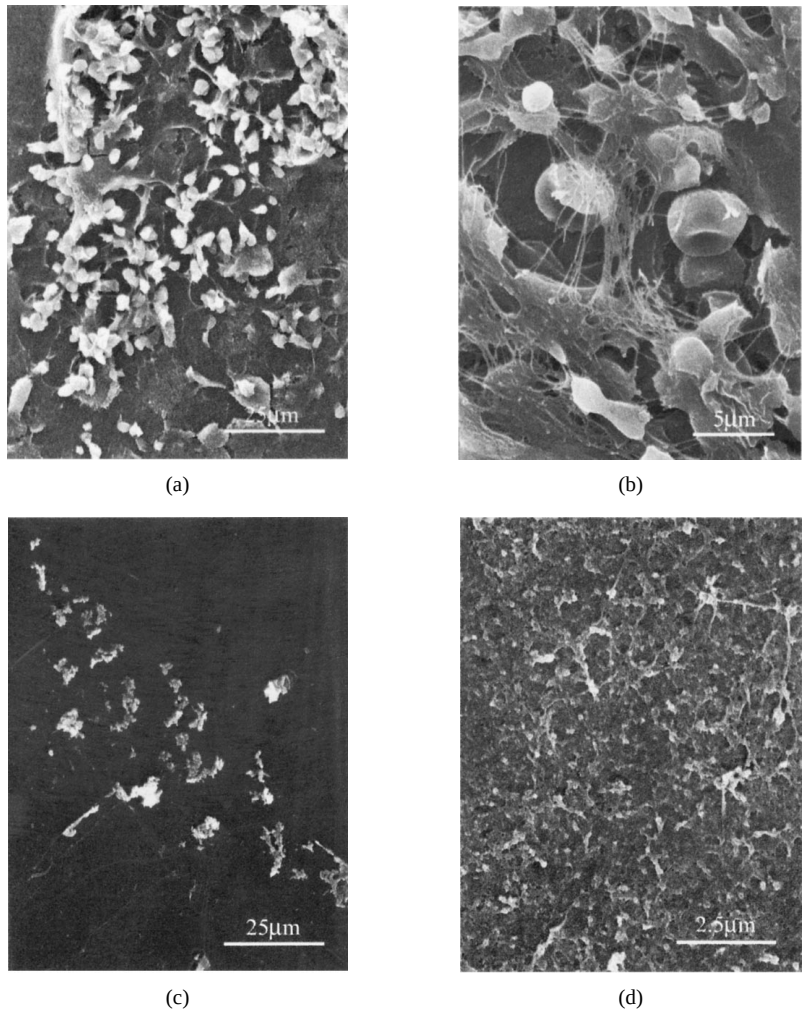
Figure 5 Illustration of the quantity of the adherent platelet on LTI-carbon and titanium oxide layers prepared at different IBED parameters.

In platelet adhesion experiment, the statistic of the adherent platelet on LTI-carbon and titanium oxide fi1ms that prepared at different IBED parameters is illustrated in Fig. 5, and the corresponding parameters are listed in Tab1e I. It shows that the quantity of the adherent platelet on LTI-carbon is almost 4 times as many as that on titanium oxide layers, whereas no significant difference exists among titanium oxide layers that synthesized at different process parameters. This suggests that titanium oxide layer has a better blood compatibility than that of LTI-carbon. *In vivo* experiment gives the same result. When the samples were taken out from the femoral artery of the dogs, no thrombus is found on both titanium oxide layer coated LTI-carbon and uncoated LTI-carbon by naked eye, which indicates that both of them have good blood compatibility. By means of scan electron microscope, magnificant difference between them is found. First of all, when observed under a magnification of 50, it is found that the quantity of the adherent stuff on the uncoated LTI-carbon is much more than that on the coated LTI-carbon, just as Fig. 6



(a) (b)

Figure 6 Surface appearance of the samples buried in the femoral artery of a dog for 1 month (a) LTI-carbon (b) titanium oxide layer coated LTI-carbon.



(a) (b)

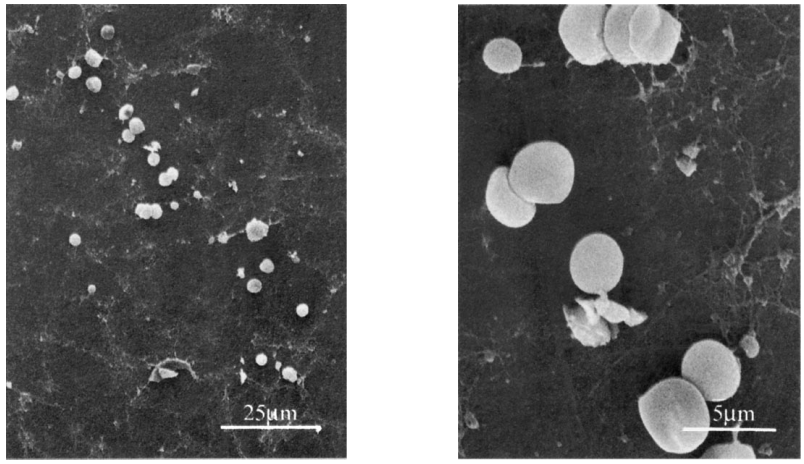
(c) (d)

Figure 7 Adhered blood red cells (a), formed fibrin (b), aggregated platelets (c), and thrombus (d) are found on the surface of LTI-carbon.

shows. Subsequently, the samples were observed under a high magnification. Fig. 7 displays the photos taken from the surface of LTI-carbon. It is found that a large amount of blood red cells adhered on LTI-carbon, most of them deformed seriously (Fig. 7a). Simultaneously, a large amount of fibrin has already formed on the surface (Fig. 7b). In addition, some aggregated and deformed platelets are found on LTI-carbon (Fig. 7c). All of these imply that coagulation was occurred on LTI-carbon. In fact, we did found thrombus on it, just as Fig. 7d shows. However, on the surface of titanium oxide coated LTI-carbon, neither platelet nor fibrin is found but only a few normal-shaped blood red cells, as shown in Fig. 8. This indicates that the blood compatibility of titanium oxide coated LTI-carbon is much better than that of uncoated LTI-carbon.

4. Discussion

The formation of thrombosis on an artificial material is correlative with charge transfer from the inactive state of the protein (e.g., fibrinogen) to the surface of the material. During the process, fibrinogen is oxidized and



(a) (b)

Figure 8 The adhered red blood cells on titanium oxide layer is very little (a) and remains a normal shape (b).

transforms to fibrinmonomer, then cross-links to the irreversible thrombus. Titanium oxide layers prepared here are lack of oxygen, and the concentration of Ti^{2+} and Ti^{3+} is very high. This makes the layer exhibit n-type semiconductor properties. After the adsorption of fibrinogen, it can inhibit or at least, delay the oxidization process of fibrinogen [10] and so that it shows good blood compatibility.

Another reason is related to the surface energy. Surface free energy can be estimated from contact angle (θ). According to Kaelble [11], following equation is established:

$$\frac{\gamma_{LV}(1+\cos\theta)}{2\left(\gamma_{LV}^{d}\right)^{1/2}} = \left(\gamma_s^d\right)^{1/2} + \left(\gamma_s^p\right)^{1/2} \cdot \left(\frac{\gamma_{LV}^{p}}{\gamma_{LV}^{d}}\right)^{1/2}$$

where, γ_{LV}, γ_{LV}^{d}, γ_{LV}^{p} is the surface energy, dispersion component and polar component of the liquid, respectively. γ_s^d, γ_s^p is the dispersion and polar component of the solid, respectively. Several kinds of liquids were separately dropped onto the surface of the titanium oxide layer coated LTI-carbon as well as uncoated LTI-carbon and the contact angles were measured. The measured value of $\frac{\gamma_{LV}(1+\cos\theta)}{2(\gamma_{LV}^{d})^{1/2}}$ and $(\frac{\gamma_{LV}^{p}}{\gamma_{LV}^{d}})^{1/2}$ were plotted and linear fitted, as shown in Fig. 9. From this figure, it can be determined that the titanium oxide coated LTI-carbon exhibit surface energy with dispersion component, $\alpha = 5.17$ (dyne/cm)$^{1/2}$ and polar component, $\beta = 2.55$ (dyne/cm)$^{1/2}$. The surface energy of LTI-carbon is measured to be $\alpha = 5.49$ (dyne/cm)$^{1/2}$ and $\beta = 2.89$ (dyne/cm)$^{1/2}$, a little different from the reported data, which is 7.38 and 2.04 respectively [12]. Accord to Kaelble and Moacanin, dispersion $\alpha = (\gamma_s^d)^{1/2}$ and polar $\beta = (\gamma_s^p)^{1/2}$ components of surface play an important role in interfacial interaction [13]. They have indicated that low dispersion high polar surface, such as surface treated stellite 21 with $\alpha =$ 5 (dyne/cm)$^{1/2}$ and $\beta = 5$ (dyne/cm)$^{1/2}$, appears to favor weak adsorption and retention of plasma proteins. Both LTI-carbon and titanium oxide layers have relatively high dispersion and low polar surface energy, which made both of them have good blood compatibility. However, the surface energy of titanium oxide is smaller than that of LTI-carbon, therefore, LTI-carbon tends to adsorb more proteins. HSA and HFG are two principal proteins in blood plasma. Through the investigation of the competitive adsorption in the HSA/HFG binary system, it is found that less HFG is adsorbed on the surface of titanium oxide layers in compartion to that on LTI-carbon [14]. It suggests that coagulation is easier to occur on the surface of LTI-carbon when contact blood, so titanium oxide layer exhibits a better blood compatibility.

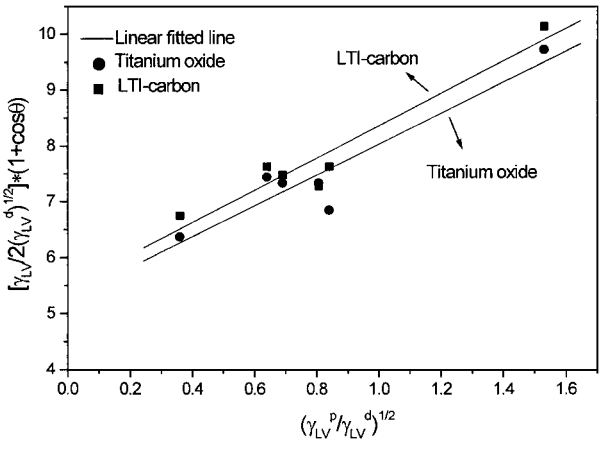


Figure 9 Surface energy measurement results of LTI-carbon and titanium oxide layer coated on LTI-carbon.

5. Summary

The titanium oxide layers deposited on LTI-carbon by IBED is polycrystalline with TiO_2, Ti_2O_3 and TiO coexist. The layer is dense with a RMS roughness of 8.7 nm.

Platelet adhesion experiment results show that the amount of the adherent platelet on LTI-carbon is about 4 times as many as that on titanium oxide layer. The difference among titanium oxide layers that prepared at different experimental conditions is not significant. *In vivo* investigation results show that the blood compatibility of titanium oxide layer coated LTI-carbon is much better than that of the uncoated LTI-Carbon, the reason is attributed to the lower surface energy of the synthesized layer.

Acknowledgement

This work is financially supported by Shanghai Science Fund and <973> program.

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Received 26 August 1999
and accepted 3 August 2000