Scanning Probe Microscopy for analysis of composite Ti/hydrocarbon plasma polymer thin films

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Composite Ti/hydrocarbon plasma polymer films were deposited on silicon by dc magnetron sputtering of titanium in an atmosphere of argon and hexane. Different titanium concentration was obtained by careful adjustment of Ar/hexane ratio. With increasing Ti content, the surface potential and hardness increase as measured by Kelvin Force Microscopy and Visco-Elastic Atomic Force Microscopy, respectively. The adhesion force against silicon increased with increasing the titanium concentration as it was evaluated from the AFM force-distance curves. Fibrinogen adsorbs on the Ti/hydrocarbon plasma polymer films as a very soft layer with fibrinogen molecules adhering stronger to the Ti-rich films. Furthermore, the increase of the surface charge due to fibrinogen adsorption has been observed.

1. Introduction

Titanium is a well known material widely used in various industrial applications. In addition, titanium is recognized as being of great importance in medicine and biotechnologies since it combines outstanding mechanical properties and biocompatibility. Ti-based materials have been clinically used in orthopedic and dental surgery fields and for production and performance improvement of various surgical instruments [1, 2].

The benefits of composite systems arise from the ability of tuning their properties by adjusting the concentration of the constituents. Composite metal (oxide)/plasma polymer films have been studied in detail with a focus on their mechanical, physical and chemical properties[3].

The performance of implants in contact with blood depends on their ability to prevent thrombus formation. It is generally recognized that biological reactions between a tissue and artificial implants are mediated by protein adsorption. Fibrinogen is a blood plasma protein playing an important role in surface induced blood clot formation (thrombosis) through the polymerization to fibrin. Hence, a research on interaction of fibrinogen with materials intended as implants is relevant.

Here, composite Ti/hydrocarbon plasma polymer (Ti/ppCH) films are studied by Scanning Probe Microscopy (SPM) with a particular interest addressed to their interaction with fibrinogen.

2. Experimental

An unbalanced magnetron equipped with Ti target was mounted in a deposition chamber 5 cm above the substrate-holder. Argon, hexane and their mixtures were used as a working gas. The depositions were performed at a working pressure of 2 Pa under a constant direct current of 0.2 A. The total flow rate of working gas was kept constant at 17 sccm.

Human plasma fibrinogen was purchased from Sigma, Inc and stored at -20° until use. Fibrinogen was dissolved by layering it on top of Dulbecco's Phosphate Buffer Saline (PBS) at room temperature. The solution was gently stirred for 30 minutes prior to the sample incubation.

The Ti/ppCH films were imaged in air at a relative humidity of 35-40% by an SPM (SPA-300HV+SPI-3800N, Seiko Instruments Inc) in Dynamic Force (DFM), Kelvin Force (KFM) and Visco-Elastic Atomic Force (VE-AFM) modes. The topography measurements were performed in the DFM mode analogous to the tapping mode. The KFM measurements were conducted by using a gold-coated silicon cantilever with a spring constant of 1.8 N/m. An alternating current voltage of 5 V was applied between the probe and sample. The VE-AFM measurements were performed in such a manner that, along with two-dimensional scanning, the vertical position of sample being in contact with cantilever was sinusoidally modulated with an amplitude of 5 nm and frequency of 5 kHz. This results in an indentation of the tip against the sample which depends on elasticity of the latter. The softer areas are indented deeper and induce less cantilever deflection. Therefore, the lower and higher cantilever amplitude measured by a position sensitive detector is indicative of softer and harder areas, respectively.

The adhesion forces between the Ti/ppCH films and silicon were assessed by taking the force-distance curves in air and in vacuum. For this purpose, the cantilevers with the silicon tips were consistently cleaned with acetone, ethanol and

water, and dried under gentle nitrogen flow. Furthermore, a series of experiments was done with fibrinogen covered tips. Fibrinogen was unspecifically adsorbed by incubating the clean silicon cantilever in solution of fibrinogen in PBS. Multiple rinsing with PBS and water was applied thereafter to remove an excess of the protein.

3. Results and discussion

Sputtering in pure Ar produces the films with 20% of Ti, 9% of C, 11% of O and 58% of H, as measured by RBS/ERDA. Titanium readily binds gases, hydrogen in particular. Even a small hydrocarbon contamination in the deposition chamber results in significant increase of atomic hydrogen concentration in the plasma, and explains such a high hydrogen concentration. The film sputtered in pure hexane contains 1% of Ti, 48% of C, 3% of O and 48% of H. Sputtering in an Ar/hexane mixture produces the films resembling those sputtered in pure hexane, with a slightly increased concentration of titanium.

The topography of Ti/ppCH scanned by the AFM in conventional DFM mode is shown in Fig. 1. The RMS roughness of the films is about 1 nm (1x1 μm scan size) and it does not change with Ti concentration.

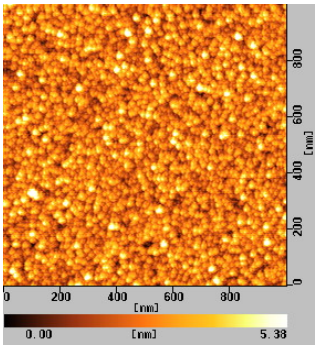

Fig. 1. The topography of the 20% Ti/ppCH film.

Figure 2 demonstrates the 5x5 μm topography images as well as the related potential (KFM) and elasticity (VE-AFM) images of the films with 20% (the left column) and 3% of Ti (the right column). The scans were acquired over the edge of the films deposited on silicon substrate so that the left part of the images corresponds to the film and the right part corresponds to silicon substrate. The topography and potential images were taken simultaneously, whereas the elasticity images were acquired over the same spot immediately thereafter. The topography pictures appear identical for both of the films (Fig. 2a, b). However, a significant difference between them can be observed in the KFM mode. The surface potential of silicon is more negative with respect to the Ti-rich film (Fig. 2c), whereas it becomes more positive with respect to the film with low (Fig. 2d, Table 1) or none (not shown) concentration of Ti. Hence, the increase of titanium concentration does not influence the surface roughness but has a significant impact on the net dipole moment of the composite films.

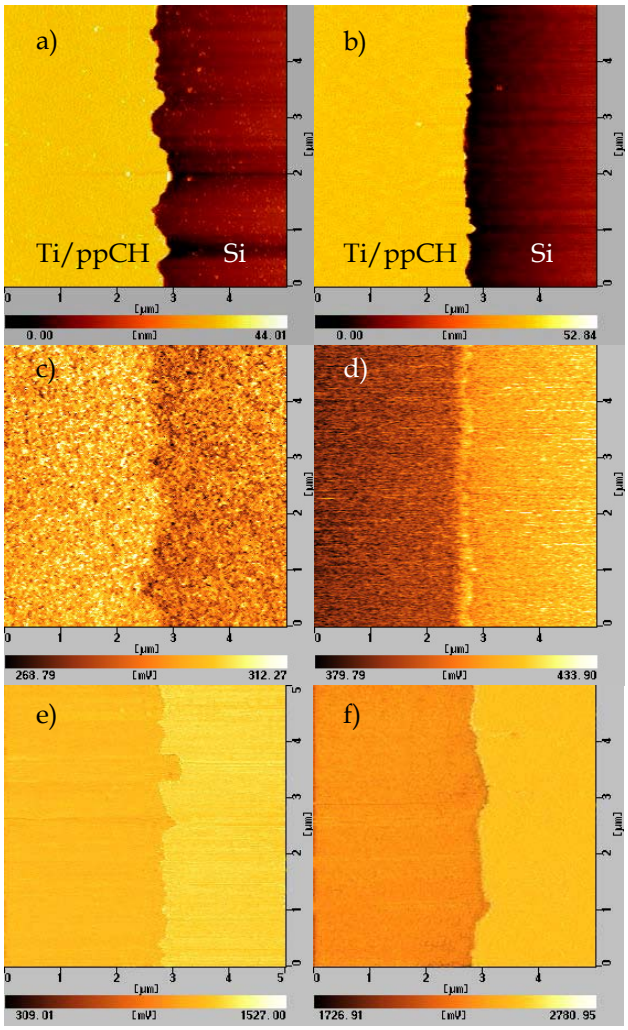

Fig. 2. The topography (a, b), potential (c, d) and elasticity (e, f) images of the Ti/ppCH films with 20% of Ti (the left column) and 3% of Ti (the right column).

Table 1 Potential, elasticity and adhesion force of the Ti/ppCH films against silicon.

Ti/ppCH	KFM V_{Si}-V_{film}, mV	VE-AFM V_{Si}-V_{film}, mV	F_{adh}, mN/m
3% Ti	+20	-140	40
20% Ti	-10	-90	120

The VE-AFM images of the same films (Fig. 2 e, f) show that amplitude of the tip in contact with both composites is lower than on underlying silicon

substrate meaning they are softer. Furthermore, the elasticity scan of the 3% Ti/ppCH film shows higher contrast with respect to silicon than that of the 20% Ti/ppCH film i. e. the absolute difference of tip amplitudes between the film and the Si substrate decreases from 140 to 90 mV with increasing Ti content. Thus, as expected the films with higher Ti content are harder.

The adhesion force of the Ti/ppCH films against silicon was measured as a pull-off force of Si tip on the retracting force-distance curve. To avoid an inconsistency between the measurements with different cantilevers the adhesion force was normalized by the tip radius. For comparison, the force-distance curves were measured against clean silicon substrate as well. The adhesion force measured in air increases with Ti content from 40mN/m for the 0 and 3%Ti/ppCH film to 120 mN/m for the 20%Ti/ppCH film (Fig. 3, Table 1).

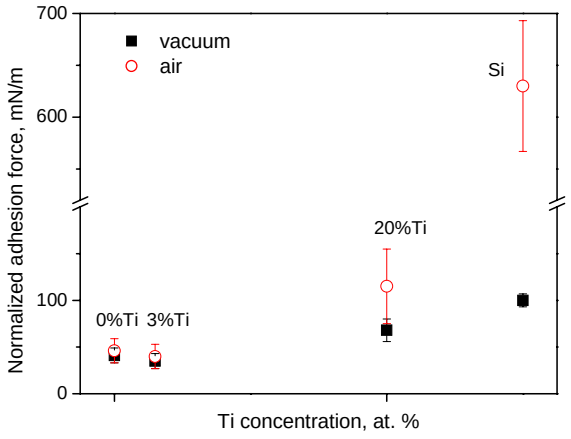

Fig. 3. The adhesion force of the Ti/ppCH films against Si.

The combination Si tip/Si substrate provides the highest adhesion force of 630 mN/m. The water contact angle on clean Si was less then 10°. It increased to 50° for the 20%Ti/ppCH film and reached 90° for the plasma polymer without titanium. Hydrophilic surfaces at humidity >30% contain a microscopic layer of water molecules adsorbed on the surface. Presence of capillary forces is responsible for enhanced adhesion between the 20%Ti/ppCH film and silicon. The measurements performed under $1x10^{-3}$ Pa vacuum allow avoiding the influence of the capillary forces. In this case, the hydrophobic samples deficient with Ti exhibit identical adhesion against silicon, whereas hydrophilic surfaces show significantly lower adhesion force. Nevertheless, the trend of increasing the adhesion with Ti content can be observed.

The interaction of the Ti/ppCH films with biomolecules was studied in terms of their ability to adsorb fibrinogen. The samples were dipped into the fibrinogen PBS solution so that only one half of the sample was immersed, the other half being kept intact. After immersion, the samples were repeatedly rinsed with pure PBS and with Mill-Q water, and blown dry by N_2. The SPM analysis was performed in air at humidity 35-40% immediately after the protein immobilization. Fig. 4 shows the topography, potential and elasticity images of fibrinogen layer adsorbed on the 3% Ti/ppCH film. The left part of the pictures corresponds to the fibrinogen coating, whereas the right part corresponds to the uncovered composite film. Because of short incubation time of 1 minute and concentration of 50 μg/ml which is much lower than the physiological concentration, fibrinogen does not form a continuous layer. The protein strands can be observed, however the individual fibrinogen molecules are hardly to be seen (Fig. 4a). The average thickness of the layer is 6-10 nm which is consistent with previously reported dimensions of fibrinogen molecule [4]. The KFM analysis shows the potential difference of +50 mV between the fibrinogen layer and the film (Fig. 4b). Fibrinogen was also adsorbed on clean Si substrate with potential difference being +30 mV (not shown). This is very well consistent with the above mentioned +20 mV potential difference between Si and the 3%Ti/ppCH film (Table 1). Fibrinogen molecule consists of the central E, two outer D domains and two αC domains extending further from the D-domains. The domains have different hydrophobic/hydrophilic properties and bear different charges. Adsorption of fibrinogen at the solid/liquid interface has a complex mechanism with multiple interaction events. Various conformations of the protein molecules are possible depending on the chemical composition, wettability, charge of the surface etc. In the case of Ti/ppCH film, the adsorption of fibrinogen proceeds with formation of multiaggregates combining to the strands. In this process the individual fibrinogen molecules take the comformations which ensure the positive net charge of fibrinogen layer with respect to both Ti/ppCH and silicon.

The VE-AFM image (Fig. 4c) shows a very strong contrast between the fibrinogen layer and the Ti/ppCH film. The difference between the tip amplitude on the composite film and on the

fibrinogen layer is +200 mV which supports the assumption that the protein layer is much softer.

The adhesion between fibrinogen molecules and Ti/ppCH films was studied by measuring the force-distance curves with fibrinogen modified tips. For this purpose, the cantilevers with silicon tip were cleaned as described above and immersed into the 1 µg/ml solution of fibrinogen in PBS for 2 hours. After the incubation, the cantilevers were carefully rinsed with pure PBS and with Mill-Q water. The force-distance measurements were conducted in air immediately thereafter.

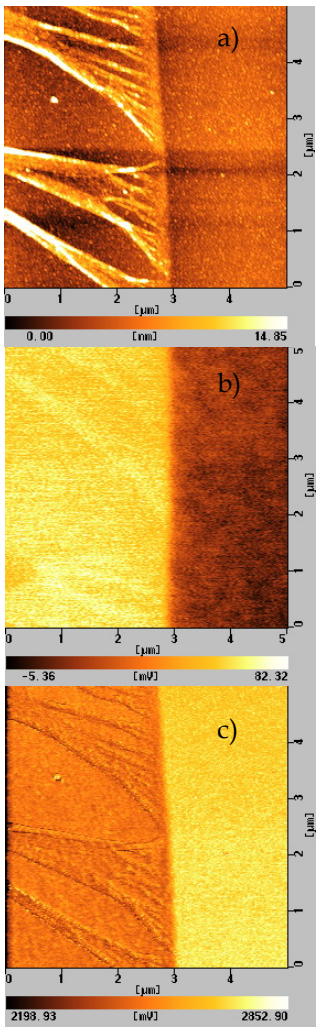

Fig. 4. The topography (a), potential (b) and elasticity (c) images of fibrinogen layer adsorbed on 3%Ti/ppCH film.

Fig. 5 shows the approaching and retracting curves for the Ti/ppCH films with 3 and 20% of titanium. The multiple adhesive jumps observed on both surfaces are typical for the measurements with protein-covered tips [5]. Such a complex interaction of proteins with the surface over a long range is attributed to unfolding of the protein molecules and breaking of the multiple contact points during the withdrawal of the tip. Due to capillary forces the absolute value of adhesion force measured here is significantly higher than that usually measured under aqueous solutions. The larger adhesive jump in the retract trace is observed for the film with 20% of titanium. Hence, adhesion of fibrinogen is stronger for Ti-rich surface.

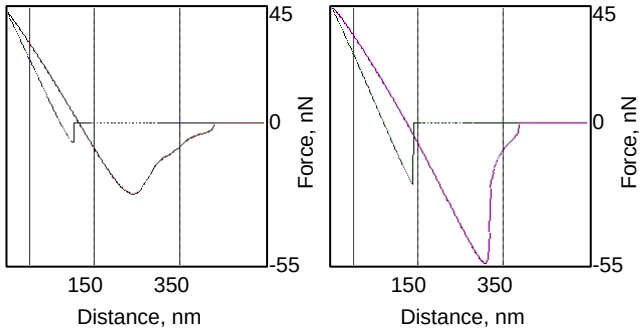

Fig. 5. The force-distance curves of fibrinogen modified tips against: a) the 3%Ti/ppCH film b) the 20%Ti/CH film.

4. Acknowledgement

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5. References

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