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HEMOCOMPATIBILITY OF SEMI-CONDUCTING BIOMATERIALS

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INTRODUCTION: Hemocompatibility of an implant is determined by its surface properties. According to recent suggestions [1], the involvement of electron exchange can occur in adsorption process from physiological media. This electron transfer may induce conformation changes of adsorbed proteins and cause their degeneration. Therefore materials with large band gaps and high work function could be advantageous and the knowledge of the surface electronic properties of biomaterials at the nanometer scale is crucial.

In addition to the physico-chemical characterization, tests to determine the interactions between the modified surface and the blood proteins and platelets are important to perform. For this the measurements of both fibrinogen adsorption and platelets adhesion are important for determining the relative thrombogenic potential of a material. Today, it is proved that synthetic materials adsorbing less fibrinogen from blood plasma do also adhere fewer platelets and thereby exhibit improved blood compatibility. The aim of this project is to characterize the surface electronic properties of different semi-conducting biomaterials using Electrostatic Force Microscopy (EFM) with AFM instrumentation, and to observe by confocal microscopy the platelet adhesion and aggregation from platelet-rich human blood plasma.

METHODS: In EFM [2], the Contact Potential Differences (CPD) are measured at the nanometer scale in an analogous manner to conventional Kelvin Probe Spectroscopy. An electrostatic force is induced between the conductive tip of an AFM and the sample surface by applying an AC bias voltage of frequency ν to the tip (already vibrating at its resonance frequency ν_0). The convoluted signal of the CPD between the sample and the tip and the gradient of the capacitance is obtained from the amplitude at ν, while at 2ν only the gradient of the capacitance is measured. The local work function of different DLC coatings was obtained by performing spectroscopy. The AFM tip was kept stationary on a desired surface point and a DC voltage was applied on either the tip or the sample. At a certain voltage, the amplitude at ν can be reduced to zero. This DC voltage corresponds to the difference of work function ($\Delta\phi$) between the sample and the tip coated with 10 nm of Pt. A commercial AFM working in controlled atmosphere (Relative humidity = 30% ±5%) was used. DLC coatings with a thickness of ca. 3 µm were deposited on Si wafers using plasma assisted CVD in a mixture of acetylene and argon. Three different types of coatings were studied. The first one is a standard DLC coating co-deposited with the sputtering of Ti. A dense DLC coating was produced using a sample potential of 75 V and a density gradient DLC coating was deposited by varying the sample potential from 25 to 75 V. These two latter coatings were deposited in a metal free procedure.

To observe the platelet adhesion on previously described materials, confocal laser microscopy (CLM) and/or epi-fluorescence measurements will be used. A custom-made set-up was thus implemented on a commercial inverted microscope (Nikon Eclipse TE300) in order to combine CLM, epi-fluorescence and classical optical imaging. The sample is scanned in a confocal arrangement on a servo-controlled x,y,z scanner. Using either a 40* (0.6 NA dry), or a 100* (1.3 NA oil immersion) objective, the lateral resolution (depth discrimination) are, respectively 0.5 µm (5 µm) and 0.3 µm (0.9 µm), as determined from response curves measured on fluorescent micro-spheres. For a high s/n-ratio, the fluorescence signal is detected by an avalanche photo-diode (EG&G) and the image constructed in a PC.

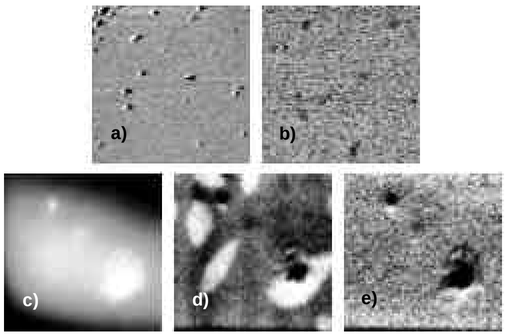

Fig. 1: Convoluted surface potential and capacitance gradient image for a) dense and b) density gradient DLC coating (scan size 2 mm); c) Topography, d) Surface potential and Capacitance gradient, e) Capacitance gradient image of standard DLC coating (scan size 10 mm).

RESULTS: The dense and the density gradient DLC coatings showed smooth topographical AFM images. Homogeneous surface potential (Figure 1a and 1b) and capacitance gradient images were found. The CPD for the different DLC coatings and the reference Si wafer are shown in Table 1. The topography of the standard DLC coating was found to be rougher than the other two. As shown in Figure 1, the surface potential image (1d) exhibited spots of ca. 2-4 μm wide with a different surface potential which was not observed on the capacitance gradient image (1e).

Table 1. CPD of DLC coatings measured by EFM.

Sample	CPD [eV]
Ref. Si wafer	-0.68±0.02 eV
Dense DLC	0.11±0.05 eV
Density Gradient DLC	0.08±0.02 eV
Standard DLC	-0.33±0.02 eV

DISCUSSION & CONCLUSIONS: The metal free coatings show similar topographical and surface electronic properties, although the coating densities are different. The inhomogeneities observed for the standard DLC coating are interpreted as a TiC clustering during the co-deposition of Ti. This is evidenced by traces of Ti detected in the Auger spectra. In conclusion, an EFM was implemented with a resolution less than 40 nm. EFM on standard DLC coating showed surface inhomogeneities (metal clusters), not seen in a metal free deposition process. The CPD of different DLC coatings was determined. By confocal microscopy of platelet-rich blood incubated samples the platelet adsorption on the surfaces will be quantified.

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