PLASMA-POLYMER MODIFICATION OF BASAL PLANE GRAPHITE SURFACES FOR IMPROVED BIOCOMPATIBILITY

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Introduction

There is current interest in producing biological implants from carbon based materials. However, there is very little information about the behaviour of carbon surfaces when exposed to physiological environments or to modifications which might be applied to these surfaces in order to manipulate their interactions with biologically active molecules, such as proteins, or with cells.

In this paper we present preliminary data for the surface modification of the graphite basal plane using a plasma-polymer deposition. We then go on to consider the adsorption interaction of these modified surfaces with serum proteins, of the type found in typical *in-vitro* tissue culture situations. This work is part of our studies to understand the behaviour of a wider range of carbon, and other materials in biological environments.

Experimental

Basal plane graphite has been surface modified by exposure to an acetone plasma in a plasma system described elsewhere [1,2]. The surfaces were treated with a RF power of 10W and acetone flow rate of 10sccm for a duration of 5, 10 and 20min. Chemical patterning of the graphite surfaces has been achieved using the same plasma system in conjunction with a simple masking technique.

The resulting changes in surface structure and chemistry have been characterized using atomic force microscopy (AFM) and X-rays photoelectron spectroscopy (XPS) techniques.

Human serum albumin (HSA) has been adsorbed onto the basal plane surfaces from solutions in the concentration range 0.001 to 1 mg/ml. The surfaces were incubated for 1h at 37°C in contact with the protein solutions at different concentrations and then washed in MilliQ water for 4 hours. One set of samples was dried overnight and another set was dried in a gentle air flow at the temperature of 37°C for only a couple of minutes. After drying, samples were immediately analyzed by AFM technique in tapping mode and under ambient conditions using a Digital Instruments Nanoscope III. XPS

was carried out using a Kratos 5-channel instrument with monochromated $Al_{k\alpha}$ radiation at a base pressure of 10^{-8} Torr and XPSPEAK 4.1 software for peak fitting.

Results and Discussion

The topography of the basal graphitic plane was modified by the acetone plasma. The AFM data shows a RMS roughness change from 0.19-0.26 nm for the untreated basal plane to 0.35-0.45 nm for the acetone plasma treated surfaces (roughness values were determined from a 1μm x 1μm area). The roughness of the deposited film as well as the thickness depends upon the deposition time and the position of the sample into the reactor. The contact angle of the modified surfaces is approximately 70±2°, which is also dependent upon the experimental conditions used. For the protein adsorption experiments only the smoothest and the thinner films were used: deposition time 5 min, sample placed closer to the monomer inlet.

The AFM topographical and phase images presented in Figure 1 clearly show a cleavage step on the graphite. The phase image presents no significant contrast, a uniform modification of the surface might be assumed.

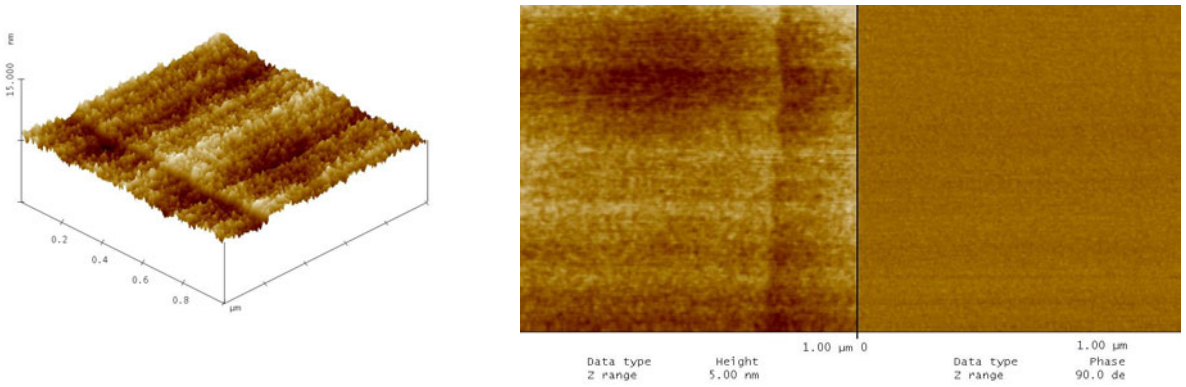

Figure 1.TM-AFM height and phase images of a plasma modified HOPG surface.

As a further development of the plasma-polymer modification of the HOPG surfaces, and for future protein adsorption and cell-attachment experiments, patterning of the graphite basal plane has been carried out using a masking technique. In this approach, freshly cleaved HOPG surfaces were covered with a copper TEM grid (250 mesh, 30μm bar width and 70μm hole width), and then placed in the plasma reactor and oxygen containing plasma species deposited through the mask. SEM images of the resulting patterned surface are shown in Figure 2, the darker squares corresponding to the polymer with a lower secondary electron field than the more conductive graphite. Previous work has already shown that cell attachment can be directed to specific hydrophilic domains on surfaces of this type [1,3].

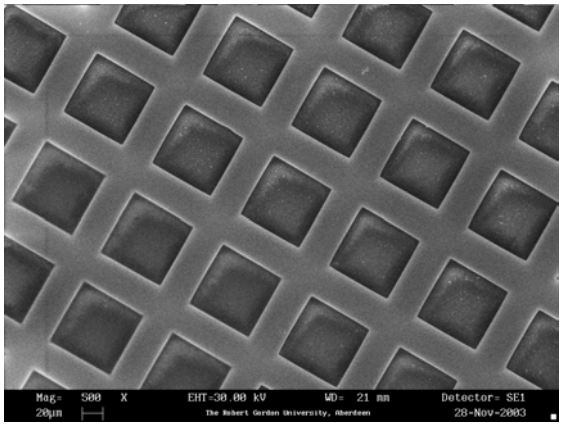

Figure 2. SEM image of a patterned graphite surface (acetone plasma treated for 10min)

XPS survey spectra from freshly cleaved HOPG surfaces show only a single C1s peak. Those from acetone plasma treated surfaces contain carbon 1s and oxygen 1s peaks indicating that oxygen containing species are deposited during plasma exposure and quantitative analysis show that oxygen levels of up to 15 atomic % have been achieved.

The high resolution carbon 1s peak from the HOPG surfaces have a FWHM of 0.55 eV which is consistent with that expected from these surfaces [4]. In contrast, the carbon 1s spectrum from the plasma treated surface is broad with a FWHM of 1.39 as shown in Figure 3, and can be resolved into a series of chemically shifted peaks by constraining the peaks to positions common for the various carbon-oxygen functionalities obtained from conventional polymers. Using this approach a main peak at 285.0 eV attributable to C-C/C-H plus peaks at ~286.5eV assigned to C-O; ~288.0eV- assigned to C=O/O-C-O; and ~288.9 eV assigned to O=C-O are present. Peak widths have been constrained to 1.5eV during fitting [4]. The relative areas as a percentage of the total C1s envelope for the resolved peaks are 82.19% for the C-C/C-H peak, 11.82% for the C-O, 4.30% for the C=O and 1.69% for the –C=O. It should be emphasized that these films are of complex chemistry and the resolved peaks correspond only approximately to the assigned functionalities and represent only an estimate of their abundance.

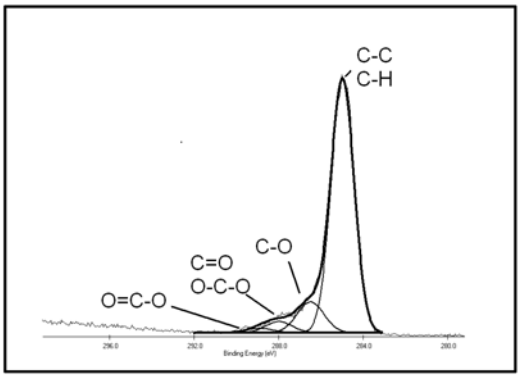

Figure 3. C1s measured for acetone plasma treated graphite surface for 20min.

When protein is adsorbed onto the modified surface the formation of a network with peaks and ridges and cavities between them was observed for the samples dried overnight (Figure 4). The height of the structure observed is in the range 2.5-3nm, with cavities between them 7 and 30nm wide while the ridges are in the range 2-40nm. The width of the cavities decreases and the width of the ridges increases with increasing protein solution concentration while the height of the ridges remain approximately the same, thus the structure appears to become more compact. The structure is similar with that reported on graphite surfaces [6] and on polystyrene surfaces [7], in similar drying conditions. The formation of this type of structure would probably be the result of a few factors such as drying on a hydrophobic surface, protein dehydration and protein-protein interactions.

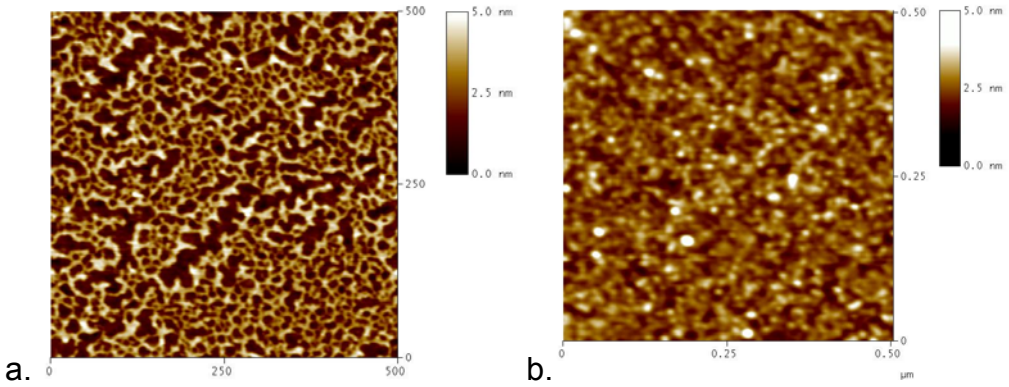

Figure 4. Topographic AFM images of HSA solution of concentration 0.001mg/ml adsorbed onto acetone plasma modified graphite surface: a. Overnight dried; b. few minutes dried

In the case of drying for only a few minutes with warm air flow, the AFM topographic images show the formation of a granular structure with an RMS roughness in the range 0.5-0.7nm, the peaks average height around 1nm. An important contribution in the ‘granular’ structure imaged for the lower protein solution concentration might be expected to be due to the swelling effects that occur in plasma polymers [8].

XPS survey spectra from surfaces which have adsorbed HSA contain a nitrogen 1s peak due to the adsorbed protein in addition to C1s and O1s peaks. Using the nitrogen 1s signals as a semi-quantitative assay for the relative amounts of protein adsorbed by the two surface types it appears that the plasma treated graphite surfaces retain more HSA than the untreated [6]. These results are also in accordance with the AFM images, which present a more compact network structure for the acetone plasma modified graphite than the freshly cleaved HOPG for adsorption from the same protein solution concentration.

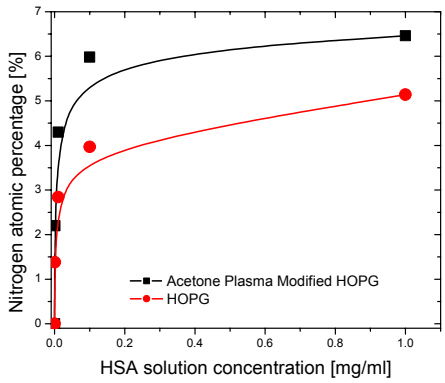

Figure 5. Nitrogen atomic percentage as measured using XPS variation function of the solution concentration

It has been shown that for polystyrene, which in its native form is a non-polar polymer, the surface interaction with HSA appears to be at a maximum and decreases upon surface oxidation i.e. with hydrophilicity [8]. A higher adsorption on the relatively hydrophobic graphite than the acetone plasma modified surface where oxygen has been added might be expected. However, the preliminary data presented here indicate that this is not the case.

The difference may be due to several factors. The slightly higher roughness of the acetone plasma modified surfaces was observed, thus an increased surface area available for adsorption would be expected. At the same time, the increase in oxygen functionality on the modified surfaces compared with the graphite may also be a factor since the data presented show an increased/preferred protein adsorption along the cleavage steps on the HOPG surfaces where polar sites might be expected [6].

Conclusions/Summary

Plasma-polymer modification of the graphite basal plane has been achieved.
AFM data show various types of protein structure after adsorption; these appear to be dependant upon the drying conditions used.
Considering the relative amounts of protein adsorbed by the surfaces studied, it appears that the plasma treated graphite surfaces retain more HSA than the untreated.

References

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