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Protein arrangement on modified diamond-like carbon surfaces – An ARXPS study

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ABSTRACT

Understanding the nature of the interface between a biomaterial implant and the biological fluid is an essential step towards creating improved implant materials. This study examined a diamond-like carbon coating biomaterial, the surface energy of which was modified by Ar^+ ion sputtering and laser graphitisation. The arrangement of proteins was analysed by angle resolved X-ray photoelectron spectroscopy, and the effects of the polar component of surface energy on this arrangement were observed. It was seen that polar groups (such as CN, CO) are more attracted to the coating surface due to the stronger polar interactions. This results in a segregation of these groups to the DLC–protein interface; at increasing take-off angle (further from to DLC–protein interface) fewer of these polar groups are seen. Correspondingly, groups that interact mainly by dispersive forces (CC, CH) were found to increase in intensity as takeoff angle increased, indicating they are segregated away from the DLC–protein interface. The magnitude of the segregation was seen to increase with increasing polar surface energy, this was attributed to an increased net attraction between the solid surface and polar groups at higher polar surface energy (γ_S^p).

1. Introduction

The use of medical implants drives the need to understand the interactions that are taking place at the interface between biological fluid and implant materials. Diamond-like carbon (DLC) coatings are commonly applied as coatings on arterial stents [1], and in this application in particular, the interactions at the interface are of key importance. This is due to adsorption of proteins – a commonplace phenomenon that occurs when an implant material is introduced, and in some cases this can lead to thrombus formation and embolism (formation of blood clots that can block blood vessels and cause tissue death) [2]. Understanding the nature of the interface and the initial protein layer is therefore an important step towards developing superior and safer implant materials.

The adsorption of BSA (bovine serum albumin) onto stainless steel surfaces has been widely studied [3–5], with results showing significant interactions between the protein and the surface. These interactions result in conformational and structural changes of the protein, and even migration of surface species. There have been few XPS (X-ray photoelectron spectroscopy) studies on protein adsorption onto DLC surfaces, however Ahmed et al. [6] found that increased Si doping increases sp^3 content, as well as decreasing adsorption. These results suggest that surface chemistry has a strong effect on both the overall adsorption, as well as the structure of the adsorbed layer. In addition, it is known that upon adsorption, protein molecules are likely to undergo conformational changes and denaturation [7], however what these changes are has not yet been identified for DLC coatings.

The polar component of surface energy appears to be the major contributor to the amount of protein adsorption [7–9]. However, the arrangement of this initial protein layer, and the effects of surface energy on this, has not been comprehensively studied. The lack of information in this area appears to be a common trend in the study of protein adsorption to surfaces, with Silva-Bermudez and Rodil identifying that more work need to be done to correlate the properties of the surface to protein adsorption mechanisms [7]. Studies by Browne et al. and Baio et al. [10,11] of protein adsorption onto polystyrene and calcium phosphate surfaces show that adsorption of protein can be significantly affected by substrate surface chemistry, and it is suggested that the changes seen in adsorption behaviour require a change in conformation of the protein molecule. Feng et al. [8] found that protein adsorption depended mainly on the polar component of surface energy and the hydroxyl groups, but there has been no investigation into

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whether this interaction causes a change in the arrangement of the adsorbed protein. In the present study, the arrangement of different protein functional groups adsorbed onto a DLC surface was investigated, and the effect of the polar surface energy component on this arrangement was analysed.

Studies on protein adsorption on DLCs have also shown that the sp^2/sp^3 bonding ratio in the DLC material has a significant effect on the haemocompatibility of the surface, with increasing sp^3 content increasing adsorption of human serum albumin relative to fibrinogen [12]. Therefore in this study the sp^2/sp^3 bonding ratio was altered by surface treatments (specifically Ar^+ ion sputtering and laser graphitisation) in order to observe the effect on protein adsorption.

In order to examine the arrangement of proteins on the biomaterial surface, a protein layer was adsorbed onto the surface of the modified and unmodified DLC coatings. BSA was used as it is often used as a model for protein adsorption from blood [13], as well as being a well-characterised protein that is often used to study protein interactions with solid surfaces [14]. This allows the present study to be relevant for applications involving interactions of blood with DLCs such as arterial stents.

2. Materials and methods

2.1. Diamond-like carbons

Diamond-like carbons (DLCs) are a form of amorphous carbon, with a mixture of both sp^2 and sp^3 bonded carbon. The properties of the DLC coating are heavily dependent on this sp^2 to sp^3 bonding ratio [15]. In this work, Ar^+ ion sputtering and laser graphitisation are used to graphitise the surface of the DLC coatings, that is, to convert some of the sp^3 bonded carbon to sp^2; the form found in graphite.

The diamond-like carbon coating used was a DLC coating with metallic interlayers to improve adhesion to the substrate, and was deposited by a combination of PACVD (plasma assisted chemical vapour deposition) and PVD (physical vapour deposition) methods.

2.2. Ar^+ ion sputtering

Ar^+ ion sputtering was carried out to induce surface graphitisation of DLC coatings, using the method developed by Viljoen et. al. [16]. The ion beam used was the Kratos Minibeam III (an accessory technique to the Kratos Axis ultraDLD XPS – see below). Samples were prepared in the same way as for XPS. For ion sputtering, the chamber pressure of the XPS was slightly higher than usual (1.5×10^{-7} Torr), due to the presence of the sputtering gas. The ion beam energy was 5.2 keV with an emission current of 6 mA. The raster size was adjusted to 3×3 mm, and the ion beam turned on for 4 min. In order to graphitise a larger area, the raster square was moved to several different positions on the sample rather than increasing the area (which would give a lower emission current density, seen by trials to result in non-optimal graphitisation).

To ensure that any changes in surface chemistry seen were a result of actual changes in the DLC coating (rather than just removal of adsorbed species), the sputtered DLC was exposed to atmosphere for 10 min, before being re-analysed. If the changes seen are simply due to removal of adsorbed species, we would expect to see the C1s peak shift back to the higher binding energy associated with sp^3 carbon after exposure to air, as adsorbed species (adventitious carbon) are usually of the sp^3 configuration [17]. However if an actual change in surface chemistry is achieved there should be little change in the C1s peak after exposure to air – it should remain at the lower binding energy associated with sp^2 (graphitised) carbon.

2.3. Laser graphitisation

Laser graphitisation experiments were carried out using a femtosecond laser micromachining system based on a Ti:Sapphire amplified femtosecond laser (Mantis (oscillator) and Legend Elite (amplifier), Coherent Inc., USA); similar to the method used by Dumitru et. al. [18]. This laser supplies 800 nm wavelength, ultrashort (100 fs) pulses at a repetition rate of 500 Hz to a JPSA IX-100 micromachining platform under atmospheric conditions. The laser was set up to produce a square spot (50×50 μm) with an energy density of 1.28 J/cm^2. Beam attenuation was set to 69% (using a waveplate and polariser variable attenuator) to give the desired energy density of 0.4 J/cm^2 to compare with the work of Dumitru et al. The micromachining platform was programmed to scan and fire across the whole sample surface, resulting in a uniformly laser treated surface.

2.4. Protein adsorption

A 1 mg/mL BSA solution was prepared by dissolving BSA powder into distilled water. The solution was prepared immediately before the adsorption test, and then analysed without delay, to prevent degradation of the protein. The sample was immersed in 20 mL of BSA solution in a plastic vial, unagitated at room temperature, for 60 min. After immersion the sample was rinsed with distilled water to remove any solution that may have dried on the surface (not adsorbed).

2.5. Analysis methods

XPS was carried out on a Kratos Axis ultraDLD, using a monochromated Al Kα X-ray source with a power of 150 W. The chamber pressure was approximately 1.5×10^{-7} Torr. Survey scans were performed at pass energy of 160 eV from 0 to 1350 eV, while core level scans were carried out at 20 eV to improve resolution. Angle resolved XPS was used to alter the analysis depth for analysis of protein arrangement – a higher takeoff angle results in a smaller analysis depth. Takeoff angles of 0, 20, 40, 60, and 80° were used to produce a depth profile of the surface (takeoff angle is defined here as the angle between the collection angle and surface normal).

Peak fitting was carried out using CasaXPS software, fitting with a Shirley-type background and Gauss–Lorentz component peak shapes. Graphitic (sp^2) carbon was fitted with the Doniach–Sunjic asymmetry known to be characteristic of graphite [19]. Components were fitted so that peak widths remained similar for all components. The sp^3 and sp^2 components of the DLC were fitted using two peaks at 285.4 eV and 284.8 eV respectively [1,17].

The components of the adsorbed protein were determined from analysis of a BSA standard sample. Samples were cleaned with isopropanol and air dried before analysis to remove surface contaminants (for samples with adsorbed proteins distilled water was used instead), and six repeats were carried out for all samples to ensure reliability and repeatability of data. Charge neutralisation was used for the BSA protein standard, which was prepared by evaporating BSA solution (1 mg/mL) in a gold-coated sample stub. The sp^2 carbon component at 284.8 eV was used as a binding energy reference.

Scanning electron microscopy was carried out using a Phillips FEI XL30S, with a field emission gun (FEG) electron source. The imaging parameters such as accelerating voltage are seen in the information bar on the image.

The surface energy of treated and untreated DLCs was calculated from measurements of contact angles of di-iodomethane and water, a polar and non-polar fluid. Prior to measurement, samples were cleaned with ethanol and distilled water. The instrument used was a KSV CAM 101 goniometer, using the sessile drop technique,