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Protein adhesion on water stable atmospheric plasma deposited acrylic acid coatings

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ABSTRACT

There is considerable interest in the application of plasma polymerised acrylic acid (ppAAc) coatings due to their ability to enhance the adhesion of cells and proteins. An issue with this coating however is its stability in water and previous studies carried out using low pressure plasmas have demonstrated that high plasma powers are required to achieve water stable coatings. In this paper the use of both helium and air atmospheric plasmas is compared for the deposition of ppAAc coatings. The deposition studies were carried out on silicon wafer substrates using the PlasmaStream™ and PlasmaTreat™ plasma jet deposition systems respectively. The coatings were characterised using contact angle, FTIR, SEM, XPS, ellipsometry and optical profilometry. While both the helium and air plasmas were successful in the deposition of ppAAc coatings, the nm thick films deposited using the PlasmaTreat system exhibited significantly higher levels of water stability, probably due to a higher level of coating cross-linking. Ellipsometry measurements demonstrated only a 0.2 nm reduction in the thickness of an 18 nm thick ppAAc coating, when immersed in an aqueous buffer solution for one hour. Protein attachment studies were carried out using a flow cell system, which was monitored using a spectroscopic ellipsometer. This study was carried out with Bovine Serum Albumin (BSA), Immunoglobulin G (IgG) and Fibrinogen (Fg) proteins. In all three cases increased levels of protein adhesion were observed for the ppAAc coating, compared to that obtained on the uncoated silicon wafer substrates.

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1. Introduction

Controlling protein adhesion is an important issue affecting many different fields including bio-processing, medical device implants, biosensors, and drug delivery devices [1,2]. When the surface of a material interacts with a biological environment one of the initial interactions is through protein adhesion [3]. Amongst the factors influencing protein adsorption, are surface chemical functionality and morphology [4]. Amongst the techniques that have been investigated to modify the surfaces of polymers prior to protein adhesion studies have been micro patterning and monomer polymerisation by free radical solution, emulsion, grafting as well as plasma processes [5–9]. It has been demonstrated by a number of authors that plasma techniques enable both the surface functionality and morphology to be tailored [10,11]. This makes it possible to create a surface which will inhibit or enhance the rate of protein adhesion onto a biomaterial surface, without changing the properties of the bulk material [12].

One surface chemistry that has received considerable attention for cell and protein adhesion has been acrylic acid. Surfaces containing this coating have been reported in applications ranging from platelet adhesion promotion [13], RGD peptide immobilisation [14], attachment of osteoblast-like [15], fibroblast [16] and keratinocyte [17] cells as well as collagen molecule grafting [18].

To-date low-pressure plasma processes have been extensively investigated for the deposition of plasma polymerised acrylic acid (ppAAc) coatings. These include: radiofrequency (13.56 MHz) parallel plate [14,15,19], glow discharge [16] and two-phase plasma polymerisation processes [20]. The coatings obtained using these techniques displayed similar properties such as the retention of a high degree of monomer structure and functionality. Coatings deposited at lower plasma powers were not found to exhibit good stability in water, the use of higher powers was required to yield coatings exhibiting greater stability in aqueous solutions [16]. These latter coatings were dominated by ester functionalities and were highly cross-linked. The enhanced stability of the ppAAc coatings however was found to be associated with a reduction in COOH functionality [14]. This is due to high fractionalisation of the acrylic acid monomer.

There have been a small number of reports on the use of dielectric barrier discharge, and atmospheric pressure plasmas for the deposition of ppAAc coatings [7,21]. Here too it was reported that as plasma power is increased the percentage of carboxyl functionalities decreases. These papers do not discuss the water stability issues associated with ppAAc, however; lower power plasma deposited coatings were reported to exhibit more hydrophilic properties (water contact angles under 20°). Coatings deposited at higher plasma powers in contrast had contact angles up to water contact angles of 45° indicating a high organic content possible due to the loss of −COOH functionality.

The objective of this study is to investigate the deposition of ppAAc coatings using both a helium and air based atmospheric plasma systems. The focus of this study is to firstly compare the properties of the coatings

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deposited using the two atmospheric plasma jet systems and in particular to evaluate the water stability of the deposited coatings. Once a relatively water stable coating is achieved the study will investigate the effect of the ppAAc coating on the adhesion of three different types of protein in an aqueous buffer solution.

2. Experimental

2.1. Coating deposition

The helium atmospheric plasma studies were carried out using the PlasmaStream™ (Dow Corning Plasma Solutions) system, which has been described previously [22,23]. In this system the discharge is formed using a modified PTI 100 W rf power supply, between two pin electrodes. This is a high voltage source that uses a flyback transformer, which produces a non-sinusoidal voltage waveform due to the rapid collapse of the magnetic circuit within the flyback transformer [23]. Liquid precursors are nebulised between these electrodes using a pneumatic Burgener Mira Mist nebuliser. The nebulised liquid is mixed with helium and nitrogen gases inside a Teflon tube, 75 mm long and 15 mm in diameter, mounted over the power source. A plasma is formed inside this tube and extends out of its base, under which the substrate is placed (Fig. 1). The plasma formed operates at a frequency of between 15 and 25 kHz, with maximum output voltages between 11.8 and 14.9 kV. The distance from the base of the tube to the substrate, as well as the speed at which the jet moves, are controlled by a computer numerical control (CNC) system. The air plasma deposition studies were carried out using the PlasmaTreat OpenAir™ system, which has also been described previously [10,22,24]. This system (Fig. 2) operates by forming a blown arc inside an arc cavity as ionised gas is expelled through a 5 mm (PFW10) jet nozzle. The 25 kHz plasma jet is driven by a positive uni-polar square wave pulse width modulation (PWM) transistor amplified circuit. A pneumatic liquid precursor delivery system is used to deliver the AAc through a heating coil and then directly into the plasma. The plasma is formed using dry compressed air at inlet pressures of between 100,000 and 300,000 Pa. The system operates at airflow rates of between 37.5 and 76.61 l.min^{-1}.

Coatings were deposited using an acrylic acid (Sigma Aldrich 99%) precursor onto silicon wafers (450 μm thick, p-type, boron doped and polished on one side). The wafers had previously been ultrasonically cleaned in methanol, dried, then cleaned ultrasonically in acetone and re-dried.

2.2. Coating characterisation

A Dataphysics Instruments OCA 20 Video Based Contact Angle Device utilised the sessile drop technique to obtain water contact
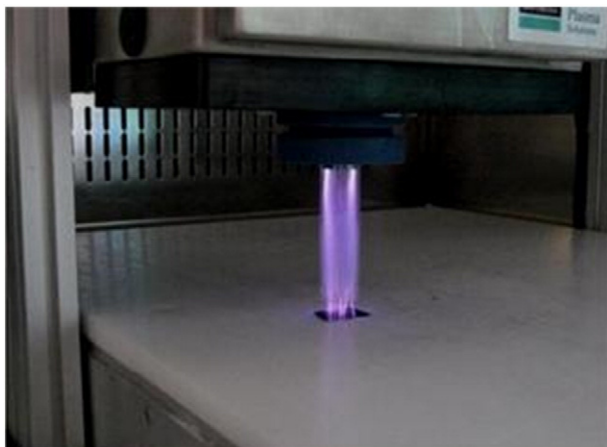

Fig. 1. Helium plasma formed using the PlasmaStream™ atmospheric plasma jet system.
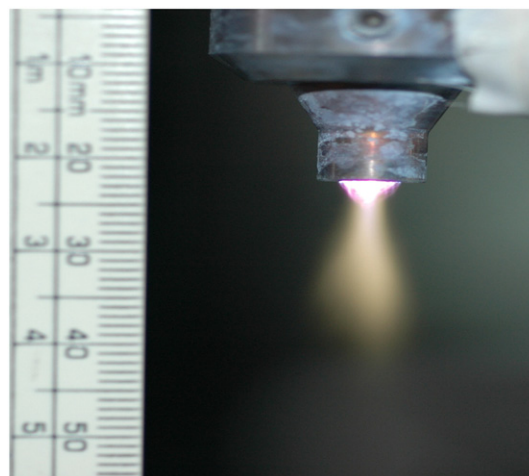

Fig. 2. Air plasma formed using the PlasmaTreat OpenAir™ atmospheric plasma jet system.

angles, using deionised water at room temperature. 1 μl drops were allowed to sit on the surface for 5 s (approx.) before contact angles were measured. Measurements were taken from three different locations on three different samples, an average of these was then calculated. These measurements were taken one week after coatings were deposited in order to minimise activation effects of the plasma on the silicon wafer substrate. Surface energies were determined using deionised water, diodomethane and ethylene glycol. The OWRK method was then employed to calculate the surface energy of the deposited coatings based on the contact angles obtained [25,26].

A Woolam M2000 (J.A. Woolam Co. Inc., USA) variable wavelength ellipsometer was used to determine film thickness on the silicon wafer. Measurements were taken at three different points on each of the test samples. Light of known polarisation reflects off and refracts through the coatings. This results in a change in the polarisation of the light. This change is recorded and a Cauchy model is fitted to the data to indicate film thickness [27]. Coating stability in phosphate buffer solution was also monitored dynamically using this technique.

Optical profilometry measurements were used to determine morphologies and roughness of the deposited coatings before and after submersion in the deionised water bath. These measurements were carried out using a Wyko NT1100 optical profilometer operating in vertical scanning interferometry (VSI) mode. The roughness values are quoted as R_q and R_a values, the former is a measure of mean square roughness, whilst R_a is an arithmetic average roughness of the surfaces. The difference between R_a and R_q is an indication of the homogeneity of surface morphology [12].

Fourier transform infrared spectroscopy (FTIR) measurements were obtained using a Bruker Vertex-70 system with a liquid nitrogen cooled MCT detector and a KBr beam splitter. Spectra were collected in the range of 400–4400 cm^{-1} using a spectral resolution of 4 cm^{-1} and an overlay of 64 scans per sample cycle.

X-ray photoelectron spectroscopy (XPS) analysis of the samples was carried out in VG Microlab 310-F electron spectrometer at base pressures, in the preparation and analysis chambers, of $2 \times 10 - 8$ and $1 \times 10 - 8$ Pa, respectively. The photoelectrons were exited with an x-ray source using MgKα ($h\nu = 1253.6$ eV) and the pass energy of the analyser was 20 eV yielding a resolution of 1.1 eV. The C1s, N1s, O1s, Cl2p & Si2p peaks were recorded along with 50–1000 eV survey scans. The intensities of the peaks were determined as the integrated peak areas were assuming the background to be linear.

In order to ascertain the water stability of the coatings deionised water immersion studies were carried out using a Clifton thermal bath (Nickel-Electro Ltd., U.K.) for 180 min at 37 °C. The samples were then air dried for 5 days and then the properties of the ppAAc coating were reassessed. This involved the use of spectroscopic ellipsometry to