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The influence of electrostatic forces on protein adsorption

G.V. Lubarsky, M.M. Browne, S.A. Mitchell, M.R. Davidson, R.H. Bradley*

Advanced Materials and Biomaterials Research Centre, School of Engineering, The Robert Gordon University, Aberdeen, AB10 1FR, UK

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Abstract

In this paper we investigate the importance of electrostatic double layer forces on the adsorption of human serum albumin by UV–ozone modified polystyrene. Electrostatic forces were measured between oxidized polystyrene surfaces and gold-coated atomic force microscope (AFM) probes in phosphate buffered saline (PBS) solutions. The variation in surface potential with surface oxygen concentration was measured. The observed force characteristics were found to agree with the theory of electrical double layer interaction under the assumption of constant potential. Chemically patterned polystyrene surfaces with adjacent 5 μm × 5 μm polar and non-polar domains have been studied by AFM before and after human serum albumin adsorption. A topographically flat surface is observed before protein adsorption indicating that the patterning process does not physically modify the surface. Friction force imaging clearly reveals the oxidation pattern with the polar domains being characterised by a higher relative friction compared to the non-polar, untreated domains. Far-field force imaging was performed on the patterned surface using the interleave AFM mode to produce two-dimensional plots of the distribution of electrostatic double-layer forces formed when the patterned polystyrene surfaces is immersed in PBS. Imaging of protein layers adsorbed onto the chemically patterned surfaces indicates that the electrostatic double-layer force was a significant driving force in the interaction of protein with the surface.

Keywords: Electrostatic double-layer force; Protein attachment; Polystyrene; AFM; Human serum albumin

1. Introduction

The interaction between proteins and the surface of a material is a fundamental phenomenon with important implications in a number of biotechnological processes. For example the irreversible binding of albumin to hydrophobic surfaces is thought to be the reason for the poor attachment of cells to many polymers. Polymers such as polyethylene and polystyrene, which are essentially non-polar in their native form, do not support cell attachment unless treated to increase the surface energy by the incorporation of heteroatoms such as oxygen or nitrogen or by the pre-adhesion of attachment proteins. Surface oxidative treatments are known to promote cell attachment on polymer surfaces with rates of attachment and subsequent proliferation being positively influenced by increasing levels of functionalisation and surface energy [1–5].

Chemically micropatterned surfaces, which have functionalised (polar) and unfunctionalised (non-polar) domains, and which are consequently adherent or abherent to cell attachment, provide a potentially useful means of directing cell growth to specific regions of device surfaces [6,7]. Indeed, for in vitro culture of a range of human primary and other cell types, distinctly different attachment and proliferation behaviour are observed on domains of different chemistry. These differential cell responses to surfaces of different chemistry are likely, in part, to result from differences in the composition and orientation of the adsorbed protein structure [8].

In a previous study, we investigated the importance of surface chemistry on the orientation and concentration of albumin adsorbed to polystyrene surfaces [9]. However, the physical size of proteins such as albumin mean that protein adsorption is essentially a colloidal process and any attempt to understand the interaction between surfaces and proteins must take surface forces into account. In the present paper, we look at the importance of surface forces as one of the key driving forces behind protein adsorption. Since interactions

* Corresponding author. Tel.: +44 1224 262822; fax: +44 1224 262837.
E-mail address: r.bradley@rgu.ac.uk (R.H. Bradley).

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between surfaces and proteins occur in aqueous solutions an electrostatic double layer will exist either through the dissociation of surface functional groups and/or through the adsorption of ions from solution. The surface charge is balanced by the accumulation of an equal number of oppositely charged counterions that are either bound to the surface to a form the Stern layer or present in an atmosphere above the surface to form an electrostatic double layer. Since proteins are usually charged molecules in aqueous solution the presence of this electrostatic double layer with its associated electric field will have an important influence on the interaction between polymer surfaces and proteins.

In this study, a UV–ozone treatment has been used to produce surfaces with adjacent regions of different chemistry that allow the study of the electrostatic forces existing above native and oxidised polystyrene surfaces under otherwise identical conditions. Atomic force microscopy will be used to measure the electrostatic forces of these chemically patterned surfaces in phosphate buffered saline—a medium widely used in cell culture and protein adsorption work. The AFM will also be used to map the topography, frictional forces and adhesion forces before and after protein adsorption.

2. Experimental

2.1. Surface preparation

The material used throughout this study was polystyrene cut from Nunc untreated cell culture dishes (VWR International Ltd). In order to produce flatter surfaces suitable for the study of electrostatic forces and adsorbed protein layers we reduced the roughness to between 0.4 and 0.5 nm by heating the polystyrene to approximately 413 K whilst pressed between two clean silicon wafers. XPS analysis of the flattened polystyrene reveals no oxidation or transfer of contaminants during this process and the XPS C1s spectrum recorded from the processed surfaces is consistent with that of pure PS [10]. The resulting surfaces contain some macroscopic features in the form of periodic steps, which presumably result from thermal stress, and viscous flow of the melted PS. These features were relatively large and did not affect the subsequent AFM imaging of the adsorbed protein layers.

The UV source for chemical modification of the polystyrene surface was a high-intensity low-pressure mercury grid lamp, which generates UV light at 184.9 and 253.7 nm (Jelight Company Inc. Irvine, USA). These wavelengths are known to excite molecular oxygen to form ozone and to photosensitise polymer surfaces [11,12].

The production of chemically patterned surfaces was achieved by irradiating the polystyrene through a copper transmission electron microscope grid. We have used this technique in previously reported tissue culture work [6,7] to control the spatial attachment of a range of cell types. However, the mask used in the present study has bar and space dimensions of 5 μm and thus produces a pattern of sub-cellular dimensions. Intimate contact between grid and sample was provided using home-built mask aligner, which effectively pressed the mask into contact with the surface. The UV irradiation was performed in an ambient air environment with an exposure time of 60 s. XPS analysis shows that this treatment incorporates approximately 7 at.% surface oxygen into the polystyrene surface. The precise chemistry of the oxidation has been discussed elsewhere [4,5,11,12].

Protein solutions of 0.1 mg/mL were prepared with human albumin, essentially fatty acid and globulin free lyophilised powder (Cat. No. A 3782, Sigma-Aldrich Company, Dorset, UK) in Dulbecco's phosphate buffered saline at pH 7.2 (PBS, Cat. No. D8537, Sigma-Aldrich). A 50 μL drop of solution was placed on chemically patterned surfaces and allowed to incubate at 37 °C. After 1 h samples were removed from the incubator and rinsed twice in Milli-Q water. Samples were then re-immersed in Milli-Q and placed on a shaker for 4 h, rinsed twice again in Milli-Q and then allowed to dry overnight at room temperature.

2.2. Electrostatic double-layer force measurement

Force–distance experiments were performed using a Digital Instruments Multimode SPM system (Nanoscope III a) in liquid media using an electrochemical fluid cell. The electrochemical hardware of the SPM was operated in the bipotentiostat regime to monitor and control the electrochemical potentials in the cell. The tip and surface were immersed in 1% Dulbecco's phosphate buffered saline (PBS, Cat. No. D 8537, Sigma-Aldrich Company, Dorset, UK) solution of concentration 3.93×10^{-3} M at 25 °C. The solution was injected into the cell, using a syringe, and the electrolyte was hydrodynamically static during measurements.

A three-electrode system (Fig. 1) was set up in the liquid cell of the AFM unit with the gold-coated cantilever as working electrode (WE), a ring-shaped gold wire as counter electrode (CE) and Ag/AgCl as the reference electrode. The

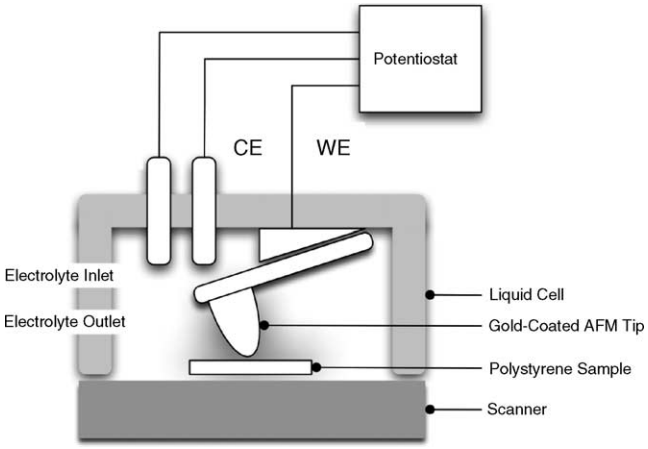

Fig. 1. Schematic of the experimental three-electrode set up.