

The attachment of catalase and poly-L-lysine to plasma immersion ion implantation-treated polyethylene

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Abstract

Plasma immersion ion implantation (PIII) treatment of polyethylene increased the functional attachment of catalase and increased the retention of enzyme activity in comparison to untreated controls. The attached protein was not removed by SDS or NaOH, while that on the untreated surfaces was easily removed. Poly-L-lysine was found to attach in a similar way to the treated surface and could not be removed by NaOH, while it did not attach to the untreated surface. This indicates that a new binding mechanism, covalent in nature, is introduced by the plasma treatment. Surfaces treated with PIII maintained the catalase activity more effectively than surfaces plasma treated without PIII. The PIII-treated surface was hydrophilic compared to the untreated surface and retained its hydrophilic character better than surfaces subjected to a conventional plasma treatment process. The strong modification of a deeper region of the polymer than for conventional plasma treatments is believed to be responsible for both the enhanced hydrophilic character and for the increase in functional lifetime of the attached protein. The results show that PIII treatment of polymers increases their usefulness for protein microarrays.

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1. Introduction

The attachment of proteins to surfaces has many applications in the food, chemical, biotechnology and medical diagnostic industries [1]. New applications in these industries using nanotechnology have been developed, including biosensors and micro-arrays for testing blood serum for the presence of proteins associated with disease [2]. Procedures that can result in increased protein attachment to surfaces and achieve longer times over which the attached proteins remain functional are therefore valuable. Polymer plastics are suitable as surfaces for attaching proteins because of their lightness, strength, ease of forming into shapes [3],

stability in air, and resistance to corrosion or breakdown in many harsh chemical environments. For example, polystyrene is used for microtitre plates which require formability, mechanical strength and impermeability [4].

Two methods have been used for increasing the attachment of proteins to surfaces, namely chemical and plasma treatments. The incorporation of oxygen-containing groups onto the surface using chemical methods has been shown to increase the density of cellular attachment [5]. An effect of chemical treatment is the introduction of oxygen-containing groups onto the surface, which may be important in the attachment process [6,7]. Plasma treatment has been used industrially to modify the surfaces of a range of biomaterials [8] including the treatment of cell culture plates for enhanced cell attachment [9]. Plasma treatment has the advantage that it can treat defined surface areas by using masks or resists. Sipehia [10] showed increased attachment of bovine serum albumin to

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polypropylene beads after treatment with ammonia plasma. Plasma methods [11] have been used to introduce oxygen-containing groups to the surface of polymers, dramatically reducing the contact angle [12,13]. Many conventional plasma treatments show a substantial hydrophobic recovery, in which the surface reverts to its prior state over hours or days [12].

Two main forms of protein attachment to surfaces are available: covalent bonding via a suitable chemical linker group, and physisorption. The main advantage of covalent bonding is that the linking of the molecule to the polymer surface is more permanent. The disadvantages of using a linker are the cost and the possibility that the binding site is affected or masked by the presence of the linker. For some applications it is necessary to maintain function for as long as possible as it is not only the total amount of protein attached to the surface that is important but the amount of protein still biologically active after repeated washing.

Catalase is used in the textile industry to remove the bleaching agent hydrogen peroxide from fabrics [14]. We selected catalase as the test protein in this study because its enzyme activity allows a convenient assay and because it denatures relatively readily compared to other enzyme candidates such as horseradish peroxidase [15,16]. It is therefore a good test of the ability of a surface to attach a protein and preserve its function.

Our aim in this paper is to develop a treatment method for polymers that has the advantages of high densities of strong binding sites that maintain the function of protein without the need for a separate linker incorporation step. We utilize energetic ion impacts, delivered through the application of high-voltage pulses in a process known as plasma immersion ion implantation (PIII) [17,18], to treat polyethylene. We use bovine liver catalase to test the ability of surfaces treated with PIII to attach protein and maintain its function. We also use a simple polypeptide, poly-L-lysine, to study the surface's binding affinity for lysine alone.

2. Materials and methods

2.1. Materials

Bovine liver catalase (EC 1.11.1.6) (C-3155, 20 mg ml⁻¹) and poly-L-lysine (MW >30,000, P-9404) were purchased from Sigma. Tween 20 was from BDH. Ultra-high molecular weight polyethylene (UHMWPE) was from Goodfellow Cambridge Ltd. (Cat. No. ET301200/1). All other reagents were of analytical quality grade. Nitrogen and argon were 99.99% pure.

2.2. Plasma treatment of polyethylene

The plasma treatment chamber used to modify the polymer surfaces has been described elsewhere [19]. It consists of an inductively coupled plasma source and a diffusion chamber in which the sample holder is placed in a face-

down orientation. The plasma is generated by supplying RF power (13.56 MHz) to a single-loop antenna wound around a borosilicate glass tube. The radiofrequency power is fed to the antenna through a CPM-2000 matching network. A magnetic field of approximately 5 mT, generated by two pairs of copper coils, then guides the plasma up into the aluminium treatment chamber. The sample holder was mounted on the axis of the chamber. Process gases were delivered to the chamber through the inlets with their flow rate regulated by MKS mass flow rate controllers.

Two kinds of plasma treatments were used. In the first treatment, the sample was exposed to the RF plasma with no electrical connections made to the sample holder. This allowed the holder to assume a floating potential determined by the potential at which the current due to the more mobile electrons is balanced by ion current drawn from the plasma. The floating potential was in the range of -20 to -40 V and this determined the energy of ion bombardment during the treatment process. In the second plasma treatment process, the sample was exposed to the same RF plasma, but 20 kV pulses lasting for 20 μs were applied to the sample holder with a repetition rate of 50 Hz, with the sample holder earthed between the pulses. This process, known as PIII, results in a fraction of the incident ions having energies of the order of tens of kilovolts. The high-voltage pulses were supplied by a pulsed power supply connected to the sample holder feedthrough.

For both types of the plasma treatment, the working gas pressure was 2 mtorr and the radiofrequency power was 100 W. The gases used were high-purity argon (22 standard cubic centimetres per minute (sccm)) and nitrogen (72 sccm) and, unless otherwise stated, the UHMWPE surfaces were treated for 800 s.

2.3. Surface morphology and contact angle measurement of polyethylene

Atomic force microscopy (AFM, Autoprobe CP) in contact mode was used to measure the topography of plasma, PIII-treated and untreated UHMWPE surfaces. Scans were taken over squares of various side lengths, ranging from 10 to 0.1 nm, and root mean square roughness was calculated over each scan.

The surface energy was studied using the sessile drop method. Kruss contact angle equipment DS10 was employed to measure the water contact angle after treatment starting at 5 min after the sample was removed from the treatment chamber and repeated at intervals for times up to 2 h. In each measurement, 50 μl deionized water was dropped on the sample and the angle between edge of water drop and the surface was measured.

2.4. Attachment of catalase to plasma-treated polyethylene

All treated samples were incubated in catalase solution within 2 h after removal from the plasma treatment chamber. Catalase was diluted to 50 μg ml⁻¹ in 10 mM sodium

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