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Increasing binding density of yeast cells by control of surface charge with allylamine grafting to ion modified polymer surfaces

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ABSTRACT

Plasma immersion ion implantation (PIII) treatment of polymers creates a biointerface capable of direct covalent immobilization of biomolecules. The immobilization of protein molecules is achieved by covalent bonds formed between embedded radicals on the treated surface and amino acid side chains and cells can be immobilized through cell-wall proteins. The attachment density of negatively charged entities on a PIII treated surface is inhibited by its negative surface charge at neutral pH. To reduce the negative charge of PIII treated surfaces in phosphate buffer (pH 7.4, 11 mM), we develop an effective approach of grafting allylamine monomers onto the treated surface. The results reveal reactions between allylamine and radicals on the PIII treated surface. One of these triggers polymerization, increasing the number of amine groups grafted. As a consequence, the PIII treated polystyrene surface after allylamine exposure becomes more hydrophobic and less negatively charged in phosphate buffer. Using yeast cells as an example, we have shown a significant improvement (6–15 times) of cell density immobilized on the PIII treated surface after exposure to allylamine.

1. Introduction

Surface immobilization of biomolecules such as antibodies, enzymes, proteins and cells is of great interest for many applications in biofuel production [1,2], environmental treatments [3,4], food technology [5,6], medical diagnostics [7,8] and prosthetics [9,10]. For example, the immobilization of enzymes on polymer surfaces has the potential to prolong catalytic function for the economic production of food and bioethanol. For surface immobilization of cells (for example, yeast cells and bacteria) in processing applications, key features are the density and strength of the attachment. Controlling the factors that influence surface immobilization will give opportunities to optimize the effectiveness of applications and is the subject of this paper.

Plasma immersion ion implantation (PIII) is a surface treatment that endows polymers with an ability to covalently attach biomolecules on their surfaces. This method has been shown to increase the density of attachment of molecules while at the same time increasing enzyme activity compared to untreated surfaces [11]. In PIII treatment, ions from plasma are accelerated under a high negative bias toward the polymer which is mounted on a conductive sample holder. The subsequent collisions as the ion penetrates the surface break the polymer chains to create unpaired electrons (also known as radicals) [12]. Surface immobilization of enzymes, proteins and yeast cells using PIII treated polymers has been described in the literature previously [11,13,14]. The immobilization process occurs in two distinct stages: first there is adsorption of the molecules onto the surface followed by formation of a covalent bond arising from the interaction from a radical within the surface [15]. The formation of covalent bonds with the biomolecules has been demonstrated by detergent washing such as with sodium hydroxide [11,14] or sodium dodecyl sulphate [13,14]. The benefits of PIII treatment in immobilizing biomolecules have been discussed in the literature [12]. The use of low cost carriers (polymers), the one step dry process to create covalent binding and the stability of immobilized biomolecules on the treated surface render the surface immobilization by PIII highly suitable for many applications.

Surface charge plays an important role in immobilization of biomolecules on the carrier [16–19]. PIII treatment makes polystyrene surface become negatively charged due to the appearance of unpaired electrons and subsequent surface oxidation [20]. In a buffer with low ionic strength (10 mM), the surface charge of the PIII treated surface is close to neutral in acidic buffer (pH 3–5)

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and becomes increasingly negative with increasing pH. By varying pH of incubation solution, we can alter the charge of PIII treated surface and hence, optimize the immobilization. Yeast cells have been found to adhere with high density on a PIII treated surface in acidic buffers (pH 3–5) and with significantly lower density in alkaline buffers (pH 6–10) [20]. Enzyme celB has a significantly higher catalytic activity when immobilized on the PIII treated polystyrene at pH 5.5 than at pH 7 [13]. These pH dependencies indicate that interactions between charges on the surface and those on the entities being immobilized play a decisive role in the immobilization process and influence the subsequent effectiveness of the immobilized layer. Varying pH to modulate charge interactions for attachment purposes may, in some cases, have a negative impact on the subsequent biological function of the immobilized layer, especially for entities which only function in a narrow pH range such as mammalian cells. Therefore, to make PIII treatment a more versatile immobilization method for cells and biomolecules, we aim to develop a method to alter the PIII treated surface charge at pH 7.4 to control the adhesion. We chose yeast cells and polystyrene as an example combination of biological entity and immobilizing surface to illustrate the outcomes.

At pH 7.4, rehydrated yeast cells have a negative charge on their cell wall [20]. The PIII treated surface is also negatively charged. It appears that the negative charge on both surfaces prevents the close approach of the yeast cell wall components to the surface, reducing the formation of covalent bonds between cell wall proteins and surface embedded radicals. These repulsive forces could be reduced by reducing the negative charge of the PIII treated surface. A possible solution is to introduce more positively charged groups, such as amine groups which can be protonated in physiological pH solution, to the surface.

Allylamine (CH_2=CH—CH_2—NH_2) is a useful chemical for modifying surface charge. Due to the presence of a double bond in its structure, allylamine can be polymerized, forming a long saturated carbohydrate back bone with many side chains of amine groups. Plasma polymerization of allylamine has been reported to create a coating with a high density of positively charged amino groups [21–24]. In this paper, we use allylamine to modify the surface charge of a PIII treated polystyrene surface. We show that the allyamine is attached covalently and that its polymerization is induced giving a significant change in surface charge. We use yeast as a model to investigate the effects on the attachment of cells.

2. Materials and methods

2.1. Sample preparation, PIII treatment and allylamine exposure

Spin-coated polystyrene films on silicon wafer samples were prepared for contact angle measurement, surface topography analysis, force measurement and yeast attachment. Droplets of 2% (by weight) polystyrene in toluene were spin-coated on silicon wafers (1.2 cm × 1.2 cm) at 33.3 Hz to get a thickness of 100–120 nm (analyzed by ellipsometry). The PIII treatments on these samples were conducted as described in our previous work [14]. In brief, samples were mounted on a conductive sample holder immersed in nitrogen plasma and periodically bombarded by energetic ions under a 20 kV bias. The treatment time varies depending on the purposes of the subsequent analysis. After removal from the plasma chamber, samples were transferred to a metal rack above allylamine liquid in containers with caps to expose the treated surfaces to allylamine vapor (the time PIII treated samples expose to ambient air is approximately 2–3 min). Allylamine solution (98% purity) was obtained from Sigma–Aldrich. Argon was blown into the container to reduce oxygen in the space above the liquid surface for 1 min before closing the cap. Samples were kept in the containers for 5, 30, 60, 180, 1440, 2880 and 4320 min; after the allylamine exposure, they were taken out of the containers, dried in argon and stored in an airtight container. Images of all samples were taken using an optical microscope (Axioplan 2 Imaging, 10× magnifications). For contact angle measurement, surface topography analysis and yeast attachment, samples were PIII treated for 400 s (corresponding to a fluence of 5×10^{15} ions/cm^2). For force measurement, samples were PIII treated for 1600 s (corresponding to a fluence of 2×10^{16} ions/cm^2).

Spin-coated polystyrene on polytetrafluoroethylene (PTFE) sheet samples were prepared for Fourier transform infrared spectroscopy (FTIR) analysis to allow better contact between samples and the germanium crystal. A mild PIII treatment on PTFE was used to enhance adhesion of the spin-coated layer of polystyrene. PTFE sheet (2 mm thick from Goodfellow) was cut into pieces (1.5 cm × 1.5 cm) and PIII treated for 100 s. After the treatment, these PTFE pieces were spin-coated with polystyrene and treated with PIII for 1600 s. Samples after PIII treatment were immediately transferred to closed containers containing allylamine as described above for 5, 30, 60 and 180 min; after the exposure, they were taken out of the containers, dried in argon and stored in an airtight container for approximately 5 min before analysis by FTIR.

For convenience, we label samples with PIII treatment P*x* (where *x* (in seconds) is the PIII treatment time) and PIII treated samples with subsequent allylamine exposure P*x*-A*y* (where *y* (in minutes) is the allylamine exposure time).

2.2. Surface characterization

Contact angle measurements using the sessile drop method were performed on PIII treated samples (15 min after the treatment) and on samples exposed to allylamine after 1 day storage in an airtight container. Kruss contact angle DS10 equipment was used to measure contact angles with two liquid probes (water and diiodo methane). Surface energies were calculated from four measurements using the Owens–Wendt–Rabel–Kaelble method as follows:

$$\gamma_{SL} = \gamma_S + \gamma_L - 2\left(\sqrt{\gamma_S^D \gamma_L^D} + \sqrt{\gamma_S^P \gamma_L^P}\right)$$

$$\gamma_S = \gamma_{SL} + \gamma_L \cos\theta$$

where θ is the contact angle of the liquid on the solid surface; γ, γ^D and γ^P are surface tension, disperse and polar fractions respectively; and the subscripts S, L and SL denote the solid–air, liquid–air and solid–liquid interfaces respectively.

Surface roughness of allylamine-exposed samples was analyzed using Molecular Force Probe (MFP3D-Bio, Asylum Research) in a contact mode. A scan size of 10 μm × 10 μm was taken at three different positions on each sample to calculate the average of root mean square roughness.

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to observe changes in the chemistry of the thin surface layer so as to clarify the reaction between radicals and allylamine monomers. Spectra were recorded using a Digilab FTS 7000 FTIR spectrometer equipped with a germanium crystal attenuated total reflectance accessory in the mid infrared range. The peaks corresponding to the PTFE were removed from the allylamine-containing spectra by subtraction of the spectrum from the corresponding PIII treated PTFE surface. The ratio of peaks at 2925 cm^{-1} (attributed to CH stretching in —CH_3 or —CH_2— groups) and 3082 cm^{-1} (attributed to CH stretching vibration in C=C group) on all allylamine-containing spectra after subtraction was calculated and compared.

Force measurements were conducted on an allylamine exposure sample and a PIII treated sample in low ionic strength buffer solution to compare the electrostatic charge (force curves do not show the repulsion force between an AFM tip and the negatively charged

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