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High-density protein patterning through selective plasma-induced fluorocarbon deposition on Si substrates

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ABSTRACT

A novel method for fabrication of protein microarrays through selective plasma-induced modification of patterned substrates is presented. Exposing Si substrates bearing SiO_2 or Si_3N_4 patterns to a c-C_4F_8 plasma in a high-density plasma reactor, a fluorocarbon (FC) film was selectively deposited on Si areas, whereas the SiO_2 or Si_3N_4 patterns were simultaneously etched. Optimizing the plasma parameters such as power, bias voltage and gas pressure, patterned substrates with highly selective protein adsorption capacities were obtained. More specifically, using fluorescently labeled protein solutions direct selective protein binding onto SiO_2 or Si_3N_4 areas versus the Si substrate was verified. In addition, model binding assays were demonstrated through proteins immobilization on the patterned substrate and their subsequent reaction with fluorescently labeled counterpart molecules. Patterned Si/SiO_2 or Si/Si_3N_4 surfaces not subjected to plasma treatment presented negligible protein adsorption. Following the proposed method, highly resolved protein spots with diameters down to 1 μm were created. The spots presented high intra-spot fluorescence homogeneity (coefficient variation (CV) ≤ 10%), inter-spot fluorescence repeatability (CV ≤ 5%) and excellent morphology. Thus, substrates prepared following the proposed method can be applied to the fabrication of high-density and high-quality protein microarrays.

1. Introduction

Recently, microarrays have become an invaluable tool for large-scale and high-throughput bioanalytical applications. They have been used for basic research, diagnostics and drug discovery, and their significance and future applications have been reviewed extensively in the literature (Wilson and Nock, 2001; Zhu and Snyder, 2003; Venkatasubbarao, 2004; Cretich et al., 2006; Yap and Zhang, 2007). Microarrays consist of immobilized biomolecules spatially addressed on solid substrates such as planar surfaces (usually modified glass slides), microchannels, microwells, and arrays of beads. They allow fast, easy, and parallel detection of thousands of addressable elements in a single experiment using minimum sample volumes. Biomolecules commonly immobilized on microarrays include oligonucleotides, polymerase chain reaction (PCR) products, proteins, lipids, peptides and carbohydrates.

The simplest way to immobilize a protein onto a surface is through physical adsorption. This approach is based on binding of the macromolecules either by electrostatic forces on charged surfaces (Haab et al., 2001) or by hydrophobic interactions (Cahill, 2001) on various polymeric substrates such as poly(methyl methacrylate) (PMMA), polystyrene, cyclic olefin polymers or polycarbonate (Feng et al., 2004; Laib and MacCraith, 2007). Another approach to immobilize proteins is through covalent binding onto appropriately activated surfaces (Guilleaume et al., 2005).

The substrate to be used for microarray fabrication must be characterized by negligible autofluorescence and minimal non-specific binding and should permit the creation of spots with high intra- and inter-spot uniformity, and good morphology. Spotting inhomogeneity that is often witnessed as "coffee ring" effect (increased signal at the spot periphery compared to its center area) (Deegan et al., 1997) has been attributed to the hydrophilicity of the substrates commonly used for the creation of microarrays. In addition, the substrate hydrophilicity results in relatively large spot size due to protein droplet spreading thus, limiting the ability to create high-density microarrays. Therefore, development of novel technologies able to produce smaller and more uniform spots is under pursuit.

Attempts towards this direction employ the use of hydrophobic substrates (Ressine et al., 2007) or hydrophobic/hydrophilic patterning of substrates (Kai et al., 2003; Hoff et al., 2004; Lee et al.,

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2004; Hozumi et al., 2004; Rucker et al., 2005). In a few cases, plasma treatment has been applied to modify the surface properties, followed by additional chemical modification of the patterned surface to induce covalent protein binding (Hoff et al., 2004).

Here we present a method based on a one-step FC plasma treatment of patterned Si/SiO_2 or Si/Si_3N_4 substrates that results to highly selective protein adsorption on SiO_2 or Si_3N_4 areas. According to the proposed method, patterned Si/SiO_2 or Si/Si_3N_4 substrates were obtained following standard lithographic/etching processes. Treatment of these substrates in c-C_4F_8 plasma under appropriate conditions (plasma power, bias voltage and gas pressure) resulted in selective deposition of a hydrophobic FC film on Si and simultaneous etching of SiO_2 or Si_3N_4 patterns. By optimizing the plasma conditions, it was possible to achieve selective protein immobilization on the etched SiO_2 or Si_3N_4 patterns over the surrounding Si surface covered by the FC film. Selective protein adsorption on the plasma modified SiO_2 or Si_3N_4 areas was demonstrated through both direct immobilization of fluorescently labeled biomolecules and model binding assays employing immobilization of rabbit gamma globulins or biotinylated bovine serum albumin and detection with fluorescently labeled anti-rabbit IgG antibody or streptavidin, respectively. The quality of the protein arrays was evaluated in terms of within-spot uniformity and between-spot repeatability. The use of the substrates prepared according to the proposed method for creation of multiple protein arrays was also demonstrated.

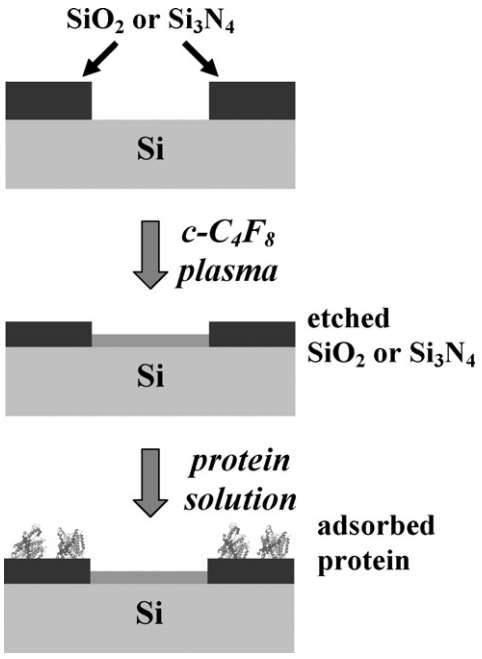

Fig. 1. Schematic of the process flow for the fabrication of protein microarrays using selective c-C_4F_8 plasma-induced FC deposition on Si followed by selective protein adsorption on hydrophilic c-C_4F_8 plasma-etched SiO_2 or Si_3N_4 patterns.

2. Experimental

2.1. Materials and reagents

Rabbit gamma-globulins (rabbit IgG) and bovine serum albumin (BSA, Cohn fraction V, RIA grade) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Goat anti-rabbit IgG antibody labeled with AlexaFluor® 488 (AF488) and streptavidin labeled with AlexaFluor® 546 (AF546) were purchased from Molecular Probes, Inc. (Eugene, OR, USA). Biotinylated BSA (b-BSA) was prepared according to a published method (Petrou et al., 2007). For lithography, AZ5214, a novolac-diazonaphthoquinone-type photoresist, was purchased from Clariant Co. (Leeds, UK). Four-inch silicon wafers were purchased from Montco Silicon Technologies, Inc. (Spring City, PA, USA). Plasma treatment was performed using the fluorocarbon gas perfluorocyclobutane (c-C_4F_8) provided by Air Liquide Hellas (Greece). All other chemicals were purchased from Merck (Darmstadt, Germany) and were used without further purification.

2.2. Patterning of silicon substrates and plasma treatment

The process flow used for substrate patterning and selective protein deposition is depicted in Fig. 1. SiO_2 or Si_3N_4 patterns on a Si substrate were created by conventional photolithography with AZ5214 resist, followed by wet etching of SiO_2 in buffered HF (BHF) solution or dry etching of Si_3N_4 areas, and photoresist removal. A 15-s O_2 plasma treatment (50 sccm, 10 mTorr, 400 W, 0 V, 15 °C) followed to remove AZ photoresist residues.

The high-density plasma reactor used in this work for selective etching/deposition was an Alcatel (MET, Micromachining Etch Tool) inductively coupled plasma (ICP) etcher, consisted of a load lock and an ultrahigh vacuum (10^{-6} mbar) main chamber. A one-loop ring-shaped antenna was supplied by a 0–2000 W rf (13.56 MHz) source, and generated the plasma through a 150 mm in diameter cylindrical alumina dome. The plasma diffused from the generation area in the process chamber, where samples were introduced for processing, loaded on a 4 in. Si substrate mechanically clamped on a chuck. The chuck was located at a distance of 20 cm downstream from the ICP source and allowed the sample to be biased and cooled during processing. The sample could be biased independently from the plasma source with a 300 W maximum rf (13.56 MHz) source. A helium pressure of 12 mbar was applied on the backside of the sample holder to provide good thermal conduction between the sample and the chuck. The temperature of the chuck was controlled by circulation of liquid nitrogen and simultaneous heat supply through six heaters, in order to achieve temperature regulation at a desired set point. Good thermal contact between the sample holder and the sample was achieved by means of thermal paste.

In this study, the FC gas perfluorocyclobutane (c-C_4F_8) was used. Before each experiment, the processing chamber was conditioned under the process conditions for 1 min. Plasma parameters such as gas pressure, rf plasma power and bias voltage were varied, whereas the electrode temperature was fixed at 0 °C.

The film thickness, as well as, etching or deposition rates on Si, SiO_2, and Si_3N_4 surfaces (blanket samples) were measured in real time by means of *in situ* ellipsometry using a M2000 Woolam spectroscopic ellipsometer (245–1000 nm, 470 wavelengths).

2.3. Surface characterization

The wettability of all treated samples was characterized by water contact angle measurements using a Digidrop Contact Angle Measurement System from GBX. The system allowed automatic loading of single droplets on the surfaces under investigation and measurements of static contact angles were performed through observation of the droplet (and its reflection) at nearly right angles with respect to the sample surface. For static water contact angle measurements in this work, the droplets volume was adjusted to 4 μl, in order to minimize the effect of gravity on droplet shape. Chemical characterization of untreated and c-C_4F_8 plasma-treated substrates was performed by means of X-ray photoelectron spectroscopy (XPS), at 0° and 60° incident angles, at the Surface Chemistry Lab., Department of Chemical Engineering, University of Patras (Prof. S. Kennou).