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ScienceDirect
Sensors and Actuators B 132 (2008) 361–367
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Micropatterning of protein-functionalized magnetic beads on glass using electrostatic self-assembly

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Available online 2 October 2007

Abstract

We demonstrate a simple and fast method for self-assembly-driven micropatterning of protein-functionalized magnetic beads on glass. We use positively charged aminosilane micro-patterns as template for selective immobilization of streptavidin-coated beads by electrostatic interactions. We show that addition of a non-ionic surfactant to the bead solution increases the selectivity of the micropatterning process. Streptavidin-coated magnetic beads (1.05 and 2.8 μm in size) are immobilized, with high reproducibility and in a very short processing time (∼30 min), in the form of stripes and dots, both on bare substrates and *in situ* in microfluidic channels. The arrangement and the number of immobilized beads can be controlled by tuning the aminosilane template size.

Keywords: Beads; Electrostatic self-assembly; Streptavidin; Micropatterned magnetic beads

1. Introduction

Numerous bio-assays are based on the patterning of proteins using micro- and nano-technologies. Protein microarray technology permits the high-throughput screening and analysis of several proteins in a single experiment [1]. Integrated microfluidic analytical systems [2] offer the advantage of strong analysis time reduction due to the small molecular diffusion distance, while consuming minute quantities of samples and reagents. However, these systems demand preparation and chemical activation of the binding surface, which impose additional treatments of the microchannel or reaction support. While printing or spotting of protein micro-patterns is a common technique, the use of protein-coated nano- or micro-particles offers additional advantages like an enhanced specific binding surface [3,4], a wide range of available bead surface chemistries and an easy recovery from the bio-analysis chip. Moreover, when microbeads incorporate a magnetic core, they can be easily manipulated using magnetic fields, irrespective of fluid flows [5]. Functionalized beads are widely commercially available and beads that are immobilized or micropatterned on surfaces are already used as platform for performing immunoassays [6,7], target DNA detection [8,9], as a scaffold for patterned cell culture studies [10], and as nucleation sites for growing and attaching permanent magnetic chains [11]. A number of bead patterning techniques on substrates has been proposed in the literature. The substrate may contain microcavities in which beads are physically trapped [12], or magnetic beads are locally immobilized using magnetic fields [9]. In the former case the trapped beads are not sticking to the surface and can be easily washed away, whereas the later method requires permanent magnets or electromagnets, making the system either bulky or complex. Also, one can use appropriate chemistries for attaching beads at selected areas of the substrate. Biotin–streptavidin interaction chemistry was used as the base for patterning streptavidin-coated beads on a template of biotinylated proteins [13], created by microcontact printing [14]. However, the mechanism of transferring proteins from a stamp to the surface is non-trivial [15] and very often the micro-contact-printed protein pattern has imperfections [16]. Alternatively, electrostatic interactions have been used as a tool for attaching charged particles to oppositely-charged surface patterns. The ease of surface charge patterning using conventional microfabrication techniques or by microcontact printing, makes this technique very attractive for

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doi:10.1016/j.snb.2007.09.076

patterning charged colloidal particles [17]. Patterned polyelectrolyte multilayers [18,19] and silane layers [20] have already been successfully used as a template for patterning a variety of charged polymer beads. Beads that were not functionalized with proteins were used in these studies.

In this paper, we show for the first time how *protein-functionalized* magnetic beads can be micropatterned on a glass surface using electrostatic interactions. Moreover, we use this technique for micropatterning protein-coated beads *in situ* in microfluidic channels with high reproducibility and in a matter of minutes. Streptavidin-coated magnetic beads are immobilized in the form of stripes and dots, whereby the exact arrangement and the number of immobilized beads can be controlled by the size of the micropatterned template layer. Our method has large potential, as the proposed electrostatic interaction technique allows the micropatterning of a wide variety of protein-coated beads on a single substrate, and may play a role in the development of future lab-on-a-chip bead-based bio-assays.

2. Experimental

2.1. Materials

(3-Aminopropyl)triethoxysilane (APTES) solution, phosphate buffered saline (PBS) solution of pH 7.4 and the non-ionic surfactant Tween-20 (which is Polyethyleneglycol (20) sorbitan monolaurate) were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). The PBS-Tween (PBST) solution (pH ∼7.4) was prepared by mixing 0.5% (v/v) of Tween- 20 with PBS. The streptavidin-coupled bead solution, Dynabeads® M-270 and Dynabeads® MyOne™ of bead diameter 2.8 and 1.05 μm, respectively, were purchased from Invitrogen AG (Basel, Switzerland). The stock concentration of Dynabeads® M-270 bead solution is 10 mg (∼6.7×10^8 beads) per ml and that of Dynabeads® MyOne™ is 30 mg (∼2.0×10^9 beads) per ml, respectively. Fluorescein isothiocyanate (FITC) conjugated IgG fraction of anti-streptavidin [Rabbit] in lyophilized powder form was purchased from Rockland Immunochemicals, Inc. (PA, USA). The negative photoresist SU8-50 and the positive photoresist AZ1512 were purchased from Microchem Corp. (MA, USA) and Shipley Europe Ltd. (Cheshire, UK), respectively. Poly-(dimethylsiloxane) (PDMS) pre-polymer and curing agent (Sylgard 184) was purchased from Dow Corning (MI, USA). Float glass wafers (Ø 4 in.) and deionized (D.I.) water of resistivity 18.2 MΩ-cm were obtained from EPFL's Center of Micro- and Nanotechnology. Ethyl vinyl acetate tubes (Microline model) of 0.51 mm inner diameter were purchased from Fischer Scientific (Wohlen, Switzerland).

2.2. Characterization

Atomic force microscopy (AFM) was performed at room temperature in tapping mode (spring constant 48 N/m) using a Nano instruments Nanosurf DT-40 to determine the thickness of the patterned APTES layer. Optical microscopy and scanning electron microscopy (SEM) was used to study the bead adsorption and bead patterning on glass. Optical micrographs were captured using a digital camera mounted on a Zeiss Axioskop 2 FS Plus. Prior to SEM imaging using a Philips XL30 FEG, the samples were coated with a 30 nm gold layer by sputtering. A stylus-based surface profiler (Tencor Alpha step-500) was used to measure the thickness and width of the SU-8 micropatterns that form the mold structure used for replicating microfluidic channels in PDMS.

2.3. Methods

2.3.1. Bead patterning on a glass substrate

The schematic in Fig. 1 illustrates the method of patterning protein-coated beads on a glass substrate using an APTES layer as the template for bead adsorption. The positive photoresist AZ1512 is spin-coated on a float glass substrate and patterned using standard photolithograpy (Fig. 1a). Then the exposed glass area is subjected to air plasma (Harrick plasma cleaner) for a minute, resulting in the creation of surface silanol groups. Following this, 1% (v/v) APTES solution in D.I. water is spin-coated at 5000 rpm, where after the wafer is baked at 100 °C for 2 min (Fig. 1b). This results in the covalent binding of the

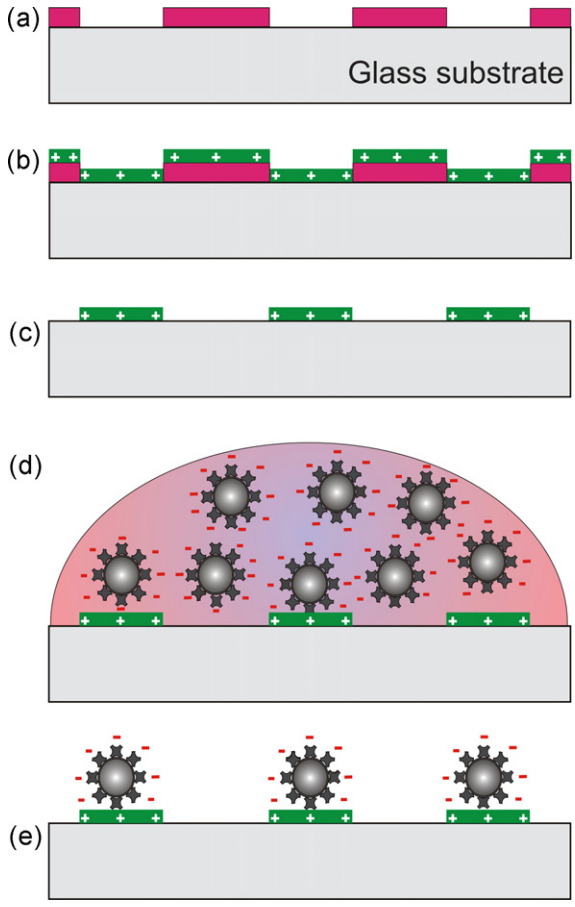

Fig. 1. Schematic illustration of the micropatterning process of streptavidin-coated beads on a APTES template using electrostatic self-assembly. (a) Positive photoresist micropattern on a glass substrate. (b) Spin-coating of the APTES layer. (c) Lift-off of the photoresist using ultrasonication. (d) A droplet of streptavidin-coated beads in PBS or PBST is incubated on the substrate. (e) Pattern of self-assembled beads after rinsing.