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Surface modification with BSA blocking based on in situ synthesized gold nanoparticles in poly(dimethylsiloxane) microchip

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ABSTRACT

A stable BSA blocking poly(dimethylsiloxane) (PDMS) microchannel was prepared based on in situ synthesized PDMS–gold nanoparticles composite films. The modified microchip could successfully suppress protein adsorption. The assembly was followed by contact angle, charge-coupled device (CCD) imaging, electroosmotic flow (EOF) measurements and electrophoretic separation methods. Contact angle measurements revealed the coated surface was hydrophilic, water contact angle for coated chips was 45.2° compared to a water contact angle for native PDMS chips of 88.5°. The coated microchips exhibited reproducible and stable EOF behavior. With FITC-labeled myoglobin incubation in the coated channel, no fluorescence was observed with CCD image, and the protein exhibited good electrophoretic effect in the modified microchip.

1. Introduction

The elastomeric polymer poly(dimethylsiloxane) (PDMS) has become one of the most popular substrates in microfluidic systems [1–5]. The intrinsic properties of PDMS can be both beneficial and disadvantageous, depending on the intended purpose of the device. The mechanical and optical properties of PDMS lend themselves to a variety of uses in microfluidics. There are also several disadvantages related to the use of PDMS, analyte absorption into PDMS has been well documented for nonpolar hydrophobic species [5–7].

Fortunately, many modification approaches have been employed on PDMS microchannels for increasing its hydrophilic property to reduce analytes adsorption and stabilize EOF. Various surface treatment methods for PDMS have been reviewed [8–10]. Generally, the surface modifications are time-consuming and hard to perform due to the difficulties in wetting and bonding for the channel with smaller diameters, bulk-modification method by adding a modifier during the fabrication process of microchips is a good approach to resolve these problems [11–15]. In recent years, many surface modification methods with colloids were reported [16–18].

As for PDMS microchips, the main aim of modification is to reduce the analytes adsorption to enhance separation efficiency, it is necessary to find suitable methods for modification to improve the surface properties. Zhang et al. reported a method for in situ synthesis of PDMS–gold nanoparticles composite films based on the special characteristics of PDMS itself [19]. In this work, we presented a simple and stable approach to suppress protein adsorption with BSA blocking on the in situ synthesized PDMS–gold nanoparticles composite films. The assembly was followed by contact angle, charge-coupled device (CCD) imaging, electroosmotic flow (EOF) measurements and electrophoretic separation methods. Contact angle measurements revealed the coated surface was hydrophilic, the water contact angle for coated chips was 45.2° compared to a water contact angle for native PDMS chips of 88.5°. The coated microchips exhibited reproducible and stable EOF behavior. With FITC-labeled myoglobin incubation in the coated channel, no fluorescence was observed with CCD image, and the protein exhibited good electrophoretic effect in the modified microchip.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade. Sylgard 184 (PDMS) silicone elastomer and curing agent were obtained from Dow Corning (Midland, MI, USA). MES, FITC (90.0%), bovine serum albumin (BSA), myoglobin (from horse skeletal muscle 95.0%) were from Sigma (St. Louis, MO, USA). Disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH) were obtained from Nanjing Chemical Reagents Factory (China). phosphate buffer saline (PBS) (20 mM pH 6.0 and 7.4), borate (20 mM pH 9.2). All solutions were prepared with doubly distilled

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water and passed through a 0.22 μm cellulose acetate filter (Shanghai Bandao Factory, Shanghai, China).

Myoglobin solution (1 mg/mL) was prepared in sodium bicarbonate (10 mM; pH 9.2). The fluorescent derivative reagent, FITC, was dissolved in DMSO at 20 mM just before use. The FITC solution was then mixed with protein solution in a molar ratio of 5:1, and the reaction was allowed to proceed at room temperature for 2 d in the dark. The labeled protein was stored at −20 °C in the dark before use. Prior to analysis, the derivatization solutions were diluted with running buffer to desired concentrations. For protein electrophoresis, 20 mM borate (pH 9.2) buffer was used, and the channels were flushed with running buffer prior to each run.

2.2. Fabrication of PDMS microchips

The master with a positive relief structure of GaAs for the channels was made using microphotolithographic technique. A cross-type channel of PDMS chip with a 3.30 cm long separation channel (effective separation length, 2.80 cm) and 1.0 cm long injection channel and a flat substrate were fabricated from PDMS as the previously described procedure [20,21]. Briefly, a mixture of elastomer precursor and its curing agent (ratio of 10:1) (sylgard 184) were degassed, poured over the GaAs master, and cured for 150 min at 80 °C. After the replica was peeled from the mold, holes (3 mm diameter) were punched. A flat PDMS substrate (0.3 mm height) was obtained via casting and curing the prepolymer mixture in a large flat glass box (5 cm × 5 cm). The PDMS layer with microchannels and the PDMS flat were ultrasonically cleaned subsequently with water, ethanol, and water, then dried under infrared lamp. Finally, they were sealed together to form a reversible PDMS microchip. The sampling channel's width and depth were 30 μm and 18 μm, the separation channel's width and depth were 50 μm and 18 μm, respectively. The laser-induced fluorescence (LIF) detection length of the separation channels was fixed at 2.80 cm from the intersection along the separation channel.

2.3. Synthesis of gold nanoparticles–PDMS composite film and BSA blocking

In situ synthesis of PDMS–gold nanoparticles composite films was performed according to the reference [19]. Briefly, $HAuCl_4$ 0.01 g/mL were syringed into each channel and incubated at 37 °C for 48 h. All resulting chips were washed with deionized water for three times, and stored at 4 °C when not in use. After the rinsing process, the chip was incubated with BSA (5%) at 37 °C for 1 h and rinsed with PBS (20 mM pH 7.4), deionized water, and dried.

2.4. Contact angle measurements

A flat piece of PDMS with BSA blocking was prepared according to the method in Section 2.3. A static contact angle measurement was performed on the plate using a CAM2000 optical contact angle analyzer (KSV Instruments, Finland). 5 μL of water droplet was placed on the material with pipette and allowed to rest on the surface for 1 min, and then an image was taken. The contact angle was recorded automatically.

2.5. Fluorescence image

Fluorescence images were obtained by means of a microscope (Nikon ECLIPSE TE2000-U, Japan).

2.6. EOF measurements

EOF measurements were performed using the current monitoring method [22]. Experiments were performed by measuring the current changes. Briefly, the wasting buffer reservoir and the channel were filled with a certain running buffer (c mM) and the buffer reservoir was filled with the same type buffer (0.9 c mM). Upon application of the voltage (1000 V), electroosmosis took place and the lower concentration electrolyte solution from the running buffer reservoir gradually displaced the higher concentration buffer in the channel, resulting in a decrease in the electrical current of the channel. Once a constant current was obtained, the potential was then applied to the reservoir with concentrated buffer and the above procedure repeated. The time required to reach a current plateau was used to calculate EOF based on Eq. (1) where L is the length of the separation channel (3.30 cm), V is the total applied voltage (1000 V), and t is the time in seconds required to reach the new current plateau.

$$m_{EOF} = \frac{L^2}{Vt} \quad (1)$$

In all cases, electrical connections to the microfluidic devices were made with platinum electrodes placed into reservoirs.

2.7. Capillary electrophoresis arrangement and electrochemical detection

The simple and effective homemade nonconfocal LIF detector was used. Briefly, an aircooled argon ion laser (Sanle Optical Company, Nanjing, China) with a 488 nm excited wavelength was adjusted to focus the laser on the microchannel with an incident angle of 45° by a precise 3D adjustor (Shanghai Lianyi Instrument Factory of Optical Fiber and Laser, China) with a precision of ±1 μm in each direction. The adjustor was fixed on a slab staged under a stereoscopic microscope (XTB-1; Jiangnan Optical Instrument Factory, Nanjing, China). The fluorescence emission signal was collected by an optical fiber of 2 mm diameter connected with an inverted microscope via a 40× objective, and then passing through a 535 nm band-pass filter amplified by a photomultiplier tube (PMT, 750 V) equipped with an amplifier. A homemade signal-recording system was used to control the power supply and the PMT. Meanwhile, it was used to record the amplified output signals from the PMT. A homemade power supply provided a stable and continuously variable high voltage ranging from 0 to 5000 V.

All the reservoirs were loaded with buffer solutions. Then the chip was placed onto the slab and the platinum electrodes were put into four reservoirs. The cross-injection was carried out by applying high voltage to the sample reservoir for several seconds through Pt electrodes connected to the power supply, with the sample waste reservoir grounded and the other two reservoirs floating. Once the injection was finished, the separation voltage was applied to the buffer reservoir with the waste reservoir grounded and the other two reservoirs floating.

3. Results and discussion

3.1. Principle of BSA blocking on PDMS–gold nanoparticles composite films

PDMS–gold nanoparticles composite films could be prepared with dropping $HAuCl_4$ solution on PDMS surface [19]. PDMS is one of the most permeable rubbery polymers, which is used in membrane-based separation applications of vapor or small molecules. The crosslinking of PDMS is based on the reaction between silicon hydride (Si–H) groups in the curing agent and vinyl groups (Si–CH–CH_2) in the monomer. In the cured PDMS, there remains some Si–H group and its concentration was controlled by the mass ratio of the curing agent and the monomer. The greater ratio results in a higher concentration of the residual