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In situ bio-functionalization and cell adhesion in microfluidic devices

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Abstract

Protein immobilization is of great importance for the development of biosensors, immunoassays and microfluidic devices. In this work, we study the in situ bio-functionalization and cell adhesion in microfluidic channels. A way of covalently linking antibodies on the channel surface while keeping their bio-functionality and its application for efficient cell adhesion were shown. Soft lithography has been used to fabricate simple microfluidic devices and all chemical and biologic materials were injected into microfluidic channels for the in situ bio-functionalization and cell adhesion. Our results show that the established bio-processing protocol significantly enhances the protein immobilization on both glass and poly-dimethylsiloxane surfaces, thereby allowing one to develop a large number of applications.

Keywords: Microfluidics; Lab on a chip; Bio-MEMS; Nanotechnology; Surface chemistry; Bio-functionalization; Cell adhesion

1. Introduction

Microfluidics is an emerging technology for advanced analytical chemistry, biology, diagnostics and biomedical research [1–4]. Since the first demonstration of microfluidic device application in 1992 [5], many works have been focused on the chip-based capillary electrophoresis to separate the species from simple chemical molecules to more specific bio-molecules [6–9]. At the same time, the development of individual components for fluidic control such as fluidic injection, valve, pump and mixer, has also received much attention [10–13]. In practice, not only device engineering issues but also biochemical processes at micro and nanoscale are important for prospect applications. For instance, the immobilization of bio-molecules should be the key step for the development of new biosensors. Previously, Tarlov and coworkers [14,15] immobilized the DNA probes in microfluidic channels by photopolymerization and developed a fluorescence based DNA diagnostic

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microfluidic assay. Delamarche and coworkers [16] used the microfluidic network pattern to fabricate the immunoassay through the nonspecific adsorption of proteins on poly-dimethylsiloxane (PDMS) substrate. In this work, we present a method of in situ bio-functionalization and cell adhesion in microfluidic devices. This method is based on covalently bonding of antibodies in microfluidic channel surfaces which is applicable for both PDMS and glass substrates. Simply, a silanized regent, 3-aminopropyltriethoxysilane (APTES) was used to generate a layer of amino group (NH_2–) on microfluidic channel surface. Then, the antibodies could be covalently bond to the amino group with their carboxyl group (–COOH) by amide bonds. Finally, antigen could be easily attached onto the channel surface through the bio-affinity between the antibody and antigen, while keeping their bio-functionality. To show its application for reliable and efficient cell adhesion, we will demonstrate the immobilization of a form of murine cancer cell, S180 cells, to the fibronectin modified microchannels.

2. Experimental

2.1. Materials and chemicals

3-Aminopropyltriethoxysilane (APTES, Sigma), *N*-hydroxysuccinimide (NHS, Sigma), *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC, Sigma), goat IgG from serum (Sigma), anti-goat IgG-FITC conjugate (Sigma), anti-fibronectin IgG (Sigma), fibronectin (Sigma), bovine serum albumin (BSA, sigma), phosphate buffered saline tablet (PBS, Sigma), poly-dimethylsiloxane (PDMS RTV615, GE) were used as received. All the solutions were prepared with 18.2 MΩ cm water from a Simplicity 185 pure water system (Millipore, USA).

2.2. Microfluidic device fabrication

Soft lithography has been used to fabricate simple microfluidic devices with channels of 100 μm width and 50 μm height. We have used a high resolution (3600 pixel per square inch) printer to pattern a transparent plastic film as mask for contact printing optical lithography. The designed fluidic channels were then replicated into a thin layer of AZ-100 photo-resist deposited on a silicon substrate by a UV light exposure. Before elastomer (PDMS) casting, the resist patterns were exposed to trimethylchlorosilane (TMCS) vapour for 1 min. Then, the liquid pre-polymer PDMS was poured on and cured at 80 °C for 1 h. After peel-off of the cross-linked PDMS structures, holes were punched through the elastomer to allow later fluidic access to the reservoirs. Afterward, the PDMS pieces were exposed to an oxygen plasma and placed on another PDMS slide or a glass plate. Finally, the ensemble was heated again at 80 °C for 1 h, which led to an irreversible PDMS–PDMS or PDMS–glass bonding.

2.3. Microchannel silanization

The microfluidic devices were surface modified with an amino group layer by injecting 2% APTES in 95% acetone into the microchannel for 2 h at room temperature. As shown in Fig. 1, the surface silanization reaction was dependent on the hydroxyl layer generated with the oxygen plasma treatment. It is reported that the hydroxyl layer on PDMS will disappear 1 h later. So this step should be carried out immediately after the PDMS layer was attached on the glass substrate with oxygen plasma treatment. The microchannels were then rinsing with acetone to remove unreacted silane. The layer of amino group (NH_2–) on the microchannel surface could be used for further bio-functionalization.

2.4. Microchannel bio-functionalization

Before injection into the microchannels, antibodies were mixed with 10 mM NHS and 20 mM EDAC to activate their carboxyl group. After 1 h of incubation at room temperature, the activated antibody could covalently bound onto the microchannels surface. For the control experiments, no antibodies were injected into the microchannels for immobilization. After washing with 1 × PBS (pH 7.4) and water by