Sensors and Actuators B 150 (2010) 478–482

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Short communication

Parylene-coating in PDMS microfluidic channels prevents the absorption of fluorescent dyes

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ARTICLE INFO

Article history:
Received 11 June 2010
Received in revised form 9 July 2010
Accepted 12 July 2010
Available online 18 July 2010

Keywords:
Poly(dimethylsiloxane)
Fluorescence
Absorption
Parylene
Microfluidic channel

ABSTRACT

Absorption of fluorescent molecules is a troublesome property of microfluidic devices fabricated with PDMS. The absorption raises background-levels of fluorescence from PDMS, and reduces concentrations of injected fluorescent molecules in PDMS channels. We report here that conformal deposition of poly-*p*-xylylene derivatives (parylenes) on the surface of PDMS substantially suppressed the absorption of Rhodamine B. In a test of this technique, we found parylene-coated PDMS channels to be useful in accurate analyses using fluorescence intensity.

1. Introduction

Poly(dimethylsiloxane) (PDMS) has been the most popular material in microfluidics community as it is optically transparent, chemically inert, biologically compatible, inexpensive and easy to pattern by soft lithography [1–3]. The porous nature of PDMS is convenient for cell-based studies, because it allows oxygen and carbon dioxide to freely diffuse through it [1,3,4]. However, this characteristic is also a drawback of PDMS, which has hampered PDMS microchannels from applications in physical sciences communities. PDMS absorbs organic solvents and small hydrophobic molecules from solution [1,4,5]. Various hydrophobic fluorescent dyes such as Nile red [4], quinine [4], BODIPY [6], and Rhodamine B (RhB) [7,8] also partition into the PDMS wall in the channel, which could raise background-levels of fluorescence from PDMS, reduce concentrations of injected fluorescent dyes [4,9], and consequently make fluorescence data quantitively poor (Fig. 1a). To solve this absorption problem, a number of efforts have been made to modify the surface of PDMS channels, such as plasma treatment [10], silanization [11,12], polymer grafting [13], adsorption of polyelectrolytes [6,14], adsorption of detergents [15], precoating with proteins [16] or phospholipid bilayers [17]. These modifications, however, have not cleared off the problem [1].

In this study, we propose an alternative approach to overcome the dye-absorption problem of PDMS channels: deposition of a non-porous polymer film on the inner surface of the channel. Poly-*p*-xylylene derivatives (parylenes) (Fig. 1b) were chosen for this purpose, because parylenes form non-porous transparent films that block the diffusion of gasses or organic solvents through it [18]. Parylenes have been used for preventing absorption of proteins and DNA onto PDMS surface [19]. However, parylene effect on the dye-absorption problem has not been reported. We systematically designed and fabricated parylene-coated straight channels with various dimensions and examined absorption of RhB. We also investigated the temperature dependence of RhB fluorescence [7,8,15,20,21] in parylene-coated PDMS channels, and demonstrated that the parylene-coating technique enables intensity-based analyses in PDMS channels.

2. Materials and methods

Straight microchannels were used in this study. The cross-sectional width and height of the channels were varied in the ranges of 15–200 μm and 25–100 μm, respectively, and the channel length was 2–20 mm. The microfluidic devices were fabricated using standard soft lithography procedures. Briefly, we casted PDMS substrate (Sylgard 184, Dow corning) on a SU-8 (Nippon Kayaku) master mold fabricated on a 2-in. silicon wafer, degassed

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doi:10.1016/j.snb.2010.07.021

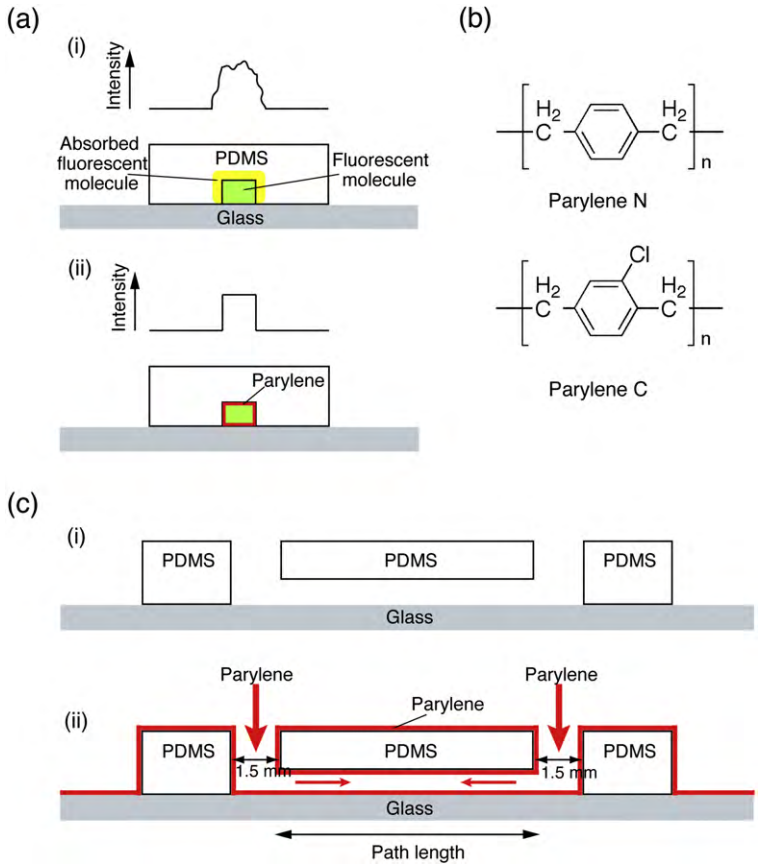

Fig. 1. (a) Schematic illustrations of parylene effect in PDMS channels. Fluorescent molecules absorbed in PDMS make channel-crossing line profiles quantitively poor (i), but parylenes deposited in PDMS channels prevent the absorption (ii). (b) Chemical structures of parylene N and C. (c) Side-view illustrations of PDMS channels without (i) and with a parylene-coat (ii).

for 20 min and baked at 75 °C for 90 min. We then punched to make two holes (1.5-mm diameter) in PDMS microchannel, and placed it on a glass slide (76 mm × 26 mm, thickness 0.8–1.0 mm; Matsunami) without O_2 plasma treatment (Fig. 1c(i)). Parylene deposition in PDMS microchannel was performed with a commercially available parylene coater (PDS2010, Parylene Japan) under 4–9 Pa. The parylene deposition was carried out after the attachment to a slide glass, because PDMS does not stick to the glass once coated with parylenes. Temperatures used for vaporization, pyrolysis and deposition of parylenes were 165–175 °C, 650–690 °C and room temperature, respectively. Vaporized parylene-monomers enter the microchannels from the two holes, and spread along the channel (Fig. 1c(ii)). To compare the amount of parylenes deposited in PDMS channels, a glass slide was placed as an external standard in the sample chamber. Microscopic images were recorded using an inverted microscope (IX-71, Olympus) mounted with a 512 × 512 pixel iXonEM + 897 EMCCD camera (Andor technology). In all fluorescence experiments, 50 μM RhB dye (Tokyo chemical industries) in 50 mM 3-morpholinopropanesulfonic acid (MOPS)–tris(hydroxymethyl)aminomethane (Tris) buffer (pH7.0) was used. RhB was excited through an appropriate filter set (U-MWIG3, Olympus), with a 100 W mercury arc lamp when capturing images. The excitation light from mercury arc lamp was weakened by 32ND25 (Olympus) filter so as to minimize photobleaching of RhB solution. The liquid temperature in PDMS channels was changed by a temperature-controllable (up to 50 °C) plate installed in the microscope stage (ThermoPlate MATS-52NLR, Tokai Hit). Samples were allowed to equilibrate at the required temperatures for at least 5 min prior to acquisition of the images. Three measurements were performed to obtain each dataset for temperature-fluorescence intensity plots.

3. Results and discussion

Like other fluorescent molecules, RhB filled in PDMS channels was absorbed in PDMS walls immediately (Fig. 2a), and remarkable background fluorescence was detected even after washing the channel (Fig. 2b). However, parylenes deposited on the surface of the channels prevented the absorption (Figs. 2c and d). We first investigated the efficiency of parylene deposition in the microchannels. We then assessed the effect of parylene-coating on the fluorescence analyses.

3.1. Maximum path length free from RhB absorption

Vaporized parylenes enter the PDMS channels from the two holes, and then spread along the channel (Fig. 1c(ii)). Because straight channels were used in our studies, parlylenes that spread in the opposite directions in the channels meet at the midpoint. Hence, the parylene-layer thickness in PDMS microchannles is the thinnest (sub-μm) at the midpoint (data not shown). If no RhB absorption is detected at the midpoint, the whole channel is free from RhB absorption. To elucidate the maximum path length that is free from RhB absorption, we prepared PDMS microchannles with different lengths (2–20 mm) (Fig. 1c), and collected fluorescence line profiles across the channel midpoints. Because the line profile with absorbed RhB has a broader spectral shape than that without absorption (Fig. 2e), spectral width of the channel-