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Original Article

Comparison of Surface Modification Techniques on Polydimethylsiloxane to Prevent Protein Adsorption

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Abstract Polydimethylsiloxane (PDMS) is commonly used in microchip fabrication due to its biocompatibility, which is essential for biological applications, as well as other properties, including transparency to visible light, controllable gas permeability, mechanical and heat stability, and elasticity. Despite these properties, adsorption of biomolecules remains a major limitation of PDMS in biochips. Methods to prevent sample adsorption have been reported, and herein we compare several surface engineering methods that do not incorporate plasma pretreatment. Three methods - Teflon coating, water-repellent spraying, and perfluorodecyltrichlorosilane (FDTS) blending - were compared by evaluating the amount of fluorescein-isothiocyanate-conjugated bovine serum albumin (FITC-BSA) adsorbed onto the biochips. FDTS-blended PDMS significantly inhibited protein adsorption and showed good oleophobicity, but provided the lowest visible light transmittance of all materials tested.

Keywords: PDMS, Protein adsorption, No plasma treatment, Teflon, Water-repellent, Perfluorodecyltrichlorosilane, FDTS, FITC-BSA

Introduction

Polydimethylsiloxane (PDMS) is a commonly used chemical in biochip fabrication due to its low cost, ease of fabrication, gas permeability, biocompatibility, and flexibility[1-4]. However, bare PDMS is easily contaminated because of its hydrophobic surface[5,6], and the inhibition of its nonspecific adsorption has been studied using various methods[5-7]. Physical adsorption of proteins makes the surface more biocompatible, but may impact the durability of the modified surface because desorption of the protein would affect the biochip operation. Chemically bound coating agents have been applied because of their good stability and durability during the fabrication process[6]. Surface activation using a plasma treatment is the most general protocol applied before chemical coating[8]. However, plasma treatment damages the PDMS surface and eventually generates cracks[8,9], which may enhance the adsorption of nano-sized biomolecules.

Several coating methods have excluded the plasma-mediated activation step to prevent damage to the PDMS. For example, Teflon can be coated on the PDMS surface without activation[10,11]. Since the Teflon coating is chemically resistant, biocompatible, and gas-permeable, it may be suitable for application in biochips. Commercially available water-repellents can also be easily coated a PDMS surface. By passing through a repellent solution, a fouling-resistant surface can be formed[12]. Furthermore, perfluorodecyltrichlorosilane (FDTS) is widely used due to the ease with which it can coat surfaces at room temperature. It is possible to form an FDTS-arrayed surface by bonding it to the surface by vapor deposition, or by blending it with PDMS before curing[13].

In this study, we compared three different surface engineering methods without a plasma treatment to identify which methods may are the most suitable for biochip applications. Fluorescent-labeled bovine se-

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rum albumin (FITC-BSA) was adsorbed after surface modification. The contact angle and transmittance were measured to evaluate the properties of the modified PDMS (Figure 1). The results described herein may help guide the choice of coating agents used for PDMS-based biochips in future studies.

Results and Discussion

Contact Angle Evaluation on Different Surfaces

Three solvents (5 μL each) typically used in microfluidic experiments, distilled water (DW), mineral oil, and phosphate buffer saline (PBS), were dropped onto the PDMS surfaces, as shown in Figure 2A. Since PDMS has a hydrophobic surface, the water-based solutions (DW and PBS) had contact angles greater than 90°, and no significant difference between the DW and PBS drops were observed. Conversely, mineral oil showed a lower surface angle of $<90°$. In particular, the FDTS-blended surface showed a significantly higher contact angle for the mineral oil drop (Figure 2B), indicating that the FDTS-blended surface may be more oleophobic. These values were also compared with standard measurement values. The standard contact angles of DW are slightly higher than the values measured experimentally, due to the imprecise control of the droplet when it came into contact with the surface (Table 1). According to ISO 15989, 5 μL of droplet solution should detach from the nozzle by the interaction energy between the surface and the droplet caused by the removal of the effect of gravity. On FDTS-blended surfaces, the DW contact angle was slightly higher and the difference from other values was significant ($p<0.01$). Diiodomethane (CH_2I_2) was used as a standard hydrophobic solution, and its contact angles on Teflon-coated and FDTS-blended surfaces were significantly higher than that on PDMS. Comparing the contact angle of two different hydrophobic solutions on each type of surface revealed that CH_2I_2 always had higher contact

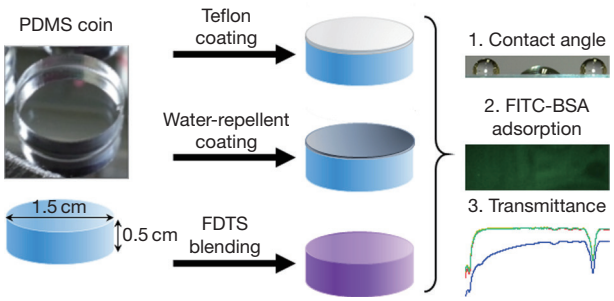

Figure 1. Preparation and evaluation of the PDMS coins with and without modification. Teflon and water-repellent was coated PDMS coin surfaces, and FDTS blended with uncured PDMS and then shaped into a coin. Surface properties such as contact angle, FITC-BSA adsorption, and transmittance of each group were compared.

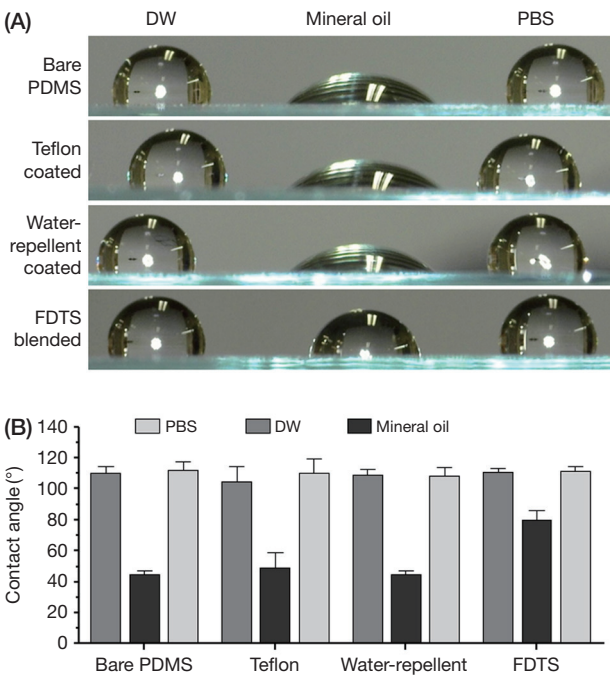

Figure 2. (A) Droplets of various solvents with a volume of 5 μL were placed on each modified surface and (B) their contact angles were measured. Similar to the bare PDMS, the water and buffer had high contact angles on the modified surfaces. For the FDTS-blended group, oleophobicity was observed.

Table 1. Contact angles on different surface types measured by two methods.

Surface type	Lab-scale measurement			Standard measurement (ISO 15989)	
	DW	PBS	Mineral oil	DW	CH_2I_2
Bare PDMS	109.9 (±4.9)	111.9 (±5.5)	44.7 (±2.7)	113.4 (±0.7)	91.8 (±2.9)
Teflon coated	104.7 (±9.8)	110.1 (±9.4)	49.1 (±9.6)	111.6 (±6.3)	103.7* (±9.6)
Water-repellent coated	109.2 (±3.6)	108.5 (±5.2)	44.7 (±2.5)	113.8 (±0.4)	91.5 (±1.4)
FDTS blended	111.0 (±2.0)	111.6 (±3.0)	79.8** (±6.3)	116.0* (±1.8)	101.5** (±1.9)

*$p<0.05$; **$p<0.01$ compared to Bare PDMS

angles. This was likely due to the higher hydrophobicity of the mineral oil (logP > 6) than that of CH_2I_2 (logP = 2.5)[14]. However, the order decreasing contact angles for both solutions were the same, FDTS-blended > Teflon-coated > water-repellent-coated ~ bare PDMS. It should be noted that the contact angle difference for mineral oil between the Teflon-coated and water-repellent-coated/bare surfaces was not significant. Consequently, the results indicate that both Teflon and FDTS are more capable of generating oleophobic surfaces compared to bare PDMS.

Protein Adsorption on the Modified PDMS Surfaces

A key factor in the development of a precise and sensitive biochip is the reduction of unwanted noise by preventing nonspecific adsorption of biomolecules. The most abundant protein found in blood is albumin, which can inhibit the detection of trace molecules in blood analysis. To mimic this problem, we adsorbed FITC-BSA to determine whether the modified surfaces had fewer interactions with the protein. As shown in Figure 3, bare PDMS showed strong adsorption of FITC-BSA even though it was applied at a relatively low concentration, 0.01% (w/v). In comparison, all the modified surfaces adsorbed less protein. The inhibition effects of albumin are significant, so all the tested modification methods should be effective in preventing protein-surface interaction and enhancing detection of trace molecules. In particular, FDTS-blended PDMS adsorbed the least amount of FITC-BSA by a factor of ~100.

From the results of contact angle measurements and protein adsorption, no clear relationship between the surface energy and adsorption ability was observed. However, the more hydrophobic FDTS-blended surface showed the lowest protein adsorption, indicating that oleophobicity is an important factor in protein adsorption. The mechanism of BSA adsorption remains ambiguous, as previous reports have been conflicting, with some indicating that the preferred surface for BSA adsorption is hydrophilic[15,16] and others concluding that a hydrophobic surface is preffered[17,18]. Fluorinated molecules have been widely used for inhibition of surface fouling, which often involves protein adsorption[11,19]. The protein adsorption would be more significantly influenced by the surface structure than the contact angle[19], and the performance of the modified surfaces is expected to differ because of the different percentage of fluorinated functional groups on PDMS. For FDTS-blended PDMS, FDTS molecules migrate to the surface of PDMS[13], which may result in a higher local concentration of fluorinated chains on the surface.

Transmittance Evaluation of Surface-modified PDMS Coins

Transparency is a key property of biochip materials, enabling the support of *in-situ* optical detection of molecules. Thus, the ideal coated or blended molecules would not resulted in transparency attenuation. When light was passed through a 5-mm-thick PDMS coin, the intensity was reduced to ~92% of its original value in the visible region. As shown in Figure 4, Teflon and the water-repellent coating did not affect the transparency of the resulting biochip. Using the FDTS-blended PDMS coin, the transmittance decreased by up to 60% compared to that of the untreated PDMS coin. This translucent property was also recognized during the experiment (Supplementary Figure 1B). The FDTS was blended but not coated, in contrast to the Teflon

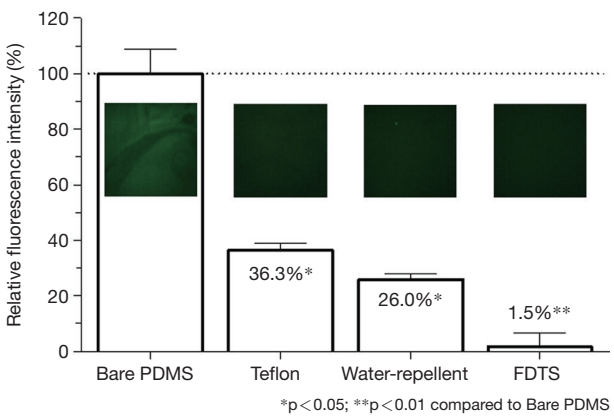

Figure 3. FITC-BSA was adsorbed on different surface types and the intensity of green fluorescence was measured. Compared to bare PDMS, every modified group showed a significant decrease in protein adsorption. The FDTS-blended PDMS performed best in inhibiting protein adsorption.

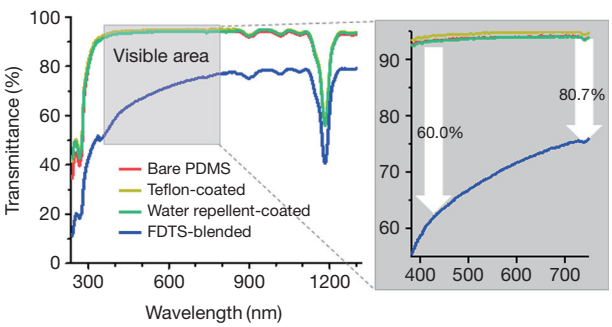

Figure 4. Transmittance change of the PDMS coins depending on their surface modification. Every modified surface except the one blended with FDTS showed similar transmittance values to that of bare PDMS. Transparency of the FDTS-blended coin decreased by approximately 60%.

and water-repellent surfaces, so the optical properties of FDTS could be affected by the ~5 mm thickness of the coins. In contrast, the other coated materials were laminated on the PDMS surfaces so that they were just sufficient to coat the inside of the micro channels (<100 μm) without blocking them[10,11,13]. If the thickness of FDTS-blended PDMS can be controlled when fabricating a surface-modified biochip, it is likely that the transmittance can be increased. However, no considerable increase of transparency is guaranteed, so the blending methods may be more suitable to engineer the inner walls of microfluidic chips with complex structures.

Summary

In this paper, we compared three different surface modification techniques to determine their applicability to PDMS-based biochips. To avoid plasma damage to the PDMS, we chose methods without plasma pretreatment steps. Through a comparison of contact angles, the FDTS-blended and Teflon-coated surfaces were determined to be more oleophobic. Protein adsorption was significantly inhibited on each type of modified surface, but no relationship could be found between the contact angle and protein adsorption. For FDTS-blended PDMS, the least amount of protein was adsorbed on the surface, but it exhibited the lowest transmittance of all surfaces, which may impact the *in-situ* observation of the microfluidic processes on the biochip. If these optical issues can be overcome, the FDTS blending method could be suitable for fabricating complex microfluidic channels. The reported results provide insight into the selection of surface engineering methods suitable for the production of novel biochips.

Materials and Methods

Fabrication of the PDMS Coin

A silicone elastomer base and curing agent (Sylgard® 184, Dow Corning, MI) were used to fabricate the PDMS coins. A mixture with a 10 : 1 ratio of elastomer to curing agent was degassed and poured into a polymethyl methacrylate mold with holes 5 mm high and 15 mm wide. The open side of the mold was sealed with a silicon wafer and held together with a clip. After curing for 40 min at 80°C in an oven, the cured PDMS coins were detached from the mold and then additional curing was performed for 18 h at 80°C in the oven.

Surface Engineering of the PDMS Coin

The procedures for surface engineering using Teflon, a water-repellent, and FDTS followed previously reported methods with only minor modifications. For the Teflon coating[10], Teflon powder (Poly(4,5-difluoro-2,2-bis(trifluoromethyl)-1,3-dioxole-co-tetrafluoroethylene, Sigma-Aldrich, St. Louis, MO, USA) was melted in Fluorinert® FC-40 (Sigma-Aldrich, St. Louis, MO) for 4 days on a 50°C hot plate to a final concentration of 3 wt%. The Teflon solution of volume 0.043 μL/mm^2 was spread on the PDMS coins, and dried at room temperature. For the water-repellent coating[12], the PDMS coins were first washed with DW for 5 s. Then, water-repellent (RAIN & Snow Repellent Agent, 3M, St. Paul, MN, USA) was sprayed onto the surface of the PDMS coins for 5 s and washed with DW again for 5 s. The engineered PDMS coins were then dried fully at room temperature. For the fabrication of FDTS-blended PDMS coins[13], FDTS (trichloro(1H,1H,2H,2H-perfluorooctyl)silane, Sigma-Aldrich, St. Louis, MO, USA), a liquid fluorocarbon chemical, was blended with an uncured and degassed PDMS mixture to a final concentration of 1.42 wt%. Then, it was poured into the mold and the FDTS-PDMS mixture was cured at 80°C for 18 h. The FDTS-blended PDMS coins were cooled for 5 min and ejected from the mold. All engineered PDMS coins were stored at room temperature before use.

Contact Angle Measurement

To measure the contact angles, three types of droplets, phosphate buffer saline (PBS, 0.01M, pH 7.4), mineral oil (Sigma-Aldrich, St. Louis, MO, USA), and distilled water (DW) were dropped onto the surface of the modified and unmodified PDMS coins. After adding 5 μL of each drop, horizontal images of the droplets were taken using a digital camera within 1 min to prevent the contact angle from changing due to decreased surface tension. The droplet images were analyzed using ImageJ to calculate the angles between the PDMS coin surface and the droplet boundary near the surface. Both side angles were measured and averaged. An additional measurement of the contact angle was performed following ISO 15989 guidelines for a more accurate evaluation.

Protein Adsorption Test

Protein adsorption was evaluated using isothiocyanate-labeled bovine serum albumin (FITC-BSA) (Molecular Probes® by Life Technologies™). To introduce the same volume of the FITC-BSA solution onto the modified surfaces, 0.6 mL of solution was carefully placed on the PDMS coins, whose volume was maximized to cover the whole area of the PDMS coin surfaces without flowing off. The concentration of FITC-BSA solu-

tion was 0.01% (w/v) and placed on the PDMS coins for 3 min to induce protein adsorption. After 3 min, the remaining solution was sucked off and the surfaces washed thoroughly with PBS for 1 min. Additional washing was performed using DW for 1 min and the remaining water droplets were removed using a blower. The adsorption test for all fabricated coins were performed in triplicate. The amount of adsorbed FITC-BSA was calculated by quantifying the fluorescent intensity of the imaged surfaces using ImageJ software. The relative intensities of each modified group were compared with the results of the bare PDMS group.

Transmittance Measurement

The surface-modified PDMS coins were placed in a UV-visible spectrophotometer (Hitachi U-4100) and their spectra were measured at 240-1300 nm. The transmittance of the light at each wavelength was recorded and compared.

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