Simple surface modification of poly(dimethylsiloxane) for DNA hybridization

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Here, we present a simple chemical modification of poly(dimethylsiloxane) (PDMS) by curing a mixture of 2 wt% undecylenic acid (UDA) in PDMS prepolymer on a gold-coated glass slide. This gold slide had been previously pretreated with a self-assembled hydrophilic monolayer of 3-mercaptopropionic acid (MPA). During curing of the UDA/PDMS prepolymer, the hydrophilic UDA carboxyl moieties diffuses toward the hydrophilic MPA carboxyl moieties on the gold surface. This diffusion of the UDA within the PDMS prepolymer to the surface is a direct result of surface energy minimization. Once completely cured, the PDMS is peeled off the gold substrate, thereby exposing the interfacial carboxyl groups. These groups are then available for subsequent attachment of 5′-amino terminated DNA oligonucleotides via amide linkages. Our results show that the covalently tethered oligonucleotides can successfully capture fluorescein-labeled complementary oligonucleotides via hybridization, which are visualized using fluorescence microscopy. © *2010 American Institute of Physics.* [doi:10.1063/1.3523055]

I. INTRODUCTION

Poly(dimethylsiloxane) (PDMS) is a moldable silicon-based elastomeric polymer with numerous advantages over other polymeric systems. These advantages include biocompatibility, gas permeability, relative chemical inertness, readily disposable, optical transparency, ease of bonding and molding into (sub)micrometer features, and low manufacturing costs.[1,2] However, the most problematic issue with native PDMS is its hydrophobicity. Therefore in order to improve the surface wettability of PDMS for, in particular, microfluidic applications it is necessary to modify the surface chemically.

To date, the wettability of PDMS surfaces has been improved by using methods such as plasma treatment,[3–6] UV treatment,[7,8] chemical vapor deposition,[9–11] layer-by-layer (LBL) deposition,[12–14] sol-gel coatings,[15,16] silanization,[17,18] dynamic modification with surfactants,[19–22] and protein adsorption.[23–25] In addition, combinations of UV or plasma treatment with chemical methods such as silanization,[26–28] graft polymerization,[29–34] and LBL methods[35,36] have been explored. A recent review by Zhou *et al.*[37] details the latest methods used to modify PDMS surfaces.

The bulk modification of PDMS is also critical in the fabrication of stable hydrophilic surfaces and in particular surfaces that do not undergo hydrophobic recovery. This has been achieved by the addition of a nonionic surfactant (TX-100),[38] an amphiphilic biocompatible copolymer poly-(lactic acid)-poly(ethylene glycol),[39] *n*-dodecyl *β*-D-maltoside,[40] or undecylenic acid[40] (UDA) into the PDMS prepolymer. Pertinent to the work presented here Luo *et al.*[40] used 0.5 wt% UDA in PDMS prepolymer and cast this onto a silicon wafer. However, they found no reduction in the water contact angle of the UDA/PDMS modified surface.

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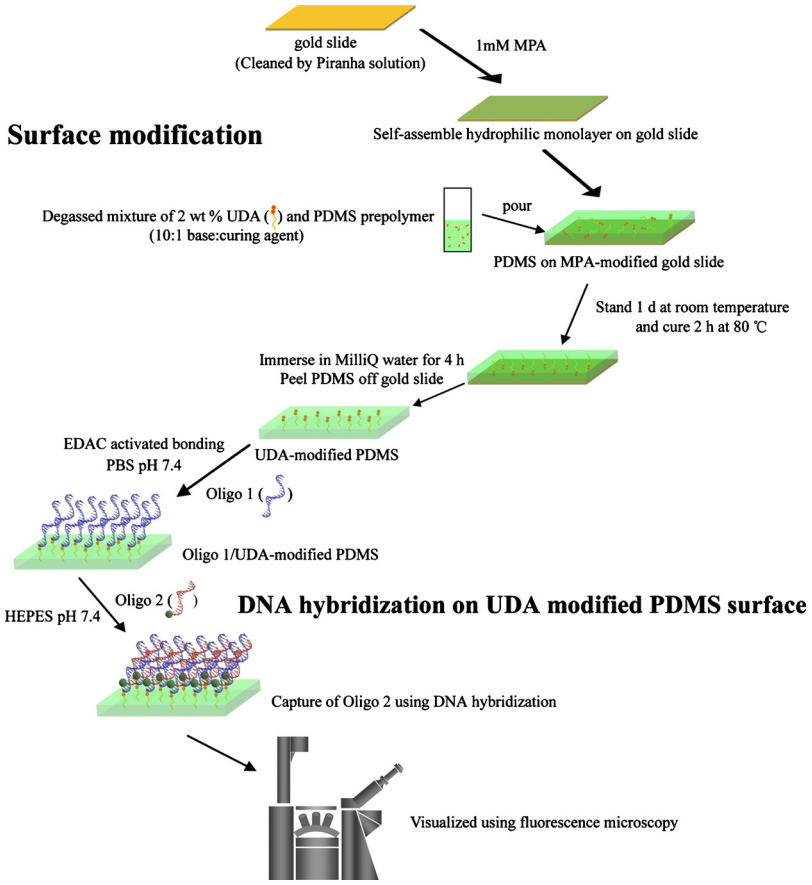

FIG. 1. Process of PDMS surface modification by UDA and DNA hybridization on UDA-modified PDMS surface. UDA: undecylenic acid; oligo1: 5′-amino AAA AAA AAA CCA CCC CTA CCA CTA ATC CCC; oligo2: 5′GGG GAT TAG TGG TAG GGG TGG-fluorescein and MPA: 3-mercaptopropionic acid; EDAC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

Here, we generate a PDMS surface with decreased hydrophobicity by adding 2 wt% UDA into the PDMS prepolymer. This mixture was then cast onto a hydrophilic 3-mercaptopropionic acid (MPA) self-assembled monolayer (SAM) coated gold slide. The UDA carboxyl moieties then migrate to the hydrophilic SAM coated gold surface. Once the UDA/PDMS is peeled off the gold the new UDA-modified PDMS surface had a water contact angle (WCA) 20° lower than native PDMS. In addition, Fourier transform infrared-attenuated total reflection (FTIR-ATR) spectroscopy showed the presence of carboxyl groups on the UDA-modified PDMS surface. These carboxyl groups remained stable at room temperature in phosphate buffered saline (PBS) for 3 and 17 h. Once the presence of the carboxyl groups had been verified by WCA and FTIR-ATR, 5′-amino oligonucleotides were attached to the carboxylated UDA-modified PDMS surface via amide linkages. Fluorescein-labeled complementary oligonucleotide strands where then passed over the modified surface and the fluorescence measured using a fluorescence microscope (Fig. 1).

II. METHODS

A. PDMS surface modification

1. Preparation of MPA-modified gold slides

Gold-coated glass slides were purchased from Platypus Technologies, USA. Each slide was cleaned with fresh piranha solution (1:3 (v/v); H_2O_2:H_2SO_4) for 5 min, then rinsed with MilliQ water (18.2 MΩ) and dried under a stream of nitrogen. The clean gold slide was then immersed

in 1 mM MPA in 100% absolute ethanol (Ajax Finechem Pty Ltd, New Zealand) for 24 h. The MPA-modified gold slides were then removed and washed with absolute ethanol. The sessile drop WCAs measured for the cleaned and MPA-modified gold slides were 72° and 20°, respectively.

2. Preparation of UDA-modified PDMS on a MPA-modified gold surface

PDMS Sylgard 184 (Dow Corning Corporation, USA) was purchased as a two-component kit, including prepolymer (base agent) and cross-linker (curing agent) components. PDMS (10:1 weight ratio of base and curing agent) and 2 wt% UDA (Sigma-Aldrich, Australia) were thoroughly mixed and degassed by applying a gentle vacuum to remove air bubbles. The degassed UDA/PDMS prepolymer mixture was subsequently poured onto the MPA-modified gold slide and cured at 80 °C for 2 h then left to stand at room temperature for one day. As a control, native PDMS was cured on a blank gold slide without MPA-modification. In addition, in order to confirm that the UDA carboxyl moieties preferentially migrate to a hydrophilic MPA modified gold slide, a mixture of UDA/PDMS was also cured on a hydrophobic 2-mercaptopyridine (2-MP)-modified gold slide which had been previously prepared by immersing the gold slide in 2% (w/v) 2-MP in absolute ethanol for 24 h.

3. Removal of UDA-modified PDMS from the gold slides

In order to remove the UDA-modified PDMS sample from the MPA-modified gold slide the entire assembly was immersed in MilliQ water for 4 h, the UDA-modified PDMS sample was then peeled off the gold slide and sequentially rinsed with MilliQ water, ethanol (75%), and then dried under a stream of nitrogen. Each surface was then analyzed using WCA and FTIR-ATR.

4. Stability tests of UDA-modified PDMS

The stability of the UDA-modified PDMS surfaces was tested in pH 7.2 PBS (50 mM phosphate, 10 mM ethylenediaminetetraacetic acid (EDTA), 0.15 M of NaCl, pH 7.2) for 3 and 17 h at room temperature. These conditions were chosen as they are typical of normal hybridization conditions. Each surface was then analyzed using FTIR-ATR.

B. DNA hybridization on the UDA-modified PDMS surface

Oligonucleotides were synthesized with an ABI 394 DNA synthesizer producing the sequences as follows: oligo1: 5′-amino AAA AAA AAA CCA CCC CTA CCA CTA ATC CCC, oligo2: 5′-GGG GAT TAG TGG TAG GGG TGG-fluorescein, and oligo3: 5′-TTT GGC CTA AGG TCT CGA AG-fluorescein (used as a noncomplementary strand control). PBS and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer were used for the attachment of oligo1 and hybridization with oligo2 and oligo3, respectively. The formulations of the PBS and HEPES buffer were as follows. PBS (pH 4.8): NaH_2PO_4 (30 mM); PBS (pH 7.4): NaCl (137 mM), KCl (54 mM), Na_2HPO_4 (10 mM), KH_2PO_4 (2 mM); and HEPES buffer (pH 7.4): HEPES (10 mM), NaCl (200 mM), EDTA (1 mM). For pH adjustment of the buffers, NaOH (0.1 M) and HCl (0.1 M) were used.

1. Attachment of the oligonucleotide capture probe

In the process of DNA attachment, the UDA-modified PDMS surface was first treated with 4 mg/mL 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) in PBS (pH 4.8) at room temperature for 2 h. Then 10 μM amino-terminated DNA (oligo1, in PBS, pH 7.4) was reacted with the carboxyl groups on the UDA-modified PDMS surface at room temperature for 2 h via condensation activation using the carbodiimide EDAC. This produced the so-called oligo1/UDA-modified PDMS.

2. Hybridization with the complementary oligonucleotide

Subsequent to the above covalent attachment of oligo1, a fluorescein-labeled complementary DNA single strand (oligo2, 10 μM in HEPES buffer, pH 7.4) was placed on the oligo1/UDA-modified PDMS surface and hybridized at 4 °C overnight (~18 h).

C. Characterization

1. WCA measurements

Static WCAs were measured using the sessile drop method by placing a small drop (2 μL) of MilliQ water onto the sample surface via a syringe, a digital image of which was taken by a Panasonic SuperDynamic WV-BP550/G camera with a macrolens. The image was processed by IMAGEJ software V1.34. All reported water contact angles are the average value of five measurements on different parts of the sample.

2. FTIR-ATR

FTIR-ATR spectra were obtained on a Nexus 870 (Thermo-Nicolet). A total of 64 scans were taken at room temperature and atmospheric pressure with a resolution of 4 cm^{-1}. The data were collected from 625 to 4500 cm^{-1} and analyzed using OMNIC version 7.0 software (Thermo-Nicolet).

3. Detection of DNA hybridization

Samples of the native PDMS and hybridized oligo1/UDA PDMS were placed on glass slides and examined under an IX 81 inverted fluorescence microscope (Olympus, Japan) through a 450–490 nm band pass excitation filter. A line scan on each sample was performed and analyzed using LS RESEARCH software (Olympus, Japan) to produce an intensity profile.

III. RESULTS AND DISCUSSION

A. Characterization of UDA-modified PDMS

The WCA of UDA-modified PDMS after removal from the MPA-modified gold was measured at $91.4 \pm 2.0°$, which is 18° lower than the WCA of native PDMS ($109.8 \pm 0.4°$). This decrease of WCA indicates that the wettability of the PDMS surface has been improved by adding UDA into PDMS prepolymer. In addition, the UDA/PDMS sample removed from the hydrophobic 2-MP-modified gold slide was measured at $104.2 \pm 1.6°$, which is only 5° lower than the WCA of native PDMS. This result showed the UDA carboxyl moieties preferentially migrate onto the hydrophilic surface to minimize the interfacial free energy.

Figure 2(a) shows the FTIR-ATR spectrum of native PDMS between 1600 and 1800 cm^{-1} indicating the absence of carboxyl moieties in this region, which is in accordance with the literature.[41,42] From Fig. 2(b), it is clear that the UDA-modified PDMS has different spectral features with the appearance of two characteristic carboxyl peaks one at 1715 cm^{-1} due to hydrogen bonded carboxylic groups and the other at 1730 cm^{-1} due to free carboxylic acid.[43] This clearly indicates the presence of UDA on the surface. Furthermore, these groups remain after storage of the samples in pH 7.2 PBS for 3 h or 17 h at room temperature [Figs. 2(c) and 2(d), respectively]. Clearly, the diffusion of the UDA to the interface has resulted in immobilized carboxyl groups, which do not undergo the typical hydrophobic recovery observed in traditional oxidized PDMS. These groups are then available for attachment of oligo1 via amide coupling. The reduction at the peak of 1730 cm^{-1} is likely due to the diffusion of unbonded UDA to the PBS.

B. DNA hybridization on the UDA-modified PDMS surface

Figures 3(a) and 3(b) show the native PDMS surface and the UDA-modified PDMS surface after exposure EDAC, then oligo1 with subsequent hybridization with oligo2, respectively. The native PDMS surface shows a slight fluorescence, which is most likely due to nonspecific DNA adsorption of the fluorescein-labeled oligo2. However, Fig. 3(b) shows an increase in the observed

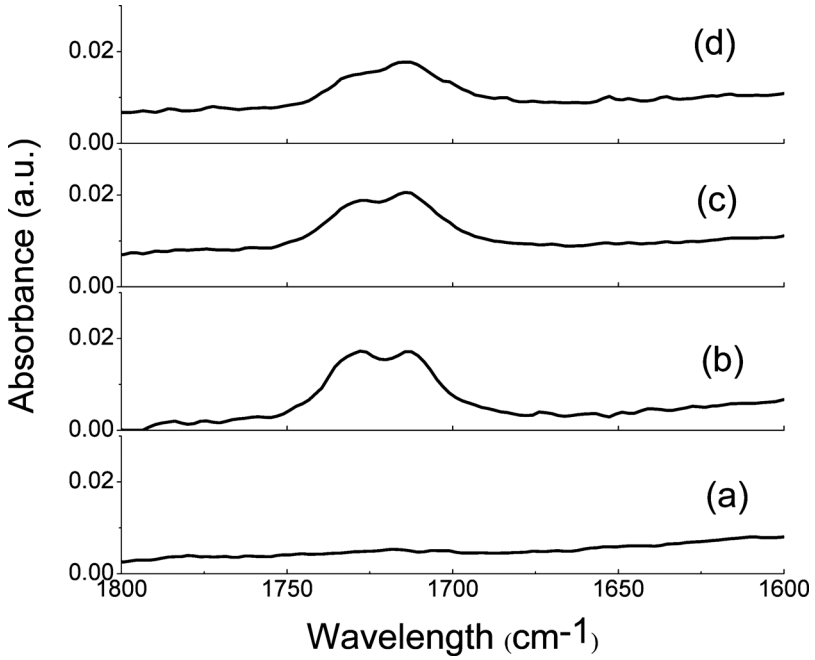

FIG. 2. FTIR-ATR spectra of (a) native PDMS, (b) UDA-modified PDMS, (c) UDA-modified PDMS after in pH 7.2 PBS for 3 h at room temperature, and (d) UDA-modified PDMS after in pH 7.2 PBS for 17 h at room temperature.

intensity of the oligo1/UDA-modified PDMS hybridized with the oligo2. Analysis of the images using a line scan of intensity profile is shown in Figs. 3(c) and 3(d) as a dotted line. For the purpose of comparing fluorescence intensity on native PDMS and UDA-modified PDMS surfaces, the fluorescence intensity values on the different glass slides were normalized. As a result, the average fluorescence intensity along the dotted line for the native PDMS surface was 30 ± 9.7 [Fig. 3(c)] while for the oligo1/UDA-modified PDMS hybridized with oligo2 this value was

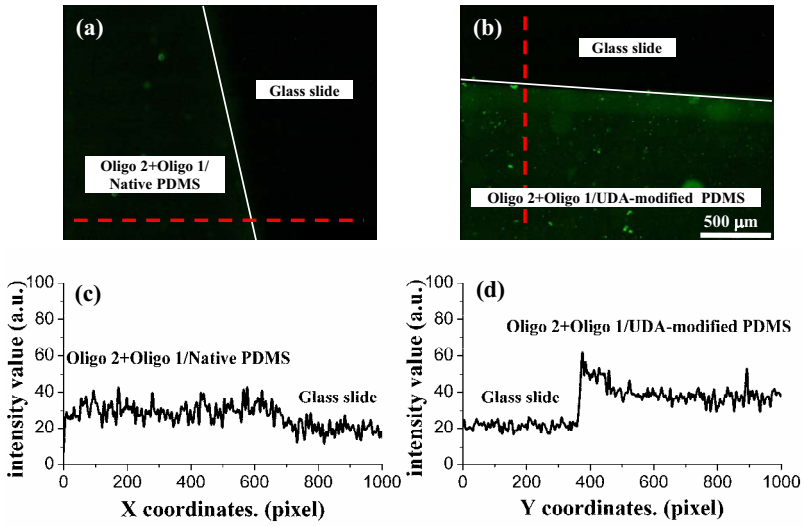

FIG. 3. Fluorescence microscopy images of (a) oligo1/native PDMS and (b) oligo1/UDA-modified PDMS after DNA hybridization after exposure to oligo2. (c) shows the line intensity profile, marked as dotted lines, of oligo1/native PDMS (from left to right) and (d) the oligo1/UDA-modified PDMS (from top to bottom) after DNA hybridization. The samples are placed on a glass slide for microscopy imaging.

44.6 ± 5.9 [Fig. 3(d)]. An increase in intensity of 1.3 times after DNA hybridization was thus observed, indicating successful PDMS surface modification and DNA hybridization. The bright spots observed in both Figs. 3(a) and 3(b) may be due to scattering centers or surface asperities with increased fluorescence in these regions.

As a control, an UDA/PDMS modified surface was reacted with EDAC, then reacting the surface with oligo2 instead of oligo1. Analysis of this surface showed fluorescence only from nonspecific adsorption (intensity value: 17 ± 12.2) (data not shown), which confirms that the covalent immobilization of oligo1 is required for sequence-specific hybridization. Furthermore, fluorescein-labeled noncomplementary DNA oligo3 was used as a control and hybridized on an oligo1/UDA-modified PDMS surface. As expected, only a small amount of fluorescence was observed from nonspecific binding (intensity value: 23 ± 9.6) (data not shown).

IV. CONCLUSION

PDMS surfaces were successfully functionalized with carboxyl groups by curing a mixture of PDMS and 2 wt% UDA on a 3-mercaptopropanoic acid modified gold slide. The pendent carboxyl groups formed at the PDMS surface were then covalently linked to an amino-terminated oligonucleotide capture probe. A fluorescently labeled complementary oligonucleotide strand was then successfully hybridized to the surface.

Typically in order to surface-functionalize native PDMS, the prepolymer is cured, then functionalized using a variety of wet and physical chemistries. Here, we show a simpler 1-step method. This method is potentially amenable to a wide range of diverse functionalities that can be introduced on the PDMS surface by simple deposition of mixtures of prepolymers and functionalized molecules on either a hydrophobic or hydrophilic template.

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