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Solution-Phase Surface Modification in Intact Poly(dimethylsiloxane) Microfluidic Channels

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An improved approach composed of an oxidation reaction in acidic H_2O_2 solution and a sequential silanization reaction using neat silane reagents for surface modification of poly(dimethylsiloxane) (PDMS) substrates was developed. This solution-phase approach is simple and convenient for some routine analytical applications in chemistry and biology laboratories and is designed for intact PDMS-based microfluidic devices, with no device postassembly required. Using this improved approach, two different functional groups, poly(ethylene glycol) (PEG) and amine (NH_2), were introduced onto PDMS surfaces for passivation of nonspecific protein absorption and attachment of biomolecules, respectively. X-ray electron spectroscopy and temporal contact angle experiments were employed to monitor functional group transformation and dynamic characteristics of the PEG-grafted PDMS substrates; fluorescent protein solutions were introduced into the PEG-grafted PDMS microchannels to test their protein repelling characteristics. These analytical data indicate that the PEG-grafted PDMS surfaces exhibit improved short-term surface dynamics and robust long-term stability. The amino-grafted PDMS microchannels are also relatively stable and can be further activated for modifications with peptide, DNA, and protein on the surfaces of microfluidic channels. The resulting biomolecule-grafted PDMS microchannels can be utilized for cell immobilization and incubation, semiquantitative DNA hybridization, and immunoassay.

Microfluidic devices have been widely used in biomedical analyses[1−4] and chemical reactions,[5−8] often with a substantial improvement in performance over conventional benchtop systems. Among various microfluidic systems, poly(dimethylsiloxane) (PDMS)-based microfluidic devices[9,10] have been gaining popularity due to advantages such as easy fabrication, low cost, practical scalability, optical transparency, and gas permeability. Additionally, the elasticity of PDMS matrixes enables the integration of pressure-driven valves[11] and pumps[12] with microfluidic channels, permitting execution and automation of complex chemical[8] or biological processes[2] within a single microfluidic chip. Despite the many advantages of PDMS-based microfluidics, certain issues with these devices remain. Due to the inherent hydrophobicity of PDMS materials, PDMS-based microfluidic channels are repulsive to aqueous solutions and can easily be contaminated (the so-called "biofouling" issue) by biological samples (e.g., peptides, proteins, serum, blood, and cells). Therefore, several approaches for surface modification of PDMS materials have been developed to confer hydrophilicity[10,13−16] and biomolecule-repelling properties[10,17,18] to PDMS surfaces. Moreover, tethering groups, e.g., amino (NH_2) groups[19] and thiol (SH) groups,[20] can also be grafted onto PDMS substrates for subsequent attachment of a variety of biomolecules. Consequently, these surface modification approaches can be utilized for chemical immobilization of probe molecules in PDMS-based microfluidic channels for applications of miniaturized biological arrays and assays.[19,20]

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In general, PDMS surfaces are treated with oxygen plasma,[13] UV radiation,[21] and UV/ozone to introduce hydrophilicity by replacing silane (Si−Me) groups with silanol (Si−OH) groups. The properties of these silanol-covered surfaces are, however, dynamic. As a result, progressive restoration[14,22] of hydrophobicity occurs within a few minutes. Silanization reactions on the freshly prepared silanol-covered PDMS surfaces [10,14−17,19,20] introduce functional groups that enable desired surface properties to be conferred to PDMS substrates. For example, poly(ethylene glycol) (PEG)[14,15,17] can be attached onto PDMS substrates by carrying out silanization reactions on the oxygen plasma-treated PDMS substrates. The resulting PEG-grafted PDMS surfaces exhibit protein-repelling characteristics[17] with relatively longer lifetime (a few weeks) against the recovery[14] of hydrophobicity. To date, the instability of silane-modified PDMS surfaces has not been fully addressed. Other approaches, such as radical graft polymerization[23] and reactive polymer coating,[24] have been developed to generate stable and durable functional polymer coatings on PDMS substrates. Nevertheless, most of the existing surface modification methods[10,13,14,17−20] are not feasible for modifying the surfaces of intact (fully assembled) microfluidic channels that are deeply embedded in PDMS matrixes. In addition, from the standpoint of device fabrication, surface-modified PDMS components often face the challenges of device assembly and microchannel sealing. These problems limit further development of PDMS-based microfluidic devices, especially for their applications in chemical and biological analysis.

Herein, we describe an improved approach composed of a solution-phase oxidation reaction in acidic H_2O_2 solution and a sequential silanization reaction using neat silane reagents for surface modification of intact microfluidic channels that are deeply embedded in PDMS matrixes. In contrast to the conventional approaches[10,14,15,17,19,20] (i.e., reactive oxidation and then silanization by diluted silane reagents), this improved approach includes advantages of (i) simple and convenient handling suitable for routine practices in both chemistry and biology laboratories, with no specialized instruments (i.e., oxygen plasma cleaner, UV light source, and ozone generator) required, (ii) great stability and fidelity of the resulting surface modifications, with no decay of surface performance, and (iii) appropriateness for intact PDMS-based microfluidic devices, with no device postassembly required. Using this improved approach, two different functional groups, PEG and amine, were introduced onto PDMS surfaces for passivation of nonspecific protein absorption and attachment of biomolecules, respectively. To characterize surface properties, X-ray electron spectroscopy (XPS) and temporal contact angle experiments[14,15] were utilized to monitor functional group transformation and dynamic characteristics of the PEG-grafted PDMS substrates. The experimental data suggest that the PEG-grafted PDMS surfaces exhibited improved short-term surface dynamics and robust long-term stability. In addition, a variety of fluorescent protein solutions were introduced into the PEG-grafted PDMS microchannels to test their protein repelling properties. The results indicate that the PEG-grafted PDMS microchannels exhibited novel protein repelling characteristics, which enhanced progressively over the initial period of 24 h and then persisted for a significant time (>2 months). Similarly, the amino-grafted PDMS microchannels are fairly stable and can be further activated for surface modifications with three types of biomolecules, including tripeptide (arginine−glycine−aspartic acid, RGD), amino-terminated single-stranded DNA, and a soluble, recombinant form of prostate stem cell antigen, PSCA.[25] Using these biomolecule-grafted PDMS microchannels, cell immobilization and incubation, semiquantitative DNA hybridization, and immunoassay were demonstrated in a miniaturized fashion, with the additional benefits of chemical/sample economy and operation efficiency. Over all, more than 200 single-channel microfluidic devices with channel height of 25 μm, channel widths ranging from 100 to 200 μm, and channel shapes both linear and circular were fabricated by soft lithography[26] and utilized in our studies.

RESULTS AND DISCUSSION

Surface Modification. Modification (Scheme 1) of PDMS-based microfluidic channels starts from the solution-phase oxidation reaction of PDMS surfaces **1**, which was carried out by continuously passing a mixture of H_2O/H_2O_2/HCl (in a volume ratio of 5:1:1) through the microchannels for 5 min. After purging the microchannels with deionized (DI) water and dry Ar, the hydrophilic silanol-covered PDMS surfaces **2** were obtained. Sequentially, neat 2-[methoxy(polyethylenxy)propyl]trimethoxysilane (**3**) was injected into the hydrophilic microchannels **2** to perform silanization reactions at room temperature for 30 min. The unreacted silane **3** was flushed from the microchannels by DI water to give the PEG-grafted microfluidic channels **4**, which were dried by Ar flow and preserved in Petri dishes under ambient environment for various periods of time (from 10 min to 8 weeks) prior to the protein repelling studies. It should be emphasized that the use of neat silane reagent **3** is critical for long-lasting protein repelling properties of the PEG-grafted microchannels **4**. Similar to the preparation of the PEG-grafted microfluidic channels **4**, the silanol-covered PDMS microchannels **2** were reacted with (3-aminopropyl)trimethoxy silane (**5**) to generate the amino-grafted PDMS surfaces **6**. The surface-grafted amino groups were converted to the isothiocyanate groups by introducing a 0.5% (v/v) thiophosgen solution in MeCN into the amino-grafted microchannels **6**. Again, after purging with DI water and dry Ar, the isothiocyanate-grafted PDMS microchannels **7** were then subjected to attachment reactions[27] with a variety of amino-terminated biomolecules, including tripeptide RGD, single-stranded DNA (5′-NH_2-$(CH_2)_6$-TTTTTTGGTT-GGTGT-GGTTGG-3′) and PSCA protein to produce the RGD-grafted PDMS surfaces **8**, the DNA-grafted PDMS surfaces **9**, and the PSCA-grafted PDMS surfaces **10**, respectively. These biomolecule-grafted microfluidic channels **8**−**10** were washed with phosphate-buffered saline (PBS, pH 7.4) or Tris−buffer solutions (pH 7.4) and preserved at 4 °C for at least 24 h prior to their respective studies.

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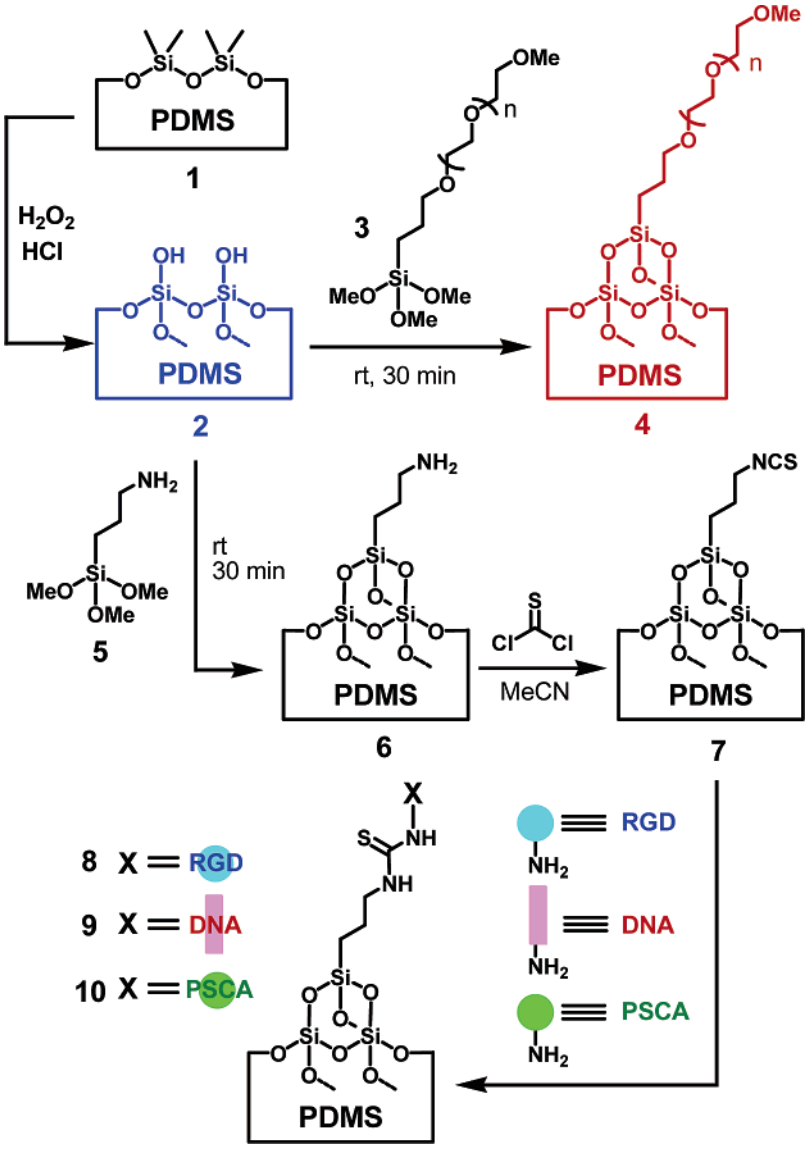
Scheme 1. Solution-Phase Surface Modification with Superior Stability and Fidelity[a]



[a] The approach was developed for preparation of the PEG-grafted PDMS microchannels **4** and amine-grafted PDMS Microchannels **6**. The amine-grafted microchannels **6** can be activated by thiophosgen to obtain the isothiocyanate-grafted PDMS microchannels **7** as a precursor for the RGD-grafted PDMS microchannels **8**, DNA-grafted PDMS microchannels **9**, and PSCA-grafted PDMS microchannels **10**.

XPS Analysis. A number of wafer-molded PDMS substrates **1** with dimensionalities of 4.0 cm × 4.0 cm × 0.5 cm were treated by the same silanization approach to give the silanol-covered substrates **2** and PEG-grafted substrates **4** for subsequent surface characterizations. XPS was utilized to validate the functional group transformation on the PDMS substrates. It is important to point out that the low-resolution XPS data (Figure 1) are semiqualitative because this analysis is unlikely to separate the photoemission contributions of the PEG-modified layers from the background of bulk PDMS. The XPS spectra of the untreated PDMS substrates **1** exhibited a ratio of 1.3 between the O 1s and C 1s photoemissions. A different O 1s/C 1s ratio of 1.6 was observed for the PEG-grafted substrates **4**, indicating[14] that the surface-grafted PEG layers changed the surface chemical compositions of the PDMS substrates. Further evidence of the surface-immobilized PEG groups are supported by the high-resolution XPS spectra (insets in Figure 1) of the PEG-grafted substrates **4**, where a new C 1s photoemission peak (280.3 eV) emerged immediately adjacent to the original Si-linked C 1s peak (279.8 eV). This new C 1s peak, centered at 280.3 eV, was identified as the O-linked carbon, which is a characteristic signal of surfaced-grafted PEG chains.

Contact Angle Analyses. Temporal contact angle experiments (Figure 2) were employed to monitor the dynamic surface characteristics of the silanol-covered substrates **2** as well as the PEG-grafted substrates **4**. For comparison, the untreated PDMS substrates **1** served as a control. Similar to the O_2−plasma-treated PDMS surfaces,[14] the silanol-covered substrates **2** obtained by the H_2O_2/HCl treatment lost their hydrophilic characters within 24 h when the substrates were stored in an ambient environment. In contrast to the H_2O_2/HCl-treated substrates **2**, the PEG-grafted substrates **4** exhibited a relative lower hydrophilicity after their surface modification. The hydrophobicity of the PEG-grafted substrates **4** was increased for the first 24 h and reached a stable state after storing these substrates in an ambient environment

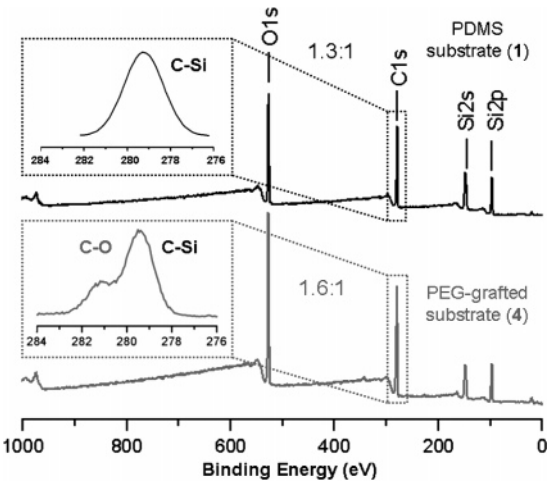


Figure 1. XPS spectra on PDMS substrates **1** and the PEG-grafted substrates **4** validating the functional group transformation before and after the PEG functionalization.

for 2−3 d. The long-term stabilization of the PEG-grafted substrates **4** is improved compared with that observed for previously reported silanization modification.[15] These results suggest that robust cross-linked PEG silane layers were created on the PDMS surfaces exhibiting the long-lasting hydrophilicity. We attribute the long-lasting hydrophilicity to the use of neat silane reagent **3**. Control experiments in which diluted silane **3** solutions were employed for the PEG modification showed poor fidelity in surface stability.

Protein Repelling Characteristics. To further study the surface dynamic property of these PEG-grafted PDMS surfaces **4**, a time-dependent investigation on the protein repelling characteristics of the PEG-grafted microchannels **4** was performed. A number of freshly prepared PEG-grafted microchannels **4** were preserved at ambient environment for 10 min, 30 min, 2 h, 1 d, 2 d, and 2−8 weeks, respectively. In this case, the intact PDMS microchannels **1** were employed as the control. The protein repelling study of each microchannel was carried out by first filling the channel with a concentrated solution (10 μL) of fluorophore-labeled proteins and incubating the microfluidic chip at 37 °C for 1 h. In our studies, three fluorophore-labeled proteins, i.e., fluorescein-labeled avidin (1.0 mg/mL solution in PBS), Alexa594-labeled fibronectin (0.5 mg/mL solution in PBS), and Alexa555-labeled bovine serum were utilized. The resulting protein-contaminated microchannel was then cleaned by flushing a PBS solution (100 μL, for 30 s) through the microchannel. Finally, the nonspecific absorption of the fluorophore-labeled protein was quantified by fluorescent microscopy (Figure 3a−c). For each microchannel, more than 30 fluorescent measurements were carried out at different locations where the channel widths and shapes are different. These measurement results showed a very small difference (<5%) through the entire microfluidic channel, suggesting that the PEG surface modification was quite homogeneous, without much influence by the channel widths and shapes. The time-dependent profiles (Figure 3d) for the protein repelling characteristics of the microchannels **4** showed consistent results for all three protein solutions. During the initial 24 h, the protein repelling property of the microchannels **4** improved progressively; thereafter, the protein repelling property lasted for more than two months. It is critical to note that the dynamic characteristics of protein repellency showed a good agreement in time scale with that (Figure 2) observed for the temporal contact angle measurements. The consistency of these observations suggests that the robust cross-linked silane layers bear well-oriented PEG chains exhibiting excellent protein-repelling property.

Cell Adhesion. Being able to specifically control cell immobilization and cell repulsion in microfluidic channels would advance many types of cell cultures,[28] cellular assays,[29] and microscale tissue engineering studies[30] in microfluidic systems. Tripeptide RGD, the smallest active fragment found in the extracellular matrix, is known[31] to be an important ligand for cell immobilization[32] through the RGD−integrin (a transmembrane protein) interactions. The RGD-grafted microchannels **8** were produced to test the feasibility to immobilize cells in PDMS-based microfluidic channels, whereas the PEG-grafted microchannel **4** was used for repelling cell adhesion. Again, the intact PDMS microchannels **1** served as the control for both types of microchannels. Here, A427 cells (colon cancer cell line, ATCC) suspended in Dulbecco's modified Eagle medium (DMEM) cell culture media (Invitrogen) were utilized in all experiments. A427 cells in culture medium were introduced into the microchannels **1**, **4**, **6**, and **8**, and the microfluidic chips were placed in the incubators at 37 °C for 4 h. Culture medium was then slowly flushed through the microchannels to remove unattached A427 cells. As shown in Figure 4a, a large number of A427 cells were immobilized on the RGD-grafted microchannels **8**. In contrast, no cells were immobilized in the PEG-grafted microchannels **4** (Figure 4d). A few cells were retained in the amino-grafted microchannels **6** (Figure 4b) and intact PDMS microchannels **1** (Figure 4c). The immobilized A427 cells survived for 4 d in the microchannels **8**, when these chips were kept at 37 °C and the cell culture medium was continuously and slowly introduced into the microchannels by gravity.

DNA Hybridization. DNA microarrays[33] are widely used tools in biomedical research. This technology requires specific attachment of probe DNA fragments onto discrete locations within a two-dimensional surface. Using the improved PDMS surface modification, probe DNA fragment (5′-NH_2-$(CH_2)_6$-TTTTTTGGTTGGTGTGGTTGG-3′) was attached onto microfluidic channels for demonstration of a semiquantitative DNA hybridization (Figure 5). A new type of PDMS-based microfluidic chip carrying parallel and individual accessible microfluidic channels (channel width, 100 μm) was fabricated and modified with the probe DNA fragment for this demonstration. These DNA-grafted microfluidic channels **9** were specifically exposed to different concentrations (i.e., 5, 25, and 50 nM, in Tris−buffer, pH 7.4) of the fluorophore-labeled target DNA (5′-5Cy3-CCAACCACA-CCAACCA-3′) solutions. As a control, an intact PDMS microchannel **1** was treated

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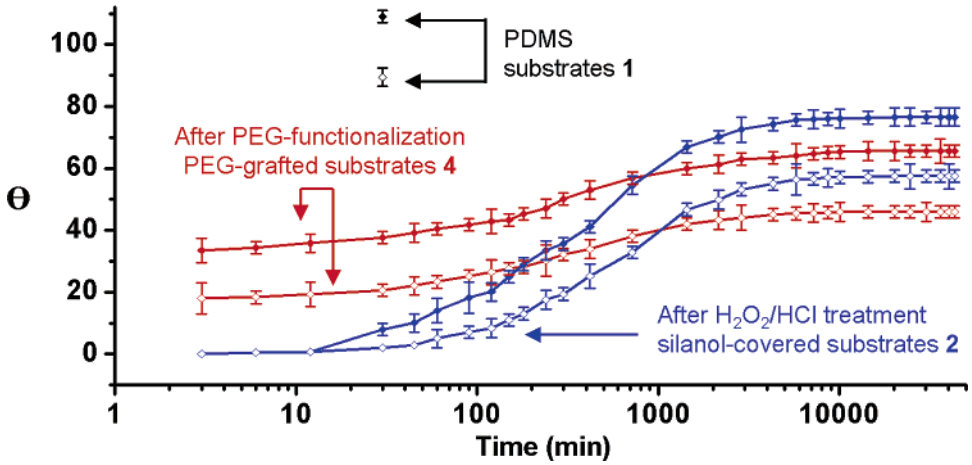

Figure 2. Temporal evaluation of the advancing (◇) and receding (◆) contact angles with DI water of the silanol-covered substrates **2** and PEG-grafted substrates **4**.

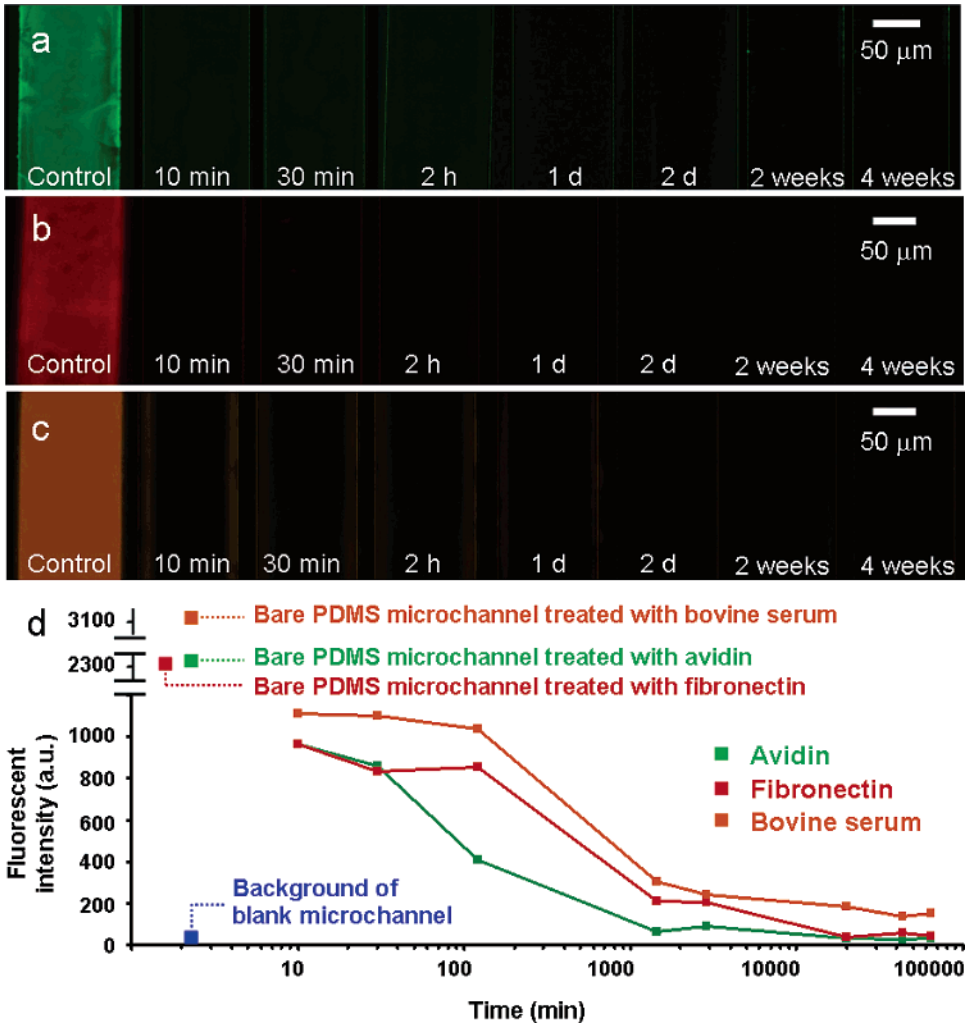

Figure 3. (a−c) Fluorescent micrographs of one intact PDMS microchannel **1** and seven PEG-grafted PDMS microchannels **4** (with preservation times of 10 min, 30 min, 2 h, 1 d, 2 d, 2 weeks, and 4 weeks after their fresh surface modifications), obtained by treating the microchannels with concentrated fluorophore-labeled protein solutions followed by PBS washing. Three separate studies were performed using (a) Fluorescein-labeled avidin (1.0 mg/mL solution in PBS), (b) Alexa 594-labeled fibronectin (0.5 mg/mL solution in PBS), and (c) Alexa 555-labeled bovine serum. (d) The time-dependent profiles summarize the protein repelling characteristics of the microchannels shown in (a) (fluorescein-labeled avidin, green line), (b) (Alexa 594-labeled fibronectin, red line), and (c) (Alexa555-labeled bovine serum, orange line).

with 50 nM DNA solution. To test specificity, another set of DNA-grafted microfluidic channels **9** were exposed to the same concentrations of the fluorophore-labeled triple-mismatch DNA (5′-5Cy3-CGAACCACTCCAAGCA-3′) solutions. After carefully washing the channels with Tris−buffer solution, the resulting DNA hybridizations were measured and quantified under fluo-

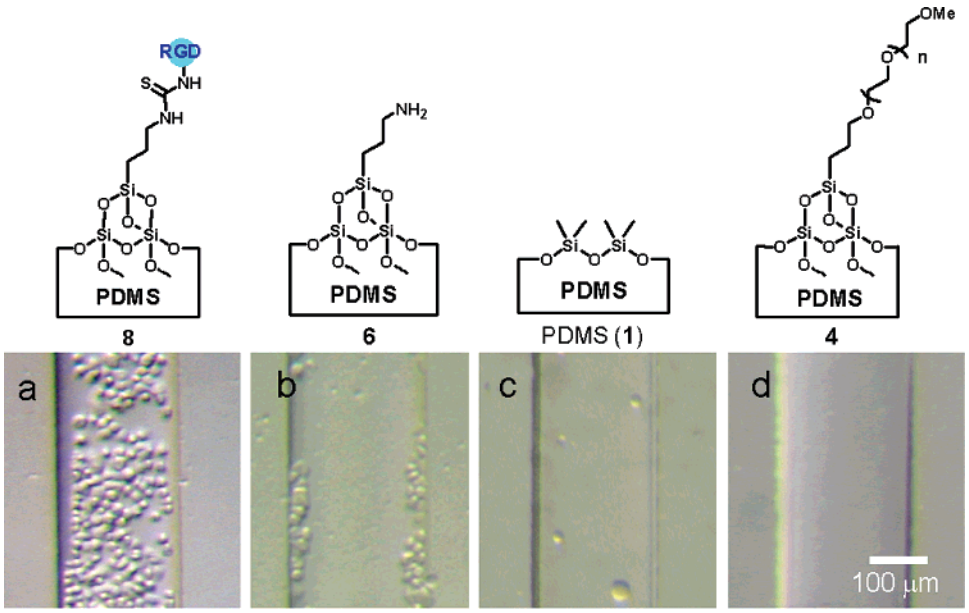

Figure 4. Optical micrographs showing cell immobilization (a) and repulsion (d) demonstrated in the RGD-modified microchannels **8** and in the RGD-modified microchannels **4**. (b) The amino-grafted microchannels **6** and (c) intact PDMS microchannel **1** served as controls, in which a few cells were retained in the microchannels. For all three cases, A427 colon cancer cell line was utilized.

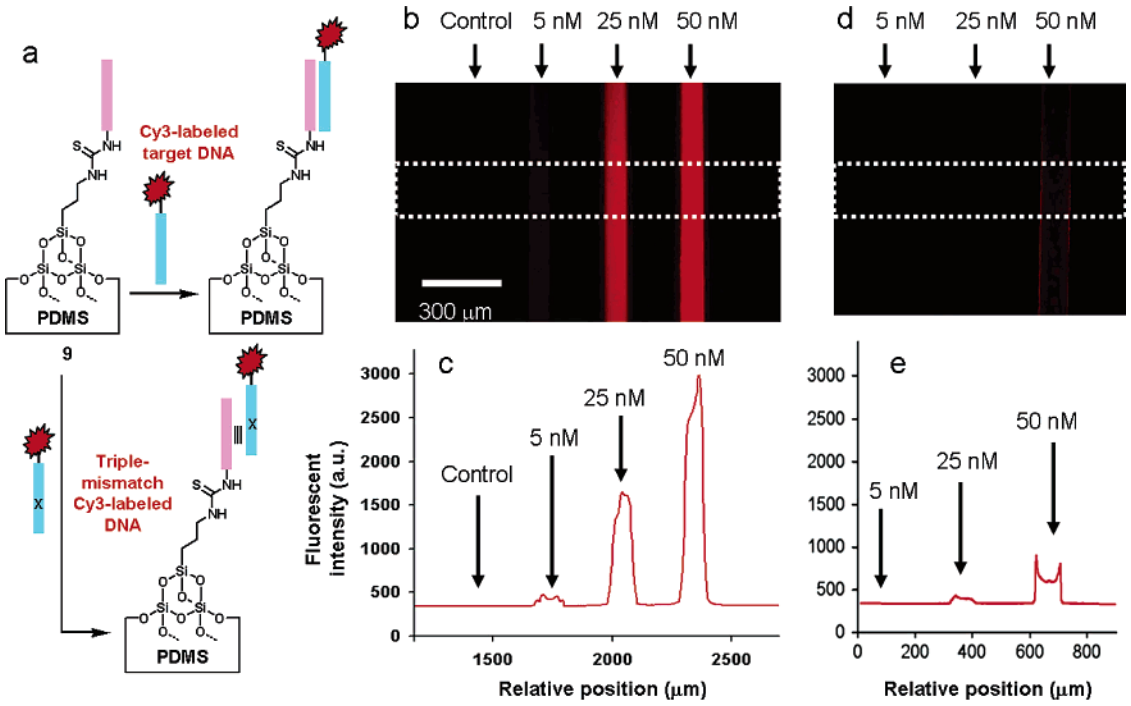

Figure 5. Demonstration of DNA hybridization in PDMS-based microfluidic channels. (a) Schematic representation of the DNA hybridization inside the DNA-grafted microchannels **9**. (b) Fluorescent micrograph of the microchannels **9** after treating with the target DNA solutions with concentrations of 5, 25, and 50 nM. In this case, a PDMS microchannel **1** was treated with 50 nM DNA solution as a control experiment. (c) Integration plot of the fluorescent micrograph obtained in (b). The integration area is indicated by dotted box. (d) Fluorescent micrograph of the microchannels **9** after treating with the triple-mismatched DNA solutions with concentrations of 5, 25, and 50 nM. (e) Integration plot of fluorescent micrograph obtained in (d).

rescent microscopy (Figure 5b and d). The integrations (Figure 5c and e) of fluorescence intensity across the microchannels suggest that the target DNA fragments can be detected semi-quantitatively with reasonable specificity.

Immunoassay. Immunoassays[34] are used to detect protein molecules with high selectivity and specificity. A conventional immunoassay is generally carried out in a 96-well plate, using microliter-level samples. Performing a miniaturized immunoassay on a microfluidic chip offers the advantages of lower sample and reagent consumption, enhanced reaction efficiency, reduced operation time, and a portable operation platform. The parallel PSCA-grafted microchannels **10** were utilized to demonstrate an immunoassay for detection and quantification of a prostate cancer biomarker, anti-PSCA, with a sensitivity of 1.0 nM. As shown in

(34) Wild, D., Ed. *The Immunoassay Handbook*, 3rd ed.; Elsevier Ltd.: Oxford, UK, 2005.

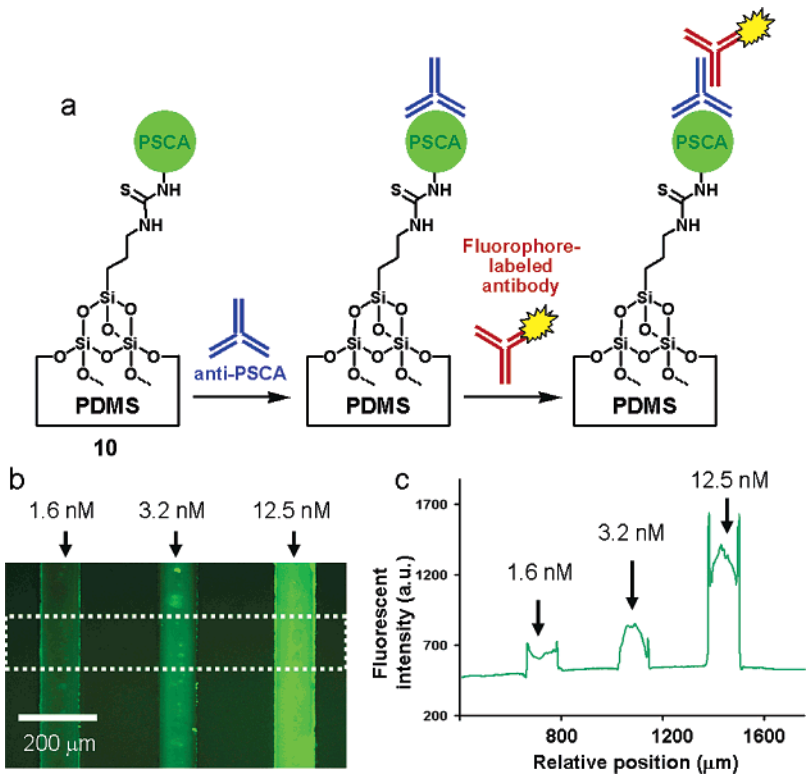

Figure 6. Demonstration of immunoassay in a PDMS-based microfluidic channels. (a) Schematic representation of immunoassay for detection and quantification of anti-PSCA using the PSCA-grafted microchannels **10**. (b) Fluorescent micrograph of the microchannels **10** after performing immunoassay, using target anti-PSCA solutions with concentrations of 1.6, 3.2, and 12.5 nM. (c) Integration plot of fluorescent intensity across the immunoassay microchannels **10**. The integration area is indicated by dotted box.

Figure 6a, The PSCA-grafted microchannels **10** trapped the target molecule, anti-PSCA,[35] from the anti-PSCA solutions (PBS, pH 7.4) of three different concentrations (1.6, 3.2, and 12.5 nM), as detected by exposure to fluorophore-labeled secondary anti-PSCA (fluorescent Ab goat anti-human IgG (H+L), Molecular Probes, 6.7 nM) for detection under fluorescent microscopy (Figure 6b). The integration (Figure 6c) of fluorescent intensity across the micrograph of three parallel microchannels indicates feasibility for semiquantitative detection of anti-PSCA.

CONCLUSION

We have successfully demonstrated an improved approach for silanization modification of intact microfluidic channels embedded in PDMS matrix. This solution-phase approach is simple and convenient for routine analytical applications in chemistry and biology laboratories. In addition, the resulting surface modifications exhibit great stability and fidelity. This improved approach is suitable for intact PDMS-based microfluidic devices, with no device postassembly required. We have also successfully introduced functional groups, including PEG, amino group, isothiocyanate, peptide, DNA, and protein on to the surfaces of microfluidic channels. These functional groups and biomolecule-grafted PDMS microchannels were utilized for protein repelling, cell immobilization and incubation, semiquantitative DNA hybridization, and immunoassay.

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EXPERIMENTAL SECTION

Materials and Reagents. DI water (Milli-Q, Millipore, Bedford, MA) was used to rinse PDMS surfaces and prepare aqueous solutions. PDMS prepolymer RTV615 (AB kit) was purchased from General Electric Co. (Waterford, NY). Hydrogen peroxide (Sigma-Aldrich, St. Louis, MO), 2-[methoxy(polyethylenxy)propyl]-trimethoxysilane (**3**) (90%, Gelest, Morrisville, PA), (3-aminopropyl)trimethoxy silane (**5**) (97%, Sigma-Aldrich), thiophosgen (Sigma-Aldrich), arginine−glycine−aspartic acid (Sigma-Aldrich), fluorescein-labeled avidin (Fisher, Pittsburgh, PA), PBS (pH 7.4, Fisher), and Tris−buffer (pH 7.4, Fisher) were used as received. Alexa594-labeled fibronectin was obtained by treating fibronectin (Invitrogen, Carlsbad, CA) with AlexaFluo 594 Labeling Kit (Invitrogen). Alexa 594-labeled serum was obtained by treating bovine serum (Invitrogen) with AlexaFluo 555 Labeling Kit (Invitrogen). The probe DNA fragment (5′-NH_2-$(CH_2)_6$-TTTTTTGGTT GGTGTGGTTGG-3′), fluorophore-labeled target DNA (5′-5Cy3-CCAACCACACCAACCA-3′), and fluorophore-labeled triple-mismatch DNA (5′-5Cy3-CGAACCACTCCAAGCA-3′) were obtained from Integrated DNA Technology Inc. (Coralville, IA). Prostate stem cell antigen, PSCA. and anti-PSCA was prepared according to published procedure.[25] The A427 human colon cell line was obtained from the American Type Culture Collection (Manassas, VA), and grown in DMEM (Invitrogen), containing heat-inactivated 10% calf serum, 0.2 mM glutamine, 100 units/mL penicillin, and 100 mg/mL streptomycin at 37 °C in a humidified incubator (95% air, 5% CO_2).

Microfluidic Chip Fabrication. The microfluidic devices utilized in these experiments were fabricated using a soft

lithography method. A 25-μm-thick negative photoresist (SU8-2025) was spin coated on to a silicon wafer (Silicon Quest, San Jose, CA). After UV exposure and development, a fluidic mold with square-profiled patterns was obtained for the fabrication of PDMS-based microchannels. Before fabricating the device, the mold was exposed to trimethylchlorosilane vapor for 2−3 min. A well-mixed PDMS (GE, RTV 615 A and B in 5:1 ratio) was poured onto the mold located in a Petri dish to give a 5-mm-thick fluidic layer containing the microchannels transferred from the mold. The fluidic layer was cured in an 80 °C oven for 50 min. After curing, the fluidic layer was peeled off the mold, and holes were introduced onto the fluidic layer for access of reaction solutions. This fluid layer was then placed on top of a glass slide that was coated (2000 rpm, 60 s, ramp 15 s) with PDMS (GE RTV 615 A and B in 20:1 ratio) and was incubated for 45 min in the oven. The device was ready for use after being baked overnight in the oven (80 °C). Metal pins (23 gauge, New England Small Tubing Corp.) were employed to connect the microchannels and Tygon tubing (Cole-Parmer) for introducing reagents and solutions.

Surface Modifications. (1) PEG-Grafted Microfluidic Channels 4. A microsyringe (Hamilton, Reno, NV) was utilized to fill the PDMS microchannels **1** with a mixture of H_2O/H_2O_2/HCl (in a volume ratio of 5:1:1) through the connected Tygon tubing. The microchannels were kept at room temperature for 5 min. After purging the microchannels with DI water and dry Ar (5 psi), the hydrophilic silanol-covered PDMS surfaces **2** were obtained. Neat 2-[methoxy(polyethylenoxy)propyl]trimethoxysilane (**3**) (90%, 60 μL) was injected into the hydrophilic microchannels **2** by a microsyringe, and the PDMS microchips were kept in ambient environment for 30 min to perform silanization reaction. A 500-μL aliquot of DI water continuously flowed through the microchannels to remove unreacted silane **3,** resulting in the PEG-grafted microfluidic channels **4**.

(2) Amino-Grafted Microfluidic Channels 4. The hydrophilic silanol-covered PDMS surfaces **2** obtained by the previously described procedure were treated by neat (3-aminopropyl)-trimethoxy silane (**5**) (50 μL). The microchannels were kept in an ambient environment for 30 min to carry out the silanization reaction. A 500-μL aliquot of DI water continuously flowed through the microchannels to remove unreacted silane **5**, resulting in the amino-grafted microfluidic channels **6**.

(3) Isothiocyanate-Grafted PDMS Microchannels 7. A thiophosgen solution (0.5%, v/v) in MeCN was introduced into the amino-grafted microchannels **6**, and the microchannels were kept in the ambient environment for 20 min at 40 °C. After sequentially purging DI water and dry Ar into the microchannels, the isothiocyanate-grafted PDMS microchannels **7** were obtained.

(4) RGD-Grafted PDMS Microchannels 8. A 5.0-μL RGD solution (1.0 mg/mL) in PBS (pH 7.4) was introduced into a freshly prepared isothiocyanate-grafted PDMS microchannels **7**, and the microchannels were kept in the ambient environment for 1 h. After rinsing with PBS (100 μL), the RGD-grafted PDMS microchannels **8** were obtained.

(5) DNA-Grafted PDMS Microchannels 9. A DNA solution (5′-NH_2-$(CH_2)_6$-TTTTTTGG-TTGGTGTGGTTGG-3′, 1.0 μM) in Tris−buffer solution (pH 7.4) was introduced into a freshly prepared isothiocyanate-grafted PDMS microchannels **7**, and the microchannels were kept in the ambient environment for 1 h. After rinsing with Tris−buffer solution (100 μL), the DNA-grafted PDMS microchannels **9** were obtained.

(6) PSCA-Grafted PDMS Microchannels 10. A 5.0-μL PSCA protein solution (1.0 μg/mL) in PBS (pH 7.4) was introduced into a freshly prepared isothiocyanate-grafted PDMS microchannels **7**, and the microchannels were kept in the ambient environment for 1 h. After rinsing with PBS (100 μL), the PSCA-grafted PDMS microchannels **10** were obtained.

General Procedure for Protein Repelling Study. The protein repelling study of each microchannel was carried out by first filling the channel with a concentrated solution (10 μL) of fluorophore-labeled protein using a microsyringe. The microfluidic chips were incubated at 37 °C for 1 h. The resulting protein-contaminated microchannel was then rinsed with PBS (100 μL) for ∼30 s using microsyringe. Alexa 594-labeled fibronectin (0.5 mg/mL solution in PBS) was obtained by labeling 1.0 mg/mL fibronectin (Sigma-Aldrich) with the Alexa 594 Fluor Labeling Kit (Invitrogen), and Alexa 555-labeled bovine serum was obtained by labeling bovine serum (Invitrogen) with the Alexa 555 Fluor Labeling Kit (Invitrogen).

Cell Immobilization and Incubation. A427 cells were suspended in DMEM cell culture medium at a density of $\sim 10^6$ cell/mL. The cell suspension was then loaded into respective microchannels **1**, **4**, **6**, and **8** with an external pressure of ∼3.0 psi. After incubating the cell-containing microfluidic chips in an incubator (37 °C, 5% CO_2) for 4 h, the unattached cells were removed gently by slowly rinsing the microchannels with DMEM culture medium. The cells survived through our experiment period (4 d).

DNA Hybrization Experiments. Three DNA-grafted microfluidic channels **9** were loaded with different concentrations (5, 25, and 50 nM) of the fluorophore-labeled target DNA (5′-5Cy3-CCAACCACA-CCAACCA-3′) solutions. As a control, an intact PDMS microchannel **1** adjacent to the DNA-grafted microfluidic channels was treated with 50 nM DNA solution. After washing the channels with Tris−buffer solution, hybridization on the resulting DNA hybridization was measured under fluorescent microscopy. DNA mismatch experiments were performed by exposing another three DNA-grafted microfluidic channels **9** with different concentrations (5, 25, and 50 nM) of the fluorophore-labeled triple-mismatch DNA (5′-5Cy3-CGAACCACTCCAAGCA-3′) solutions. After washing the channels with Tris−buffer solution, the resulting mismatched DNA hybridization was measured under fluorescent microscopy.

Immunoassay. The three PSCA-grafted microchannels **10** were first loaded with anti-PSCA solutions at three different concentrations (1.6, 3.2, and 12.5 nM), respectively. The microchannels were kept at room temperature for 1 h and then washed with 100 μL of PBS buffer to remove the unattached anti-PSCA. The microchannels were then loaded with fluorophore-labeled secondary anti-PSCA (fluorescent Ab goat anti-human IgG (H+L), Molecular Probes, 6.7 nM) for 1 h at room temperature and washed with 100 μL of PBS buffer for each individual microchannel before the measurement and quantification under fluorescent microscopy.

Instruments. XPS was performed in a UHV chamber. Experiments were performed at room temperature, with 1486.6-eV X-ray from the AlKR line and a 35° incident angle measured from the

sample surface. ESCA-2000 software was used to collect the data. Dynamic contact angle measurements were performed on PDMS specimens using FTA100 equipment (First Ten Ångstrons, Portsmouth, VA), with the sessile drops technique. DI water (18.4 MΩ) was used. Each data point given is based on 10 contact angle measurements at 5 different positions on the PDMS specimen. Advancing and receding contact angles were measured with the needle remaining in the water droplet. Light and fluorescent micrographs were taken by Nikon TE-2000 fluorescent microscopy. All fluorescent images employed for protein repelling investigation were taken using the same objective (10×), with an identical exposure time of 5 s. The fluorescent intensities were quantified by MetaMorph (Molecular Devices Inc., Downingtown, PA).

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