Biocompatible Hydrophilic Modifications of Poly(dimethylsiloxane) Using Self-Assembled Hydrophobins

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Wettability improvement and bioactive interface designing of poly(dimethylsiloxane) (PDMS) are keen for its application in biodevices. Herein we developed a fully biocompatible approach for achieving stable hydrophilic surfaces by modifying PDMS with hydrophobins. X-ray photoelectron spectroscopy (XPS), water contact angle (WCA), and the force curve measurements demonstrated the conversion of the surface properties from hydrophobic to hydrophilic. The patterning of antigen molecules and the following immunoassays illustrated that this method should be a feasible strategy for bioactive surface modification in biosensing devices.

1. Introduction

The designing of a bioactive interface with appropriate surface wettability[1] is key to miniaturization of biosensors, clinical immunological assay, and many other research applications, ranging from mapping the genome and proteome to examining interactions between biomolecules. As a kind of flexible, inexpensive, and nontoxic polymer, poly(dimethylsiloxane) (PDMS) has been widely used in microfluidic devices[2−4] and microcontact printing technology.[5−7] Furthermore, PDMS is known to be transparent down to the wavelength of 280 nm, which makes it suitable for detection of a wide range of wavelengths.[8−12] However, much effort has been dedicated to overcoming the difficulties to immobilize biomolecules onto the PDMS surface directly because of its intrinsic hydrophobicity. A number of methods have been reported, such as oxidation of the surface by ultraviolet,[13−17] plasma treatment,[18−20] CO_2 pulsed laser,[21] adsorption of polymers on the surface,[22,23] corona discharge,[24] and polymer grafting on PDMS surface.[25] These methods have demonstrated appreciable wettability improvement for the PDMS surface, while also revealing that additional efforts are needed to improve the related important issues including the recovery of hydrophobicity by rotation of surface polar groups Si−OH into the polymer bulk due to surface energy minimization,[26−29] the physical damage to the surface of PDMS by producing "cracks" or other microstructures on the surface,[30,31] and limited biocompatibility of the PDMS surface after chemical treatments.

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(1) Yam, C. M.; Mayeux, A.; Milenkovic, A.; Cai, C. Z. *Langmuir* **2002**, *18*, 10274.
(2) Wang, B. Z.; Abdulali-kanji, E.; Dodwell, J.; Horton, H. R.; Oleschuk, D. *Electrophoresis* **2003**, *24*, 1442.
(3) Li, C. W.; Cheung, C. N.; Yang, J.; Tzang, C. H.; Yang, M. *Analyst* **2003**, *128*, 1137.
(4) Eteshola, E.; Leckb, D. *Sens. Actuators, B-Chem.* **2001**, *72*, 129.
(5) Abbasi, F.; Mirzadeh, H.; Katbab, A. A. *Polym. Int.* **2001**, *50*, 1279.
(6) Delamarche, E.; Donzel, C.; Kamounah, F. S.; Wolf, H.; Geissler, M.; Stutz, R.; Schmidt-Winkel, P.; Michel, B.; Mathieu, H. J.; Schaumburg, K. *Langmuir* **2003**, *19*, 8749.
(7) Wang, L. K.; Feng, X. Z.; Hou, S.; Chan, Q. L.; Qin, M. *Surf. Interface Anal.* **2006**, *38*, 44.
(8) Ng, J. M. K.; Gitilin, I.; Stroock, A. D.; Whitesides, G. M. *Electrophoresis* **2002**, *23*, 3461.
(9) Sia, S. K.; Whitesides, G. M. *Electrophoresis* **2003**, *24*, 3563.
(10) Makamba, H.; Kim, J. H.; Lim, K.; Park, N.; Hahn, J. H. *Electrophoresis* **2003**, *24*, 3607.
(11) Donzel, C.; Geissler, M.; Bernard, A.; Wolf, H.; Michel, B.; Hilborn, J.; Delamarche, E. *Adv. Mater.* **2001**, *13*, 1164.
(12) Lee, S.; Vörös, J. *Langmuir* **2005**, *21*, 11957.
(13) Genzer, J.; Efimenko, K. *Science* **2000**, *290*, 2130.
(14) Phely-Bobin, T. S.; Muisener, R. J.; Koberstein, J. T.; Papadimitrakopoulos, F. *Adv. Mater.* **2000**, *12*, 1257.
(15) Phely-Bobin, T. S.; Muisener, R. J.; Koberstein, J. T.; Papadimitrakopoulos, F. *Synth. Met.* **2001**, *116*, 439.
(16) Ouyang, M.; Klemchuk, P. P.; Koberstein, J. T. *Polym. Degrad. Stab.* **2000**, *70*, 217.
(17) Ouyang, M.; Yuan, C.; Muisener, R. J.; Boulares, A.; Koberstein, J. T. *Chem. Mater.* **2000**, *12*, 1591.
(18) He, Q.; Liu, Z.; He, N.; Xiao, P.; Lu, Z. *Proc. SPIE-Int. Soc. Opt. Eng.* **2001**, *4601*, 406.
(19) Wilson, D. J.; Pond, R. C.; Williams, R. L. *Interface Sci.* **2000**, *8*, 389.
(20) Xia, Y.; Whitesides, G. M. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 550.
(21) Khorasani, M. T.; Mirzadeh, H.; Sammes, P. G. *Radiat. Phys. Chem.* **1999**, *55*, 685.
(22) Barker, S. L. R.; Tarlov, M. J.; Canavan, H.; Hickman, J. J.; Locascio, L. E. *J. Anal. Chem.* **2000**, *72*, 4899.
(23) Linder, V.; Verpoorte, E.; Thormann, W.; de Rooij, N. F.; Sigrist, H. *Anal. Chem.* **2001**, *73*, 4181.
(24) Wang, B.; Chen, L.; Abdulali-Kanji, Z.; Horton, J. H.; Oleschuk, R. D. *Langmuir* **2003**, *19*, 9792.
(25) Okaniwa, M.; Ohta, Y. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 2607.
(26) Kim, J.; Chaudhury, M. K.; Owen, M. J.; Orbeck, T. *J. Colloid Interface Sci.* **2001**, *244*, 200.
(27) Elidiane, C. R.; Giovana, Z. G.; Nilson, C. C. *Plasmas Polym.* **2004**, *9*, 35.
(28) Yan, Y. H.; Chan-Park, M. B.; Yue, C. Y. *Langmuir* **2005**, *21*, 8905.
(29) Chen, L.; Ren, J. C.; Bi, R.; Chen, D. *Electrophoresis* **2004**, *25*, 914.
(30) Diaz-Quijada, G. A.; Wayner, D. D. M. *Langmuir* **2004**, *20*, 9607.
(31) Xiao, D. Q.; Zhang, H.; Wirth, M. *Langmuir* **2002**, *18*, 9971.

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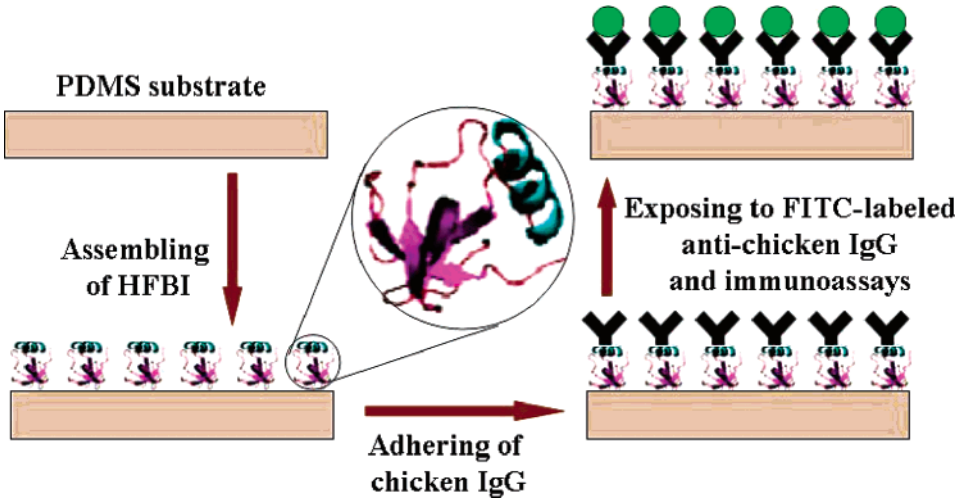
Scheme 1. Immobilization Process of Chicken IgG[a]



[a] The secondary structure model of HFBI is shown in the middle circle.

Recently, modification of surfaces with biomaterials has been drawing much attention because of their intrinsic biocompatibility and designable structures. Among the biomaterials, hydrophobins show unique self-assembling behavior due to their remarkable structural characteristics for pharmaceutical and other medical applications.[32−35] Hydrophobins are a family of small, cysteine-rich and amphipathic fungal proteins which are highly abundant in the hyphal cell walls and secreted to the culture medium.[36,37] The secreted proteins can be self-assembled into amphiphilic membranes at hydrophilic−hydrophobic interfaces (for example, at air−water interfaces or water−hydrophobic solid surfaces) to convert surfaces from hydrophobic to hydrophilic or hydrophilic to hydrophobic.[38,39] The molecular weight of hydrophobins is about 7−15 kDa, and the amino acid sequences include very specific hydrophilic and hydrophobic regions.

In this contribution, an approach is reported to modify the PDMS surface with a class II hydrophobin, HFBI (7.5 kDa), which was distilled from the filamentous fungus *Trichoderma reesei*. The water contact angle (WCA) and the force curve measurements demonstrate the significant improvement of surface hydrophilicity. Furthermore, micropatterning of antigen molecules and the following immunoassays illustrate the bioactive properties of the modified PDMS surfaces.

2. Experimental Section

2.1. Materials. The hydrophobin HFBI was obtained from VTT Biotechnology (Finland). The detailed process for the cultivation and isolation of HFBI can be found in the Supporting Information. PDMS elastomer kit (SYLGARD 184; http://www.dowcorning-.com/DataFiles/090007b280e2dbb0.pdf) and curing agent were purchased from Dow Corning. The chicken IgG used as antigens, the fluorescein isothiocyanate (FITC)-labeled anti-chicken IgG developed in rabbit, and the bovine serum albumin (BSA) used as blocking reagent were all purchased from Sigma.

2.2. Fabrication of PDMS. PDMS elastomer and the curing agent (w/w = 10:1) were poured on the glass substrate after being mixed sufficiently and cured at 60 °C for overnight. After being peeled off from the glass substrate, the PDMS substrate was rinsed with water several times and ultrasonicated in water for 10 min, followed by washing in an ethanol solution (75 vol % ethanol in water) and drying with nitrogen.[40,41]

2.3. Micropatterning of HFBI on the PDMS Substrates. A simple method was utilized to pattern HFBI on the PDMS substrates. First, cleaned copper transmission electron microscopy (TEM) grids were gently pressed into PDMS surfaces, and then the surface was incubated in an HFBI aqueous solution (100 μg/mL) at 20 °C for 20 min. After being rinsed by pure water and dried with nitrogen, the patterned surface was immersed in the solution of chicken IgG (200 μg/mL) for 5 min followed by water rinsing and N_2 drying. Then the copper TEM grids were peeled off from the HFBI−PDMS surface carefully to avoid damage to the chicken IgG patterns on the surface. When the substrate was dried, the HFBI−PDMS surface was immersed in the BSA solution (10 mg/mL) to block the unpatterned areas. About 15 min later, the substrate was rinsed with phosphate buffer solution (PBS, pH = 7.4) and purified water three times and dried with nitrogen. Finally the HFBI−PDMS surface was covered with droplets of FITC-labeled anti-chicken IgG solution (200 μg/mL, FITC is a reactive fluorescein molecule, which is typically conjugated to proteins via primary amines) for 2 min, and then the substrate was rinsed again with PBS and distilled water for several times. After being dried with nitrogen, the grid pattern could be observed with fluorescence microscopy.

2.4. X-ray Photoelectron Spectroscopy (XPS) Measurements. The modification of PDMS substrate by self-assembled HFBI was

(32) de Vocht, M. L.; Reviakine, I.; Wösten, H. A. B.; Brisson, A.; Wessels, J. G. H.; Robillard, G. T. *J. Biol. Chem.* **2000**, *275*, 28428.
(33) Wessels, J. G. H. *Adv. Microb. Physiol.* **1997**, *38*, 1.
(34) Wösten, H. A. *Annu. Rev. Microbiol.* **2001**, *55*, 625.
(35) Wösten, H. A.; de Vocht, M. L. *Biochim. Biophys. Acta* **2000**, *1469*, 79.
(36) Palomo, J. M.; Penas, M. M.; Fernandez-Lorente, Mateo, G.; C.; Pisabarro, A. G.; Fernandez-Lafuente, R.; Ramirez, L.; Guisan, J. M. *Biomacromolecules* **2003**, *4*, 204.
(37) Wessels, J. G. H. *Mycologist* **2000**, *14*, 153.
(38) Bilewicz, R.; Witomski, J.; Van der Heyden, A.; Tagu, A.; Palin, B.; Rogalska, E. *J. Phys. Chem. B* **2002**, *105*, 9772.
(39) Corvis, Y.; Walcarius, A.; Rink, R.; Mrabet, N. T.; Rogalska, E. *Anal. Chem.* **2005**, *77*, 1622.
(40) Bernard, A.; Michel, B.; Delamarche, E. *Anal. Chem.* **2001**, *73*, 8.
(41) (a) Bernard, A.; Renault, J. P.; Michel, B.; Bosshard, H. R.; Delamarche, E. *Adv. Mater.* **2000**, *12*, 1067. (b) Bernard, A.; Delamarche, E.; Schmid, H.; Michel, B.; Bosshard, H. R.; Biebuyck, H. *Langmuir* **1998**, *14*, 2225.

Table 1. Elemental Compositions of the Surface of Native PDMS and HFBI Modified PDMS

	C	N	Si	O	S
native PDMS	50.09%	0	22.30%	27.61%	0
HFBI modified PDMS	51.87%	5.80%	13.48%	28.68%	0.18%

confirmed by XPS (PHI-5300, Phi, U.S.A.) employing a monochromatic Mg Kα radiation source ($h\nu = 1253.6$ eV). The binding energy was swept from 0 to 1100 eV, and the electron take off angle was fixed at 45°. The energy resolution of the analyzer is 0.8 eV, and the sensitivity is 80−1600 KCPS. The peaks in the elemental core-level spectra were fitted using UNIX on an Apollo Domain series 3500.

2.5. WCA Measurements. The WCAs were measured on a 5 μL water droplet under ambient conditions with an optical contact angle meter (Dataphysics, Inc., OCA20). The WCA values were averaged from three measurements at different locations.

2.6. Atomic Force Microscopy (AFM) Measurements. The force curve measurements were conducted on a multimode atomic force microscope (Nanoscope IIIA, Veeco Metrology, U.S.A.) in an aqueous solution, and the Si_3N_4 tips ($k = 0.12$ N/m, Veeco Metrology, U.S.A.) used in the experiment were immersed in the BSA aqueous solution (10% by weight) for 12 h prior to the force curve measurements. In addition, the root mean square roughness (Rq) of the film was obtained according to the AFM images by using the Nanoscope software.

2.7. Fluorescence Measurements. The fluorescence measurements were performed with the Nikon TE2000-U fluorescence microscope with CCD-Rtke (Japan).

3. Results and Discussion

The process for the assembly of HFBI for wettability improvements and the immobilization of IgG and FITC-labeled anti-chicken IgG for evaluation of biocompatibility is illustrated in Scheme 1. The PDMS surface, after being coated with HFBI, was patterned with chicken IgG. Then the patterned surface was exposed to FITC-labeled anti-chicken IgG for immunoassays. The secondary structure model of a HFBI hydrophobin is illustrated in the middle circle of Scheme 1. The blue part represents the α helix structure of HFBI formed by residues in the middle part of the primary amino acid sequence. In the tertiary structure, this α helix lies outside of the protein and forms the hydrophilic part of HFBI hydrophobin. The red part represents the β sheet structure of HFBI formed by two β hairpins. It is reported that most of the hydrophobic patch consists of two loops of hairpins,[42] and the β sheet structure is the hydrophobic region of the HFBI hydrophobin. It is reasonable to propose that the hydrophobic β sheet structure of HFBI should be attached to the hydrophobic PDMS surface as shown in Scheme 1, while the α helix was exposed to the outside to convert the surface to a hydrophilic one.

Table 1 shows the XPS analysis of elemental compositions of native PDMS and HFBI modified PDMS. The native PDMS showed a surface consisting of 50.09% carbon (C), 27.61% oxygen (O), and 22.30% silicon (Si), which is in agreement with the expected composition. The modification of HFBI leads to the decrease of the Si signal (103 eV, Figure S2, Supporting Information) to 13.48%, and the N 1s peak (400 eV) increases from 0 to 5.8%. The Si element only exists in the $-Si-CH_3$ groups of PDMS, and the N element only exists in the $-NH_2$ groups of the HFBI protein. So the increase of the N 1s peaks and the decrease of the Si 2p peaks indicated the originally outward $-Si-CH_3$ groups of PDMS were partially covered by $-NH_2$ groups of HFBI proteins. Therefore, it could be considered that the hydrophobins have been coated onto the PDMS surface with the amino groups exposed outward. Low standard deviations were observed for all elements when five randomly selected measurement spots were compared, indicating homogeneous coating of HFBI. The AFM characterizations showed the Rq value of the modified film was 0.83 ± 0.55 nm, which showed the homogeneous film formation by the HFBI adsorption. In addition, the sectional profile indicated that the film thickness was around 7.3 ± 1.1 nm (Figure S3, Supporting Information).

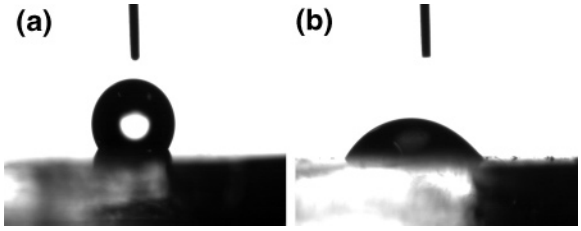


Figure 1. Micrographs of 5 μL water droplets on (a) the PDMS substrate and (b) the PDMS substrate modified with HFBI.

The wettability improvement of the HFBI modified PDMS substrate was investigated with WCA measurement. Figure 1 shows the micrographs of 5 μL water droplets on the two types of PDMS surfaces. The WCA of the PDMS surface shown in Figure 1a is $123.9 \pm 0.7°$, which indicates that the PDMS surface is rather hydrophobic. Upon self-assembling of hydrophobins on the surface, the WCA of HFBI modified PDMS surface is dramatically reduced to $51.0 \pm 0.5°$, indicating a hydrophilic surface associated with HFBI modification (Figure 1b). As illustrated in Scheme 1, the improvement of wettability could be attributed to the adhering of hydrophobic regions of HFBI to the PDMS surface to minimize the system energy. While the hydrophilic region was exposed to the outside of the surface, the assembled membrane converted the hydrophobic PDMS surface to a hydrophilic surface.

In a separate experiment, we employed AFM force curve measurements to investigate the interactions between a tip and a sample surface. The force−displacement curve reveals the cantilever deflection (or force when calibrated using the cantilever spring constant) versus substrate displacement. The force at the pull-off point corresponds to the adhesion force between the tip and the sample, which can be applied to the identification of surface properties.[43,44]

The adhesion forces in Figure 2a,b demonstrate a weak interaction of the BSA modified Si_3N_4 tip with the PDMS surface, while a stronger one was revealed between the tip and the HFBI modified PDMS surface. Because of the

(42) Linder, M. B.; Szilvay, G. R.; Nakari-Setälä, T.; Penttila, M. E. *FEMS Microbiol. Rev.* **2005**, *29*, 877.

(43) Nakagawa, T.; Ogawa, K.; Kurumizawa, T.; Ozaki, S. *Jpn. J. Appl. Phys.* **1993**, *32*, L294.

(44) Frisbie, C. D.; Rozsnyai, L. F.; Noy, A.; Wrighton, M. S.; Lieber, C. M. *Science* **1994**, *265*, 2071.

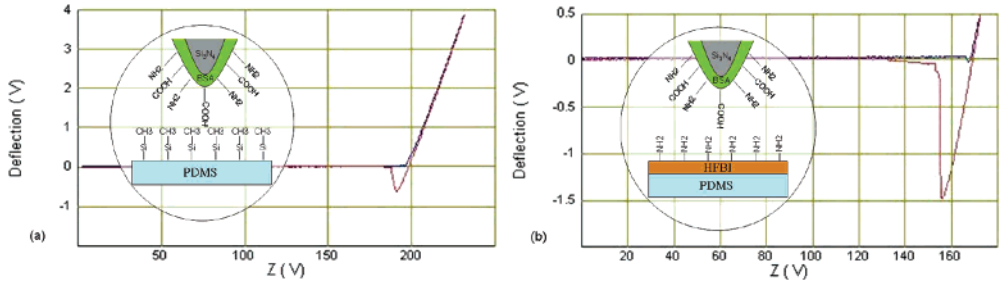


Figure 2. Typical force curves on (a) the PDMS surface and (b) the HFBI modified PDMS surface.

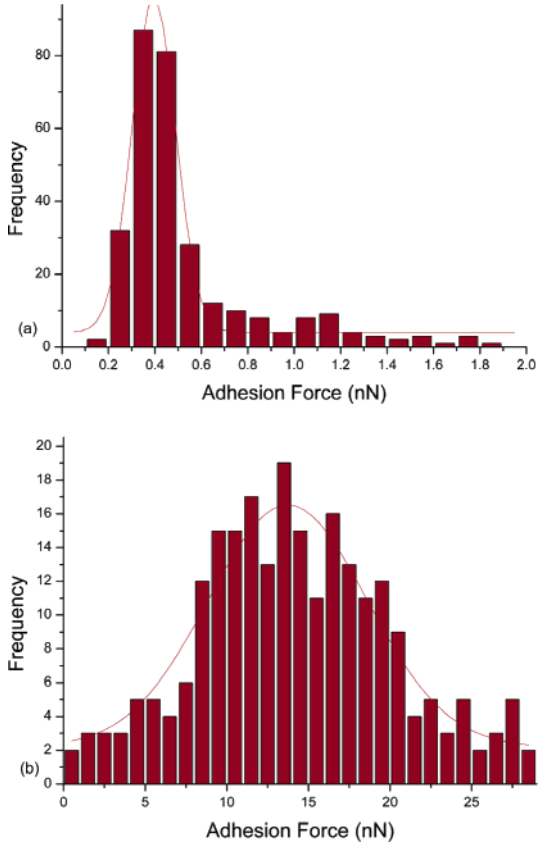


Figure 3. Histograms showing the distribution of adhesion forces on (a) the PDMS surface and (b) the HFBI modified PDMS surface.

hydrophilic nature of the BSA molecules, the stronger adhesive force between the BSA modified tip and the HFBI modified PDMS surface further demonstrated the enhanced hydrophilic properties of the modified surface. This adhesive force increase may be attributed to the conversion of surface groups from methyl to amine.

Shown in Figure 3 are the histograms indicating the distribution of the adhesion force. Figure 3a,b illustrates the distribution map of the adhesion forces on pristine and HFBI modified PDMS surfaces, respectively. The statistics of the adhesion force using Gaussian distribution yield an adhesion force on the PDMS surface of 0.39 ± 0.01 nN, which can be attributed to the weak interactions between −COOH or $-NH_2$ groups on AFM tip and the $-CH_3$ groups on the PDMS substrate. After modification with HFBI, the average adhesion force is increased to 13.69 ± 0.36 nN, which is resulted from the hydrogen bond formation between the BSA modified tip and the amino-functionalized PDMS surface. The statistic results are consistent with the above XPS and WCA measurements. The investigations demonstrated the effective modification of PDMS surface with HFBI proteins from a hydrophobic to a hydrophilic nature.

The above results have demonstrated that the HFBI assembly on PDMS surfaces could improve the wettability from hydrophobic to moderately hydrophilic. In addition, the bioactivity of the modified PDMS surface was investigated by observing the adsorption stability of biomolecules on it. Figure 4 shows the fluorescent patterns with high contrast and resolution. The green circles are the patterns of chicken IgG with blocked FITC-labeled anti-chicken IgG on the HFBI-coated PDMS substrates, and the gray dark regions are the uncovered PDMS surface caused by peeling of TEM grid. Because the FITC-labeled anti-chicken IgG could be easily bound with patterned chicken IgG as compared to the native PDMS, the fluorescence patterns in Figure 4 confirmed the immobilization of chicken IgG on the solid surfaces with self-assembled HFBI as the linking layer and clearly showed the biocompatibility of HFBI modified PDMS substrate.

The high contrast and resolution of the fluorescent images confirmed the good mechanical stability of the pattern and little surface diffusion of the adsorbed proteins. Previous studies have indicated the stability of the hydrophobin films and the improvement of the protein binding efficiency on hydrophobin modified surfaces.[45−47] Our repeated experiments revealed the stability of the adsorbed proteins upon washing by water solution. Such extended retention time of the wettability property is also a significant improvement compared with the previous reports.

In addition, the HFBI modified surface has no indication of hydrophobic recovery even exposing the modified PDMS surfaces in air for 20 days. The stability of hydrophobin coating was verified by WCA on modified surfaces, which indicated that WCA on modified surfaces (Figure S4, Supporting Information) was not changed even after storing in air or in distilled water for several days. Moreover, as seen in Figure S5 (Supporting Information), the fluorescent patterns were still observable after more than 10 days, which showed highly improved stabilities. We wish to note that for the future studies, rigorous and quantitative measurements should be developed to characterize the strength and durability of the binding of the biolayer to the PDMS surface. Also, this method is convenient and inexpensive to achieve protein patterning, so the application of this modification method could be adapted to a number of related research topics, such

(45) Linder, M. B.; Szilvay, G. R.; Nakari-Setä lä, T.; Söderlund, H.; Penttilä, M. E. *Protein Sci.* **2002**, *11*, 2257.
(46) Carpenter, C. E.; Mueller, R. J.; Kazmierczak, P.; Zhang, L.; Villalon, D. K.; Van Alfen, N. K. *Mol. Plant−Microbe Interact.* **1992**, *5*, 55.
(47) Wesseles, J. G. H. *Annu. Rev. Phytopathol.* **1994**, *32*, 413.

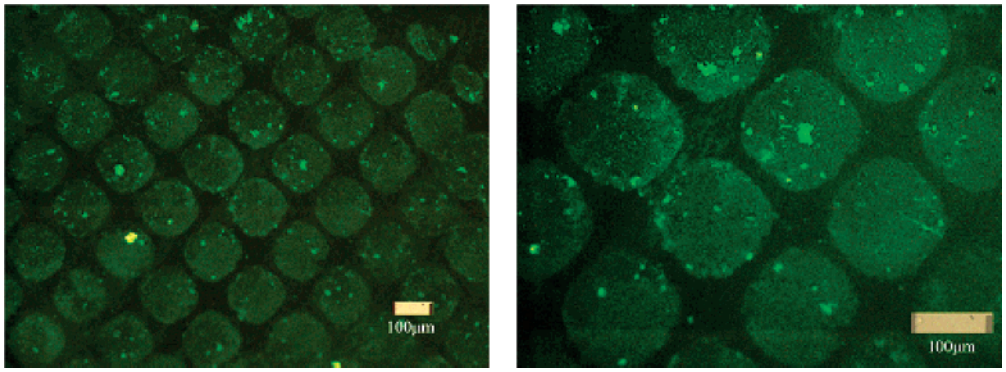


Figure 4. Fluorescent images of patterned chicken IgG with blocked FITC-labeled anti-chicken IgG on the PDMS substrate patterned with HFBI. The right picture is the magnified image of the left one, and the corresponding scale bars are presented in the images.

as microfluidic devices, biosensors, and other clinical immunological assays.

4. Conclusion

A bioactive surface with significantly improved wettability was developed by the modification of the PDMS surfaces with HFBI assemblies. The XPS and WCA results illustrated the effective conversion of the wettability of PDMS surface from hydrophobic to moderate hydrophilic with HFBI modification, which is suitable for the immobilization of proteins. The force curve measurements confirmed this wettability improvement. The following immunoassays demonstrated that HFBI coating was a convenient way to immobilize proteins effectively and preserve their bioactivity on modified PDMS substrates with minimal surface damages and *improved* stabilities. These advantages enable it to be a useful method for the bioactive modification of the PDMS surface for the development of biosensing devices and immunoassays.

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Supporting Information Available: Detailed processes for the cultivation and isolation of HFBI are provided (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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