Cellulose Paper Sensors Modified with Zwitterionic Poly(carboxybetaine) for Sensing and Detection in Complex Media

ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 2014

Impact Factor: 5.64 · DOI: 10.1021/ac500467c · Source: PubMed

9 AUTHORS, INCLUDING:

Yan Deng
Tsinghua University
14 PUBLICATIONS **57** CITATIONS
SEE PROFILE

Jiaqiang Xu
Shanghai University
144 PUBLICATIONS **3,753** CITATIONS
SEE PROFILE

Qiuming Yu
University of Washington Seattle
59 PUBLICATIONS **1,851** CITATIONS
SEE PROFILE

analytical chemistry

Letter

pubs.acs.org/ac

Cellulose Paper Sensors Modified with Zwitterionic Poly(carboxybetaine) for Sensing and Detection in Complex Media

Yongheng Zhu,[†,‡,§] Xuewei Xu,[†,§] Norman D. Brault,[†] Andrew J. Keefe,[†] Xia Han,[⊥] Yan Deng,[†] Jiaqiang Xu,[‡] Qiuming Yu,[†] and Shaoyi Jiang*[,†]

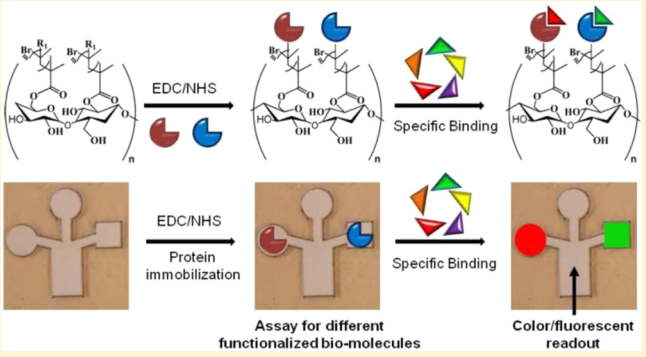
[†]Department of Chemical Engineering, University of Washington, Seattle, Washington 98195, United States

[‡]Department of Chemistry, College of Science, Shanghai University, Shanghai 200444, China

[⊥]Key Laboratory for Advanced Materials and Department of Chemistry, East China University of Science & Technology, Shanghai 200237, China

Supporting Information

ABSTRACT: Poly(carboxybetaine) (PCB) functionalized cellulose paper was used as a paper-based microfluidic device. The results showed that the PCB modified paper sensor was able to achieve (a) more rapid and sensitive glucose detection from undiluted human serum compared to bare cellulose and (b) specific antigen detection via covalently immobilized antibodies.



Diagnostic devices using patterned paper are the subject of growing interest due to their development as research tools and environmental monitoring devices and for low-cost point-of-care clinical analysis in limited-resource settings.[1−4] Cellulose has recently attracted attention as an excellent paper-based diagnostic platform.[5−7] Its advantages include low production cost, portability, and durability, along with excellent wicking properties of aqueous solutions via capillary action.[8,9] However, for practical applications of paper-based analytical devices such as for the detection of specific diseases or for monitoring selective interactions of cells or biomolecules, especially in complex media such as blood, unmodified cellulose is inadequate at resisting nonspecific adsorption,[10] leading to high background noise, loss of target analyte, and a reduction in assay reproducibility.[11−14] Detection from undiluted complex media is highly desirable as it permits the best assay sensitivity to be achieved for real-world applications as opposed to using diluted samples. Additionally, the physical adsorption of molecular-recognition elements (MREs) typically used for immobilization on cellulose often results in ligand drift, signal distortion, and loss of ligand bioactivity, all of which can be significantly improved or eliminated via covalent attachment while also further enhancing the limit of detection (LOD).[15] By enabling applications in undiluted complex media as well as the convenient covalent attachment of MREs, one would significantly improve paper-based detection platforms.

Over the past two decades, numerous attempts have been made to improve the nonspecific properties of cellulose by surface modification with poly(ethylene glycol) (PEG)- and phosphorylcholine (PC)-based polymers or the use of cellulose composites containing synthetic polymers and biopolymers.[16−19] However, very few chemistries allow the conjugation of biomolecules or can be used in complex media. Other approaches for improving sensitivity have focused solely on signal amplification.[20] However, the use of significant signal enhancement, for example, via gold nanoparticles, still faces all of the same issues associated with nonspecific binding and hence still limit the true potential of paper-based sensors. In order to more fully develop these diagnostic devices for real-world applications, improvements over detection sensitivity and specificity from complex media is highly necessary, thus requiring a unique approach to the current limitations.[21]

Zwitterionic poly(carboxybetaine) (PCB) is an attractive material for paper-based devices due to its dual capacity for enabling fouling resistance and functionalization.[22] Super-hydrophilic PCB has demonstrated ultralow fouling levels (less than 5 ng/cm^2 of nonspecifically adsorbed protein) in the presence of 100% human blood plasma and serum.[23,24] Furthermore, unlike other nonfouling materials, PCB has abundant functional groups (COOH) which can be employed for the convenient immobilization of MREs via conventional 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide and *N*-hydroxysuccinimide (EDC/NHS) coupling chemistry.[25] Most im-

Received: February 2, 2014
Accepted: February 26, 2014
Published: February 26, 2014

   dx.doi.org/10.1021/ac500467c | *Anal. Chem.* 2014, 86, 2871−2875

portantly, unreacted NHS-ester groups in the PCB film can be converted back into negatively charged carboxylate ions upon hydrolysis, rendering the original ultralow fouling properties to postfunctionalized PCB films.[26,27]

In this paper, we report a robust and effective surface modification method for the formation of PCB brushes onto cellulose paper (CP) using surface-initiated atom transfer radical polymerization (SI-ATRP). This platform presents an extremely low fouling background to undiluted complex media with abundant functional groups for the immobilization of bioactive molecules. To demonstrate the potential of the PCB modified cellulose paper as a highly sensitive diagnostic sensor, we constructed a high-performance microfluidic paper-based analytical device (μ-PAD). Compared with unmodified CP,[28,29] the PCB coated cellulose paper was superior for its more rapid response as well as its specificity for sensing target molecules. The very low fouling background of PCB led to a significant improvement in the availability of target molecules within the test zone leading to improved detection sensitivity even in the presence of undiluted human serum, indicating this platform to have a great potential for the rapid, sensitive, and convenient detection of analytes in real-world applications.

RESULTS AND DISCUSSION

XPS surface analysis was used to track the surface composition differences of PCB modified and unmodified CP substrates. As shown in the XPS elemental composition results (Table S1, Supporting Information), only carbon and oxygen were detected at the interface of unmodified cellulose membranes. After PCB modification using atom transfer radical polymerization (ATRP) and subsequent washing with PBS, the appearance of a nitrogen signal in the XPS spectrum confirmed that PCB was successfully and covalently modified onto the cellulose substrate. As nonspecific protein adsorption on surfaces is thought to be the first step of many undesired biological responses and can severely affect the limit of detection for sensing devices.[30] FITC-BSA (in PBS) was selected to measure the protein fouling for coated and uncoated substrates, as shown in Figure 1b–e. It can be clearly seen that the amount of adsorbed proteins is drastically decreased after surface modification with PCB. Fibrinogen adsorption on different surfaces was also measured by ELISA (Figure 1f). Here, the protein fouling from an unmodified CP sample was used as the reference. As shown in Figure 1f, fouling on all of the PCB modified CP samples was significantly reduced as compared with that of the unmodified control. An ATRP polymerization time of 12 h yielded a PCB coated CP substrate with the lowest fouling (relative protein adsorption of 6.5%).

We then prepared a paper-based analytical device using a method previously reported by the Whitesides group but with several modifications.[2] Originally, cellulose paper was coated with a photoresist that could be modified into a specific pattern using UV-light. However, this left the paper slightly hydrophobic, presumably due to residual photoresist bound to the paper, even though the surface was exposed to oxygen plasma in an effort to increase the hydrophilicity of the paper. Here, we developed a method which does not compromise the performance of the cellulose paper. Instead, due to the PCB coatings, the modified paper was superhydrophilic and exhibited very low fouling. This enabled fluids to move faster in the flow channel (shown in Figure S1, Supporting Information) and also allowed more analytes to reach the test zones, particularly in the presence complex media such as

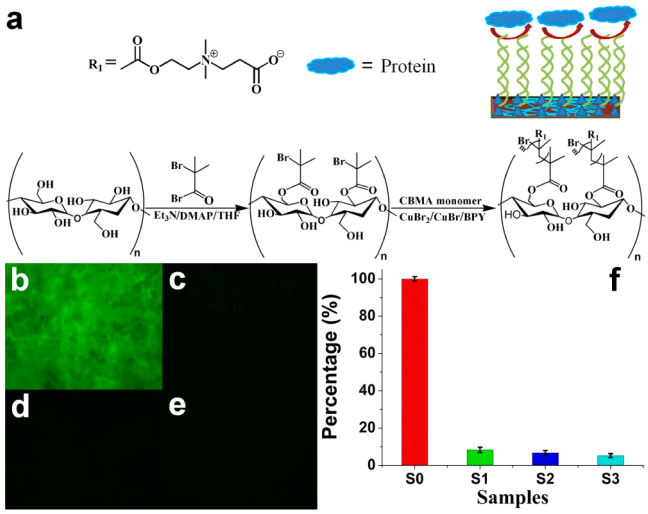


Figure 1. (a) The reaction scheme for grafting protein-resistant PCB onto cellulose substrates. (b–e) Representative fluorescence microscopy images of FITC-BSA adsorption onto untreated cellulous paper (b, S0) and PCB coated cellulous paper using 3 h (c, S1), 6 h (d, S2), and 12 h (e, S3) ATRP polymerization times. (f) Amount of adsorbed human fibrinogen (Fb) measured from ELISA on the four surfaces corresponding to b, c, d, and e.

undiluted human serum. The protocol is demonstrated in Figure S1, Supporting Information: (a) glass slides were first modified with allyltrimethoxysilane; (b) the substrates were then coated with PDMS; (c) laser-cutting was used to pattern sensing channels into the PDMS; (d) patterned paper was then loaded onto the patterned PDMS channels and used to perform detection assays. Figure S1e, Supporting Information, shows the hydrophobic properties of the bare patterned PDMS, indicated by the beading-up and lack of wetting of water droplets. The patterned PDMS could be reused by simply replacing the CP substrate for each detection. Thus, the final configuration was composed of a porous matrix of hydrophilic cellulose fibers (modified or unmodified with PCB) bounded by a hydrophobic PDMS barrier to contain the liquid sample being analyzed. The central channel wicks fluid into three independent test zones, each of which can be used for separate bioassays. To compare the capillary behavior of the devices and the benefit of PCB modified CP relative to the unmodified substrate, Waterman blue ink was loaded onto the bottom of each test strip and the rate of liquid movement was monitored as a function of time. The results shown in Figure S1f, Supporting Information, indicate that the fluid filled the entire microfluidic CP analytical device modified with PCB coatings faster than those without. The PCB modified and unmodified paper-based analytical devices were then used to perform real-life bioassays.

We first compared the detection performance of the CP and the PCB modified substrate for glucose using a relatively high analyte concentration (5.0 mM) from both buffer and undiluted human serum. Figure 2a shows the response for the PBS samples monitored as a function of time (see Supporting Information for experimental details). In a typical assay, a 0.6 M solution of potassium iodide was spotted onto the left detection zone followed by spotting a 1:5 horseradish peroxidase/glucose oxidase solution. After the droplets containing the detection reagents were fully dried in air at room temperature, the test solution containing 5.0 mM glucose

dx.doi.org/10.1021/ac500467c | Anal. Chem. 2014, 86, 2871–2875

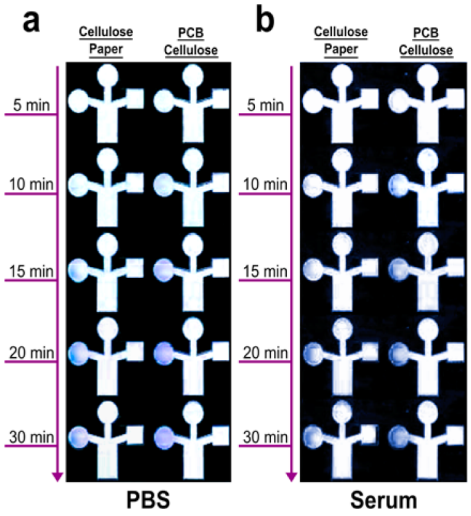


Figure 2. Glucose detection as a function of time using PCB modified and unmodified cellulose paper-based analytical devices. Here, glucose was spiked (5.0 mM) into PBS (a) and undiluted human serum (b). The circular region on the left was used for the glucose test while the square region on the right was used as the control spot. The circular region on the top was not used for these experiments. Detection reagents to react with and detect glucose were spotted only in the left detection zone and were allowed to dry before analyzing the samples. Each glucose-spiked solution (in PBS or serum) was dropped onto the bottom of each substrate (time = zero) and subsequently wicked into all three detection zones. The color was adjusted to better enable visualization of the detection spots using the naked eye.

(in PBS) was then spotted onto the bottom of each test strip. Figure 2b shows the same experiment except glucose (5.0 mM) was spiked into undiluted human serum. A comparison of the two devices as shown in Figure 2a revealed that the PCB coated substrate responded slightly more rapidly (i.e., the sensing spot became darker in a shorter time period), but both devices achieved similar signal intensities after 30 min. The similar end-result (at 30 min) was expected due to analyzing a simple solution of analyte in PBS. Analysis from buffer, while convenient for testing the basic performance of a sensing device, does not represent the true performance in real-life applications involving complex media. The subsequent analysis of glucose detection from undiluted human serum revealed significant differences between the bare and PCB modified devices (Figure 2b). Here, not only did the detection occur noticeably more rapidly for the PCB coated device, but also the signal intensity after 30 min was significantly darker and more uniform. These results confirm that PCB functionalized paper-based sensors are able to achieve a faster response, particularly in complex media, which is attributed to the superhydrophilic properties of PCB which increase the rate of transport through the analytical device. Furthermore, the larger signal intensity (i.e., darker color) for the PCB substrate was a result of the extremely low fouling of the zwitterionic coating, enabling more analyte to be present in the detection zone. In order to account for the differences in the transport properties and enable a fair comparison between the two devices, it was important to start the detection time immediately after the spiked media was spotted onto the bottom of each test strip and to allow enough time for the detection reagents to fully develop.

The robustness and sensitivity of the PCB modified CP and the bare substrate was further compared by detecting multiple concentrations of glucose (0.5 mM to 5.0 mM) from both PBS and undiluted human serum as shown in Figure 3a,b, respectively. The resulting changes in the color of each image

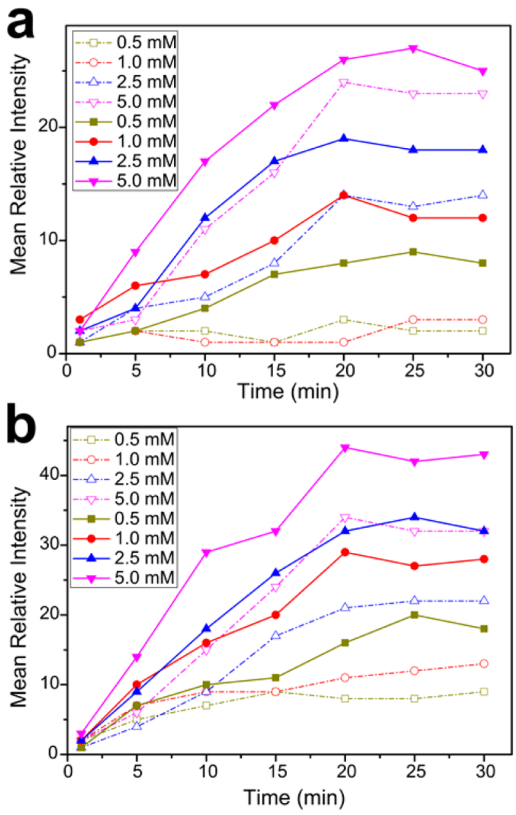


Figure 3. The mean relative intensity of the glucose detection zone as a function of time and concentration (0.5 to 5.0 mM) for analysis from PBS (a) and undiluted human serum (b). Closed symbols and solid lines represent PCB modified cellulose paper while open symbols and dotted lines represent the unmodified substrate. The detection time was started immediately after the glucose spiked media was spotted onto the bottom of each test strip.

(shown in Figure S2, Supporting Information), for each concentration, were quantitatively analyzed using ImageJ as described in the Supporting Information. This enabled the calculation of the mean relative intensity which was plotted as a function of time. A comparison of Figure 3a, b with Figure S2, Supporting Information, revealed that, while detection was sometimes difficult to determine visually using the naked eye for all glucose concentrations analyzed from either media, it was always possible to differentiate the signal using ImageJ. Furthermore, the color intensity of the PCB modified CP was always higher than that of unmodified substrate for all glucose concentrations, time points, and media. For practical applications, this again indicates that the glucose concentration can be obtained faster using the PCB modified substrates particularly in the presence of undiluted human serum. The ability to achieve better detection sensitivities is also apparent from Figure 3a,b. This was indicated by the overlapping signals for bare CP substrates at lower concentrations (0.5 and 1.0 mM for both PBS and serum) which were not observed for PCB modified substrates. The difference in detection performance between coated and uncoated cellulose was more apparent for the serum assay (Figure 3b). PCB enabled the different concentrations to be significantly more differentiated, even in the presence of the crude complex media, while also obtaining

dx.doi.org/10.1021/ac500467c | Anal. Chem. 2014, 86, 2871−2875

higher overall responses. This was accredited to the super-hydrophilicity of PCB which provided low fouling properties and increased the amount of target analyte available in the detection zone. It was further noticed that, for the same glucose concentration, the color observed for serum was generally darker than that from PBS. This is most likely due to the natural yellowish color of the serum.

To further examine the capabilities and versatility of the PCB modified CP for conjugating bioactive molecules, anti-BSA (the circular region on the left) and anti-fibrinogen (the square region on the right) were covalently immobilized using EDC/NHS coupling chemistry. Antigen detection was then studied via fluorometric binding assays, as demonstrated in Figure 4a. A

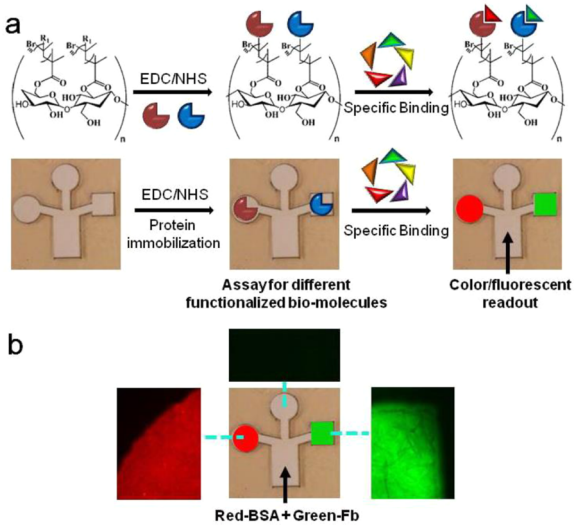


Figure 4. (a) A schematic representation of the activation and functionalization of PCB modified cellulose paper with desired biomolecules (anti-BSA and anti-Fb) and the visualization of immobilized proteins via the detection of a fluorometric antigen probe. (b) A mixture of fluorescently tagged BSA (red, 50 μg/mL) and fibrinogen (Fb, green, 50 μg/mL) proteins in PBS was wicked through the microfluidic device followed by washing with buffer. The circular region on the left revealed only red fluorescence, the square region on the right revealed only green fluorescence, and the circular region on the top was black due to the absence of nonspecific binding.

mixture of fluorescently tagged BSA (red, 50 μg/mL) and fibrinogen (green, 50 μg/mL) proteins in PBS was wicked through the microfluidic device followed by washing with buffer. The circular region on the left revealed only red fluorescence while the square region on the right revealed only green fluorescence, each corresponding to the expected antigen. The circular region on the top, used as a reference without any immobilized antibody, was black (Figure 4b). These results indicate that the PCB coated CP sensor is able to specifically detect analytes while resisting nonspecific adsorption using covalently coupled ligands. Specifically, the lack of any observed signal drift during detection confirms the strong covalent attachment of the antibodies. Thus, PCB offers the ability to eliminate signal drift and signal distortion, both frequently observed using typical physical adsorption for ligand attachment, thereby further improving the performance of paper-based diagnostic devices in addition to being able to perform in the presence of undiluted complex media (Figure 3a,b).

CONCLUSIONS

To summarize, PCB modified cellulose paper was demonstrated as a potential paper-based microfluidic diagnostic device. Due to the excellent fouling resistance and super-hydrophilic properties of PCB, devices with PCB coatings have the advantages of faster diffusion as well as a significant increase in the amount of target analytes present in the detection zone which thereby increases assay sensitivity, particularly in complex media such as undiluted human serum. Due to the functionalization properties of PCB, we further demonstrated the ability of the PCB modified cellulose paper for antigen–antibody detection via the covalent immobilization of protein ligands using EDC/NHS coupling chemistry. Previous work using PCB modified glucose sensors has demonstrated highly sensitive and long-term (42 days) detection from whole blood (containing cells).[31] Thus, for real-life applications in which a fingerstick will be used to obtain a sample of blood, it is anticipated that the performance of the PCB modified paper sensors, especially for use in a low resource setting, will not be reduced in any way.

While this work has demonstrated the detection of glucose from undiluted human serum as well as covalent antibody immobilization, additional efforts will be necessary before the PCB modified sensors can be used in clinically relevant situations. This includes more in depth stability studies, investigating the potential for interfering substances, and establishing the performance on clinical specimens. While more work lies ahead, the results presented here provide a basis for the development of low-cost, portable, and simple multiplexed bioassays with high specificity and low background noise for real-world applications in complex media.

ASSOCIATED CONTENT

Supporting Information

Experimental details and additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: sjiang@uw.edu.

Author Contributions

[§]Y.Z. and X.X. contributed equally.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (CBET 1264470). Y.H.Z was supported by the Chinese Scholar Program with additional support from the National Nature Science Foundation of China (No. 61371021). X.H. acknowledges the support from the National Nature Science Foundation of China (No. 21176065) and the Shanghai Municipal Education Commission.

REFERENCES

(1) Yager, P.; Edwards, T.; Fu, E.; Helton, K.; Nelson, K.; Tam, M. R.; Weigl, B. H. *Nature* **2006**, *442*, 412–418.

(2) Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1318–1320.

(3) Yager, P.; Domingo, G. J.; Gerdes, J. *Annu. Rev. Biomed. Eng.* **2008**, *10*, 107–144.

(4) Paroloa, C.; Merkoçi, A. *Chem. Soc. Rev.* **2013**, *42*, 450−457.
(5) Pelton, R. *Trends Anal. Chem.* **2009**, *28*, 925−1036.
(6) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E. *Anal. Chem.* **2010**, *82*, 3−10.
(7) Kwon, H.; Samain, F.; Kool, E. T. *Chem. Sci.* **2012**, *3*, 2542−2549.
(8) Tobjörkand, D.; Österbacka, R. *Adv. Mater.* **2011**, *23*, 1935−1961.
(9) Zhao, W. A.; Ali, M. M.; Aguirre, S. D.; Brook, M. A.; Li, Y. F. *Anal. Chem.* **2008**, *80*, 8431−8437.
(10) Liu, P. S.; Chen, Q.; Liu, X.; Yuan, B.; Wu, S. S.; Shen, J.; Lin, S. C. *Biomacromolecules* **2009**, *10*, 2809−2816.
(11) Stollner, D.; Scheller, F. W.; Warsinke, A. *Anal. Biochem.* **2002**, *304*, 157−165.
(12) Brumer, H.; Zhou, Q.; Baumann, M. J.; Carlsson, K.; Teeri, T. T. *J. Am. Chem. Soc.* **2004**, *126*, 5715−5721.
(13) Su, S. X.; Nutiu, R.; Filipe, C. D. M.; Li, Y. F.; Pelton, R. *Langmuir* **2007**, *23*, 1300−1302.
(14) Araujo, A. C.; Song, Y.; Lundeberg, J.; Stahl, P. L.; Brumer, H. *Anal. Chem.* **2012**, *84*, 3311−3317.
(15) Yu, A.; Shang, J.; Cheng, F.; Paik, B. A.; Kaplan, J. M.; Andrade, R. B.; Ratner, D. M. *Langmuir* **2012**, *28*, 11265−11273.
(16) Idris, A.; Yet, L. K. *J. Membr. Sci.* **2006**, *280*, 920−927.
(17) Ishihara, K.; Takayama, R.; Nakabayashi, N.; Fukumoto, K.; Aoki, J. *Biomaterials* **1992**, *13*, 235−239.
(18) Linder, A.; Bergman, R.; Bodin, A.; Gatenholm, P. *Langmuir* **2003**, *19*, 5072−5077.
(19) Ye, S. H.; Watanabe, J.; Iwasaki, Y.; Ishihara, K. *Biomaterials* **2003**, *24*, 4143−4152.
(20) Fu, E.; Liang, T.; Spicar-Mihalic, P.; Houghtaling, J.; Ramachandran, S.; Yager, P. *Anal. Chem.* **2012**, *84*, 4574−4579.
(21) Rusling, J. F.; Kumar, C. V.; Gutkinde, J. S.; Patele, V. *Analyst* **2010**, *135*, 2496−2511.
(22) Jiang, S. Y.; Cao, Z. Q. *Adv. Mater.* **2010**, *22*, 920−932.
(23) Krause, J. E.; Brault, N. D.; Li, Y. T.; Xue, H.; Zhou, Y. B.; Jiang, S. Y. *Macromolecules* **2011**, *44*, 9213−9220.
(24) Yang, W.; Zhang, L.; Wang, S. L.; White, A. D.; Jiang, S. Y. *Biomaterials* **2009**, *30*, 5617−5621.
(25) Vaisocherova, H.; Yang, W.; Zhang, Z.; Cao, Z. Q.; Cheng, G.; Piliarik, M.; Homola, J.; Jiang, S. Y. *Anal. Chem.* **2008**, *80*, 7894−7901.
(26) Li, Y. T.; Keefe, A. J.; Giarmarco, M.; Brault, N. D.; Jiang, S. Y. *Langmuir* **2012**, *28*, 9707−9713.
(27) Huang, C. J.; Li, Y. T.; Jiang, S. J. *Anal. Chem.* **2012**, *84*, 3440−3445.
(28) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 19606−19611.
(29) Zhao, W.; van der Berg, A. *Lab Chip* **2008**, *8*, 1988−1991.
(30) Seo, J. H.; Matsuno, R.; Konno, T.; Takai, M.; Ishihara, K. *Biomaterials* **2008**, *29*, 1367−1376.
(31) Yang, W.; Bai, T.; Carr, L. R.; Keefe, A. J.; Xu, J. J.; Xue, H.; Irvin, C. A.; Chen, S. F.; Wang, J.; Jiang, S. Y. *Biomaterials* **2012**, *33*, 7945−7951.