Journal of Applied Polymer Science

# New method for fabricating an α-fetoprotein affinity monolithic polymer array

**Li Li,[1] Li-Ru Xia,[1] He-Ye Wang,[1] Xiao-Dong Bi[2]**

[1]Institute of Facilities and Equipment in Agriculture, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China
[2]Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China
Correspondence to: L. Li (E-mail: muzishuiqudou@163.com)

**ABSTRACT:** Bisphenol A based epoxy acrylate (BABEA), a commercial UV-curable material, was introduced as a crosslinker for the fabrication of an epoxy-functionalized monolithic polymer array through UV-initiated copolymerization with glycidyl methacrylate as the functional monomer and poly(ethylene glycol) 200 as the porogen. Scanning electron microscopy images showed that the monolithic poly(bisphenol A based epoxy acrylate-*co*-glycidyl methacrylate) [poly(BABEA-*co*-GMA)] exhibited a well-controlled skeletal and well-distributed porous structure. The α-fetoprotein (AFP) immunoaffinity monolithic polymer array prepared by the immobilization of AFP on epoxy-functionalized monolithic arrays was used as an immunosensor for chemiluminescent AFP detection. X-ray photoelectron spectroscopy results indicate that the AFP antibody was successfully immobilized on the monolithic poly(BABEA-*co*-GMA) array. With a noncompetitive immune-response format, the proposed AFP immunoaffinity array was demonstrated as a low-cost, flexible, homogeneous, and stable array for AFP detection. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41792.

**KEYWORDS:** photopolymerization; porous materials; sensors and actuators

Received 13 July 2014; accepted 22 November 2014
**DOI: 10.1002/app.41792**

## INTRODUCTION

The enzyme-linked immunosorbent assay (ELISA) is widely used in medical diagnostic and biochemical analyses to detect proteins based on their binding to immobilized antibodies. A primary antibody immobilized on a solid surface is used to bind the antigen, and a secondary labeled antibody is used to detect the captured antigen. The binding of the secondary antibody is quantified by the measurement of the activity of an enzyme bound to the secondary antibody.

Most ELISAs today are performed in 96-well plates. Dedicated instruments have been developed to automate the assay, including robotic pipetters, plate washers, and optical colorimetric detectors. However, the assay is slow (e.g., several hours) and requires large volumes of samples and reagents. Miniaturized chemical systems on immunoassays have been garnering great interest.[1–8] Miniaturized immunoassays, nevertheless, place greater demands on handling accuracy, and attempts to address this with more precise machinery naturally translate to a higher cost. This has led to the development of alternative approaches for handling small liquid volumes without the need for complex or precise machinery.[9–13]

Polymeric monoliths have already developed into important materials in the areas of separation science.[14,15] Monoliths consist of a single continuous network of porous materials that provides a high surface area, tunable functionality, easy fabrication, and low cost. Because of these advantages, polymeric monoliths can be excellent supports for immunoaffinity materials. Two methods that are commonly used to prepare monoliths are thermal polymerization[16] and UV-initiated polymerization.[17,18] UV-initiated polymerization is more desirable for the fabrication of monolithic arrays because it can provide spatial resolution with a photomask.

Bisphenol A based epoxy acrylate (BABEA) is one of the most widely used commercially available UV curable oligomers because of its fast curing speed, low cost, good pigment wetting, high gloss, hardness, and chemical resistance of the cured films.[19,20] In this study, BABEA was introduced as a crosslinker for the facile manufacturing of epoxy-functional monolithic arrays through on-suit UV copolymerization with glycidyl methacrylate (GMA) as the functional monomer and poly(ethylene glycol) 200 (PEG 200) as the porogen. A fabrication procedure was proposed to fabricate epoxy-functionalized monolithic polymer arrays. As compared with poly(bisphenol A

Additional Supporting Information may be found in the online version of this article.

**Table I.** Compositions of the Prepolymer Solutions Used for the Preparation of the Monolithic Polymer Arrays

| | A | B | C | D |
|---|---|---|---|---|
| PEG 200 (mL) | 0 | 20 | 50 | 80 |
| GMA (mL) | 3 | 3 | 3 | 3 |
| BABEA (mL) | 10 | 10 | 10 | 10 |
| Irgacure 184 (mg) | 1 | 1 | 1 | 1 |

based epoxy acrylate-*co*-glycidyl methacrylate) [poly(BABEA-*co*-GMA)] microzone plates,[21] the poly(BABEA-*co*-GMA) monolithic arrays exhibited two significant differences. First, the monolithic poly(BABEA-*co*-GMA) exhibited a well-controlled skeletal and porous structure, which provided a high surface area and increased the binding amount of biological molecules. Second, the complementary photomask was used, so the monolithic poly(BABEA-*co*-GMA) was polymerized at the test zone.

α-Fetoprotein (AFP), an oncofetal glycoprotein with a molecular weight of approximately 70,000 Da, is well known as a tumor marker. The concentration of AFP in healthy adults is typically below 25 ng/mL. Increased serum AFP levels have been considered as an early indication of some cancerous diseases, including hepatocellular cancer, yolk sac cancer, liver cancer, and nasopharyngeal cancer. Thus, it is very important to explore a rapid detection method for AFP. In this study, epoxy groups located within porous monoliths permitted binding with AFP antibodies through an epoxy–amino group reaction, which was applied for construction of AFP immunoaffinity monolithic polymer arrays.[22]

## EXPERIMENTAL

### Reagents and Materials

BABEA photoresist (EBECRYL 600) was purchased from UCB (Belgium). 1-Hydroxyl cyclohexyl phenyl ketone (Irgacure 184) from Ciba Specialty Chemicals (Switzerland) was used as a photoinitiator. Human AFP, Human carcino-embryonic antigen (CEA), AFP antibodies, CEA antibodies, horseradish peroxidase (HRP) conjugated AFP antibodies, and HRP-conjugated CEA antibodies were purchased from Shuangliuzhenglong Chemical and Biological Articles Co., Ltd. (China). Bovine serum albumin was purchased from Sigma and was prepared in a 0.1 mol/L phosphate buffer solution (PBS; pH = 7.0). Luminol and *p*-iodophenol (PIP) were purchased from Nanjing Searchbio Co., Ltd. (China). Dimethyl sulfoxide and GMA were purchased from Aladdin-Reagent (Shanghai, China). Other chemicals were analytical-reagent grade. Water was purified with a Milli-Q Advantage A10. A series of photomasks with different designs were purchased from the Fifty-Fifth Research Institute of China Electronic Science & Technology Group Co. (China).

### Instruments

A UV-curing chamber equipped with a 365-nm UV light source was obtained from Zhongtian Coating (Baoding, China). The X-ray photoelectron spectroscopy (XPS) measurement was performed on a Thermo ESCALAB 250 spectrometer (Waltham, MA) with an Al Kα X-ray source (1486.6 eV). All binding energies were referred to the C1s neutral carbon peak at 284.6 eV. Chemiluminescence (CL) detection was performed on an MPI-A luminescence analyzer (Ruimai Analytical Instrument, Xi'an, China). Scanning electron microscopy (SEM) analyses were performed on a Hitachi field emission scanning electron microscopy (S-4800, Tokyo, Japan).

### Preparation of Stock Solutions

The prepolymer solution was prepared by the dissolution of BABEA, the monomer GMA, and the photoinitiator Irgacure 184 in the porogen PEG 200. The compositions of the solutions used for the preparation of the monolithic poly(BABEA-*co*-GMA) are shown in Table I.

A luminol stock solution (0.01*M*) was prepared by the dissolution of 177 mg of luminol (Nanjing Searchbio, China) in 100 mL of 0.1 mol/L NaOH (0.1*M*) and was kept in the dark. A PIP stock solution (0.01*M*) was prepared by the dissolution of 110 mg of PIP (Nanjing Searchbio, China) in 5 mL of dimethyl sulfoxide and then dilution with water to 50 mL and was kept in the dark. CL substrates were freshly prepared by the addition of 50 μL of luminol stock solution, 50 μL of PIP stock solution, and 3.4 μL of $H_2O_2$ (3% v/v) to 900 μL of phosphate buffer (0.2*M*, pH 8.5).

### Pretreatment of the Glass Substrates

The glass substrates were pretreated with a vinyl silanizing agent that anchored the monolithic polymers onto the surface. After treatment with 0.1*M* NaOH and 0.1*M* HCl for 1 h each and rinsing with water until neutralization (pH 7.0), the glass slides were dried on a heating plate at 60°C for 30 min. Subsequently, the glass slides were immersed in a 1:9 v/v mixture of γ-methacryloxypropyltrimethoxysilane (γ-MAPS) and methanol at 60°C for 12 h. Finally, the glass slides were rinsed with methanol and water to flush out residual reagents.

### Fabrication and Characterization of the Monolithic Poly(BABEA-*co*-GMA) Array

The proposed fabrication procedure was simple, as shown in Figure 1. Briefly, the stock prepolymer solution was first spin-coated onto the pretreated glass substrates at a speed of 3000 rpm for 20 s. Then, a photomask of a designed pattern printed on a transparency sheet was attached on top of the substrate surface. Finally, UV exposure was performed with a 300

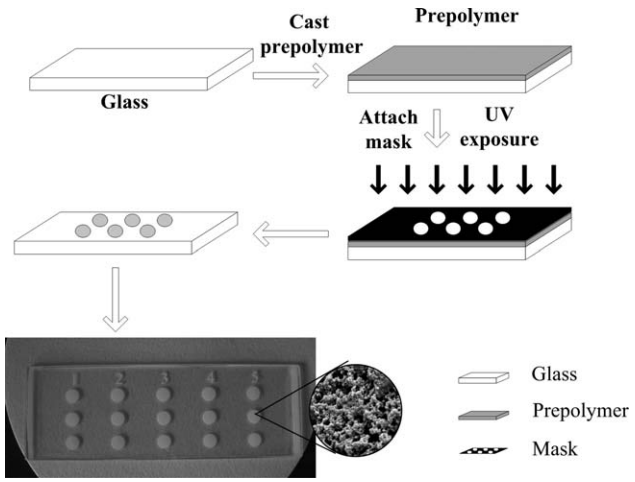


**Figure 1.** Scheme of the epoxy-functionalized monolithic polymer array fabrication process.

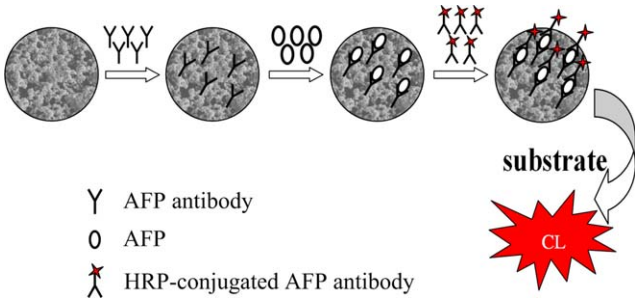


**Figure 2.** Scheme of the noncompetitive immune response method of AFP detection. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mJ/cm$^2$ dose at a wavelength of 365 nm for 30 s. Clear areas of the photomask allowed transmission of the UV light and the curing of the mixture on illuminated sites, whereas the dark areas of the mask blocked the UV light and left the covered regions unpolymerized. The unpolymerized mixture was removed by washing with acetonitrile–water solution (50:50 v/v). Then, the monolithic polymer arrays were dried *in vacuo*.

### Fabrication of the AFP Immunoaffinity Monolithic Poly(BABEA-*co*-GMA) Array and Detection

A volume of 10 $\mu$L of 0.1 mg/mL AFP antibody was added to each array point, and the array was incubated for 12 h at 20°C with frequent vibrating. After three washes with 0.1*M* PBS (pH 7.0), the left epoxy groups were blocked with 5 $\mu$L of 1% bovine serum albumin for 2 h. Finally, the AFP immunoaffinity monolithic poly(BABEA-*co*-GMA) arrays were washed with 0.1*M* PBS (pH 7.0) and stored in 0.1*M* PBS (pH 7.0) at 4°C when not in use.

The detection of AFP was based on a noncompetitive immune response method (Figure 2). First, 10-$\mu$L portions of AFP standard solution were added to the AFP immunoaffinity monolithic array points. After preincubation for 30 min at room temperature, the monolithic polymer array points were washed three times with 3 × 5 mL of 0.1*M* PBS (pH 7.0) containing 0.05% Tween-20. Then, 10-$\mu$L portions of HRP-conjugated AFP antibodies were added to the points. After preincubation for 30 min at room temperature, the AFP immunoaffinity monolithic polymer array points were washed again three times with 3 × 5 mL 0.1*M* PBS (pH 7.0) containing 0.05% Tween-20. Finally, the AFP immunoaffinity monolithic polymer array was placed on the photomultiplier, and 10 $\mu$L of CL substrate was dropped into the monolithic array points. The CL signal was captured and recorded by the detector.

## RESULTS AND DISCUSSION

### Physicochemical Properties

Under UV light radiation, the photoinitiator generated active free radicals and thus initiated the polymerization reaction. According to the copolymerization reaction, there were some epoxy groups on poly(BABEA-*co*-GMA). The presence of these epoxy groups allowed the monolithic poly(BABEA-*co*-GMA) to be made of the proposed materials for the easy immobilization of proteins, such as AFP antibodies.[21]

The ratios of porogens have great effects on the internal structure of monoliths. In our experiments, PEG 200 was selected to

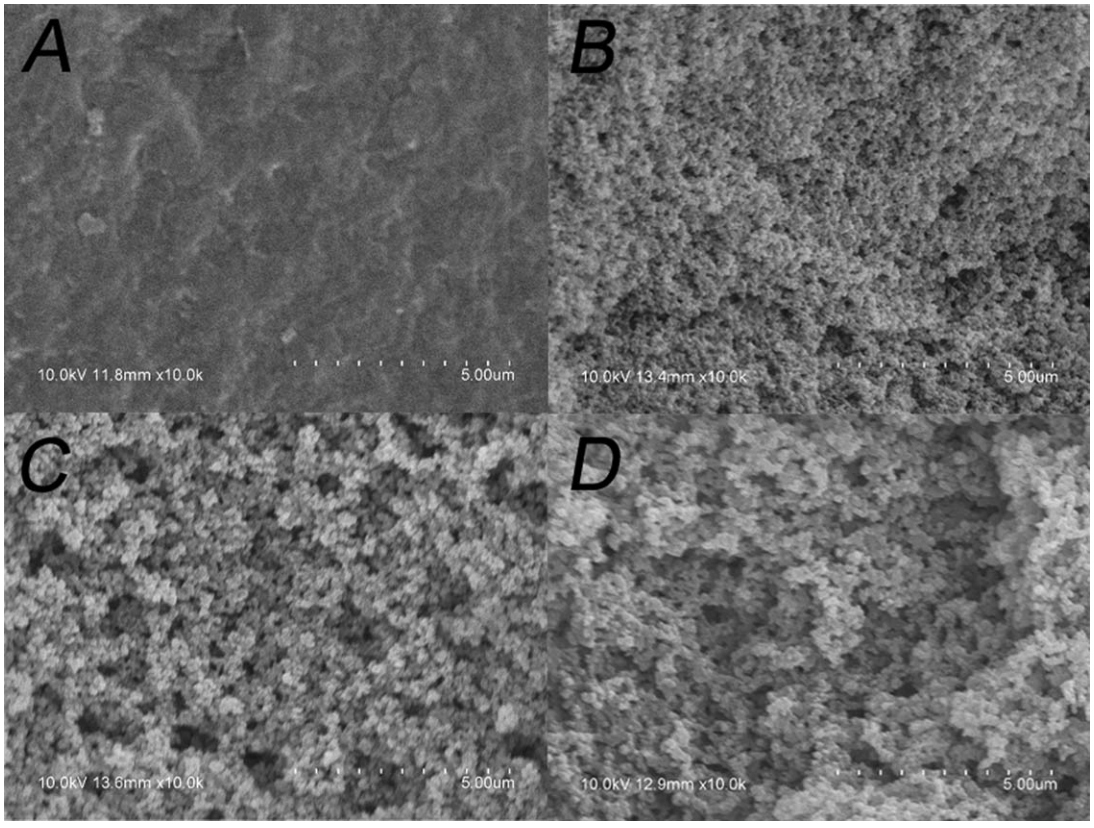


**Figure 3.** SEM images of internal structures of polymers A, B, C, and D.

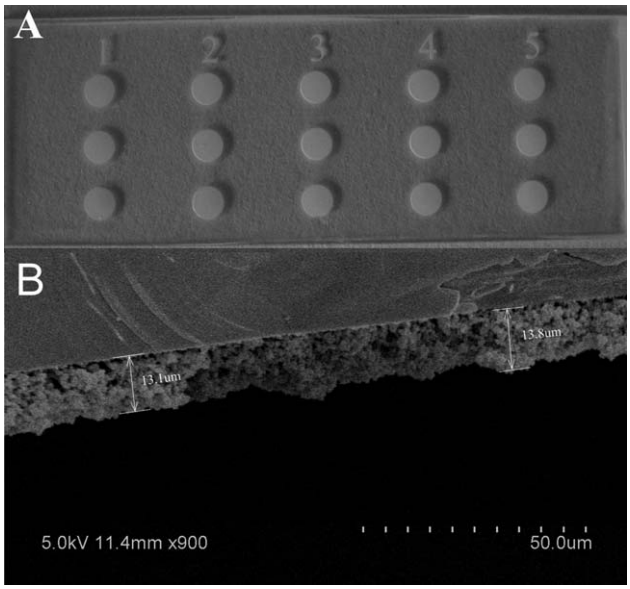


**Figure 4.** (A) Photograph of the monolithic poly(BABEA-*co*-GMA) array and (B) SEM image of a cross section of the poly(BABEA-*co*-GMA) monolith.

improve the homogeneity and penetrability of the monolithic matrix. The effect of the ratio of PEG 200 to BABEA on the porous structure of the monoliths was investigated by SEM and the mercury intrusion method. Figure 3 shows the SEM micrographs of the internal structure of the polymeric monoliths where A, B, C, and D correspond to the prepolymer compositions A, B, C, and D in Table I. Figure 3(A) shows the lucent polymer A has a dense structure. With PEG 200 added, a nonporous structure was obtained in the translucent polymer B [Figure 3(B)]. A well-controlled skeletal and well-distributed porous structure was obtained in polymer C [Figure 3(C)]. However, with more PEG 200 added, the porosity of polymer D was improved [Figure 3(D)], but the polymer rigidity became poor.

The average size of the micropores and the total porosity were measured by the mercury intrusion method to be 11.24 nm and 35.1% for monolith A, 693.57 nm and 64.91% for monolith B, 1.17 $\mu$m and 76.3% for monolith C, and 1.57$\mu$m and 71.4% for monolith D, respectively.

As could be observed by a comparison of monoliths C and D, the optimized monolith (monolith C) had a uniform pore distribution and well-controlled skeletal structure. Meanwhile, it also had good polymer rigidity for the following experiments. Thus, in the following experiment, prepolymer solution C was selected as working solution.

The monolithic poly(BABEA-*co*-GMA) array C was fabricated according to the proposed procedure. The photograph of the fabricated monolithic array C is shown in Figure 4(A). The monolithic array C was designed to have three rows and five columns, and each monolith point was 2 mm in diameter.

To evaluate the homogeneity of the monolithic array C, SEM characterization was carried out. Figure 4(B) shows a vertical angled top-view image of the monolithic wall on array C; this indicates that the monolithic walls were homogeneous and had a uniform thickness of about 13.5 $\mu$m.

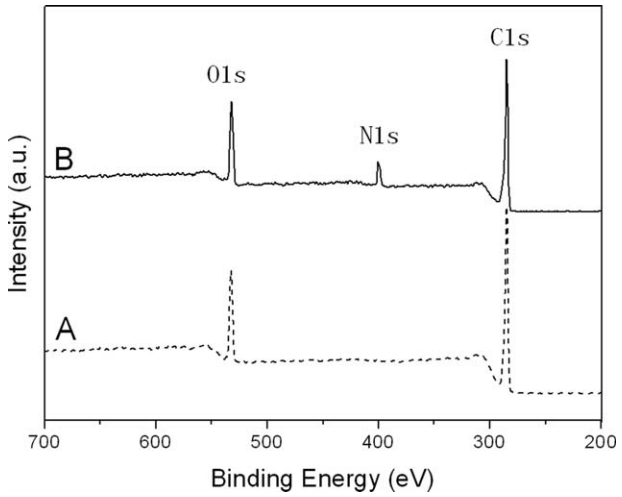


**Figure 5.** XPS overview spectra: (A) poly(BABEA-*co*-GMA) and (B) AFP antibody-immobilized poly(BABEA-*co*-GMA).

### Immobilization of the AFP Antibodies

Information on the immobilization of the antibodies on the poly(BABEA-*co*-GMA) monoliths was obtained by XPS (Figure 5). In the XPS overview spectra, in addition to those of the carbon and oxygen, an N1s peak was detected at the binding energy of 400 eV; this showed the presence of AFP antibodies on the surface of poly(BABEA-*co*-GMA).

### Performance of the AFP Immunoaffinity Monolithic Poly(BABEA-*co*-GMA) Array

The uniformity of the AFP immunoaffinity monolithic poly (BABEA-*co*-GMA) array was assessed by intra-assay coefficients of variation (CVs). The intra-assay CV was the relative difference between the CL measurements of the same sample (20ng/mL AFP) on five different monolithic array points (Figure 6). The intra-assay CVs obtained at the AFP concentration of 20 ng/mL were 5.12%. The low value of the CV indicated that the immunoaffinity monolithic array was homogeneous and stable.

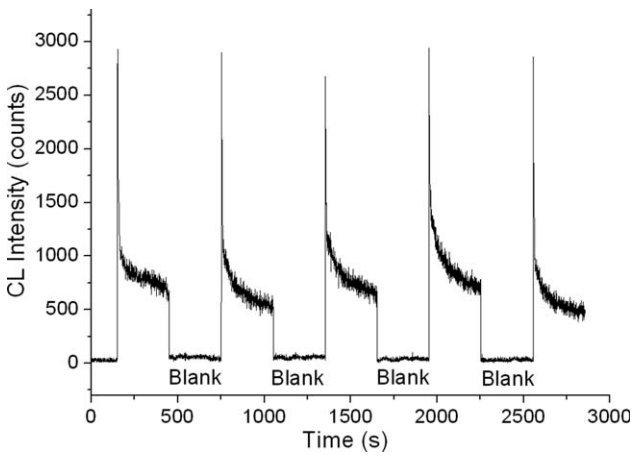


**Figure 6.** CL signal of an intramonolithic polymer array point.

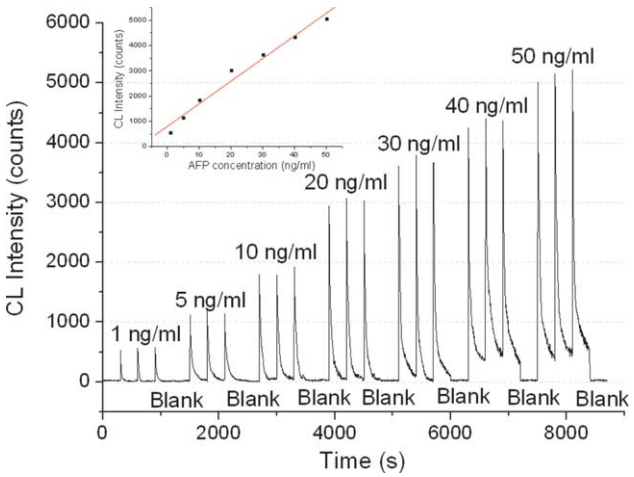


**Figure 7.** Dose–response curves for the AFP array. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The performance of the AFP immunoaffinity monolithic poly(BABEA-*co*-GMA) array was demonstrated by the measurements of the AFP samples of different concentrations. The CL intensity ($I$) increased with increasing AFP concentration ($C$). The dose–response curves for AFP showed a linear range from 1.0 to 50 ng/mL with a correlation coefficient of 0.99 (Figure 7). The regression equation was as follows:

$$I = 803.1 + 89.6C$$

The limit of detection was 1 ng/mL (signal/noise ratio = 3); this met well the requirement for early clinical diagnosis. The obtained limit of detection for AFP was lower than those of the previously reported multiplex immunoassay methods.[22–24]

## CONCLUSIONS

In this study, a simple fabrication procedure was proposed to fabricate epoxy-functionalized monolithic polymer arrays. BABEA was introduced as a crosslinker for the facile manufacturing of porous epoxy-functionalized monolithic polymer arrays through photopatterned UV-initiated copolymerization with GMA as the functional monomer and PEG 200 as the porogen. The method provided the poly(BABEA-*co*-GMA) monoliths with several excellent properties, including a fast curing, high replication fidelity, hydrophilicity, well-distributed porous structure, and existence of epoxy groups, on the copolymer surface for the later immobilization of proteins. These favorable features make the monolithic poly(BABEA-*co*-GMA) a promising porous material for the fabrication of immunoaffinity arrays. Although these advantageous features were exemplified with the fabrication of an AFP immunoaffinity monolithic poly(BABEA-*co*-GMA) array, the monolithic poly(BABEA-*co*-GMA) arrays are applicable also for other protocols. Figure 1 in the Supporting Information shows the application of the poly(BABEA-*co*-GMA) monolithic material for the fabrication of the CEA immunoaffinity monolithic poly(BABEA-*co*-GMA) array for detecting CEA in serum. These results demonstrate that the monolithic poly(BABEA-*co*-GMA) arrays can provide a sensitive, low-cost, and reliable tool for detecting various biomarkers in biological fluids.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support of the Jiangsu Provincial Natural Science Foundation of China (contract grant number BK20130707) and the Jiangsu Agricultural Science and Technology Innovation Foundation [contract grant number CX(13)5064].

## REFERENCES

1. Joshua, B.; Miguel, O.; Yordan, K.; Hugh, A. B.; Avraham, R. *Lab Chip* **2011**, *11*, 941.
2. Hong, C.; Assem, A.; Mohammad, F. *Microfluid. Nanofluid.* **2011**, *10*, 593.
3. Parween, S.; Nahar, P. *Biosens. Bioelectron.* **2013**, *48*, 287.
4. Mirasoli, M.; Guardigli, M.; Michelini, E.; Roda, A. *J. Pharm. Biomed. Anal.* **2014**, *87*, 36.
5. Alves, N. J.; Mustafaoglu, N.; Bilgicer, B. *Biosens. Bioelectron.* **2013**, *49*, 387.
6. Yang, M.; Kostov, Y.; Bruck, H. A.; Rasooly, A. *Anal. Chem.* **2008**, *80*, 8532.
7. Sapsford, K. E.; Sun, S.; Francis, J.; Sharma, S.; Kostov, Y.; Rasooly, A. *Biosens. Bioelectron.* **2008**, *24*, 618.
8. Sun, S.; Yang, M. H.; Kostov, Y.; Rasooly, A. *Lab Chip* **2010**, *10*, 2093.
9. Cheng, C. M.; Martinez, A. W.; Gong, J. L.; Mace, C. R.; Phillips, S. T. *Angew. Chem. Int. Ed.* **2010**, *49*, 4771.
10. Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E. *Anal. Chem.* **2010**, *82*, 3.
11. Horak, J.; Dincer, C.; Bakirci, H.; Urban, G. *Sens. Actuators B* **2014**, *191*, 813.
12. Lu, L. L. Y.; Bie, Z. J.; Chen, H. Y.; Liu, Z. *Angew. Chem. Int. Ed.* **2013**, *52*, 7451.
13. Carrilho, E.; Martinez, A. W.; Whitesides, G. M. *Anal. Chem.* **2009**, *81*, 7091.
14. Liu, Y. C.; Lu, Y.; Liu, Z. *Chem. Sci.* **2012**, *3*, 1467.
15. Cafer, C.; Jerome, P. F.; James, P. L.; Perihan, C. *Anal. Bioanal. Chem.* **2010**, *398*, 1909.
16. Liu, Y. C.; Ren, L. B.; Liu, Z. *Chem. Commun.* **2011**, *47*, 5067.
17. Cafer, C.; Jerome, P. F.; James, P. L.; Perihan, C. *Anal. Chim. Acta* **2011**, *690*, 94.
18. Li, L.; Bi, X. D.; Yu, J. Z.; Ren, C. L.; Liu, Z. *Electrophoresis* **2012**, *33*, 2591.
19. Bajpai, M.; Shukla, V.; Habib, F. *Prog. Org. Coat.* **2005**, *53*, 239.
20. Li, L.; He, Z. H.; Wang, W. W.; Fan, Y. M.; Mao, X. P.; Chen, D. H.; Chen, C. *Prog. Org. Coat.* **2006**, *56*, 126.
21. Li, L.; Bi, X. D.; Feng, M.; Zhu, J. T. *J. Appl. Polym. Sci.* **2014**, *131*, 39787.
22. Fu, Z. F.; Hao, C.; Fei, X. Q.; Ju, H. X. *J. Immunol. Methods* **2006**, *312*, 61.
23. Fu, Z. F.; Yan, F.; Liu, H.; Yang, Z. J.; Ju, H. X. *Biosens. Bioelectron.* **2008**, *23*, 1063.
24. Zhao, Y. J.; Zhao, X. W.; Hu, J.; Xu, M.; Zhao, W. J.; Sun, L. G.; Zhu, C.; Xu, H.; Gu, Z. Z. *Adv. Mater.* **2009**, *21*, 569.