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UV-Initiated Copolymerization Route for Facile Fabrication of Epoxy-Functionalized Micro-Zone Plates

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ABSTRACT: Bisphenol A-based epoxy acrylate (BABEA), a commercial ultraviolet (UV)-curable material, was introduced as a new manufacturing material for facile fabrication of epoxy-functionalized micro-zone plates through UV-initiated copolymerization using glycidyl methacrylate (GMA) as the functional monomer. The poly (BABEA-*co*-GMA) was highly transparent in visible range while highly opaque when the wavelength is less than 295 nm, and of high replication fidelity. X-ray photoelectron spectroscope (XPS) results indicated the existence of epoxy groups on the surface of the poly (BABEA-*co*-GMA), which allowed for binding protein through an epoxy-amino group reaction. A fabrication procedure was proposed for manufacturing BABEA based epoxy-functionalized micro-zone plates. The fabrication procedure was very simple; obviating the need of micromachining equipments, wet etching or imprinting techniques. To evaluate the BABEA-based epoxy-functionalized micro-zone plates, α-fetoprotein (AFP) was immobilized onto the capture zone for chemiluminescent (CL) detection in a noncompetitive immune response format. The proposed AFP immunoaffinity micro-zone plate was demonstrated as a low cost, flexible, homogeneous, and stable assay for α-fetoprotein (AFP). © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39787.

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INTRODUCTION

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 quantitated by measuring the activity of an enzyme bound to a 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 are 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 higher cost. This has led to the development of alternative approaches to handle small liquid volumes without complex or precise machinery needed.[9–13]

UV curable technology has several advantages, such as rapid prototyping with short curing time, low energy consumption, room temperature operation, excellent resistance toward organic solvents/chemicals/heats, and tunable properties of the polymers.[14] With these advantages, miniaturized chemical devices have been successfully fabricated by using UV curable resins such as thermoset polyester,[15,16] polyurethane-related optical adhesive,[17] polyurethane-methacrylate,[18] and polyurethane acrylate.[19] However, these materials have not been evaluated for surface properties such as functional groups and hydrophilic property. With increasing interest in applying miniaturized chemical devices in biological applications, it is important to develop new alternatives to provide more desirable properties.

Bisphenol A-based epoxy acrylate (BABEA) is one of the most widely used commercially available UV curable oligomers for its fast curing speed, low-cost, good pigment wetting, high gloss, hardness, and chemical resistance to the cured films.[20,21] These

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

interesting and unique properties have made BABEA as a good candidate for protective resin. In this article, BABEA was introduced as a new material for the facile manufacturing of epoxy-functional micro-zone plates through UV-initiated copolymerization using glycidyl methacrylate (GMA) as the functional monomer. The poly (BABEA-*co*-GMA) as a miniaturized immunoassay-fabricating material exhibited several excellent properties, including fast-curing, high replication fidelity, excellent optical transparency in the visible range, and existence of epoxy groups on the copolymer surface for later immobilization of antibodies. A fabrication procedure was proposed to fabricate epoxy-functionalized micro-zone plates.

α-Fetoprotein (AFP), an oncofetal glycoprotein with a molecule 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, yolksac cancer, liver cancer, and nasopharyngeal cancer. Thus, it is very important to explore a rapid detection method for AFP. Here, surface of the micro-zone plates have much epoxy groups; anti-AFP antibody was then immobilized onto the capture zone through an epoxy-amino group reaction[22] for construction of an AFP immunoaffinity micro-zone plate.

MATERIALS AND METHODS

Reagents and Materials

Bisphenol A-based epoxy acrylate photoresist (EBECRYL 600) was purchased from UCB (Belgium). 1-Hydroxy cyclohexyl phenyl ketone (Irgacure 184) from Ciba Specialty Chemicals (Switzerland) was used as a photoinitiator. Propylene-glycol-monoether-acetate (PGMEA) developer was obtained from Microchem (USA). AFP diagnostic kit and anti-AFP antibody were purchased from Shuangliuzhenglong Chemical and Biological Articles (China). The diagnostic kit consisted of AFP (20 ng/mL) standard solutions and a solution of horseradish peroxidase (HRP)-labeled horse polyclonal AFP antibody used as enzyme tracer. Bovine serum albumin (BSA) was purchased from Sigma (USA) and prepared in 0.1 mol/L phosphate buffer solution (PBS, pH = 7.0). Luminol and p-Iodophenol (PIP) were purchased from Nanjing Searchbio (China). Dimethylsulfoxide (DMSO) and GMA were purchased from Aladdin-Reagent (Shanghai, China). Other chemicals were of analytical-reagent grade. Water was purified with a Milli-Q Advantage A10 (USA). A series of photomasks with different design were purchased from the Fifty-fifth Research Institute of China Electronic Science & Technology Group Company (China).

Instruments

A UV curing chamber equipped with a 365-nm UV light was obtained from Zhongtian Coating (Baoding, China). The X-ray photoelectron spectroscopic (XPS) measurement was performed on a Thermo ESCALAB 250 spectrometer (Waltham, MA, USA) with an Al Kα X-ray source (1486.6 eV). All binding energies were referred to C1s neutral carbon peak at 284.6 eV. Optical transmission spectra were collected using a Beckman Coulter DU720 UV-VIS spectrophotometer (Fullerton, CA). Differential scanning calorimetry (DSC) was conducted with a DSC-60 (Shimadzu) at heating rate of 20°C/min, under nitrogen atmosphere. Samples of the glass, quartz, and poly (BABEA-*co*-GMA) for the optical transmission measurements were all 2 mm thick. Optical microscope images of the poly (BABEA-*co*-GMA) microstructures were obtained using a Leica DMIL inverted epi-fluorescence microscope (Wetzlar, Germany). Chemiluminescence (CL) detection was performed on a MPI-A Luminescence Analyzer (Ruimai Analytical Instrument, Xi'an, China).

Synthesis of Copolymer

Fabrication of Poly (BABEA-co-GMA). The BABEA (0.5 g) and the photo-initiator Irgacure 184 (0.005 g) were dissolved in GMA (300–700 μL). The obtained prepolymer solution was coated onto PDMS slide and prebaked on an electric heating plate at 50°C for 10 min. After the PDMS slide cooled to room temperature, UV exposure was performed with a 300 mJ cm^{-2} dose at a wavelength of 365 nm for 5–30 s. The poly (BABEA-*co*-GMA) was peeled off from the PDMS slide and washed with PGMEA developer to remove the unpolymerized mixture.

Characterization of Monomer Content. To measure the monomer content of the copolymer, the dried samples were immersed in acetonitrile for 6 h. The monomer content (MC, gram per gram) of the copolymers was calculated according to the following equation:

$$\text{Monomer content} = (W_0 - W_1)/W_0 \quad (1)$$

where W_0 is the weight of as-prepared sample and W_1 is the weight of sample after extraction in acetonitrile. All the experiments were carried out in triplicate, and the average values were reported.

Swelling Behaviors of Copolymer. To measure the swelling ratio (SR) of the copolymer, the dried samples were immersed in water for 48 h. The swelling ratio (SR, gram per gram) of the copolymer was calculated according to the following equation:

$$\text{SR} = (W_t - W_0)/W_0 \quad (2)$$

where W_0 is the weight of as-prepared sample and W_t is the weight of the swollen polymer. All the experiments were carried out in triplicate, and the average values were reported.

Fabrication of Poly (BABEA-*co*-GMA) Micro-Zone Plates

The proposed fabrication procedure was simple, as shown in Figure 1. First, prepolymer was spun onto the poly (BABEA-*co*-GMA) layer, followed with prebaking at 50°C for 10 min. After the substrate cooled to room temperature, a photomask printed on a transparency sheet was attached to the substrate surface, followed with UV exposure under the same conditions as above. Clear areas of the photomask allowed transmission of the UV light, curing the mixture while dark areas of the mask blocked the UV light and left the covered regions unpolymerized. Post-exposure bake was performed at 80°C for 15 min. After cooling naturally to room temperature, the unpolymerized mixture was removed by washing with the PGMEA developer.

Preparation of CL Substrates

The luminol stock solution (0.01 mol/L) was prepared by dissolving 177 mg of luminol (Nanjing Searchbio, China) in 100 ml of 0.1 mol/L NaOH and kept in the dark.

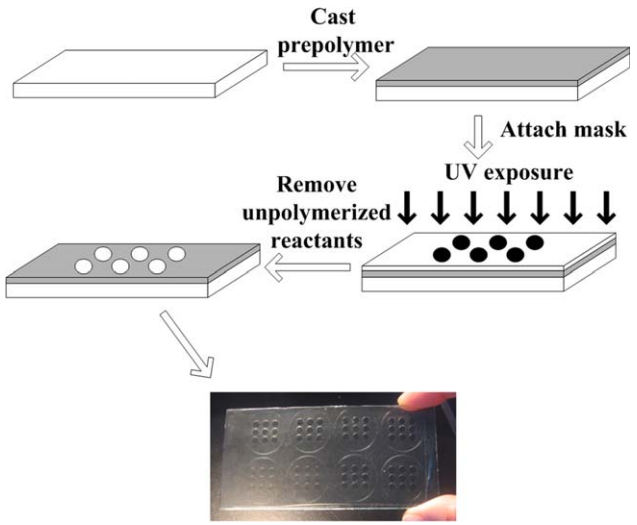

Figure 1. Schematic of the epoxy-functionalized micro-zone plate fabrication processes. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The p-Iodophenol (PIP) stock solution (0.01 mol/L) was prepared by dissolving 110 mg of PIP (Nanjing Searchbio, China) in 5 mL of dimethylsulfoxide and then diluted with water to 50 mL and kept in the dark.

Immobilization of AFP

Totally, 5 μL of AFP (100 ng/mL) were added to capture zones and incubated for 12 h at 20°C with frequent vibrating. After washing three times with 0.1*M* PBS (pH 7.0), the left epoxy groups were blocked with 5 μL of 1% BSA for 2 h. Then, each capture zone was rinsed with 0.1M PBS (pH 7.0) and stored in 0.1*M* PBS (pH 7.0) at 4°C.[23]

Preparation of AFP Immunoaffinity Micro-Zone Plates

Totally, 5 μL of anti-AFP (100ng/mL) were added to capture zones and incubated for 12 h at 20°C with frequent vibrating. After washing three times with 0.1*M* PBS (pH 7.0), the left epoxy groups were blocked with 5 μL of 1% BSA for 2 h. Finally, the micro-zones were washed with 0.1*M* PBS (pH 7.0) and stored in 0.1*M* PBS (pH 7.0) at 4°C.

The detection of AFP serum samples were based on a noncompetitive immune response method. 5 μL of HRP-labeled anti-AFP antibody was first mixed with the 5 μL AFP serum samples of certain concentrations. After preincubation for 30 min at room temperature, the mixture was dropped into capture zones. The capture zones were washed with 3 × 5 μL 0.1 M PBS (pH 7.0), containing 0.05% Tween-20 (PBST) in order to remove the physically adsorbed enzyme tracer. Then the epoxy-functionalized micro-zone plates were placed on the photomultiplier (PMT), and 5 μL of CL substrates were dropped into the capture zones. The CL signal was captured and recorded by the detector.

RESULTS AND DISCUSSION

Copolymerization Reaction

The copolymerization reaction between BABEA and the functional monomer (GMA) is depicted in Figure 2. Exposed under UV light, the photo-initiator generates active free radicals and thus initiates the polymerization reaction. According to the reaction, there will be some epoxy groups on the poly (BABEA-*co*-GMA). The presence of these epoxy groups allows the micro-zone plates made of the proposed materials for easy immobilization of protein such as α-fetoprotein (AFP).

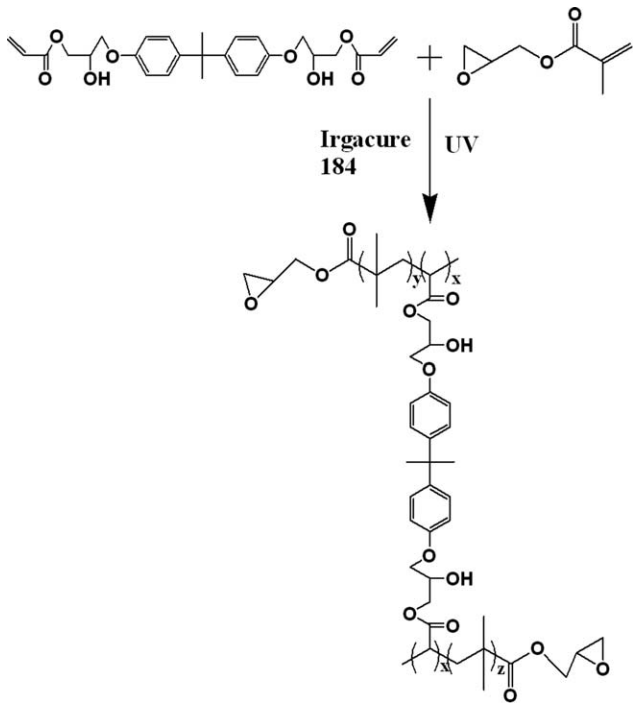

Figure 2. The polymerization reaction between the BABEA and GMA.

The feeding ratios of poly (BABEA-*co*-GMA) samples are also shown in Table I. For all the copolymers, the weight of the crosslinker (BABEA) and photo-initiator (Irgacure 184) were the same. But the copolymer obtained showed different compositions [Figure 3(A)]. P3 (BABEA : GMA= 1 : 1, mg/μL) contained 4.7% of MC, while P5 (BABEA : GMA= 5 : 7, mg/μL) contained 17.3% of MC. It can be concluded that functional monomer (GMA) participated in the polymerization reaction. And 1 mg BABEA mostly copolymerized with 1 μL GMA. Figure 3(B) shows the MC of copolymer (BABEA : GMA= 1 : 1, mg/μL) in different UV exposure times. T1 (5 s exposure times)

Table I. Feed Ratio and Compositions of Poly (BABEA-*co*-GMA) Samples

	Feed ratios				
Samples	Irgacure 184 (mg)	BABEA (mg)	GMA (μL)	UV exposure times (s)	MC% (w/w)[a]
P1	5	500	300	30	4.4
P2	5	500	400	30	4.8
P3	5	500	500	30	4.7
P4	5	500	600	30	11.7
P5	5	500	700	30	17.3

[a] Weight content of MC in dry copolymer, calculated from eq. (1).

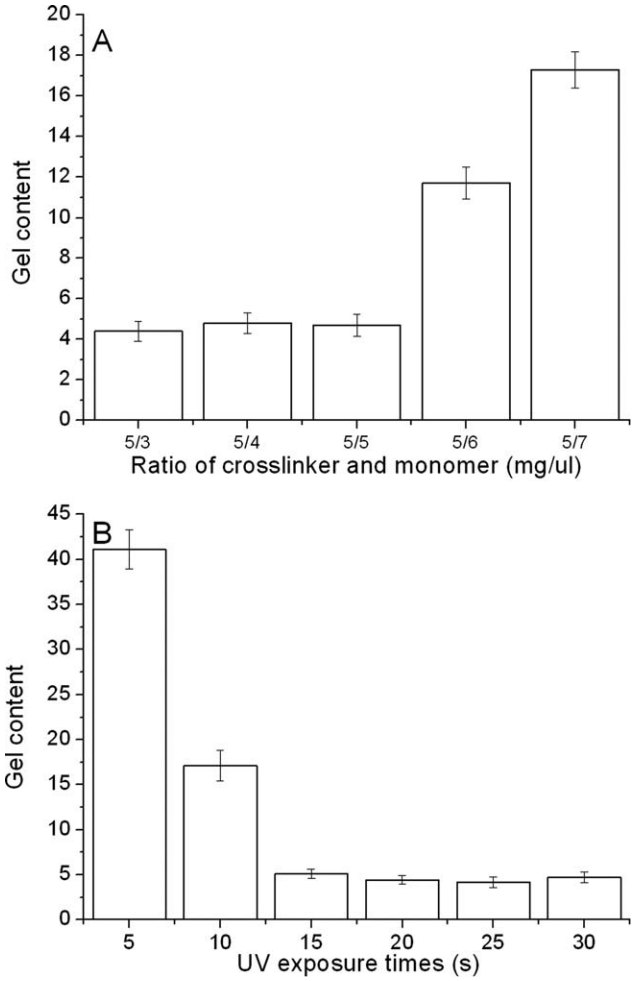

Figure 3. The monomer content of the poly (BABEA-*co*-GMA) (A) different ratio of BABEA and GMA, (B) different UV exposure times.

contained 41.1% of MC, while T3 (15 s exposure times) contained 5.1% of MC. Within 15–30 s exposure times, the MC ratios changed little due to the functional monomer (GMA) total overall reaction with BABEA. In consideration of energy consumption, this paper used 15 s as the UV exposure times and P3 (BABEA : GMA= 1 : 1, mg/μL) as the prepolymer solution.

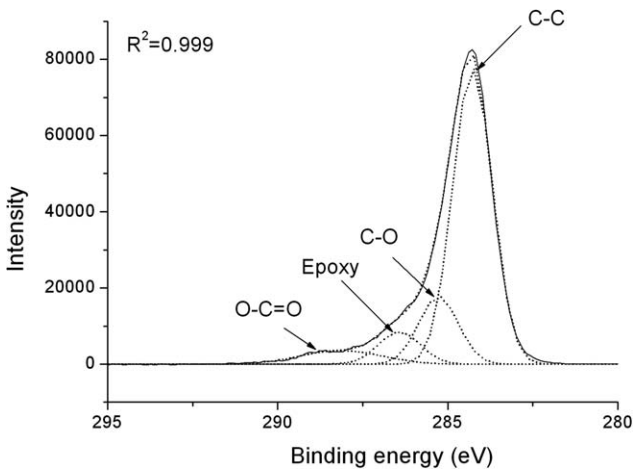

Figure 4. XPS spectrum for poly (BABEA-*co*-GMA). Four peaks at 284.4, 285.3, 286.3, and 288.4 eV were observed, which can be assigned to C—C/C—H, C—O, epoxy group and O—C=O, respectively.

Physical and Chemical Properties

The epoxy groups were investigated by XPS photoelectron spectroscopy. The typical deconvoluted C_{1s} core-lever spectra of the poly (BABEA-*co*-GMA) were shown in Figure 4. Four peaks at 284.4, 285.3, 286.3, and 288.4 eV were observed, which can be assigned to C—C/C—H, C—O, epoxy group and O—C=O, respectively.[24,25] The presence of these epoxy groups allows the micro-zone plates made of the proposed materials for easy immobilization of protein such as α-fetoprotein (AFP).

The distinct optical properties of the poly (BABEA-*co*-GMA) make them compatible for CL detection. Supporting Information Figure 1 shows the optical transmission spectrum for the poly (BABEA-co-GMA) in the range of 200–800 nm, along with those for borosilicate glass, and quartz. Quartz exhibited the best UV transparency among these materials, with the highest transmittance at 200 nm (84%). Very similar to glass, the poly (BABEA-*co*-GMA) was completely opaque at wavelength less than 290 nm (the transmittance was less than 0.02%).

The thermal behavior of the poly (BABEA-*co*-GMA) was investigated by TGA at rate of 20°C/min. Supporting Information Figure 2(A) shows that the temperature at 3% weight loss of the poly (BABEA-*co*-GMA) under nitrogen was about 150°C, indicating the interaction among the BABEA and GMA. A glass

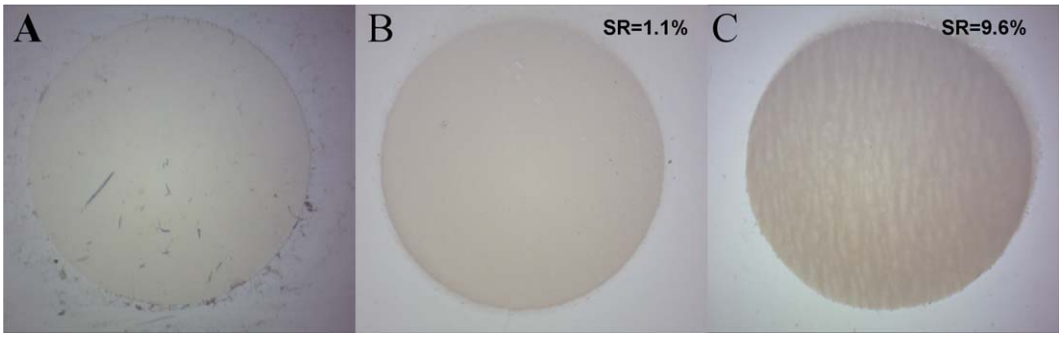

Figure 5. Poly (BABEA-*co*-GMA) disc (2mm in diameter) (A), Poly (BABEA-*co*-GMA) disc submerged for 48 h in water (B), Poly (BABEA-*co*-GMA) disc submerged for 8 h in water at 60°C (C). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

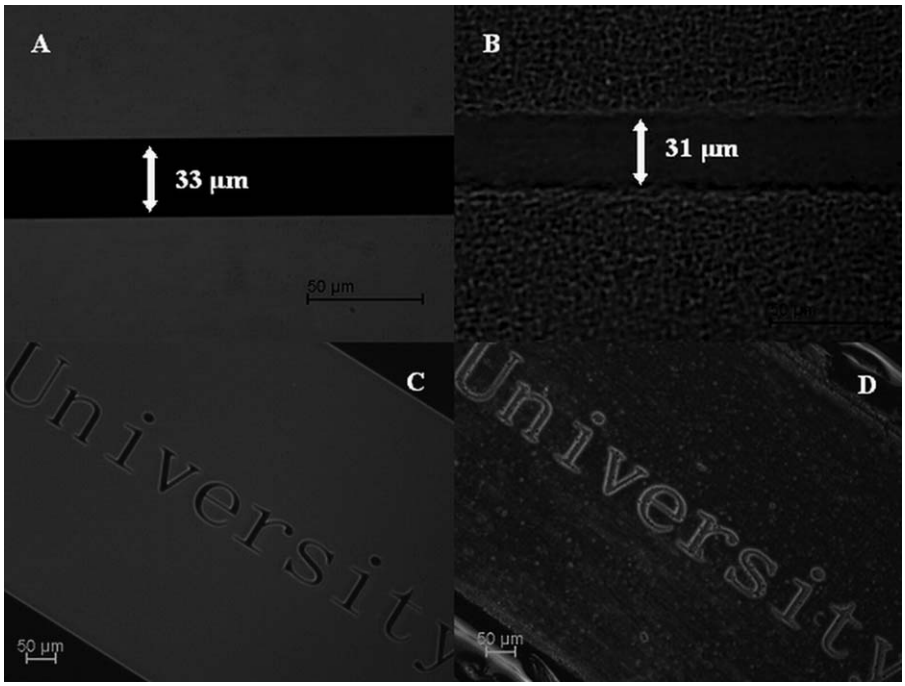

Figure 6. Microscopic images of a transparent mask with line structure (A), the corresponding poly (BABEA-*co*-GMA) replica (B), a transparent mask with letters (C), and the corresponding poly (BABEA-*co*-GMA) replica (D).

transition behavior was observed at 99°C in the DSC curve of poly (BABEA-*co*-GMA) [Supporting Information Figure 2(B)].

Figure 5(B) shows the images of poly (BABEA-*co*-GMA) discs (2 mm in diameter) after immersion for 48 h in water to illustrate the effect of immersion. Water had no effect on poly (BABEA-*co*-GMA) discs [Figure 5(A)]. In water environment, the poly (BABEA-*co*-GMA) (BABEA : GMA = 1 : 1, mg/μL) exhibited lower swelling ratios (SR = 1.1%). We also conducted additional testing of poly (BABEA-*co*-GMA) by heating sample in water up to 60°C for 10 h [Figure 5(C)]. The image shows that the poly (BABEA-*co*-GMA) disc had no change in the circle area except slight swelling (SR=9.6%).

Replication Fidelity

Replication fidelity is a critical factor for the poly (BABEA-*co*-GMA) to be excellent materials for fabrication of micro-zone plates. The replication fidelity was assessed using two photomasks; one with a simple pattern (straight line) as shown in Figure 6(A), and the other with a relatively complex pattern (English letters for University) as shown in Figure 6(C). The patterns on the photomasks were respectively duplicated to two poly (BABEA-*co*-GMA) layers through UV-exposure, and the obtained poly (BABEA-*co*-GMA) replicas are shown in Figure 6(B,D). For the simple straight line pattern, the line width was 33 μm while the obtained poly (BABEA-*co*-GMA) replication width on the replica was 31 μm. For the complex pattern, the letters were well transferred to the replica, with well-preserved curve details. These results demonstrated the excellent replication fidelity of the poly (BABEA-*co*-GMA).

The above three aspects have already exhibited the desired characteristics for the poly (BABEA-*co*-GMA) to be excellent microplates fabricating materials. These advantageous features are

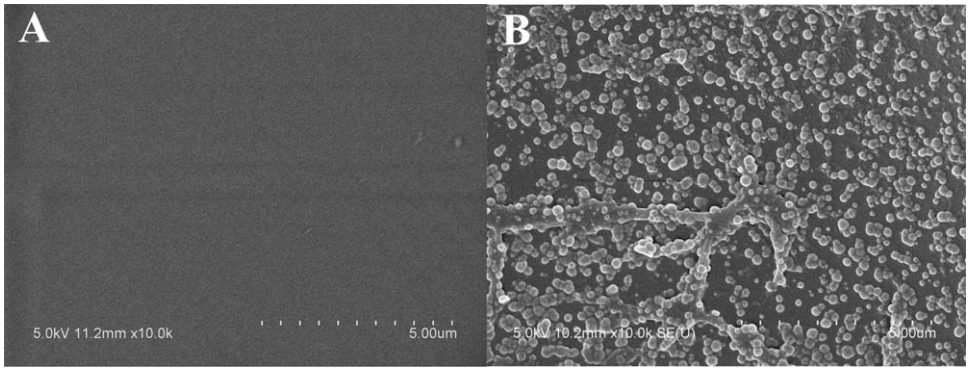

Figure 7. SEM photograph of the BABEA based epoxy-functionalized micro-zone plates (A), AFP immobilized epoxy-functionalized micro-zone plate (B).

further exemplified with the fabrication of epoxy-functionalized micro-zone plates. The photograph of the fabricated micro-zone plates is shown in Supporting Information Figure 3. The micro-zone plate was designed to have eight circulars, and every circular has nine test zones was 2 mm in diameter.

Preparation and Characterization of Micro-Zone Plates

AFP was immobilized onto the capture zone through the epoxy-amino reaction. SEM (Figure 7) showed that the AFP was successfully linked to the surface of the epoxy functionalized capture zone.

The CL reaction further proved that the AFP was immobilized onto the capture zone. The intensity of CL emission form epoxy-funztionalized capture zone increased quickly and trended to its maximum value within 1 s. The uniformity of the epoxy functionalized micro-zone plate was evaluated by measurements of 20 ng/mL AFP on five different capture micro-zones. Figure 8 shows the CL signal of the intra capture zone, which indicates that the epoxy-funztionalized micro-zone plates were homogeneous and stable.

Performance of the AFP Immunoaffinity Micro-Zone Plates

The performance of the AFP immunoaffinity micro-zone plates was demonstrated with the measurement of AFP in human serum. AFP has been routinely used as a biomarker in clinical screening. Combined with CL detection, an AFP immunoaffinity micro-zone plates permitted the specific detection of the trace AFP in serum (Figure 9). The sample consumption was 5 μL. The limit of detection was 5 ng/mL (signal/noise ratio = 6), which meets the requirement for early clinical diagnosis (threshold for positive: 5 ng/mL).

CONCLUSIONS

The commercially available UV curable material, BABEA, has been introduced as a new material for the manufacturing of micro-zone plates combined with GMA. The poly (BABEA-*co*-GMA) exhibited several significant advantages, including optical transparency, fast-curing speed, availability of epoxy functionalities, and high replication fidelity. These favorable features make poly (BABEA-*co*-GMA) as a promising material for facile fabrication of functionalized micro-zone plates. Although these advantageous features were only exemplified with the fabrication of epoxy-functionalized micro-zone plates and immobilization of AFP, the poly (BABEA-*co*-GMA) is applicable for other protocols.

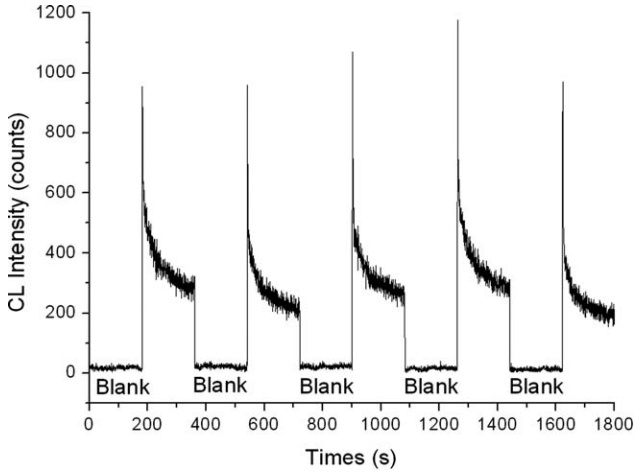

Figure 8. The CL signal of the intra capture zone. The results indicate that the epoxy-funztionalized micro-zone plates were homogeneous and stable.

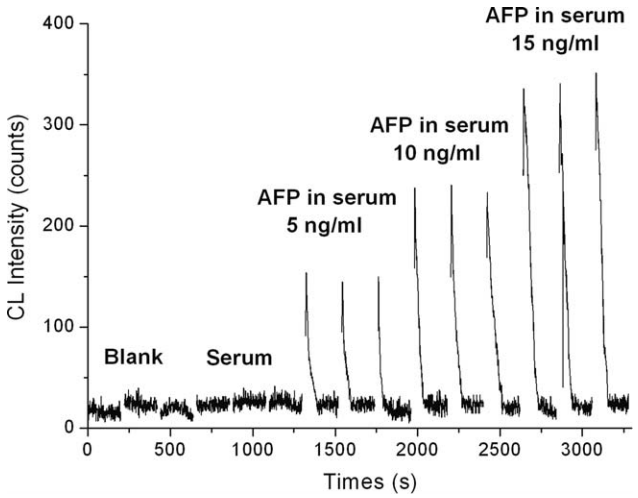

Figure 9. AFP immunoaffinity micro-zone plates of human serum samples spiked with different concentrations of AFP.

ACKNOWLEDGMENTS

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