Analytica Chimica Acta 706 (2011) 143–148

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Streptavidin-functionalized capillary immune microreactor for highly efficient chemiluminescent immunoassay

Zhanjun Yang[a,b], Chen Zong[a], Huangxian Ju[a,*], Feng Yan[c,*]

[a] State Key Laboratory of Analytical Chemistry for Life Science, Department of Chemistry, Nanjing University, Nanjing 210093, PR China
[b] College of Chemistry and Engineering, Yangzhou University, 88 South University Avenue, Yangzhou 225002, PR China
[c] Jiangsu Institute of Cancer Prevention and Cure, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 19 April 2011
Received in revised form 1 August 2011
Accepted 4 August 2011
Available online 2 September 2011

Keywords:
Immune microreactor
Capillary
Chemiluminescence
Immunoassay
Streptavidin
α-Fetoprotein

ABSTRACT

A streptavidin functionalized capillary immune microreactor was designed for highly efficient flow-through chemiluminescent (CL) immunoassay. The functionalized capillary could be used as both a support for highly efficient immobilization of antibody and a flow cell for flow-through immunoassay. The functionalized inner wall and the capture process were characterized using scanning electron microscopy. Compared to conventional packed tube or thin-layer cell immunoreactor, the proposed microreactor showed remarkable properties such as lower cost, simpler fabrication, better practicality and wider dynamic range for fast CL immunoassay with good reproducibility and stability. Using α-fetoprotein as model analyte, the highly efficient CL flow-through immunoassay system showed a linear range of 3 orders of magnitude from 0.5 to 200 ng mL^{-1} and a low detection limit of 0.1 ng mL^{-1}. The capillary immune microreactor could make up the shortcoming of conventional CL immunoreactors and provided a promising alternative for highly efficient flow-injection immunoassay.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Flow-injection immunoassay (FIIA), due to its small sample consumption, simplified handling step, acceptable reusability, good reproducibility, and easy automation for high sample throughout [1–4], has attracted considerable interest in environmental monitoring [5], food safety [6,7], pharmaceutical analysis [8,9], and clinical diagnosis [10–12]. Chemiluminescent (CL) detection has extensively been used in sensitive immunoassay due to its wide calibration range, need of simple instrumentation and suitability for miniaturization [13–16,7,17]. In flow-injection CL immunoassay, a support-based immunoreactor is usually employed in performing the separation step. The most commonly used supports for immobilization of antibody or antigen are bead or membrane materials [15,18]. So far two dominant immunoreactors have been used to carry out flow-injection CL immunoassay.

The first immunoreactor is the packed tubing reactor fabricated by packing antibody immobilized bead materials into transparent tube [19–23], which suffers major disadvantages associated with the packed tube as an immunoreactor for FIIA [24]. Firstly, light-scattering by the packing material seriously compromises the detection of CL. Secondly, the tube with small diameter is usually desired for FIIA (e.g. 1 mm), which makes it difficult pack uniformly the bead in the tube, and thus weakens the performance of the immunoreactor. This leads to significant variation of reactor sensitivity. Furthermore, the fabrication reproducibility also depends on the packing uniformity. Another immunoreactor is thin-layer cell immunoreactor designed by sandwiching antibody-immobilized membrane or solid substrate between two plates (for example Plexiglas slice). Since Wilson and co-workers [24] reported the first thin-layer cell reactor for flow injection CL immunoassay, many kinds of improved thin-layer cell immunoreactors have been used to perform FIIA [25–28]. Although this immunoreactor can avoid the shortcomings of the packed tubing immunoreactor, it shows some disadvantages, such as limited antibody-immobilized capacity and relatively strong nonspecific absorption. Alternatively, other CL immunoreactors based on relatively high-cost paramagnetic microspheres (PMs) have been proposed for FIIA [29–31]. In order to lower the cost of detection and improve the shortcomings of conventional immunoreactors, it is very necessary to develop novel CL immunoreactors.

Capillary can offer the advantages of large surface-to-volume ratio, laminar flow, and reduced band-broadening effect compared to packing tube or thin-layer cell [32]. Thus this work designed a functionalized capillary immune microreactor to overcome the drawbacks existed in conventional immunoreactors,

* Corresponding authors. Tel.: +86 25 83593593; fax: +86 25 83593593.
E-mail addresses: hxju@nju.edu.cn (H. Ju), yanfeng2007@sohu.com (F. Yan).

0003-2670/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2011.08.011

with which an automated flow-through immunosensing system was proposed for highly efficient CL detection of tumor markers. The inner wall of capillary was firstly treated with 3-glycidoxypropyltrimethoxysilane (GPTMS) and then functionalized with streptavidin. Based on highly selective recognition of streptavidin to biotin labeled antibody, the antibody could be captured on the inner wall of capillary. The use of streptavidin for antibody immobilization increased the antigen binding capacity by orienting the antigen-specific sites away from the matrix, thus allowing minimum interference. Moreover, the proposed capillary immune microreactor showed obvious advantages over conventional immunoreactors, including small inside diameter and large reactive surface area for accelerating immunoreaction and CL reaction, and enhanced binding capacity for capture of antibody, which improved the sensitivity and linear range. The resultant immunoassay method was simple, low-cost and convenient. Using α-fetoprotein (AFP) as a model analyte, the highly efficient flow-through immunoassay system showed a wide linear range, fast CL immunoassay, high sensitivity, good reproducibility and stability.

2. Experimental

2.1. Materials and reagents

AFP ELISA reagent kits were purchased from CanAg Diagnostics, which consisted of a series of AFP standard solutions from 0 to 500 $ng\,mL^{-1}$, the stock solutions of horseradish peroxidase (HRP)-labeled mouse monoclonal anti-AFP (20 $\mu g\,mL^{-1}$, dilution of 1:20 ratio in use with tracer diluent) and biotinylated mouse monoclonal anti-AFP (1 $\mu g\,mL^{-1}$). Electrochemiluminescent immunoassay reagent kit used for reference detection of AFP was provided by Roche Diagnostics GmbH (Germany). HRP substrate solution (luminol–*p*-iodophenol–H_2O_2) was supplied by Autobio Diagnostics Co., Ltd. (China). GPTMS (98%), streptavidin and bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO). Ultra-pure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs. The clinical serum samples were from Jiangsu Institute of Cancer Research. All other reagents were of the best grade available and used as received.

Coupling buffer for streptavidin immobilization was 0.01 M pH 7.4 phosphate buffer solution (PBS). Blocking buffer was PBS containing 1% BSA. To minimize unspecific adsorption, 0.05% Tween-20 was spiked into 0.01 M pH 7.4 PBS as wash buffer (PBST). Regeneration buffer was 0.1 M glycine–HCl at pH 2.2.

2.2. Apparatus

The automated flow-through CL immunoassay system was constructed as illustrated in Fig. 1. Teflon tubes (0.8 mm i.d.) and silicon rubber tubes (1.0 mm i.d.) were used to connect all components in this system. All fluids were delivered with a multichannel bidirectional peristaltic pump of an IFFM-D Luminescent Analyzer (Remax, China). The introduction of different solutions into the channel was performed using a multiposition valve with five inlets and one outlet. A glass capillary of about 30 μL (i.d. 1.0 mm, length 3.8 cm) for anti-AFP immobilization was positioned in front of photomultiplier (PMT). The CL signal produced in the immune microreactor was measured with the PMT operated at −800 V. Instrument control and data recording were performed using IFFM software package run under Windows 2000.

The reference electrochemiluminescent immunoassay (ECLIA) was performed with a Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics GmbH) according to the instruction and assay procedure in the manual of the Roche diagnostic kits for AFP. The planar scanning electron micrographs (SEM) for inner surface of the modified glass capillary were obtained by characterizing the original fragments of glass capillary with a Hitachi S-3000N scanning electron microscope (Japan) at an acceleration voltage of 10 kV.

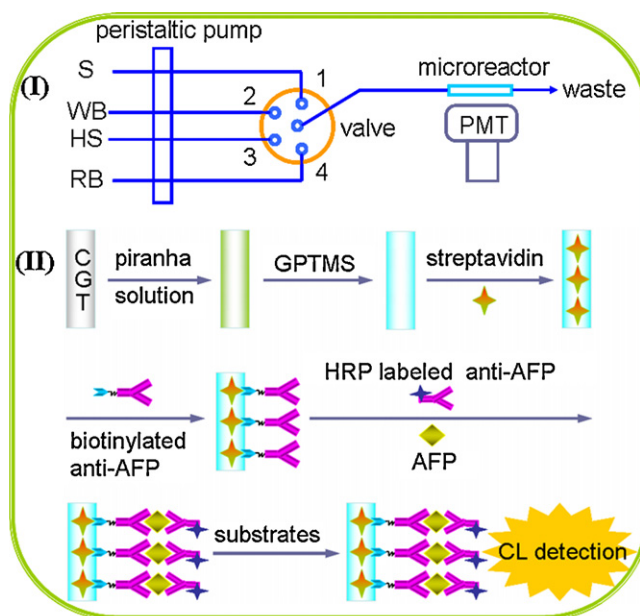

Fig. 1. Scheme of the automated flow-through CL immunoassay system: (I) flow-through analysis system. S, incubation mixture; WB, wash buffer, HS, CL substrate for HRP; RB, regeneration buffer; (II) biofunctionalization of inside wall of glass capillary and one-step sandwich immunoassay procedure for AFP.

2.3. Preparation of streptavidin functionalized glass capillary

The glass capillary was firstly immersed in piranha solution (H_2SO_4/30% H_2O_2, 7:3 in volume) for 12 h. After washing thoroughly with ultra-pure water, its inner wall was dried under a stream of nitrogen and silanized by immersing it in 1% GPTMS toluene solution overnight at room temperature (RT, 25 °C) [33]. After the coupling reaction, the modified glass capillary was rinsed several times with pure toluene and ethanol to remove the physically absorbed silane from the inner surface and dried under a stream of nitrogen. The exposed active epoxy groups, formed on the inner wall of capillary, further reacted with streptavidin by injecting 30 μL of 1 $mg\,mL^{-1}$ streptavidin solution in the glass capillary at RT for 2 h and followed by storage at 4 °C overnight. After washing three times with 0.01 M pH 7.4 PBST and blocking the remaining epoxy groups with blocking buffer for 12 h at 4 °C, the streptavidin functionalized glass capillary was obtained.

2.4. Preparation of capillary immune microreactor and immunoassay protocol

Antibody was introduced into the capillary microreactor by injecting 30 μL of 1 $\mu g\,mL^{-1}$ biotinylated antibody into the streptavidin functionalized capillary and reaction at room temperature for 3 h. The resulting immune microreactor was washed three times with PBST and then connected into flow-through CL immunoassay system as a flow cell (Fig. 1). The detailed description of detecting AFP was demonstrated in Fig. 1 and Table 1. The assay process was performed at RT and controlled automatically by a personal computer. A mixture of AFP and HRP-labeled anti-AFP antibody (15 μL for each) was firstly delivered into the immune microreactor and incubated under stop flow at RT for 20 min. PBST was then delivered into the system at a flow rate of 0.5 $mL\,min^{-1}$ to

Table 1
Details of flow-through chemiluminescent immunoassay for AFP.

Step no.	Valve position	Step	Starting time (min:s)
1	1	Introduce the mixture of 15 μL sample and 15 μL AFP tracer antibody into capillary immune microreactor	00:00
2	1	Stop flow and incubation at room temperature	00: 30
3	2	Wash the immune microreactor with PBST at a flow rate of 0.5 mL min^{-1}	20: 30
4	3	Introduce 30 μL HRP substrate into immune microreactor and stop flow to collect CL signal	22: 30
5	4	Introduce regeneration buffer to regenerate immune microreactor at a flow rate of 1.0 mL min^{-1}	26:30
6	2	Introduce PBS to recondition immune microreactor at a flow rate of 1.0 mL min^{-1}	28:30
7	1	Ready for the next assay cycle	29:00

wash the microreactor. At a time of 22.5 min, HRP substrate solution (luminol–*p*-iodophenol–H_2O_2) was then introduced into the immune microreactor. When the HRP catalyzed CL reaction was triggered under stop flow, the CL signal from the immune microreactor was collected. The whole procedure from sample injection to signal detection could be completed within 27 min. Thereafter, regeneration buffer (0.1 M pH 2.2 glycine–HCl) and PBS passed through the immune microreactor respectively for two cycles to regenerate the immune microreactor. At a time of 29 min, the analytical system was ready for the next assay cycle.

2.5. Specimen collection and safety consideration

According to the rules of the local ethical committee, blood spemicroreactorens were collected using a standard venipuncture technique and the sera were centrifugally separated from the cells, without hemolysis. The serum samples could be directly assayed with the proposed and reference methods. If necessary, the samples could be stored at 4 °C for less than 48 h or frozen at −20 °C for less than 2 months. Prior to use, the samples were allowed to come to RT and mixed by gently swirling. For safety consideration, all handling and processing were performed carefully, and all tools in contact with patient spemicroreactorens and immunoreagents were disinfected after use.

3. Results and discussion

3.1. Characterization of immune microreactor

Compared to conventional immunoreactors, the streptavidin functionalized capillary immune microreactor used in CL immunoassay possesses some advantages: small inside diameter for rapid immunoreaction or CL reaction, and large reactive surface area to accelerate reaction and allow binding more capture antibody for enhancing the sensitivity and extending the linear range. From the SEM of capillary treated with piranha solution, a complete, smooth and homogeneous inner surface was observed (Fig. 2a). The SEM of GPTMS-silylanized capillary showed a homogeneous epoxysilane layer film formed on the inner surface (Fig. 2b). After streptavidin was bound to capil-

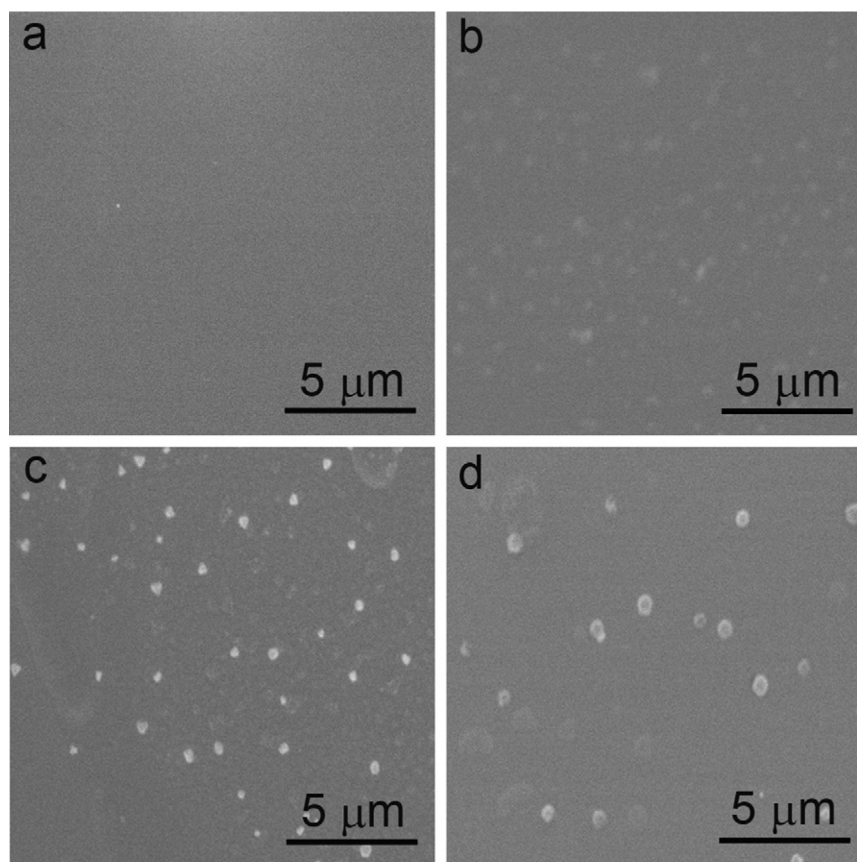

Fig. 2. SEM images of bare (a), GPTMS-silylanized (b), streptavidin-functionalized (c) and anti-AFP immobilized (d) capillary.

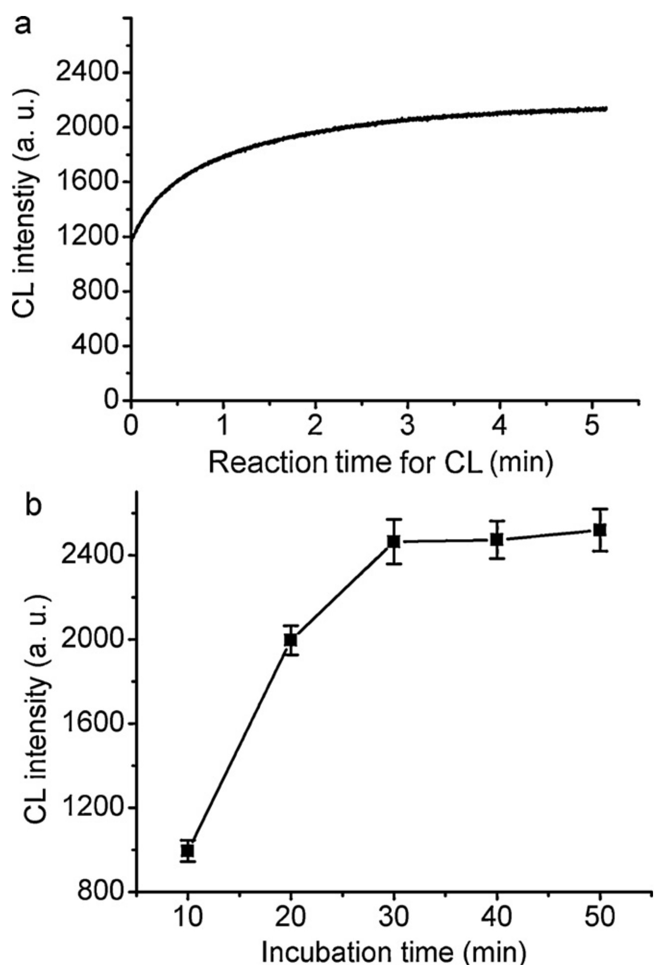

Fig. 3. Effects of CL reaction time (a) and incubation time (b) on CL intensity at 25 ng mL^{-1} AFP concentration (PMT: −800 V; n = 5 for each point; incubation time for a: 20 min; CL reaction time for b: 4 min).

lary through the epoxy group of GPTMS and the amino group of protein, the functionalized film displayed obviously different surface morphology from the unbound capillary (Fig. 2c). While the anti-AFP antibody was immobilized on the inner surface of streptavidin functionalized capillary, the SEM showed bigger aggregates of the coated proteins than that of streptavidin-functionalized capillary (Fig. 2d), suggesting AFP antibody was successfully linked to inner surface of streptavidin-functionalized capillary.

3.2. Kinetic curve of CL reaction

The kinetic behavior of the CL reaction catalyzed by HRP labeled to the sandwich immunocomplex in immune microreactor was examined with a static method. Upon injection of CL substrate the CL reaction immediately occurred on inner wall of immune microreactor. The CL intensity from immune microreactor increased quickly and reached its maximum value within 4 min (Fig. 3a). The CL intensity slowly decreased after 4 min due to the inhibition of HRP bioactivity by H_2O_2 after a long time exposure [34] (not shown here). Therefore, the CL measurement was performed at 4 min for obtaining the highest sensitivity. Obviously, the capillary immune microreactor could lead to faster mass transfer of the enzyme substrate, which shortened the CL reaction time achieving the highest detection signal.

3.3. Incubation time

The incubation time is a key factor to improve the whole assay speed in the immunoassay, which is usually contributed to mass transport of immunoreagents and kinetics of immunoreaction [12]. Compared to the microwell used in conventional ELISA, the immune microreactor prepared using a glass capillary (i.d. 1.0 mm) has larger reactive surface area and can reduce the diffusion distance, which was beneficial to accelerating both mass transport of immunoreagents and the formation of sandwich immunocomplex. The results obtained in a study of CL intensity as a function of incubation time were shown in Fig. 3b. With the increasing incubation time the CL signal from immune microreactor for 25 ng mL^{-1} AFP increased and reached 81% of the maximum value at the incubation time of 20 min (Fig. 3b). Considering the high sensitivity of CL detection and the low detection limit enough for clinical diagnosis, 20 min of incubation time was used in the proposed immunoassay for higher sample throughput.

3.4. Regeneration of immune microreactor

The capillary immune microreactor could be regenerated for reuse by a simple and fast regeneration step with the regeneration buffer. This is very important for developing low-cost automation immunoassay. The regeneration of the covalent binding of antibodies for reuse must avoid loss of the immobilized protein activity and leakage of the protein out of the support surface. Different regeneration reagents, including buffer with low pH value (0.1 M glycine/HCl, pH 2.2), diluted alkali solution (50 mM NaOH) and organic solvent (CH_3OH–H_2O, 1:1), were used to regenerate the microreactor. The regeneration efficiency (RE) was calculated according to the following equation [35]:

$$\text{RE}\ \ (\%) = \left[1 - \frac{A}{A_0}\right] \times 100 \qquad (1)$$

where A represents the CL signal obtained after the regeneration cycle and A_0 is the signal before applying the regeneration step. The most efficient dissociating reagent was found to be 0.1 M glycine–HCl (pH 2.2), which allowed a fast and complete dissociation of the immunocomplexes with a RE of 98.7% for AFP. After 10 times repeated use, the CL signal for 25 ng mL^{-1} AFP standard or serum sample decreased by less than 6%, showing acceptable the activity of the regenerated anti-AFP immobilized capillary immune microreactor. Thus glycine–HCl buffer was chosen as the regeneration buffer for the regeneration step.

3.5. Analytical performance

Under the optimum conditions, the CL intensity for AFP linearly increased with the increasing concentration of analyte (Fig. 4). The dose–response curve showed the wide linear range from 0.5 to 200 ng mL^{-1} with a correlation coefficient of 0.9981 for AFP. The coefficients of variations (CVs) for every concentration of AFP were less than 5.9%. At a signal-to-noise ratio of 3, the detection limit for AFP was calculated to be 0.1 ng mL^{-1}. The comparison between quantitative performance indicators of the proposed method and literature methods with conventional packed tube or thin-layer cell immunoreactor for flow injection CL immunoassay of AFP were illustrated in Table 2. The proposed capillary immune microreactor showed the lowest detection limit and the widest linear range. Moreover, the detection limit of 0.1 ng mL^{-1} was also lower than those of 1.7, 20 and 0.68 ng mL^{-1} reported values for AFP in other immunoassay methods [11,40,41]. The cutoff value of the tumor marker in clinical diagnostic is 25 ng mL^{-1}, so the detection limit of the proposed flow-through immunosensing system was enough to meet practical application. When the capillary immune microre-

Table 2
Comparison between capillary immune microreactor and conventional immunoreactor for flow injection CL immunoassay of AFP.

Immunoreactor	Immobilized support	Linear range (ng mL^{-1})	Detection limit (ng mL^{-1})	Reference
Capillary microreactor	Inside wall	0.5–200	0.1	This work
Thin-layer cell reactor	UltraBind membrane	5.0–150	1.5	[36]
Packed tubing reactor	Glass microbeads	10–100	2.4	[37]
	Sepharose beads	2.0–70	0.5	[38]
PMs based reactor	PMs	1.0–40	0.6	[39]

actor was not in use, they could be stored in PBS containing 0.1% NaN_3 at 4 °C for at least 20 days without obvious signal change. The acceptable retention of immunoreactivity was very important for the development of the proposed system in low-cost application.

Using samples containing 25 ng mL^{-1} AFP, the reproducibility of the proposed microreactor was evaluated by the intra- and inter-assay CV with five determinations. The intra- and inter-assay CVs obtained were 5.5 and 8.7% for AFP, respectively, showing acceptable detection and fabrication reproducibility.

3.6. Detection of tumor marker AFP in clinical serum samples

The potential interferences for the proposed CL immunoassay system were nonspecific adsorption and cross-reactivity. In this work, the capture antibody was immobilized on the inner surface of glass capillary through high selective streptavidin–biotin system. Thus using a simple and fast wash step, the nonspecific adsorption to the capillary wall could be significantly avoided. The cross-reactivity from other tumor markers could be examined according to our previous works [36,39,42]. With the increasing concentration of other tumor markers (such as CEA or CA 125 generally coexisted in serum of cancer patient) up to 10-fold concentration of AFP, the change in CL signal for AFP was less than 5.9%, indicating that the interference for CL immunoassay system was negligible.

To examine the accuracy of the microreactor-based immunoassay system, 20 clinical serum samples were assayed using the proposed immunoassay system as well as the reference ECLIA method. The latter were carried out by Jiangsu Institute of Cancer Prevention and Cure. When the levels of tumor markers were over the calibration ranges, serum samples were appropriately diluted with 0.01 M pH 7.4 PBS prior to assay. All samples were detected for five times, showing the CV values varied form 2.7 to 7.9%. The results showed an acceptable agreement between the two methods (P < 0.0001) with the relative errors less than 11.5% (Fig. 5).

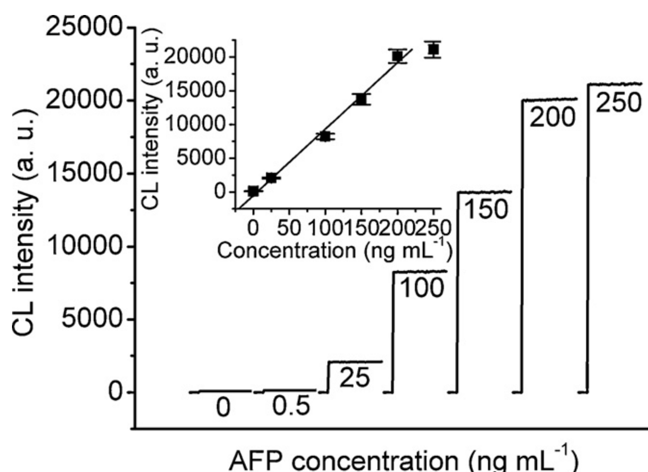

Fig. 4. Dose–response curve for AFP (PMT: −800 V; incubation time: 20 min; CL reaction time: 4 min). Inset: calibration curve (n = 5 for each point).

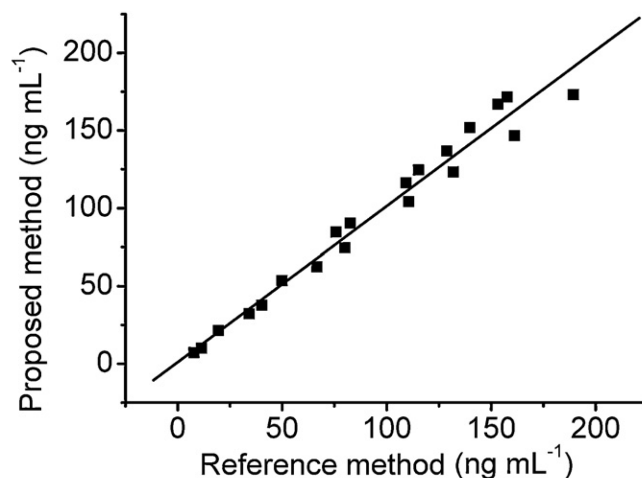

Fig. 5. Comparison between the proposed (PMT: −800 V; incubation time: 20 min; CL reaction time: 4 min) and reference methods for immunoassay of AFP in serum samples.

4. Conclusions

A novel streptavidin-functionalized capillary microreactor has been developed for highly efficient flow-through chemiluminescent immunoassays. The designed microreactor combines the highly efficient immobilization of capture antibody on support and an efficient reaction cell for flow-though immunoassay. Compared to the conventional immunoreactors, the capillary immunoreactor shows the advantages as follows: (i) the use of streptavidin for antibody immobilization can orient the antigen-specific sites away from the matrix, thus allowing minimum interference and remarkably increasing the immunoreaction efficiency; (ii) small inside diameter increases the rate of immunoreaction or CL reaction; (iii) large reactive surface area allow binding more capture antibody for improving the sensitivity and linear range. Moreover, the proposed capillary microreactor can also be used disposable, thus avoiding the necessity of system regeneration and further shortening the assay time. Coupled with our proposed channel resolved strategy [29], the resultant immune microreactor can be used for multi-analyte CL immunoassay. The streptavidin-functionalized microreactor is simple, convenient, low-cost, and possesses a promising practicality in automated FIIA.

Acknowledgments

This work was financially supported by the National Science Fund for Creative Research Groups (20821063) and the projects (20875044, 21075055, 21005070) from National Natural Science Foundation of China.

References

[1] C.H. Pollema, J. Ruzicka, G.D. Christian, Anal. Chem. 64 (1992) 1356–1361.
[2] P.C. Gunaratna, G.S. Wilson, Anal. Chem. 65 (1993) 1152–1157.

[3] K. Kronkvist, U. Lovgren, J. Svenson, L.E. Edholm, G. Johansson, J. Immunol. Methods 200 (1997) 145–153.
[4] R. Nandakumar, M.P. Nandakumar, B. Mattiasson, Biosens. Bioelectron. 15 (2000) 241–247.
[5] S.R. Jain, E. Brorowska, R. Davidsson, M. Tudorache, E. Ponten, J. Emneus, Biosens. Bioelectron. 19 (2004) 795–803.
[6] J.B. Delehanty, F.S. Ligler, Anal. Chem. 74 (2002) 5681–5687.
[7] B.G. Knecht, A. Strasser, R. Dietrich, E. Märtlbauer, R. Niessner, M.G. Weller, Anal. Chem. 76 (2004) 646–654.
[8] H.H. Yang, Q.Z. Zhu, H.Y. Qu, X.L. Chen, M.T. Ding, J.G. Xu, Anal. Biochem. 308 (2002) 71–76.
[9] A.V. Eremenko, C.G. Bauer, A. Makower, B. Kanne, H. Baumgarten, F.W. Scheller, Anal. Chim. Acta 358 (1998) 5–13.
[10] D.P. Tang, R. Niessner, D. Knopp, Biosens. Bioelectron. 24 (2009) 2125–2130.
[11] J. Wu, F. Yan, J.H. Tang, C. Zhai, H.X. Ju, Clin. Chem. 53 (2007) 1495–1502.
[12] Z.F. Fu, H. Liu, H.X. Ju, Anal. Chem. 78 (2006) 6999–7005.
[13] Y. Zhou, Y.H. Zhang, C.W. Lau, J.Z. Lu, Anal. Chem. 78 (2006) 5920–5924.
[14] C. March, J.J. Manclus, A. Abad, A. Navarro, A. Montoya, J. Immunol. Methods 303 (2005) 92–104.
[15] Z.J. Yang, Z.F. Fu, F. Yan, H. Liu, H.X. Ju, Biosens. Bioelectron. 24 (2008) 35–40.
[16] L.X. Zhao, J.M. Lin, Z.J. Li, X.T. Ying, Anal. Chim. Acta 558 (2006) 290–295.
[17] E. Yacoub-George, L. Meixner, W. Scheithauer, A. Koppi, S. Drost, H. Wolf, C. Danapel, K.A. Feller, Anal. Chim. Acta 457 (2002) 3–12.
[18] S. Morais, A. Maquieira, R. Puchades, J. Immunol. Methods 224 (1999) 101–109.
[19] G. Shellum, G. Gubitz, Anal. Chim. Acta 227 (1989) 97–107.
[20] J.A.A. Ho, H.W. Hsu, M.R. Huang, Anal. Biochem. 330 (2004) 342–349.
[21] J.A.A. Ho, M.R. Huang, Anal. Chem. 77 (2005) 3431–3436.
[22] F. Yan, J.N. Zhou, J.H. Lin, H.X. Ju, X.Y. Hu, J. Immunol. Methods 305 (2005) 120–127.
[23] M.A. Gonzalez, S. Morais, R. Puchades, A. Maquieira, A. Abad, A. Montoya, Anal. Chim. Acta 347 (1997) 199–205.
[24] H.J. Liu, J.C. Yu, D.S. Bindra, R.S. Givens, G.S. Wilson, Anal. Chem. 63 (1991) 667–669.
[25] Z. Dai, S. Serban, H.X. Ju, N.E. Murr, Biosens. Bioelectron. 22 (2007) 1700–1706.
[26] J.H. Lin, F. Yan, X.Y. Hu, H.X. Ju, J. Immunol. Methods 291 (2004) 165–174.
[27] Z.J. Yang, Z.Y. Xie, H. Liu, F. Yan, H.X. Ju, Adv. Funct. Mater. 18 (2008) 3991–3998.
[28] Y.F. Wu, C.L. Chen, S.Q. Liu, Anal. Chem. 81 (2009) 1600–1607.
[29] K. Tanaka, H. Imagawa, Talanta 68 (2005) 437–441.
[30] H. Kuramitz, M. Dziewatkoski, B. Barnett, H.B. Halsall, W.R. Heinemana, Anal. Chim. Acta 561 (2006) 69–77.
[31] P.W. Stege, J. Paba, G.A. Messina, Electrophoresis 31 (2010) 3475–3481.
[32] C.F. Poole, S.K. Poole (Eds.), Chromatography Today, Elsevier Science, New York, NY, 1991.
[33] L. Luzinov, D. Julthongpiput, A. Liebmann-Vinson, T. Cregger, M.D. Foster, W. Tsukruk, Langmuir 16 (2000) 504–516.
[34] M. Dequaire, B. Limoges, J. Moiroux, J.M. Savéant, J. Am. Chem. Soc. 124 (2002) 240–253.
[35] A.E. Botchkareva, F. Fini, S. Eremin, J.V. Mercader, A. Montoya, S. Girotti, Anal. Chim. Acta 453 (2002) 43–52.
[36] Z.F. Fu, F. Yan, H. Liu, Z.J. Yang, H.X. Ju, Biosens. Bioelectron. 23 (2008) 1063–1069.
[37] Y.F. Wu, Y.F. Zhuang, S.Q. Liu, L. He, Anal. Chim. Acta 630 (2008) 186–193.
[38] J.H. Lin, F. Yan, H.X. Ju, Appl. Biochem. Biotechnol. 117 (2004) 93–102.
[39] Z.F. Fu, F. Yan, H. Liu, J.H. Lin, H.X. Ju, Biosens. Bioelectron. 23 (2008) 1422–1428.
[40] B. Zhang, X. Zhang, H.H. Yan, S.J. Xu, D.H. Tang, W.L. Fu, Biosens. Bioelectron. 23 (2007) 19–25.
[41] Y.J. Zhao, X.W. Zhao, X.P. Pei, J. Hu, W.J. Zhao, B.A. Chen, Z.Z. Gu, Anal. Chim. Acta 633 (2009) 103–108.
[42] Z.J. Yang, H. Liu, C. Zong, F. Yan, H.X. Ju, Anal. Chem. 81 (2009) 5484–5489.