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Vertical flow immunoassay (VFA) biosensor for a rapid one-step immunoassay†

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A highly rapid, one-step immunoassay of high sensitivity C-reactive protein (hsCRP) using a biosensor with a vertical flow immunoassay (VFA) was developed. The VFA biosensor was primarily composed of a sample pad, conjugate pad, FTH film and nitrocellulose (NC) membrane, which were all vertically stacked upon one another. Anti-hsCRP and secondary antibodies were consecutively immobilized on the NC membrane at the position below the holes. Gold nanoparticles (AuNPs) conjugated with another anti-hsCRP antibody were encapsulated in the conjugation pad. Various assay conditions, including the size of the hole and the sample volume, were optimized. Under optimized conditions, hsCRP concentrations from 0.01 to 10 μg mL^{-1} were detected within 2 min. In comparison with a lateral flow assay (LFA) system, the VFA sensor showed a gradual increase of signal in a concentration-dependent manner without a hook effect in the tested range.

Point-of-care (POC) biosensors are widely used in the clinical diagnosis of conditions, such as cardiac diseases,[1] inflammation/infection[2] and cancer.[3] In general, POC biosensors provide assays that are simple to apply, rapid, accurate, precise, inexpensive and exhibit a low limit of detection.[4] POC biosensors are particularly useful in urgent situations, such as a heart attack or serious inflammations, as they provide important patient diagnostic information within minutes.[5]

The lateral flow immunoassay (LFA) (Fig. S1†) biosensor is a one-step POC biosensor that provides relatively rapid assay times (approximately 10–20 min for results), low-cost analysis, simple handling, stability and ease of mass production.[6,7] Because of these advantages, the LFA biosensor is the most widely used commercially available POC diagnostic assay.[6] Conversely, the limitations of the LFA biosensor include relatively low sensitivity, limited sample volume and the inability to acquire simultaneous measurements.[7] However, in recent years, the performance of LFA biosensors has significantly improved due to manufacturing with new materials, advanced reagents technologies, development of new LFA-based methods and the availability of hand-held optical detection systems.[7]

In addition to improvements in LFA biosensors, microfluidic-based POC biosensors are becoming increasingly available, and these miniaturized devices can analyse small sample volumes and rapidly deliver results.[8,9] Despite the successful commercialization of microfluidic POC biosensors by several companies (*e.g.*, i-STAT®, Abbott, and Triage®, Alere), the field of microfluidics, compared with LFA, still has several obstacles to overcome, including issues with implementation, the development of facilitative production technologies and performance.[9] Comparatively, LFA-based immunoassay systems can be improved more simply and can be rapidly developed for a commercial use with lower costs than other POC biosensor systems.[7]

Several approaches have been investigated to improve the sensitivity, multi-detection capability and the abilities of the LFA biosensors. Previous research in our laboratory led to the development of a cardiac troponin I detection LFA system capable of detecting a minimum of 0.1 ng mL^{-1} by using a signal enhancement method involving dual size gold nanoparticles.[10] Worsley *et al.*[11] combined 2 quantitative analyses in a single test line by using a fluorescent microspheres system. Abe *et al.*[12] fabricated fluidic channels on filter paper by using inkjet printing for a lateral flow immunoassay. Other examples include microfluidic integrated LFA biosensors,[13] chemiluminescence LFA,[14] multiple line measurement LFA to expend the measuring range for C-reactive protein (CRP)[15] and 2D LFA test strip fabrication methods for multiple bioassays.[16] However, these approaches have focused on improving 1 or 2 performance factors of the conventional LFA, but new LFA-based measuring systems are

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† Electronic supplementary information (ESI) available: Experimental details, each component of the VFA sensor, microscope images of asymmetric membrane, photographic images. See DOI: 10.1039/c2lc41016h

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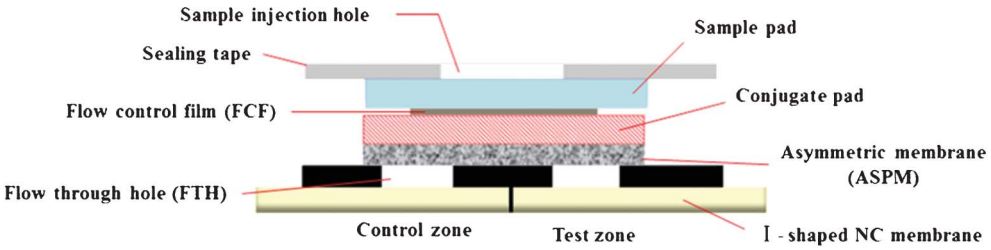


Scheme 1 The structure of the vertical flow immunoassay (VFA) biosensor. Individual components and sensor photographs are further described in the ESI (Fig. S1†).

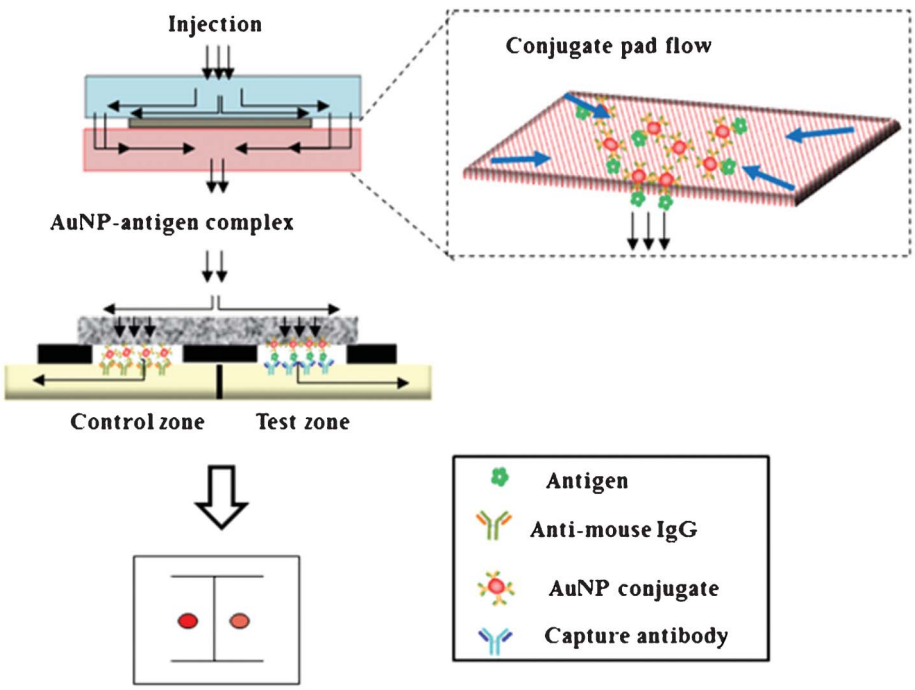
required to overcome the fundamental LFA limitations, including rapid commercialization.

C-reactive protein (CRP) is a well-known marker for acute inflammation. The 114-kDa protein, consisting of five repeated structures, is synthesized by the liver upon stimulation by interleukin-1 and interleukin-6. Recently, high-sensitivity CRP (hsCRP) has been widely accepted as a reliable biomarker and potential risk predictor for cardiovascular disease (CVD).[17] The precise and rapid measurement of hsCRP assists in monitoring the risk of serious consequences for patients with a CVD risk. The CRP level is considered normal up to 3 μg L^{-1}, and abnormal at levels higher than 5 μg L^{-1}.[18–20] When compared to different markers for acute myocardial infarction (MI), such as cardiac troponin and myoglobin, the assay for hsCRP requires measurement of rather high concentrations of the protein ($\geqslant$1 μg L^{-1}).[21,22]

In this study, we report the development of a new vertical flow one-step immunoassay (VFA) system (Scheme 1) that provides rapid results (within 2 min, to complete the reaction), requires relatively small sample volumes and provides a wide detection range with sensitivity similar to that of conventional LFA methods.

The feasibility of this VFA biosensor was evaluated using hsCRP, and the performance of VFA was compared with that of conventional LFA methods.

For measurements using the LFA biosensor, samples flow only in a horizontal direction through the NC membrane to the absorbent pad. This horizontal flow causes 3 major limitations in LFA. One limitation is assay time. To complete the immunoassay using LFA, all of the metal conjugates should pass completely through the test or control line on the NC membrane. Therefore, the total reaction time depends on the NC membrane flow rate and absorption capacity of the absorbent pad. For this reason, most LFA sensors require approximately 10 min to complete analyses. Another limitation is the "hook effect",[23] which is interference related to excess antigens that do not bind to the detection antibody conjugate. The last limitation is line interference. It is difficult to use multiple lines in an LFA sensor



Scheme 2 A sample flow diagram of the VFA biosensor.

because the front-line reaction interferes with the behind-line reaction. To overcome these LFA limitations, the VFA biosensor is used to create a rapid vertical flow assay system with controlled vertical flow and a separate measuring area using laser patterning.

The VFA sensor is produced by simple stacking of all the components. When a serum sample is incubated on the sample injection hole, the solution sequentially contacts and wets the sample pad, conjugation pad, ASPM and NC membrane through the VFAs. In brief, the sample flow can be divided into 4 steps. First, the samples are initially absorbed vertically by the sample pad, before flowing horizontally to the outside due to the hydrophobic property of the flow control film (FCF). Second, the samples flow to the conjugate pad vertically. The AuNP–antibody conjugate reacts with the antigen and is then concentrated in the middle of the conjugate pad. Third, the samples are transferred to the asymmetric membrane (ASPM). Because the conjugate pad is in contact with the smaller pore side of the ASPM, the sample flows downward from the horizontal flows. Finally, the samples pass through the submicron size flow-through holes (FTH) and then wash to the outside of the NC membrane. The antigen–antibody–AuNP-conjugate flows through the FTH, resulting in a sandwich antigen–antibody reaction with the capture antibody on the NC membrane. The NC membrane is lined by the laser system with an "I" shape to allow the sample solution to flow separately. The overall flow steps are briefly described in Scheme 2.

FCF, ASPM and FTH are critical flow control components for improving sensor performance, including signal intensity, precision and dynamic range. The FCF, which is smaller than the conjugation pad (ESI, Fig. S2†), serves to draw off the flow of the sample solution in the conjugation pad from the outside to the inside (Scheme 2). Thus, AuNP–antibody conjugates are efficiently released without loss from the conjugation pad. The use of the FCF in the VFA sensor resulted in higher signal intensity and improved reproducibility based on lower CV% (Fig. 1, test 2).

The ASPM between the conjugation pad and FTH film is also an important component. The 2 faces of the ASPM have different pore sizes (ESI, Fig. S3†). Inserting the ASPM between the conjugation pad and the FTH film by positioning the smaller pore face upwards provided a higher signal intensity than that obtained by removing or inverting the membrane. When the small-pore face is in contact with a solution, the asymmetric structure permits the aqueous solution to flow in the horizontal direction first, and then, in the vertical direction due to the reverse capillary action of the ASPM. It is hypothesized that the ASPM plays a role in promoting the uniform vertical flow speed of the sample through the holes and onto the NC membrane by delaying the vertical flow from the conjugate pad. Conversely, when the large pore was placed in contact with the conjugation pad, the non-uniformity of the vertical flow increased more significantly than was observed on removing the ASPM (Fig. 1, test 3). In case of using a symmetric membrane instead of the ASPM, the VFA sensor did not work properly (data not shown).

In addition, the ASPM membrane layer improved binding efficiency between the antigen and detection antibody–AuNPs due to the vertical flow delay effect (Fig. 1, test 3). Fig. 1, clearly confirms the roles of FCF and ASPM.

The FTH film promotes the reaction between the sample solution and the antibodies in a small area. We investigated the effect of the size of the hole on the signal intensities. As shown in Fig. S4A†, the signal intensity increased with decreasing hole size, up to 0.5 mm. As the sample solution containing the AuNP–antibody conjugate flows only through the holes, the immunoreactivity signal will increase with decreasing hole diameter due to concentration effects.

However, analysis performed using the 0.35 mm hole, showed that the sample solution did not consistently flow uniformly into the NC membrane (data not shown). Additionally, in the VFA biosensor, the total flow time of the actual injected sample takes about 10 min, while the flow time of the AuNP–conjugate takes less than 1 min to pass through the FTH. Therefore, residual samples do not affect the sensor performance except having a greater self-washing effect. For this reason, as the sample volume increased, more self-washing was observed and the reproducibility would also improve. Therefore, sample volumes that exceed the critical volume (>50 μL, Fig. S4 (B)†) do not affect signal intensity. However, increasing the sample volume does increase the effect of

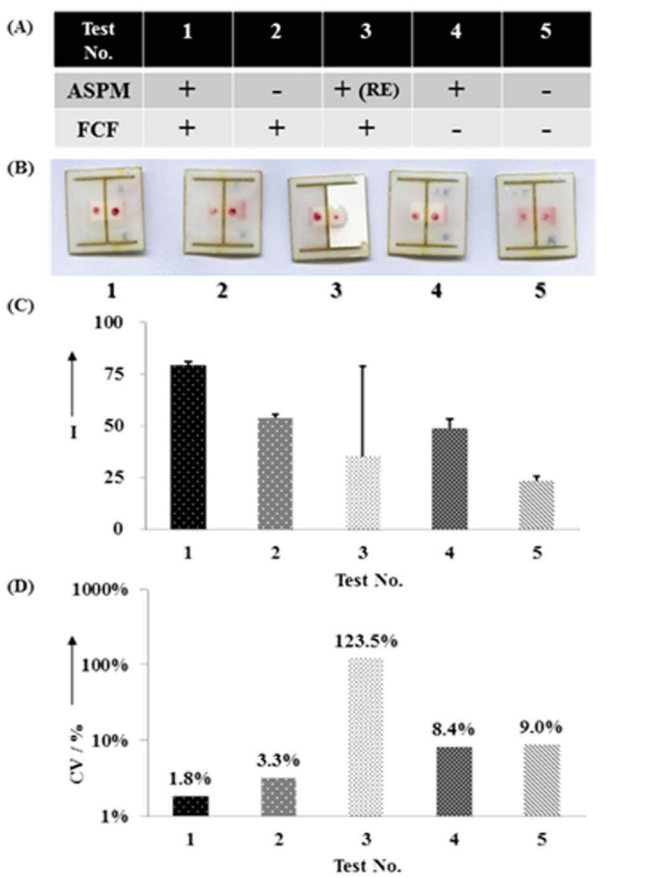


Fig. 1 The effect of inserting (+) or deleting (−) the flow-control film (FCF) and/or asymmetric membrane (ASPM) on signal intensity and reproducibility; (A) test information table, (B) photographic images of each test, (C) a comparison of signal intensities for each test, (D) coefficient of variations (CV/%) of each test. (+RE) signifies that the ASPM is inverted.

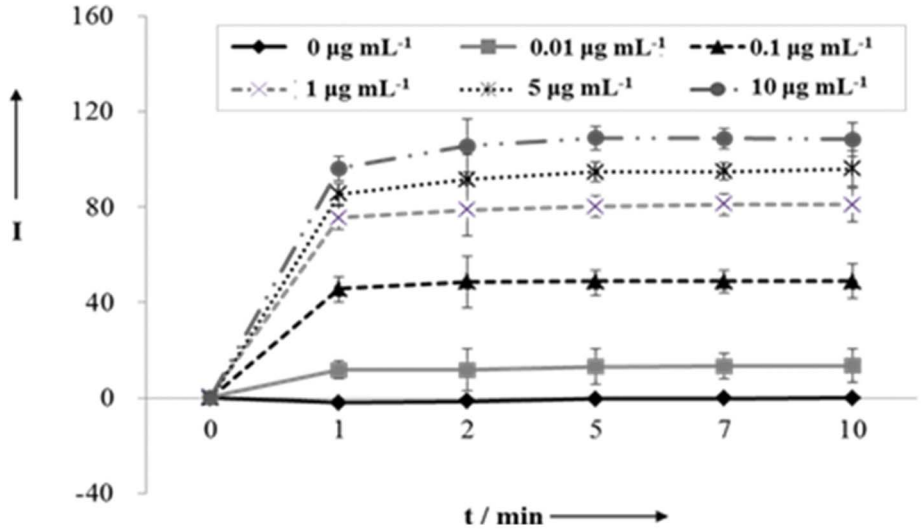


Fig. 2 Time-dependent signal intensities for various concentrations of CRP. The data points were obtained in triplicate, with error bars representing standard deviations.

self-washing, which resulted in a lower standard deviation (Fig. S4 (B)†).

The CRP analysis was performed at various concentrations (0.01–10 μg mL^{-1}) using the VFA, and the signal intensities were measured at several assay time points (Fig. 2). The signal intensities increased with increasing concentration of the CRP, and the immunoreactions were completed within 2 min, suggesting that the AuNP–antibody conjugates passed through the FTHs within that period. It was further observed that the time required to completely wet the NC membrane was approximately 4 min. This technical observation is important, because a rapid immunoassay is sometimes required in POC diagnoses. These results indicate that the VFA biosensor provides faster analysis than the LFA biosensor.

For comparison, a conventional LFA system was fabricated and tested. Fig. 3 shows the CRP concentration-dependent intensities for the FTH and LFA biosensors. The VFA sensor shows a gradual signal increase with the concentrations in the tested range, whereas the LFA sensor shows an increase in signal up to 1 μg mL^{-1}, with signal decrease at 5 μg mL^{-1}. In contrast in the VFA system, the hook effect is not observed up to the CRP concentration of 10 μg mL^{-1}.

Conclusions

In conclusion, to implement a rapid one-step VFA immunosensor, we controlled vertical flow by specifically using a combination of

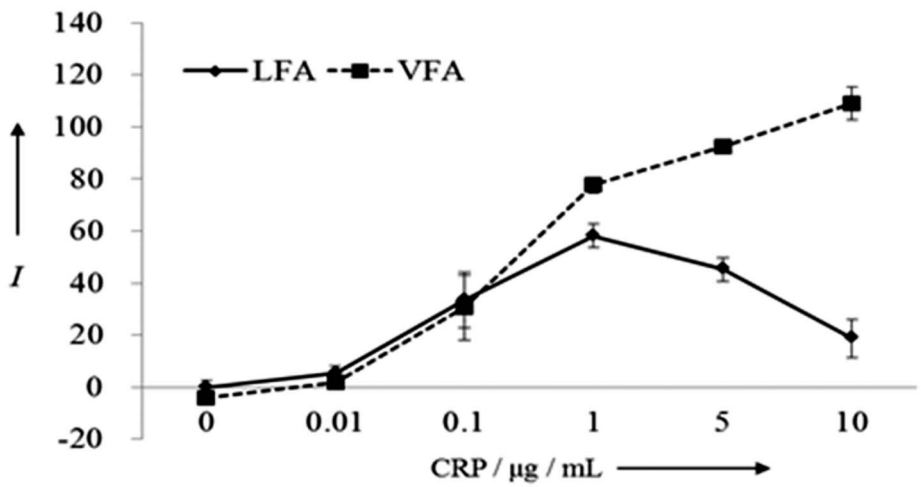


Fig. 3 A comparison of the conventional LFA and the VFA sensor for the detection of CRP. The data points were obtained in duplicate, with error bars representing standard deviations.

methods and materials, such as double-sided tape (FCF, FTH) and an asymmetric membrane (ASPM).

In addition, most of the components used were previously used as LFA materials, which means that the VFA sensor is easy to produce at a lower cost and is more suitable for commercialization than the microfluidic-based POC biosensor. In the performance comparison between VFA and LFA, the VFA sensor could complete rapid assays covering a wide dynamic range and using low sample volumes with similar sensitivity. Although several vertical flow-based sensors, including the immunofilteration[24] method and the flow through system[25] have been already reported for a simple and rapid measurement, compared to these systems, our one-step VFA sensor shows a rapid and specific flow control, as well as quantitative results.

To the best of our knowledge, the VFA sensor, composed of commercially available and inexpensive materials, is the most rapid one-step immunosensor that can be used for serum sample tests. It is expected that the VFA biosensor will be applicable for rapid immunoassays in point-of-care testing.

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Notes and References

1 B. McDonnell, S. Hearty, P. Leonard and R. O'Kennedy, *Clin. Biochem.*, 2009, **42**, 549–561.
2 A. Pfafflin and E. Schleicher, *Anal. Bioanal. Chem.*, 2009, **393**, 1473–1480.
3 S. A. Soper, K. Brown, A. Ellington, B. Frazier, G. Garcia-Manero, V. Gau, S. I. Gutman, D. F. Hayes, B. Korte, J. L. Landers, D. Larson, F. Ligler, A. Majumdar, M. Mascini, D. Nolte, Z. Rosenzweig, J. Wang and D. Wilson, *Biosens. Bioelectron.*, 2006, **21**, 1932–1942.
4 P. von Lode, *Clin. Biochem.*, 2005, **38**, 591–606.
5 G. J. Fermann and J. Suyama, *J. Emerg. Med.*, 2002, **22**, 393–404.
6 G. A. Posthuma-Trumpie, A. van Amerongen, J. Korf and W. J. van Berkel, *Trends Biotechnol.*, 2009, **27**, 652–660.
7 S. Zapata, L. Mejía, F. Le, R. León, B. Pesson, C. Ravel, L. Bichaud, R. Charrel, C. Cruaud, G. Trueba and J. Depaquit, *Parasites Vectors*, 2012, **5**, 144.
8 F. B. Myers and L. P. Lee, *Lab Chip*, 2008, **8**, 2015–2031.
9 C. D. Chin, V. Linder and S. K. Sia, *Lab Chip*, 2012, **12**, 2118–2134.
10 D. H. Choi, S. K. Lee, Y. K. Oh, B. W. Bae, S. D. Lee, S. Kim, Y.-B. Shin and M.-G. Kim, *Biosens. Bioelectron.*, 2010, **25**, 1999–2002.
11 G. J. Worsley, S. L. Attree, J. E. Noble and A. M. Horgan, *Biosens. Bioelectron.*, 2012, **34**, 215–220.
12 K. Abe, K. Suzuki and D. Citterio, *Anal. Chem.*, 2008, **80**, 6928–6934.
13 L. Gervais and E. Delamarche, *Lab Chip*, 2009, **9**, 3330–3337.
14 C. H. Yoon, J. H. Cho, H. I. Oh, M. J. Kim, C. W. Lee, J. W. Choi and S. H. Paek, *Biosens. Bioelectron.*, 2003, **19**, 289–296.
15 W. Leung, C. P. Chan, T. H. Rainer, M. Ip, G. W. Cautherley and R. Renneberg, *J. Immunol. Methods*, 2008, **336**, 30–36.
16 E. M. Fenton, M. R. Mascarenas, G. P. Lopez and S. S. Sibbett, *ACS Appl. Mater. Interfaces*, 2009, **1**, 124–129.
17 W. Koenig, N. Khuseyinova, J. Baumert and C. Meisinger, *Clin. Chem.*, 2008, **54**, 335–342.
18 R. Siebenhaar, P. B. Mushholt, T. Forst, M. M. Weber, R. Maurer and A. Pfutzner, *J Diabetes Sci Technol*, 2010, **4**, 717–722.
19 N. J. Wijnstok, J. W. Twisk, I. S. Young, J. V. Woodside, C. McFarlane, J. McEneny, T. Hoekstra, L. Murray and C. A. Boreham, *J. Adolesc. Health*, 2010, **47**, 346–351.
20 M. H. Meyer, M. Hartmann, H. J. Krause, G. Blankenstein, B. Mueller-Chorus, J. Oster, P. Miethe and M. Keusgen, *Biosens. Bioelectron.*, 2007, **22**, 973–979.
21 A. S. Jaffe, L. Babuin and F. S. Apple, *J. Am. Coll. Cardiol.*, 2006, **48**, 1–11.
22 F. S. Apple, M. M. Murakami, L. A. Pearce and C. A. Herzog, *Clin. Chem.*, 2004, **50**, 2279–2285.
23 S. A. Fernando and G. S. Wilson, *J. Immunol. Methods*, 1992, **151**, 47–66.
24 A. Y. Kolosova, S. D. Saeger, S. A. Eremin and C. V. Peteghem, *Anal. Bioanal. Chem.*, 2007, **387**, 1095–1104.
25 S. Wang, C. Zhang, J. Wang and Y. Zhang, *Anal. Chim. Acta*, 2005, **546**, 161–166.

