Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supplementary Information

Carbon nanotubes for highly sensitive colorimetric immunoassay biosensor

Jing-Huei Huang [a], Ying-Jhan Hong [a], Yun-Tzu Chang [a], Pin Chang and Tri-Rung Yew*[a]

[a] National Tsing Hua University, Department of Materials Science and Engineering, 101, Sec. 2, Kuang-Fu Road, Hsinchu, Taiwan 30013

* Corresponding author: Tri-Rung Yew. Fax: +886-3-5722366;

E-mail: tryew@mx.nthu.edu.tw

1. Experimental

1.1 Fabrication of the sensing-substrate

The sensing-substrate was fabricated with the method similar to that reported earlier.[1] First, the glass substrate was functionalized with hydroxyl groups by piranha solution (1:3 H_2O_2-concentrated H_2SO_4). The amino-group layer of APTES, was self-assembled on the glass for 2 h. The glass was then rinsed with DI water for several times, dried by N_2 flow, and baked at 120 °C for 30 min to form a stable APTES film.[2] Second, the APTES-modified glass was then incubated in 0.1 M phosphate buffered saline (PBS) containing 8 μg ml^{-1} of mAHSA and shaken at 35 °C for 1 h. Third, the mAHSA/APTES-modified glass was incubated in 1 wt% BSA and shaken at 35 °C for 1 h to block untreated and non-specific sites. Moreover, the specimen was washed with PBS for several times after each step of process.

1.2 Fabrication of the CNT-label

The pAHSA was covalently bound on CNTs with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) reaction,[3-4] and the detailed fabrication process is described as following. First, the 0.12 g L^{-1} carboxylic group modified with CNTs (COOH-modified CNTs) (Golden Innovation Business, CDH-AMC SW2012) in DI water was prepared under ultra-sonication for at least 30 min. Second, the 0.5 ml COOH-modified CNTs were mixed with 0.5 ml 0.1 M buffer solution, *i.e.*, the PBS with KH_2PO_4 (0.2 g L^{-1}) and Na_2HPO_4 (1.16 g L^{-1}), containing 250 mM

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Alfa Aesar), and 100 mM N-hydroxysuccinimide (NHS, Sigma-Aldrich), and 0.9 μg ml^{-1} pAHSA. The solution was then kept at 28 °C with ultra-sonication for 2 h for the cross-linking of pAHSA on CNTs to become CNT-label. Third, the CNT-label was extracted from centrifugation at 9000 rpm for 5 min. The supernatant including excess reagents was then disposed of and the precipitate was redispersed in buffer solution. These wash steps were repeated for three times, and the final CNT-label solution was kept in buffer solution with ultra-sonication about 30 min before used.

1.3 HSA detection

The biosensor fabricated in this work was used to detect the sensing-target HSA with the concentration ranging from 2×10^{-7} to 2×10^{-1} mg ml^{-1}, including one control sample without HSA. First, the sensing-substrate was immersed in HSA solution and shaken at 35 °C for 1 h, followed by the wash in PBS for several times for the specific bonding of HSA on the sensing-substrate. Second, the HSA-bonded sensing-substrate was immersed in a pAHSA modified CNT-label solution under ultra-sonication at 28 °C for 1 h for the bonding of CNT-label onto the detected HSA on the sensing-substrate (CNT-labeled sensing-substrate). The CNT-labeled sensing-substrate was then by rinsed with PBS to remove unbound CNT-label for several times and dried with N_2 flow, followed by the optical transmission measurement by using UV-Vis (Cary 60)

2. Verification of surface modification and antibody/antigen immobilization on the sensing-substrate

To ensure the specific HSA detection of the biosensor, the sensing-substrate was functionalized with mAHSA and BSA. Therefore, the immobilization of the mAHSA on the APTES-modified glass, and the blocking of BSA on untreated and non-specific bonding sites before HSA sensing should be confirmed. This can be determined by applying anti-IgG-FITC (Abcam, ab6785) (goat polyclonal secondary antibody to mouse IgG with fluorescein isothiocyanate, FITC) to the sensing-substrate. Although the fabrication process was similar to that was used in previous works,[1] the sensing-substrate and HSA immobilization was maintained at 35 °C in this work instead of at room temperature to enhance the quality of HSA bonding. Therefore, the antibody and antigen immobilization needs be verified again.

Fig. S1a and S1b show the pre- and post- conjugation of anti-IgG-FITC on the sensing-substrate, respectively. Fig. S1a indicates that no fluorescence on the sensing-substrate under a fluorescence microscope. Fig. S1b reveals green fluorescence, which represents that the mAHSA has been conjugated on APTES-modified glass successfully. Furthermore, the blocking effect of BSA was also confirmed by fluorescence microscope. Fig. S1c and S1d show the conjugation of anti-IgG-FITC on APTES-modified glass without- and with BSA blocking, respectively. According to the fluorescence images in Fig. S1c and S1d, BSA could block the untreated and non-specific sites as anti-IgG-FITC could not bind on the APTES-modified glass with BSA blocking while anti-IgG-FITC could directly bind on the APTES-modified glass without BSA blocking. In brief, the APTES-modified substrate has been functionalized with mAHSA and the APTES substrate without mAHSA bounded has been blocked by BSA successfully.

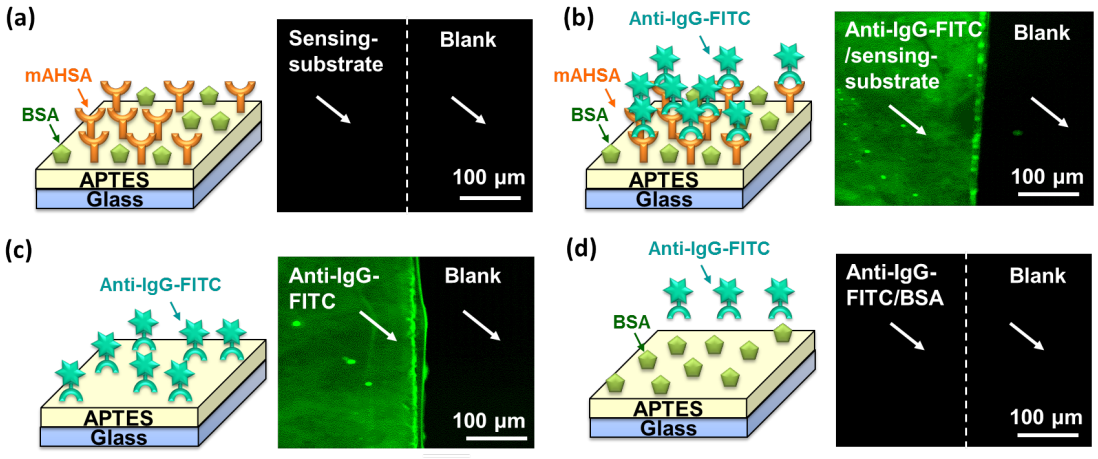

Fig. S1. The (a) pre- and (b) post- conjugation with Anti-IgG-FITC on the sensing -substrate. (c) without- and (d) with BSA blocking and then conjugation of Anti-IgG-FITC on APTES-modified glass.

3. CNTs dispersion in buffer solution

CNTs as a label material in this study needs to be immobilized with pAHSA for specific binding to the detection target, HSA, via antibody-antigen specific binding mechanism. In addition, pAHSA should be stored in buffer solution with pH = 7.4 to maintain its activity. Therefore, CNTs need to be dispersed well in buffer solution. Four common buffer solutions used in biology were tested in this work. The same amount of COOH-modified CNTs was dispersed in 0.1 M PBS and buffer solution, a solution with KH_2PO_4 (0.2 g L^{-1}) and Na_2HPO_4 (1.16 g L^{-1}), Hank's Balanced Salt Solution (HBSS), and tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), respectively. Then the COOH-modified CNTs with buffer solutions were treated with ultra-sonication for 30 min. After being stored statically for 24 h, only COOH-modified CNTs mixed with buffer solution still dispersed well as shown in Fig. S2. The results were consistent to previous work,[5] which reported that CNTs

were dispersed better in the solution with fewer ions. Consequently, buffer solution was chosen as the buffer solution for the cross-linking of pAHSA in the following study.

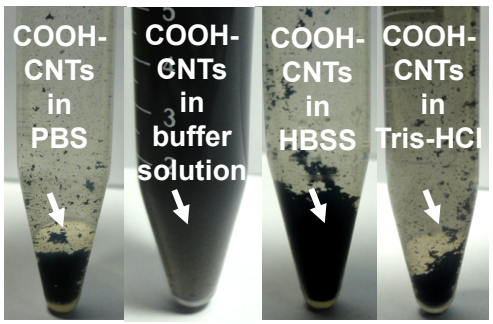

Fig. S2. The photos images of COOH-modified CNTs dispersed in the buffer solution of PBS, buffer solution, HBSS and Tris-HCl, after being stored statically for 24 h.

4. The cross-linking of pAHSA with CNT using EDC and NHS

The immobilization of pAHSA onto COOH-CNTs for CNT-label fabrication was via EDC-NHS reaction. The amount of pAHSA cross-linking on CNTs was determined by the concentrations of EDC and NHS. Therefore, one set of experiments was using both EDC and NHS concentrations of 100 mM, 200 mM and 500 mM, respectively, *i.e.*, with a EDC/NHS ratio of 1:1. Another set of experiments was by using NHS concentrations at 100 mM and 200 mM, while with the ECD of 250 mM and 500 mM, respectively, to keep a EDC/NHS ratio of 5:2. The anti-IgG-FITC was then applied onto each CNT-label specimen to verify the cross-linking of pAHSA onto COOH-CNTs.

Fig. S3a-S3e show the fluorescence images of the anti-IgG-FITC conjugated the CNT-label in buffer solution dropped on glass for the samples with various EDC and NHS concentrations. The green fluorescence indicated that the anti-IgG-FITC can be conjugated on the CNT-label directly, showing that the pAHSA has been bound on COOH-modified CNTs via EDC-NHS reaction. The results indicated that better protein immobilization was observed in the EDC/NHS ratio of 5:2 than that of 1:1 from the brighter green fluorescence image. However, the high concentrations of EDC and NHS would lead to the loss of activity for protein owing to the deviated pH value of buffer solution. The failure of pAHSA conjugation on COOH-CNTs could be inferred from the failure of anti-IgG-FITC binding on pAHSA as shown in Fig. S3c. Therefore, the optimum cross-linking of pAHSA onto COOH-CNTs occurs to the condition with 250 mM EDC and 100 mM NHS in this study as shown in Fig. S3d. Hence, this condition was utilized to fabricate biosensor.

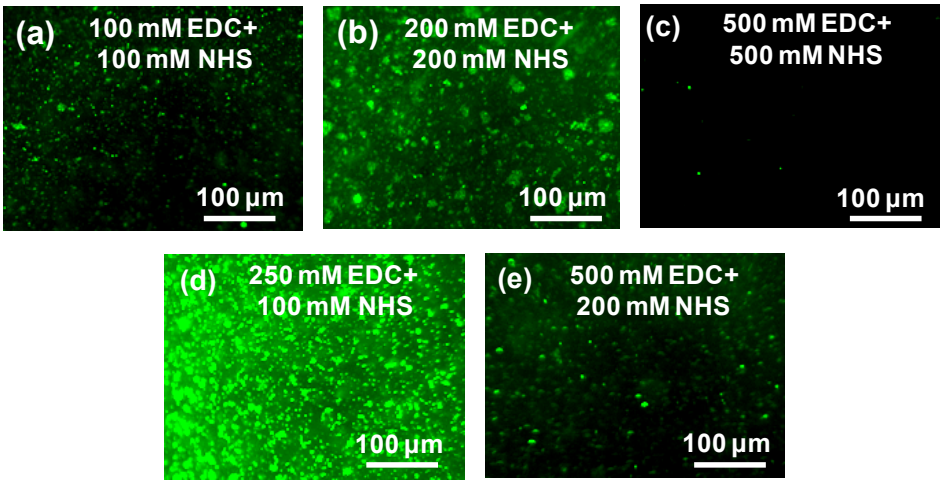

Fig. S3. The fluorescence images of anti-IgG-FITC conjugation on the CNT-label in buffer solution applied on glass at various concentrations of EDC and NHS (a) 100 mM and 100 mM (b) 200 mM and 200 mM (c) 500 mM and 500 mM

by keeping a EDC/NHS ratio of 1:1, and (d) 250 mM and 100 mM (e) 500 mM and 200 mM, respectively, by keeping a EDC/NHS ratio of 5:2.

References

(1) M. C. Tu, Y. T. Chang, Y. T Kang, H. Y. Chang, P. Chang and T. R. Yew, *Biosens. Bioelectron.*, 2012, **34**, 286.

(2) N. Crampton, W. A. Bonass, J. Kirkham and N. H. Thomson, *Langmuir*, 2005, **21**, 7884.

(3) N. Nakajima and Y. Ikada, *Bioconjugate Chem.*, 1995, **6**, 123.

(4) Y. T. Chang, J. H. Huang, M. C. Tu, P. Chang and T. R. Yew, *Biosens. Bioelectron.*, 2013, **41**, 898.

(5) E. Heister, C. Lamprecht, V. Neves, C. Tilmaciu, L. Datas, E. Flahaut, B. Soula, P. Hinterdorfer, H. M. Coley, S. R. Silva and J. McFadden, *ACS Nano*, 2010, **4**, 2615.