Paper-Based Microfluidic Analytical Device Based on Molecularly Imprinted Polymer for Detection of Carcinoembryonic Antigen

Ji Qi\textsuperscript{a}, Lin Zhang\textsuperscript{a}, Dongmei Deng\textsuperscript{a}, Yuanyuan Li\textsuperscript{a,b}, Liqiang Luo\textsuperscript{a,*}

\textsuperscript{a}College of Sciences, Shanghai University, Shanghai 200444 P. R. China.
\textsuperscript{b}Shanghai Applied Radiation Institute, School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, P. R. China

Liqiang Luo: luck@shu.edu.cn

Abstract:
This paper describes a novel strategy to fabricate a low-cost microfluidic paper-based analytical device (\muPAD) readily based on surface molecularly imprinted technology for clinical detection of carcino-embryonic antigen (CEA). The movable valve fabrication was realized using hollow rivets as the holding center to control the electrode and channel of the \muPAD in different layer movement, so the entire process of synthesis and testing could be completed easily. Graphene oxide, chitosan and CEA templates were assembled on the \muPAD as a substrate, and subsequently the molecularly imprinted membranes were formed by electropolymerization of dopamine. Finally, the novel \muPAD was obtained after removal of CEA templates. Because the entire manufacturing process was done on the \muPAD without any external treatment, the \muPAD has the characteristics of easy synthesis, low cost and low toxicity. Moreover, the \muPAD gave a flexible and easy way to operate the entire detection process conveniently. Experimental parameters such as equilibration time, scan cycles, template concentration and pH value were optimized. A good linearity was obtained in the range of 1 – 500 ng mL\textsuperscript{-1} ($R^2=0.994$) with the limit of detection (LOD) of 0.21 ng mL\textsuperscript{-1}.

Key words: microfluidic paper-based devices, electrochemical sensor, molecularly imprinted technology, carcinoembryonic antigen

Introduction
Microfluidic paper-based analytical device (\muPAD) technology has been developing quickly like the bamboo shoots after a spring rain. However, most studies require the help of external conditions to synthesize complex materials to support the operation of the \muPAD. Therefore, finding a simple and low-carbon synthesis method has become a major challenge for \muPAD development. On the other hand, accurate and sensitive determination of CEA by the application of \muPAD in human body is very important in early diagnosis, screening disease recurrence, and healing the patients with certain tumor-associated disease [1].

Fabrication of \muPAD
Firstly, we designed a structure that contains hydrophobic and hydrophilic areas on the paper chip, as shown in Fig. 1. Secondly, graphene oxide (GO), chitosan and CEA templates were assembled on the working electrode pool of \muPAD to synthesize molecularly imprinted polymer by electropolymerization of dopamine [2]. Lastly, \muPAD was obtained after removal of CEA templates.
Electrochemical characterization

Cyclic voltammetric responses at bare paper electrode and modified paper electrode are shown in Fig. 2. The bare paper electrode showed a couple of redox peaks at 0.11 and 0.27 V in a solution of 5 mM [Fe(CN)₆]³⁻/⁴⁻ (0.1 mol/L PBS, pH 7.4). An obvious increase of peak current could be observed when GO was used on bare paper due to the large surface area and high conductivity of GO. Then a further increase was obtained after chitosan was coated. This could be attributed to that the electrostatic attraction between positively charged chitosan amino group and negatively charged [Fe(CN)₆]³⁻/⁴⁻ accelerated electron transfer on the electrode surface. The peak current of molecularly imprinted polymer modified paper was very weak before elution, but recovered after templates removal.

Performance of µPAD

Because CEA was not electrochemically active, a blocking effect was caused by the presence of the CEA, resulting in the reduction of electron transfer. The relationship between the peak current variation and the CEA concentration was detected using differential pulse voltammetry (DPV) under the optimal conditions. As shown in Fig. 3, the differences of peak current were proportional to the logarithm concentration of CEA in the range of 1–500 ng mL⁻¹ and the regression equation was ΔI (µA) = 19.342 lg C (ng mL⁻¹) + 6.933 with correlation coefficient of 0.994.

Conclusion

This paper describes a novel strategy to fabricate a low-cost electrochemical µPAD readily based on surface molecularly imprinted technology. The electrochemical µPAD is successfully applied for detection of CEA, showing promising prospect in the field of clinical diagnostics and healthcare in the future.

Acknowledgements

This research is supported by the National Natural Science Foundation of China (Nos. 61571278, 61571280).

References
