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Ultra-sensitive electrochemical detection of single nucleotide polymorphisms based on an electrically controllable magnetic gold electrode†

Jing Zhang,[ab] XiaoYan Wu,[b] WeiJuan Yang,[b] JingHua Chen*[c] and FengFu Fu*[b]

A novel controllable magnetic electrochemical biosensor for the ultra-sensitive detection of single nucleotide polymorphisms (SNPs) of trace HIV-related salivary DNA is first described here based on a home-made electrically controllable magnetic gold electrode (ECM-GE).

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in individual genomes and are considered a primary factor in genetic diseases.[1] Therefore, considerable attention in genetic studies has been focused on the genotyping and detection of SNPs, which are implicated in the mechanisms of infectious diseases such as the human immunodeficiency virus (HIV).[2] In particular, HIV is the most serious infectious disease that challenges public health worldwide.[3] So far, the popular diagnostic tests for HIV are serologic tests such as enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA) and Western blot tests.[4] These methods are sensitive but have the disadvantages of complex instrumentation and elaborate sample preparation, as well as the risk of false positive and false negative results.[5] These immunoassays are also time-consuming since they indirectly detect the presence of antibodies of HIV. Currently, more than 90% of new HIV infections occur in developing countries where there is a lack of adequate medical facilities due to destitution.[1] This poses a new challenge to develop a mass-scale point-of-care diagnostic method, which is characterized as fast, sensitive, specific and cost-effective, for the HIV.[6]

Recently, it was reported that HIV-related DNA exists in the infectee's saliva.[7] Accordingly, it is expected that HIV-infection can be diagnosed by detecting HIV-related DNA in saliva, which has notable merits such as simple sample collection, no requirement for a phlebotomist and low risk of HIV infection for healthcare workers. However, due to the low concentration (~fM) and the complex matrix, an ultra sensitive, selective and stable method for the specific detection of HIV-related DNA in saliva is required. Electrochemical biosensors are considered the most promising method for point-of-care diagnosis due to the fact that they are sensitive, cost-effective and portable.[8] To date, various electrochemical biosensors have been developed for the sensitive and specific detection of DNA.[9] However, the application of these sensors may be hampered by one or more of following problems: poor stability and specificity, complexity of fabrication, lower sensitivity, long assay time and poor resistibility to the matrix.

Magnetic beads (MBs) can be easily collected with a magnet. They have been widely used as a carrier for the pre-concentration of analytes,[10] as well as for the specific capture of different molecules in nucleic acid testing due to their bio-affinity.[11] Recently, a disposable magnetic electrochemical biosensor was proposed for the specific detection of DNA.[12] The biosensor showed a high sensitivity even without PCR amplification. However, it has obvious limitations, such as, the strength and direction of the magnetic field cannot be regulated due to the utilization of a permanent magnet, the hybridization and detection processes are performed separately making the biosensor unsuitable for on-line analysis, high analysis cost due to the use of a single-use screen printed electrode, low discrimination ability for the single-base mismatch, *etc.* In an effort to solve the aforementioned problems, we herein develop a novel controllable magnetic electrochemical biosensor based on an electrically controllable magnetic gold electrode (ECM-GE, see Fig. S1 in ESI†). The ECM-GE combines the merits of a heated electrode and a magnetic electrode, and has notable advantages such as the strength and direction of the magnetic field, and the temperature of the electrode's surface can be regulated according to the experimental strategy. These features give our biosensor, which is based on an ECM-GE, ultra-high sensitivity by combining MB-based amplification, better discrimination ability for single-base mismatch by controlling

[a] *Faculty of Life Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China*
[b] *Key Lab of Analysis and Detection for Food Safety (MOE and Fujian Province), Department of Chemistry, Fuzhou University, Fujian 350108, China. E-mail: fengfu@fzu.edu.cn; Fax: +8659122866135*
[c] *Faculty of Pharmacy, Fujian Medical University, Fuzhou, Fujian 350004, China. E-mail: cjh_huaxue@126.com*

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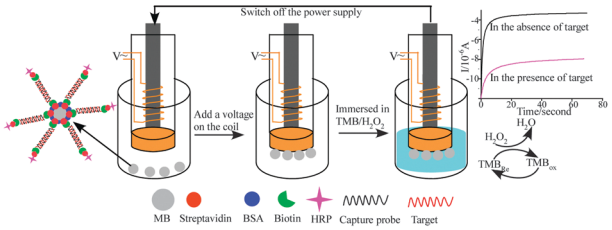

Scheme 1 Experimental principle of the controllable magnetic electrochemical biosensor.

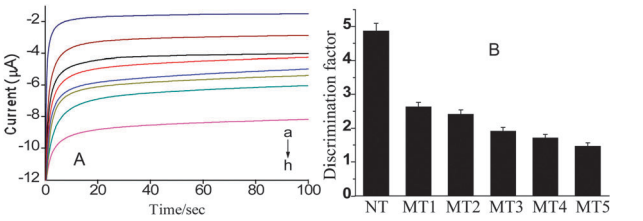

Fig. 1 (A) Current–time curves of C_p-modified MBs after they were hybridized with different sequences: (a) nothing, (b) NT, (c) MT_1, (d) MT_2, (e) MT_3, (f) MT_4, (g) MT_5 and (h) target DNA. (B) The discrimination factors of the biosensor for single-base mismatch. Data shown in Fig. 1A were used for the calculation.

the temperature of the electrode's surface, short analysis time since the separation and detection can be performed simultaneously, and low analysis cost due to the good re-usability of the ECM-GE.

The principle of the proposed biosensor is illustrated in Scheme 1. The capture probes (C_p, colored red), which were functionalized with biotin groups at one end, were attached on the surface of streptavidin-modified MBs by the interaction of biotin and avidin. In the presence of target DNA, which was labeled with a biotin group (T, colored blue), the C_p could hybridize with T to form a duplex structure on the surface of the MBs. The hybrid-attached MBs could capture streptavidin–horseradish peroxidase by the specific interaction of streptavidin and biotin, and the resulting HRP-tagged MBs were then adsorbed onto the surface of the ECM-GE by applying a voltage to its coil. The electrode was then used for the analysis of chronoamperometry in H_2O_2–3,3′,5,5′-tetramethylbenzidine sulfate media. It is well known that HRP can catalyze the H_2O_2-mediated oxidation of TMB and leads to an obvious change in electrochemical signal.[13] Therefore, the electrochemical current responding to the H_2O_2-mediated oxidation of TMB directly reflects the amount of HRP on the surface of the MBs, and indirectly reflects the amount of T on the surface of the MBs as well. As shown in Scheme 1, before electrochemical measurement, the HRP-tagged MBs were completely captured on the surface of the ECM-GE. The capture of HRP-tagged MBs greatly increased the density of HRP on the surface of the working electrode, which led to a much bigger electrochemical current, *i.e.* a much higher sensitivity.

A 21 base complementary target DNA T (HIV-related salivary DNA) and its one-base mismatch DNA MT_1, MT_2, MT_3, MT_4 and MT_5 (MT_1 with an A-to-T substitution at the center, MT_2 with an A-to-C substitution at the center, MT_3 with an A-to-G substitution at the center, MT_4 with an A-to-T substitution at the 3′ end, and MT_5 with an A-to-T substitution at the 5′ end), and non-complementary DNA NT were used to test the discrimination ability of our biosensor (see Table S1 in ESI†). The chronoamperometry results are shown in Fig. 1A. The lowest current signal was observed for NT (curve b), implying that non-complementary DNA cannot be hybridized with C_p to form HRP-tagged MBs. Whereas the highest signal was observed for T (curve h), indicating that T hybridizes with C_p to form a duplex structure on the surface of the MBs and the duplex structure captures the streptavidin–HRP to form HRP-tagged MBs. As a result, the amount of HRP adsorbed on the electrode's surface increased, which resulted in a much bigger current signal. In comparison with T, a much lower signal was detected for the one-base mismatch DNA and the signal decreased in the order MT_5, MT_4, MT_3, MT_2, MT_1, indicating that our biosensor has an excellent discrimination ability for single-base mismatch and can be used to detect SNPs.

To quantitatively evaluate the discrimination ability of our biosensor, we defined the discrimination factor (DF) as the ratio of net catalytic current (current after subtracting the background) obtained with complementary target DNA T to that obtained with MT_1, MT_2, MT_3, MT_4 and MT_5. A larger discrimination factor is thus indicative of improved specificity for single-base mismatch. From Fig. 1A, we calculated that the DF was in the range of 1.47–2.63 for single-base mismatch (in a 21 base DNA sequence). The best DF of 2.63 was observed for MT_1 (T–T mismatch at the center), followed by 2.41 for MT_2 (T–C mismatch at the center), 1.92 for MT_3 (T–G mismatch at the center), 1.71 for MT_4 (T–T mismatch near 3′) and 1.47 for MT_5 (T–T mismatch at the edge of 5′) (Fig. 1B). Fig. 1B clearly suggests that the discrimination ability of the biosensor is highly dependent on the position of the mutation. The mutations that occurred at the center had a larger DF, whereas the mutations that occurred near/at the edge of the DNA strand had a smaller DF. This is probably because the mismatched base at the center could "cut" the oligonucleotide into shorter sequences more effectively, which lead to a significant decrease in the melting temperature and stability in comparison to the fully matching double-strand. Moreover, the central mismatched bases also have an "ortho effect", which means they have a greater influence on hydrogen bond formation of the adjacent nucleotide than the end-on mismatched bases. The discrimination ability is also dependent on the base involved in the mutation. MT_1, MT_2 and MT_3 have different DFs although they all have only one mismatched base at the center, indicating that our sensor can recognise the genotype of different mismatches. To sum up, compared with previous "duplex strategy"-based biosensors,[12,14] our sensor has better discrimination ability or higher sensitivity since the surface temperature of the ECM-GE is high in comparison with that of the common electrode. The DF of 2.63 for one-base mismatch is close to that of biosensors using the "junction-probe" strategy and the deoxyinosine probe (see Table S1 in ESI†).[8c]

The sensitivity of the biosensor was also investigated. The current signals after the biosensor hybridized with different concentrations of target DNA were measured with chronoamperometry. As shown in Fig. 2, the average current shows a good

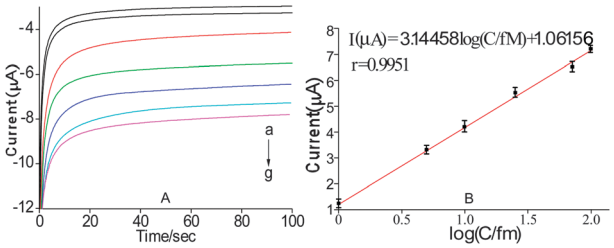

Fig. 2 (A) Current–time curves of C_p-modified MBs after hybridization with different concentrations of the target DNA. From a–g: 0 fM, 1 fM, 5 fM, 10 fM, 25 fM, 75 fM and 100 fM. (B) The relationship between the current and the concentration of the target DNA (*C*).

Table 1 Determination of target DNA in artificial saliva samples (n = 5)

T added (fM)	Detected conc. (fM)	Recovery (%)	RSD (%)
1.00	1.06	106	6.3
5.00	4.79	96	5.3
10.0	9.88	99	4.9

linear correlation with the logarithm of the target DNA concentration in the range of 1–100 fM (see Fig. 2B). The regression equation is: I/μA = 3.1446 log(C/fM) + 1.0616, r = 0.9951.

The detection limit ($3\sigma/S$) was calculated to be 0.37 fM (3.7×10^{-16} M) for the target DNA. The relative standard deviation (RSD) for 8 replicate detections of 10 fM of target DNA is 6%. Compared to the previous methods, which were based on the ''duplex strategy'',[12,14] the proposed biosensor has a much higher sensitivity and specificity.

In order to evaluate the applicability, the saliva samples spiked with different concentrations of target DNA (T) were detected with our biosensor. The results shown in Table 1 indicate that our biosensor has a strong resistance to the complex matrix of saliva, and can be used to detect ultra-trace amounts of target DNA in real saliva samples.

In summary, a controllable magnetic electrochemical biosensor for the detection of SNPs was first developed based on an ECM-GE. The ECM-GE combines the merits of a heated electrode and a magnetic electrode, and has notable advantages including the fact that the strength and direction of the magnetic field and the temperature of the electrode's surface can be easily regulated, *etc.* These features, as well as the utilization of MB-based enzymatic catalysis amplification, give our biosensor ultra-high sensitivity and specificity for the detection of single-base mismatches. It can be used to detect concentrations of target DNA (HIV-related salivary DNA) as low as 0.37 fM (3.7×10^{-16} M) with satisfactory recovery and reproducibility, as well as to determine the genotype of single-base mismatches with a discrimination factor of 1.47–2.63. The high sensitivity and selectivity, as well as the ease of fabrication, operational convenience, short analysis time, stability and reusability, make it a promising candidate for the non-invasive and point-of-care early diagnosis of HIV.

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