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Multiplex electrochemiluminescence DNA sensor for determination of hepatitis B virus and hepatitis C virus based on multicolor quantum dots and Au nanoparticles

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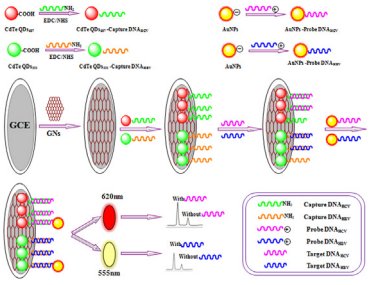
HIGHLIGHTS

- A novel electrochemiluminescence DNA sensor has been developed for the determination of target DNA_{HBV} and target DNA_{HCV}.
- The DNA sensor shows good sensitivity, reproducibility and stability.
- The ECL provided a convenient, low-cost, sensitive, and specific method for target DNA_{HBV} and target DNA_{HCV} determination.
- Target DNA_{HBV} and target DNA_{HCV} in human serum samples were detected with good accuracy and precision.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, a novel multiplex electrochemiluminescence (ECL) DNA sensor has been developed for determination of hepatitis B virus (HBV) and hepatitis C virus (HCV) based on multicolor CdTe quantum dots (CdTe QDs) and Au nanoparticles (Au NPs). The electrochemically synthesized graphene nanosheets (GNs) were selected as conducting bridge to anchor CdTe QDs_{551}-capture DNA_{HBV} and CdTe QDs_{607}-capture DNA_{HCV} on the glassy carbon electrode (GCE). Then, different concentrations of target DNA_{HBV} and target DNA_{HCV} were introduced to hybrid with complementary CdTe QDs-capture DNA. Au NPs-probe DNA_{HBV} and Au NPs-probe DNA_{HCV} were modified to the above composite film via hybrid with the unreacted complementary CdTe QDs-capture DNA. Au NPs could quench the electrochemiluminescence (ECL) intensity of CdTe QDs due to the inner filter effect. Therefore, the determination of target DNA_{HBV} and target DNA_{HCV} could be achieved by monitoring the ECL DNA sensor based on Au NPs-probe DNA/target DNA/CdTe QDs-capture DNA/GNs/GCE composite film. Under the optimum conditions, the ECL intensity of CdTe QDs_{551} and CdTe QDs_{607} and the concentration of target DNA_{HBV} and target DNA_{HCV} have good linear relationship in the range of 0.0005–0.5 nmol L^{-1} and 0.001–1.0 nmol L^{-1} respectively, and the limit of detection were 0.082 pmol L^{-1} and 0.34 pmol L^{-1} respectively (S/N = 3). The DNA sensor showed good sensitivity, selectivity, reproducibility and acceptable

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stability. The proposed DNA sensor has been employed for the determination of target DNA_{HBV} and target DNA_{HCV} in human serum samples with satisfactory results.

1. Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are single-stranded virus. HBV and HCV infection induce a number of hepatic diseases, Which including chronic hepatitis, hepatocirrhosis and liver cancer [1]. In spite of its seriousness, no effective therapy is currently available for HBV and HCV. An epidemiological survey has shown that at least 800 million and 170 million individuals all over the world have been infected with HBV and HCV respectively [2,3]. Therefore, it is urgent to develop simple, rapid and reliable methods for the determination of HBV and HCV. Up to now, a large amount of methods for the determination of DNA_{HBV} and DNA_{HCV} have been reported including high performance liquid chromatography [4], real-time polymerase chain reaction (PCR) [5,6], electrochemical detection [7–9] and mass spectrometric analysis [10]. However, some of these methods are complicated, expensive and time consuming for DNA_{HBV} and DNA_{HCV} diagnosis. In addition, compared to parallel single detection, multiplex analyses enable the detection of two or more target DNA in a single analyze with remarkable advantages, including high assay efficiency, low sample requirement, shorter assay time and low expenditure [11,12]. Thus, there is an intense demand for simple and rapid DNA sensor for determination of target DNA_{HBV} and target DNA_{HCV} with high sensitivity and selectivity.

Electrochemiluminescence (or electrogenerated chemiluminescence, ECL) is a versatile analysis method that combines the simplicity of electrochemistry with inherent sensitivity and wide linear ranges of chemiluminescence method. Most importantly, ECL DNA sensor have a lot of advantages, including high sensitivity, low backgound, easy to control and simple equipments [13,14]. Quantum dots (QDs), a popular nanostructured material with numerous advantageous features such as broad excitation spectra for multicolor imaging, high quantum yield, robust and narrowband emissions, good chemical stability and feasibility for surface modification, have been widely used as ECL labels for DNA assay [15–18]. Furthermore, all colors of QDs can be excited by a single excitation source, that makes they become an ideal candidate than the traditional luminophor to realize multiplex detection [19]. Graphene, a “rising star” material, is a single layer of carbon atoms in a densely packed honeycomb two-dimensional lattice. It has recently attracted enormous attention in constructing DNA sensor due to its novel properties such as high carrier mobility, excellent biocompatibility, large specific surface area, good mechanical strength, zero band gap and outstanding electrical conductivity [20,21]. It has been proved that the electrochemical reduction of the exfoliated graphene oxide (GO) is a green and facile way to synthesize large-scale graphene film in high quality [22,23]. Herein, the electrochemically reduced graphene nanosheets (GNs) were selected as a supporting material to anchor dual-color CdTe QDs, which exhibited a significantly amplified ECL signal of CdTe QDs [24–26]. Gold nanoparticles (Au NPs) have been widely used because of the excellent properties, such as high biocompatibility, distinctive size-related electronic and optical behavior, good conductivity and the availability of versatile bioconjugation means [27–29]. Hence, Au NPs are suitable potential candidate nanomaterials for the sensitive electrical transduction of different biomolecular recognition events [30]. Au NPs can quench the ECL intensity of CdTe QDs due to the inner filter effect. The inner filter effect for AuNPs to quench CdTe QDs was due to AuNPs could be incorporated into the CdTe QDs. The ECL signal of CdTe QDs was largely decreased by the addition of AuNPs, due to the efficient charge transfer between CdTe QDs and AuNPs [31,32].

In this paper, we report a new and simple method for the fabrication of ECL DNA sensor by using Au NPs-probe DNA/target DNA/CdTe QDs-capture DNA/GNs/GCE composite film. Scheme 1 described the preparation of composite film and the mechanism of ECL system for the detection of target DNAHBV and target DNAHCV. GNs connect CdTe QDs_{551}-capture DNA_{HBV} and CdTe QDs_{607}-capture DNA_{HCV} onto GCE to enhance the ECL intensity of CdTe QDs. After the addition of target DNA, some capture DNA on the surface of CdTe QDs hybridized with complementary target DNA and only the unreacted capture DNA could hybridize with complementary Au NPs-probe DNA. Au NPs can quench the ECL intensity of CdTe QDs due to the inner filter effect. So, the more target DNA added, the less unreacted capture DNA on the surface of CdTe QDs left, and the less complementary Au NPs-probe DNA hybridized with capture DNA, thus the higher ECL signals emitted. So, the ECL signals of dual-color CdTe QDs increased with the increase of the concentration of target DNA_{HBV} and target DNA_{HCV} added. The major scientific merit and novelty of this DNA sensor are: (i) GNs not only displays excellent adsorption ability and good adhesion to load the dual-color CdTe QDs onto the GCE surfaces, but also promote the electron transfer and enhance the ECL intensity of CdTe QDs. (ii) The ECL DNA sensor provided a convenient, low-cost, sensitive, and specific method for target DNA_{HBV} and target DNA_{HCV} determination. (iii) To the best of our knowledge, this is the first report of the detection of target DNA_{HBV} and target DNA_{HCV} with the ECL DNA sensor. (iv) The ECL DNA sensor has great potential to expand its application to the early diagnosis of liver cancer and even can be used to detect other pathogenic DNA by using different capture DNA, probe DNA and target DNA. The proposed Au NPs-probe DNA/target DNA/CdTe QDs-capture DNA/GNs/GCE system is a novel ECL DNA sensor with good selectivity and stability. And it was successfully applied to determination of target DNA_{HBV} and target DNA_{HCV} in samples of human serum with good accuracy and precision.

2. Experiment section

2.1. Materials and chemicals

All chemicals and reagents were analytical grade and used directly without further purification. All synthetic oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The sequences of oligonucleotides were listed in Table 1 [33,34]. 3-Mercaptopropyl acid (MPA), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (NHS) were purchased from J&K Chemical. Tellurium powder (~200 mesh, 99.8%), $CdCl_2$ (99%) and $NaBH_4$ (99%) were purchased from Aldrich Chemical Co. $HAuCl_4$ was purchased from Acros Organics. Bovine serum albumin (BSA) and other materials were purchased from Beijing Dingguo Biotechnology Co. Ltd. Phosphate buffer solution (0.1 mol L^{-1} NaH_2PO_4–Na_2HPO_4–Na_3PO_4; PBS) containing 0.1 mol L^{-1} KCl and 0.08 mol L^{-1} $K_2S_2O_8$ (pH 7.4) as coreactant was