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An efficient electrochemiluminescence amplification strategy *via* bis-co-reaction accelerator for sensitive detection of laminin to monitor overnutrition associated liver damage

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ABSTRACT

With the world wildly improvement in dietary and nutrition status, it couldn't be ignored that the chronic liver disease (CLD) resulted from the overnutrition. In order to estimate nutrition status for healthy living, an efficient and sensitive electrochemiluminescence (ECL) sandwich immunosensor of laminin (LN), a marker of CLD, was proposed for early diagnosis of CLD. In this work, the anodic ECL behavior of perylene derivative using H_2O_2 as co-reactant was demonstrated and the possible ECL mechanism was proposed. Furthermore, a significantly amplified ECL response could be obtained *via* Ag and Fe-Fe_2O_3 nanoparticles as bis-co-reaction accelerator. As a result, the proposed ECL immunosensor performed good sensitivity and accuracy with a detection limit down to 0.03 pg/mL. Moreover, this immunosensor was successfully employed to monitor patient serum, which exhibited an alternative avenue for the early diagnosis of other diseases *via* proteins, nucleotide sequence, microRNA and cells.

1. Introduction

With the great improvement in dietary and nutrition status in the last decades (WHO and FAO, 2003), the morbidity of chronic liver disease (CLD) caused by overnutrition cannot be ignored (Tsukita et al., 2012; Vilstrup et al., 2014). It's worth pointing out that early liver damage could recover though the diet adjustment and drug assistance. Therefore, early detection and assessment of CLD presents a practical significance on human healthy living (Liu et al., 2013). On the basis of the CLD research, human laminin (LN) can be used as available serum marker of CLD (Körner et al., 1996). Thus, a sensitive and simple LN detection method is a significant way for early treatment of liver diseases. Recently, electrochemiluminescence (ECL) has become a fascinating analytical technique owning to the merits of low background noise, high sensitivity, low detection limit and simple operations (Richter, 2004; Valenti et al., 2015; Hu and Xu, 2010), which is an alternative strategy for trace LN detection. Lately, the ECL emitter of perylene and its derivatives with outstanding electro-photoactive properties and excellent functional flexibility (Liu et al., 2015; Krieg and Rybtchinski, 2011; Zhan et al., 2015), is a rising star in ECL assays. Due to the structure of perylene ring, the earlier research on the ECL of perylene derivatives showed the dependence of organic solvents (Williams and Murrat, 1998; Werner et al., 1970), which would restrict its further application in bio-detection of ECL sensor. In later research, our group had elaborated the ECL of perylene derivatives in aqueous solutions by decorating with hydrophilic groups to improve the water-solubility (Lei et al., 2015, 2016; Zhao et al., 2016), which could improve the biocompatibility of perylene derivatives. Nevertheless, almost all the perylene-based research of ECL analytical applications had depended on cathode potential solely, the anodic ECL behavior of perylene has never been uncovered in current research report, to date.

It was generally known that a high-efficiency signal amplification strategy was the critical process to improve the sensitivity of a "signal-on" sensor for disease diagnosis and treatment (Liu et al., 2010; Fang et al., 2015). Currently, nucleic acid amplification strategy was the mainstream signal amplification method for the improvement of the high efficiency and specificity (Du and Dong, 2016; Shuai et al., 2016a; Huang et al., 2016), such as hybridization chain reaction (HCR) (Shuai et al., 2017), catalyzed hairpin assembly (CHA) (Shuai et al., 2016b) and polymerase chain reaction (PCR) (Shen et al., 2012). Nevertheless, it would encounter the problems of complex operations and high cost of DNA as well as complicated experimental design. Lately, our groups have proposed co-reaction accelerator strategy for signal amplification, which could be alternative signal amplification method due to its low

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cost, high efficiency, good stability, simple operation and short operating time. Specifically, semicarbazide (Ma et al., 2015) or PTC-lysine (Yu et al., 2016) were used as co-reaction accelerator to promote the ECL reaction rate between luminophor and its co-reactant of peroxydisulfate ($S_2O_8^{2-}$) for signal amplification, respectively. And then, Lei and co-workers used aniline as a co-reaction accelerator to amplify the initial "signal-on" state signal response in luminophor/$S_2O_8^{2-}$ system (Lei et al., 2016). Especially, co-reaction accelerator could react with co-reactant instead of luminophor, which would improve the ECL reaction rate and further enhance the ECL response. Accordingly, in this work, the *in situ* obtained Ag nanoparticles (Ag NPs) and Au nanoparticles covered Fe-Fe_2O_3 (Au@Fe-Fe_2O_3) were employed as bis-*co*-reaction accelerator to acquire signal amplification.

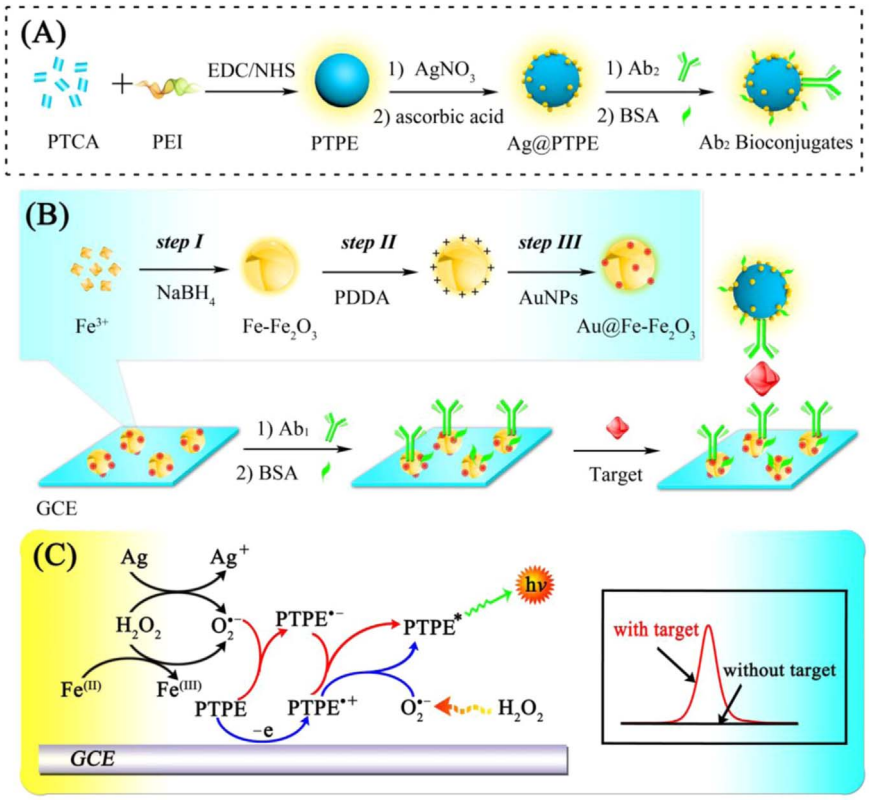
Herein, nano-sphere of perylene derivatives, was obtained by covalently crosslinked PTCA with polyethyleneimine (PEI) (abbreviated as PTPE), and then its anodic ECL behavior in aqueous solution was observed. It's worth stressing that the anodic ECL emission of PTPE exhibited higher ECL emission efficiency than that of the cathodic ECL emission of other perylene derivatives (Lei et al., 2015, 2016; Zhao et al., 2016). In this present study, a sandwich ECL "signal-on" pattern for LN detection was designed based on an ECL system with PTPE as anodic ECL emitters and H_2O_2 as co-reactant (Scheme 1). Although this work only used ordinary sandwiched model, the results exhibited wide response range and low detection limit, compared with other ECL immunosensors for LN detection (Wu et al., 2017; Jiang et al., 2016), which could be attributed to the introduce of bis-*co*-reaction accelerator signal amplification strategy. Totally, the sensing interface was established by immobilizing primary antibodies (Ab_1) on the glassy carbon electrode which decorated with Au@Fe-Fe_2O_3, using the Ag@PTPE complex labeled LN second antibodies (Ab_2) as signal probes. More importantly, the success in the establishment of this ECL assay for the detection of LN revealed an alternative avenue for early disease diagnosis with higher efficiency which without any expensive setup and tedious processes.

2. Experiment

2.1. Reagents and material

Perylene-3, 4, 9, 10-tetracarboxylic dianhydride (PTCDA) was acquired from Liaoning LianGang Pigment and Dyestuff Chemicals Co. Ltd. (Liaoning, China). Poly-(diallyldimethylammonium chloride) (PDDA), L-ascorbic acid (AA), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimidehydrochloride (EDC), N-hydroxysuccinimide (NHS), L-cysteine, hydrogen peroxide, (H_2O_2, 30%), sodium borohydride ($NaBH_4$), bovine serum albumin (BSA, 95–99%), potassium ferricyanide [$K_4Fe(CN)_6$] and gold chloride tetrahydrate ($HAuCl_4{\cdot}4H_2O$, 99.9%) were brought from Sigma-Aldrich Co. (St. Louis., MO., USA). Superoxide Dismutase (SOD) was supplied by Bailingwei technology Co. Ltd. (Beijing, China). Ferric chloride ($FeCl_3{\cdot}6H_2O$) was obtained from Qiangshun Chemical Reagent Co. Ltd. (Shanghai, China). Silver nitrate ($AgNO_3$) was supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Polyethylenimine (ethylenediamine branched, average Mn ~600 by GPC and average Mw ~800 by LS, PEI) was purchased from Fluka Co. (Buchs, Switzerland). Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were of analytical reagent grade and bought form Chemical Reagent Co. (Chongqing, China). Human Laminin (LN) ELISA Kit was provided by HuaYi Bio-lab Co. Ltd (Shanghai, China). Collagen type IV (Col IV) antigen was purchased by Shanghai HuaYi Bio-technology Co., Ltd. (Shanghai, China). Carcinoembryonic (CEA) and α-1-fetoprotein (AFP) antigen were provided by Biocell Company (Zhengzhou, China).

The deionized water (18.2 MΩ/cm) was used in this experiment, which was controlled by ultrapure water system. Phosphate buffer solutions (PBS, pH = 8) were used as detection solution containing 0.1 M K_2HPO_4, 0.1 M NaH_2PO_4 and 0.1 M KCl (All PBS are air-saturated unless otherwise specified). The AuNPs was prepared by citrate reduction of $HAuCl_4{\cdot}4H_2O$ according to the classic procedure (Enustun and Turkevich, 1963).



Scheme 1. (A) The preparation of Ab_2-BSA-Ag@PTPE bioconjugate. (B) The preparation of Au@Fe-Fe_2O_3. (C) Reaction mechanism of ECL immunosensor.