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Ultrasensitive electrochemiluminescence immunosensor for 5-hydroxymethylcytosine detection based on Fe_3O_4@SiO_2 nanoparticles and PAMAM dendrimers

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ABSTRACT

An ultrasensitive sandwiched electrochemiluminescence (ECL) immunosensor was developed for 5-hydroxymethylcytosine (5hmC) detection in genomic DNA by using Fe_3O_4@SiO_2 core–shell magnetic nanomaterial as a immobilization matrix for anti-5hmC antibody, PAMAM conjugated avidin and $Ru(bpy)_2(phen\text{-}5\text{-}NH_2)(PF_6)_2$ as signal amplification unit. Importantly, Fe_3O_4@SiO_2 nanoparticles were verified to not only possess enormous surface for loading antibody by amido link, but also exhibit excellent bioactivity. With the dual signal amplification strategy, the ECL immunosensor showed wide detection range from 0.1 to 30 nM with low detection limit of 0.047 nM (S/N = 3). Based on the specific immunoreaction, the developed method also illustrated excellent detection selectivity. The fabricated immunosensor was also applied to detect the 5hmC in genomic DNA of cancer tissue, which indicated that the immunosensor possess potential applications in clinical detection.

1. Introduction

DNA hydroxymethylation, one of the most notable parts of epigenetics, has been attracted enormous attention owing to its relationship with a variety of diseases (Tan and Shi, 2012). In addition, some researchers have demonstrated that 5-hydroxymethylcytosinewas formed by the oxidation of ten-eleven translocation (TET) proteins to 5-methylcytosine (5mC) (Tahiliani et al., 2009). Up to now, many works of DNA hydroxymethylation detection have been constructed, such as liquid chromatography–mass spectrometry (LC–MS) (Tang et al., 2013), immunofluorescence microscopy (Haffner et al., 2011), photoelectrochemical method (Yang et al., 2016), UV detection (Liutkevičiūtė et al., 2009), electrochemical method (Cui et al., 2017; Yang et al., 2015) and electrochemiluminescence (ECL) (Ma et al., 2016). Among them, ECL has been widely employed and played a crucial role in biological electroanalytical chemistry, because of its specificity, simple equipment, low cost and high sensitivity (Qi et al., 2013). For instance, Yu et al. presented a strategy for thrombin (TB) detection based on perylene derivative functionalized graphene-CdTe nanocomposites as signal probe (Yu et al., 2016). Xu et al. designed an ECL immunosensor for α-fetal protein detection based on CdZnSe nanocrystals (NCs) (Xin et al., 2016). Sun et al. (2016) developed a method for the discrimination of DNA hydroxymethylation based on gold nanoparticles (AuNPs)/Nafion film and β-glucosyltransferase treatment, after glucosylated, the modified electrodes were treated by MspI endonuclease, leading to an increased signal.

Polyamidoamine (PAMAM) dendrimers, a newfangled kind of macromolecules possessing multiplebrached structure, which functioned with lots of groups at the periphery, such as amine, or carboxylic acid (Sadekar and Ghandehari, 2012). Because of their unique structure, they have been widely applied in some areas, such as biosensor designing (Borisova et al., 2016; Dervisevic et al., 2017; Li et al., 2016). Yuan et al. presented a sandwich-type immunosensor based on dendrimer functionalized reduced grapheme oxide as a signal platform for thrombin detection to acquire an obvious signal enhancements (Yuan et al., 2013). In addition, aminated-terminated PAMAM often used as a coreactant of $Ru(bpy)_3^{2+}$, owning to their plenty of amino groups (Yuan et al., 2014). For example, Xiong et al. (2015) fabricated a solid-state ECL immunosensor for α-fetoprotein detection, where the poly(ethylenimine) and PAMAM severed as co-reactant to $Ru(bpy)_3^{2+}$ to obtain an amplified ECL signal. Meanwhile, carboxyl-terminated PAMAM (G3.5) have amount of carboxyl groups at the periphery, which was benefited to functionalization molecules through hydrogen bonding (Luo et al., 2012). Thus, they were employed to build the biosensor for improving the sensitivity.

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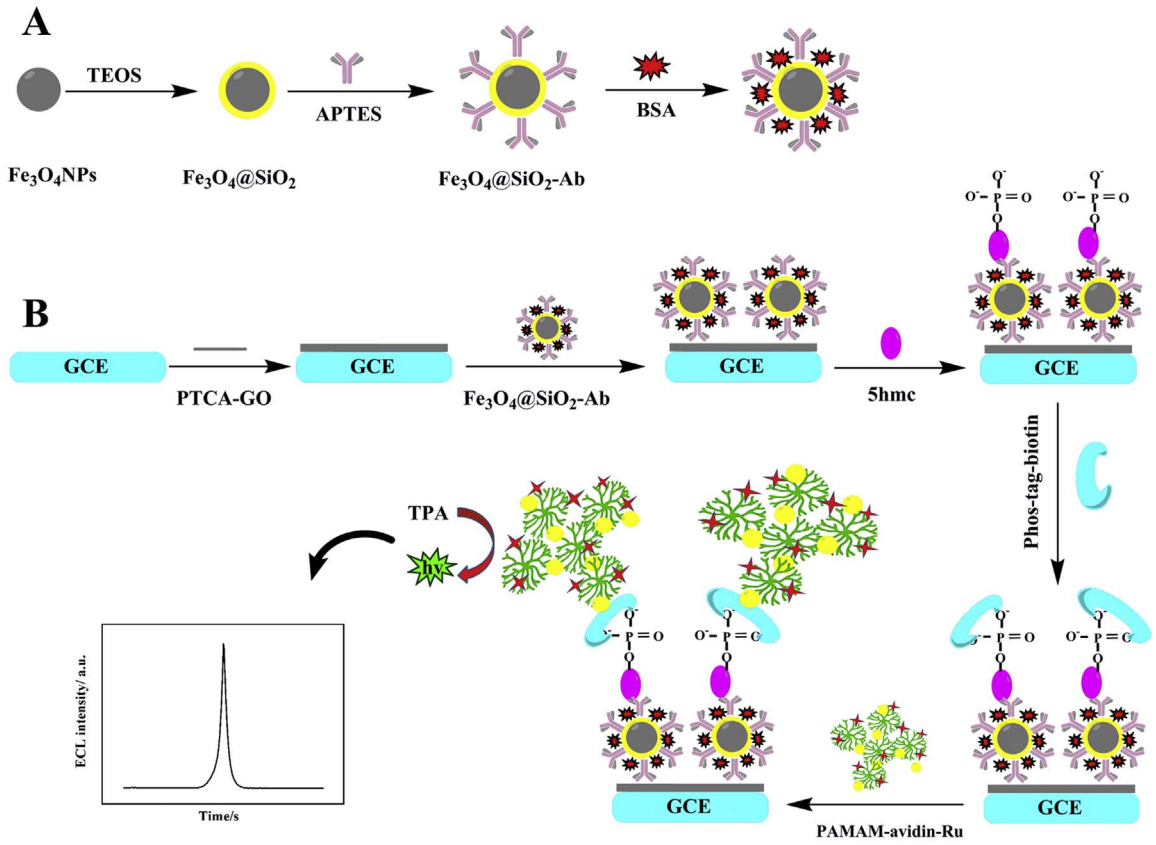

Scheme 1. Schematic diagram of the preparation of Fe_3O_4@SiO_2-Ab (A), and the fabrication procedure of the ECL immunosensor (B).

Silica-coated Fe_3O_4 nanoparticle (Fe_3O_4@SiO_2 NPs), a popular core–shell magnetite nanomaterial, has been shown great potentials in many fields, such as drug delivery (Wu et al., 2010), catalysis (Maleki, 2014) and biosensor (Wang et al., 2014), owning to their excellent separation ability, good biocompatibility and low toxicity (Lu et al., 2002). For example, Feng et al. fabricated an amperometric biosensor for hydrogen peroxide detection based on Fe_3O_4@SiO_2 for the modification of hemin (Zhang et al., 2014). Moreover, because of their specific surface area, lower mass transfer resistance, they have already shown attractive prospects for immobilizing biomolecules (Kouassi and Irudayaraj, 2006). Additionally, the SiO_2 coated shell chemistry is famous, which was convenience for conjugation of various biomolecules (Chang and Tang, 2014). Therefore, it turns out to be an excellent platform for antibody modification with good stability and bioactivity.

In this work, we constructed a new sandwich-type ECL sensor for 5-hydroxymethylcytosine (5hmC) detection in genomic DNA of cancer tissue, where core–shell structure of Fe_3O_4@SiO_2 NPs was employed as platform for antibody immobilization (Scheme 1A), and the activated bioconjugation of Ru-PAMAM-adivin for signal amplification unit. It should be commented that the $Ru(bpy)_2(phen\text{-}5\text{-}NH_2)(PF_6)_2$-TPA system has been applied in bioanalysis (Wei et al., 2012). As illustrated in Scheme 1B, PTCA-GO was assembled on the bare glass carbon electrode, serving as a nanocarrier to capture plenty of Fe_3O_4@SiO_2-Ab via amido bond owning to its specific surface area (Liu et al., 2017). After that, 5hmC was modified on the electrode surface by the specific recognition between 5hmC and anti-5hmC antibody. Subsequently, the ECL signal was produced by the reaction of Ru-PAMAM-adivin towards tripropylamine (TPA), which was connected by Phos-tag-biotion. The advantages are as exhibited: (i) the Fe_3O_4@SiO_2 NPs not only have the great surface that make them easily capture lots of antibodies, but also improve the stability of the immunosensor. (ii) The PAMAM with density of carboxyl groups provide larger space for catching more Ru by the formation of amide link. Inspired by the several amplification factors, the ECL signal of the immunosensor was dramatically enlarged as well as the detection sensitivity. Thus, we present a strategy for signal amplification of 5hmC detection, which has potential application in the early detection of cancer.

2. Experimental

2.1. Reagents and apparatus

Anti-5-hydroxymethylcytosine (5hmC) antibody (Ab) was purchased from Abcam (Cambridge, UK).100 mM 2′-deoxycytidine-5′-triphosphate (dCTP) solution, 100 mM 2′-deoxycytidine-5′-triphosphate (dCTP) solution, 100 mM 2′-deoxyadenosine-5′-triphosphate (dATP) solution, 100 mM 2′-deoxyguanosine-5′-triphosphate (dGTP) solution, 100 mM 2′-deoxythymidine-5′-triphosphate (dTTP) solution and avidin were purchased from Sangon Biotech (Shanghai). Phos-tag-biotin was provided by Wako Pure Chemical Industries, Ltd. (Japan). 5-methyl-2′-deoxycytidine-5′-triphosphate (5-m-dCTP) and 5-hydroxymethyl-2′-deoxycytidine-5′-triphosphate (5-hm-dCTP) were acquired from TriLink BioTechnologies (California, USA). PAMAM dendrimer (ethylenediamine core, generation 3.5 solution) and bis(2,2′-bipyridine)-(5-aminophenanthroline)ruthenium bis(hexafluorophosphate)($Ru(bpy)_2(phen\text{-}5\text{-}NH_2)(PF_6)_2$) were obtained from Sigma-Aldrich (USA). Graphene oxide (GO) was provided by XFNANO (Nanjing, China). Tri-n-propylamine, perylene-3, 4, 9, 10-tetracarboxylic dianhydride (PTCDA), N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride (EDC) were received from Aladdin (Shanghai, China). The stock solutions were as followed. Anti-5-hmC-antibody buffer, 10 mM PBS containing 30% glycerinum (pH = 7.4). Phos-tag-biotin reaction buffer, 10 mM Tris–HCl containing 0.1 M NaCl, 0.1% Tween-20, 0.4 mM $Zn(NO_3)_2$ (pH = 7.0). All oligonucleotides were stored in TE buffer,