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The Ru complex and hollow gold nanoparticles branched-hydrogel as signal probe for construction of electrochemiluminescent aptasensor

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ABSTRACT

In this work, a novel Ru complex and hollow gold nanoparticles branched-poly(N-(3-aminopropyl)methacrylamide) hydrogel composites (pNAMA-Ru-HGNPs) were prepared and used as electrogenerated chemiluminescence (ECL) signal probe to construct aptasensor for ultrasensitive detection of thrombin (TB). Herein, $[Ru(phen)_2(cpaphen)]^{2+}$ linked N-(3-aminopropyl)methacrylamide and hollow gold nanoparticles functionalized N-(3-aminopropyl)methacrylamide were used as two polymer monomers to prepare pNAMA-Ru-HGNPs composites via free-radical polymerization. The obtained hydrogel composite, containing amount of Ru complex and HGNPs, were used as effective tag-carriers for the immobilization of thrombin binding aptamer II (TBA II) to form the pNAMA-Ru-HGNPs labeled TBA II (pNAMA-Ru-HGNPs-TBA II). For building the interface of the aptasensor, dendritic gold nanoparticles reduced by poly(ethyleneimine) (PEI@DGNPs) were modified on the carbon nanotube–nafion (CNTs–Nf) coated electrode through electrostatic adsorption, which was used not only as matrix for immobilization of thrombin binding aptamer I (TBA I) but also as enhancer to amplify the ECL signal because PEI is an efficient co-reactant of Ru complex. Target TB was sandwiched between pNAMA-Ru-HGNPs-TBA II and TBA I, resulting in the ECL signals relevant to the TB concentrations. Combining the novel pNAMA-Ru-HGNPs containing amount of Ru complex as the ECL signal probe and PEI@DGNPs as the enhancer for signal amplification, the sandwich ECL aptasensor was constructed for the detection of TB with a wide range of 1.0 fM to 10 pM and a low detection of 0.54 fM.

1. Introduction

Hydrogels are polymers crosslinked via chemical bonds, ionic interactions, hydrogen bonds, hydrophobic interactions, or physical bonds, which have the structure of three dimensional polymeric networks (Montiel-Herrera et al., 2015; Haraguchi et al., 2005). Because of their high water content and biocompatibility, hydrogels are appealing for biological applications (Choi et al., 2015). However, the mechanical properties of hydrogels make them very weak and brittle, and triggered release or disintegration because of the composition (large proportion of water) and the randomly cross-linked network structure. Recently, nanoparticle–hydrogel composite, consisting of metal nanoparticles embedded in polymer hydrogels, have attracted broad interest, which could overcome the limitations of conventional polymeric hydrogels to impart desirable responsive properties and to improve their stability (Haraguchi and Li, 2006; Jones and Lyon, 2003).

Gold nanoparticles, as one of noble metal nanoparticles, have attracted considerable attention owing to their unusual several remarkable advantages such as extremely large surface area to volume ratio, flexibility in surface functionalities, high porosity and superior mechanical performance and potential applications in optics, catalysts, sensors and biology (Shan et al., 2008; Gong et al., 2015). A number of methods have been developed to incorporate gold nanoparticles and various polymer molecules to realize the potential applications in sensors or microreactors. For example, Zhang et al. (2013) have reported novel gold nanoparticles functional poly(N-isopropylacrylamide) via reversible addition-fragmentation chain transfer polymerization. Boyer et al. (2009) have used the "grafting-to" procedure to design a gold nanoparticles/polymer hybrid displaying a broad range of lower critical solution temperature values. So far as we known, the nanoparticle–polymer composites applied in electrogenerated chemiluminescence (ECL) sensing are relatively rare because examples of electrochemically active polymers are scarce (Balogh et al., 2011; Yuan et al., 2011). Pinaud group have reported an ECL and photoluminescence of thermores-ponsive redox microgels by

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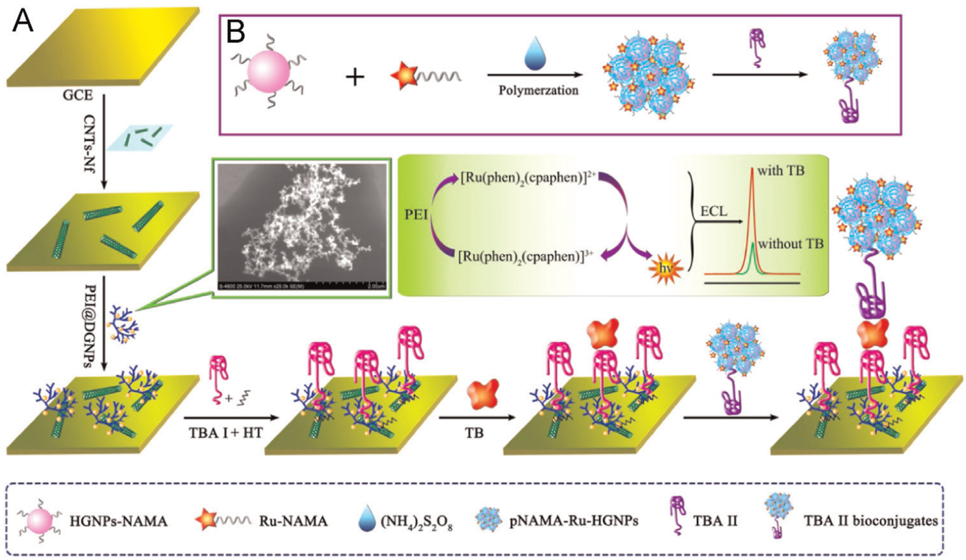

Scheme 1. (A) The schematic diagrams of the ECL aptasensor and signal amplification mechanism. (B) The preparation of TBA II biocojugates.

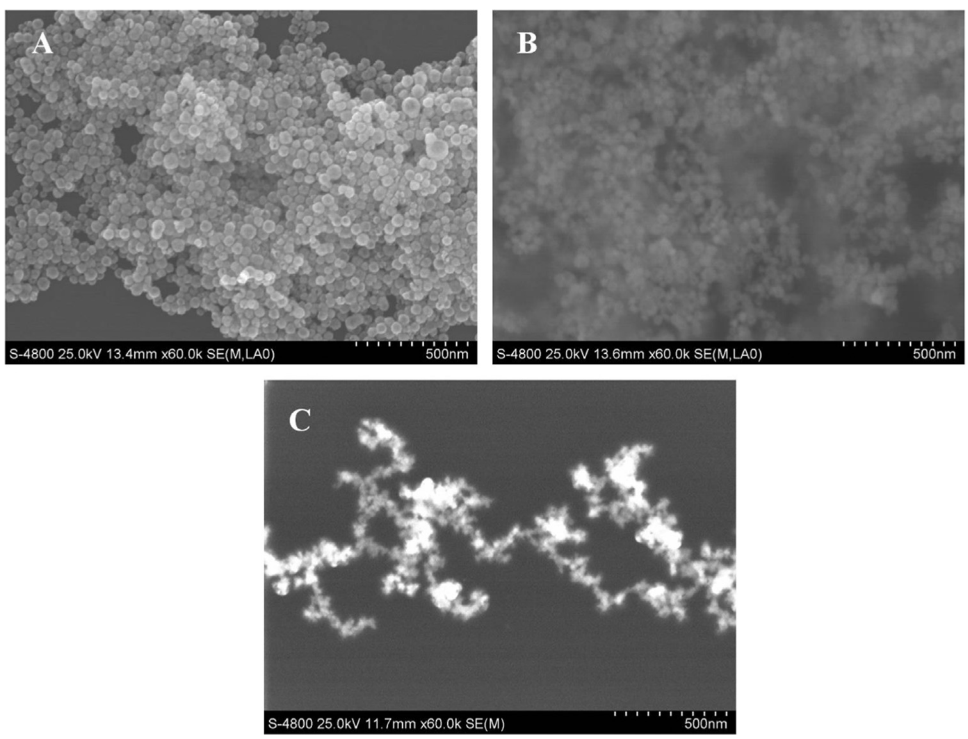

Fig. 1. SEM of HGNPs (A), pNAMA-Ru-HGNPs (B) and PEI@DGNPs (C).

the combination of ECL reagents of Ru(II) with stimuli-responsive poly(N-isopropylacrylamide) polymers (Pinaud et al., 2013). Inspired by this, hollow gold nanospheres (HGNPs), owing to their advantage of their high specific surface (Li et al., 2015; Skrabalak et al., 2008), were chosen to functionalize with the ECL hydrogel polymer side chains, which was also used as signal tag to construct ECL aptasensor.

In this work, HGNPs functioned N-(3-aminopropyl)methacrylamide (NAMA) acted as one polymer monomer and [Ru(phen)$_2$(cpaphen)]$^{2+}$ linked NAMA acted as another polymer monomer. When the two polymer monomers were mixed, a free-radical polymerization was initiated by ammonium persulfate to form the Ru complex and HGNPs branched-poly(N-(3-aminopropyl)methacrylamide) hydrogel composites (pNAMA-Ru-HGNPs). The obtained pNAMA-Ru-HGNPs were used as effective tag-carriers for the immobilization of thrombin binding aptamer II (TBA II) to form TBA II bioconjugate (pNAMA-Ru-HGNPs-TBA II). For building the ECL thrombin (TB) aptasensor, glass carbon electrodes were first immersed in carbon nanotubes–Nafion (CNTs–Nf) homogeneous suspension to obtain CNTs–Nf film, which could make the surface of electrodes carry negative charges. Then, dendritic gold nanoparticles (PEI@DGNPs), reduced by poly(ethyleneimine) (PEI), were fabricated on the CNTs–Nf modified electrode by electrostatic adsorption, which could not only provide attractive candidates for immobilization of thrombin binding aptamer I (TBA I) but also enhance the ECL efficiency (Song et al., 2010; Wang et al., 2015). At the present of target TB, signal probe of pNAMA-Ru-HGNPs-TBA II was linked on the modified electrode via sandwich assay. Here, an ECL aptasensor was constructed based on the functionalized hydrogel composite as ECL signal