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Manufacturing of an electrochemical biosensing platform based on hybrid DNA hydrogel: Taking lung cancer-specific miR-21 as an example

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ABSTRACT

DNA hydrogel garnered increasing attention in the sensing and medical field owing to its native biocompatibility and mechanical stability. While electrochemistry serves as a quantitative and sensitive detection technique, electrochemical DNA hydrogel biosensor is rarely reported. Here, for the first time, we report an electrochemical biosensor based on hybrid DNA hydrogel immobilized on indium tin oxide/polyethylene terephthalate (ITO/PET) electrode for the detection of lung cancer-specific microRNA, miR-21. The biosensor is capable of detecting miR-21 at a concentration as low as 5 nM (1 pmol) and linear read-out from 10 nM to 50 μM. Ferrocene-tagged recognition probes were cross-linked with DNAs grafted on the polyacrylamide backbones to form the hybrid DNA hydrogel, which was further immobilized on 3-(trimethoxysilyl)propyl methacrylate (KH 570) treated ITO electrode. When the recognition probe was hybridized with the target miR-21, the hydrogel dissolved, producing a loss of ferrocene tags and a reduction in current, detected by Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV). The material characteristics of the biosensor were verified using contact angle meter and Energy Dispersive Spectrometer (EDS). This novel biosensor holds great promise in early sensitive clinical diagnosis for a variety of cancer-specific biomarkers due to the flexible sequence design of the recognition probe.

1. Introduction

DNA hydrogel has received a substantial amount of attention as a promising biomaterial for sensor and actuator applications (Cai et al., 2017; Huang et al., 2016; Liu, 2011; Wei et al., 2008), owing to its biocompatibility, large specific surface, high diffusivity of small molecules, and mechanical stability (Huang et al., 2014; Liu et al., 2011). According to the hydrogel composition, DNA hydrogels are categorized into pure and hybrid DNA hydrogel. Pure DNA hydrogel was firstly constructed from entirely branched DNAs through ligase-catalyzed reaction in 2006 (Um et al., 2006). The development of pure DNA hydrogel was limited by poor mechanical properties and high cost due to the massive DNAs required to form the hydrogel. To reduce the amount of DNA and enhance the stability of hydrogel, hydrophilic polymers like polyacrylamide (Lin et al., 2004; Zhu et al., 2014), polypeptide (Chen et al., 2012; Shao et al., 2015), poly(phenylenevinylene) (Tang et al., 2009) and other materials (Cheng et al., 2011; Xu et al., 2010) have been reported to be involved as the backbones, and DNA strands grafted onto these polymers were cross-linked with probes to build the hybrid hydrogel (Huang et al., 2016; Liu et al., 2017; Yang et al., 2008). DNA sequences of proper design can both act as the cross-linkers to build hydrogel and as the recognition probes to realize different stimuli-responses, which offer the hybrid DNA hydrogel a great possibility to be applied to multi-target diagnosis due to the flexible sequence design of DNA strands (Shao et al., 2017).

While colorimetry has been used by the majority of the hybrid DNA hydrogel biosensors for detecting metal ion (Lin et al., 2011; Wei et al., 2015), cocaine (Wei et al., 2015, 2016; Zhu et al., 2010) and adenosine (Wei et al., 2015, 2016; Yang et al., 2008), it suffers from semi-quantification and relatively low sensitivity. Electrochemistry on the other hand has shown great promise since it exhibits numerous advantages such as high sensitivity and selectivity, minimal sample preparation, and simple usage for continuous in situ analysis. Due to these advantages, electrochemical biosensor based on hydrogel constructed with graphene oxide (GO) and fish sperm DNA was developed for nucleic acid detection (Sun et al., 2015). Polyethylenimine (PEI) and DNA probes were adsorbed to the GO/DNA hydrogel electrode by electrostatic attraction for target detection. Once the complementary DNA was hybridized with the DNA probe, the impedance will obviously decrease, showing great potential in sensitive and quantitative biomolecule detection.

MicroRNAs are small, noncoding RNAs (19–25 nucleotides) with regulatory functions and are highly stable compared to RNAs. Rapid and quantitative detection of microRNAs can be applied to early cancer

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diagnostics and provide information on cancer progression (Calin and Croce, 2006; Chen et al., 2008; Esquela-Kerscher and Slack, 2006; Labib et al., 2013). However, the intrinsic properties of microRNAs, such as low abundance, short and highly homologous sequences, make it extremely challenging to develop a rapid, low-cost and sensitive microRNA detection platform. Over the past few decades, extensive research efforts have been made toward rapid and sensitive microRNA detection techniques (Catuogno et al., 2011). Northern blotting is the golden standard but it is time-consuming and limited by low-sensitivity. Other techniques such as the RT-PCR (Cissell and Deo, 2009) and the Surface Enhanced Raman Spectroscopy (SERS) (Pang et al., 2016) are restricted by sophisticated instruments and false positive problems. Electrochemical biosensors are auspicious methods for sensitive and simple detection of microRNA without labeling or amplification.

In this paper, the hybrid DNA hydrogel was immobilized on the silanized ITO electrode surface to develop a novel electrochemical biosensor to detect lung cancer-specific microRNA, miR-21. The hybrid hydrogels were scaffolded with the DNA-grafted linear polyacrylamide (PAA) and the ferrocene-tagged DNAs as the recognition probes. The recognition probes were cross-linked with the DNA strands grafted onto two PAAs with sequences that are complementary to adjacent areas of the recognition probes. The polymer solution transformed into a hydrogel as the hybridization proceeds. To enhance the experimental results, the formation time of the hybrid DNA hydrogel and the immobilization time of hydrogel on the ITO electrode have been optimized. The fabrication process of the electrochemical biosensor was verified using Cyclic Voltammetry (CV), contact angle meter, and Energy Dispersive Spectrometer (EDS). Finally, the developed DNA hydrogel biosensor was applied to detect microRNA of different concentrations utilizing the Differential Pulse Voltammetry (DPV) technique. The stability and selectivity of the developed DNA hydrogel biosensor were further inspected to evaluate the potential commercial application for clinical diagnosis.

2. Experimental

2.1. Materials and apparatus

The DNAs and microRNAs involved in this study were synthesized by Takara (Dalian, PRC) and purified by HPLC (Table 1). Acrylamide, 3-(trimethoxysilyl) propyl methacrylate (KH 570), ammonium persulfate (APS) and N,N,N,N-tetramethylethylenediamine (TEMED) were all analytical grade and purchased from Acros (USA), while other reagents were purchased from Sinopharm Chemical Reagent (Shanghai, PRC). Aqueous solutions were prepared with Milli-Q water (18.2 MΩ•cm resistivity) from a Milli-Q Academic system (Millipore, USA). Tris-EDTA buffer solution (TE, pH 8.0) was purchased from Sigma-Aldrich (USA). Phosphate buffered saline (PBS, 0.0067 M, pH 7.4) was purchased from Thermo Fisher Scientific (USA). Solutions involved in microRNA-related experiments were prepared with DEPC-treated water (Sigma, Switzerland) to minimize the degradation of microRNAs.

Electrochemical measurements were performed with a CHI 660E electrochemical workstation (CH Instruments, USA) and a conventional three-electrode system consisting of an ITO/PET working electrode, a platinum counter electrode, and an Ag/AgCl (3 M KCl) reference electrode (Chenhua, PRC).

Table 1
Sequences of DNAs and MicroRNAs Used in This Work.

Name	Sequence (5′-3′)
Strand-A	acrydite-AAAATGTTGATATAT
Strand-B	acrydite-AAAATATCAGACTGA
DNA probe	ATATATCAACATCAGTCTGATAAGCTA-Fc
hsa-miR-21	UAGCUUAUCAGACUGAUGUUGA
hsa-miR-16	UAGCAGCACGUAAAUAUUGGCG
hsa-miR-122	UGGAGUGUGACAAUGGUGUUUG
hsa-miR-141	UAACACUGUCUGGUAAAGAUGG

2.2. Construction of electrochemical hybrid DNA hydrogel biosensor

An electrochemical biosensor, based on the hybrid DNA hydrogel that utilizes the ferrocene-tagged DNAs as the recognition probes, was designed and constructed as shown in Fig. 1.

Two oligonucleotides modified with acrydite (strand-A, and strand-B) at the 5′-end were separately dissolved in TE buffer solution containing 4% acrylamide and pretreated in vacuum desiccator as stock solutions (3 mM). The oligonucleotide stock solutions were then placed in the vacuum oven at 37 ℃ for 5 min. 0.01 g APS and 5 μL TEMED were separately added in 100 μL deionization water to prepare fresh APS solution and TEMED solution. 1.4 μL APS solution and 2.8 μL TEMED solution were added to both stock solutions and then placed in the vacuum oven at 37 ℃ for 10 min to initiate the polymerization (Fig. S1 in the Supporting Information). Polymer strands A and B (P-A and P-B) were mixed with 3 μL recognition probe tagged with ferrocene (100 mM). The mixed solution was incubated at 60 ℃ for 15 min and then cooled down to 37 ℃. After hybridization, the sequences were cross-linked to form the DNA hydrogel with ferrocene tags trapped inside (Wei et al., 2016; Yang et al., 2008).

The ITO electrodes were ultrasonically cleaned in water and ethanol for 5 min, respectively. Oxygen plasma was utilized to pretreat the electrodes to increase the number of hydroxy groups on ITO surface. Then, the electrodes were immersed in 10% (v/v) KH 570/ethanol solution overnight to produce a silanized ITO electrode. The silanized electrodes were cleansed using ethanol and kept at 120 ℃ for 20 min. The self-assembled DNA hydrogels were immobilized on the prepared ITO electrodes at 37 ℃ for 1 h.

2.3. MicroRNA detection with the electrochemical hybrid DNA hydrogel biosensor

MicroRNA analyte solutions of different concentrations (5 nM – 50 μM) were prepared by diluting microRNA stock solution (100 μM) with DEPC-treated water. The developed hydrogel biosensors were initially incubated in miR-21 analyte solutions for 30 min so that DNA recognition probes could sufficiently hybridize with the target miR-21. The hydrogel biosensors hybridized with the target miR-21 were then rinsed with PBS to remove the physical adsorption molecules and supervised by DPV from 0 to 0.6 V with the pulse period of 0.5 s in PBS. Five pieces of the prepared hydrogel biosensors were utilized for the detection of each microRNA concentration. MicroRNAs with highly homologous sequences like miR-122, miR-16, and miR-141 (50 μM) were used as interfering sequences to investigate the specificity of the developed hydrogel biosensor. A mixture solution containing bovine serum albumin (BSA, 500 μM), glucose (500 μM), lactic acid (500 μM), miR-122 (500 μM), miR-16 (500 μM), miR-141 (500 μM), and miR-21 (50 μM) was used to evaluate the selectivity.

3. Results and discussion

3.1. Preparation of electrochemical hybrid DNA hydrogel biosensor

To further improve the detection results, the self-assembled time of DNA hydrogel was optimized to 9 h (Fig. S2A in the Supporting Information) and the time of immobilization on the silanized ITO electrode surface was optimized to 1 h (Fig. S2B in the Supporting Information). The preparation process of the DNA hydrogel electrode was characterized by CV in PBS at a scan rate of 100 mV/s. Bare and silanized ITO electrode exhibited no redox peak (Fig. 2A, black and red line). However, there was a pair of obvious redox peaks generated from the ferrocene tags (Fig. 2A, blue line), indicating that the hybrid DNA hydrogel was successfully immobilized on the ITO electrode.