Biosensors and Bioelectronics 99 (2018) 564–570

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# Gold nanoparticles superlattices assembly for electrochemical biosensor detection of microRNA-21

Liang Tian, Kun Qian, Jinxu Qi, Qinyao Liu, Chen Yao, Wei Song, Yihong Wang*

*School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, PR China*

ARTICLE INFO

*Keywords:*
Electrochemical biosensor
Breast cancer
AuNPs superlattice
Toluidine blue
MicroRNA-21

ABSTRACT

Gold nanoparticles (AuNPs) superlattice and small molecule dyes such as toluidine blue have remarkable effect on signal amplification. In this report, a label-free and simple electrochemical microRNA biosensor is developed by employing toluidine blue (TB) as a redox indicator and AuNPs superlattice as a support material. Conductive polymer, polypyrrole coated AuNPs was self-assembled to form a superlattice which exhibited the most close-packed type thereby producing the maximum current. The successful immobilization of the single strand RNA (ss-RNA) probe and hybridization with the target microRNA sequence were confirmed by electrochemical cyclic voltammetry (CV) methods as well as differential pulse voltammetry (DPV) technique, which was used to determine the oxidation peak current of TB under optimal condition. TB with efficient signal amplification was applied in microRNA biosensor for the first time. By employing this strategy, microRNA can be detected in a range from 100 aM to 1 nM with a relatively low detection limit of 78 aM. Alongside the outstanding sensitivity and selectivity, this nanobiosensor had great reproducibility and showed a remarkable response in the real sample analysis with serum samples. In conclusion, the proposed electrochemical nanobiosensor could be clinically useful in the early detection of the breast cancer by direct detection of the serum microRNA-21 in real clinical samples without sample preparation, RNA extraction and/or amplification.

## 1. Introduction

Cancer afflicts all communities in a worldwide range and is known as one of the leading causes of death, with breast cancer being one of the most common invasive type in women globally and its early detection is the key to the high potential, easy, inexpensive and most effective therapy. Early detection of cancer biomarkers could timely diagnose specific diseases as well as provide treatment for such before it develops into its later period, thereby increasing the survival rate of patients. In vitro diagnostic (IVD) test is a crucial component of clinical care that performs a diagnostic test on biological samples that have been taken from a living body, such as blood, urine, and tissue (Zhou et al., 2015). Such tests are usually conducted to confirm the presence and determine the level of some diseases in an individual.

MicroRNAs are short single-stranded ribonucleic acid (RNA) molecules of about 19–23 nucleotides in length, which can play important regulatory roles in animals and plants by targeting microRNAs for cleavage or translational repression (Dong et al., 2013). As an important circulating microRNA, microRNA-21 is a robust oncogenic microRNA whose overexpression plays a significant role in the process of carcinogenesis and can be used as a biomarker for diagnosis, staging, progression and prognosis of the breast cancer (Shen et al., 2015). In addition, they are perfectly stable in serum, hence their sampling would be easy and non-invasive. Furthermore, it can also be used as a perfect biomarker for early detection of breast cancer. Due to their intrinsic properties of short length, low abundance and sequence homology among family members, it is difficult to realize sensitive and selective detection with economical use of time and cost. The traditional methods for microRNA analysis are northern blotting (Lagos-Quintana et al., 2001), microarray analysis (Lim et al., 2005) and real-time quantitative polymerase chain reaction (qRT-PCR), but they are very time-consuming, sample-consuming, and semi-quantitative with low sensitivity and throughput.

In recent years, electrochemical biosensor technology has received greater attention by the virtue of its unique detection, analysis methods and the potential applications in clinical diagnostic. Electrochemical nanobiosensors are emerging field of biosensors which combines the advantages of electrochemical biosensing with nanotechnology, thereby generating a new class of low cost, robust, reliable, easy-to-use and ultrasensitive diagnostics (Turner, 2013; Xia et al., 2013; Hou et al., 2015). Nanomaterials hold unique physicochemical properties that offer desirable and unmatched characteristics for chemical and

* Corresponding author.
*E-mail address:* yihongwang@seu.edu.cn (Y. Wang).

http://dx.doi.org/10.1016/j.bios.2017.08.035
Received 29 April 2017; Received in revised form 13 August 2017; Accepted 13 August 2017
Available online 15 August 2017
0956-5663/ 

biological detection such as significant surface area to volume ratio, strong signal intensities, and finely tunable surface chemistries (Miao et al., 2015). To date, many studies based on various nanomaterials, such as carbon nanotubes, silica nanowires, quantum dots (QDs), magnetic nanoparticles, AuNPs, just to mention a few, have been targeted at creating high-sensitivity in vitro diagnostic (IVD) systems for biomarkers (Li et al., 2016c). Due to their excellent conductivity, high surface area, good biocompatibility and catalytic properties, AuNPs are ideal platforms for electrochemical biosensor detection. While the electrodes of most biosensors are constructed by simply dropping nanoparticles onto the electrodes' surface, random pack of the nanoparticles may block many active sites, thereby reduce the number of biomolecules that bind to nanoparticles (Ye et al., 2015). Thus, there is an immediate need for ordered array of nanoparticles and more active sites in specific area of electrode to provide highly sensitive biomarker detection.

Nowadays nanoparticles superlattices have shown a variety of applications in biosensing, catalysis, photonic crystals, nanoelectronics and energy conversion and storage (Paik et al., 2015). Spontaneous organization of inorganic nanoparticles (NPs) into superlattices is of great interest due to the new and advanced collective properties of the assemblies, exhibiting a wide range of stoichiometries and lattice symmetries (Li et al., 2016b). Ordered arrays of NPs demonstrate a tunable structure and property. The patterned nanoparticles are closely packed, hence eliminate the block of many active sites and provide efficient active sites on specific area of the electrode surface. In particular, interparticle interactions in NPs superlattices can lead to new, collective properties, which are significantly different from isolated NPs. More importantly, the NPs superlattices are capped with pristine or polymer, hence exhibit physical properties which are strongly dependent on inorganic core size of the NPs, the packing ratio of NPs and their dimensionality (Li et al., 2015). At a specific size ratio and concentration ratio of nanoparticles, the assembled superlattices become most densely packed which could improve conductivity and also accelerate electronic transmission. The most densely packed superlattices were suitable for use as an electrode surface support material due to their excellent electrical conductivity and large surface area, thereby providing more active sites in combination with capture probes.

Toluidine blue (TB) is an aromatic heterocyclic dye which interacts with nucleic acid sequence and can be employed as hybridization indicator in the design of electrochemical nucleic acid biosensor (Rafiee-Pour et al., 2016). The possible mechanism of TB and microRNA interaction has been established via electrostatic interaction with negatively charged backbone phosphate groups. Then toluidine blue interacted with small groove of single stranded DNA or RNA through hydrophobic effect, and intercalated into large groove of the duplex DNA or RNA through π-π stack, forming hydrogen bonds with DNA or RNA bases (Tavallaie et al., 2014; Peng et al., 2015; Nguyen et al., 2016). The behavior of TB linked to single- and double-stranded oligonucleotides has been studied using both spectroscopy and electrochemistry methods (Azimzadeh et al., 2016). In this work TB was employed to interact with microRNA in microRNA biosensor for the first time. Depending on the binding mode of TB-microRNA complex, either decrease or increase in the voltammetric response after hybridization is reported for quantification of nucleic acid sequences. The main advantage of TB linking mode is that microRNA can be determined without any laborious labeling and operation complexity. Furthermore, with the advantages of molecular structure, TB has better flatness and could form hydrogen bonds easier with RNA skeleton, hence promotes more intercalation into RNA skeleton than previously reported methylene blue (Bai et al., 2014).

Herein, AuNPs superlattices was modified onto the electrode to enlarge the specific surface area and electrical conductivity for the first time. A novel convenient and label-free platform for sensitive detection of microRNA was presented, which is based on TB as a hybridization indicator. Due to the excellent electron transport capability and tunable structures capacity of AuNPs superlattices, the remarkable improvement of the immobilizing amount of probe molecules on the electrode surface and a great enhancement of the electrical signal of the substrate were achieved, after which a label-free electrochemical biosensor for detecting microRNA-21 is prepared.

## 2. Experimental

### 2.1. Chemicals and materials

Synthetic capture probes ss-RNA and microRNA sequences used in this work were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All chemicals were purchased from Sigma Aldrich and used without further purification. The buffer solutions used in this work are as follows. Buffer for ss-RNA immobilization was 10 mM $K_2HPO_4$-citric acid containing 1.0 M NaCl (pH 5.4). Hybridization buffer was prepared with 1.0 SSC (Saline-Sodium Citrate) solution containing 15 mM tri-sodium citrate and 150 mM NaCl, while washing buffer solution was 0.1 SSC. A pH 7.4 phosphate-buffer solution (PBS, 20 mM phosphate buffer + 0.15 M NaCl) was used as the supporting electrolyte for differential pulse voltammetry (DPV) quantification. All solutions were treated with diethyl pyrocarbonate (DEPC) and all sample tubes, microtips and glassware were autoclaved to minimize the effect of RNases on the stability of microRNAs. The oligonucleotide sequences are listed in Table S1. Artificial human serum samples used for the addition and recovery experiments of microRNA-21 were purchased from Sigma Aldrich (St. Louis, MO), and the human serum samples of breast cancer patients and healthy individuals were supplied by the affiliated hospital of Qingdao University. qRT-PCR data were supplied by KeyGEN-BioTECH (Nanjing, China).

### 2.2. Electrochemical apparatus and measurements

Electrochemical experiments were carried out with a CHI660E electrochemical workstation (Shanghai Chenhua Instruments, China). A conventional three-electrode system, consisting of a 3.0-mm diameter glassy carbon electrode (GCE), a saturated calomel electrode (SCE) as reference electrode and a platinum wire counter electrode were used in all electrochemical measurements. The electrochemical techniques are cyclic voltammetry (CV), AC impedance (EIS) and differential pulse voltammetry (DPV). Transmission electron microscopy (TEM) images were obtained from a Hitachi H-800 microscope (Japan), while UV–vis absorption spectrum was analyzed by Hitachi UH4150 UV–vis–NIR Spectrophotometer.

### 2.3. Gold nanoparticles synthesis and characterization

AuNPs were prepared by reducing $HAuCl_4$ with trisodium citrate according to previous reports (Hill et al., 2006; Liu et al., 2006). Briefly, 5 mL of 38.8 mM trisodium citrate was added rapidly into a stirred boiling aqueous solution containing 50 mL of 1 mM $HAuCl_4$ while stirring. The solution turned from clear to black, purple and deep red in sequence within 2 min. The solution was kept boiling with continuous stirring for 15 min, after which it was naturally cooled down to room temperature. The final colloidal solution was stored at 4 °C for further use. The concentration of AuNPs was calculated to be 13 nM using Beer-Lambert Law.

### 2.4. Synthesis of oligomer polypyrrole (ppy)

PPy were prepared according to previous reports (Wen et al., 2013; Mondal et al., 2015). The detail of the procedure is given as Supplementary information.