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Ratiometric fluorescence biosensor based on DNA/miRNA duplex@CdTe QDs and oxidized luminol as a fluorophore for miRNA detection

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ABSTRACT

Breast cancer is the second most diagnosed cancer in women worldwide. Hence, the detection of prognostic and diagnostic biomarkers can improve the patient's quality of life during the course of illness and treatment. Most recently, microRNAs (miRNAs) have been widely studied for non-invasive prediction of prognosis markers in the diagnosis of breast cancer. Herein, we reported a ratiometric fluorescence nanobiosensor for detection of miR-155. The biosensor comprises of 3-mercapropionic acid-coated cadmium telluride (CdTe) quantum dots and oxidized luminol (Lum_{ox}) exhibiting emission peaks at 550 and 440 nm, respectively, under single-wavelength excitation (350 nm). In the presence of miR-155, CdTe QDs were aggregated and their fluorescence was quenched via interacting strongly at their metal centers, Cd, with heteroduplex formed between the DNA probe and miR-155. Then, by addition of blue-emitting Lum_{ox} with their constant fluorescence emission, a ratiometric means of miR-155 detection was developed. The relative fluorescence intensity ration is directly proportional to the concentration of miR-155 between 20.0 and 100.0 pM. The detection limit is 12.0 pM. This novel assay is "light-on" and has been successfully applied for the detection of miRNA in MCF-7 and HEK 293 cell lysates as real samples.

1. Introduction

MicroRNAs (miRNAs) are a large family of non-coding single stranded RNAs (usually19–23 nucleotides) that regulate gene expression at the post-transcriptional level via direct binding to target mRNAs. The roles of miRNAs in disease, particularly in cancer, have made miRNAs attractive tools for diagnosis and therapy. For instance, MiR-155, located in chromosome 21q21, is encoded with B cell integration cluster (BIC) gene, which is up-regulated in different types of human cancers such as breast cancer [1,2]. The miR-155 binds directly to RhoA, a prometastatic gene, and plays a key role in breast cancer metastasis. In another research it has been shown that inflammatory stimulation of breast cancer cells increased the level of miR-155 which then they would bind to SOCS1 [3,4]. Also, it was found that there is an overexpression of miR-155 in invasive tumors but not in noninvasive cancer samples and it was shown that miR-155 is associated with the proliferation, invasion and apoptosis due to its action on caspase 3 [5]. These facts show that there miRNAs could have many potential applications in the diagnosis, prognosis and forecasts in therapeutic [6]. Therefore, accurate and rapid quantification of miRNAs is important. Common methods used for this purpose include microarray, next-generation sequencing, NanoString nCounter and quantitative reverse transcriptase real-time (qRT-PCR); however, each of these methods has its own advantages and limitations [7]. For example, a major limitation of qRT-PCR, which is used as a gold standard method is that it will not generate quantitative results at the limits of its sensitivity [8].

Recently, the use of photoluminescent nanoparticles like semiconductor quantum dots (QDs) as fluorescent probes in biosensing (such as DNA, proteins, peptides, and drugs) and bio-imaging has been very much considered because of their advantages such as size-dependent tunable emission, photostability and broad excitation spectra, in comparison to organic dye. QDs are nanocrystals, which are comprised of a few hundreds to a few millions of atoms, and only a small number of free electrons ($\leq$ 100) [9–12].

In this study for the first time, oxidized luminol (Lum_{ox}) was used as a blue-emitting fluorophore probe in the green-emitting 3-mercaptopropionic acid-capped CdTe QD-based dual-emission ratiometric fluorescence biosensor for miRNA detection. Initially, miR-155 aggregated and quenched the fluorescence of CdTe QDs [13–17] via strong interaction with DNA/miR-155 heteroduplex [18–24] switching the green fluorescence into an "off" state at 550 nm with a short response time. Meanwhile, Lum_{ox} molecules were insensitive to both CdTe QDs and DNA/miR-155 duplex and maintained constant fluorescence intensity at 440 nm, thereby serving as a reference for

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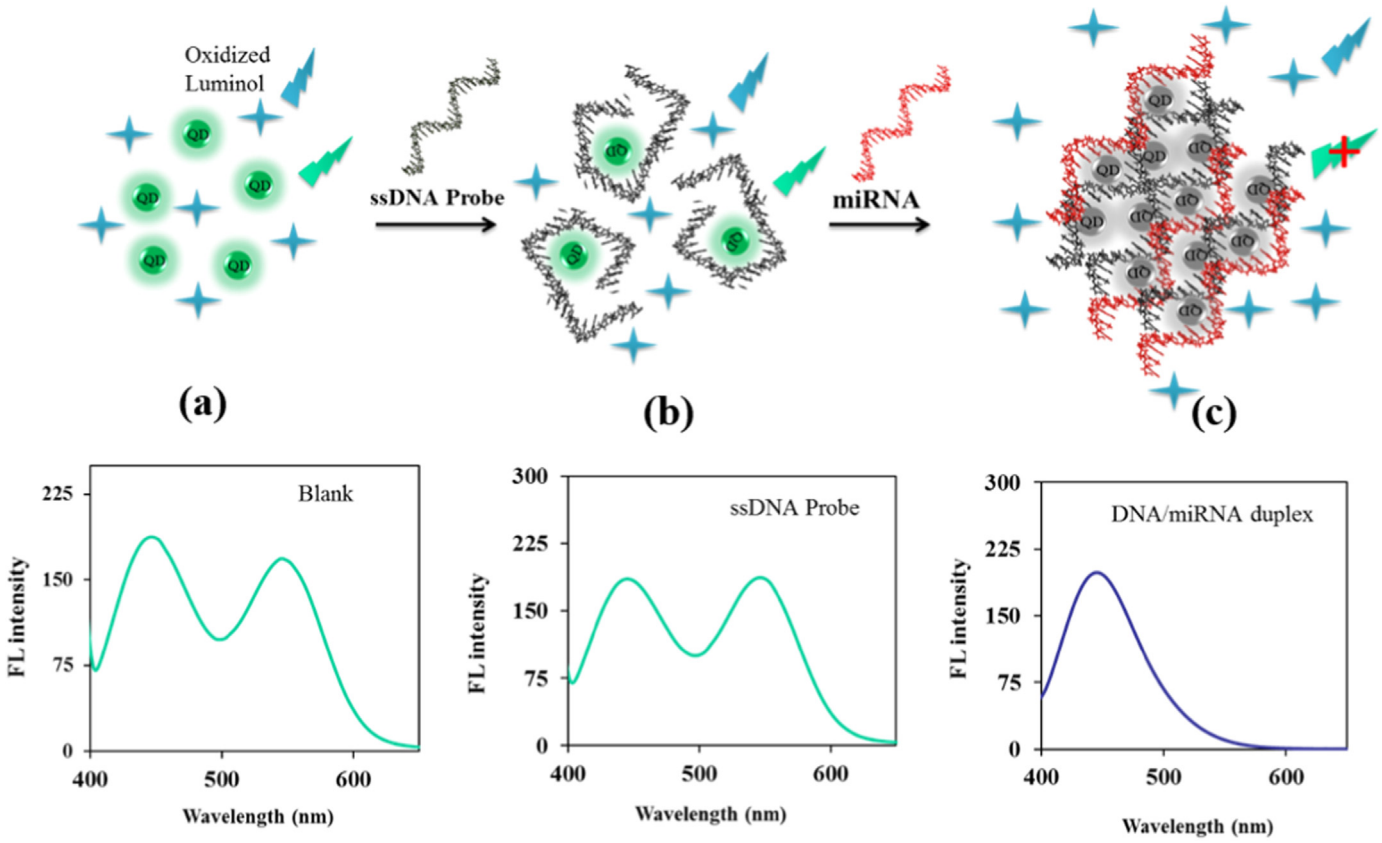

Scheme 1. Schematic representation of the fluorescence behavior of green CdTe QDs (a), DNA probe@CdTe QDs (b) and dsDNA/miR-155@CdTe QDs (c) in the presence of blue-emitting Lum_{ox}.

ratiometric detection of miRNA. On the other hand, in the absence of miR-155, CdTe QDs and Lum_{ox} exhibited emissions peaks at 550 nm and 440 nm, respectively (Scheme 1). This biosensor was successfully used for detection of miR-155 in human breast carcinoma cells (MCF-7) and human normal cells (HEK 293).

2. Experimental

2.1. Apparatus

All fluorescence measurements were carried out using a Perkin Elmer LS-55 fluorescence spectrometer with a xenon lamp as source of excitation while the spectral band widths of monochromators for excitation and emission were 10 nm. (Buckinghamshire,UK). The size and morphology of bare QDs were measured by transmission electron microscopy (TEM) (Zeiss, EM10C, 80 kV, Germany) and atomic force microscopy (AFM) (NT-MDT, Zelenograd, Russia). UV–vis spectroscopy was performed by a Specord 250 spectrophotometer (Analytik Jena, Germany).

2.2. Materials and reagents

Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), and penicillin/streptomycin were purchased from Gibco (USA). Oligonucleotides were synthesized and purified by Shanghai Generay Biotech Co (Shanghai, China), and their sequences were listed in Table.S1. All oligonucleotide samples were purified by PAGE and prepared with TE buffer (1 M Tris-HCl, 0.5 M EDTA). $Cd(NO_3)_2$, tellurium powder, thioglycolic acid (TGA) and sodium borohydride ($NaBH_4$) were purchased from Merck and cell culture lysis reagent (CCLR) was purchased from Sigma Aldrich. All other reagents were of analytical reagent grade and ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout the reactions.

MCF-7 cells (human breast cancer cell line) and HEK 293 cells (from normal human embryonic kidney cell line) were used in this study.

2.3. Preparation of samples for atomic force microscopy (AFM) imaging

AFM imaging was performed on DNA probes and DNA/miR-155 heteroduplex samples deposited on freshly cleaved mica sheets. For this, 1 × 1 cm mica slides soaked in 5 mM $MgCl_2$, and 2 min after, the surface was dried at room temperature. Then, 30 µL of samples in 1 mM $MgCl_2$ were spotted onto mica plates and dried at room temperature. After washing the samples with deionized water and drying, AFM imaging was done on a Solver PRO AFM system (NT-MDT, Russia), in semi-contact (tapping) mode, using Si-gold-coated cantilevers (NT-MDT, Zelenograd, Russia) with a resonance frequency of 375 kHz. Images were recorded in height mode, and Nova image processing software (NT-MDT, Zelenograd, Russia) software was used for data processing and particle analysis.

2.4. Preparation of oxidized luminol

Reduced luminol was prepared by dissolving luminol (10^{-4} M) in to 10 ml of 0.1 M NaOH. In the next step, Blue-emitting luminol (Lum_{ox}) or aminodiphthalate is formed by adding a suitable amount of hydrogen peroxide (H_2O_2, 10^{-4} M).

2.5. Preparation of MAA capped-CdTe Quantum Dots

The experimental procedure was based on previous works [11, 25, 26]. In summary, Cd solution (0.4 mmol) and thioglycolic acid (TGA) (1.4 mmol) were solvated in 80 ml distilled water with pH adjusted to 10.0 using NaOH solution. Next, sodium borohydrate (0.8 mmol) and