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Fluorescent sensor for detection of miR-141 based on target-induced fluorescence enhancement and PicoGreen

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ABSTRACT

Studies have shown that microRNAs affect the development of tumors. In many cases, they can be applied as biomarkers for the diagnosis of cancer; therefore, simple and sensitive analytical methods for detection of miRNAs are necessary. In this study, miR-141, which is used to diagnose several types of cancer, was detected in water and serum samples using a biosensor designed based on streptavidin-coated magnetic beads (SMBs), complementary sequences of miR-141 and PicoGreen as the fluorescent dye. The method is relatively fast and simple. Briefly, in the presence of miR-141, the complementary sequence forms a DNA:RNA double–strand on the surface of SMBs with intercalated PicoGreen. Upon attachment of the PicoGreen, the fluorescence intensity increased significantly (1000-fold). In the absence of a target, only single-stranded DNA (complementary strand of miR-141) existed on the surface of the SMBs. The fluorescence of the PicoGreen was low. The results revealed that the detection limits of the biosensor for miR-141 were 70 and 113.8 nmol L^{-1} in deionized water and serum samples, respectively.

1. Introduction

Colon cancer is the third most common cancer worldwide with 1.23 million people diagnosed and about 607,800 deaths each year [1,2]. It has been shown that early detection of colon cancer could increase the five-year survival rate in 90% of patients. Because colon cancer shows no phenotypic symptoms in early stages, it is often diagnosed late [3–5]. Therefore, there is an essential need for analytical methods that can detect colon cancer in an early stage is essential.

The microRNAs (miRNAs) are composed of 19–25 noncoding short nucleotides. Recently, their importance for cancer cells has been recognized [6–8]. Proper control of miRNA expression is crucial for maintaining a steady state of the cellular machinery [9,10]. It has been established that inappropriate expression of miRNA occurs in cancer and that miRNAs play role in the occurrence, progression and metastasis of cancer. Recent studies have shown that miRNA can be used as a potent biomarker in serum for diagnostic screening [11]. It is hoped that miRNAs can be a promising candidate for early detection of cancer [7]. For example, Zhaohui Huang et al. [12] measured miR-29a and miR-92a in the plasma of patients with colon cancer and showed that these types of miRNA are potential non-invasive biomarkers for early diagnosis of the disease. It has been suggested that tumor suppressor miRNAs or antagonists of miRNAs can be used as effective treatments for cancer [13,14].

The short length of miRNA makes its amplification difficult and many are lost during separation and purification [10,15,16]. One factor that challenges their detection is that they are very similar in their sequences [15–17]. Different detection methods have been used and several commercial products have been developed to measure miRNA, but most of them have disadvantages such as being of low sensitivity, time-consuming and costly [18]. Thus, a standard and reliable analytical method is needed for detection of miRNA to overcome these problems [18].

The fluorescence method is one of the approaches for measurement of miRNAs with high precision and repeatability. PicoGreen is one of the fluorophore dyes which is used in this method [19,20]. PicoGreen

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Table 1
Oligonucleotide sequences used in this study.

	Sequence
Anti-miR-141	5′-C CAT CTT TAC CAG ACA GTG TT-Biotin- 3′
MiR-141	5′-UAA CAC UGU CUG GUA AAG AUG G-3′
MiR-429	5′- UAA UAC UGU CUG GUA AAA CCG U- 3′
Hg aptamer	5′-TTC TTT CTT CCC CTT GTT TGT T-3′
MUC1aptamer	5′-GCA GTT GAT CCT TTG GAT ACC CTG G-3′

in free form does not emit fluorescence, but upon binding to dsDNA increases 1000-fold fluorescence intensity [21]. It is an ultrasensitive fluorescent dye that is used to detect dsDNA [22].

In this study, a simple, inexpensive and fast method was designed to measure miR-141 in water and serum samples that uses magnetic beads and PicoGreen. MiR-141 was chosen as the target because of its occurrence in many cancers such as prostate, colon, and lung cancer. It is a good biomarker for metastatic colon cancer [6]. In this work, streptavidin magnetic beads (SMBs) were used to concentrate and facilitate the washing steps and for miR-141 isolation from buffers. *Anti*-miR-141 was conjugated to the SMBs by a strong biotin-streptavidin bond. The miR-141 first was separated from water and serum samples by the SMBs, followed by addition of the complementary sequence. The miR-141 then was measured using fluorometric method following the addition of PicoGreen.

2. Materials and apparatus

The miR-429, miR-141 and its complementary strand (*anti*-miR-141), miR-200a, Hg aptamer and MUC1 aptamer were purchased from Bioneer (South Korea) (Table 1). Streptavidin magnetic beads were purchased from New England Biolabs (UK). PicoGreen (PG, 200 ×) was ordered from Invitrogen (USA). Acetone was purchased from Merck (Germany). The fluorescence spectrums were recorded using a Synergy H4 microplate reader (BioTeK; USA). The serum was prepared from rat blood.

2.1. Preparation of SMB-anti-miR-141 complex

The SMB-*anti*-miR-141 complex was prepared by mixing 25 µL SMB with 5, 10 or 20 µL *anti*-miR-141 (5 µmol L^{-1}). The TE buffer 1X (pH = 7.4) then was added to adjust the sample volumes to 50 µL. The final concentrations of *anti*-miR-141 in the vials were 0.5, 1 and 2 µmol L^{-1}, respectively. The mixtures were incubated at room temperature for 45 min. The formation of the complex was characterized by 2.5% agarose gel electrophoresis.

2.2. Selection of best concentration of PicoGreen

In order to optimize the concentration of PicoGreen, 40 µL miR-141 (5 µmol L^{-1}) was added to 20 µL SMB-*anti*-miR-141 complex and the volume was adjusted to 90 µL with TE buffer. The mixture was incubated for 1 h at room temperature. After that, the SMB-*anti*-miR-141 complex was separated and washed twice with TE buffer using a strong magnet (DynaMag™). Thereafter, 5, 15 and 20 µL PicoGreen (diluted 100-fold with TE buffer) were added to the complexes and the volumes were adjusted to 90 µL with TE buffer. After 10 min of incubation, the complexes were separated, washed twice with TE buffer and then was diluted with TE buffer. The fluorescence spectra (λ_{Ex} = 485 nm) were recorded at room temperature.

2.3. Quantitative detection of miR-141 using fluorescence analysis

In the first step, 40 µL of the different concentrations of miR-141 (0–2000 nmol L^{-1}) and 30 of µL TE buffer were added to 20 µL SMB-*anti*-miR-141 complexes. After 1 h of incubation at room temperature, the complexes were separated and washed with TE buffer. In the next step, 15 µL of PicoGreen and 75 µL of TE buffer were added to the complexes and incubated for 10 min. In the final step, the complexes were pelleted and washed with TE buffer, then again diluted with 90 µL TE buffer. The fluorescence spectra (λ_{Ex} = 485 nm) were recorded at room temperature.

2.4. Determination of miR-141 in serum samples

MiR-141 was spiked into the serum samples (diluted 100-fold with water). A 3-fold solution of acetone/water (2:1) was added to the diluted serum samples (final concentrations of miR-141 were 0–2000 nmol L^{-1}). Next, the samples were placed at −20 °C for 3 h. After that, the samples were centrifuged at 14500 g for 10 min at 4 °C. About 40 µL of supernatant was measured using the proposed fluorescent method.

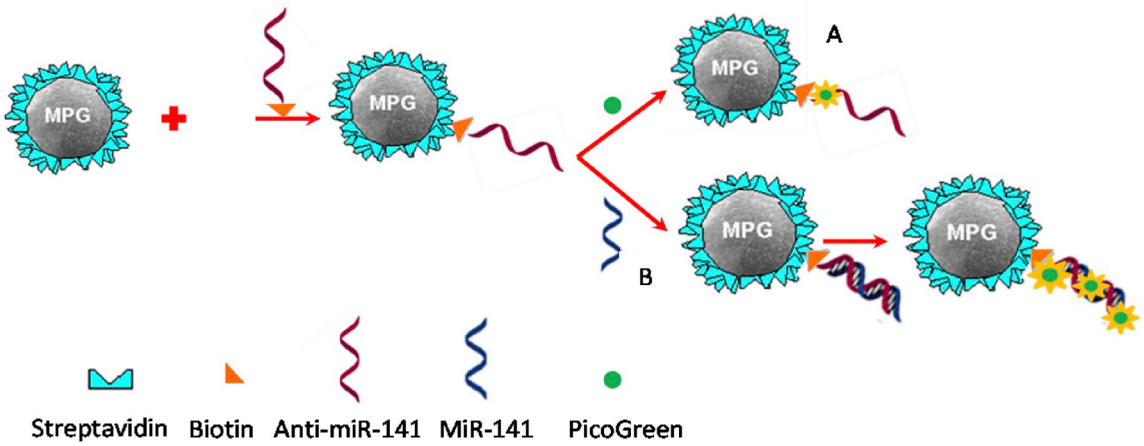
2.5. Selectivity assay

The selectivity was analyzed for 1000 nmol L^{-1} miR-141, miR-429, Hg aptamer and MUC1 aptamer as mentioned before.

3. Results and discussion

3.1. Sensor function

As shown in Scheme 1, the SMBs were bound to *anti*-miR-141



Scheme 1. The schematic shows that in the presence of miR-141, a double-stranded structure formed on the surface of magnetic beads and upon attachment of PicoGreen a strong increase in fluorescence intensity is observed. In the absence of miR-141, a weak fluorescence was recorded.

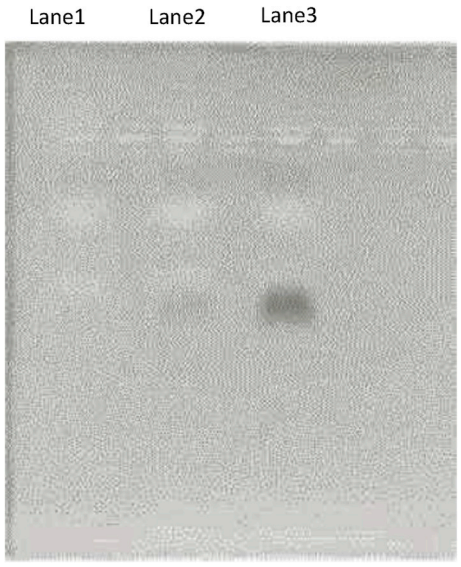


Fig. 1. Agarose gel electrophoresis of SMB-*anti*-miR-141 complexes. Lane 1: SMBs interacting with *anti*-miR-141 (0.5 μmol L^{-1}); Lane 2: SMBs interacting with *anti*-miR-141 (1 μmol L^{-1}); Lane 3: SMBs interacting with *anti*-miR-141 (2 μmol L^{-1}).

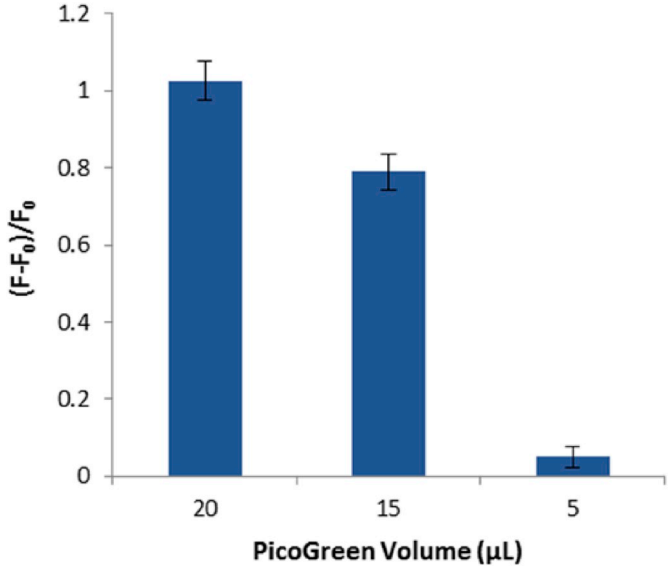


Fig. 2. Relative fluorescence spectra in the presence and absence of miR-141 after addition of 5, 15 and 20 μL PicoGreen (F is fluorescence intensity with a target and F_0 is fluorescence intensity without a target).

through the interaction of *anti*-miR-141 biotin and the streptavidin in the SMBs. The SMBs help to easily separate, concentrate and wash the miR-141 and its complementary sequence from the residual material in the reaction medium. Without the miR-141 sequence, no double strand formed on the SMBs, but a weak fluorescence signal was observed due to the low affinity of the PicoGreen to ssDNA (*anti*-miR-141). With miR-141, the *anti*-miR-141 formed a ssDNA:RNA double strand on the surface of the SMBs. Upon addition of PicoGreen, the fluorophore interacted with the double strand sequence and its fluorescence emission intensity increased significantly.

3.2. SMB-antimir-141 complex characterization

In order to find the best concentrations of SMBs and *anti*-miR-141, three solutions with different concentrations of *anti*-miR-141 were incubated with SMBs. Fig. 1 shows that the sample with a concentration of 1 μmol L^{-1}, no band of free *anti*-miR-141 was observed. This means that there were no free sequences in the sample and all *anti*-miR-141 was connected to the SMBs. Thus, the 1 μmol L^{-1} concentration of *anti*-miR-141 was used for the subsequent experiments.

3.3. Optimum concentration of PicoGreen

Free PicoGreen does not fluoresce; however, when it interacts with DNA, its fluorescence intensity increases up to 1000-fold [21]. The time required for the interaction of PicoGreen was 2–5 min, and the time required is much shorter than the time required for other fluorescent dyes [23]. To determine the best PicoGreen concentration, the SMB-*anti*-miR-141 complex was incubated with miR-141 and subjected to three volumes of 100-fold diluted PicoGreen for 10 min. Fig. 2 shows a large difference in fluorescence between the samples containing 5 and 15 μL PicoGreen, but the fluorescence intensity variation between samples containing 15 and 20 μL PicoGreen was small. Thus, 15 μL PicoGreen was chosen for subsequent works.

3.4. Analytical detection of miR-141 in water

In order to verify the sensor function, the target concentration was measured in deionized water. Several solutions containing different

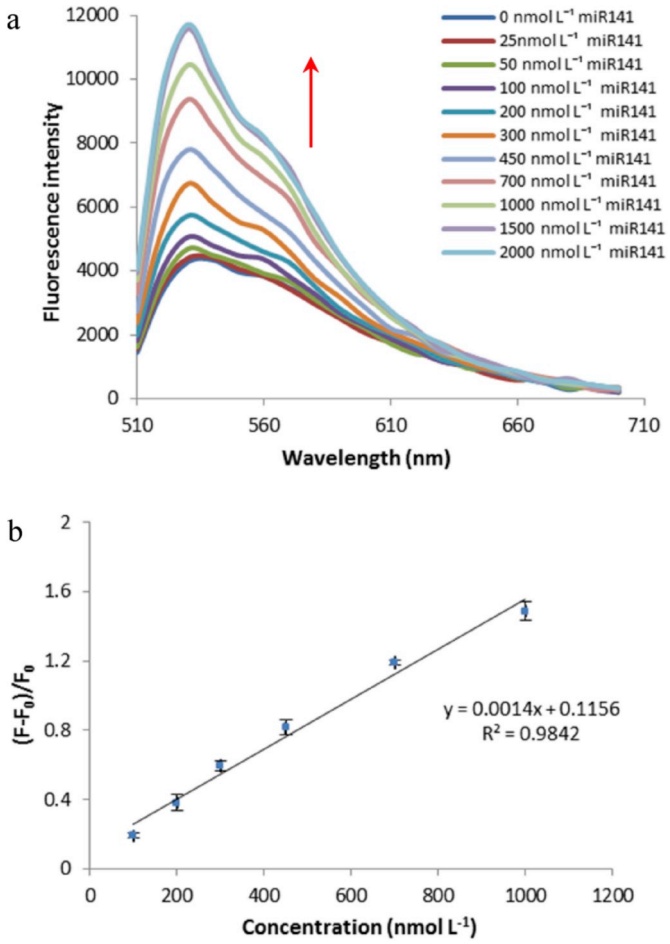


Fig. 3. (a) Increase in fluorescence spectra of SMB-*anti*-miR-141 complexes after addition of different concentrations of miR-141 (0–2000 nmol L^{-1}) in water; (b) miR-141 standard curve in water (F is fluorescence intensity with a target and F_0 is fluorescence intensity without a target).

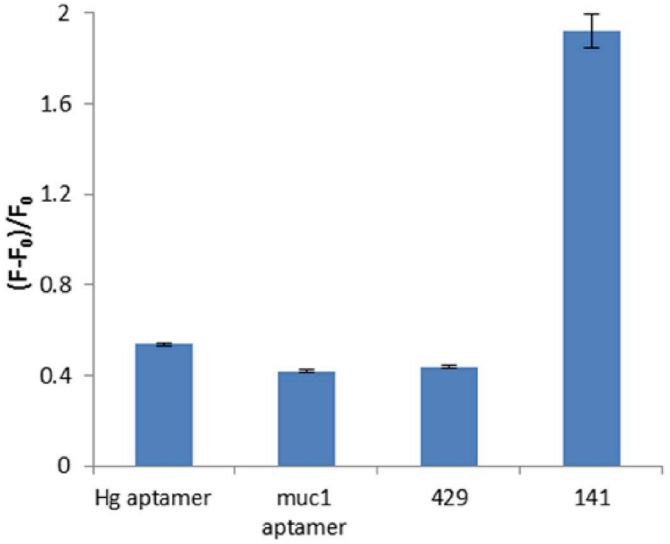


Fig. 4. Relative fluorescence intensity of the analytical sensor with 1000 nmol L^{-1} miR-141, miR-429, Hg aptamer and MUC1 aptamer (F is fluorescence intensity with a target and F_0 is fluorescence intensity without a target).

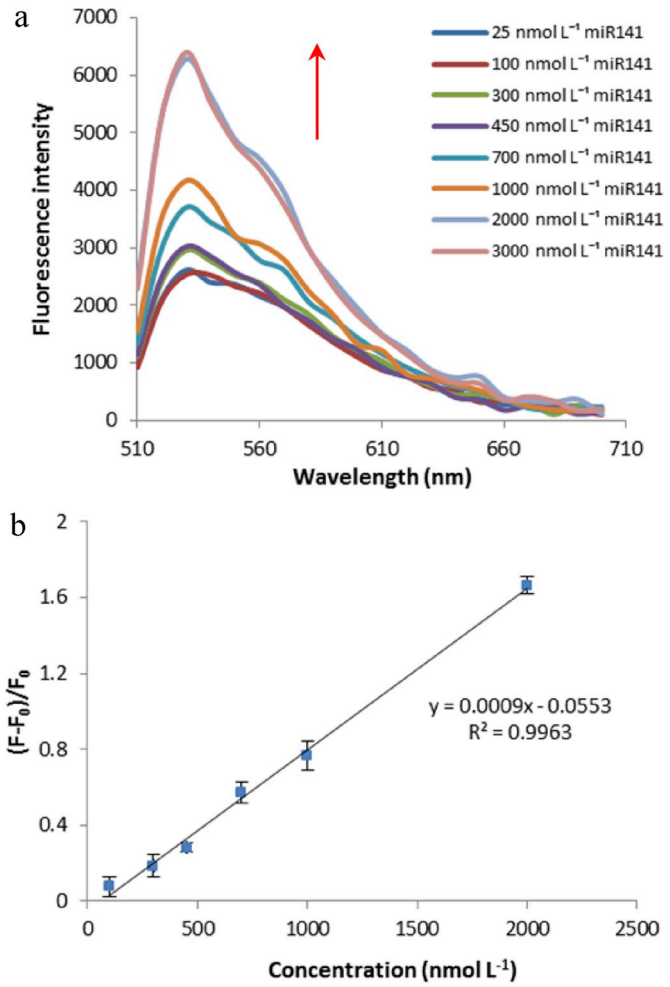


Fig. 5. (a) Fluorescence spectra of SMB-*anti*-miR-141 complexes after addition of different concentrations of miR-141 (0–3000 nmol L^{-1}) in serum; (b) miR-141 standard curve in serum (F is fluorescence intensity with a target and F_0 is fluorescence intensity without a target).

Table 2
Recovery of miR-141 from serum samples (n = 3). Data are mean ± standard deviation (SD).

Added miR-141 (nmol L^{-1})	Found (nmol L^{-1})	Recovery (%)	RSD (%, n = 3)
300	342	114	7.16
700	624	89.14	2.72
1000	1125	112.5	8.25

concentrations of miR-141 were prepared. Fig. 3(a) shows an increase in fluorescence emission intensity with an increase in the concentration of the target. When plotting the $(F–F_0)/F_0$ ratio against miR-141concentration (70–1000 nmol L^{-1}) (where F is the fluorescence intensity with a target and F_0 is the fluorescence intensity without a target), a calibration plot was obtained (Fig. 3(b)). The detection limit was calculated by dividing the three-fold standard deviation of the blank response by the slope to obtain a result of 70 nmol L^{-1}.

3.5. Specificity performance of sensor

One of the most important properties of a biosensor is its selectivity, especially in the presence of similar examples. The miR-249 sequence is similar to miR-141, only four nucleotides are different. Two sequences of mercury aptamer having the same length and MUC1 aptamer having a similar length were used. The results (Fig. 4) indicate that the difference in relative fluorescence between miR-141 and other sequences was meaningful and the sensor showed good specificity to miR-141.

3.6. Detection of miR-141 in serum

One valuable characteristic of an analytical method is its ability when used on complicated biological samples; thus, the sensor was used to measure miR-141 in the serum samples (Fig. 5(a)). Acetone was used to remove protein from the serum samples. Using the method described in the sensor function, the fluorescence intensities of the samples were recorded. The detection limit was 113.8 nmol L^{-1} with a linear range of 113–2000 nmol L^{-1} (Fig. 5(b)).

3.7. Recovery assay and accuracy

In order to verify the accuracy and reliability of the sensor, the recovery assay was used for serum containing different concentrations of miR-141 Table 2 shows the results of recovery assay was from 89.14% to 114% and RSD was from 2.72% to 8.25%. The small standard deviation values and good recovery verifies that the proposed method can be used to detect miR-141 in serum.

4. Conclusion

A fluorescent sensor was fabricated for detection of miR-141 using streptavidin magnetic beads, their complementary strand and the fluorescence emission of PicoGreen. The proposed fluorescent sensor recognized the miR-141 in the water and serum samples with LODs of 70 and 113.8 nmol L^{-1}, respectively. Overall, the proposed sensor is simple to operate and shows good selectivity and sensitivity. This analytical system can be expanded to the sensors of other possible miRNAs.

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