ARTICLE

QCM Sensing of miR-21 by Formation of MicroRNA-DNA Hybrid Duplexes and Intercalation on Surface-Functionalized Pyrene

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(1) Synthesis of 1-pyrenebutyric acid N-hydroxysuccinimide ester

Firstly, 30 ml of THF solution dissolved with 0.58 g of 1-pyrenebutyric acid (2.0 mmol) and 0.23 g of NHS (2.0 mmol) was added drop-wide to the 20 ml of THF solution of dissolved with 0.45 g of DCC (2.2 mmol) under vigorous stirring. After overnight reaction at room temperature, the solution was filtered and the filtrate was evaporated using rotary evaporator. The solid residue was washed with ethanol several times and dried under vacuum to obtain a light yellow crystal (0.69 g, 94%). ^{1}H NMR (400MHz, $CDCl_3$) 1.72 (q, 2H), 2.30 (7, 2H), 2.64 (s, 4H), 3.06 (t, 2H), 7.68-8.12 (m, 9H)

(2) Synthesis of gold nanoparticles (AuNPs)

We synthesized the gold nanoparticles using citrate reduction following the reported procedure.[1,2] Briefly, we boiled 50 mL of 0.01% (w/w) $HAuCl_4$ aqueous solution with vigorous stirring in a round-bottomed flask fitted with a reflux condenser and then rapidly added 1.5 mL of 0.1% (w/w) trisodium citrate aqueous solution to the boiling solution. We maintained the resulting solution at its boiling point with continuous stirring for 15 min; the color of the solution changed from light yellow to wine red, indicating the formation of AuNPs. Then we cooled the solution at room temperature for about 15 min with stirring and stored the suspension in a refrigerator until further use. We characterized the synthesized AuNPs by transmission electron microscopy. We estimated the sizes of the citrate-stabilized AuNPs to be 17 ± 6.3 nm and found the maximum absorbance wavelength to be 520 nm.

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2. H. J. Park and S. S. Lee, *Sens. Actuators, B-Chem*., 2018, **258**, 836-840.

(3) QCM measurement

All QCM measurements were taken using a Princeton Applied Research QCM922A and silicon dioxide coated QCM resonators (5 mm in diameter; 9 MHz resonance frequency). The instrument was operated using the WinQCM and QCMAdm control software. The method for performing the analysis was to inject analyte solution over a modified surface of a QCM sensor chip from the instrument. PBS buffer was used as a running buffer for the QCM assays. Each sample was taken and placed in the Eppendorf tube and stored at room temperature. The QCM system was set at 25℃, and the flow rate was kept at 1.0 ml/min. Each sample (20 μL) was injected into the injection inlet using a peristaltic pump. Fig. S1 shows the detection module and the custom-made fluidic module for QCM sensing.

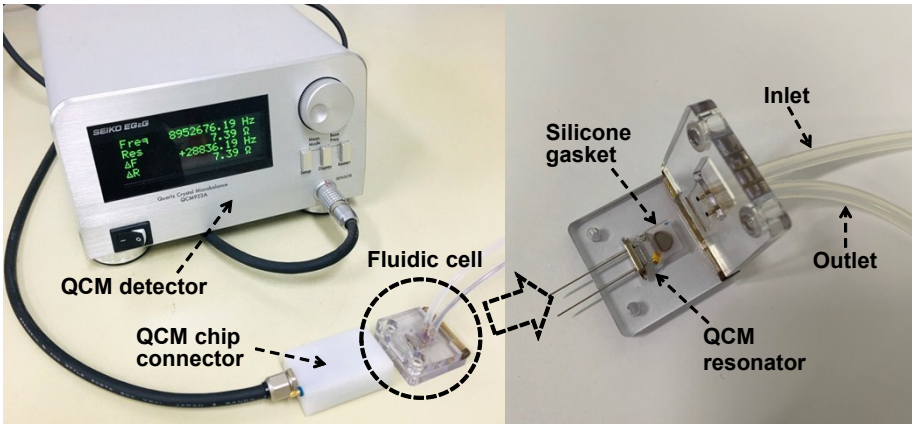

Fig. S1 The detection module and the custom-made fluidic module for QCM sensing.

(4) RT-qPCR assay for detecting miR-21 on total RNA extracted from A549 cell

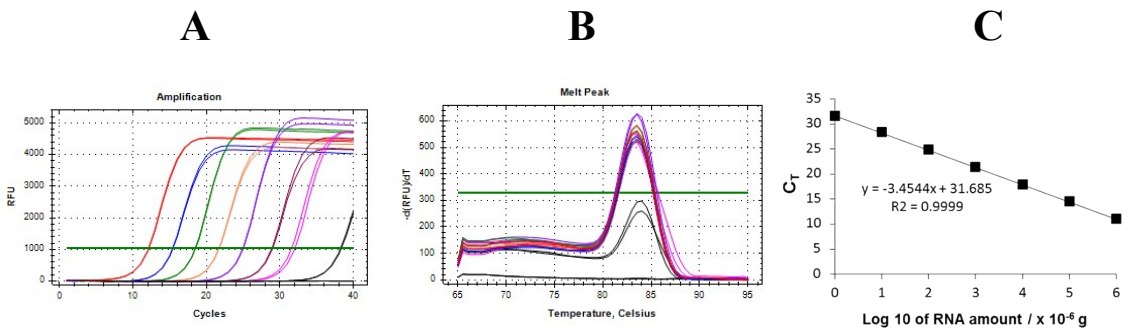

Fig. S2 Evaluating miR-21-specific RT-qPCR in total RNA extracted from A549 cells. (a) Amplification curves of a seven–log 10 dilution series of human miR-106b in total human brain RNA RT-qPCR assays. (b) Melting curve analysis performed from 65°C to 95°C. (c) Extrapolation of C_T as a function of the log10 of the amount of target miRNA was a straight line (R^2 = 0.9999) with a slope of -3.454 (PCR efficiency = 96.0%) over a seven-log 10 dilution of the target. Three measurements were carried out for each amount of target miRNA.

(5) Results of miR-21 detection assay with and without AuNP

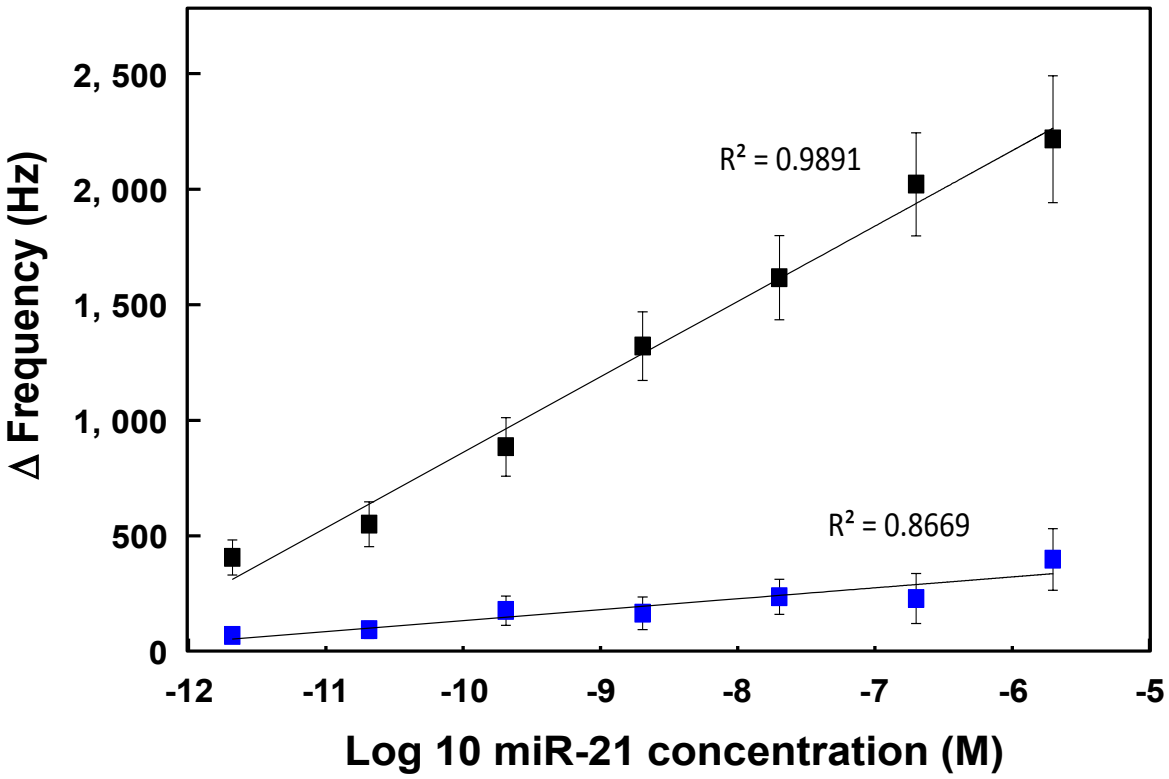

Fig. S3 Changes in resonance frequency of QCM biosensors according to synthetic miR-21 concentrations in the range of 2.5 pM to 2.5 μM. Black squares show the results by the use of gold nanoparticles and gold staining signal amplification process, and blue squares show the results by no gold nanoparticles, respectively.

(6) LODs for miRNA biosensor

Table S1. Comparison of the LODs for miRNA detection with similar methods reported in the literature

Target miRNA	LODs	Analytical techniques	References
miR-21	3.6 pM	QCM	this study
mIR-21	28 fM	SPR	Krishnan et al, 2017 [1]
miR-21	400 pM	QCM	Liedberg et al., 2018 [2]
miR-21	29 fM	Electrochemical	Raouafi et al., 2018 [3]
miR-21	330 pM	Fluorescence	Ouyang et al., 2016 [7]
miR-21	500 pM	Fluorescence	Tong et al., 2018 [9]
miR-21/miR-155	10 aM	SPR	Zhang et al., 2019 [4]
miR-107	100 aM	Electrochemical	Shiddiky et al., 2018 [5]
miR-107	10 fM	Electrochemical	Trau et al., 2016 [6]

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6. K. M. Koo, L. G. Carrascosa, M. J. A. Shiddiky and M. Trau, *Anal. Chem*., 2016, **88**, 2000-2005

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(7) The condition optimization of concentrations

a) Synthetic miR-21: miR-21 were obtained from Bioneer (Daejeon, Korea) in lyophilized form. A lyophilized form of miR-21 was dissolved with RNase-free water and prepared to a 100uM stock solution. Then, we applied the experiment using samples prepared by 10-fold serial dilution.

b) Human brain total RNA: Human brain total RNA (AM7962) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The samples obtained by serial dilutions of 100 ug / ml of this total RNA were used for miR-21 detection assays.

c) Total RNA extracted from cancer cell: A549 human epithelial lung cancer cell line (ATCC® CCL-185™) was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells are first lysed and homogenized in the presence of a highly denaturing guanidine-thiocyanate–containing buffer, which immediately inactivates RNases to ensure purification of intact RNA. Ethanol is added to provide appropriate binding conditions, and the sample is then applied to an RNeasy Mini spin column, where the total RNA binds to the membrane and contaminants are efficiently washed away. High-quality RNA is then eluted in 100 µl RNase-free water. The resulting total RNA was quantified using NanoDrop spectrophotometer (Thermo Scientific NanoDrop 2000) and diluted to 10 ug/ml with RNase-free water to make stock solution. Then, we also applied the experiment using samples prepared by 10-fold serial dilution.

In all experiments, finally, the minimum concentration was determined to be the lowest concentration at which the signal intensity is at least three times that of the blank sample.

(8) Results of miR-107 detection assay

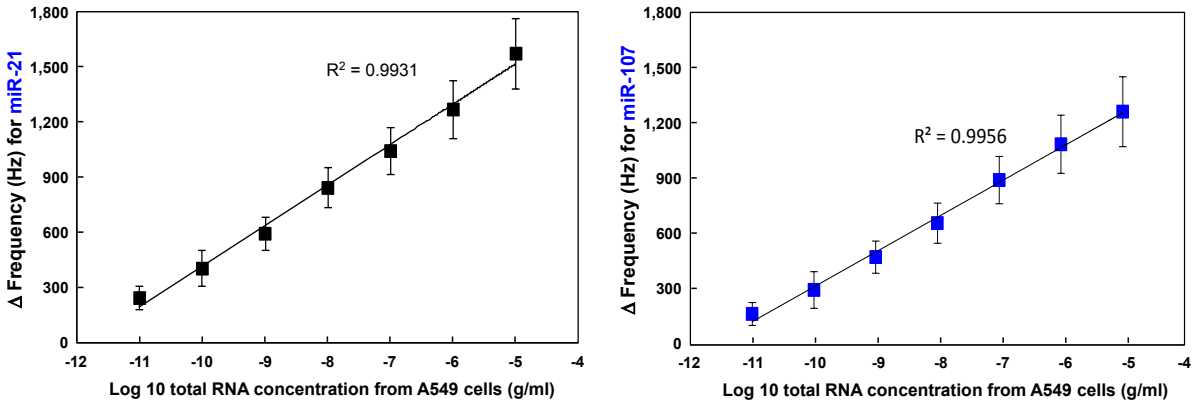

Fig. S4 Change in the resonance frequency of QCM biosensors as a function of log 10 (total RNA extracted from A549 cells) concentrations in the range of 10 pg/ml to 10 μg/ml. Left black squares shows the results for the detection of miR-21 and right blue squares for miR-107, respectively.