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Surface Plasmon Resonance Biosensor for Rapid Label-Free Detection of Microribonucleic Acid at Subfemtomole Level

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Microribonucleic acids (miRNAs) have been linked with various regulatory functions and disorders, such as cancers and heart diseases. They, therefore, present an important target for detection technologies for future medical diagnostics. We report here a novel method for rapid and sensitive miRNA detection and quantitation using surface plasmon resonance (SPR) sensor technology and a DNA*RNA antibody-based assay. The approach takes advantage of a novel high-performance portable SPR sensor instrument for spectroscopy of surface plasmons based on a special diffraction grating called a surface plasmon coupler and disperser (SPRCD). The surface of the grating is functionalized with thiolated DNA oligonucleotides which specifically capture miRNA from a liquid sample without amplification. Subsequently, an antibody that recognizes DNA*RNA hybrids is introduced to bind to the DNA*RNA complex and enhance sensor response to the captured miRNA. This approach allows detection of miRNA in less than 30 min at concentrations down to 2 pM with an absolute amount at high attomoles. The methodology is evaluated for analysis of miRNA from mouse liver tissues and is found to yield results which agree well with those provided by the quantitative polymerase chain reaction (qPCR).

Over the past decade, microRNA has emerged as a new modality in medical diagnostics. These short ribonucleic acid (RNA) molecules (on average only 22 nucleotides long) play important roles in modulating various biological functions through the interaction between microribonucleic acids (miRNAs) and messenger ribonucleic acid (mRNA).[1] Several thousand miRNAs have been identified in species from algae to animals. Even though the understanding of their complex roles is in its infancy, expression levels of specific miRNAs in tissues have already been correlated with cell fate decisions and outcome of serious diseases, such as heart diseases[2] and various cancers.[3,4] Recently, we have also shown that the levels of miR-122 in plasma and tissue can be used to detect and monitor drug-induced liver injury,[5] the principal side effects of various drugs.

Measuring miRNAs holds many advantages over detecting traditional protein biomarkers. Complexity and modifications make protein detection with sufficient sensitivity, specificity, and reliability a rather challenging task. Unlike proteins, there are far fewer miRNA species, and the sensitivity of existing methods is already superior to protein detection methods. However, accurately measuring microRNAs has also posed numerous new challenges to the analytical technologies. Given the miRNA's size and levels in cells, the detection methods need to be extremely sensitive. In addition, they need to be specific to accurately measure the levels of specific analytes in small amounts of complex RNA sample.

The Northern blot is still considered to be a gold standard for miRNA detection.[6] In this method, labeled complementary probe binds to a target miRNA captured on nitrocellulose membrane. Major drawbacks of this method are low throughput, semiquantitative data, and time-consuming experimental process (often taking several hours). The sensitivity of the method has been partly enhanced by the use of locked nucleic acid[7] and carbodiimine cross-linking,[8] but large amounts of sample are still required for the analysis. Another commonly used method to assess the levels of specific miRNA is the real-time quantitative polymerase chain reaction (qPCR), which claims high sensitivity and relative short detection time.[9] The high sequence similarity and short sequence length of mature miRNAs makes the accurate assessment of miRNA levels by qPCR-based methods rather difficult. In addition, modifications of miRNA sequence by adding an additional tag sequence at the 3′ end of miRNAs prior to qPCR is generally required, adding an additional variable in quantitation.

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(1) He, L.; Hannon, G. J. *Nat. Rev. Genet.* **2004**, *5*, 522–531.

(2) Ikeda, S.; Kong, S. W.; Lu, J.; Bisping, E.; Zhang, H.; Allen, P. D.; Golub, T. R.; Pieske, B.; Pu, W. T. *Physiol. Genomics* **2007**, *31*, 367–373.

(3) Iorio, M. V.; Ferracin, M.; Liu, C. G.; Veronese, A.; Spizzo, R.; Sabbioni, S.; Magri, E.; Pedriali, M.; Fabbri, M.; Campiglio, M.; Menard, S.; Palazzo, J. P.; Rosenberg, A.; Musiani, P.; Volinia, S.; Nenci, I.; Calin, G. A.; Querzoli, P.; Negrini, M.; Croce, C. M. *Cancer Res.* **2005**, *65*, 7065–7070.

(4) Lu, J.; Getz, G.; Miska, E. A.; Alvarez-Saavedra, E.; Lamb, J.; Peck, D.; Sweet-Cordero, A.; Ebet, B. L.; Mak, R. H.; Ferrando, A. A.; Downing, J. R.; Jacks, T.; Horvitz, H. R.; Golub, T. R. *Nature* **2005**, *435*, 834–838.

(5) Wang, K.; Zhang, S. L.; Marzolf, B.; Troisch, P.; Brightman, A.; Hu, Z. Y.; Hood, L. E.; Galas, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 4402–4407.

(6) Lagos-Quintana, M.; Rauhut, R.; Yalcin, A.; Meyer, J.; Lendeckel, W.; Tuschl, T. *Curr. Biol.* **2002**, *12*, 735–739.

(7) Valoczi, A.; Hornyik, C.; Varga, N.; Burgyan, J.; Kauppinen, S.; Havelda, Z. *Nucleic Acids Res.* **2004**, 32.

(8) Pall, G. S.; Hamilton, A. J. *Nat. Protoc.* **2008**, *3*, 1077–1084.

(9) Benes, V.; Castoldi, M. *Methods* **2010**, *50*, 244–249.

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The extensive miRNA end region sequence heterogeneity may also affect the accuracy of qPCR-based miRNA measurement.[10] Similar to mRNA assessment, one of the technologies for miRNA measurement is the microarray method, which is well suited to parallelized detection of multiple miRNAs.[11] Again, due to the short length of mature microRNA, it is difficult to design very specific probes for a number of microRNAs. Even though it is possible to detect and monitor nonspecific hybridization since it displays different hybridization kinetics, the current microarray detection methods rely on the end-point hybridization intensity; which makes it difficult to distinguish real signals from nonspecific hybridization. Therefore, the levels of miRNA measured by microarrays may not be accurate. In addition, microarray methods usually require labeling of the miRNA with fluorescence probe or biotin resulting in rather complex multistep detection protocols.[12,13] This limits the use of this high throughput technology to a controlled and well equipped laboratory environment. Several alternative approaches including ligation of specific oligonucleotide reporter group[14] and rolling circle amplification[15] have been developed to address some of the limitations on microRNA measurement (for review, see ref 16).

Biosensors, such as those based on surface plasmon resonance (SPR), can offer rapid, sensitive, and on-site analysis; therefore, they present an attractive alternative to conventional techniques.[17] In the past decade, SPR biosensors have been used for detection of a wide variety of biomolecules (proteins, peptides, nucleic acids, etc.) and intact micro-organisms (bacteria, viruses).[18] In addition to detection of individual analytes, SPR biosensors have also made substantial advances toward high-throughput screening.[19] Short oligonucleotides have been directly detected down to femtomole levels by SPR.[20] Even lower limits of detection may be achieved using a more complex assay format.[21,22] For instance, Fang et al. developed a nanoparticle-based approach with an enzymatic reaction for signal amplification capable of detecting nucleic acids at attomole levels.[23] This approach, however, requires complex protocol, which involves multiple preparation and detection steps.

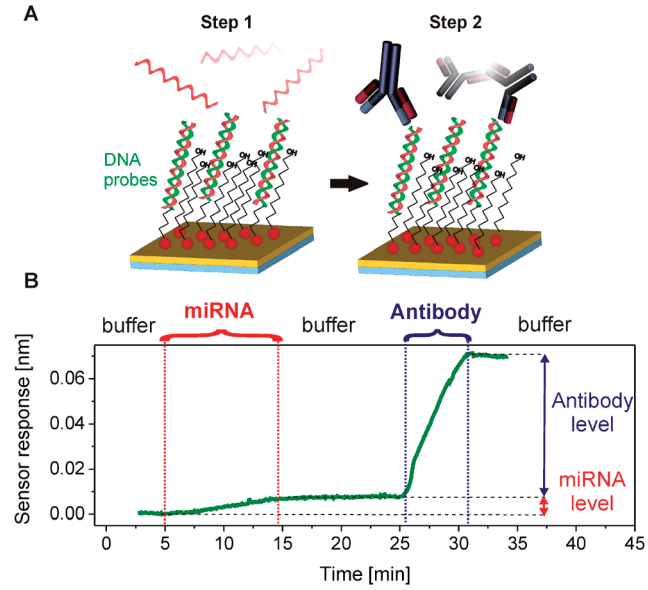

Figure 1. (A) Schematic representation of the antibody-based assay for detection of miRNA. (B) Example of temporal sensor response to the miRNA and antibody in the two-step assay.

In this paper, we present a novel method for rapid and sensitive detection of miRNA. This method combines a novel high-performance portable SPR sensor platform based on spectroscopy of surface plasmons on a special diffraction grating (SPRCD) and assay utilizing antibody recognizing the DNA*RNA hybrid duplexes.[24] The method is demonstrated for detection of synthetic miR-122 in buffer and quantification of mature miR-122 in complex mouse liver tissue samples. The results are compared with the results obtained using the quantitative polymerase chain reaction.

MATERIALS AND METHODS

Assay Principle. A scheme of the reported microRNA assay principle of operation is shown in Figure 1A. The assay is performed in two simple steps. In the first step, surface of the SPRCD sensor functionalized with covalently attached thiolated DNA probes, having sequence complementary to the targeted miRNA, is incubated with the sample containing miRNA. In this step, the miRNA is captured by the DNA probes. In the second step, the sensor surface is incubated with solution containing a special antibody that specifically recognizes the RNA*DNA hybrid duplex and binds to the miRNA*DNA duplex. The corresponding sensor response is shown in Figure 1B. Even though the second step is not strictly necessary, it significantly increases the sensitivity of the assay due to the enhancement of the captured miRNA signal by the presence of the antibody.

SPR Sensor. In this work, a recently developed portable six-channel SPR sensor based on a novel approach to spectroscopy of surface plasmons was employed.[25] This approach uses a special diffraction grating structure (SPRCD) to couple light into surface

(10) Lee, L. W.; Zhang, S.; Etheridge, A.; Ma, L.; Martin, D.; Galas, D.; Wang, K. *RNA* **2010**, *16*, 2170−2180.
(11) Thomson, J. M.; Parker, J.; Perou, C. M.; Hammond, S. M. *Nat. Methods* **2004**, *1*, 47–53.
(12) Liu, C. G.; Calin, G. A.; Meloon, B.; Gamliel, N.; Sevignani, C.; Ferracin, M.; Dumitru, C. D.; Shimizu, M.; Zupo, S.; Dono, M.; Alder, H.; Bullrich, F.; Negrini, M.; Croce, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9740−9744.
(13) Schetter, A. J.; Leung, S. Y.; Sohn, J. J.; Zanetti, K. A.; Bowman, E. D.; Yanaihara, N.; Yuen, S. T.; Chan, T. L.; Kwong, D. L. W.; Au, G. K. H.; Liu, C. G.; Calin, G. A.; Croce, C. M.; Harris, C. C. *JAMA, J. Am. Med. Assoc.* **2008**, *299*, 425–436.
(14) Geiss, G. K.; Bumgarner, R. E.; Birditt, B.; Dahl, T.; Dowidar, N.; Dunaway, D. L.; Fell, H. P.; Ferree, S.; George, R. D.; Grogan, T.; James, J. J.; Maysuria, M.; Mitton, J. D.; Oliveri, P.; Osborn, J. L.; Peng, T.; Ratcliffe, A. L.; Webster, P. J.; Davidson, E. H.; Hood, L. *Nat. Biotechnol.* **2008**, *26*, 317–325.
(15) Li, N.; Jablonowski, C.; Jin, H. L.; Zhong, W. W. *Anal. Chem.* **2009**, *81*, 4906–4913.
(16) Wark, A. W.; Lee, H. J.; Corn, R. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 644–652.
(17) Cosnier, S.; Mailley, P. *Analyst* **2008**, *133*, 984–991.
(18) Homola, J. *Chem. Rev.* **2008**, *108*, 462–493.
(19) Piliarik, M.; Párová, L.; Homola, J. *Biosens. Bioelectron.* **2009**, *24*, 1399–1404.
(20) Špringer, T.; Piliarik, M.; Homola, J. *Sens. Actuators, B* **2010**, *145*, 588–591.
(21) He, L.; Musick, M. D.; Nicewarner, S. R.; Salinas, F. G.; Benkovic, S. J.; Natan, M. J.; Keating, C. D. *J. Am. Chem. Soc.* **2000**, *122*, 9071–9077.
(22) Okumura, A.; Sato, Y.; Kyo, M.; Kawaguchi, H. *Anal. Biochem.* **2005**, *339*, 328–337.
(23) Fang, S. P.; Lee, H. J.; Wark, A. W.; Corn, R. M. *J. Am. Chem. Soc.* **2006**, *128*, 14044–14046.
(24) Hu, Z. L.; Zhang, A. X.; Storz, G.; Gottesman, S.; Leppla, S. H. *Nucleic Acids Res.* **2006**, 34.
(25) Piliarik, M.; Vala, M.; Tichý, I.; Homola, J. *Biosens. Bioelectron* **2009**, *24*, 3430–3435.

plasmons and disperse the spectrum of light over a position sensitive detector. This concept integrates the detection surface with the light handling process and eliminates the need for a spectrograph. This idea has allowed development of a very compact yet high-performance sensing platform. The SPRCD diffraction grating is integrated with microfluidics to a compact SPRCD cartridge. The microfluidics supports six independent sensing channels and allows for the SPRCD chip to be independently functionalized in six sensing areas. The refractive index resolution of the SPRCD sensor platform was demonstrated to be as low as 3×10^{-7}.[25]

Reagents. Oligonucleotides and synthetic microRNA were obtained from Integrated DNA Technologies, USA. The thiolated DNA oligonucleotide P122 SH-5′-d(CAA ACA CCA TTG TCA CAC TCC A)-3′ was used as a capture probe for miR-122 detection. DNA probe R1 5′-d(TGC GTG TTT GAT TAT T)-3′-HS was used for functionalization of reference sensing surfaces. The synthetic target of the sequence of mature miR-122 5′-r(UGG AGU GUG ACA AUG GUG UUU G)-3′ was used for development, optimization, and calibration of the assay. RNA−DNA hybrids were detected using the S9.6 mouse monoclonal antibody that is available as a hybridoma cell line from ATCC (HB-8730). Synthetic miR-192 of the sequence 5′-r(CUG ACC UAU GAA UUG ACA GCC)-3′ was used in control experiments as miRNA model for investigation of specificity of detection.

Immobilization of DNA Probes. SPRCD chips were functionalized by attaching the thiol-derivatized DNA probes to the surface of gold layer on the SPRCD chip. Initially, the sensor chip was rinsed with ethanol and Milli-Q water and dried with a stream of nitrogen. The sensor chip cartridge was then assembled and mounted in the external immobilization unit. The 4 μM solution of thiolated DNA probes in PBS buffer (137 mM NaCl, 1.4 mM KH_2PO_4, 8 mM $Na_2HPO_4.12H_2O$, 2.7 mM KCl, pH 7.4 at 25 °C) was injected in the flow-cell and flowed through at a flow rate of 5 μL/min for 20 min. The sensor surface was incubated in 1 μM blocking alkanethiol solution (HS-C_{11}-$(EG)_4$-OH; Prochimia, Poland) in PBS for 1 h. Then, the flow rate was increased to 20 μL/min, and PBS buffer was injected and flowed through the flow-cell for 5 min. Finally, the surface was flushed with 2 mM NaOH for 5 min and then with the PBS.

Assay Protocol. First, the running buffer (PBS; $Tris_{Mg}$ [10 mM Tris−HCl, 15 mM $MgCl_2$, pH 7.4 at 25 °C] or $Tris_{Na}$ [10 mM Tris−HCl, 1 M NaCl, pH 7.4 at 25 °C]) was introduced to generate a baseline. Then, the solution of miRNA in the running buffer was injected and flowed through the flow-cell for 10 min. The sensor surface was then flushed with buffer for 5 min. Subsequently, the solution of antibody in the running buffer at a concentration of 0.7 μg/mL was injected and flowed through the flow-cell for 5 min. Finally, the sensor surface was incubated with the running buffer. All the detection experiments were carried out at a flow rate of 20 μL/min and a temperature of 25 °C. To compensate for changes in refractive index and the nonspecific adsorption, the measurements were performed simultaneously in the sensing channel and reference channels (functionalized with reference probe R1) and the response in the reference channel was subtracted from that in the sensing channel.

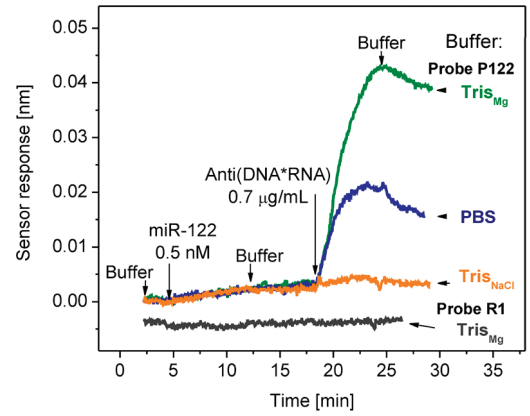

Figure 2. Reference-compensated responses to miRNA and antibody for three different reaction buffers. Arrows indicate injection of the respective solutions. The bottom line shows sensor response in reference channel.

Tissue Samples. Liver samples were harvested from six-month-old male C57/B mice 24 h after been injected (IP) with a single dose of acetaminophen at 150 mg/kg (S1). PBS was used as control (S2). Total RNA, including miRNA, was extracted from 100 mg of liver tissue using the mirVana total RNA extraction kit and following the instructions from the manufacturer (Applied Biosystems/Ambion, Foster City, CA, USA). RNA concentration was determined with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

qPCR. The cDNA was generated using the miScript Reverse Transcription kit (Qiagen, Germantown, MD, USA). In brief, miRNAs were polyadenylated by poly(A) polymerase and converted to cDNA using oligo-dT and random primers with reverse transcriptase. Polyadenylation and reverse transcription were performed in parallel in the same tube. The oligo-dT primers have a universal tag sequence on the 5′ end. This universal tag allows amplification in the real-time PCR step. The cDNA served as the template for the real-time PCR analysis using the Human miScript Assay (Qiagen, Germantown, MD, USA). qPCR was performed on a 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). All of the end-point data were analyzed by SDS Enterprise Database (Applied Biosystems, Foster City, CA, USA).

RESULTS AND DISCUSSION

Optimization of miRNA Assay. The miRNA assay was optimized in terms of the composition of the reaction buffers to achieve maximum sensitivity. The following buffers were tested: the high magnesium buffer ($Tris_{Mg}$: Tris with 15 mM $MgCl_2$), sodium chloride buffer ($Tris_{Na}$: Tris with 1 M NaCl), and standard phosphate buffer PBS, which is often used for SPR-based detection of proteins. Figure 2 shows the sensor response to synthetic miRNA hybridization (miR-122, concentration −500 pM) and subsequent sensor response to the binding of antibody (concentration −0.7 μg/mL). Our experiments revealed that the sensor response to the miRNA hybridization varies slightly with the salt content and is somewhat lower in PBS. This can be attributed to less efficient compensation of negative charges on the DNA surface due to a lower concentration of positive ions in

the PBS.[26] There was no observable difference in the efficiency of miRNA hybridization between $Tris_{Na}$ and $Tris_{Mg}$. We have shown in our previous work, that both buffers have an excellent stabilization effect on DNA*DNA duplex at a room temperature, the $Tris_{Mg}$ being slightly more efficient.[27] In contrast, the composition of buffer was found to have a profound effect on the antibody binding to RNA*DNA duplexes. The sensor response to the antibody solution in $Tris_{Na}$ was very low, indicating that the high content of NaCl results in the loss of antibody binding activity. Sensor response to antibody in $Tris_{Mg}$ was equal to about a double of that observed in PBS. Because of the strong antibody binding it facilitates, $Tris_{Mg}$ buffer was used in further experiments. The nonspecific binding of the miRNA and the antibody to the sensor surface was also investigated by monitoring the sensor response in the reference channel. Respective sensor response is presented in the bottom of Figure 2. As follows from Figure 2, no increase in the sensor response is observed during the injection of synthetic miRNA and the antibody. This suggests that neither miRNA nor the antibody exhibit significant nonspecific adsorption to the sensing surface in the $Tris_{Mg}$.

A regeneration procedure was developed to allow for removal of both the miRNA and antibody from the sensor surface and for repeated use of the SPRCD chip. The procedure consists of two steps. To strip off the antibody, the sensor surface was incubated with 2 mM sodium hydroxide (NaOH) for 2 min. Then, hydroxide was replaced with running buffer ($Tris_{Mg}$). To remove the bound miRNA, a 2 min injection of 2 mM hydrochloric acid (HCl) was performed. The regeneration was confirmed by a short injection of the antibody which produced no sensor response. The regeneration protocol was demonstrated to preserve activity of the DNA probes for at least five detection/regeneration cycles (data not shown).

Detection of Synthetic miRNA in Buffer. To evaluate performance of this approach to miRNA detection, the assay condition was employed with synthetic miR-122 in buffer. Figure 3A shows the reference-compensated sensor response to various concentrations of the synthetic miR-122 in two independent flow channels. Detail of sensor response to low concentrations is displayed in Figure 3B. Clearly, sensor response can be observed for miR-122 concentrations higher than 0.2 nM. For miR-122 concentrations higher than 50 nM, a 10 min injection of miR-122 was found to be sufficient for the sensor response to reach the equilibrium value. Almost no dissociation of miR-122 is observed when the buffer is injected in the flow-cell. This is caused by high content of Mg^{2+}. Mg^{2+} diminishes the repulsion between negatively charged DNA and RNA strands and, hence, stabilizes the duplex. This stabilizing effect has been observed in DNA*DNA duplexes.[27] Figure 3C shows the sensor response to nontarget miRNA (upper part) and the response of the reference channel to miR-122 (lower part). Both these nonspecific responses are negligible which indicates that the direct binding of miR-122 is indeed very specific.

In the second detection step, the sensor surface is incubated with antibody that exhibits affinity toward the RNA*DNA hybrids

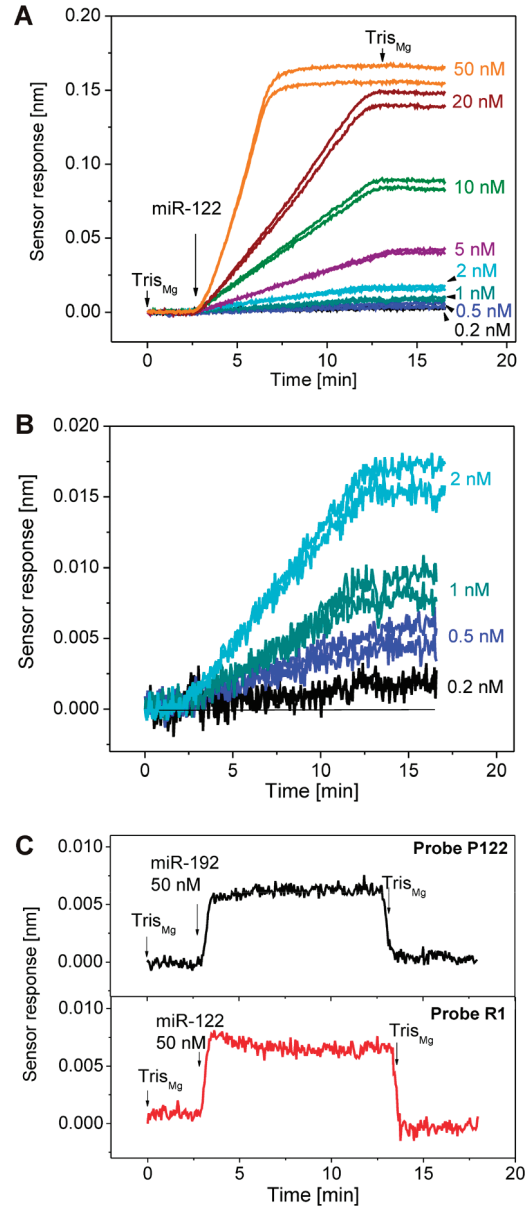

Figure 3. Direct detection of the miRNA122. Reference-compensated sensor response to (A) high and (B) low concentrations of miRNA binding to the surface with DNA probe P122. (C) Measurement of nonspecific binding of the miRNA to the SPR surface coated with reference probes. Arrows indicate injection of respective solutions.

(Figure 4). Figure 4A displays the response of the sensing channels that have been functionalized with the S122 probe and incubated with miR-122 at low concentrations (10−100 pM); the concentrations are too low to produce detectable sensor response upon hybridization (in the first step of detection). As a fixed concentration of the antibody is used in all the measurements in Figure 4A (0.7 μg/mL), the initial binding rate is rather similar. In contrast, the equilibrium sensor response to the antibody is directly proportional to the concentration of RNA*DNA hybrids formed in the first step and can be used for quantification of miR-122. Clearly, antibody binding to RNA*DNA hybrids significantly enhances the sensitivity of miR-122 detection. Figure 4B displays the sensor response in the reference channels, which were not preincubated with the miR-122 solutions. Apparently, the nonspecific adsorption of the antibody to the sensor surface coated with

(26) Peterson, A. W.; Heaton, R. J.; Georgiadis, R. M. *Nucleic Acids Res.* **2001**, *29*, 5163−5168.
(27) Špringer, T.; Šípová, H.; Vaisocherová, H.; Štěpánek, J.; Homola, J. *Nucleic Acids Res.* **2010**, *38*, 7343−7351.

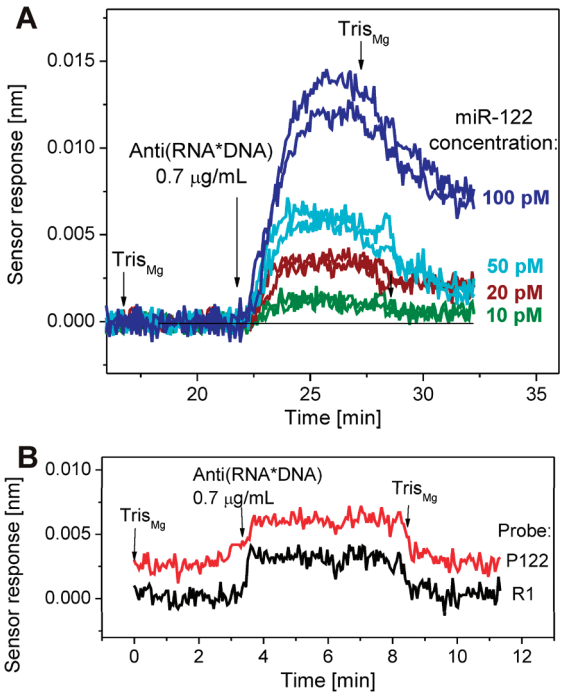

Figure 4. MiR-122 detection with specific antibody of fixed concentration. (A) Sensor surface preincubated with standard solutions with different concentrations of miRNA (reference-compensated data). Straight line indicates the baseline level before the injection of the antibody. (B) Nonspecific sensor response to the antibody solution in sensing channels with two different probes. Arrows indicate injection of respective solutions.

S122 and S192 probes is negligible. This indicates that the antibody binds specifically to the RNA*DNA hybrid duplexes.

A calibration curve was established using sensor response obtained for various concentrations of synthetic miR-122 and four-parameter logistic function $f(c) = (A_1 - A_2)/(1 - (c/c_0)^p) + A_2$. For each of the concentrations, the SPRCD sensor response was measured on at least three chips and in two replicas. Calibration curve of the first step of the assay (direct detection of microRNA) was calculated from the initial slope of the sensor response to miR-122 hybridization (Figure 5A). This approach is well suited to direct detection, as it provides rapid and sensitive sensor output, which is rather insensitive to the surface concentration of immobilized probes. The parameters of the logistic fit demonstrate that the calibration curve is linear in the used operating range and intersects the zero value. The calibration curve for the detection of antibody was determined from the equilibrium values of the sensor response, which was shown to be proportional to the surface concentration of heteroduplexes (Figure 5A). As both the nonspecific binding of miRNA and the antibody have been determined to be negligible (Figure 3C and 4B), the limit of detection (LOD) is in both cases determined as a concentration of microRNA that results in sensor response equal to three standard deviations of the baseline noise (5×10^{-5} nm/min for binding rate and 5×10^{-4} nm for equilibrium sensor response). As follows from Figure 5, the LOD for direct and antibody-amplified detection are 100 and 2 pM, respectively. Considering the sample volume required per analysis (~440 μL), this concentration corresponds to the smallest detectable absolute amount of less than 900 attomoles.

Detection of miRNA in Biological Samples. To demonstrate the utility of this label-free detection method, the SPRCD sensor-based assay was used to quantify the miR-122 levels in total RNA isolated from mouse liver tissue. Two tissue samples were examined, one originating from liver tissue of acetaminophen-treated (24 h post 150 mg/kg IP) mice (S1) and the other from control mouse (24 h post PBS IP; S2). The RNA including microRNA was extracted using a commercial RNA isolation kit. The total concentration of RNA in the sample was determined with UV spectroscopy as 1045.81 ng/μL (control, S2) and 1124.4 ng/μL (treated, S1). The samples were diluted by a factor of 7.3 (S1) or 7.72 (S2) to a volume sufficient for the analysis. Figure 6 shows SPRCD sensor responses to the sample S1 in both the sensing and reference channels. The injection of sample S1 resulted in a large increase in the sensor response. This increase is mainly due to the difference in the refractive indices between the sample and running buffer (Tris$_{Mg}$). When the sample was replaced with the Tris$_{Mg}$ buffer, the sensor response exhibited a quick drop, as expected (due to the refractive index change in the buffer). However, no significant difference was observed between baseline level in sensing and reference channel, indicating the concentration of miR-122 in the sample probably was lower than the limit of detection as described in an earlier section. As follows from Figure 6, during a few minutes in Tris$_{Mg}$ buffer, gradual dissociation of the nonspecifically adsorbed molecules from the sensing surface was observed. When the sensor response became stable (after ~10 min), the antibody was injected and flowed through the flow-cell for 5 min followed

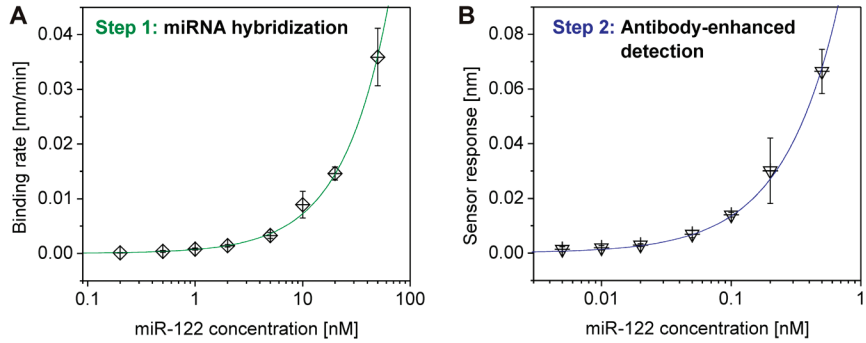

Figure 5. Calibration curves of SPRCD sensor in semilog scale. (A) Direct detection (parameters of logistic fit: $A_1 = (-4 \pm 5) \times 10^{-5}$ nm/min, $A_2 = (0 \pm 8) \times 10^8$ nm/min; $c_0 = (0 \pm 1) \times 10^{12}$ nM; $p = (0.99 \pm 0.07)$ and (B) antibody-enhanced detection of miR-122 (parameters of logistic fit: $A_1 = (1.1 \pm 0.1) \times 10^{-3}$ nm, $A_2 = (0.18 \pm 0.03)$ nm; $c_0 = (0.79 \pm 0.15) \times 10^{12}$ nM; $p = (1.24 \pm 0.04)$. Each point was measured at least three times on different SPR chips; error bars represent the standard deviation.

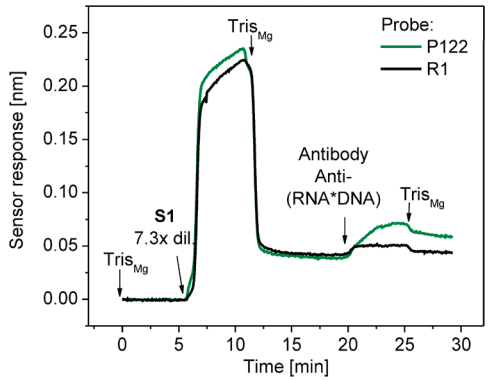

Figure 6. Analysis of the real samples containing total RNA isolated from mouse liver tissues. Sensor responses in reference and measuring channels to the diluted S1 sample and subsequent binding of the detection antibody.

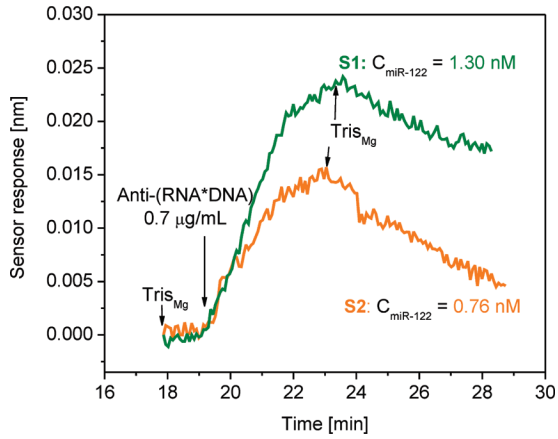

Figure 7. Determination of miRNA concentration in the tissue samples. Reference-compensated sensor response to the antibody after sensor chip incubation with two different liver tissue samples S1 (acetaminophen-treated mouse) and S2 (control mouse).

by Tris$_{Mg}$ buffer without antibody. While the response to antibody was clearly observed in the sensing channel, no response was observed in the reference channel after washing the channels with Tris$_{Mg}$ buffer.

Figure 7 demonstrates the reference-compensated sensor responses to the RNA*DNA antibody injected after the sensor surface incubation with the tissue samples S1 and S2. Using the corresponding calibration curve (Figure 5B), the concentrations of miR-122 in diluted samples S1 and S2 were estimated to be 167 pM (S1) or 104 pM (S2), respectively. The original concentration for the miR-122 in the undiluted samples can be, therefore, determined as 1.30 and 0.76 nM, respectively. Analysis of the tissue samples using qPCR provided the following concentrations: 1.46 nM (S1) and 1.05 nM (S2). Clearly, the results obtained using the SPR biosensor show an excellent agreement with those obtained with the qPCR. The observation of a slightly higher miR-122 concentration in liver from 150 mg/kg acetaminophen treated animal is different from our previous report.[5] This probably is caused by a lower acetaminophen dosage, 150 vs 300 mg/kg, used in this study.

The results demonstrate the potential of the SPR biosensor technology to detect miRNAs. The method requires less than a femtomole amount of the targeted miRNA, which is much less than needed for Northern blotting[28] and comparable with the amounts that are required by microarrays.[11] The microarray technology provides global profiling of miRNA; however, it requires additional labeling steps, overnight hybridization, and posthybridization processing. The SPR biosensor-based approach is not suitable for global profiling, but with selected miRNA targets, the method outlined here can be performed in less than 30 min and can be, therefore, used for on-site analysis.

CONCLUSIONS

In this report, we demonstrated a new label-free method for rapid and sensitive measurement of microRNA levels in biological sample. This approach utilizes a novel high-performance portable SPR sensor and RNA*DNA antibody. We demonstrated the measurement of microRNA with miR-122, which is a prospective marker for drug-induced liver injury. The assay is optimized in terms of reaction buffer. At optimal conditions, a high attomole limit of detection (LOD) is achieved in the assay which requires less than 30 min. The obtained results are determined to be in excellent agreement with the results obtained using quantitative polymerase chain reaction. The method is, therefore, demonstrated to provide a simple and cost-effective alternative to conventional methods to measure microRNA levels in biological samples.

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