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Measuring Ultra-low Levels of Nucleotide Biomarkers Using Quartz Crystal Microbalance and SPR Microarray Imaging Methods: A Comparative Analysis

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Abstract

Circulating serum nucleotide biomarkers are useful indicators for early diagnosis of cancer, respiratory illnesses, and other deadly diseases. In this work, we compared detection performances of a quartz crystal microbalance (QCM), which is a mass sensor, with that of a surface plasmon resonance (SPR) microarray for an oligonucleotide mimic of a microRNA-21 biomarker. A surface immobilized capture oligonucleotide probe was used to hybridize with the target oligonucleotide (i.e., the microRNA-21 mimic) to facilitate selective detection. To obtain ultra-low femtomolar (fM) detection sensitivity, gold nanoparticles (50 nm) were conjugated with the target oligonucleotide. We achieved detection limits of 28 and 47 fM for the target oligonucleotide by the QCM and SPRi microarray, respectively. We also conducted sample recovery studies and performed matrix effect analysis. Although the QCM had a lower detection limit, the microarray approach offered better throughput for analysis of up to 16 samples. We confirmed that the designed assay was selective for the target oligonucleotide and did not show signals for the control oligonucleotide with five mismatch sites relative to the target sequence. Combination of the QCM and microarray methods that utilize the same assay chemistry on gold are useful for overcoming clinical sample matrix effects and achieving ultra-low detection of small nucleotide biomarkers with quantitative insights.

Graphical Abstract

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Keywords

SPR microarray; mass sensor; gold nanoparticles; serum biomarker; oligonucleotides; comparative analysis

Introduction

In recent years, DNA-based biosensors have received immense attention due to their applicability in the fields of gene sequencing,¹ therapeutic and delivery systems,² pathogen detection,^{3–5} and forensic studies.⁶ Single stranded DNA molecules can be used as inexpensive molecular analogs of RNA for assay methodology development for complex sample matrices. Recently, microRNAs (miRNAs) have become important genetic biomarkers for early diagnosis of various diseases, including cancer,^{7,8} and lung diseases.^{9,10} miRNAs are a group of small RNAs approximately 21–25 nucleotides in length that function mainly by binding the 3'-untranslated regions of specific target messenger RNAs (mRNAs) to repress protein translation or cleave mRNAs.¹¹ In particular, miRNA-21 has been found to be relevant to infectious diseases, such as viral¹² and bacterial¹³ infections, as well as non-infectious diseases such as cardiovascular disorder¹⁴ and cancer.¹⁵ Hence, ultra-sensitive and selective detection of miRNA biomarkers present in a complex clinical matrix such as blood, plasma, or serum is significant for diagnostic applications. Suitable structural features of a capture oligonucleotide probe is essential for selective hybridization with a target miRNA in the presence of other nucleotides.

A quartz crystal microbalance (QCM) is a mass sensor that provides both qualitative and quantitative measurements by translating changes in the oscillation frequency of the quartz crystal to the corresponding mass changes.¹⁶ Surface plasmon resonance imaging (SPRi)^{17–19} is an emerging technique that is superior to conventional SPR for *in situ* detection of biomolecular interactions in real-time in a single microarray platform.^{20,21} SPRi offers simplicity and higher throughput compared to the conventional single sample analysis by allowing measurements at a fixed angle of incidence to directly collect reflectivity changes ($R\%$) across an array of gold spots.²² Furthermore, the SPRi microarray requires less assay time than conventional SPR to analyze several replicates at once leading to high precision.^{23,24} The hybridization of oligonucleotides with the SPR surface-immobilized

capture probe results in an increase in the refractive index. The use of gold nanoparticles (AuNPs) has been shown to increase the reflectivity changes of a hybridization reaction due to surface plasmon signal enhancement.^{25,26,27}

In this study, an inexpensive target oligonucleotide containing a similar number of nucleotides to that of the miRNA-21 marker with thymine in the place of uracil and with a poly-T tail and a free thiol group was designed to covalently attach to AuNPs via the well-known Au-thiol chemisorption.²⁸ The AuNP-linked mimic oligonucleotide was hybridized with a complementary surface immobilized capture probe. The effect of serum matrix percentage on the resulting detection signals for the miRNA-21 mimic was evaluated. Selectivity of the assay also was assessed using a five base pair mismatch-containing sequence as the control. Furthermore, we measured the changes in SPR reflectivity with an image output in the designed hybridization assay along with quantitative QCM mass analysis. Such oligonucleotide models are cost-effective way to develop sensitive assay strategies for complex sample matrices, compare two analytical methods, and evaluate the efficiency of a hybridization strategy that may be useful for measuring ultra-low levels of miRNA markers in circulating body fluids.

Experimental Methods

Reagents

Thiol-modified custom-designed oligonucleotides (sequences are presented in Table 1), dithiothreitol (DTT), 6-mercapto hexanol (MHOH), and NAP-10 columns were obtained from Sigma-Aldrich (Woodlands, TX, USA). AuNPs(50 nm diameter) were purchased from BBI Solutions (Cardiff, UK). Human serum samples were purchased from Fitzgerald Industries International Inc. (North Acton, MA, USA). SpotReady-16 gold spotted glass microarray chips (spot size 1 mm diameter, SPR-1000-016) were obtained from GWC Technologies (Madison, WI, USA). Gold disc infused quartz crystals (gold diameter 0.2 inch) used for the QCM were purchased from International Crystal Manufacturing Co, Inc. (Oklahoma City, OK, USA). All other chemicals were of high-purity analytical grade. To prepare all solutions, ultrapure distilled water (DNase and RNase free) purchased from Life Technologies (Carlsbad, CA, USA) was used.

The hybridization sequences for probe, target, and control are highlighted. Base pair mismatches of the control with respect to the target are highlighted in red. The addition of Poly-T₅₀ segments to the target and control sequences provides stability and flexibility to the strands.²⁹

Instrumentation

The optical imaging experiments were performed using a SPRImagerII array instrument at room temperature (GWC Technologies). The experiments were conducted using a dual-channel set-up equipped with two syringe pumps (100 μ L sample loop, New Era Pump System, Inc., Farmingdale, NY, USA) in order to assess hybridization of the target and control oligonucleotides with the surface capture probe. The Digital Optics V++ software package provided with the instrument was used to collect SPRi difference images (i.e.,

differences of the pixel intensities before and after hybridization events), and the images were visualized using ImageJ 1.49v software (National Institutes of Health, USA).

The mass changes resulting from hybridization were measured using a QCM instrument (Gamry Instruments Inc., Warminster, PA, USA). Each modification step on the gold quartz crystal was performed offline, and the frequency changes were measured in the QCM system at a sample measurement interval of 0.1 s until a constant frequency response with time was reached. The fundamental oscillation frequency of the crystals was 10 MHz, and the QCM sensor surface was immersed in 0.1 M NaCl/0.1 M sodium phosphate buffer (pH 7.4) during measurements.

A Nanodrop ND1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) was used to quantify the surface capture probes immobilized on the SPR array or quartz crystal surface and additionally to quantify the target and control oligonucleotides linked to AuNPs. Surface characterization of the gold surface of the SPR microarray before and after coating with the surface capture probe was conducted by scanning electron microscopy (SEM, Model: FEI Quanta 600FE). An accelerating voltage of 20 kV was applied. The images were acquired using the FEIxt Microscope Control Software.

AuNP-linked oligonucleotide preparation

The disulfide-modified oligonucleotide reduction protocol provided by the manufacturer (Sigma-Aldrich) was followed to activate the oligonucleotides to link with the AuNPs. For thiol activation, the dry oligonucleotide samples were treated with an aqueous solution of DTT (100 mM) at a 10:1 ratio (μL DTT to A_{260} units of oligonucleotides), which is sufficient to activate the thiol groups of the oligonucleotides.³⁰ The reaction mixture was incubated for 1 h at room temperature. DTT and other reaction byproducts were removed using a NAP-10 column that was pre-equilibrated with approximately 15 mL of 50 mM sodium phosphate buffer (pH 6.0). The purified oligonucleotides were quantified by measuring absorbance at 260 nm using a Nanodrop spectrophotometer (1 mm path length light source; CCD array detector).^{31,32} The molar extinction coefficient of each oligonucleotide sequence was used to calculate concentrations (ϵ_{probe} : 698.2, ϵ_{target} : 637.9, $\epsilon_{\text{control}}$: 622.7 $\text{mM}^{-1} \text{cm}^{-1}$, Sigma-Aldrich).

AuNP-linked oligonucleotides were prepared with slight modifications to previously described methods.^{33–} Briefly, 250 μL of test concentrations (50 fM – 50 pM) of the target and control oligonucleotides were prepared in sodium phosphate buffer (pH 6.0), mixed with 100 μL of 50 nm AuNP solution, and incubated for 4 h at 37 °C. At the completion of incubation, 150 μL of 1 M NaCl/0.1 M sodium phosphate buffer (pH 7.4) and 350 μL of distilled water were added, and the samples were left to age in an incubator for 24 h at 37 °C. The samples then were centrifuged at 12,500 rpm for 45 min to remove the supernatant, and the precipitate obtained was rinsed twice with 0.1 M NaCl/0.1 M sodium phosphate buffer (pH 7.4). Samples were resuspended to the original volume of 250 μL using the same buffer solution for both SPRi and QCM analysis. The calibration plot generated for the AuNP-target oligonucleotide conjugates was used to assess the recovery when the target oligonucleotide was dissolved in various percentages of human serum.

Quartz crystal surface modification and detection

New gold-coated quartz crystals were washed with ethanol followed by distilled water and dried under N₂ gas prior to surface modification steps. Each crystal was incubated with 20 µL of thiol-activated probes for 4 h in a moist and cold environment (4 °C) following a wash with phosphate buffer (pH 7.4). The free surface was blocked using 25 µL of 5 mM MHOH, followed by a wash with distilled water after a 30 min incubation. The initial oscillation frequency was measured by QCM in solution mode. The surface capture probe-modified quartz crystals were exposed to 20 µL of AuNP-linked control oligonucleotide for 45 min and then washed with 0.1 M NaCl/0.1 mM sodium phosphate buffer (pH 7.4) to remove any unbound material from the crystal surface. Decrease in the oscillation frequency was measured on the modified quartz crystal surface. The same crystals were exposed to 20 µL of AuNP-linked target oligonucleotide (miRNA-21 mimic) and incubated for 45 min. Decrease in the frequency of the quartz crystal was measured after washing with 0.1 M NaCl/0.1 M sodium phosphate buffer (pH 7.4) to remove any unbound target oligonucleotide. Separate experiments were conducted using the target and control oligonucleotides that were devoid of AuNP conjugation to assess the signal amplification resulting from the AuNP linkage strategy.

Modification of the SPR microarray surface and detection

After an initial wash step of the SPR gold array chip with ethanol, 20 µL of thiol-activated capture probes (Scheme 1) were added to 12 spots and incubated for 4 h in a moist and cold environment (4 °C), followed by washing with phosphate buffer (pH 7.4). The free surface of the whole array was blocked by incubation with 25 µL of 5 mM MHOH for 30 min to minimize nonspecific binding interactions. The modified chip was immediately placed on the SPRi dual flow channel system. The chip-prism contact surface was filled with a layer of index matching fluid (Cargille Labs, Cedar Grove, NJ, USA). Two sample injector units and two syringe pump systems were connected to the flow channels. Real-time measurements were taken using the running buffer, 0.1 M NaCl/0.1 M sodium phosphate buffer (pH 7.4).

The running buffer was pumped at a flow rate of 50 µL/min, and a reference image of the array was taken. The dual sample injector loops (100 µL volume) then were separately filled with suspensions of target or control oligonucleotide-conjugated AuNPs in 0.1 M NaCl/0.1 M sodium phosphate buffer (pH 7.4) and allowed to pass onto the capture probe-modified array surface for 45 min. Upon completion of the incubation, the chip surface was washed for 10 min with the running buffer to remove all unhybridized oligonucleotides present in solution. The final images of the microarray after introducing target and control oligonucleotides were acquired using a CCD camera. The difference between the images taken before and after the hybridization events were obtained as SPR pixel intensity changes, which can be represented in a 3D format using ImageJ software. Gold spots treated with only the blocking reagent were used to assess nonspecific signals. Two control spots and six test spots each for detecting the target and control nucleotides were designed within the same microarray chip, which minimized spot-to-spot variations among different chips. The average SPR pixel intensities and standard deviations were calculated from the results obtained for six test spots with three replicate measurements.

Results and Discussion

Spectroscopic analysis of AuNP-linked oligonucleotides

Figure 1 shows the UV-vis spectra of unmodified AuNPs in aqueous solution and AuNPs linked with 5'-thiol modified control and target oligonucleotides in 0.1 M NaCl/0.1 M sodium phosphate buffer (pH 7.4). After linking AuNPs to the target and control oligonucleotides, the Soret band absorbance shifted from 517 nm to 526 and 524 nm, respectively. The shift could be a result of surface modifications that occurred on the AuNPs and/or the centrifugation step used to separate the AuNP-linked oligonucleotides affecting the particle size distribution.³⁶ The oligonucleotide linking to AuNPs was free of aggregate formation, as no drastic changes in the absorption band or decrease in peak intensity was detected. Aggregation of 13 nm AuNPs upon conjugation with thiolated oligonucleotides has been shown to red shift the Soret band.³⁷ Thus, the absence of notable aggregation in our system was possibly facilitated by the relatively larger AuNPs (50 nm) and/or negatively charged citrate stabilization of the AuNPs that repel particle aggregation.³⁸

Table 2 presents the results of quantitation of thiol-activated oligonucleotides before and after attachment to gold surfaces, which was performed to estimate binding efficiency. The immobilization efficiency of the surface capture probe was slightly greater for the QCM than for the SPR microarray. This can be attributed to the larger surface area provided by the gold disc of the QCM crystal (0.2 cm²) compared to the gold spots of the SPR microarray (0.008 cm²). Binding of the surface capture probe to the gold surface of the quartz crystal resulted in a frequency change of 979 ± 18 Hz, which corresponded to a mass change of 662 ± 12 ng. This correlates with the results obtained from Nanodrop analysis of the surface capture probes that were bound to the quartz crystal.

Capture efficiencies of > 80% suggest that the design of the surface capture probe on the gold surface of the quartz crystal and microarray was efficient and that the strategy for AuNP conjugation of target and control oligonucleotides was effective. The globular morphologies that appeared after modifying the SPR microarray surface with capture probes are presented as SEM images (Fig. 2A–C).

Assessment of target hybridization and signal amplification by the mass sensor

We quantified the mass changes that occurred on the quartz crystal (m) based on the observed decrease in oscillation frequency for successive modification steps and by using the Sauerbrey equation:

$$\Delta f = \frac{-2 f_o^2 \Delta m}{A \sqrt{\mu \rho}}$$

where Δf is the frequency change from the mass adsorbed, f_o is the fundamental frequency of oscillation (10 MHz), A is the geometric area of the gold disc bonded to the quartz crystal (0.2 cm²), μ is the shear modulus of the quartz crystal (2.947×10^{11} dyn cm⁻²), and ρ is the density of the quartz crystal (2.648 gcm⁻³).³⁹ The Sauerbrey equation is used to calculate

the mass bound on to the quartz crystal at a thousand times greater sensitivity compared to an electronic balance with 0.1 μg sensitivity.⁴²

The QCM showed a significant mass change when the crystal was exposed to MHOH, suggesting that this reagent has an efficient free surface blocking ability to minimize non-specific interactions. The AuNP bioconjugation with the target oligonucleotide amplified the mass changes by ~ 2.5 times in comparison to the hybridization signals without the AuNPs strategy (Fig. 3A–B). The small QCM signal responses for the control oligonucleotide suggest good selectivity and specificity of the surface capture probe to the target miRNA-21 mimic sequence. Even after linking with AuNPs, the responses were quite negligible in the control, which had five base pair mismatches relative to the target (~ 13 times less signal than the target). With the signal amplification strategy, we were able to detect the target concentrations from 0.05 to 50 pM.

Pixel intensity changes in the SPR microarray in response to target or control nucleotide hybridization with the surface capture probe

Selective hybridization of the target over the control oligonucleotide with the surface capture probe coated on the SPR gold microarray was observed, similar to the mass sensor results. Figure 4 shows the real-time response plots of the SPR imager. Measurements were made from the new steady state baseline observed after washing the microarray surface with phosphate-buffered saline (PBS) for 10 minutes, which was sufficient to remove bulk and unhybridized oligonucleotides in solution.

The blocking agent-treated SPR spots that contained only MHOH (Fig. 5) showed no nonspecific hybridization signals after exposure to the AuNP-linked target or control oligonucleotide. The dual-channel SPR microarray allowed measurement of the pixel intensity changes with the simultaneous introduction of similar concentrations of the target and control oligonucleotides. The observed changes in the pixel intensities for various concentrations of AuNP-linked target oligonucleotides (0.1 – 50 pM) are summarized in Figure 5 as 3D image representations, and line profiles. These data show that the small gold spot array surface with about 0.008 cm^2 of geometric area allows sufficient immobilization of surface capture probe molecules to hybridize with the low concentration of target oligonucleotide.

The surface capture probe demonstrated excellent selectivity for the target oligonucleotide over the control oligonucleotide (with five base pair mismatches). The observed changes in the pixel intensities for control spots were very low, illustrating that there was minimal non-specific interaction between the control oligonucleotide and the surface capture probe. The spots that were completely blocked with MHOH showed negligible non-specific binding to the target or the control oligonucleotide, suggesting no false-positive signals in the absence of the surface capture probe.

Comparison of QCM and SPRi methods

QCM is an excellent quantitation method, and it can provide high sensitivity and selectivity with use of an appropriate signal amplification strategy and surface chemistry. Results described herein are similar to those of our prior demonstration of an electrochemical QCM

immunosensor for serum insulin measurements based on magnetic nanoparticle conjugation.³⁵ The double logarithmic response plot shown in Figure 6A shows a linear correlation ($R^2=0.99$) between the mass change and concentration of the target oligonucleotide. The limit of detection (LOD)^{43,44} for QCM-based quantification was 28 fM, which is a promising detection level and indicates the uniqueness of the designed experimental conditions in achieving ultra-low detection resulting from minimal nonspecific and false-positive signals.

Although the QCM method provides sensitivity, quantitative details, and is useful for optimization of assay conditions, it is a low throughput approach (i.e., one sample at a time, and the most commonly available form is single quartz crystals). This is because designing a QCM crystal array is challenging due to engineering difficulties arising from the need to control piezoelectric properties across the array.^{45,46} Better throughput can be achieved by using the SPR microarray strategy. The advantage of using the SPR microarray is that several concentrations can be studied on the same array platform without the need for multiple experiments. Thus, samples and controls can be analyzed in the same array in one experiment. Another advantage is that the required volume of samples is low (200 nL per SPR array spot are sufficient, whereas a QCM crystal needs several μ L of sample). The use of AuNPs contributes to enhancement of the SPRi reflectivity changes due to the electronic coupling between the plasmons of AuNPs and that of the gold sensor surface upon hybridization of the nucleotides.^{47,48} The LOD for the SPRi method in this study was 47 fM. Although this LOD is slightly higher than that of QCM, our assay in a microarray format displays a better throughput and similar sensitivity.

Assessment of selectivity

A recovery test was performed to evaluate the efficiency of the QCM and the SPRi microarray in detecting the AuNP-linked target oligonucleotide marker prepared in various concentrations of human serum. Prior to conjugation with AuNPs, 1 pM of the target and control oligonucleotides were suspended in different concentrations of human serum (10, 25, 50, and 75%) diluted in PBS solution (pH 7.4). A protocol similar to that described under the Experimental methods was followed to prepare AuNP-linked target or control oligonucleotides. Table 3 presents the sample recovery results for analyzing serum samples. Recovery was calculated as a percentage from the fraction of spiked nucleotide concentration detected after subtracting the control signals. The SPR microarray offered a reasonably good recovery until 25% serum was reached (> 90%), whereas for the QCM sensor analyte recovery (> 90%) was significantly affected above 10% serum. The results obtained suggest the likely applicability of calibration plots generated from buffer nucleotide standards for the determination of nucleotide concentrations present in 10 to 25% serum samples. A lower dilution factor for serum sample analysis is preferred, as it would minimize dilution of the nucleotide marker in serum and facilitate easier detection when applied to patient samples.

Table 4 provides a short comparative summary of previous studies that measured various nucleic acids using AuNPs as a source of signal amplification. Our SPR microarray (LOD:

47 fM) and QCM (LOD: 28 fM) results are better than or comparable with the detection levels reported in prior studies.

Conclusions

We designed highly sensitive sensors that provide a platform for detecting specific oligonucleotide biomarkers at femtomolar levels by linking them with AuNPs to enhance plasmonic sensitivity. The SPR microarray offered better throughput with a comparable LOD than a QCM sensor provided. Compared to the QCM sensor, the SPR microarray allows multiple experiments to be run with suitable controls using the dual-channel syringe pump system, minimizing assay time and offering improved precision. Thus, the SPR microarray approach is promising for clinical applications to accomplish early diagnosis, treatment, and monitoring of recurrence of diseases based on circulating miRNAs as biomarkers.

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Research Highlights

- Ultra-sensitive miRNA-21 mimic nucleotide mass sensor/imaging arrays
- Comparative analysis of QCM and SPR microarray imager
- High selectivity and good sample recovery demonstrated
- Gold nanoparticles based signal amplification upon hybridization
- Microarray method development for sample analysis in complex matrices

Figure 1. UV-visible spectra of (a) AuNPs before conjugation to oligonucleotides and AuNPs linked to (b) target or (c) control oligonucleotide present in 0.1 M NaCl/0.1 M sodium phosphate buffer (pH 7.4).

Figure 2. SEM images of (A) a bare gold spot of the SPR microarray before modification and (B) the gold spot self-assembled with the thiol-activated surface capture probe. (C) Higher magnification image of (B).

Figure 3.

A. Mass changes for the gold-coated quartz crystals modified with the surface capture probe upon addition of increasing concentration (in pM) of (a) target oligonucleotide captured onto AuNPs, (b) target oligonucleotide not linked to AuNPs, (c) control oligonucleotide linked to AuNPs, and (d) the control not linked to AuNPs. **B.** Mass changes shown for (a) control and (b) target oligonucleotides (0.5 pM) with or without linking to AuNPs. Data shown are mean $\pm$ standard deviations represented by error bars for N = 3 replicates. Relative standard deviations (RSDs) for the control were 17% with no AuNPs and 22% with AuNPs linkage. The RSDs for the target were 11% (no AuNPs) and 7% (with AuNPs).

Figure 4. Real-time SPR response (average of eight array spots) for the binding of 0.5 pM of (a) target or (b) control oligonucleotide linked with AuNPs onto the surface immobilized capture probes at a flow rate of 50 $\mu\text{L}/\text{min}$. Sample injection and buffer wash points are labeled.

Figure 5.

Representations of SPRi responses: the 3D representation (left) and the line profile (right) that depicts the SPR pixel intensities for various concentrations of target and control nucleotides. The spot labeled as (a) is the bare gold surface treated with 5 mM MHOH and that of (b) are the test spots containing the immobilized capture probes. (A)-b: control oligonucleotide of (i) 0.1, (ii) 0.5, (iii) 1, (iv) 10, (v) 30, and (vi) 50 pM concentrations conjugated with AuNPs; and (B)-b: target oligonucleotide of concentrations similar to those of the control allowed to bind with the surface capture probe.

Figure 6. Double logarithmic calibration plots of (A) QCM response (control response is subtracted) and (B) SPRi response (control response is subtracted) upon hybridization of various concentrations of the AuNP-conjugated target oligonucleotide with the surface capture probe. (mean $\pm$ standard deviation for three replicates).

Scheme 1.

Fabrication procedure of the SPR microarray chip for selective detection of the target miRNA-21 mimic (T) from the control (C) oligonucleotide sequence by hybridization in a dual-channel SPRi system.

Table 1

Sequences of designed oligonucleotides.

Surface capture Probe	5'-HS-TGTCAGACAGCCCATCGACTGGTGTGCCATGAGATTCAACAGTCACATCAGTCTGATAAGCTACCCGACA-3'
Target	5'-HS-TTATGCTTATCAGACTGATGTTGA-NH ₂ -3'
Control	5'-HS-TTGGATATCACACTGATGAAGA-NH ₂ -3'

Table 2

Quantitation of oligonucleotides immobilized on the QCM or SPR gold surface and conjugated to AuNPs (N = 3 replicates).

Molecule type	Surface used to bind	Amount introduced (pmoles)	Amount bound (pmoles)	Average percentage binding
Surface capture probe (9.3 nM)	SPR microarray	461.4 ± 26.8	375.3 ± 39.6	81%
	Quartz crystal	461.4 ± 26.8	406.5 ± 41.7	88%
Target (10 nM)	AuNPs	2.48 ± 0.35	2.17 ± 0.23	87%
Control (10 nM)	AuNPs	2.52 ± 0.41	2.27 ± 0.16	91%

Table 3

Analysis of the target oligonucleotide in different percentages of serum samples (diluted in PBS, pH 7.4) using the QCM and SPR microarray.

Serum %	[Actual] (pM)	[Detected] from calibration (pM)	Recovery (%)
<u>QCM</u>			
10	1.00	0.93	93%
25	1.00	0.82	82%
50	1.00	0.76	76%
75	1.00	0.67	67%
<u>SPR microarray</u>			
10	1.00	0.95	95%
25	1.00	0.93	93%
50	1.00	0.84	84%
75	1.00	0.64	64%

Table 4

Summary of recent studies related to nucleic acid detection.

Technique	Tested molecules	Sample matrix	Limit of detection	Ref.
Multiplexed miRNA detection using AuNP-modified poly-T DNA hybridization SPR microarray	miRNA-16, miRNA-122b, miRNA-23b	0.3 M NaCl/10 mM phosphate buffer (pH 7.4)	10 fM	Fang <i>et al.</i> , 2006 ⁴⁹
DNA sensing using catalytic growth of AuNP-enhanced SPR	cDNA, ssDNA	0.3 M NaCl/10 mM phosphate buffer (pH 7.0)	4.8 pM	Yang <i>et al.</i> , 2007 ⁵⁰
AuNP-amplified SPR interfaces for the detection of mercury ions using hairpin DNA probes containing a 21mer T-rich Hg ²⁺ binding sequence loop	Hairpin DNA	TE buffer (10 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, pH 8)	1 nM	Chang <i>et al.</i> , 2011 ⁵¹
Label free multiplexed detection of miRNA using silicon photonic microring resonator arrays	miRNA-24-1	PBS	150 fM	Qavi <i>et al.</i> , 2010 ⁵²
Nanopore-based programmable oligonucleotide probe for circulating miRNA quantification	miRNA-155	1 M KCl buffered with 10 mM Tris (pH 8.0)	100 fM	Wang <i>et al.</i> , 2011 ⁵³
Gold nanoprism based localized SPR sensor for miRNA detection	miRNA-21, miRNA-10b	PBS buffer (pH 7.4) and 40% human plasma	50 fM	Joshi <i>et al.</i> , 2014 ⁵⁴
3D tetrahedral DNA nanostructure for DNA recognition and amplification by hybridization chain reaction using QCM	Synthetic DNA targets	TM buffer (10 mM Tris-HCl and 50 mM MgCl ₂ , pH 8.0)	0.1fM	Ge <i>et al.</i> , 2014 ⁵⁵
Carboxy-polyethylene glycol microarray for AuNP-based detection of miRNA using differential interference contrast microscopy	let-7a let-7b let-7f let-7g	Saline sodium citrate	10 fM	Roy <i>et al.</i> , 2016 ⁵⁶
Mass sensor	miRNA-21 mimic	Phosphate buffer (pH 7.4)	28 fM	This study
SPR microarray			47 fM	