CMOS-Compatible Silicon Nanowire Field-Effect Transistors for Ultrasensitive and Label-Free MicroRNAs Sensing

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***M**icroRNAs (miRNAs) have been regarded as promising biomarkers for the diagnosis and prognosis of early-stage cancer as their expression levels are associated with different types of human cancers. However, it is a challenge to produce low-cost miRNA sensors, as well as retain a high sensitivity, both of which are essential factors that must be considered in fabricating nanoscale biosensors and in future biomedical applications. To address such challenges, we develop a complementary metal oxide semiconductor (CMOS)-compatible SiNW-FET biosensor fabricated by an anisotropic wet etching technology with self-limitation which provides a much lower manufacturing cost and an ultrahigh sensitivity. This nanosensor shows a rapid (< 1 minute) detection of miR-21 and miR-205, with a low limit of detection (LOD) of 1 zeptomole (ca. 600 copies), as well as an excellent discrimination for single-nucleotide mismatched sequences of tumor-associated miRNAs. To investigate its applicability in real settings, we have detected miRNAs in total RNA extracted from lung cancer cells as well as human serum samples using the nanosensors, which demonstrates their potential use in identifying clinical samples for early diagnosis of cancer.*

1. Introduction

MicroRNAs (miRNAs) are a class of highly conserved short noncoding RNA molecules with approximately 18–25 nucleotides. They play an important role in a wide range of biological processes including metastasis, cell proliferation, and cell development.[1–3] More recent studies have demonstrated that some miRNAs also serve as the regulators of multiple gene expression, which is directly involved in human cancer,[4–7] such as leukemia, breast, lung, liver, and colon cancer, and function as tumor suppressors or oncogenes.[8] Interestingly, some miRNAs are found to be released from the primary tumor into the bloodstream in a stable form; therefore, circulating miRNAs in the bloodstream are considered to be promising diagnostic and prognostic biomarkers for early cancer detection and new targets for basic biomedical research.

However, because of their short length, similarity among family members, and low abundance, it has been challenging to develop rapid and label-free methods to identify the miRNA expression level.[9] Although northern blotting analysis[10–13] is regarded as the gold standard method in early miRNA profiling studies, there are several technical limitations of this method, including low throughput analysis, semiquantitative data, and requirement of a large amount of total RNA sample. Quantitative real-time polymerase chain reaction

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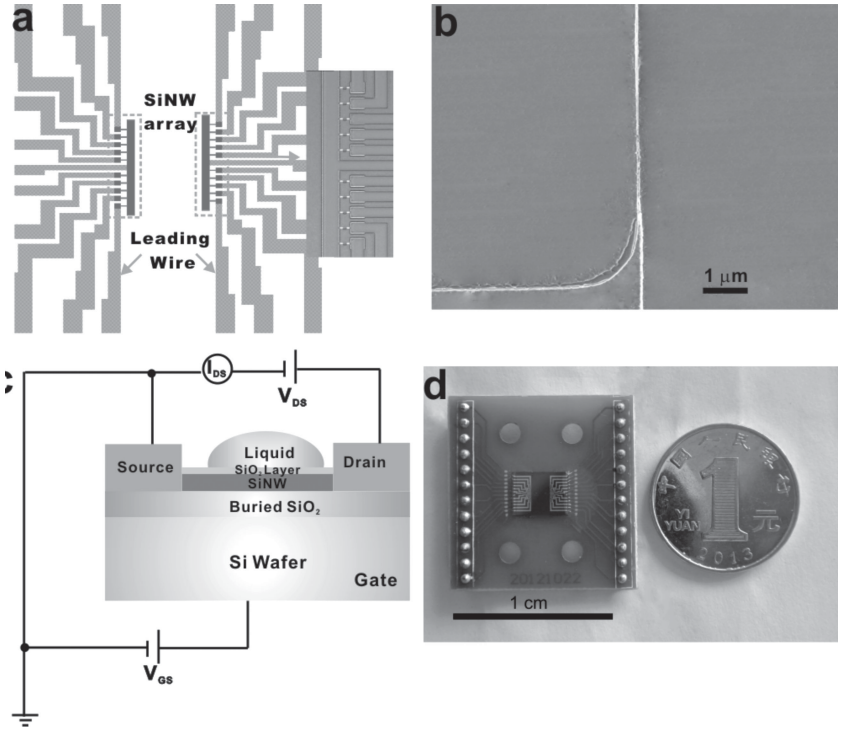

Figure 1. a) Scheme of a fabricated SiNW array on the chip. Each chip consists of two individually addressable SiNW arrays. The optical image is one addressable SiNW array highlighted by the green box. Thick blue lines represent leading wires. b) SEM image of a SiNW with the width of 80 nm, and the length of 16 μm, respectively. c) Schematic illustration of the biosensing measurement setup of a single SiNW FET device. When a voltage bias V_{DS} is applied across to the SiNW, the current I_{DS} is continuously monitored. The gate voltage (V_{GS}) is applied to the liquid on top of the SiNW. Once the charged molecules are bound onto the surface of the nanowire, it results in a variation of the current. d) Photograph of a complete SiNW-FET chip, compared to Chinese 1 Yuan coin.

(qRT-PCR)[14–16] and microarray technology[17–19] have been developed for gene expression quantification; however, they suffer from error-prone amplification, time-consuming steps, and poor sensitivity and inaccuracy resulting from cross hybridization and nonspecific adsorption. Recently a variety of new strategies have been devoted to developing analytical methods for miRNA analysis with higher sensitivity and better reliability, including electrochemical transduction,[20,21] surface plasmon resonance (SPR),[22] nanotechnology-based amplification,[23–26] and enzymatic-based methods.[27] However, most of these methods still require enzymatic labeling or nanoparticle conjugations that involve complicated and labor-intensive experimental steps.

Field-effect transistors (FETs) provide an attractive platform to analyze biological samples owing to their ability to directly translate the interaction with target molecules taking place on the FET surface into readable electronic signals (detected directly), which allows for a more rapid and convenient sensing detection. One-dimensional silicon nanowires, configured with FETs, have emerged as promising biosensors because of their high sensitivity and selectivity, real-time response, and label-free detection capabilities,[28] coupled to their manufacturability for mass production and benefiting from commercial semiconductor developments. A diversity of FET-based biosensors has been employed for the specific and sensitive detection of nucleic acids,[29–31] proteins,[32,33] viruses,[34] protein interactions,[35] and cells.[36,37] Thanks to their excellent properties, SiNW FETs are especially appropriate for miRNA analysis allowing for direct detection at their expression levels. Zhang et al. have demonstrated a concept of a label-free miRNAs assay with silicon nanowire biosensors fabricated using deep ultra-violet (DUV) lithography,[38,39] which achieved a detection limit of 1 fM. However, it is challenging to develop even better miRNA sensors and simultaneously reducing the manufacturing cost and improving the sensitivity. Herein, we develop a complementary metal oxide semiconductor (CMOS)-compatible SiNW-FET biosensor fabricated by an anisotropic wet etching technology with self-limitation that can lower the production cost as well as enables production at the large scale. This device displays ultrasensitive, label-free, and rapid detection for cancer-associated miRNAs (miR-21 and miR-205, upregulated in human cancerous tissues) with an ultrahigh sensitivity at the 1 zeptomole level (ca. 600 copies) and excellent selectivity for distinguishing single-nucleotide mismatched sequences. This nanosensor also enables the direct detection of miRNAs in lung cancer cells and total human blood serum.

2. Results and Discussion

SiNWs were manufactured on silicon-on-insulator (SOI) wafers by a top-down fabrication process based on our previously published protocol, in which conventional photolithography was combined with anisotropic wet etching with a self-limitation of Si<111> plane that has a much slower etching rate in tetramethylammonium hydroxide (TMAH) or other alkaline solution.[30] As illustrated in **Figure 1**a, a multi-channel SiNW chip, containing two groups of individually addressable SiNW arrays, was patterned by photolithography and anisotropic wet etching using TMAH. The microscopic image shows an individual SiNW array, consisting of 10 nanowires, which shared the same contact line at one side. The nanowire is 80 nm in width and 16 μm in length (Figure 1b). At the topside, a 20-nm thick thermally oxidized silicon oxide was deposited as an electrically insulating layer on the transistors.

Figure 1c shows a sketch of the biosensing measurement setup of a SiNW FET device. A voltage bias is used to apply the source-drain voltage V_{DS}, and the current I_{DS} is continuously monitored. The gate voltage (V_{GS}) is applied to the liquid on top of the SiNW, so that as the charged molecules bind onto the surface this results in a variation in the current. After the fabrication processes, the wafer was cut and each

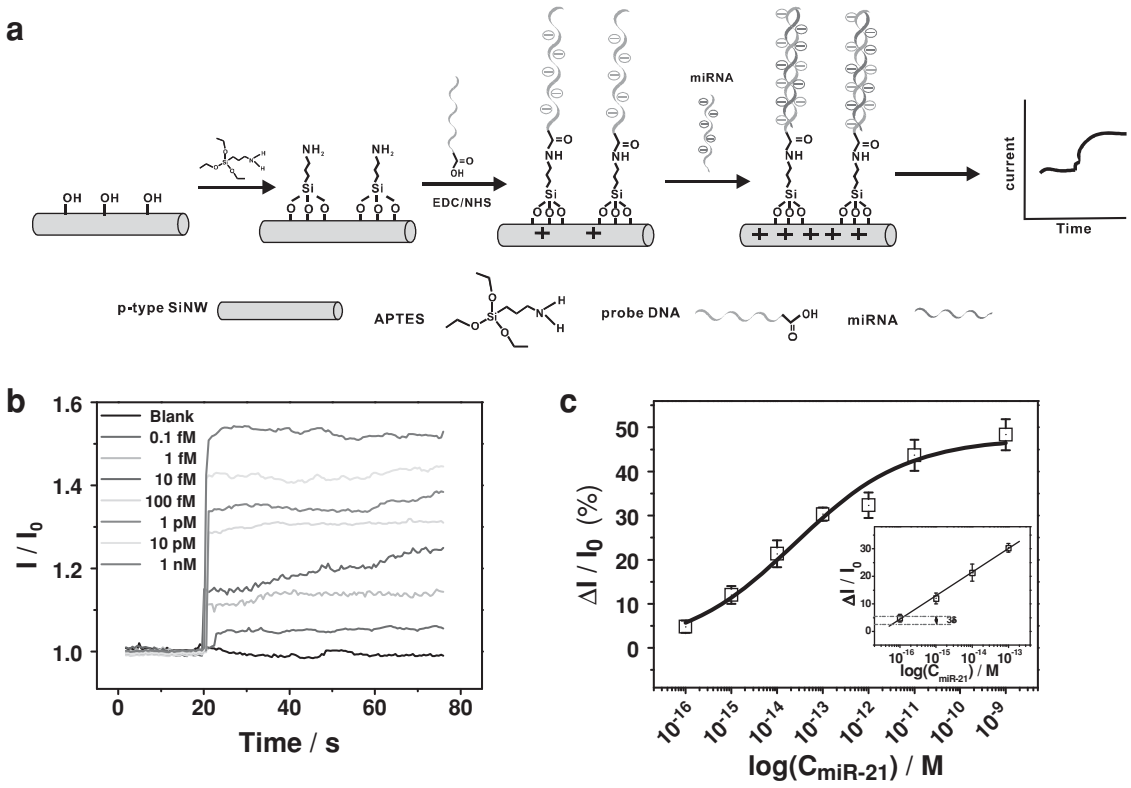

Figure 2. Detection of miRNA using a SiNW-FET device. a) Schematic illustration of rapid, label-free, and electrical direct detection of miRNA molecules. b) Real-time measurements of miR-21. Current vs.time data recorded for the concentrations of blank, 0.1 fM, 1 fM, 10 fM, 100 fM, 1 pM, 10 pM, and 1 nM solutions, respectively. c) A calibration curve for miR-21 detection. The error bars represent standard deviations for measurements obtained from three experiments. The inset shows a linear calibration curve for target concentrations lower than 100 fM. The red dashed lines indicate the limit of detection (LOD) obtained from three-times the standard measurement error bar of the baseline noise. The SiNW arrays used were both p-type and boron doped.

chip was glued and gold-wire-bonded into a printed circuit board (PCB), which was almost the size of a Chinese 1 Yuan coin (Figure 1d).

Figure 2a illustrates the sensing mechanism of the SiNW nanosensors for the detection of miRNA molecules. By using saline chemistry, silanol groups were converted to amino groups in an (3-Aminopropyl)triethoxysilane (APTES) solution. After the condensation with the help of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-Hydroxysuccinimide (NHS), a monolayer of carboxyl-modified DNA capture probes was covalently assembled onto the surface of SiNWs via amino groups. Under optimal conditions, the target miRNA hybridized with the probe DNA, which increased the negative charge on the SiNW surface, leading to an accumulation of carriers and corresponding increase in the conductance in the p-type SiNWs.

Prb21 with a complementary sequence to miR-21 (Table S1 in Supporting Information) was used as the probe to miR-21, a series of different concentrations of miR-21 were measured in real time in the buffer solution, ranging from 0.1 fM to 1 nM (Figure 2b). The current increased with each addition of miR-21 at all concentrations, primarily owing to the increased negative charge induced by DNA/miRNA hybridization, which resulted in the depletion of carriers in the case of p-type nanowires. Here, we define a relative change in current for the response of the device to the target miRNA: $\Delta I/I_0$, where ΔI is the change of the current in the assay, and I_0 represents the value of the initial current ($t = 0$). A calibration curve for the miR-21 detection was obtained (Figure 2c), in which the relative change of the current was plotted as a function of the logarithm of miR-21 concentration. From the inset of Figure 2c it can be seen that the curve was linear for miR-21 concentrations lower than 100 fM. We calculated the limit of detection (LOD) to be 0.13 fM from a sensor response that was equal to three times the standard deviation of the baseline noise. Considering the sample analysis volume (ca. 8 μL), this concentration corresponds to a smallest detectable absolute amount of about 1 zeptomole, which is equal to about 600 copies of target miRNAs. This sensitivity is almost 20 times higher than that of a previously reported FET-type miRNA sensor.[38] One reason for this is that the modulation efficiency of the structure of our triangular nanowire was much higher than that of other structures, for instance, that of a rectangular solid (Figure S1, Supporting Information). Moreover, our device was used in the subthreshold regime, which has been confirmed to retain the highest signal response for back-gated SiNW-FET sensors.[40]

Also, importantly, the nanosensor response was less than one minute for miRNA detection, which is more suitable for point-of-care testing as opposed to other assays that take much longer (e.g., PCR, microarray technology). We also detected miR-205 with our SiNW nanosensor using

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the probe Prb205 separately (Figure S2a, Supporting Information). Figure S1b shows the signal response as a function of the miR-205 concentration range from 0.1 fM to 1 nM (Figure S2b), which demonstrated the capability of multiplex detection.

From over 1500 human miRNA genes that have been identified,[41] many of the miRNA family have similar sequences with only one or two base mismatches. For example, miR-15 and miR-16 are structurally closely related to miR-21. The alignment of their sequences has a strong similarity in the 5′ region (seeding region), which is important for binding to the 3′UTRs (untranslated regions) of the mRNA targets. We investigated the specificity of our nanosensor with miR-15, miR-16, and one-base mismatched sequence of miR-21 (miR-21–1MM). When miR-16 was flowed through the sensor surface, there was essentially no significant change of the electrical current (**Figure 3**a). However, when a mixture of 1 nM miR-15, miR-16, and miR-21 was introduced, there was a rapid decrease in the electrical current. Similarly, in the presence of only miR-15, the device also did not lead to an obvious current response (Figure 3b). When one-base mismatched sequence miR-21–1MM was introduced instead of miR-21, the relative current decreased by as much as 17% (Figure 3c), indicating that the nanosensor can effectively discriminate single-base mismatches (Figure 3d).

To further investigate the precision of the sensor, we surveyed dehybridization and subsequent rehybridization of miR-205 on the sensor by a continuous fashion. As illustrated in **Figure 4**a, hybridization of miR-205 leads to a rapid increase in the current, in good agreement with our previous results (Figure S2a, Supporting Information). After the response stabilized, an urea solution was introduced to effect dehybridization and remove the dehybridized strands from the surface. This caused a rapid decrease in the current owing to the denaturation of the urea solution. In order to confirm the reusability of the sensor, we switched between hybridization with the target miR-205 and dehybridization with urea for three cycles and found no sensor damage, suggesting that the sensor could be used repeatedly.

This ultrasensitive and label-free nanosensor should be well suited for rapid, direct, and PCR-free sensing of miRNA expression profiled in biological samples. We therefore went on to perform miRNA detection in real samples by analyzing miR-21 in total RNA extracted from lung cancer cell lines and normal human lung tissues. The total RNA samples were diluted with ribonuclease (RNase)-free water and then applied to a Prb-21 functionalized SiNW device. As shown in **Figure 5**a, the signal response of A549 cell lines was obvious larger than that of normal human lung tissue when total RNA was flowed through the surface. By using the SiNW device, different amounts of total RNA were analyzed. The expression level of miR-21 in the A549 cell was significantly higher than that in normal human lung tissue (Figure 5b). The finding of upregulated expression of miR-21 is consistent with that found in previously reported literature.[5,7] We found that the SiNW device could detect miR-21 levels as low as 10 ng of total RNA from lysed cells.

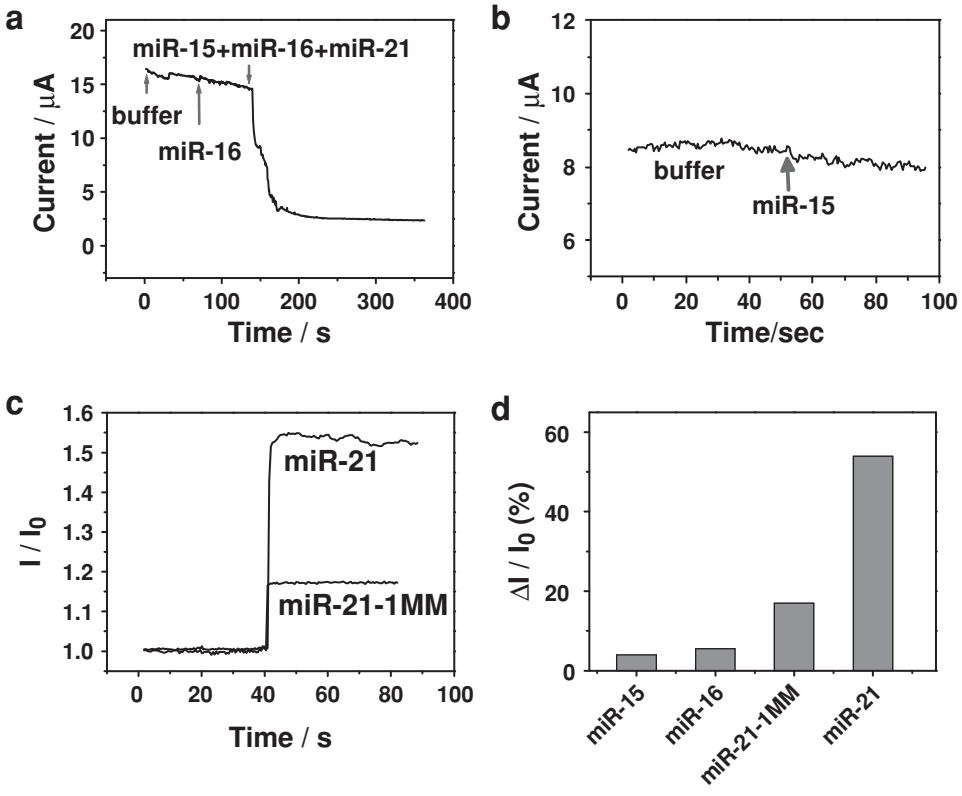

Figure 3. Specificity of miRNA detection. a) Current versus time curve recorded when different solutions flowed through the sensor surface. The injected solutions were buffer, miR-16, and a mixture of miR-15, miR-16 and miR-21, respectively. The red arrows correspond to the points where the solutions were introduced. b) Plot of current versus time for miR-15. c) Selectivity of SiNW-FET biosensor for one-base mismatched sequence of miR-21 (miR-21–1MM) and full-complementary sequence (miR-21). d) The relative change of electrical signals obtained for miR-15, miR-16, miR-21, and miR-21–1MM. The nanowires used were n-type and phosphorus doped in (a,b), and p-type and boron doped in (c).

We next challenged the SiNW sensor for the analysis of miRNA in serum samples. miR-205 was diluted to different concentrations with 100% healthy human serum. As shown in Figure 5c, the nanosensor's response to serum showed little change as compared to that in buffer solution, suggesting the high robustness of the nanosensor in the high protein-containing background in serum. Subsequently, miR-205 at concentrations of 0, 100 fM, 10 pM, and 1 nM was introduced onto the surface of the device, and the current was monitored in real time. In the p-type sensor, the

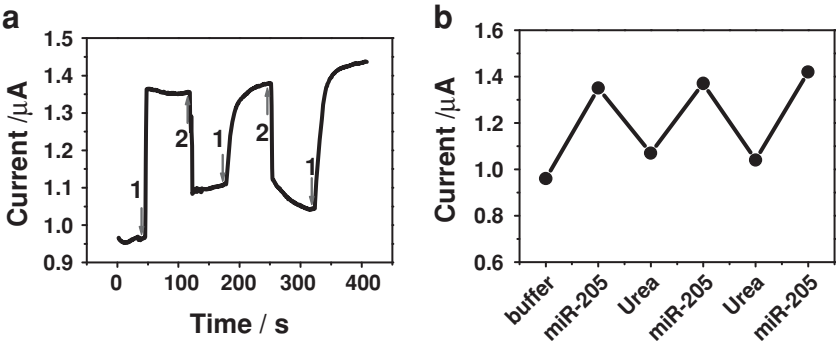

Figure 4. a) Real-time measurement of hybridization, dehybridization, and rehybridization of miRNA continuously with the SiNW sensor, where region 1 represents 1 nM of miR-205, and region 2 represents 8 M of urea. b) The recyclability of the sensor. The nanowires used were p-type.

current increased stepwise after the injection of serum samples (Figure 5c). We found that this SiNW sensor could detect levels as low as 100 fM miRNA in serum samples (Figure 5d), which clearly demonstrated its applicability in real-world assays.

Our SiNW-based biosensor provides an ultrahigh sensitivity for miRNA sensing at the zeptomole level (ca. 600 copies), improving the analytical sensitivity by almost 20 times that previously reported for a FET-type miRNA sensor.[38] That is to say, our sensor permits the direct detection of low-abundance miRNA biomarkers (down to around 600 copies), which is a desirable quality for miRNA-based diagnostics. Moreover, our biosensor is more favorable as it requires only a one-step fabrication without the need for multiple self-assembly steps in amplification-based assays or complicated operations in enzyme-based methods.[23,24,27,42] In addition, the experimental cost is an essential factor that must be considered when designing and fabricating nanoscale biosensors. Our SiNW devices are fabricated by conventional optical lithography combined with anisotropic wet etching, which is much cheaper than other technologies (e.g., DUV lithography). The dynamic range is also a significant factor for practical diagnosis. Many sensitive miRNA sensors are intrinsically limited by their small dynamic ranges (even as low as 1–2 orders of magnitude);[43] however, the large dynamic range of our sensor spanning 8 orders of magnitude from the 0.1 fM level to 1 nM or even higher offers an opportunity to screen miRNAs that often exist in diverse cells.[15]

The most widely used formats for miRNA detection and profiling includes northern blotting, microarray, and qRT-PCR; however, these methods are limited to RNA labeling, amplification experimental steps, or data normalization. In contrast with the other sensing technologies, our SiNW-FET sensor reported in this study shows several combined advantages. First, it provides an ultrahigh sensitive approach

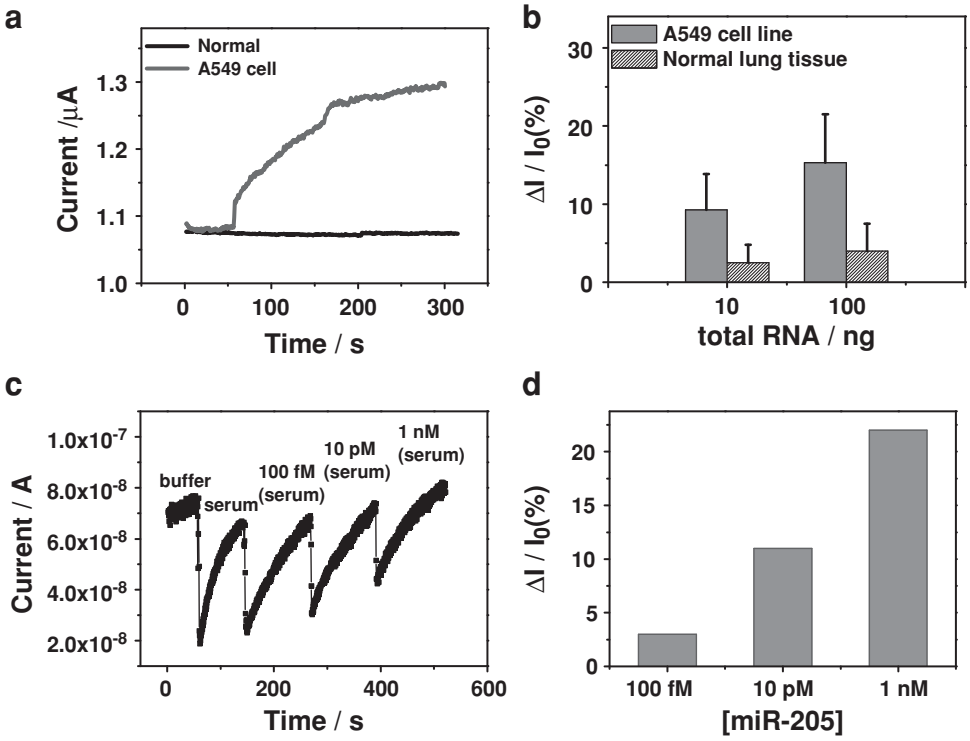

Figure 5. Analysis of miRNA in biological samples. a) Real-time current response to the total RNA (100 ng) extracted from A549 cell lines and normal lung tissue with the Prb21-functionalized SiNW device. b) Detection of miR-21 with different amounts of total RNA from A549 cell lines and normal lung tissue. c) Sensor real-time response to miR-205 in 100% human serum. d) The relative changes of electrical signals to different levels of miR-205 in 100% human serum. The nanowires used were n-type.

for quantitative detection of zeptomole miRNAs with good sequence specificity. Importantly, the extremely low LOD can be achieved without any signaling groups or PCR amplification steps, which opens new avenues for miRNA detection. Second, this sensor can be implemented into rapid and direct electrical sensing chips, coupled with its excellent measurement properties, which could be beneficial for future biomedical applications (e.g., point-of-care testing). Third, our SiNWs can be manufactured in high yield via reproducible conventional silicon technology, allowing for large-scale production and the integration of complex sensor applications; which, again, is low-cost and commercially available. Overall, the SiNW-FET biosensor can provide a solution to directly detecting miRNA members and has the capacity to advance the applications of miRNA biomarkers in the early diagnosis of diseases.

3. Conclusion

We developed CMOS-compatible SiNW-based biosensors fabricated by an anisotropic wet etching technology with self-limitation and at much lower fabrication costs. It enabled an ultrasensitive, label-free, and rapid (<1 min) detection of miRNA members and family members, which demonstrated an ultrahigh sensitivity (a LOD of 1 zeptomole, ca. 600 copies) of miRNA detection, which has an enhancement of about 20 times that previously reported for a FET-type miRNA sensor.[38] Our sensors also showed good specificity of discriminating between one-base mismatched miRNA. We also analyzed miRNA in total RNA extracted from lung cancer cells and in total human serum samples using this device. In the future, we expect that these SiNW nanosensors can be incorporated in a more complex design of multiplexed arrays for high-throughput miRNA detection, which holds promise for future biomedical applications and early screening and diagnosis of cancers.

4. Experimental Section

Materials: Probe DNA samples were custom-synthesized and purified by Shanghai Sangon Bioengineering Technology and Services Co., Ltd. (Shanghai, China). All miRNA sequences were synthesized and purified by Life Technologies Co. (Shanghai, China). The sequences of nucleic acids are listed in Table S1 in the Supporting Information.

Surface Functionalization of SiNWs: Based on the protocol described in our previous work,[36] surface functionalization of the SiNW array was performed by employing APTES to convert the surface silanol groups to amines followed by reaction with carboxyl-modified single-stranded DNA (ssDNA).

Electrical Detection: DNA/miRNA hybridization was incubated in a buffer of 0.01× phosphate buffered saline (PBS) (pH 7.4) at room temperature. Electrical measurements for all sensing experiments were carried out using a Keithley 4200 semiconductor parameter analyzer (Keithley Instruments Inc., Cleveland, OH). In all sensing experiments, we used V_{DS} = +1 V, V_{GS} = −1 V for n-type SiNW devices, and V_{DS} = −1 V, V_{GS} = +1 V for p-type SiNW devices, respectively. The devices used for functionalized-sensing experiments were similar, with widths of approximately 100 and 200 nm. In the concentration experiments, the current was monitored in real time when a series of different concentrations of target miRNAs were introduced into the SiNWs device.

Dehybridization of DNA-RNA Duplexes: An 8 M urea aqueous solution was applied to dehybridize DNA-RNA duplexes on our biosensor.

Total RNA Samples: Total RNA of A549 cells was extracted using TRIzol Reagent according to the literature's protocol by Invitrogen (Shanghai, China). Human lung total RNA as a control sample was purchased from Life Technologies Co. (USA).

Serum Assays: Sera were taken from healthy donors. In serum assays, miR-205 was spiked to 100% human serum and diluted to different concentrations, 100 fM, 10 pM, and 1 nM. Measurements were carried out using the same protocol as in the buffer solution.

Supporting Information

Materials and reagents, surface functionalization of SiNWs, and detection of miR-205 are available as Supporting Information. Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

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