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Label-Free and Rapid Electrical Detection of hTSH with CMOS-Compatible Silicon Nanowire Transistor Arrays

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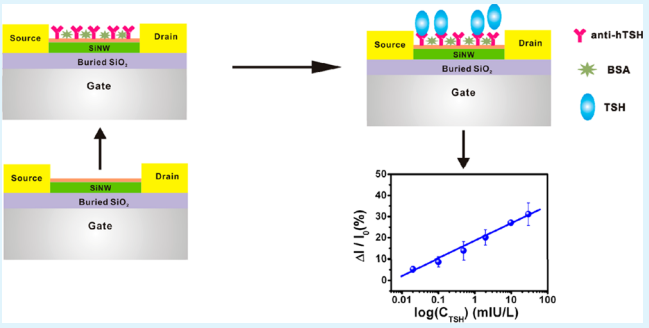
ABSTRACT: Now a human thyroid stimulating hormone (hTSH) assay has been considered as a screening tool for thyroid disease. However, some existing methods employed for in-hospital diagnosis still suffer from labor-intensive experimental steps, and expensive instrumentation. It is of great significance to meet the ever growing demand for development of label-free, disposable, and low-cost productive hTSH detection biosensors. Herein, we demonstrate a novel sensing strategy for highly sensitive and selective immunodetection of hTSH by using a CMOS-compatible silicon nanowire field effect transistor (SiNW-FET) device. The SiNW chips were manufactured by a top-down approach, allowing for the possibility of low-cost and large-scale production. By using the antibody-functionalized SiNW-FET nanosensors, we performed the label-free and rapid electrical detection of hTSH without any nanoparticle conjugation or signal amplifications. The proposed SiNW biosensor could detect hTSH binding down to a concentration of at least 0.02 mIU/L (0.11 pM), which is more sensitive than other sensing techniques. We also investigated the influence of Debye screening with varied ionic strength on hTSH detection sensitivity, and real-time measurements on various concentrations of the diluted buffer. The simple, label-free, low-cost, and miniaturized SiNW-FET chip has a potential perspective in point-of-care diagnosis of thyroid disease.



KEYWORDS: *human thyroid stimulating hormone (hTSH), silicon nanowires (SiNWs), biosensor, COMS-compatible, label-free*

1. INTRODUCTION

Disease prevention is becoming increasingly important because of the worldwide rise in prevalence of chronic diseases and deaths from these diseases.[1] Affordable healthcare becomes a global issue with special concern. Diagnostics play crucial roles in healthcare in the developing countries and source-poor regions in the developed countries, and the probability of the potential utility for point-of-care (POC) diagnostics is even higher.[2] Therefore, the demand for rapid, cost-effective, miniaturized, and portable biosensing devices is ever growing. During the past decades, some diagnostic techniques have widely been developed for biomedical applications, such as qRT-PCR, enzyme-linked immunosorbent assay (ELISA), and nanomaterials-based biosensors.[3−5] However, most of them suffer from the requirements of target-labeling, expensive instrumentation, as well as unminiaturization. It is challenging to develop low-cost, label-free, disposable, and portable biosensing chips for POC diagnostics.

As a high-effective manner, biosensors that can directly transduce a biological binding event or biological interaction into an electronic signal are favorable for digital readout in sensing technologies. Electrical transducers, such as electrodes, piezoelectrics, and field effect transistors (FETs), offer a series of qualities of miniaturizability, low-power requirement, and mass production and are attractive in biomedical sensing applications.[6−8] Among these transducers, FETs alone provide some further advantages, such as rapid and real-time monitoring in label-free electrical detection, multiparameter readout, and signal amplification.[9−11]

One-dimensional (1D) semiconducting nanomaterial-based FET biosensors, such as silicon nanowire (SiNW) and carbon nanotube (CNT) devices (referred to SiNW-FETs and CNT-FETs), have attracted tremendous attention as a promising tool for biosensors because of their capabilities of excellent high sensitivity, good selectivity, label-free, and real-time response. Although CNT-FETs were at the forefront of the exploration of biological applications,[8,12−14] some shortcomings were encountered in the fabrication and applications of CNT-FETs. Benefiting from mature silicon industry fabrication technologies, SiNWs have been proven to be a promising material for well-controlled technique, high reproducibility, as well as its manufacturability for mass production. To date, a variety of SiNW-FET biosensors have been used for application of the broad fields, such as detection of nucleic acids and proteins,[15−18] characterization of small-molecule−protein interactions,[19] cell detection,[20] and virus detection.[21] Because of

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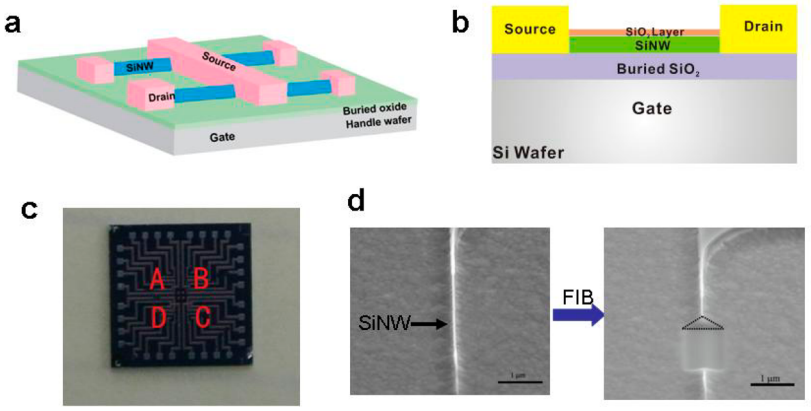

Figure 1. (a) Schematic diagram of one SiNW array biosensor. Each cluster contains four nanowires with shared source electrode and individual drain electrode. The back-gate voltage is applied to the handle wafer. (b) Schematics of a cross section of SiNW-FET device. (c) Optical image of a SiNW biosensor chip with four clusters. (d) SEM micrograph of a 60 nm wide SiNW with the length of 10 μm (left). After FIB etching, a triangular cross section of the nanowire was observed (right).

the ultrahigh sensitivity attributed to their small size and large surface-to-volume ratio, SiNW-FETs are an optimal tool for sensing of abnormal concentrations of biomarkers.[22−26] For instance, Zheng and co-workers developed multiplexed electrical detection of protein cancer markers, including prostate specific antigen (PSA), carcinoembryonic antigen (CEA), and mucin-1 with SiNW-FET sensor arrays, with detection to at least 0.9 pg/mL in undiluted serum samples.[22] Stern et al. reported a microfluidic purification chip system to pre-isolate blood samples and release them, followed by detection of PSA and carbohydrate antigen 15.3 (CA15.3).[23] Chua and co-workers used a complementary metal−oxide semiconductor (CMOS)-compatible SiNW array to detect human cardiac troponin-T as low as 30 fg/mL in an undiluted human serum environment.[24]

Thyroid stimulating hormone (TSH), as an α/β heterodimeric glycoprotein hormone synthesized and secreted from the anterior pituitary gland, regulates the edocrine function of the thyroid gland. The TSH level reflects the metabolic activities in the human body. Now, a TSH assay has been considered as a screening tool for thyroid disease.[27] So far, there have been some traditional analytical techniques used for TSH analysis, including radioimmunoassay (RIA),[28] time-resolved immunofluorometric assay (TRIFA),[29] electrochemiluminescence immunoassay (ECLIA),[30] chemiluminescence immunoassay (CLIA),[31] ELISA,[32] homogeneous particle based immunoassays,[33] and other techniques.[34,35] Despite that some of them have been used for in-hospital analysis, they still suffer from long assay time, labor-intensive experimental steps, and expensive instruments, which cannot meet the need for POC testing and quick screening applications. Therefore, it is of great significance to develop new methods for label-free, low cost, and disposable TSH detection.

In this work, we have developed a novel sensing strategy for rapid and simple immunodetection of human thyroid stimulating hormone (hTSH) without any nanoparticle conjugations and signal amplifications by using CMOS-compatible SiNW-FET devices. The proposed devices were manufactured by a top-down approach with anisotropic wet etching allowing for the possibility of large-scale production. By using the antibody-functionalized SiNW-FET nanosensors, we performed the label-free, rapid, and real-time electrical detection of hTSH with the high sensitivity and specificity. Furthermore, we also investigated the effect of Debye screening with varied ionic strength of buffer solution on hTSH detection. In this study, the label-free, low-cost, and miniaturized SiNW-FET chip has demonstrated a promising perspective in quick screening and POC diagnostics of thyroid disease.

2. MATERIALS AND METHODS

2.1. Materials. 3-Aminopropyltriethoxysilane (APTES), glutaraldehyde, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, Inc. and used as received. hTSH was obtained from Scripps Laboratories Inc. (USA) and diluted to 100 mIU/L with 7.5% BSA solution. Antibody anti-hTSH was purchased from Medix Biochemica (Finland). The proteins were diluted to the desired concentrations with the assay buffer.

2.2. Fabrication of the SiNW Device. The SiNW array chips were fabricated by silicon-on-insulator (SOI) wafers with light boron adulteration of 5×10^{15} cm^{-3} (SIMOX) using a top-down approach. Conventional optical lithography and anisotropic wet etching with tetramethylammonium hydroxide (TMAH) as previously published protocols[16] were used to etch SiNWs. Ion implantation of boron or phosphorus with a concentration of 10^{19} cm^{-3} was performed to form an effective contact region. A structure of a nitride-oxide-nitride stack formed by plasma enhanced chemical vapor deposition (PECVD) was deposited to enable operation of liquid solution onto the device. The fabricated silicon chip was mounted on a testing printed circuit board (PCB), and the electrical interconnections between the chip and the PCB were realized through gold wire bonding.

2.3. Functionalization of the SiNW Surface. The SiNW chips were first cleaned with a Piranha solution at 90 °C, and then, 2% APTES was used to generated amino groups onto the surface of nanowire. After being thoroughly washed with absolute ethanol, the chips were immersed in 2.5% glutaraldehyde, converting the amine-terminated surface to an aldehyde-terminated one. Next, freshly prepared specific antibodies (anti-hTSH) were covalently immobilized onto the surface via an amino−carboxyl reaction. To reduce nonspecific absorption, 1% BSA was used to block active sites. Finally, hTSH was introduced for real-time sensing measurement.

2.4. Electrical Measurements. Electrical measurements of SiNWs currents were performed by a Keithley 4200 semiconductor parameter analyzer (Keithley Instruments Inc., Cleveland, Ohio). In the sensing, a drain−source voltage of 1 V and a gate voltage of −1 V were applied to the SiNW devices. hTSH was incubated in a buffer of 0.01 × PBS (pH 7.4) at room temperature.

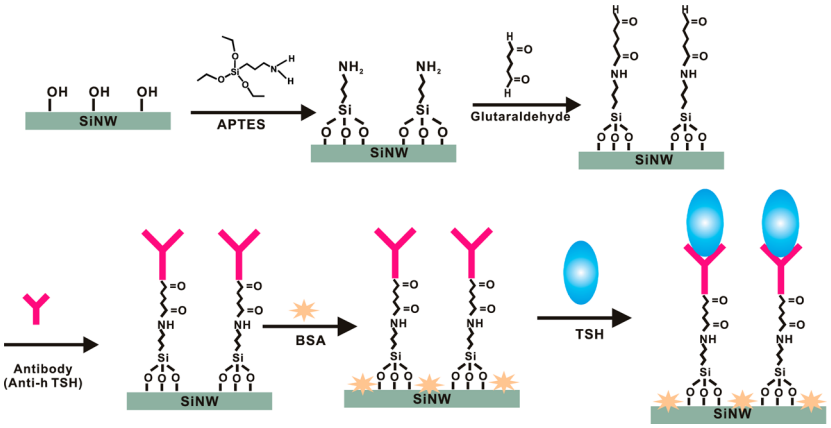

Figure 2. Schematic representation of functionalization and detection procedure of SiNW-FET sensing platform for detection of hTSH.

3. RESULTS AND DISCUSSION

3.1. Fabrication of SiNW Devices. In the work, SiNWs biosensor chips were fabricated by a top-down fabrication approach as previously published protocols[16] using silicon on insulator (SOI) wafers, which are fully compatible with CMOS technology. SiNWs were developed by conventional optical lithography combined with anisotropic wet etching using tetramethylammonium hydroxide (TMAH), employing a self-stop limitation owing to the slower etching speed of the Si(111) plane than other planes. As illustrated in Figure 1a, each cluster consists of four nanowires allowing for multiplexed detection, sharing the common source electrode (D) and individual drain electrode (S). The back-gate voltage is applied to the handle wafer. Figure 1b shows the schematics of the cross section of a device. Figure 1c shows an optical image of a SiNW sensor chip, which contains four arrays (A, B, C, and D), each has four nanowires. Figure 1d shows an SEM image of SiNW with the width of about 60 nm and the length of 10 μm. After etching of the focused ion beam (FIB), we clearly observed the triangular cross section, in agreement with anisotropic etching with self-stop limitation.

3.2. Detection of hTSH with SiNW Chips. In this present study, the SiNW nanosensors for detection of hTSH were demonstrated by covalently immobilizing antibodies anti-hTSH on the surface of the FETs, as shown in Figure 2. The native oxide layer coating on nanowire surfaces is usually used to be functionalized with bioreceptor molecules. First, amino termination was produced by conventional saline chemistry using APTES. Second, glutaraldehyde was modified onto the nanowire surface that converts the amine-terminated surface to an aldehyde-terminated one. Subsequently, freshly prepared specific antibodies (anti-hTSH) were covalently immobilized onto the surface via an amino−carboxyl reaction. In order to reduce nonspecific absorption of the surface, 1% BSA was used to block nonspecific sites. Finally, hTSH in diluted buffer solutions were used for electrical testing. The functionalized SiNW chips were employed for electrical testing by a semiconducting parameter analyzer.

In immunologically modified FET devices, the antibodies anti-hTSH immobilized onto the surface of the FETs were employed to measure the concentrations of the corresponding antigen hTSH. In an n-type SiNW-FET, upon the introduction of the target antigen hTSH, the interactive binding between the negatively charged analytes and the corresponding antibody will result in the depletion of charge carriers in the current channel and give rise to the increasing current in the device. The magnitude of the current variation indicates the concentration of the target antigen hTSH.

In order to develop a label-free SiNW biosensor with ultrahigh sensitivities of hTSH detection, various concentrations ranging from 0.02 to 30 mIU/L of hTSH diluted with 0.01 × PBS buffer were performed, while the current (I_{DS}) was monitored in real-time. As shown in Figure 3a, the current

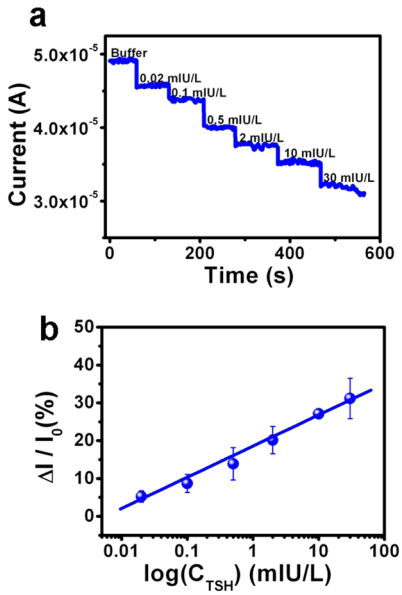

Figure 3. (a) Time course of current for an anti-hTSH-modified SiNW-FET device. The target hTSH were introduced at various concentrations of 0, 0.02, 0.1, 0.5, 2, 10, and 30 mIU/L. (b) Sensitivity vs logarithm of hTSH concentration.

decreased stepwise upon the injection of each concentration of hTSH. The decrease of the current upon hTSH addition indicates a binding event of negatively charged species to the nanowire, which is consistent with the negative charge of hTSH (PI ~ 6.9)[36] at the pH of 7.4 in our experiment.

To allow for a more quantitative analysis, we plotted the calibration curve of our device, with the sensitivity of the device being defined as

$$\text{sensitivity} = \frac{I_0 - I}{I_0} \quad (1)$$

Figure 3b shows that the net change of the current has a linear relationship with the logarithm of hTSH concentration. 0.02, 0.1, 0.5, 2, 10, and 30 mIU/L solutions of hTSH resulted in a current decrease of 6, 10, 17, 21, 28, and 33%, respectively. It was found that the biosensor can detect hTSH down to a concentration of at least 0.02 mIU/L of hTSH. Moreover, the nanosensor shows a rapid response of hTSH at a short time less than 1 min, showing good potential in the high-throughput screening.

From the experimental results in Figure 4a, the concentration above 10 mIU/L demonstrated a saturated amount of net

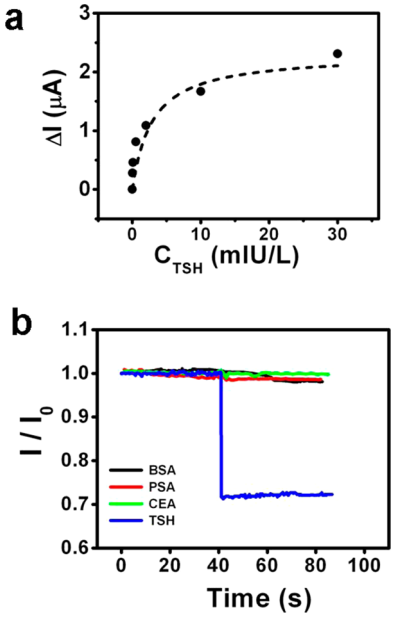

Figure 4. (a) Amount of net change of the current as a function of hTSH concentration. The dashed curve shows a fit to the Langmuir adsorption isotherm. (b) Specificity and background effect of noncognate proteins on the surface. Time course of current for 10 mg/mL of BSA, 100 μg/mL of PSA, 100 μg/mL of CEA, and 1.6 ng/mL (10 mIU/L) of TSH.

source−drain current change. This indicated that the absorption of hTSH molecules onto anti-hTSH antibodies on SiNW surfaces follows the Langmuir adsorption isotherm, which is given by

$$\Delta I = \frac{\Delta I_{max} C_{hTSH}}{K_d + C_{hTSH}} \quad (2)$$

We estimate that the dissociation constant, K_d, according to the Langmuir adsorption isotherm, where ΔI is the net change of source−drain current, ΔI_{max} is the amount of saturated net change in the current, K_d is the dissociation constant of the reaction between hTSH molecules and antibodies, and C_{hTSH} is the concentration of hTSH protein, respectively. From fitting in Figure 4a, we got K_d and ΔI_{max} were estimated to be 7 pM (1.23 mIU/L) and 2.1 μA, respectively. Pekary et al. developed a radioimmunoassay for hTSH using radiation labeling, and their dissociation constant was 2.3 pM.[37] Compared with the reported results, the dissociation constant of the reactions between antibodies and antigens in our nanosensor is very close to the reported one, indicating a good affinity of hTSH immuno-reaction on the nanowire channels.

To explore the specificity and background effect of nonspecific binding of noncognate proteins on the surface, some control experiments of real-time responses were carried out by using anti-hTSH functionalized SiNW biosensors. As shown in Figure 4b, a negligible response was observed after introduction of 10 mg/mL BSA, whereas subsequent addition of hTSH solution produces an obvious current drop, suggesting that the nonspecific absorption of BSA molecules, even at a very high concentration level, could be neglected in the measurement. Another two glycoproteins, 100 μg/mL of PSA and CEA, were also applied to the hTSH antibodies-modified SiNW chips, respectively. Similarly, an unobvious response was observed while PSA and CEA were injected to the functionalized nanowire surface, respectively (Figure 4b). The results indicate that the anti-hTSH functionalized SiNW biosensor shows nonspecificity to BSA, PSA, and CEA, but good specificity to hTSH.

3.3. Effect of Ionic Strength on hTSH Detection. After hTSH had been successfully detected, we analyzed the effect of buffer ionic strengths, as one of the most crucial considerations on the sensitivity of the sensor on device sensitivity. It has already been demonstrated that the charge screening distance, more commonly known as Debye length (λ_D), is proportional to the inverse square root of the ionic strength of the buffer. For aqueous solutions at room temperature, the Debye length is given by

$$\lambda_D = \sqrt{\frac{\varepsilon_r \varepsilon_0 k_B T}{2 N_A e^2 I}} \quad (3)$$

where ε_r is the dielectric constant, ε_0 is the permittivity of free space, k_B is the Boltzmann constant, T is the absolute temperature in kelvins, N_A is the Avogadro number, e is the charge of an electron, and I is the ionic strength of the buffer solution, which is defined as

$$I = \frac{1}{2} \sum_{i=1}^{n} b_i z_i^2 \quad (4)$$

where i is the ion identification number, $\sum$ is the sum over all ion species, b_i is the molar concentration of the ion, and z_i is the charge of the ion. The Debye length is estimated by eq 4. Although many biological activities are optimized at physiological buffer (1 × PBS), the ionic strength of this buffer yields a λ_D of ∼0.7 nm, screening most of the intrinsic charge of the target protein, which returns the current signal close to the value of the baseline. A diluted concentration with a 10-fold decrease in the ionic strength (0.1 × PBS) produces a Debye length (∼2.3 nm), partially screening the protein's charge. Therefore, a longer Debye length is expected to retain significant charges unscreened. A λ_D of ∼7.3 nm, over which most charge separation can take place on the nanowire surface, is obtained with a further decrease in buffer ionic strength (0.01 × PBS) (Figure 5a). However, too much diluted electrolyte may not ensure the biological reactions between antibody−antigen.

To study the effect of different ionic strength on detection sensitivity, real-time measurements on various concentrations of the buffer over a range from 1 × PBS to 0.01 × PBS were conducted while the other conditions were held constant. Introduction of 2 mIU/L of hTSH in 0.01 × PBS caused a response of ∼20% after establishing a baseline in the same buffer solution, and a slight current decrease (∼7%) was observed when 2 mIU/L of hTSH in 0.1 × PBS was

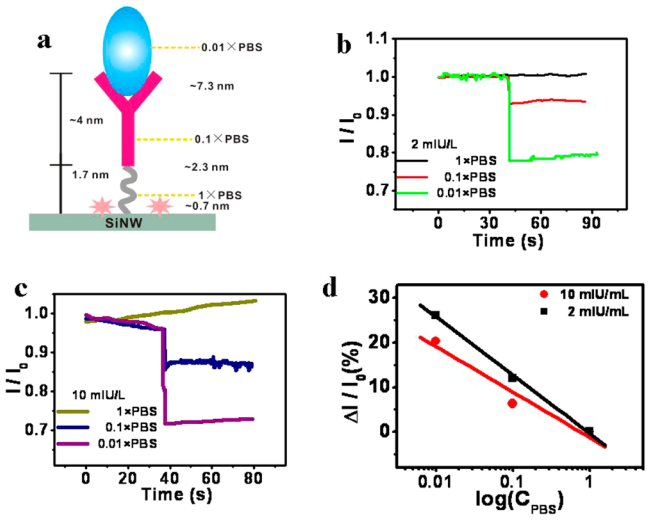

Figure 5. Effect of ionic strength variation on hTSH detection. (a) Schematics of the Debye length with varying ion strengths of PBS buffer on SiNW device surface (not to scale). (b, c) Real-time hTSH detection in 0.01 × PBS, 0.1 × PBS, and 1 × PBS buffer. In (b), hTSH was 2 mIU/L, and in (c), hTSH was 10 mIU/L. (d) Device sensitivity vs logarithm of PBS concentration.

introduced, whereas addition of 2 mIU/L of hTSH in 1 × PBS only gave rise to a negligible response (Figure 5b). The similar results were obtained in Figure 5c when 10 mIU/L hTSH was injected. Figure 5d shows the relationship between the sensitivity of the device and the logarithm of the concentration of PBS buffer solution. The largest response in a low ionic strength buffer was observed. In high ionic strength buffer, a shorter Debye length was yielded and most of the hTSH intrinsic charge was screened; hence, the charge detection was limited due to the formation of an electrostatic double layer. Therefore, most of the FET experiments are carried out under extremely diluted buffer or other systems that are insensitive to ionic strength.

We demonstrated that the CMOS-compatible SiNW-FETs could be used in the label-free and rapid electrical detection of hTSH with extremely high sensitivity. The comparison of this present work with other TSH assays in terms of detection limit and dynamic range was performed, as shown in Table 1. As can be seen, our proposed method was more sensitive than other techniques, so that not only is this method more sensitive than other sensing techniques but also the calibration curve obviously shows that the dynamic range covers more than 5 orders of magnitude and entirely covers the normal concentration range of hTSH in serum (0.3−3.0 mIU/).[38] What's more, most of the other sensing technologies require biomolecule labeling or nanoparticle conjugation to generate or amplify the signal, which is limited to multistep, time-consuming, and complicated experimental process. In contrast to the other sensing technologies, the proposed label-free and simple method only requires one-step reaction and also does not need any reporter tagging or signal amplifications. In addition, as to the total assay time of hTSH detection, the SiNW-FET biosensor has a faster response than other techniques, which showed good potential in quick screening and POC testing of thyroid disease (Table 1).

Using the standard silicon processing technology, fabrication of CMOS-compatible SiNWs by the proposed top-down approach makes it possible to produce them with uniform and reproducible device characteristics with a high yield, as well as directed integrated with electrical readout circuits. In contrast to the previously reported CMOS-compatible SiNW protein sensors,[39,40] our SiNWs were fabricated by conventional photolithography and anisotropic wet etching employed, instead of other expensive techniques, such as the electron beam lithography technique, or deep UV lithography, which allows for fabricating SiNW arrays with a much lower cost in the future. This proposed method has demonstrated great potentiality in hTSH analysis of complicated samples, such as human serum sample, or plasma sample. It could certainly act as a good tool in the clinical practice for patients suspected of hyperthyroidism or deficiency hypothyroidism in the future. Therefore, SiNW-FETs, as a simple electrical sensing module, would be of great significance and offer an integrated a portable system to enable thyroid diagnostics.

4. CONCLUSION

In summary, a CMOS-compatible SiNW-FET biosensor was demonstrated for the first time for rapid and label-free immunodetection of hTSH. The nanoelectronic SiNWs were fabricated by a top-down approach with photolithography and anisotropic wet etching technology, allowing for massive production and integration of complex application. The SiNW biosensor enabled rapid sensing of hTSH through direct electrical measurement, providing a method of label-free detection of biomolecules that will eliminate labor-intensive labeling experimental steps, and reduce the cost for expensive instrumentation in diagnostic screening tests. By using the antibody-functionalized SiNW chips, the ultrasensitive antibody-functionalized SiNW chips, capable of probing hTSH as low as 0.02 mIU/L, can be used to detect hTSH at the

Table 1. Comparison of Detection Limit, Dynamic Ranges, and Total Assay Time Obtained for TSH Assays

detection techniques[a]	methods	detection limit (mIU/L)	dynamic range (mIU/L)	total assay time	references
commercial eLISA	immunoenzymometric assay (ELISA kit)	1.6	1.6−28	>75 min	32
TRFIA	Eu and Sm labeling	0.028	0.21−80	>45 min	29
ECLIA	Tb-1 chelate at oxide-coated silicon electrodes	1	1−100	>1 h	30
CLIA	biotin-streptavidin amplification	0.01	0.1−40	>65 min	31
CLI-ELISA	polymer lab-on-a-chip	1.9	1.9−55	30 min	32
SFMIA	gold-labeled conjugates	0.075		>1 h	35
EC	copolymer membrane with immuno-electrode matrix	1.4	1.4−17.5	>1 h	34
SiNW-FETs	immunodetection	0.02	0.02−30	<15 min	This work

[a]TRFIA, time-resolved fluorescent immunofluorometric assay; ECLIA, electrochemiluminescence immunoassay; CLIA, chemiluminescence immunoassay; SFMIA, scanning force microscopic immunoassay; EC, electrochemistry.

extremely low concentration level in thyroid disease. Furthermore, we studied the effect of different ionic strength on detection sensitivity, using real-time measurements on various concentrations of the diluted buffer. Finally, the SiNW-FETs that could be used as label-free, low-cost, disposable, and portable chips demonstrated a promising potential in application for quick screening tests and point-of-care of neonatal hypothyroidism.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

hTSH, human thyroid stimulating hormone; SiNW, silicon nanowires; FETs, field effect transistors; POC, point-of-care; 1D, one-dimensional; CNT, carbon nanotube; PSA, prostate specific antigen; CEA, carcinoembryonic antigen; CA15.3, carbohydrate antigen 15.3; CMOS, complementary metal−oxide semiconductor; TRFIA, time-resolved fluorescent immunofluorometric assay; ECLIA, electrochemiluminescence immunoassay; CLIA, chemiluminescence immunoassay; SFMIA, scanning force microscopic immunoassay; EC, electrochemistry; APTES, 3-aminopropyltriethoxysilane; BSA, bovine serum albumin; TMAH, tetramethylammonium hydroxide; SOI, silicon-on-insulator; PCB, printed circuit board

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