FET-Based Aptasensor for Multi-Analyte Colorectal Cancer Screening

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Abstract

Proposed in this report is the chosen transducer element for an aptamer-based biosensor for early detection of colorectal cancer biomarkers. This transducer is an array of Silicon nanoribbon (Si-NR)-based field effect transistors (FET) which can be easily multiplexed by functionalizing the surface of each nanoribbon with a different aptamer. Nanoribbons are highly sensitive and allow for a limit of detection (LOD) at approximately 1 pg/mL of cancer biomarkers in blood serum samples. The ability to multiplex with this device also allows for more reliable diagnostics as many cancers have multiple important biomarkers to monitor.

I. Introduction

Colorectal cancer (CRC) is the third most common type of cancer worldwide, affecting approximately 2.2 million people. Currently, the only reliable method for detection of CRC is by performing a colonoscopy; however, this is a very invasive procedure and it is only performed once every five to ten years. The other available screening method is a stool test, but they deliver a high false positive rate so they are not reliable enough [1]. Thus, having reliable and non-invasive detection method is important as it would be used as a yearly screening process for patients over 50 years of age. A potential solution is a biosensor device to monitor levels of CRC biomarkers in the blood.

Most cancer biosensors today generally are not used for early detection, but they are used for monitoring biomarker levels during treatment instead. The most common biomarker to detect is the carcinoembryonic antigen (CEA) which is tracked for monitoring patients undergoing chemotherapy in advanced cancer stages [2]. Readily available cancer biosensors are skewed towards prostate, breast, and cervical cancers [2], so there is a need for point-of-care biosensors for early CRC detection. The main biomarkers to look for in early CRC diagnosis are LRG1, EGFR, ITIH4, HPX, and Superoxide dismutase 3 [3]. Concentration of these biomarkers for patients at risk of CRC are shown in Table I [3]. CEA can also be monitored, but should not be prioritized for early detection.

TABLE I
Healthy versus At-Risk Concentrations of CRC Biomarkers

	LRG1	EGFR	ITIH4	HPX	SOD3
CRC Level	47.6 μg/mL	66.88 ng/mL	0.211 μg/mL	TBD	TBD
Healthy	39.3 μg/mL	62.7 ng/mL	0.134 μg/mL	0.5-1.15 mg/mL	53.6 ng/mL

introduce semiconductors / **semiconductor industry**The transducer proposed here is an array of Si-NR based FETs that would allow for multiplexed detection of CRC biomarkers. Semiconductor materials have a wide range of band gap energies which control their ability to conduct electricity [4]. By nature, Silicon is a semiconductor which allows it to act as a conductor under certain conditions and an insulator in others. Typically semiconductors have band gaps in the range of 0.7 eV to 3 eV [5]. Due to the small band gap of Silicon, the device is highly sensitive to small changes in gate voltage from binding events.

II. Goals

As previously stated, there is a growing need of biosensors for early cancer detection. The goal of this device is to offer reliable early detection of CRC in the form of a non-invasive test. Healthy levels of some of the important biomarkers are quite low, so the device must have a low LOD. As such, high sensitivity and selectivity is also important. Multiple biomarkers are to be monitored, so the ability for the device to multiplex is required.

III. Silicon Nanoribbon FET

A. Device Structure

Figure 1 depicts a basic schematic of a Si-NR structure along with the proposed device schematic and an example constructed device. The Si-NR is fabricated using a top-down approach using a chemical vapor deposition (CVD) process, and this is followed by a lithography and etching process to add the Si-NR. At the end, the metal contacts are deposited and annealed to obtain an ohmic contact [6]. The surface of the Si-NRs are functionalized using the chemical covalent method. This method makes it easy to functionalize the surface with any bioreceptor. To prevent corrosion of the metal contacts while the solution flows onto the chip, the surface is passivated with an SiO_2 layer through a plasma-enhanced CVD process.

As mentioned previously, Si-NR FETs allow for easy multiplexing of the outputs. For this biosensor, the multiplexing is performed by constructing an array of Si-NR FETs. Figure 1 depicts a schematic and an optical microscope image of an example proposed structure.

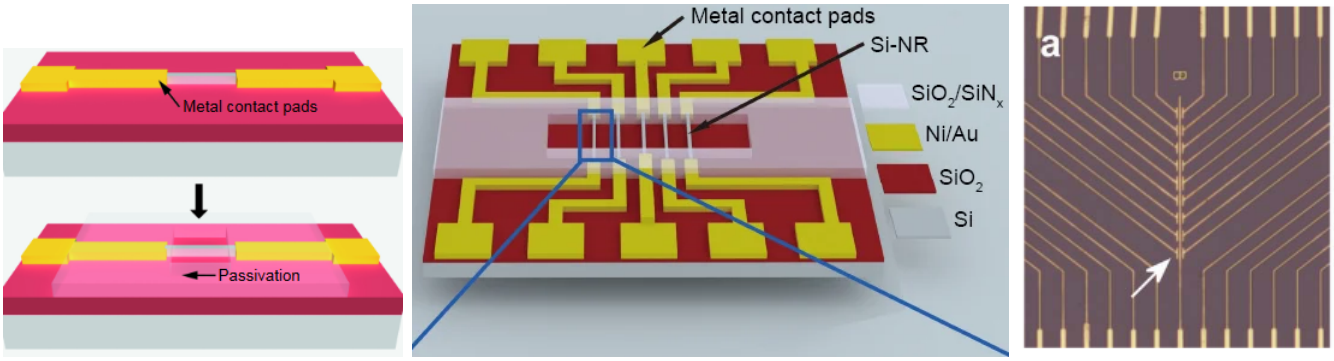

Fig. 1. (Left) Schematic depicting the basic structure of a Si-NR based FET. Reprinted from [6]. (Middle) Schematic showing the proposed array of Si-NR FETs [6]. (Right) Example structure of the proposed array of Si-NR FETs. The white arrow points to the junction between the metal contacts and the Si-NR. An SEM image of the junction can be seen in supplemental figures. Reprinted from [7].

B. Operating Principles

The basic working principle of the Si-NR FET is based on the gating effect. In the case of this semiconductor nanoribbon structure, there is an ohmic contact at the metal-semiconductor junction. Ohmic contacts occur at the junction of a metal and semiconductor when the metal work-function is less than that of the semiconductor [8]. Applying a bias voltage either forces electrons to flow from the metal to the semiconductor or vice versa. Typically I-V curves for these juctions are linear, which can also be observed in the device's I-V curves in different analyte concentrations (Figure 2).

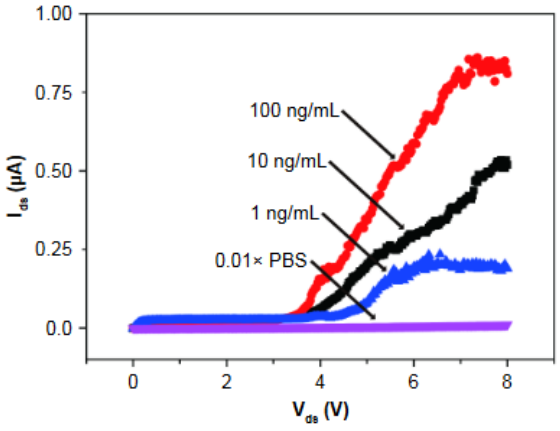

Fig. 2. I-V curves of the Si-NR device in varying concentrations of CEA. The 0.01xPBS solution serves as a control in which no CEA is present. Reprinted from [6].

Si-NRs are chosen as the transducer due to their high sensitivity. This high sensitivity comes from the small band gap of Silicon at room temperature, which is approximately 1.1 eV [9]. Having this small band gap means that even small changes in bias voltage will have an observable effect on output current. Binding events on the surface of the Si-NRs induce a small change in gate bias voltage, thus a noticeable change can be seen with the output current of the device.

This device operates with a gate bias voltage of -5 V, and the V_{DS} is varied from 0 V to 8 V. In Figure 2 a current saturation can be observed after the V_{DS} reaches the range of 6-7 V. A direct correlation between increasing concentration and increasing current can also be seen [6]. From extensive testing, the Si-NRs have a LOD as low as 0.9 pg/mL [7].

Having an array of Si-NRs in this device also allows for easy multiplexing. This array is able to detect multiple analytes at once from a single blood sample, because each Si-NR is functionalized with a different bioreceptor. Figure 3 shows how the multiplexing of this device functions when various concentrations of different analytes are added over time. In this figure, the analyte concentration can be detected with changes in conductance of the Si-NRs. The number labeling at the top of the figure correspond to points in time where different concentrations of analytes are added. Since each Si-NR is functionalized with a different bioreceptor, clear differences in measured conductance of each Si-NR can be observed. Results from this multiplexing setup also demonstrate a signal-to-noise ratio around 3:1, at concentrations in the range of 50 fg/mL to 100 fg/mL [7].

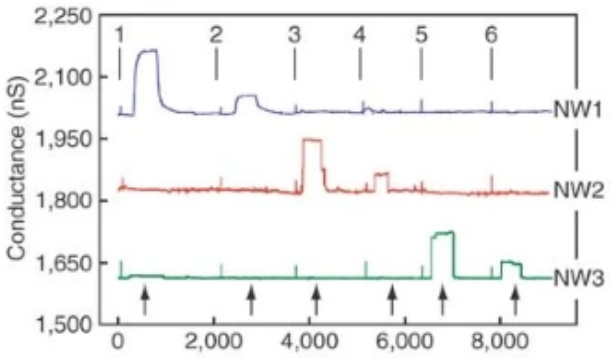

Fig. 3. Graph showing the multiplexing ability of a Si-NR FET array. Numbers 1-6 at the top correspond to addition of different analyte concentrations - (1) 0.9 ng/ml PSA, (2) 1.4 pg/ml PSA, (3) 0.2 ng/ml CEA, (4) 2 pg/ml CEA, (5) 0.5 ng/ml mucin-1, (6) 5 pg/ml mucin-1. Reprinted from [7].

IV. Conclusion

This proposal of a Si-NR FET-based biosensor demonstrates real-time multiplexing and the ability to reliably detect cancer biomarker concentrations above 1 pg/mL. Excellent signal-to-noise ratios even at concentrations as low as 50-100 fg/mL demonstrate great reliability of the device. The low LOD of 1 pg/mL and high sensitivity of the device allow for accurate measurements of important CRC biomarkers from Table I.

Tests have been carried out mainly on CEA as the target analyte; however, the important biomarkers for CRC screening can be easily targeted thanks to using the chemical covalent method for surface functionalization. Multiplexing tests have successfully used mucin-1 (lung cancer biomarker) as a target analyte, showing that any cancer biomarker can potentially be used as a target analyte.

Due to the extremely small size of Si-NRs, variations in the dimensions had potential to affect reliability; however, results of device testing show excellent reproducibility. A total of 60 devices were tested, and error levels for output current were maintained under 1% [6]. Variations in the Si-NR size were shown to have negligible effect on measurements.

Most of the semiconductor industry revolves around CMOS chips, so Si-NR FETs are still novel in terms of device availability on the market. Although the novelty may be questionable for commercial availability, these Si-NR FETs are easy to mass produce thanks to many similarities with the CMOS fabrication process. Therefore this device will also be much cheaper to develop than other readily available biosensors.

References

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V. Supplemental Figures

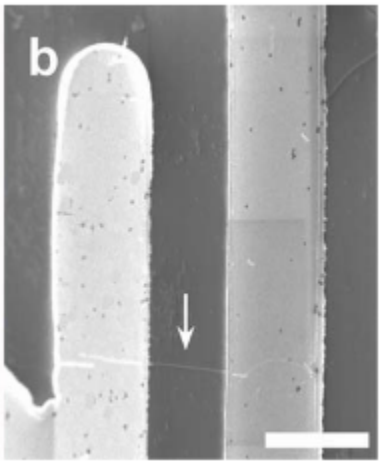

Fig. 4. An SEM image of the junction bewteen the Si-NR and metal contacts. The arrow is pointing to the Si-NR, and the scale bar corresponds to 2 μm.

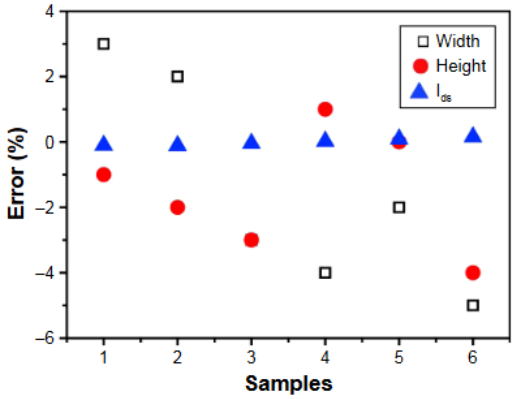

Fig. 5. Graph showing the relationship between variability in Si-NR dimensions and output current.