Label-free Detection of Sex determining Region Y (SRY) via Capacitive Biosensor

Shilpa Sivashankar, Christos Sapsanis, Sumeyra Agambayev, Ulrich Buttner and Khaled Nabil Salama

Abstract— **In this work, we present for the first time, the use of a simple fractal capacitive biosensor for the quantification and detection of sex-determining region Y (SRY) genes. This section of genetic code, which is found on the Y chromosome, finds importance for study as it causes fetuses to develop characteristics of male sex-like gonads when a mutation occurs. It is also an important genetic code in men, and disorders involving the SRY gene can cause infertility and sexual malfunction that lead to a variety of gene mutational disorders. We have therefore designed silicon-based, label-free fractal capacitive biosensors to quantify various proteins and genes. We take advantage of a good dielectric material, Parylene C for enhancing the performance of the sensors. We have integrated these sensors with a simple microchannel for easy handling of fluids on the detection area. The read-out value of an Agilent LCR meter used to measure capacitance of the sensor at a frequency of 1 MHz determined gene specificity and gene quantification. These data revealed that the capacitance measurement of the capacitive biosensor for the SRY gene depended on both the target and the concentration of DNA. The experimental outcomes in the present study can be used to detect DNA and its variations in crucial fields that have a great impact on our daily lives, such as clinical and veterinary diagnostics, industrial and environmental testing and forensic sciences.**

I. Introduction

Biosensors have contributed significantly to a broad range of applications and have gained attention for detecting and quantifying biomolecules connected to several sectors, including diagnostic chips, food safety, agriculture, environmental safety monitoring, bioterrorism and health care [1-4]. Many detecting mechanisms may require the sample to be labeled [5-7]. This is not desirable, as labeling is both tedious and expensive and the necessary equipment is neither portable nor feasible for use in on-site detection. However, capacitive sensors [8-11] are gaining more attention due to their many merits: they require no such complex sample handling or labeling phenomena and are comparatively cheap and portable with high sensitivity and selectivity. The passivation layer on these sensors prevents charge transfer at the electrode surface and the measured change in capacitance relates to the number of biological species that are specifically bound on the bioreceptors that are grafted to the sensors. Therefore choosing a right passivation layer for sensing would greatly enhance the performance of the sensor.

In the present study, we utilize the SRY gene [5'-ATAAGTATCGACCTCGTCGGAAG-3'], which is a small single-exon gene that is located on the shorter arm of a Y chromosome (Yp) at region 1, band 1, sub-band 3 and sub-sub-band 1 (denoted as Yp11.31) and that gets expressed in the precursors of the supporting cells (which are called Sertoli). The SRY and other genes are together responsible for the development of the testicles, which is only possible when this gene controls other chromosomes [12]. So far, efforts have been made to study the mechanisms of this gene [13] and to amplify it using a microfluidic platform [14], but not to give quick measurements that can be used as a biosensor. We used this gene for our pilot study, as the detection of one such gene would provide medical practitioners with the opportunity and information that they need to identify gene-mutated disorders. To the best of our knowledge, this is the first time that the SRY gene is being used on a biosensing platform for the prognosis and detection. A part of this gene (23 mer) was utilized for our model study. A study using dilutions of this gene was also analyzed to recheck the reliability of the sensors that were used in our study.

The system used in this study provided a label-free detection method that is preferable as it is not tedious and measures the change in capacitance on the hybridization of genes. The microfluidic channel incorporated onto the device allowed easy handling of samples and on-site measurement. Surface modification for the immobilization of biomolecules greatly improved the experimental outcomes. Our device generated information on both the specificity and sensitivity of the hybridized gene. This study can also be used to detect DNA variations in crucial fields that have a great impact on our daily lives, such as clinical and veterinary diagnostics, industrial and environmental testing and forensic sciences.

II. Materials and Methods

A. Fabrication

In order to minimize the sample volume, a microchannel was positioned over the sensor to create a direct and atmospheric-independent zone. A PDMS microchannel with an inlet and outlet located between two PMMA stacks was utilized, as shown in Figure 1(a).

*Research was funded by King Abdullah University of Science and Technology.

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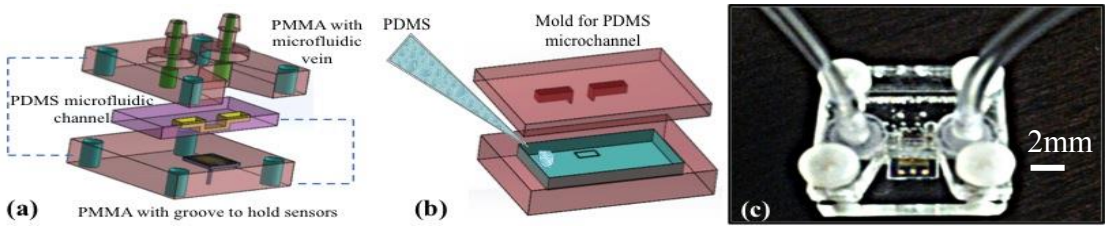

Figure 1: (a) Illustration of the PDMS microfluidic channel sandwiched between PMMA blocks: (b) the schematic PDMS mold used to fabricate this microfluidic channel; (c) virtual chip set-up.

The lower PMMA block was engraved using a Universal Laser system to hold the 2 mm capacitive transducer in position. The engraved well was 2×2 mm with a depth of 0.5mm. A separate PDMS microchannel mold was fabricated using PMMA (see Figure 1(b)). The PDMS microchannel fabricated from this mold covered the sensor area of 1.5×1.5 mm at a height of 0.05mm with an inlet and outlet tubing of 0.5mm ID. The total volume of reagents required to fill the chamber of the large sensor area was about 112 µl. The upper PMMA block had a trapezoidal cut for easy and convenient probing of the sensor electrode pads. The upper and lower blocks had through-holes and threads, and screws at four ends held the PDMS channel between the PMMA blocks. The overall microchannel and stage used in the experiments are shown in Figure 1(c).

B. Experiment setup

The capacitance of the fabricated structure was measured before the analyte deposition. The sensor was then surface treated for immobilization of the DNA template. Sensor electrode pads were probed using a Semiprobe MA-8005 probe station that was connected to an LCR meter (Agilent Technologies). A LabVIEW interface was then used to measure sensor capacitance while the experiment was progressing. Template DNA of 23 mers were customized and purchased from Eurofins MWG operon. The exactly matching complementary and non-matching non-complementary strands for the purchased template DNA were sequenced in the KAUST biocore labs and then utilized throughout the experiments.

C. *Surface treatment of PDMS channel*

Before the utilization of the fabricated microchannel, the microchannels were surface treated by flushing in 0.1N HCl and 0.1 NaOH for five mins each. Subsequent to this an acid/base treatment; the channels were injected with DI water to clean the inner surface of the microchannels completely. These microchannels were also introduced to BSA to coat the inner surface and thereby limit the attachment of other biomolecules to the walls. These PDMS channels were then re-cleaned with DI water to remove any remnants of the BSA. This incorporation of PDMS channels and treatment greatly enhanced experimental outcomes without changing solution concentrations. With this ionic treatment and coating of the BSA surface, no free PDMS surfaces were available to bind with the biomolecule that we used.

III. RESULTS AND DISCUSSION

The Hilbert curve was selected based on the high periphery and thus the high capacitance density that it can achieve. It was patterned using a subtractive process, which was preferred to lift-off due to the structure complexity [15]. Each capacitive structure was coated with a thick layer of 350 nm film of Paralyne C by chemical vapor deposition (CVD). Parylene C has a useful combination of electrical and physical properties plus a very low permeability to moisture and other corrosive gases. Along with its ability to provide a true pinhole free conformal insulation, Parylene C is the material of choice for coating electric assemblies. The coating was conformal with the thickness to be controllable by the recipe and inserted Paralyne C powder. In our case, 0.6 grams of Parylene C powder were used in order to achieve the thickness of 350 nm. We preferred Parylene C deposition over Hafnium oxide (HfO_2) and silicon nitride (SiN_x) due to many merits (a) quick, non-tedious process (b) the thickness of deposit is controllable for sensing whereas, the maximum thickness that can be obtained for HfO_2 and SiN_x is maximum 100 nm to 150 nm which is not preferable in this kind of sensors (c) the thickness is uniform when deposited on large area (4 inch wafer).

With reference to previous study [16, 17], it showed that a system without an enclosed microchannel was not well suited, as the evaporation of molecules during the analysis was time dependent and influenced the experimental results. Efforts were therefore made in this study to minimize evaporation using microchannels as shown in Figure 1. Although the probing was quite challenging, the system was reliable as it predominantly reduced the evaporation factor. The microchannel was fabricated from PDMS, and in this process biomolecules tend to attach to the walls [18, 19]. The PDMS microchannels therefore were surface treated before mounting them onto sensors. The fabrication and surface treatment of PDMS is detailed in the experimental section.

Figure 2: Schematic representation of immobilization technique.

We took advantage of the aldehyde group that was formed during the interaction between cysteamine dihydrochloride and glutaraldehyde to attach the template DNA strands to the surface of the sensors, Figure 2. Many immobilization techniques on sensor surfaces [20, 21] have been employed and still require some modification to enhance the immobilization of template strands. In this study we try to show that the sensors were more efficient by employing some chemical treatments to the surfaces (detailed in experimental section) and channels: first, the surface modification of the sensor paved the way for active sites for the binding of template DNA, and second, surface treatment to PDMS paved the way for an efficient use of the target DNA that was used for detection. The capacitance measurement is mainly achieved by an interplay between the template DNA and the complementary strand. When an AC signal is applied, the capacitor will accumulate a specific amount of charge before the sign of the potential difference changes and the charge dissipates. The capacitance measurements are frequency dependent. The higher the frequency, the less charge will accumulate and the smaller the opposition will be to the current. Reading capacitance in low frequencies provides a long time for water dipoles in buffer to orient to a large extent when complementary DNA is added. Upon the addition of complementary DNA, the water molecules (used for in preparing buffer) can hence be more polarized at lower frequencies. For this reason, the capacitance readout in higher frequencies is more reliable without significant fluctuations; a frequency of 1 MHz was thus selected. Microscopic and AFM image of fractal sensors are represented in Figure 3(a). When non-complementary strand was added the change in capacitance

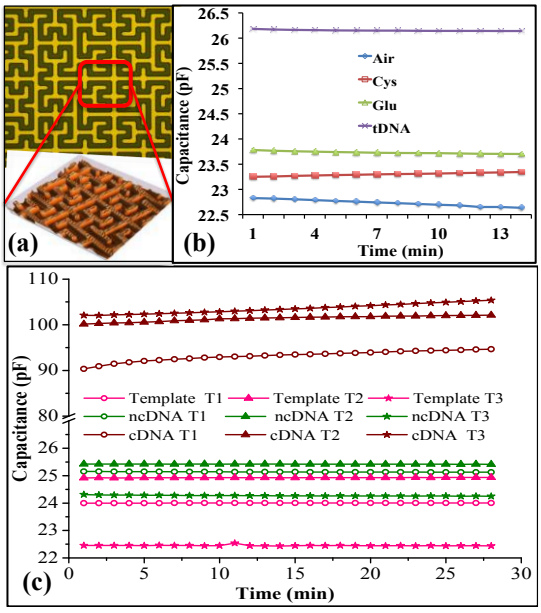

Figure 3: (a) Microscopic and AFM image of the enclosed area of fractal sensors (b) capacitance of sensors in air, on adding cysteamine, on adding glutaraldehyde and after immobilization of template DNA (tDNA) (c) Represents specificity of the sensors. Sensors showed a 4- to 5-fold increase in capacitance, which indicates that the strands were hybridized when [Complementary (cDNA) was added. Non-complementary (ncDNA).

change was about two pF, which indicates that the sensors were specific to the target genes. After immobilization of sensors the capacitance measured was around 23 pF and when non-complementary DNA was added the capacitance measured was around 26 pF. As hybridization never occurred, the capacitance has not changed to a great extent, and this change is negligible. The capacitance was measured when in the air, adding cysteamine, adding glutaraldehyde, after immobilizing of the template DNA and is plotted in Figure 3(b). All the sensors used in the experiments were of the same type and hence an average of three trials is plotted for control experiments. The change in capacitance for all sensors after immobilization and after hybridization is shown in the respective plots thereupon. It is evident from Figure 3(c) that when complementary DNA was used, a substantial change in capacitance was measured. Here again, after immobilization of the template DNA, when non-complementary DNA was used the average capacitance of three trials was around 26 pF. In contrast, after introducing complementary DNA strands, the average of three trials was around 90 pF on hybridization, which clearly confirms that the template DNA were specific to a given sequence of DNA strand and sensor were highly sensitive.

Further, to quantify, complementary strands were diluted with 0.1 mM PB pH 7.2 to concentrations of 100 ng, 75 ng, 50 ng and 25 ng. The results of quantifying these concentrations are enumerated in Figure 4(a). The entire experimental setup utilizes 0.1µg template DNA prepared with 0.1 mM PB at pH 7.2. Figure 4(a) shows that the capacitance decreased as the concentration decreased, which proves the nature of quantification. The experiment was monitored for about 10 min to observe if the change in capacitance was stable for a certain period of time. We calculated the average value of capacitance of three trials and the standard deviation of the specific concentration revealed that the range of a given sample to be quantified. The range and value of the quantification of a sample are depicted in Figure 4(b). However, to ensure that the sensors

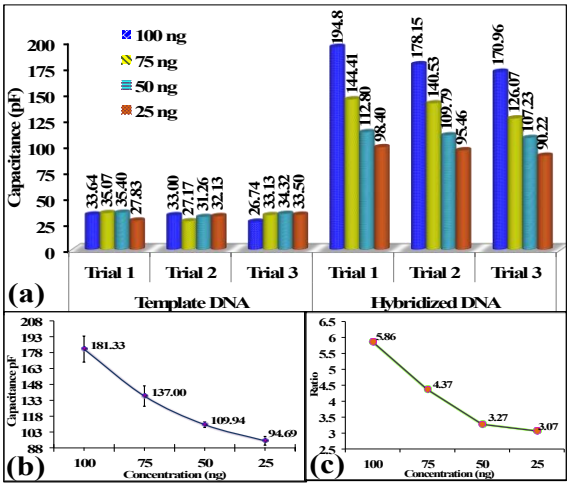

Figure 4: (a) Quantification of target genes using the capacitive gold IDE biosensor (b) The average of 3 trials and provides a range of the quantification of target genes; (c) the ratio of variation of concentrations.

could be useful for quantifying a given sample, we calculated the ratio both before and after hybridization of the samples of the samples; the corresponding data is plotted in Figure 4(c). This ratio gave us insight into how capacitance varied with consecutive concentrations, which also yielded a measure of quantification between consecutive lower concentrations. Although the difference between concentrations of 50 ng and 25ng is not significant, it is noticeable and shows the trend. Many interdigitated microelectrodes have employed detection of oligonucleotides [22]. However, these methods used surface acoustic wave resonators that suffer from increased viscosity related attenuation and can be more difficult to manufacture. On the contrary, we propose a simple, label-free, easy-to-use sensor that could be used for many applications, such as the detection of DNA in disease diagnosis and the detection of harmful bacteria in food and drink [23]. The detection of tiny quantities of DNA could also be used to test for bacteria in beer [24] and short tandem repeat profiling.

IV. CONCLUSION

In conclusion, we propose a simple label-free fractal capacitive biosensor for the detection and quantification of the SRY gene. We demonstrate the reliability of using such as sensor by quantifying the genes when they are introduced at different concentrations. Parylene C and SAMs formed on gold paved the way for an optimal immobilization of the template DNA. SAMs formed in this way set an ease to functionalize and form an organic film that resembles a bio-membrane microenvironment for the attachment of a template DNA to the substrate. The modification that entailed introducing the PDMS microchannel greatly reduced the wastage of biomolecules, thereby yielding substantially good experimental outcomes. The sensor being proposed is not only useful for detecting the SRY gene; it can also be used in other health care applications that involve anticipating or identifying disease in a pre-symptomatic stage.

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