A Fast, Sensitive and Label Free Electrochemical DNA Sensor

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Abstract. A label free and sensitive DNA/RNA silicon based electrochemical microsensor array was developed by using thin film of the conducting polymer polypyrrole doped with an oligonucleotide probe. The electrochemical potential pulse amperometry technique was used for a biowarfare pathogen target DNA detection. The electrical potential assisted DNA hybridisation method was applied. The sensor signal was increased by increasing the electrical potential assisted DNA hybridisation time. It was possible to detect 0.34 pmol and 0.072 fmol of complementary oligonucleotide target in 0.1 ml in seconds by using unpolished and polished gold electrode respectively. The probe preparation was also in seconds time, comparing indirect electrochemical DNA sensor, it has a fast sensor preparation as well as sensor response and label free advantages. The silicon microfabrication technique was used for this sensor array fabrication, which holds the potential to integrate with sensor electrical circuits. The conducting polymer polypyrrole was electrochemically deposited on each electrode respectively which has a possibility to dope the different DNA probe into the individual electrode to form a sensor array.

Keywords: electrochemical sensor; label free; DNA sensor; polypyrrole; pulsed amperometry

1. Introduction

The detection and quantification of specific DNA/RNA sequences is of great importance in numerous applications, such as in medical research and clinical diagnosis. The challenge is to develop simple, reliable, fast and economical tests for large-scale and routine diagnostics purposes. The need for only basic electrochemical equipment in electrochemical detection of hybridization makes it attractive.

Electrochemical indirect methods¹,²,³ involving labels and markers require longer steps and time, although they generally have better detection limits than direct methods⁴,⁵,⁶, which require no additional reagents or pre-/post-hybridization steps. Recent works on direct methods are promising. The amperometry method with ultra thin films of oligonucleotide-doped polypyrrole is reported with detection limit as low as 1.6 fmol in 0.1 ml⁴. The pulsed amperometry with platinum electrodes modified by single stranded DNA is reported with detection limit of 0.37 ng/ml⁵.
This research is focused on the direct electrochemical DNA detection method. A label free and sensitive DNA/RNA silicon based electrochemical microsensor array was developed by using thin film of the conducting polymer polypyrrole doped with an oligonucleotide probe. The electrochemical potential pulse amperometry technique was used for a biowarfare pathogen target DNA detection. The silicon microfabrication technique was applied for this sensor array fabrication, which holds the potential to integrate with sensor electrical circuits.

2. Experimental

2.1 Design and microfabrication of electrochemical sensor array

A silicon based electrochemical sensor array integrated chip has been designed with 12 disc electrodes with a 3x4 layout shown in Fig. 1. The electrode is with 90µm diameter and 250µm spacing (electrode centre to centre). All the electrodes are located on the bottom of the electrochemical reaction chamber and each electrode is individually controlled. Microchip structure and packaged device has been shown in Fig. 2.

Two pieces of 6” silicon wafer were used as substrates to make this electrochemical sensor array. Au was used as electrode material. The microelectrodes integrated microchips were fabricated based on two of 6” silicon wafers, which were about 670 µm thick with chip size of 1cm x 1cm and chamber volume of 0.5cm x 0.5cm x 0.67 cm (W x L x H). 5000A of Au disk microelectrodes with 200A Ti adhesion layer were sputtered as sensing electrode. The lithography process was used to define the electrode sensing area, connecting leading wires and bond pads. The 3 x 4 electrode array ware spaced on the center of the silicon base plate, which is insulated by 5000A thermal dioxide layer. The diameter of microelectrode is 90µm and the distance between the centers of two neighbor electrodes was 250 µm. Each electrode was individually controlled and connected by a metal line to a bonding pad. After the metal deposition, an insulating layer with 2000A silicon dioxide, 2000A silicon nitride and 2000A silicon dioxide sandwich structure was deposited by PECVD. The electrode sensing area and bond pads are finally opened by plasma etching. To form an electrochemical reaction chamber and DNA hybridization chamber, the second silicon wafer is used and the cavities are etched through to form reaction chamber and bond pad opening. To form a biocompatible surface for the bioreaction, both sides of the cover silicon plate were deposited by an insulating silicon dioxide layer. The insulated cover plate was then aligned with the base plate and these two plates were bound together by polydimethylsiloxane (PDMS) at room temperature. The electrode surface was initially polished with a fine polish (0.05 alumina powder), rinsed thoroughly with D.I. water and blow dried with nitrogen for comparation testing.
2.2 Chemicals and DNA probes

Pyrrole, glycine, potassium ferricyanide and sodium chloride were purchased from Sigma-Aldrich. Oligonucleotides were synthesized and purchased from Invitrogen. Deionized DNase/RNase-free water was purchased from Invitrogen. The buffer solution, doped polypyrrole, Dopant15, complementary target20 and Non-complementary target41 are listed in the Table.1.

| Material             | Composition                                      |
|----------------------|--------------------------------------------------|
| Buffer               | 0.1 M NaCl/ 0.1 M glycine in DNase/RNase-free water |
| Potassium ferricyanide | 0.5M in                                          |
| Doped-polypyrrole    | 0.359 ug/ul dopant15 in 0.05M polypyrrole         |
| Dopant15             | Primer with sequence: (5’)<br>GCAATAGTAATCAGG(3’) |
| Complementary target20 | Primer with sequence: (3’)CGTTATCATTAGTCCATCTC(5’) |
| Non-complementary target41 | Primer with sequence:<br>(5’)<br>AATTGTTGTAACGGAAGATGCAATAGTAATCAGGTAATCAGGAGAC(3’) |

2.3 Electrochemical characterization and DNA detection

The cyclic voltammetry was firstly used for the sensor array electrochemical basic characterization. The voltage scan is done from −0.4v to 0.1v with a scan rate of 0.1v/s in 5 mM potassium ferricyanide solution.

The electrochemical deposited doped-polypyrrole was performed by the constant potential electropolymerisation method, which a constant potential of 0.7V is supplied for 15 seconds in 0.05M pyrrole and 0.359 ug/ul of 15-mer dopant in DNase/RNase-free water.

DNA hybridization was done by added the target DNA solution to the buffer solution and a positive voltage of 0.4V was applied, possibly facilitating the diffusion of negatively charged DNA target to the sensor surface for hybridization.

Pulsed amperometry was used for DNA detection. A sequence of 10 potential steps with pulse profile at 0mV and 600mV was applied. The magnitudes of the last 5 peak anodic and cathodic current in the detection buffer before and after hybridisation was recorded and average reading was obtained. The percentage of the difference of the average magnitudes was used as sensor response to corresponded target DNA. The potential steps after target DNA hybridisation was reordered in fresh buffer solution. Three measurements were repeated for each condition and the overall average reading was as the sensor final signal to presented here.

The control run was experimented in the target DNA free buffer solution. The Non-complementary DNA was tested by adding 0.780 uM non-complementary target DNA into buffer solution.

3. Results and Discussions

3.1 Basic electrochemical characterization

The basic electrochemical characterization for the micro sensor array was tested for each electrode and the internal Au electrodes were performed as reference and counter electrodes for the CV measurements. A typical cyclic voltammogram of 5 mM potassium ferricyanide using 90 µm gold electrodes (working, counter and reference) is shown in Fig.3.
3.2 Polypyrrole electrochemical deposition

The typical polypyrrole electrochemical deposition current response is shown in Fig. 4. The use of constant potential polymerisation showed a polymer growth with an increase in current with time.

![Fig. 3 Cyclic voltammetry of sensor microarray](image1)
![Fig. 4 Current response of polypyrrole electrochemical deposition](image2)

3.3. Brief characterization of some parameters on DNA electrochemical hybridisation detection

3.3.1. Pulsed amperometry response. The change in resistance/capacitance of the polypyrrole film following hybridization of the DNA target with the DNA probe provides the basis for detection. Pulsed amperometry performed in the detection buffer showed an increase in the magnitude of the signal after hybridization with complementary DNA for one hrs (Fig. 5a-b). This observation was absent when the control testing was done (Fig. 5c-d)

![Fig. 5 Pulsed amperometry response](image3)

3.3.2. Target DNA concentration effect. The magnitude of the signal increases with the increase in the copies of target DNA added (Fig. 6 & 7). Fig. 6 is presented an unpolished Au electrode response to different DNA concentration. It is possible to detect 0.34pmol of complementary oligonucleotide target in 0.1ml in seconds.

3.3.3. Hybridization time and non-complementary DNA testing. An increasing in hybrization time from 1 to 1.5 hrs resulted in an increase in the magnitude of signal as well (Fig. 7). There was no sensor response to 4x10^{12} copies non-complementary DNA in 0.1ml solution for 1 hr hybridisation, but negative sensor response was obtained once the hybridisation time was increased to 1.5hrs (Fig. 7). Fig. 7 also presents an unpolished Au electrode response.
3.3.4 Electrode surface effect. It was found that the condition of the gold electrode affected the lowest detection limitation significantly. When micro-polished gold electrode was used, 0.072 fmol target DNA could be detected in 0.1 ml solution. This is shown in Table 2.

Table 2. Polished microelectrode response to target DNA

| Copy $(10^{10})$ | Signal change(%) after 1 hr hybridisation |
|------------------|----------------------------------------|
| 31.6             | 54.9                                   |
| 0.264            | 24.4                                   |
| 0.0476           | 9.9                                    |
| 0.00432          | 8.9                                    |

To further evaluate the target DNA interference effects on the doped-polypyrrole electrode, which was without hybridisation potential supplied, but it was in the target DNA solution and at same time, another doped-polypyrrole electrode was supplied hybridisation potential for the hybridization detection. It was found that the electrode without hybridisation potential supplied, which was no magnitude response to target DNA. This result indicated that by using doped-polypyrrole microelectrode method, which holds a potential for doping different dopants into different electrodes to form an electrochemical sensor array for simultaneously perform multi target detection without cross interference.

4. Conclusions
The developed silicon based electrochemical microchip shows the possibility to be integrated with the DNA/RNA sample preparation chips to form a Lab on a chip and with array detection. The simplicity in application is the draw of this approach.
Acknowledgments
This work was supported by research funds of the Agency for Science, Technology and Research (A*STAR) to Yu Chen through Institute of Microelectronics. This research is also supported by Miss Jong Ming Ching for the mask layout and Mr. Hui Wing Cheong for the various supports.

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