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A miniaturized silicon based device for nucleic acids electrochemical detection

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ABSTRACT

In this paper we describe a novel portable system for nucleic acids electrochemical detection. The core of the system is a miniaturized silicon chip composed by planar microelectrodes. The chip is embedded on PCB board for the electrical driving and reading. The counter, reference and work microelectrodes are manufactured using the VLSI technology, the material is gold for reference and counter electrodes and platinum for working electrode. The device contains also a resistor to control and measuring the temperature for PCR thermal cycling. The reaction chamber has a total volume of 20 μL. It is made in hybrid silicon–plastic technology. Each device contains four independent electrochemical cells.

Results show HBV Hepatitis-B virus detection using an unspecific DNA intercalating redox probe based on metal–organic compounds. The recognition event is sensitively detected by square wave voltammetry monitoring the redox signals of the intercalator that strongly binds to the double-stranded DNA. Two approaches were here evaluated: (a) intercalation of electrochemical unspecific probe on ds-DNA on homogeneous solution (homogeneous phase); (b) grafting of DNA probes on electrode surface (solid phase).

The system and the method here reported offer better advantages in term of analytical performances compared to the standard commercial optical-based real-time PCR systems, with the additional incomes of being potentially cheaper and easier to integrate in a miniaturized device.

1. Introduction

Nucleic acid analysis has been an attracting topic in fields such as gene analysis, pathogen detection, environmental and forensic analysis [1–3]. In this scenario polymerase chain reaction (PCR) has become a common method for nucleic acids detection. In this field, scientists had developed a series of technologies, such as multiplex PCR, isothermal PCR, real time PCR and reverse transcriptase PCR. Recently the scientific community has focused its attention on the development of miniaturized microfluidic chips, made of silicon or plastic material, suitable to perform PCR on a small sample volume (<25 μL) [4]. The main advantages of these chips include low the cost of analysis due to the low volume of reagent and sample, the low response time and the ability to integrate upstream and downstream process such as sample preparation and detection directly on chip [5]. Several microfluidics chips are describes in the literature performing nucleic acids amplification. However, only few examples that include nucleic acids detection by real time PCR are reported [6] and the most of these are based on optical detection methods.

The immobilization of oligonucleotides onto surfaces has been reported as one of the most successful strategy to enhance the sensitivity for biosensor systems [7–9]. In 2008, Hsing demonstrated, for the first time, the possibility to electrochemically monitor DNA during the amplification process on a solid phase PCR, through the incorporation of redox-labeled base during the amplification [10,11]. Limoges et al. proposed a novel electrochemical detection method that indirectly detects DNA polymerization in homogeneous phase [12]. In particular, PCR process is detected in real time by monitoring the electrochemical signal of a intercalating redox probe, based on osmium complexes [13], that remains free in solution in presence of amplified DNA: the final result is an exponentially decreasing of the signal of the redox intercalation with the increasing of the amplified PCR sample by the PCR cycles.

Intercalating molecules such as metal complexes based on ruthenium, osmium, iridium, platinum, cobalt [14–17] or organic compounds [18] may be a good method for electrochemical DNA probing because of their advantages such as reversibility of the redox reaction, chemical

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stability and simple functionalization. Recently researcher reported the use of bypyridine (bpy) and dipyridophenazine (DPPZ) osmium (II) as luminescence and electrochemical probes for real-time method. However it is difficult to employ it as electrochemical probe since its high redox potential can destroy the species immobilized on the electrode (guanine and adenine oxidation).

In this paper we reported a novel silicon based device for nucleic acids detection based on the electrochemical monitoring of a unspecific DNA intercalating probe based on osmium complex. The miniaturized silicon chip integrates planar microelectrodes together with temperature sensors and heaters manufactured by using the standard VLSI technology. A PCR chamber is defined by a polycarbonate structure, so that a total reaction volume of 20 μL is achieved. The chip is embedded on PCB board for the electrical driving and reading.

To demonstrate the ability to electrochemically detect DNA, experiments using HBV (Hepatitis B virus) clone as target and $Os[(bpy)_2DPPZ]^{2+}$ as probe were performed. Two approaches were here evaluated: (a) intercalation of electrochemical unspecific probe on ds-DNA on homogeneous solution (homogeneous phase); (b) grafting of DNA probes on electrode surface (solid phase). In the first approach the detection is proven by the decrease of redox signal due to the less easily electrochemically detectable probe intercalated into ds-DNA, while in the second one the DNA detection is confirmed by the increasing of redox signal with the increasing of PCR cycles.

2. Materials and methods

2.1. Chemicals

The osmium complex [Os(2,2′-bipyridine)(dipyrido[3,2-a:2′,3′-c]phenazine)]Cl_2 ($Os(bpy)_2(DPPZ)^{++}$) was synthesized according to published procedures [17]. According to literature [12], the complex can reversibly exchange one-electron at a standard potential ($E°$ ranging from 0.1 to 0.8 V vs SCE).

Hepatitis B virus (HBV) clone (ref. product CLO-05960116 HBV Complete Genome) an all the reagents for the HBV real time PCR were purchased from Clonit (kit ref. product CLO-FO2 HBV MMIX KIT 48) and used according to the *Instruction for Use*.

Human Genomic DNA (10 ng/μL) was purchased from Jena Bioscience.

Thiolated HBV capture probe 25-mer long was supplied form Clonit.

2.2. Amperometric device

The silicon electrochemical device has been manufactured using the VLSI technology on a 6″ silicon substrate. To electrically isolate the electrodes from the substrate a silicon oxide layer has been firstly thermally grown (first passivation layer). Then a platinum film has been sputtered and lithographically defined in the electrodes areas and contact zones (PAD). A second passivation layer was then deposited (Silicon oxide by PECVD) to isolate the first metallization (Pt) form the second one (Au). A dry etch was performed to connect the first and the second metallization (i.e., on interconnection tracks). The second metallization (Au) was then sputtered and lithographically defined in complementary electrodes regions and contacts areas. Fig. 1 shows a schematic cross section of the electrochemical cell structure.

The chip is then assembled with a second silicon device containing integrated temperature sensors and heaters (Fig. 2a). The final silicon device is mounted on a polycarbonate ring to create 4 reaction chambers of 20 μL each that contains on their floor the electrochemical electrodes. The complete structure is fixed on a plastic holder for easily handling (Fig. 2c).

Each electrochemical cell is composed by three planar electrodes: a working electrode in platinum with size 1000 × 2000 μm, a counter and a reference electrode made in gold with size 800 × 500 and 800 × 1250 μm, respectively. The electrode distances are 100 μm and 200 μm. Fig. 2b reports a scheme of the electrochemical cell layout.

2.3. Homogenous phase experiment (real time PCR)

The real time electrochemical PCR experiments were performed using a master mix solution of 20 μL containing Clonit buffer (1×) and Hot start polymerase, 0.5 μM of each forward and reverse primers, 2 μL of HBV-clone (10^5 copies/μL). Different amount of $Os(bpy)_2(DPPZ)^{++}$ ranging from 0.1 to 1 μM were added. The PCR cycling was performed in a portable thermalcycler (Q3-thermocycler developed by STMicroelectronic – Fig. 2d) by using the following thermal program: preheating period of 10 min at 95 °C, followed by a maximum of 45 cycles of 95 °C for 15 s and 60 °C for 60 s.

The square wave voltammograms were recorded at the end of the PCR cycles.

Same real time PCR experiments (20 μL of the above reported master mix) were executed on standard tube in Applied Biosystem 7500 real time PCR equipment.

2.4. Solid phase experiment (hybridization on immobilised capture probe)

The working electrode of the electrochemical device has been functionalized by spotting of a solution containing 10 μM of thiolated HBV capture probe (25-mer long). The chip was incubated for 4 h at 30 °C (90% RH) and washed by deionized water and dried by nitrogen flow.

The functionalized working electrode was hybridized (60 min at 55 °C) with solutions (20 μL) of HBV PCR-cycled at 0, 20 and 40 PCR cycles, respectively. After hybridization, a solution (2 μL) containing 0.1 μM of redox probe was added and square wave curves recorded.

2.5. Electrochemical measurement

The square wave voltammetry measurements were recorded by a Parstat 2273 (Princenton Applied Research) equipment with the follow conditions: square-wave (SW), scan rate 10 mV/s, pulse high/pulse

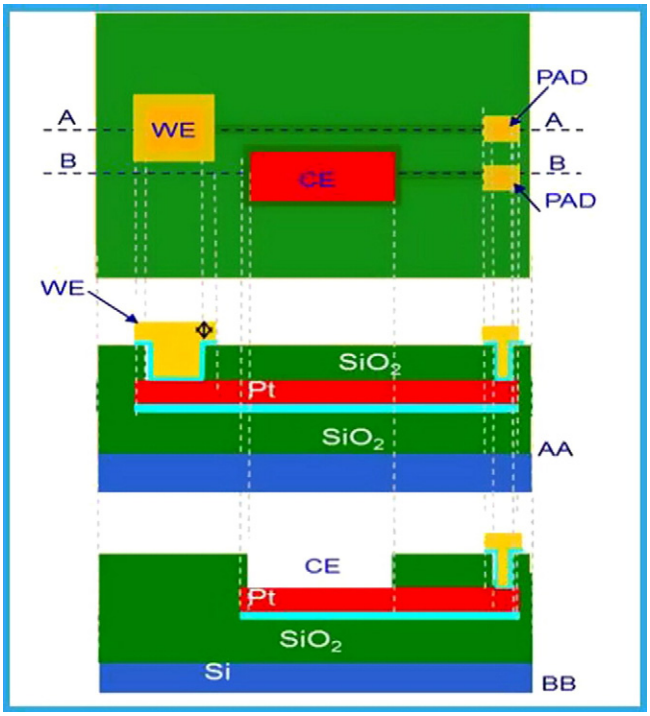

Fig. 1. Schematic cross section of the electrochemical cell.