Sub-micro-liter Electrochemical Single-Nucleotide-Polymorphism Detector for Lab-On-Chip System

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1. Introduction
A SNP (Single Nucleotide Polymorphism) is a single nucleotide variation in the consensus sequence of DNA, which can result in differences in people’s reaction to pathogens, chemicals and drugs. Existing methods to detect SNPs use dedicated equipment, are often rather slow and need relatively large blood samples; also limited SNP specificity and sensitivity is sometimes an issue [1]. We have recently proposed a small Lab-on-Chip (LoC) system that enables fast SNP detection with high sensitivity and specificity [2]. The operation principle and a schematic drawing of the LoC are shown Fig. 1a. Since the detector volume determines total solution volume of the LoC, a smaller SNP detector is better for realizing a compact sensor. However, there is a limit for miniaturizing sensor, because a small electrode area leads to low signal to noise ratio. In this contribution, we fabricated a newly designed SNP detector, which had a chamber as small as 0.5 µL and encapsulated structure. The sensor, the volume of which is 40 times less than what reported before [3], could successfully detect the typing of a SNP within the ABO gene using blood.

2. Detector fabrication and characterization
(a) Fabrication
Our detection system is shown in Fig. 1b. The miniaturized detector chip is fabricated on a separate Si wafer; it is integrated into the total system after filling with suitable reagents for SNP detection. Electrodes are made of gold and the cavities are molded in PDMS.

(b) SNP detection principle
The detection flow is shown in Fig. 2. Prior to the electrochemical detection of the SNP, an allele specific PCR reaction is conducted. Due to the use of allele specific primers (ASP), extension is only initiated when the SNP is present [3] and pyrophosphoric acid (PPi) is produced. Then, through chemical reactions involving three enzymes (Pyrophosphase, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and Diaphoraze), PPi gives electrons to potassium ferricyanide, a component of the detector chemical mixture, transforming it into potassium ferrocyanide. This ferrocyanide is then detected electrochemically: it is oxidized back to ferricyanide, electrons are released and a current flows in the detector circuit.

(c) Detector structure optimization
In our previous report [2], we determined that a detector having a cavity depth of 300 µm and a volume of 0.5 µL has sufficiently large electrodes area to guarantee the required sensitivity. However, the solution evaporation severely affected the stability of measurements in the range of sub-micro-liter volume. The newly developed detector features an on-chip reference electrode (Fig. 3a) thus allowing encapsulation of the cavity. By this encapsulation, the cyclicvoltammetry loop is stabilized (Fig. 3b) and the current drift decreases by a factor of 4 (Fig. 3c), hence detection robustness in sub-micron-liter volume is greatly improved.

(d) PPi detection
The detector has been loaded directly with PPi and the reactions shown in the bottom of Fig. 3 occurred. Fig. 4a shows the time dependence of the current vs. PPi concentration. Fig. 4b shows that the current is proportional to the PPi concentration in the typical range of DNA replication. For the concentration above 0.5 mM, saturation occurs due to reactant depletion. This indicates that the detector has sufficient potential for SNP detection in LoC systems, as demonstrated by the following typing experiment.

(e) SNP typing
SNP detection was demonstrated by typing of human gene, experiments were conducted on AB and O type blood, which, in the ABO gene, differ by only one nucleotide. The detection principle is shown in Fig. 5. In a first step, the DNA fragment in exon 6 of ABO gene was selected and amplified by a PCR, as indicated by electrophoresis patterns, (Fig. 6a lane 2, 3). After filtering and diluting the mixture obtained from the first PCR, a second allele specific PCR reaction using an ABO261-ACG primer was conducted [4]. The use of this primer gives amplification only if the AB sequence is present. The selectivity was confirmed by gel electrophoresis (Fig. 6a lane 6) and by using our detector with a 0.5 µL volume (Fig. 6b and 6c). The current from AB is always higher than that from O.

3. Conclusions
Functionality of an electrochemical detector with a 0.5 µL volume is achieved by appropriate cavity/electrode structure and by encapsulation. PPi detection and also SNP detection was demonstrated by typing of human gene, SNP detection in ABO gene from blood are successfully demonstrated in this improved structure.

References
Fig. 1. (a) Functional blocks of the SNP detection system and its schematic cross-section. Not visible reservoirs containing chemical reagents and enzymes, valves and microchannels. Most of the components are fabricated on a Si substrate using CMOS/MEMS processing techniques. Sealing for fluidic operation is achieved by Si-Pyrex anodic bonding. Pumps, valves and detectors are fabricated separately and later embedded in the chip. Through DNA extraction, amplification, purification and separation, DNA segments of interest with respect to SNP detection are isolated and routed to the SNP detector. The system can simultaneously detect multiplex SNPs. (b) Schematic view of SNP micro-detectors.

Fig. 2. SNP detection flow based on ASP and electrochemical reaction. (Ferricyanide:[Fe(CN)]$_3^{3+}$; ferrocyanide:[Fe(CN)]$_3^{4+}$)

Fig. 3. (a) Photograph of the fabricated chip. (b) Cyclic voltammetry curves. Measurements are repeated 4 times for determining current drift. (c) Current variations in open and encapsulated cavities.

Fig. 4. (a) Current vs. time for different concentration of PPI (0, 0.1, 0.3, 1 mM) (b) Current value at t=30s vs. PPI concentration.

Fig. 5. Detection flow of a SNP in exon 6 of ABO gene.

Fig. 6. SNP detection results in ABO gene from AB and O type blood. (a) Gel electrophoresis patterns. 50base pair (bp) pitch reference markers from 50 to 500 bp are shown in lane 1, 5 and 9. Extracted AB and O DNA fragments of 135 and 134 bp and negative control (NC) fragment after amplification are shown in lane 2, 3 and 4, respectively. AB and O fragments and NC fragment after amplification with ASP are shown in lane 6, 7, and 8 respectively. DNA fragment of 134 bp from O type blood (lane 7) is not amplified. (b) Current vs. time for AB, O and negative control after amplification using ASP. AB signal is always larger than O signal. (c) Values of current at t=30 sec for AB and O fragment after baseline subtraction.