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A Lab-on-Chip System for Direct SNP Detection from Human Blood

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1. Introduction

A Single Nucleotide Polymorphism (SNP) is a change in only a single base pair in the genome relative to the consensus genome. An individual's SNP can influence the probability of developing certain diseases and can affect the therapeutic response to certain drugs. Hence, detecting their presence/absence is very important in the framework of personalized healthcare [1]. SNP detection performed using conventional laboratory techniques is time-consuming, expensive, and requires skilled personnel. Various research groups [2] are working on developing microsystems for SNP detection. These "sample-to-answer" systems offer the possibility for fast and inexpensive detection directly from the doctor's office.

We have recently proposed a silicon based Lab-on-Chip (LoC) that enables fast SNP detection with high sensitivity and specificity [3]. Some of its components, like pumps [4], mixers and microreactors [5], and electrochemical (EC) detector [6], have already been developed and characterized. In this paper, we report the full integration of all components into a single microsystem and we validate their combined functionality, thus realizing a proof of concept of a sample-to-answer SNP detection device.

2. Chip design, working principle and fabrication

Figure 1 shows the architecture of the LoC system comprising two building blocks. The first block is a silicon-Pyrex chip. Microfluidic structures are etched in a Si wafer and sealed by anodic bonding to a Pyrex wafer. The silicon chip is designed using a modular philosophy; meaning that the modules developed for the present application can be reused for different purposes later on. The second block is made of non-Si components gathered together in a plastic frame.

Figure 2 shows the functional layout of the LoC. Blood and reagents are loaded into reservoirs and transported using pumps and valves. After mixing, thermal lysis of cells occurs. A polymerase chain reaction (1st PCR) is then performed to amplify a DNA fragment containing the SNP of interest. The fluid is then transported through the filter to remove cellular debris. Next, the filtrate containing the amplified DNA is mixed with reagents for performing an allele-specific amplification (2nd PCR). In the latter PCR reaction primers are designed in such a way that amplification only occurs in presence of a SNP. Afterwards the sample is transferred to the EC detector to detect pyrophosphoric acid (PPi), a by-product of the PCR reaction. Hence the detector measures a sizeable signal only in the presence of a SNP.

Photographs of the Si-Pyrex chip and assembled device are shown in Fig. 3a and 3b, respectively. A schematic cross-section of the device is shown in Fig. 3c. Reservoirs, pumps, valves and detector are shown in the blue areas of Fig. 2, and are placed in a plastic support which is clamped and connected with the microfluidic circuit via though-holes fabricated in the silicon. An external thermal solution is attached to the PCR for temperature control (see Fig. 3c).

3. Results

DNA extraction and amplification directly from blood

After mixing the blood with PCR reagents, the cells are thermally lysed at 98 ^{o}C for 4 minutes. Thermal cycling commences to amplify the DNA for a total time of 17 minutes (30 cycles). KOD polymerase is used for the direct amplification from whole blood. The solution is then purified by pumping it through a micropillar filter, (see Fig. 4a) which entraps the cellular debris (see Fig. 4b). Figure 4c shows successful micro-PCR amplification of the target fragment.

Allele-specific amplification and SNP detection

The LoC functionality is demonstrated by detecting three SNPs: CYP2C9, K-ras and CYP2D6. These SNPs are related to the therapeutic response to the drugs warfarin, cetuximab, and tamoxifen, respectively. An allele-specific 2nd PCR amplification is performed after the 1st PCR. Careful optimization of both amplification reactions is necessary for reliable SNP detection (see Table 1). In particular, the 1st PCR protocol requires a low primer concentration so that i) the first amplification process will not continue during the second allele-specific amplification, and ii) the amount of PPi produced during the 1st PCR is below the detection threshold of the EC detector. Figure 5 illustrates the detection of the amplicons from the 1st PCR. The amplified DNA is below the detection limit for the gel electrophoresis (Fig. 5a) but can be detected using liquid chromatography (Fig. 5b). Optimization of the 2nd PCR optimization is required to limit the amplification of non-specific products . Optimum conditions vary between the different SNPs (see Table 1). In Fig. 6, gel electrophoresis and EC detection results of the 2nd PCR are shown. A positive band is observed in presence of SNP (mutant) and no band is observed in its absence (wild type)(see Fig. 6a). Fig. 6b shows a clear current difference between mutant and wild fragments using the EC detector and confirms we can specifically detect SNPs using our LoC SNP detection system.

4. Conclusion

A LoC system for SNP detection has been fabricated and tested. DNA extraction, amplification and purification directly from blood were performed in less than 21 minutes. The presence of SNP was successfully detected using a sim-

ple electrochemical cell and an optimized protocol. It is expected that upon process automation, SNP detection can be performed in less than one hour which is necessary in situations where a fast time to result is desired.

References

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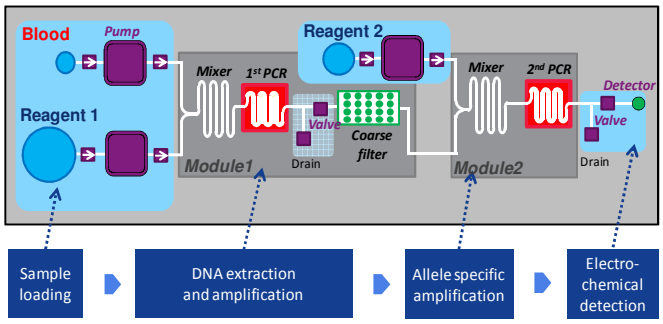

Fig. 2 Functional layout and blocks of the LoC system.

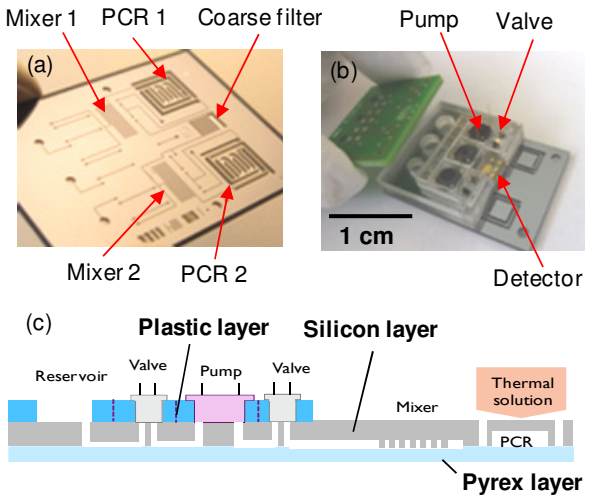

Fig. 3 Photographs of (a) the Si-Pyrex chip and (b) the assembled device. (c) Schematic of the chip x-section.

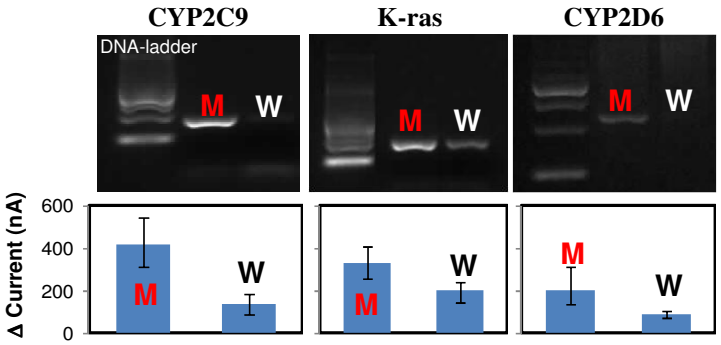

Fig. 6 Gel electrophoresis (top) and EC detection (bottom) results after 2nd PCR for mutant (M) and wild (W) in CYP2C9, K-ras, and CYP2D6, respectively.

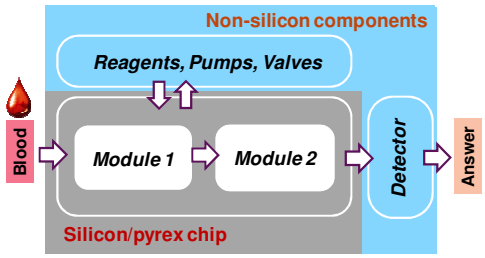

Fig. 1 Schematic of architecture concept.

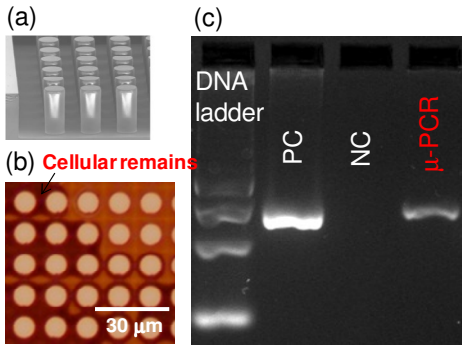

Fig. 4 Photographs of pillar filter (a) x-section, (b) top view after filling 1st PCR amplicon. (c) Gel electrophoresis from the purified products.

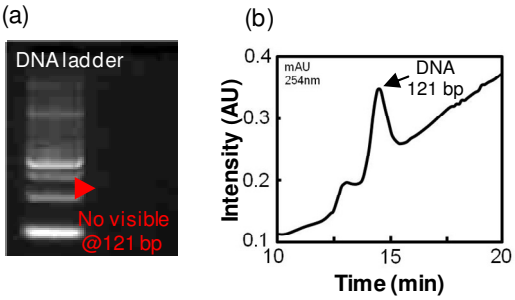

Fig. 5 Detection of optimized 1st PCR amplicon with (a) gel electrophoresis and (b) liquid chromatography.

Table 1 Optimized PCR protocols for detection of the 3 SNP's. Template is blood for 1st PCR and 1st PCR products are the template for 2nd PCR.

	1st PCR	2nd PCR		
		CYP2C9	K-ras	CYP2D6
Polymerase	KOD	TAQ		
Fwd Primer (nM)	300	500	300	300
Rev Primer (nM)	300	500	300	300
dNTPs (nM)	200		200	
Template (mL)	0.2	0.5	0.5	1
T-anneal (°C)	60	60	58	57
Cycles	30	20	16	30