A NOVEL CONTAMINATION FREE PCR WELL ARRAY DEVICE FOR CLINICAL APPLICATIONS

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ABSTRACT

We developed a novel contamination free PCR well device, which enables all procedures of PCR assay -sample preparation, dispensing, assay and measurement- in an isolated package. In order to dispensing small volume of sample reagent to reaction wells in the enclosed system, "Drip-into-oil" injection method is proposed. A prototyping device having 12 reaction wells was fabricated and sample dispensing of 2.5μL into each wells was performed. In addition, using the prototype device, SNP genotyping was demonstrated by PCR-Invader® method successfully.

KEYWORDS: Sample Injection, Passive Valve, PCR, Contamination-Free

INTRODUCTION

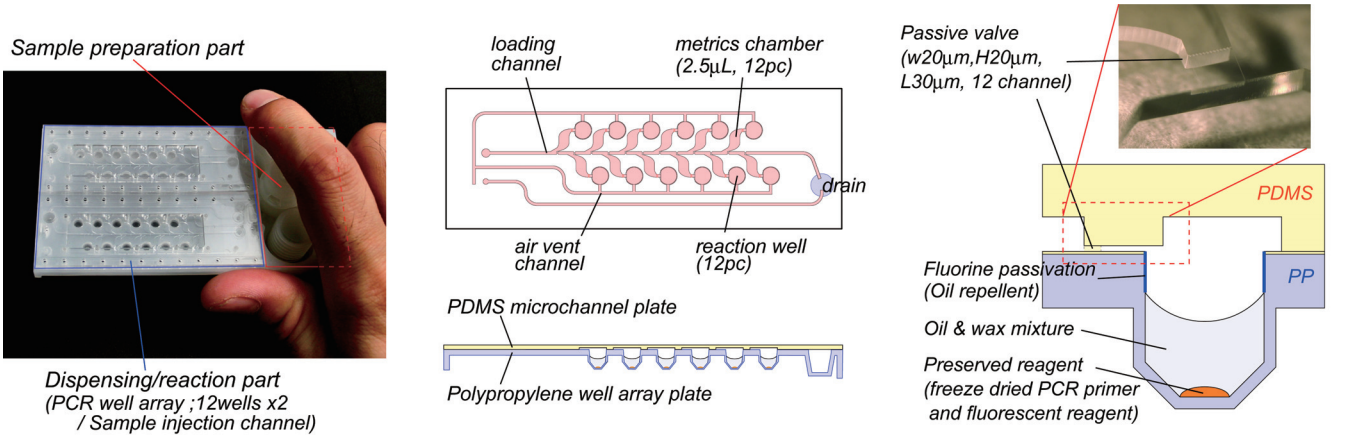
In many previous studies, sample dispensing and PCR reaction on PDMS devices are demonstrated[1]. These devices are desirable for clinical applications, because all procedures of PCR assay, that is sample preparation, dispensing, assay and measurement, can be completed in an isolated package. The isolating package can avoid contamination from environment such as laboratory air. However, due to high vapor permeability of PDMS and bubble formation during thermal cycles, there are fundamental issues of reliability[2]. To ensure the reliability, in our proposed device, precise sample dispensing of small volume is performed in PDMS microchannel, on the other hand PCR thermal cycles are carried out in commonly-used polypropylene(PP) wells with overlaid oil. In order to dispensing small volume of sample reagent to the PP wells, "Drip-into-oil" injection method is proposed.

DEVICE DESIGN

Figure 1 shows a photograph and schematic overview of the developed device. The device consists of sample preparation part and dispensing/reaction part. On the sample preparation part, an on-chip syringe and a bellows air tank are equipped. In this device, sample liquid is driven by pressurized air generated by the on-chip syringe. In order to avoid contamination from environmental, the driving air is supplied from the bellows air tank, in which clean air is packed preliminarily. Consequently, the risk of DNA contamination is extremely low, so that the device is adaptable for clinical uses.

The dispensing/reaction part consists of PDMS microchannel plate and PP well array plate. A dozen metrics chambers that branched from a sample loading channel are formed on the PDMS microchannel plate. Sample reagent is introduced through the loading channel and is segmented to 2.5μL in 12 metrics chambers. Subsequently, the segmented sample is dispensed to reaction wells overcoming each passive valve. On the bottom of the reaction wells, freeze-dried PCR primer and fluorescent reagent is preserved.

As shown in Fig.2(a), if one intends to dispense small volume of aqueous liquid less than several micro liters into a PP well via microchannel, the droplet sticks to lid or edge of the well, because the surface tension is dominant compared to the



a) the well array device b) Schematics of dispensing/reaction part c) Magnified schematics of reaction well

Figure 1: Photograph and schematics of the prototype device

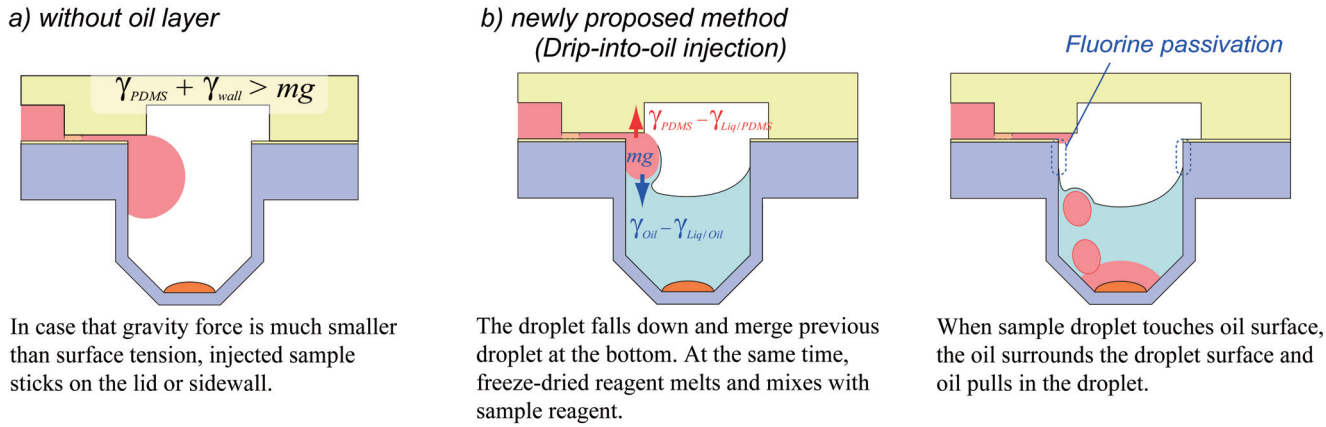


Figure 2: Schematic concept of Drip-into-oil injection

small gravity force. In our proposed method as shown in Fig.2(b), what we call "Drip-into-oil" injection method, oil/wax mixture(oil) is prepared in the well. To define the level of oil surface, fluorine passivation coating is applied on the edge of the well. In the first step of sample injection, droplet is formed on the edge of the well. When the droplet touches the oil surface, oil surrounds the droplet surface and pulls droplet into oil by the surface tension. Then the droplet falls down to the bottom and following droplet is formed and drips into oil successively. The dripped droplets are merged and mixed with preserved reagent at the bottom. In this device, oil acts as moisture barrier for freeze-dried reagent, bridging between microchannel to reaction well.

EXPERIMENTAL

Using human whole blood, we demonstrated sample injection in the prototype device having 12 wells. The human whole blood mixed with a PCR inhibition neutralizing reagent, Ampdirect® (Shimadzu Corp., Japan)[3]. Figure 3 shows captured images of sample injection sequence. The sample reagent is introduced through the loading channel and is distributed to each metrics chamber of 2.5μL (Fig.3a). The loading channel is narrowed in downstream part each bifurcation. Therefore, sample loading along the loading channel is paused in each bifurcation, until each connected metrics chamber is filled. After filling all metrics chambers, pressurized air purges residual sample reagent in the loading channel(Fig.3b). In this case, sample reagent of 40μL was introduced into the device. As the result, sample loss during sample dispensing is only 10μL. With outlet of the loading channel close, segmented sample in each metrics chamber is injected to each reaction well simultaneously by "Drip-into-oil" method. Total pressurized air consumption to accomplish whole sample injection sequence is 130μL(1atm), which can be supplied from the bellows air tank.

Figure 4 shows captured images of "Drip-into-oil" injection. In order to supply pressurized air, the syringe pump infused 30μL air at a flowrate of 80μL/min. Sample reagent segmented in the metrics chamber overcomes the passive valve driven by the pressurized air, then forms droplet on the edge of the reaction well(Fig.4a). With growth of the droplet, the droplet touches preserved oil surface. When the droplet touches the oil surface, the droplet is pulled into oil and falls down to the bottom(Fig.4b). The droplet forms repeatedly until the metrics chamber is empty.

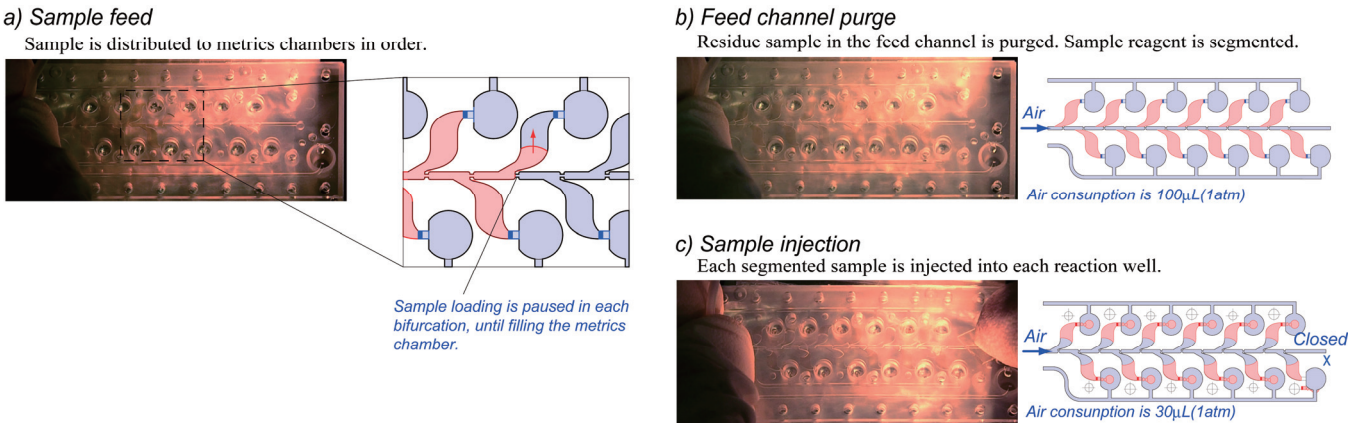
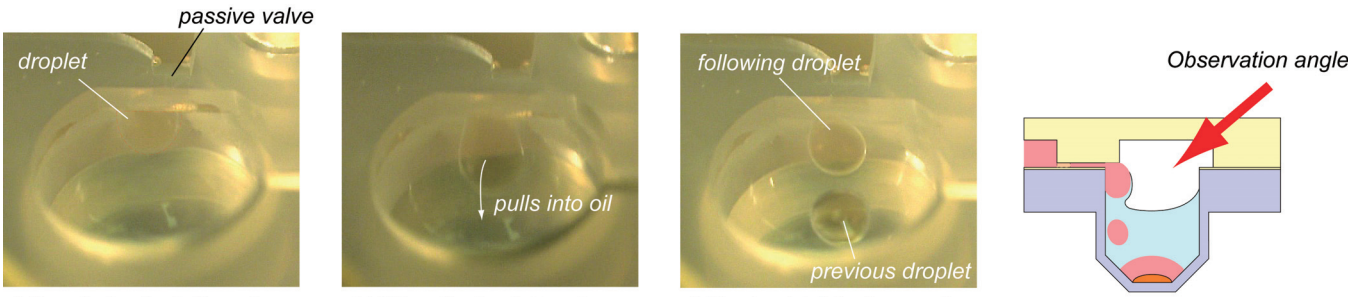


Figure 3: Diagram of sample injection sequence



a) Sample droplet is formed on the edge of reaction well

b) When the drpolet touches oil surface, oil surface pulls the droplet into oil.

c) The droplet falls down and following droplet is formed subsequently

Figure 4: Captured images of Drip-into-oil injection

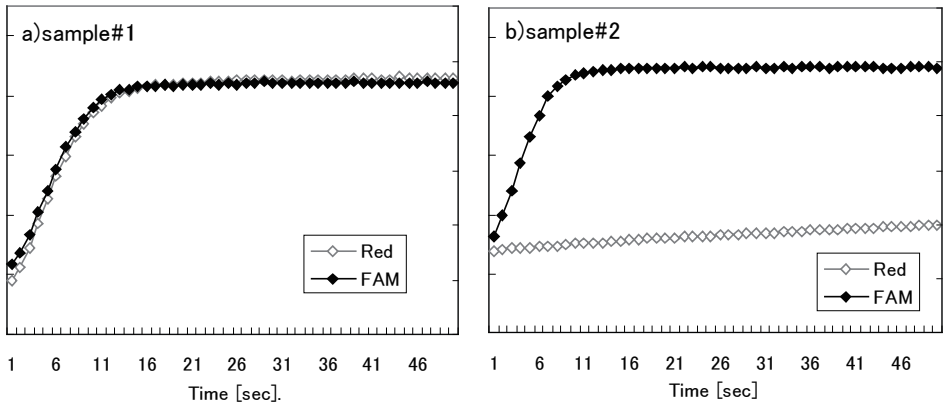


Figure 5: Fluorescent intensity of PCR-Invader® assay

Using the prototype device, SNP genotyping (CYP2C9) was performed by PCR-Invader®(Hologic Inc., USA) method [4]. Pretreatment human blood sample was introduced into the device, and was dispensed into each well. In each well, the freeze-dried reagent was dissolved with the sample, then PCR-Invader® assay was performed according to the standard protocol. Figure 5 shows fluorescent intensity of two FRET probes. As shown in the results, different alleles were identified successfully.

CONCLUSION

In this paper, we proposed new concept of "Drip-into-oil" injection method. The new injection method helps dispensing small volume of aqueous liquid less than several micro liters into a PP well via microchannel. Facilitating the new injection method, We developed a novel contamination free PCR well device, which enables all procedures of PCR assay -sample preparation, dispensing, assay and measurement- in isolating package. Using the fabricated prototype device having 12 wells, sample dispensing of 2.5μL into each wells and SNP genotyping (CYP2C9) were demonstrated. The developed device is useful for clinical application.

REFERENCES

[1] Research review paper "PCR microfluidic devices for DNA amplification" C. Zhang, et.al. Biotechnology Advances, 24 (2006) pp.243–284

[2] "ON-CHIP LIQUID DEGASSING WITH LOW WATER LOSS", J.M. Karlsson, et.al., 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences 2010, pp.1790-1792

[3] "Direct polymerase chain reaction from whole blood without DNA isolation" ,N. Nishimura., et al. Ann. Clin. Biochem, 37(2000), pp. 674-680

[4] "Detection and differentiation of wild-type and vaccine mutant varicella-zoster viruses using an Invader Plus® method" ,Tang, Y., et al. J Clin Virol. 40(2007), pp.129-134.

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