SILICON MICROCHAMBER ARRAY FOR SEQUENCE-SPECIFIC DNA AMPLIFICATION AND DETECTION USING A NOVEL DISPENSING METHOD

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Abstract
This paper describes on-chip DNA amplification in a highly integrated microchamber array. The 40 nL of PCR mixture was introduced into each chamber of the microarray precisely by using nL dispensing system through the oil layer that served as a coverlid. The amplified DNA was then detected with CCD camera built-in fluorescence microscope by using SYBR Green and TaqMan chemistry.

Keywords: Microchamber array, TaqMan PCR, nanoliter dispenser, Mineral oil

1. Introduction
During the past decade, there have been many reports about miniaturized DNA amplification devices by several other groups in the literature [1, 2]. Recently, on-chip DNA amplification using highly integrated and precise dispensing system at nL level has been reported by our group [3]. We have fabricated a highly integrated and precisely addressable DNA amplification system. The main advantage of this system is the introduction of small volumes (several nL) of amplification mixture through an oil layer, which avoids evaporation that brings an undesirable change in the concentration of a sample mixture. In order to achieve this goal, firstly a mineral oil layer was coated on the microchamber array as a coverlid and then the sample mixture was introduced through the oil layer using a nL dispensing system. Combining these two steps led to the development of a highly integrated microchamber array system. In this research, the feasibility of microchamber array was improved by using TaqMan PCR, which does not require different thermal cycling protocols. Three different target DNA samples were amplified and detected in the same microchamber array for the first time. Therefore, this system proves to be a promising device for the low-cost high-throughput DNA amplification and detection for point-of-care clinical diagnosis, which can also be handled by non-specialist users.

2. Experiments
The silicon microchamber array chip was fabricated by photolithography and chemical etching. The dimensions of each microchamber were about 650 × 650 × 200 μm, and could accommodate approximately 50 nL of sample volume. The chip has 1248...
chambers in total (Fig. 1). The chip feature is 1 inch x 3 inch. In order to achieve precise introduction of sample mixture into the microchamber, only the chamber surface was prepared as hydrophilic by leaving an oxidized layer on it with photolithographic techniques. The principle of the novel dispensing method is shown in Figure 2. This method can prevent evaporation during not only dispensing but also amplification reactions such as PCR. First, the chip was soaked in the 5% (w/v) bovine serum albumin (BSA) solution in order to prevent the non-specific adsorption by coating the chamber wall. Then, several kinds of specific primers and TaqMan probes related to beta-actin [4], sex determining region Y (SRY) [5], rhesus D gene (RhD) [6] were introduced in the certain area of microchambers respectively using nL dispenser commercialized from Cartesian Technologies (Michigan, USA), and dried in room temperature (Fig. 2-a). The probe of beta-actin was purchased from Applied Biosystems (California, USA). The other probes were purchased from Fasmac Co. (Kanagawa, Japan). The mineral oil was coated onto the primer modified chip (Fig. 2-b), and PCR mixture which included target DNA (human male DNA, Rh (-) human female DNA, negative control DNA) was dispensed into the microchambers respectively (Fig. 2-c). After preparing this setup, the chip was placed onto a conventional thermal cycler to achieve PCR reaction. The amplified DNA was observed using a CCD camera (Hamamatsu photonics, Japan) which was mounted on a Leica fluorescence microscope (Germany).

Figure 1. A photograph of the microchamber array chip.

Figure 2. Schematic illustration of the novel dispensing method.

3. Results and discussion

Figure 3 demonstrates that the target DNA was amplified specifically and fluorescence intensity increased by TaqMan PCR using a microchamber array chip. This result clearly demonstrates that successful on-chip DNA amplification of a sample mixture in
Figure 3. left: The fluorescence image of the chip after DNA amplification.
right: An illustration for the outcome of the fluorescence responses from the areas of the microchamber, where positive or negative samples from different sources were dispensed.

microchambers of the array was achieved. The contents of the amplification could also be fully retrieved by a micromanipulator, in the presence of oil layer, and these contents could be employed for further analysis. Since the probe for beta-actin was purchased from a different source, the background fluorescence intensity was found to be much lower than the other two probes. Thus, the different fluorescence intensities background shown in Figure 3 were attributed the utility of three different probes. Comparing the positive fluorescence intensity signals with the negative ones makes it very simple to distinguish, which chamber contained the target DNA. If the target DNA sequence is present in the dispensed sample, TaqMan PCR takes place and a high fluorescence signal is easily obtained. The fluorescence intensity was also scanned and evaluated using a DNA microarray scanner and its analysis system. Figure 4 shows the average fluorescence intensity values obtained from 16 chambers. The remarkable

Figure 4. Histograms for the average fluorescence intensity values obtained from 16 chambers.
The difference between the fluorescence intensities of the positive and negative controls is clearly presented for three target DNA sequences. Since the amplification of SRY sequence was relatively more difficult than the amplifications of actin and RhD sequences in the microchamber, the difference between the SRY (+) and SRY (-) samples were observed to be smaller than the other values.

4. Conclusions

These results show that the microchamber array chip could amplify multiple target DNA in combination with a nL dispenser. Theoretically, the chip could amplify and detect around 1200 target DNA at once. Moreover, this system can be adopted to SYBR Green PCR to detect DNA amplification. However, it is important to consider about the thermal protocol to amplify the target DNA. The thermal setting for each target DNA should have the same condition, otherwise the chip cannot be put on a conventional thermal cycler. Therefore, the TaqMan PCR is more suitable for this microchamber array, because the thermal cycling control of the TaqMan PCR is almost the same for the most of the samples. Overall, this system is a potential candidate for mass-microfabrication due to its low-cost and high through-put detection ability.

References