DIFFUSION-BASED MICROFLUIDIC PCR FOR “ONE-POT” ANALYSIS OF CELLS

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ABSTRACT

Genetic analysis starting with cell samples often requires multi-step processing including cell lysis, DNA purification, and polymerase chain reaction (PCR) based assays. When conducted on a microfluidic platform, the compatibility among various steps often demands complicated procedure. Here we present a microfluidic device that permits “one-pot” strategy for multi-step PCR analysis starting from cells. Taking advantage of the diffusivity difference, we replace the smaller molecules in the reaction chamber by diffusion while retaining DNA molecules inside. This simple scheme effectively removes reagents from the previous step to avoid interference and thus permits multi-step processing in the same reaction chamber.

KEYWORDS: Micro-reactor, Polymerase chain reaction, Single cell analysis

INTRODUCTION

Analysis of genes from targeted cells based on polymerase chain reaction (PCR) is routinely required for illustrating the fundamental molecular biology involved in cellular events and detecting abnormal pathways involved in disease development. Microfluidic devices offer potential for genetic analysis based on tiny amounts of cell samples with high sensitivity and high degree of integration. Genetic analysis of cells often starts with cell lysis which releases genes from cells. The released genes typically require isolation and purification before they are analyzed by PCR-based amplification [1]. The integration of these steps on a microfluidic platform warrants careful consideration and arrangement. The entire process involves various chemical and biological reagents, as such some reagents may strongly interfere with the functions of others. Most notably, chemical reagents used for cell lysis (such as sodium dodecyl sulphate and Triton X-100) may inhibit PCR by reducing polymerase activity. Furthermore, the intracellular molecules such as proteins, polysaccharides, and ions (including Ca²⁺, Fe³⁺ and EDTA) in the cell lysate may also interact with a polymerase and affect the PCR result.

So far there have been three strategies to alleviate the impact of cell pretreatment on PCR: 1. Institute some type of isolation step to remove lysis reagent and undesired intracellular molecules while preserving nucleic acids. The involved isolation step increase the complexity of the procedure and chip design. 2. Use alternative lysis methods, such as freeze-thaw [2] or heating [3], that interfere with PCR to a less degree than surfactants. These methods are less efficient than surfactant-based lysis [4]. 3. Use Direct PCR kit based on Phusion polymerase that is tolerant to surfactant-based lysis reagents, but this commonly-used fluorescence-based quantification is impossible with the Phusion polymerase system [5]. To summarize, microfluidic strategies that permit simple operation and device design and are compatible with Taq polymerase are still in high demand.

EXPERIMENTAL

In this work, we demonstrate a simple scheme for conducting microfluidic PCR starting from cells, taking advantage of the difference in the diffusivity between genomic DNA and various reagent/intracellular molecules. Our microfluidic device had a simple structure that included a reaction chamber connected with two loading chambers on both sides (Figure 1a). The connections between the reaction chamber and the two loading chambers could be cut off by closing two-layer valves. The lysis buffer and the PCR mix were introduced into the chamber by concentration-gradient driven diffusion. During such diffusion, the new solution replaces the solution and molecules from the previous step without removing the slow-diffusing genomic DNA (Figure 1b).
RESULTS AND DISCUSSION

As an initial test, we used our platform for PCR of purified human genomic DNA. To verify that the PCR result was dictated by the diffusion process, we used COMSOL to model the molecular transport during loading. We then conducted on-chip PCR in the reaction chamber containing PCR mixes with the COMSOL calculated concentrations (Figure 2). We found that the amount of PCR product was very similar in these two cases. We also show that this scheme allows highly efficient genetic analysis of a low number of cells (including single cells) (Figure 3). The single chamber (“one-pot”) design drastically minimizes the complexity of the microfluidic device. We envision that this may be a general approach for on-chip multi-step assays on genomic DNAs.

Figure 1: Principle of solution replacement based on diffusion. (a) The design of the microfluidic device. The device consists of two PDMS layers. Control and hydration are implemented in the same control layer by actuating the valves and supplying water under pressure (~25 Psi). The fluidic layer has a small reaction chamber connected with two large loading chambers. When the valves are open, molecules in the solution A may enter the reaction chamber by diffusion, effectively replacing solution B. (b) The entry (solid lines) and release (broken lines) of various molecules into/out of the reaction chamber, modeled by COMSOL Multiphysics. The initial concentration in the loading chamber (in the cases of entry into the reaction chamber) or that in the reaction chamber (in the case of release out of the reaction chamber) was used as the reference (i.e. having a value of 100%).

Figure 2: Diffusion-based PCR in a 24 nl microfluidic chamber starting with genomic DNA purified from GM12878 cells. The detection targeted GAPDH gene and the amplification run for 30 cycles. The copy number of the PCR product was quantified using qPCR. The results with various loading times (grey bars, 0, 1, 3, 10 and 30 min) are compared with those of on-chip PCR with various Taq polymerase concentrations (0, 0.2, 2, 13 and 38 U/ml) and primers concentrations (0, 3, 20, 120, 400 nM) (striped bars, these values were generated by COMSOL modelling of the diffusion process for the corresponding loading times) to confirm the impact of the diffusion on PCR results.
Figure 3: Combined lysis and PCR starting with GM12878 cells in a 3 nl reaction chamber. (a) A schematic on the procedure. Triton X lysis buffer replaces the original cell buffer by diffusion. Then the triton X lysis reagents are replaced by PCR mix by diffusion. Finally PCR amplification occurs for 45 cycles. Triton X lysis buffer and PCR mix had loading times of 10 and 30 min, respectively. (b) The cell lysis observed under differential interference contrast (DIC) microscope. Cells are completely lysed after the loading of Triton X lysis buffer for 3 min. (c) The combined lysis and PCR procedure on cells of various numbers (0-50). The PCR signal resulted from the control sample (0 cells) is from primer dimerization as shown by the melting curves.

CONCLUSION
The use of diffusion for changing small-molecule reagents in a microfluidic reactor is universally applicable to assays involving large DNA molecules isolated from cells. Our approach efficiently eliminates DNA isolation and purification steps and drastically simplifies the design of the microfluidic device.

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REFERENCES

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