DROPLET-BASED POLYMERASE CHAIN REACTION (PCR) USING INFRARED-MEDIATED HEATING SYSTEM

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ABSTRACT

We report progress towards a droplet-based microfluidic platform that enables fast, accurate, and integrated genetic analysis. Discrete nano- and pico-liter sized droplets can be quickly generated using a custom generation kit and moved to an area on a microchip where our patented ‘infrared (IR) heating system’ allows for the amplification of the target DNA for down-stream interrogation of the mutations found in genetic and infectious-agent mediated disorders. As an essential first step towards this platform, we demonstrate the potential for our integrated platform with a simple DNA amplification inside droplets using our IR heating system.

KEYWORDS: Infrared-Mediated PCR, Droplet Microfluidics, Digital PCR, High-throughput Genomic Analysis, Molecular Diagnostics, Genomics, Genetic and Infectious diseases, Portable Genetic Analyzer

INTRODUCTION

Diseases including rare genetic disorders, cancers, neurodegenerative diseases, and diabetes, are associated with inherited genomic variance and casual genetic mutations in the functional region of human chromosomes. Moreover, infectious disease diagnosis involves the detection of low copy numbers of exogenous DNA in the human body. With recent advances in molecular diagnostics, rapid and early detection of these diseases is becoming a reality [1, 2]. In order to enable the rapid interrogation of DNA, we are developing a droplet-based microfluidic platform which has high-throughput, multiplexed capabilities to perform a integrated genomic analysis. Furthermore, the properties of this microchip constructed from a polymeric substrate, poly(methy methacrylate), PMMA, allow us to easily and quickly generate droplets and perform droplet-based PCR. Finally, we describe that our IR heating system enables the simple amplification of λ-phage DNA for down-stream interrogation of the mutations found in genetic and infectious-agent mediated disorders.

THEORY

High-throughput molecular diagnostics on microfluidic devices can lead to a fast and accurate interpretation of biological and chemical phenomena in miniaturized devices. As an example of multi-diagnostic approaches, we are adapting a droplet-based microfluidics [4]. While constantly flowing liquid through the device for one continuous reaction, water-in-oil droplets can be easily generated to contain many distinct and small volumes (nano and pico-liter) of biochemical reactions for any application inside the same microchip. Moreover, in order to achieve rapid genetic analysis using a simple device, we are utilizing a non-contact heating approach (infrared mediated heating system) which can perform rapid PCR with a fast ramping rate and assist in the miniaturization of PCR instrumentation for portability [3]. Figure 1 illustrates a flowchart for droplet-based microfluidic platform and highlights our research.

Figure 1: Flowchart of droplet-based microfluidic platform. After packing each single cell into nano- or pico-liter droplets and trapping them within very small areas, the polymerase chain reaction (PCR) can be performed in a high-throughput manner. Each droplet can be sorted based on physical properties of components inside droplets. Finally, DNA amplification can be verified using fluorescent detection or micro-electrophoresis.
EXPERIMENTAL

In our approach to droplet microfluidics (Figure 1), we have focused on developing fast and simple fabrication with the low cost of PMMA and ease of CO2 laser etching. The PMMA, etched by a CO2 laser, provides a hydrophobic channel surface where droplets can be easily generated with a custom generation kit (Figure 2) and give a constant heating into the thermally bonded (three layers; 1.5 mm, 0.2 mm, and 1.5 mm of thickness) PMMA microchip. Strongly-bonded PMMA can decrease the air bubble formation around PCR heating region and the laser-cutting through a thin PMMA gives us a smooth and precise microchannel; the depth (200 μm) can be determined by the thickness of PMMA layer and the width (200 μm) by CO2 laser cutting. The droplets containing PCR master mix are moved through the device into the infrared-mediated PCR region where IR heating and forced air cooling are performed. In order to achieve this in a high-throughput manner, a tungsten lamp is used for larger heating area where each droplet is exposed to the irradiated area for the time needed for rapid PCR (Figure 2F and G).

Figure 2: Multi-layer PMMA microchip with a custom generation kit for droplet generation. A. CO2 laser etching 200μm PMMA to generate a precise channel. B. Thermal bonding [4] with 1.5mm PMMA covers using a hot plate and pressure. C. 3D drawing for PMMA droplet generation kit. D. Droplet flow inside chip. E. Process of making small droplets inside microchannels (200μm). Silicon tubing was attached for flow of oils and samples, using this kit, tubing can be attached without the need of nanopore. F and G show a high-throughput capability and infrared-mediated heating system [3] patented in our lab for droplet PCR applications. F. In order to confirm DNA amplification inside small droplets, many droplets were generated with syringe pumps and the chip was transferred on infrared heating system. When droplets pass PCR heating region, PCR will be performed. G. Tungsten lamp (5V) directly heats an 1cm area on the chip and is cooled by fan (12V). All equipment can be controlled by LabVIEW program through a DAQ card (USB-6008).

RESULTS AND DISCUSSION

Stable temperature profiles are maintained with maximum ramping rates found to be 18 °C/s and temperature holds at 68 °C, 72 °C, and 95 °C, that varied an average of only ±0.2 °C (Figure 3A) on the multi-layer PMMA microchip. Based on previous studies of our infrared-mediated heating system [3], this temperature profile indicated that we could perform proper thermocyclings for PCR applications. Furthermore, we attempted a simple DNA amplification in a single layer PMMA chip to evaluate optimized conditions for droplet PCR. With accurate temperature profiles being obtained in the device, amplification of DNA in individual droplets was verified. A more simplistic PMMA device was conceived containing several distinct chambers where droplets can be easily transferred and PCR performed (Figure 4A). Using a simple, initial amplification strategy to test the system, we achieved rapid amplification of λ-phage DNA in ~10 minutes (Figure 4B and C). The total time for one cycle was 22 s, with 2 s denaturation, 5 s annealing, and 5 s extension. Using a glass chip for PCR as a reference (30 minute-amplification with 15 s denaturation, 15 s annealing, and 15 s extension), we verified that the PCR products detected in the droplets were consistent with a 500 bp λ-phage DNA fragment (Figure 4C). This indicates the potential of the infrared heating system for fast amplification of droplets inside oil for the capability of high-throughput analysis.
Figure 3:  A. Temperature measurement and profiling (30 cycles) using infrared-mediated heating system using multilayer PMMA chips. B. In order to exactly measure the temperature of droplets, we inserted a thermocouple directly into the PCR heating area through a micro-channel filled with oil. Using this chip, we performed 30 thermal cycles (95 °C, 68 °C, and 72 °C) for PCR applications.

Figure 4: Droplet PCR (around 10 minutes) in a single layer PMMA microchip with one example of thermal cycles (30 cycles). A. One layer PMMA chips having several chambers where we can transfer each droplet to perform DNA amplification. B. Thermal profiles for DNA amplification around 10 minutes inside the reference microchamber where a thermocouple was inserted. One cycle takes 22 seconds. C. Electropherograms from DNA amplification inside droplets using a single layer PMMA microchip and a stationary chamber in glass chip. After performing the fast PCR using a single layer PMMA microchip around 10 minutes, we measured DNA amplification using Bioanalyzer 2100. As a comparison to DNA amplification inside droplets, we show 500bp λ-phage amplification (30min) in a glass chip according to previous research [3]. PCR mixture; MgCl₂ (25mM), PCR buffer, dNTPs (10mM), Primers (20µM), λ DNA (25ng/µL), Taq Polymerase (5U/µL), BSA (1µg/µL).

CONCLUSION
In conclusion, we demonstrate that small volume droplets are capable of simple and rapid amplification of target DNA in PMMA microchips using an IR-mediated heating system. Moreover, these microdevices have the potential for wider use in droplet microfluidics, potentially allowing for integrated high-throughput analysis devices for rapid detection of genetic and infectious diseases.

REFERENCES

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