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DETECTION AND APPLICATION OF MICROFLUIDIC ISOTHERMAL AMPLIFICATION ON CHIP

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Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method. Compared with the widely utilized polymerase chain reaction (PCR), LAMP has higher speed and efficiency as well as lower requirement for system temperature control because the whole amplification process is isothermal and no efforts are needed to switch between different temperatures. In this paper, we designed and fabricated different kinds of polycarbonate (PC) microfluid chips, explored appropriate reaction condition for LAMP in microenvironment ($1\,\text{nL} \rightarrow 10\,\mu\text{L}$), and developed a microfluidic isothermal amplification detection system. The DNA optimal amplification temperature is obtained; the starting time of exponential amplification of DNA is put forward farther. The optimal condition of DNA amplification in microenvironment, with a little reaction materials and early starting exponential amplification time of DNA are very important for clinic DNA detection and the application of Lab-on-a-Chip.

Keywords: Loop-mediated isothermal amplification; Lab-on-a-Chip; microfluid chips; polymerase chain reaction; DNA amplification.

1. Introduction

Nucleic acid amplification and analysis has, so far, been employed in various fields. The first-developed amplification protocol is the polymerase chain reaction (PCR),[1] which is also the most widely used method nowadays. With this method, we can amplify a specific nucleic acid sequence from a complex sample in a cyclic process, and generate a large number of identical copies, which is available for further analysis.

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method, which was first described by Notomi *et al.*[2] Since this first application, LAMP has been employed in various fields, including the detection of virus[3–5]

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and bacteria[6–8] and so on. Compared with conventional amplification methods such as PCR, LAMP has several advantages: first, the whole amplification reaction can be carried out under isothermal conditions, which makes the temperature control system relatively easy to design and reduces cost; second, the reaction is highly specific for the target; furthermore, because no extra time is needed for LAMP reaction to wait for thermal changes, the amplification efficiency is greatly enhanced.

Microfluidic chips have already been widely utilized in PCR, for their fast speed and small-sample volume requirement, as well as their potential to be integrated in micro total analysis or "Lab-on-a-Chip" systems.[9] Temperature control systems for PCR microfluidic chips have also been developed.[10] However, the combination of microfluidic chips and LAMP has not been reported so far.

In this paper, we designed and fabricated different kinds of PC (polycarbonate) microfluidic chips, explored appropriate reaction condition for LAMP in microenvironment, and developed a microfluidic isothermal amplification and detection system. The system also passed performance testing and feasibility analysis. Using this system, we successfully carried out real-time fluorescence detection of LAMP. The optimal amplification temperature is obtained, the time of starting exponential amplification of DNA is put forward farther. An application of test clinic sample was performed. Different from the initial micro-tube based LAMP technology where a limited reaction volume $\geq 5\,\mu$L for detection and a later starting time of exponential amplification of DNA after 34 minutes were observed and obtained, our developed microfluidic chips LAMP technology yields very little efficient reaction volume 1 nL for detection and an early starting time of exponential amplification of DNA after 18 minutes, which is very important to Lab-on-a-Chip.

2. Materials and Methods

2.1. *Materials*

Thermo buffer, $MgSO_4$, RPM_0691-Sau_femA_FIP, RPM_0691-Sau_femA_BIP, PM_0691-Sau_femA_F3, RPM_0691-Sau_femA_B3, dNTPs, dUTP, BSA, EvaGreen, Bst polymerase, UNG, Template (Methicillin-resistant *Staphylococcus aureus* gene-TR558).

2.2. *Amplification temperature-control methods*

The process of DNA amplification temperature control in microfluid chips with a microenvironment ($1\,\text{nL} \rightarrow 10\,\mu$L) is as shown in Table 1.

In Table 1, T is a changed temperature from 50°C to 70°C for different microenvironment ($1\,\text{nL} \rightarrow 10\,\mu$L) of microfluid chips. When the microenvironment of microfluid chips is set as, for example $1\,\mu$L, we can change the DNA amplification temperature T and explore the optimal condition of DNA amplification.

From Table 1, it is obvious that LAMP technology for DNA amplification is at an isothermal condition T, but the conventional PCR needs a lot of repeated changed

Table 1. The control process of DNA amplification.

Temperature (in°C)	Time (min)	Remark
37	2	For ready
T	3	For preparation
T	60	For test in real-time
80	5	For stop-life activity

temperature as shown in the *following steps*: *Initialization step* with a temperature of 94°C–96°C for 1–9 minutes; *Denaturation step* with a temperature of 94°C–98°C for 20–30 seconds; *Annealing step* with a temperature of 50°C–65°C for 20–40 seconds; *Extension/elongation step* with a temperature of 72°C for 45 seconds, and *Final elongation* with a temperature of 70°C–74°C for 5–15 minutes after the last cycle.

2.3. *Microfluid chip fabrication*

The microfluid chips are designed as in Fig. 1(a).

At first, the microfluid chips with different structure and microenvironment 1 nL → 10 μL was designed as a masking diagram by machine-design software solidworks 2006. Second, these designed masking diagrams were fabricated in PC materials with thicknesses of 0.5 mm, 1.0 mm and 1.5 mm etc., and the PC masking was obtained. Third, two PC films with thickness 0.1 mm were bonded to PC masking from up-and-down directions by using heat-compression techniques with a heating temperature of 150°C and a compressive stress of 100 kg for about 30 minutes. Finally, the edges of microfluid chips were smoothened or cut out to have the correct size for the chip encapsulation, and one small hole at the top of the outlet and another small hole at the bottom for the inlet was added respectively, where sample is injected into the microenvironment of microfluid chip as shown in Fig. 1(b).

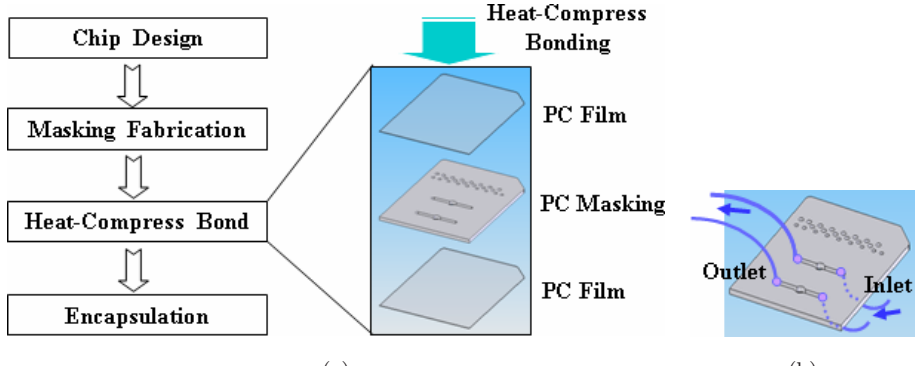

Fig. 1. The fabrication of microfluid chips.

2.4. *Microfluid chip isothermal amplification detection system*

In order to detect a real-time fluorescent signal of DNA amplification with high sensitivity and high resolving power, a confocal optical system was designed and optimized by using rays-tracing calculation. The optical geometry structure of the system was described in Fig. 2, and the full-set of optical design structure parameters were given in Table 2 (units in mm).

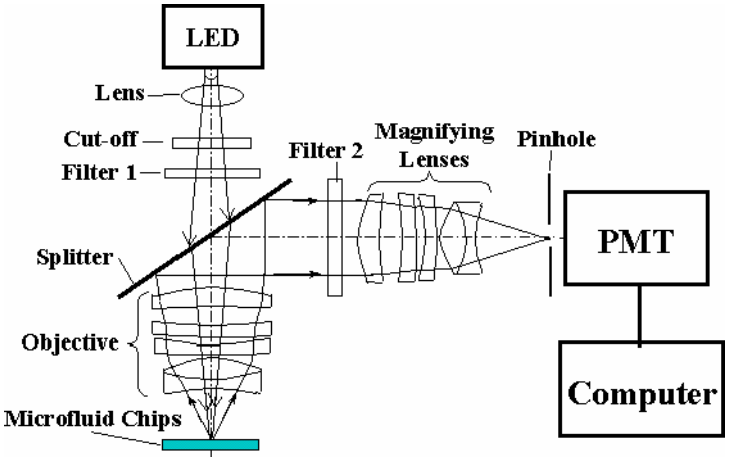

Fig. 2. The optical geometry structure of the system.

Table 2. The whole optical design structure parameters (units in mm).

Surface	Radius of curvature (mm)	Thickness (mm)	Glass
1	−26.3	2.20	ZF2
2	20.1	6.10	ZK7
3	−12.7	0.21	
4	79.3	2.70	ZK7
5	32.5	2.00	
6	183.5	3.10	ZF2
7	73.2	4.20	
8	−354.5	5.50	ZK7
9	−25.5	100.00	
10	25.5	5.50	ZK7
11	354.5	4.20	
12	−73.2	3.10	ZF2
13	−183.5	2.00	
14	−32.5	2.70	ZK7
15	−79.3	0.21	
16	12.7	6.10	ZK7
17	−20.1	2.20	ZF2
18	26.3	16.50	

In Fig. 2, the optical imaging system is composed of a set of objective and magnifying lenses. The objective set is a combination of five lenses including one doublet and uses only two glass materials, ZF2 and ZK7 as shown in Table 2. The objective has a high numerical aperture of 0.4 for collecting signals from the object, a resolution of higher than 1 μm, a focal length of 32.0 mm, and a front focal length of 16.5 mm to provide a long working distance. There is a parallel ray path between the objective and the magnifying lenses, to which it is also convenient to add the filter and other optical elements. The magnifying lenses consist of five lenses with one set of doublets also. The light from the LED was assembled by the lens and filtered by the filter 1 with a center wavelength of 532 nm (or 470 nm) and width of 20 nm, and an irradiated beam with power of 5 mW was obtained. The irradiated beam passed the splitter which was focused on microfluid chips and the fluorescent signal was excited. A shutter was used to control the exposure time of 2 seconds per minute and reduced the photo-bleaching from the irradiated beam. The fluorescence of DNA amplification with labeled dyes CY3 (or EvaGreen) at the double-strand DNA on the microfluid chips was collected by the objective, reflected by splitter, and filtered by the emission filter 2 with a center wavelength of 570 nm (or 520 nm) and width of 50 nm, and focused by the magnifying lens set on the pinhole with diameter 100 μm. The photomultiplier tube (PMT) collected the fluorescence and sent to the computer for digital-image processing by a data-acquisition unit as shown in Fig. 3.

Based on the above optical design and microfluid chips, a microfluidic isothermal amplification and detection system was set as in Fig. 3, which integrates XYZ-stage positioning unit, temperature control unit, heating plate, microfluid chip,

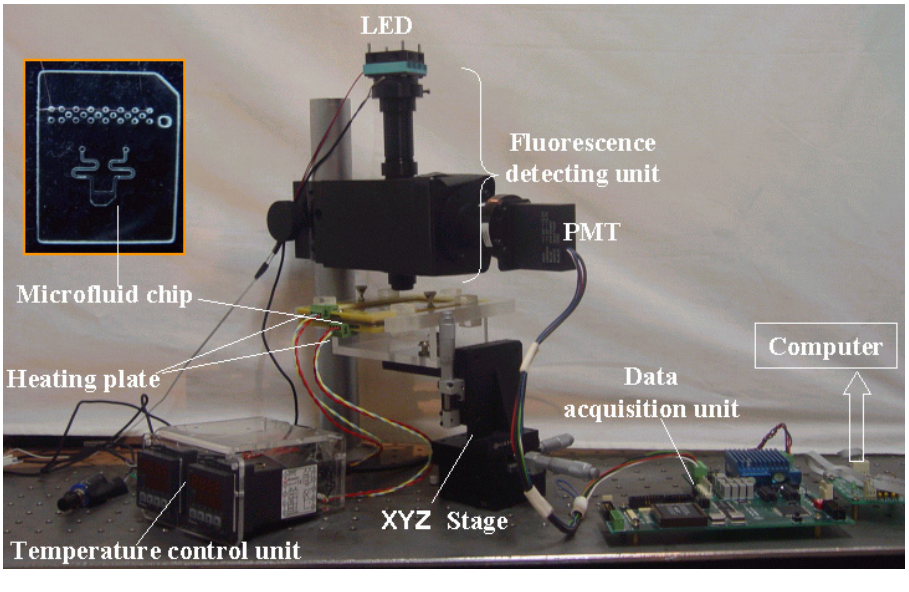

Fig. 3. The microfluidic isothermal amplification and detection system.

fluorescence-detecting unit and data-acquisition unit. Temperature control and real-time fluorescent signal detection of isothermal amplification in microfluid chip were supervised by a computer. In order to reduce the noise from PC and background, a confocal system as shown in Fig. 2 is needed to detect fluorescence from a limited diameter 100 μm at the center of reaction cell in microfluidic chip, which is very important for microfluidic chip LAMP application. The temperature in microfluid chips was measured by a temperature resistor and controlled by temperature control unit with a negative feedback as shown in Table 1.

As the process of DNA amplification temperature control shown in Table 1, when the microenvironment of microfluid chips is set in Fig. 3, T is changed with different temperatures from 50°C to 70°C for DNA amplification, and the optimal condition of DNA amplification is explored.

3. Results and Discussion

With the above developed microfluid chips and microfluidic isothermal amplification and detection system, the clinic sample of Methicillin-resistant *S. aureus* gene-TR558 was identified. The whole process of temperature control and real-time fluorescent signal detection of isothermal amplification in microfluid chip were obtained as shown in Fig. 4.

When the microenvironment of microfluid chips with an efficient detection volume of 1 nL $\rightarrow$ 10μL was set, DNA amplification at temperature T was also changed from 50°C to 70°C, we explored the optimal condition of DNA amplification. For example, when the microenvironment of microfluid chips was set with an efficient detection volume of 1 μL, the real-time fluorescence signal of DNA amplification

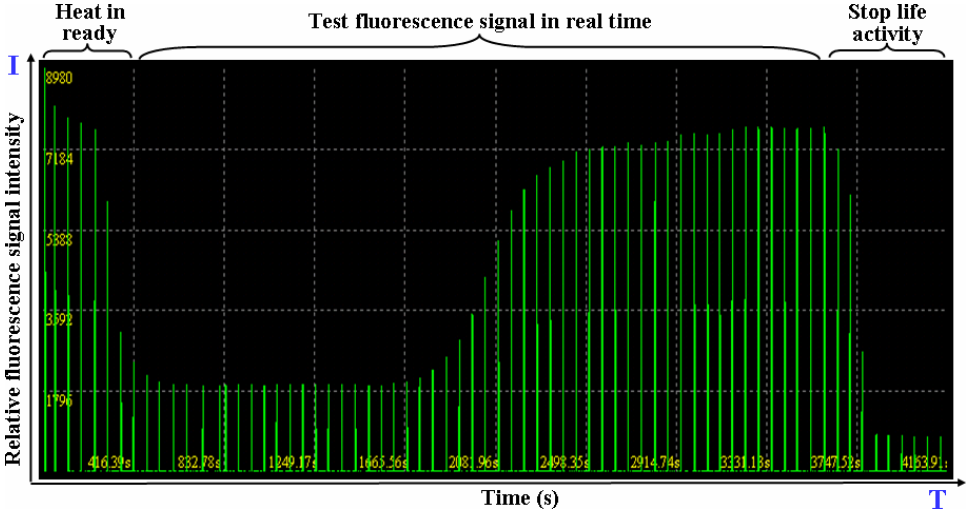

Fig. 4. The process monitor of DNA isothermal amplification in microfluid chips.

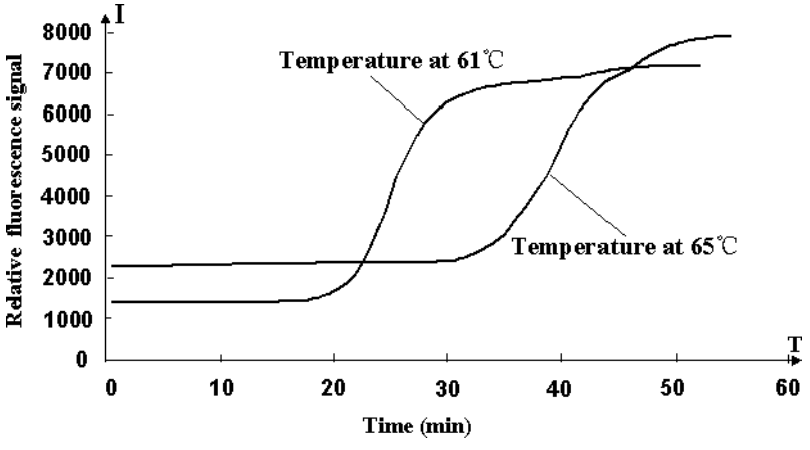

Fig. 5. The real-time fluorescence signal of DNA amplification at different temperatures.

for clinic sample of Methicillin-resistant *S. aureus* gene-TR558 at temperature $T = 65°C$ and 61°C were obtained as shown in Fig. 5. In Fig. 5, the starting time of the exponential amplification of DNA at temperature 65°C is at about 32 minutes after the start of the reaction and the ratio of the maximum relative fluorescence signal to the original relative fluorescence signal is about 4.2. When amplification temperature was changed to 61°C, the starting time of the exponential amplification of DNA is about 18 minutes after the start of the reaction, the ratio of the maximum relative fluorescence signal to the original relative fluorescence signal is about 5.1, the shape of the real-time fluorescence signal of DNA amplification is as good as the exponential function curve.

As a comparison, a commercial real-time fluorescence PCR instrument (Capitalbio Corporation, Beijing, China) was used for testing DNA isothermal amplification of the same clinic sample of Methicillin-resistant *S. aureus* gene-TR558 with a volume of 20 μL, the real-time fluorescence signal of DNA amplification was obtained as shown in Fig. 6. In Fig. 6, the starting time of the exponential amplification of DNA is about 34 minutes after the start of the reaction and the ratio of the maximum relative fluorescence signal to the original relative fluorescence signal is about 5.

From Figs. 5 and 6, while using the microfluid chips and optimal condition of DNA amplification, with a little reaction materials used, an early starting exponential amplification time of DNA can be seen obviously.

4. Conclusion

In this paper, we have demonstrated a detection and application of microfluidic isothermal amplification on chip. The method of designing and fabricating PC microfluid chips was introduced, a new microfluidic isothermalamplification and

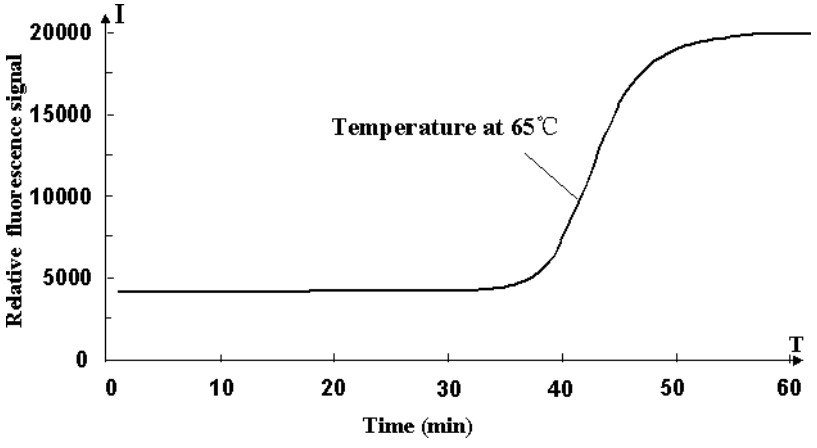

Fig. 6. The real-time fluorescence signal of DNA amplification by using RT-PCR instrument.

detection system was developed, and DNA optimal amplification temperature for LAMP in microenvironment ($1\,\text{nL} \rightarrow 10\,\mu\text{L}$) was explored. The experiment showed that the starting time of the exponential amplification of DNA is put forward when reaction materials were reduced. Under a $1\,\mu$L efficient detection volume, the optimal temperature condition of DNA amplification is at 61°C. Using the microfluid chips and optimal condition of DNA amplification, the reaction materials and testing time of DNA amplification are very economical, which are very important for clinic DNA detection and the application of Lab-on-a-Chip.

Acknowledgments

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