Microfluidic polymerase chain reaction

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We implement microfluidic technology to miniaturize a thermal cycling system for amplifying DNA fragments. By using a microfluidic thermal heat exchanger to cool a Peltier junction, we have demonstrated rapid heating and cooling of small volumes of solution. We use a miniature K-type thermocouple to provide a means for *in situ* sensing of the temperature inside the microrefrigeration system. By combining the thermocouple, two power supplies controlled by a relay system, and computer automation, we reproduce the function of a commercial polymerase chain reaction thermal cycler and demonstrate amplification of a DNA sample of about 1000 base pairs. © *2008 American Institute of Physics*. [DOI: 10.1063/1.3046789]

The polymerase chain reaction (PCR) has emerged as one of the most sensitive analysis tools available to researchers in molecular diagnostics.[1] DNA genotyping, virus identification, and forensic applications are a few of the applications that rely on PCR amplification and analysis.[1–3] Clearly, thermal cycling PCR is one of the leading techniques for the amplification of DNA fragments.[1] It is a rapid and straightforward method for generating many copies of DNA molecules. PCR amplification typically consists of thermally cycling DNA fragments through many heating (denaturation), cooling (annealing), and extension temperatures of target DNA mixed with a primer and a polymerase (involved in the catalysis of DNA).

To obtain accurate amplification of the desired DNA chains, it is necessary to carefully control the three temperatures and to ensure good temperature uniformity in the thermal cycling process. Traditionally, resistive heating or forced air heating has been used to control the temperature. More recently, Peltier junctions have been the refrigeration and heating system of choice for PCR applications.[4] This choice is a result of the relative simplicity of integrating electrically controlled Peltier junctions within electronic feedback loops using thermocouple measurement data.[4]

Herein, we compare two alternatives of combining thermocouple device with thermovoltaic solid-state devices (a Peltier junction) to form a miniaturized PCR cycling system within a microfluidic and glass capillary system with rapid ramp rates. One of the advantages of developing such a microfluidic cooling system is that liquid cooling can be used for effective heat sinking of the Peltier junctions. The other advantage of integrating PCR cyclers within microfluidic systems is that rather complex sample preparation can be performed microfluidically and the transfer of amplicon from the fluidic chip into a capillary-based thermal cycler can be avoided. However, more conventional glass capillary tubes offer the great advantage of well-defined surface chemistries and the avoidance of evaporation through porous elastomer channels—a major problem with previous polydimethylsiloxane (PDMS) approaches.[5]

Two Peltier junction systems were constructed: the first was based on a multilayer replication molded PDMS microfluidic chip developed for the purpose of integrating fluidic sample delivery, where both the junction and the thermocouple are assembled to run a DNA sample using the PCR technique described above.[6,7] Therefore, the process of incorporating different elements such as the junction and the thermocouple into the chip can be thought as a "lab-on-a-chip" device that can both miniaturize and reduce costs when dealing with the nowadays-expensive techniques of PCR. An alternative system, in which a commercially available glass microcapillary tube (Polymicro Technologies TSG320450) with a 320 μm inner diameter is inserted into a copper heat exchanger, was also constructed. PCR amplification was demonstrated on large DNA molecules, and the ramping speed of the system was characterized. The microfluidic device was able to perform very fast cycling that consisted of a rise in temperature from 22 to 95 °C and then holding at 95 °C for 900 s, followed by a 35-cycle PCR amplification reaction.[3] The control thermocouple was calibrated to actual sample temperatures by performing a dummy run with a

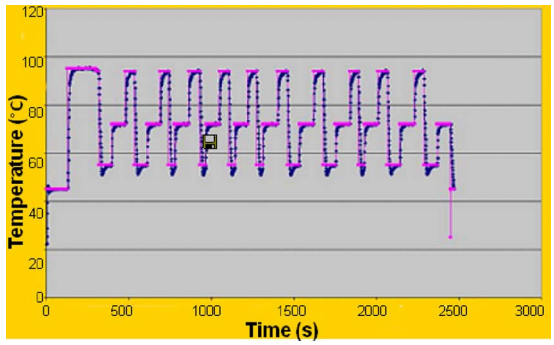

FIG. 1. (Color online) A graph of temperature vs time showing the typical temperature control temperature we can achieve. The actual temperature control in the tube is better as the glass tube behaves as a low pass filter. We calibrate the control temperature to a thermocouple in the liquid during a dummy run.

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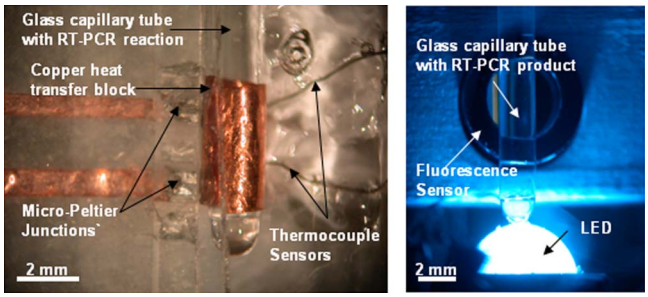

FIG. 2. (Color online) An optical micrograph of our microfluidic PCR system. The glass capillary tube is heated and cooled by the *P* and *N* Peltier junctions attached to the copper jacket. Also visible to the right is the control thermocouple.

thermocouple placed in the reaction chamber, which was filled with PCR mastermix (Fig. 1). A calibration between the control temperature and the sample temperature was then performed. We noticed little need for recalibration of the system once this procedure was carried out. The schedule for this experiment is summarized as follows: 50 °C for 1200 s and 95 °C for 900 s; PCR (35 cycle): 94 °C for 15 s, 55 °C for 20 s, 72 °C for 40 s.

A solution with 1000 base pair DNA molecules was run through 35 cycles, and the amplified product was passed through a gel using the gel electrophoresis technique to determine whether or not the PCR run amplified the appropriate DNA sample. A silica capillary tube was prepared with the amplicon, and mineral oil was used to avoid evaporation of the sample at the high denaturation temperature. When single or double Peltier junctions were used on the copper heat sink holding the capillary tube, 20 °C/s ramp rates were measured, whereas the introduction of four parallel Peltier junctions resulted in ramp rates of approximately 100 °C/s, measured in the amplicon mixture. Figure 2 shows a time-temperature plot in which heating rates of above 100 °C/s and cooling rates of 90 °C/s were measured. In this system, the direction of current flow was reversed for heating and cooling of the primer/sample mixture. The 0.32 mm capillary enabled the amplification of 0.4 μl DNA+primer samples. A 1000 base pair beta-actin DNA mixture, as well as a DNA positive control, and a DNA ladder were tested in parallel by using agarose gel electrophoresis after the DNA sample was amplified. Figure 3 shows the gel electrophoresis results indicating that the amplified product did consist of 1000 base pair molecules.[3] Furthermore, once the DNA sample was cycled and gel electrophoresis tests were completed, the same sample was used to obtain a melting curve to determine the melting temperature of the amplified DNA mixture. Figure 4 shows the resulting melting curve, indicating a very sharp melting temperature at approximately 85 °C, indicative again of the amplification of rather large DNA fragments. It is of interest to note that no primer-dimer signals were detected from this melting curve, indicating a uniform temperature within the amplification system.[8]

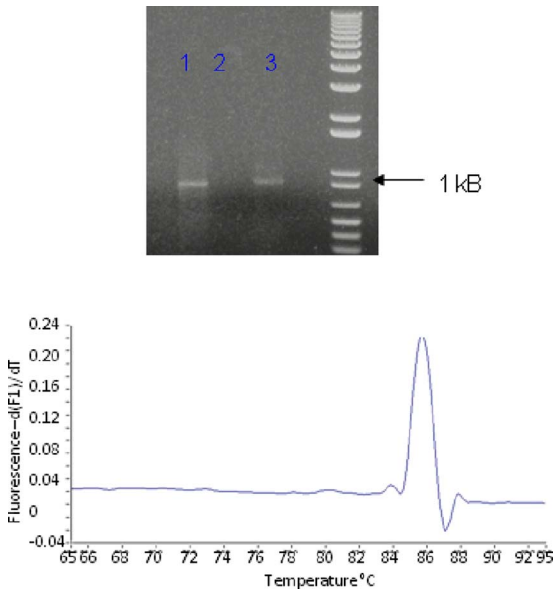

FIG. 3. (Color online) (a) Electrophoresis gel of the amplified product (2 μl PCR products were loaded into each lane. Lane 1: beta-actin amplified with Roche Lightcycler. Lane 2: negative control with template. Lane 3: beta-actin amplified with Caltech device.) and (b) melting curve of the amplified product on the Roche Lightcycler.

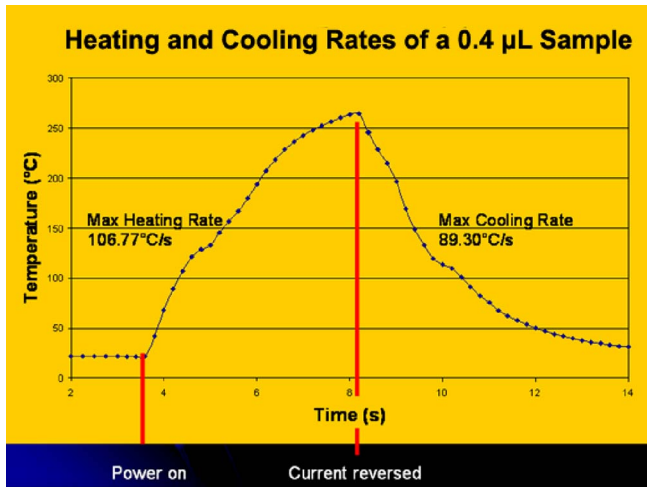

FIG. 4. (Color online) Maximum heating and cooling rates of a 400 nl sample in our microfluidic thermal cycler.

We have described a simple and small PCR-on-a-chip system to amplify DNA fragments. Recent work on PCR technologies has focused on reducing the size of PCR systems. For on-chip PCR amplification, the amplicon is often pumped over constant temperature heaters located in the path of the fluidic channels. However, three important problems arise when such microfluidic flow-controlled PCR systems are to be used: (1) the flow rate of DNA molecules within a channel depends on the distance between the molecule and the side walls of the channel, (2) the PDMS commonly employed is porous in air and steam, and water evaporation at high temperatures requires careful designs to avoid fluid loss during the PCR amplification, and (3) the surface-to-volume ratios of such fluidic systems are very large and not well understood. The system described herein is based on chambers that can be rapidly heated and cooled rather than fluid flow based thermal cycling systems and on the use of Peltier junctions. We show that modern Peltier junctions can be used for rapid thermal control with heating and cooling rates of 100 °C/s—with temperature control accuracies of 0.1 °C.

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