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Evaluation of microheaters for stationary miniaturized PCR thermocyclers

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Summary:

PCR, as a very important and basic method in microbiology, is used to amplify DNA sequences and is applied worldwide, especially for the detection of diseases and viruses. Today, PCR devices are in most cases still laboratory-based, complex instruments. We present the use of microheaters with the aim to miniaturize PCR devices, making them faster, more energy efficient, mobile and cost-efficient.

Keywords: PCR, simulation, microheater, thermocycler, thermodynamics

Motivation

Polymerase **C**hain **R**eaction (PCR) has been a method used for decades to amplify nucleic acids (DNA and RNA) for the detection of hereditary diseases or viral infections. In PCR, a periodic repetition of three reaction steps is usually performed: A denaturation step to separate the two DNA strands at 92-96 °C (1. stage), primer hybridization at 55-68 °C (2. stage), and an amplification step at ~72 °C (3. stage), as outlined in Figure 1. The basic requirement for efficient amplification is a rapid heat transfer or heat dissipation into or out of the sample. Consequently, a heating element with low heat capacity and high thermal conductivity is desirable. In most traditional stationary PCR instruments, the heating and cooling rates of the heating elements used are relatively low due to components with a large thermal mass (cf. Figure 1). Miniaturization of conventional PCR instruments using novel heating elements could significantly accelerate the amplification reaction and also save reagents [1].

Our aim is to develop a thermal cycler for PCR devices that is faster, more energy efficient and smaller than previous conventional thermal cyclers, essentially by reducing the thermal mass. For this purpose, a new heating element technology for micro-PCR is evaluated. These novel heating elements have a great potential to perform PCR reactions in a small space without the need for Peltier or other coolers, thus minimizing the disadvantages of the currently available solutions for PCR.

Microheater

The most frequently used heater metallization in microsystems technology is platinum. There are several reasons in favor of platinum. The material enables a simple realization of heater structures and has a low tendency to oxidation. Platinum exhibits a linear temperature dependence of electrical conductivity and can therefore be used not only as a heater but also as a temperature sensor. The microheater is manufactured by depositing platinum on its carrier substrate, e.g. Al_2O_3, by vapor deposition or sputtering over the entire surface of the substrate. Subsequent patterning is usually done with aqua regia using a photoresist mask or alternatively with ion beam etching [2, 3].

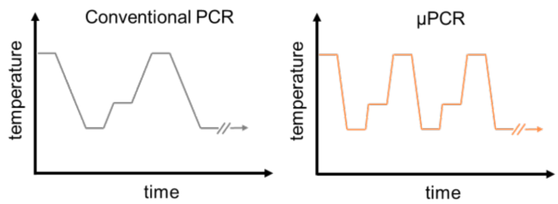

Fig. 1. PCR is based on cyclic repeating steps: Denaturation at 92°C – 96°C, hybridization at 55°C – 68°C, and polymerization at 72°C. This procedure is repeated several times. On the left, the temperature curve of a conventional PCR device with slow heating and cooling rates is shown. On the right, the µPCR is shown with faster heating and cooling rates.

Figure 2 shows the simulation of a microheater which consists of a 2.1 x 1.3 x 0.4 mm aluminum oxide substrate and a 2 mm thick platinum meander structure between two platinum pads lying on the substrate. The line width of the meander

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structure is 56 µm, while the gaps between them are 15 µm. The substrate including the platinum structure is passivated with 15 µm thick borosilicate glass to prevent chemical reactions with the environment. The resistance of the microheater is 14.75 Ω. When an electrical voltage is applied to the pads, a current flows through the platinum meander structure.

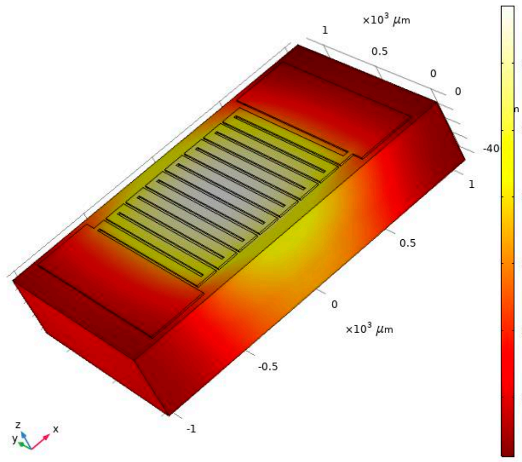

Fig. 2. The microheater consists of an aluminum oxide substrate and a meander structure made of platinum. The temperature distribution is shown in color.

The resulting Joule heating describes the conversion of electrical energy into thermal energy due to the ohmic resistance of the conducting material. Figure 2 shows the temperature distribution of the microheater in color, with maximum temperatures of over 500 K being reached at the meander structures for a voltage of 5 V.

PCR chamber

The chamber (Figure 3) is used to heat the DNA sample, which is located on the upper borosilicate glass layer of the microheater with a cylindrical volume of 10.6 µl. Compared to conventional thermal cyclers, this has the advantages that the contact resistance between heater and chamber is minimal, less energy is required for temperature cycling, and expensive reagents can be saved. The comparison of different chamber materials shows that especially metals such as aluminum allow a homogeneous temperature distribution within the chamber. This is due to the better thermal conductivity of metals with electrons as the dominant heat carrier in the particle model. Aluminum (237 W/mK) has for example a much better thermal conductivity than common ceramics such as Alumina (30 W/mK), where heat is mainly transferred via lattice vibrations. In addition, heating rates of over 20 K per second are possible with conductive metals, which is faster than commercially available thermal cyclers [4-6].

Conclusion

The use of microheaters in PCR offers new set-up possibilities. By reducing the thermal capacity and sample volume, low voltages are sufficient to generate high temperatures. The simulations confirm these assumptions. Materials as well as

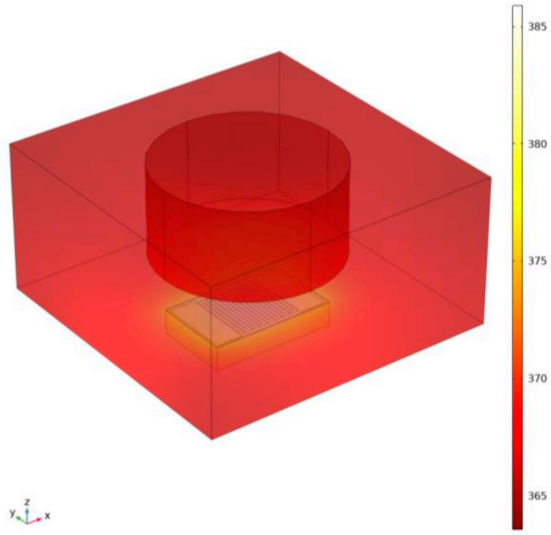

Fig. 3. The cylindrical sample chamber is placed on the microheater and has a volume of 10.6 µl.

reagents can be saved and enable cost-effective production and operation of thermal cyclers. The simulations serve as the basis for a possible battery-powered PCR solution, which enables the mobile design of thermal cyclers.

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