PCR Microsystem for Fast Cycling

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In this paper we present the design, fabrication and test of silicon-glass based PCR chips which amplify specified DNA strands. The design of the chip was optimized to ensure a fast PCR process in terms of thermal cycling speed, biocompatibility, size of reaction chamber and simplicity of fabrication. First tests using a conventional setup for thermocycling show successful DNA amplification in the micro PCR chip.

Introduction

A lot of effort is put into the development of integrated microsystems for analyzing blood and especially DNA. The aim of the integration is to design small devices for fast and cost efficient analysis. Polymerase Chain Reaction (PCR) is a proven technique for amplifying DNA. This method allows multiplying a single DNA molecule up to a billion times. The DNA of the target gene is copied leading to an exponential increase of the number of the gene. The whole process consists of 20 to 50 heat cycles. One PCR cycle contains three different temperature steps. First, the double stranded DNA is heated up to 95 °C to break up the hydrogen bonds (denaturation) and separate the two DNA strands from each other. Next, at a temperature of approximately 55 °C (annealing) gene-specific oligonucleotide primers, which flank the DNA region to be amplified, hybridize to the single strands. Finally these DNA strands are extended in the presence of the thermostable Thermus aquaticus polymerase (Taq) and deoxynucleotide triphosphates (dNTPs) at a temperature of 72 °C (extension) [1].

After first tests on a silicon-glass PCR chip where the filling ports were fabricated in the silicon part, in this paper a modified design of silicon-glass PCR chips is demonstrated and tested. The PCR chip is a single chamber chip with filling ports through the glass. The realization of the filling ports through glass has the advantage that a heating unit can be attached on the silicon side, which improves the thermal behavior of the device and simplifies the injection and extraction of the PCR sample from the chip. Practical issues related to chip design, fabrication process and PCR tests will be discussed in the next sections.

Concept and Design

Several different PCR microsystems have been published [2], which are generally grouped into two principles; (i) the continuous flow PCR microsystems and (ii) the micro chamber PCR systems. In the continuous flow PCR microsystems the reaction mixture is moved between three well-defined temperature zones. The mixture is transported in rotary, serpentine or back and forth motion. In the micro chamber PCR systems the reaction mixture is kept stationary, while the temperature of the reaction chamber is cycled between three different temperatures. Advantages of the chamber PCR are the freedom in the number of cycles to be made and the possibility to test the

device in a conventional thermocycler. This allows first PCR tests of the device without any heating and sensing elements on chip and the conventional PCR results can be compared with those of the PCR chip. Therefore, we decided in favor for the chamber PCR concept. To achieve high recovery of the PCR sample the chamber has to be constructed like a channel, where the filling ports are located near the chamber walls. In Fig. 1 on the left hand side the designed PCR chip is shown. The chamber consists of a (wide) channel with funnel shape at the side of the filling ports. The volume of the chamber is 25 µl. The chip dimensions are 15 x 20 mm and the distance between the two filling ports is about 13 mm. The access holes are drilled in the Pyrex glass with a diameter of 1 mm. The reason for realizing the filling ports through glass is that a heating unit can be attached on the silicon side for thermocycling the chamber, which, because of the better thermal conductivity of silicon compared to glass, improves the thermal behavior of the device.

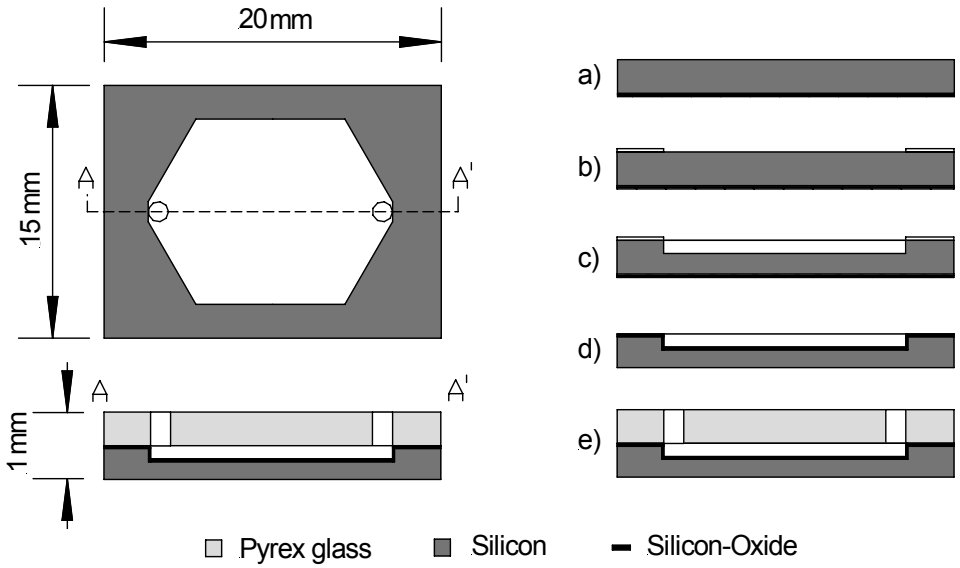

Fig. 1: Design of the PCR chip (top view and cross section) with a reaction volume of about 25 µl (left); Fabrication process of the PCR chip (right)

For fast thermocycling on chip the device has to be small, so that less thermal mass has to be heated and cooled. As a further term the material has to have a high thermal conductivity and a low heat capacity. Silicon fulfills both criteria and additionally, it has excellent structuring characteristics. The heating unit will be realized on the silicon side of the PCR microsystem. As lid a Pyrex glass is used, which has the advantage of allowing insight into the chamber and the possibility of irreversible anodic bonding to the silicon. It can also be machined (through-holes).

Surface chemistry plays a dominant role in PCR reactions within a micro fabricated environment due to the high surface-to-volume ratio. Silicon as such is not very well compatible with DNA and Taq polymerase, since it provokes adhesion effects that can inhibit standard PCR reactions. An oxidized surface however was shown to give consistent amplification that is comparable to those performed in conventional PCR tubes [3]. Thus an oxide layer will be thermal grown on the silicon side in the chamber.

Fabrication Process

The PCR chip is fabricated using photolithography, combined with DRIE (deep reactive ion etching) process, glass drilling and glass-to-silicon dioxide anodic bonding (Fig. 1, right hand side). We used double side mirror polished silicon wafers (100 mm diameter, 525 µm thick, <100>, n-type) coated with an insulating layer consisting of 250 nm thermally grown silicon dioxide and 70 nm LPCVD (low pressure chemical vapor deposition) silicon nitride on one side (a). To fabricate the cavities for the chamber the positive photoresist AZ6624 was coated and patterned on the silicon side of the wafers (b). The reaction cavities were plasma etched to the depth of 180 µm using a DRIE reactor (SF_6/O_2, so called Bosch process) (c). Afterwards a silicon oxide layer with the thickness of 40 nm was thermally grown on the side of the reaction cavities to achieve biocompatibility. The oxide and nitride layers were removed from the wafers with RIE in order to achieve electrical contact during anodic bonding (d). Holes with 1 mm in diameter are drilled in the 500 µm thick Pyrex 7740 glass wafers. Afterwards the wafer is anodically bonded with the silicon wafer to cap the cavities (e). Finally the wafers were diced with a conventional wafer saw.

Fig. 2: Photo of the fabricated PCR chip

Proof of Functionality

The functionality of the PCR chip is tested by placing it into a thermocycler (PTC-200 slide cycler from MJ research). For the thermal cycling a standard PCR program can be run. The temperature of the hot air in the cycler is set at 95 °C for denaturation, 55 °C for primer annealing and 72 °C for extension. Each step is performed for 1 min and to achieve a high yield of DNA amplification 50 cycles are carried out. A standard PCR mastermix is used, where gene-specific primers, deoxynucleotide triphosphates (dNTPs), Taq polymerase and E. coli DNA are included.

The ready-made mastermix is manually injected into the PCR chamber with a conventional pipette. At one port the mastermix is filled in whereas at the other port the air escapes. Before the chip is placed into the thermocycler the filling ports are sealed properly with the PCR tape “ARseal 90404” from Adhesives Research. This prevents gradual evaporation of the sample at denaturation temperature and avoids generating undesired air bubbles in the chamber.

After running the PCR in the conventional cycler the detection of the amplified DNA product is performed off chip. For this the amplified DNA sample is pulled out from the chip with a conventional pipette and afterwards analyzed on an agarose gel. The volumes of the recovered PCR product and the mastermix initially applied to the chip are almost identical due to the chip design. Only tiny amounts of the DNA sample remain at the corners of the reaction chamber.

Figure 3 shows the result of the on chip DNA amplification.

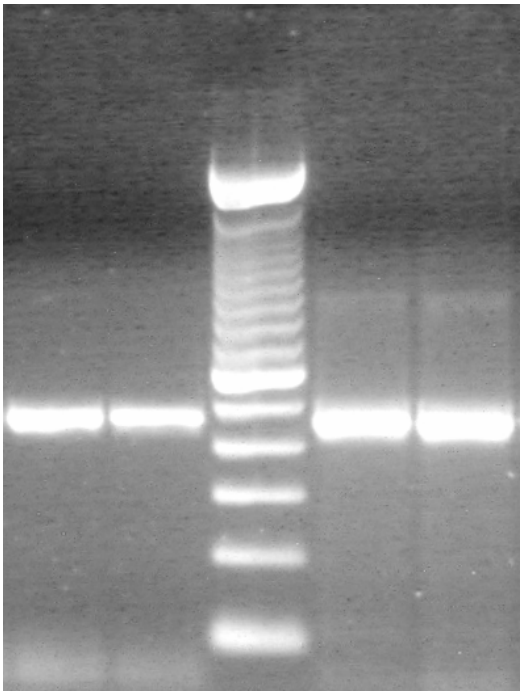

Fig. 3: Agarose gel electrophoresis of 16S rRNA PCR product (497 bp fragment) amplified from E. coli DNA; Lane 1 and 2: single chamber design; in comparison to conventional PCR reaction tubes (Lane 4 and 5). Lane3: 100bp ladder

Discussion

The design of PCR chips with filling ports through the glass has been presented and the functionality of the PCR of these devices has been successfully proven. The device promises miniaturized, fast and effective PCR performance. As a next step a heating unit and thermal sensing elements will be added on the chip to achieve a standalone PCR microsystem.

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