Convective polymerase chain reaction around micro immersion heater

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Polymerase chain reaction (PCR) is performed in the thermal convection created by a micro immersion heater. Instead of repetitive heating and cooling, the temperature gradient induces thermal convection which drives the reaction liquid between hot and cold parts of the chamber. The convection triggers DNA amplification as the DNA melts into two single strands in the hot region and replicates with the use of proteins into twice the amount in the cold region. The constant heater is simply dipped into the reaction solution. Compared to previous experiments, we demonstrate that convective PCR is possible in a robotically accessible open vessel. Our approach compares well with fast PCR cyclers and replicates DNA 500 000 fold within 20 minutes. We reduce the necessary components for PCR to cheap, single-use components and therefore increasing the prospects of bringing PCR to point of care applications—even in third world countries. © *2005 American Institute of Physics*. [DOI: 10.1063/1.2051787]

DNA-based biochemistry expanded widely with the invention of the polymerase chain reaction (PCR).[1,2] PCR allows to exponentially amplify a piece of DNA of specific sequence to exceedingly high amounts. The power of PCR is based on the combination of two main characteristics. First, the tremendous sensitivity of PCR is based on the exponential amplification of DNA in a chain reaction. PCR at its best doubles the targeted DNA sequence in each temperature cycle. Therefore, PCR can detect DNA down to single DNA molecules as initial DNA concentration is an exponential measure of the amplification time. PCR does detect DNA concentrations over nine orders of magnitude. Second, PCR is very specific. It amplifies only if its short DNA fragments (primers) exactly match and bracket the sequence of the DNA to be amplified. Primers have a typical length of 20–30 base pairs.[3]

To reach fast temperature changes, complicated and delicate PCR instrumentation is required. For example, thin capillaries are heated and cooled by air.[4] Several microfluidic approaches of PCR have been pursued.[5,6]

Here we show that convective PCR is possible in an open vessel format at high reaction speeds. Convective PCR, although being only in its infancy, was proved to work at intermediate to high speed in three very different geometries.[7–13] A compilation of techniques is available as short review.[14] The idea is that a constant temperature gradient across a suitably chosen chamber geometry induces thermal convection between temperatures of about 65 and 95 °C. The reaction liquid rises in the hot regions of the chamber due to thermal expansion and moves downward into colder regions. The geometries are chosen such that the reaction liquid oscillates along laminar trajectories. A good thermal convection design shuttles most of the volume with similar temperature characteristics.

The polymerase chain reaction proceeds in three steps that are periodically repeated: (i) heating a DNA double helix at 95 °C to dissociate it into two single strands (melting); (ii) decreasing the temperature to 50–65 °C in the presence of a large excess of primers, which bind to the terminal target sequence (annealing); (iii) raising the temperature to 72 °C in the presence of a thermostable polymerization enzyme called polymerase that elongates each of the two short double helices to twice the amount of target DNA (elongation). The temperature cycling can be simplified in many cases to two temperatures for melting and annealing/elongating.

If the biochemistry of the PCR reaction could be speeded up, convective PCR is expected to take over conventional PCR machines. For example, if the biochemistry were ten-times faster, current PCR machine designs would be too slow due to their large thermal mass. However, in convection PCR only the liquid itself is heated and cooled, not the chamber. Therefore the thermal mass is minimal. Convection cycler could easily keep up with increased cycling speed.

Our open vessel convective PCR can be simply made from disposables. The micro immersion heater consists of a standard medical cannula (diameter 0.4 mm) and a thin lacquer insulated copper wire (diameter 0.08 mm) as shown in Fig. 1(a). The wire is threaded through the cannula and fixed at the tip by a droplet of super glue (Pattex Blitz Glas, Henkel, Germany). The cannula's cusp is bent in a right angle, the copper wire coiled around and fixed with super glue. The wire is connected to a DC power supply.

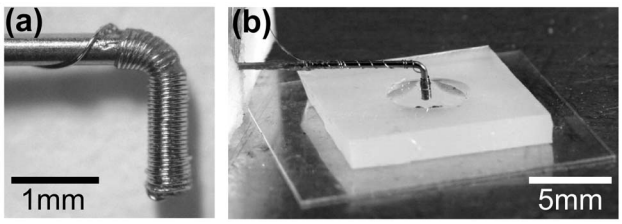

FIG. 1. PCR Convection Setup. The DNA amplifying PCR reaction is performed in a thermal convection flow formed around a microscopic immersion heater (a) which is dipped into 20 μl of reaction solution (b). Constant heating drives a steady state convection that shuttles the reaction molecules regularly between the hot center and the cold periphery. DNA is exponentially amplified as it melts in the center and is duplicated by proteins in the periphery. The open design of the chamber allows simple liquid exchange.

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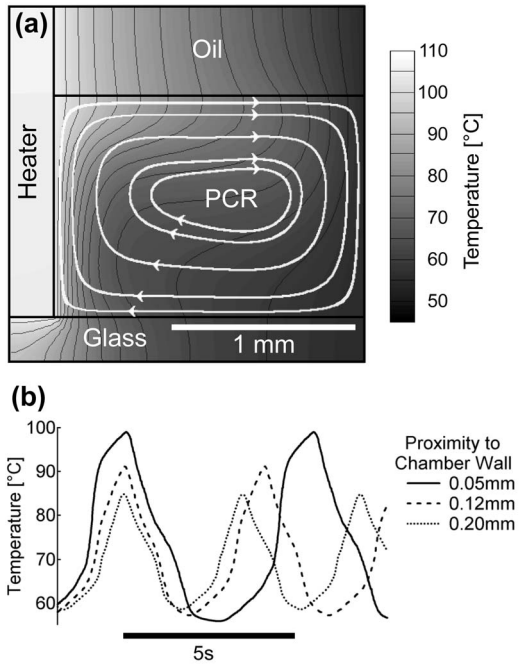

FIG. 2. Flow Simulation. (a) Temperature profile and convection flow is simulated in radial coordinates using finite element methods (FEMLab). Temperature contour lines have a distance of 5 K. More than half the chamber volume cycles through regions capable of melting the product. (b) Temperature over time along three different flow lines show that convection return times lie between 4 and 6 s. Fast DNA amplification is the result of this high speed temperature cycling.

The convection chamber into which the heater is dipped consists of 1.75 mm thick silicone rubber sheet in which a 4 mm hole is blanked out with a ticket-punch [Fig. 1(b)]. The rubber sheet sticks to the glass cover slip naturally; however, we secure the contact from outside with super glue. The chamber bottom is thermally connected to a temperature controlled aluminum stage.

To optimize convective PCR for an open chamber design, we modelled convection with a finite element solver (FEMLab).[15] Calculation of Navier-Stokes and heat transfer equations were performed in cylinder coordinates with cross-terms to account for convection from thermal expansion and cooling from fluid flow (Fig. 2). Boundaries in contact with air were considered thermally isolated. Outer contacts at radius $r=2$ mm were fixed to $T_0=55$ °C due to good thermal contact to the stage. Nonslip boundary conditions are used for water-glass and water-oil interfaces. The heater is modelled by homogeneous heat deposition with 8×10^8 W/m^3.

The material constants were as follows: water with density $\rho=10^3$ kg/m^3, heat capacity $c_P=4.2\times10^3$ J/(kg K), thermal heat conductivity $\lambda=0.65$ W/(mK) with linear approximation for viscosity between $\eta(50\,^\circ\mathrm{C})=0.55\times10^{-3}$ Ns/m^2 and $\eta(100\,^\circ\mathrm{C})=0.3\times10^{-3}$ Ns/m^2 and volume expansion coefficient between $\alpha(50\,^\circ\mathrm{C})=0.43\times10^{-3}$ and $\alpha(100\,^\circ\mathrm{C})=0.7\times10^{-3}$. Fixed tabulated constants were used for glass bottom, stainless steel heating rod and covering silicone oil.

The temperature profile is radially dominated and induces toroidal convection along flow lines that become rectangular-shaped near the chamber walls [Fig. 2(a)]. The chamber geometry was optimized for the standard 25 μl reaction volume. Due to the cylindrical geometry, temperature rises predominantly near the heated center and yields fast temperature spikes which are needed for PCR. Water rises in the hot center with a maximal speed of 4 mm/s.

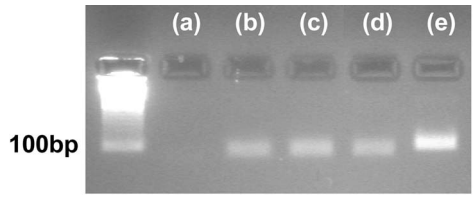

FIG. 3. Gel electrophoresis of PCR product. Agarose gel electrophoresis reveals single products after 20 minutes of convection. (a) No product for heating powers of 34 mW. (b)–(d) Near-optimal convection PCR for stage temperatures 50, 55, 60 °C and heating power 75, 60, 60 mW. Amplifications are 5.6×10^5, 5.7×10^5, and 5.5×10^5-fold. (e) Standard PCR results in a twofold higher amplification after 120 minutes—however, with an exceedingly more complex machinery.

Temperature profiles along flow lines over time [Fig. 2(b)], show fast and nearly symmetric oscillations between hot and cold. The shape of the convection flow is robust against changes in heating power. Return times are between 4 and 6 s. Interestingly, return times are comparable to laser-heated designs[14] where no heater imposes nonslip boundaries in the center of the convection. Such fast temperature changes are well suited for PCR of smaller DNA products that is mainly used to detect sequences, i.e., viruses, from their genetic fingerprint. To amplify larger products, we could use thinner and broader chambers, as previously shown in closed laser-heated designs.[14]

We use a standard PCR protocol and amplify 96 bp out of 48 502 bp λ-DNA, a protocol we used in a previous report.[11] Primer sequences were 5′-GATGAGTTCGTGTCCGTACAACTGG-3′ and 5′-GGGCAATCAGTTCATCTTTCGTCATGG-3′ (melting temperatures 61 and 62 °C) to amplify 96 bp piece out of 48 502 bp long λ-DNA. The reaction mix was prepared according to manufacturer's protocol: 5 pmole of each primer, 500 pg of λ-DNA (GibcoBRL), 0.2× SYBR Green I (S-7563, Molecular Probes) in 25 μl water (degassed by boiling) were used to dissolve a hotstart PureTaq-PCR bead (#27-9559-01, Amersham Biosciences). PCR yield dropped about twofold if in contact with glass and stainless steel. Silicone oil did not affect PCR whereas Zeiss immersion oil #518F inhibited PCR. However, to ensure reproducibility, we avoided coating of the chamber components with blocking agents such as bovine serum albumine (BSA) or polyethylene glycol (PEG). The final solution was pipetted into the reaction chamber and closed with a 10 μl-layer of silicone oil (Fluka 85409) to avoid evaporation.

PCR was first performed with a heating power of 75 mW, driven by a current of 450 mA. After 20 min 5 μl of the PCR product is removed by simply pipetting through the oil layer. It is analyzed on a 2% agarose gel. A single product with the expected length is found (Fig. 3). Quantification against 100 ng marker of 100 bp DNA marker shows 95 ng of PCR product. We therefore have amplified the initial DNA 560 000-fold.

Very similar results are found for 60 mW heating power and stage temperatures 55 and 60 °C, shown in Figs. 3(c) and 3(d). Amplifications are 5.7×10^5 and 5.5×10^5-fold, respectively. Therefore, effective doubling time of immersion heated convective PCR is at least 60 s. This value is identical to the 55 s measured for the more sophisticated laser heated approach.[11] For a heating power of 34 mW and stage tem-

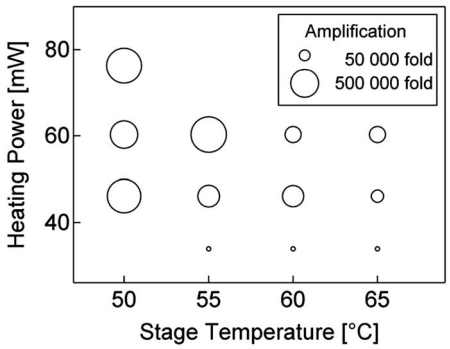

FIG. 4. Power and temperature needed for amplification. DNA amplification after 20 min, plotted against heating power and stage temperature. A broad range of convection conditions allow strong DNA amplification. Optimal amplifications are found for 50–70 mW heating power and 50–55 °C stage temperature.

perature of 50 °C no amplification was found [Fig. 3(a)].

We compared with cycling performed by a standard PCR-machine. Here, the reaction vessel is iterated through well-controlled temperature steps for 2 h and 40 cycles. We used the following temperature steps: 1 min at 95 °C, 1 min at 60 °C, and 2 min at 72 °C. We found only a twofold higher DNA amplification [Fig. 3(e)] as compared to convective PCR [Figs. 3(b)–3(d)]. The difference is insignificant since PCR amplification doubles the target in each cycle. However, a twofold drop in efficiency could be attributed to the presence of immersion heater or glass surfaces.

We varied heating conditions to determine optimal conditions for convection PCR. Amplifications are plotted logarithmically against stage temperature and heating power in Fig. 4. We find that the reaction is remarkably stable against variations of temperature and heating. For 50–70 mW heating power and 50–55 °C stage temperature, optimal amplifications are found.

Previously demonstrated convective PCR machines used closed designs. Whereas closed chambers are useful in fluorescence-detected real-time PCR, filling requires manual work. If the PCR product is needed for subsequent gel analysis, opening the chambers is again nontrivial. Here, convective PCR is performed by simply dipping a constantly heated wire into the reaction vessel. Therefore, convective PCR is robotically accessible and filling the chamber is as easy as for a standard PCR tube.

We demonstrate a convective PCR machine with cheap disposables without computer control. Disposable PCR minimizes cross-contamination and enhances reliability. As previously shown,[9] the simplicity of convective PCR minimizes power consumption. In our case, convective PCR around an immersion heater needs only 60 mW for 20 min. This yields a total power consumption for PCR of 20 mWh which can be easily driven by small batteries or solar cells.

Convective PCR was performed recently in a variety of geometries.[7–13] In contrast to competing approaches, we gain speed and specificity by using a most unrestricted convection flow. In the chosen toroidal flow, return times between different flow lines are comparable as no nonslip boundary conditions restrict the convection flow. Moreover, convection reaches maximum speeds.

Previously,[11,12] we heated the reaction mixture with a focussed infrared laser. Whereas this type of heating does not need to touch the reaction liquid, IR lasers amount to $1000. It is, therefore, important to prove that much cheaper designs were possible using fast toroidal flow convection PCR.

In closing we want to note that very similar convective conditions are capable of strongly accumulating DNA below the heater due to directed movements along temperature gradients.[16] The driving force is called thermophoresis,[17,18] which is not yet understood in liquids.[19–21] We predict thermophoretic trapping in a thin gap below the immersion heater, comparable to previously studied laser heated geometries.[16] In 1 mM monovalent salt, 100 bp DNA would be trapped 5× and 1000 bp DNA 120×. Trapping ratios increase exponentially with DNA length. However, for salt concentrations found in the PCR mix, thermophoretic drift is reduced. Trapping will drop to insignificant levels of about 1.5× and 4×, respectively.

In conclusion we have shown that convective PCR can be driven by simply dipping a constantly heated wire into reaction solution. This approach is simple to handle and compatible with automated liquid handling. It uses cheap disposables, no computer and needs only minimal heating power. The demonstrated 0.5×10^6-fold amplification within 20 min is competitive and compares well with complex high-speed PCR machines. We envisage disposable convective PCR machines in point of care applications.

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[1] R. K. Saiki, S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim, Science **230**, 1350 (1985).
[2] R. K. Saiki, D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich, Science **239**, 487 (1988).
[3] B. H. Tan, E. A. Lim, J. C. Liaw, S. G. Seah, and E. P. Yap, Expert Rev Mol Diagn. **4**, 219 (2004).
[4] R. Higuchi, C. Fockler, G. Dollinger, and R. Watson, Bio/Technology **11**, 1026 (1993).
[5] P. Belgrader, W. Benett, D. Hadley, G. Long, R. Jr. Mariella, F. Milanovich, S. Nasarabadi, W. Nelson, J. Richards, and P. Stratton, Clin. Chem. **44**, 2191 (1998).
[6] P. Belgrader, W. Benett, D. Hadley, J. Richards, P. Stratton, R. Jr. Mariella, and F. Milanovich, Science **284**, 449 (1999).
[7] W. J. Benett, J. B. Richards, and F. P. Milanovich, United States Patent US 6,586,233 B2.
[8] E. K. Wheeler, B. Benett, P. Stratton, J. Richards, A. Christian, A. Chen, T. Weisgraber, K. Ness, J. Ortega, F. Milanovich, in *Proceedings of Micro Total Analysis Systems 2003*, Vol. 2, edited by M. A. Northrup, K. F. Jensen, and D. J. Harrison, 5–9 October 2003, pp. 1122–1135.
[9] E. K. Wheeler, W. Benett, P. Stratton, J. Richards, A. Chen, A. Christian, K. D. Ness, J. Ortega, L. Li, T. H. Weisgruber, K. Goodson, and F. Milanovich, Anal. Chem. **76**, 4011–6 (2004).
[10] M. Krishnan, V. M. Ugaz, and M. A. Burns, Science **298**, 793 (2002).
[11] D. Braun, M. L. Goddard, and A. Libchaber, Phys. Rev. Lett. **91**, 158103 (2003).
[12] D. Braun and A. Libchaber, Phys. Bio. **1**, P1 (2004).
[13] M. Krishnan, N. Agrawal, M. A. Burns, and V. M. Ugaz, Anal. Chem. **76**, 6254 (2004).
[14] D. Braun, Mod. Phys. Lett. B **18**, 775 (2004).
[15] The Femlab model file can be obtained from the authors.
[16] D. Braun and A. Libchaber, Phys. Rev. Lett. **89**, 188103 (2002).
[17] C. Ludwig, Sitzber. Akad. Wiss. Wien, Math.-naturw. Kl. 20, 539 (1856).
[18] C. Soret, Arch. Sci. Phys. Nat. **2**, 48 (1879).
[19] J. Rauch and W. Köhler, Phys. Rev. Lett. **88**, 185901 (2002).
[20] R. Piazza and A. Guarino, Phys. Rev. Lett. **88**, 208302 (2002).
[21] S. Duhr, S. Arduini, and D. Braun, Eur. Phys. J. E **15**, 277 (2004).