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PCR in a Rayleigh-Bénard Convection Cell

Madhavi Krishnan,[1]* Victor M. Ugaz,[1]* Mark A. Burns[1,2]

Rayleigh-Bénard convection is caused by buoyancy-driven instability in a confined fluid layer heated from below (*1*). The dimensionless Rayleigh number $Ra = g\alpha(T_2 - T_1)h^3/\nu\kappa$ expresses the interplay between buoyant forces driving the instability and diffusive restoring forces acting in opposition. Here, α is the coefficient of thermal expansion of the fluid, g is the acceleration due to gravity, T_1 and T_2 are the temperatures of the top and bottom surfaces of the cavity, respectively, h is the height of the cavity, ν is the kinematic viscosity, and κ is the thermal diffusivity.

The inherent structure of Rayleigh-Bénard convection—steady circulatory flow between surfaces maintained at two fixed temperatures—is ideally suited for performing thermally activated chemical reactions that require temperature cycling. We have developed a device that uses Rayleigh-Bénard convection to perform polymerase chain reaction (PCR) amplification of DNA inside a 35-μl cylindrical cavity. Instead of the external temperature control of conventional thermocyclers, temperature cycling is achieved as the flow field continually shuttles fluid packets vertically through the temperature zones associated with denaturation (≈95°C) and annealing/extension (60° to 70°C). The steady circulatory flow field must engage the entire reaction volume yet be slow enough to allow the reaction within each temperature zone to reach completion. The parameters available to control the fluid motion are the Rayleigh number and the aspect ratio h/d, where d is the diameter of the cavity. In the case of PCR, the required reaction efficiency constrains the reaction solution and the temperature difference; thus, Ra can only be changed by varying the height of the cavity, leaving geometry as the primary flow control parameter.

We constructed a series of Rayleigh-Bénard convection cells by drilling holes in Plexiglas cubes (Fig. 1A) and imaged the resulting flow patterns by recording the motion of an aqueous suspension of fluorescent latex microspheres (Fig. 1B). At values of Ra on the order of 10^5, the convective motion is characterized by steady flow within a single well-defined axisymmetric cellular pattern. Increasing Ra to the vicinity of 10^6 while holding h/d constant causes the flow velocity to increase, ultimately inducing a transition to an unsteady flow regime. Holding Ra constant in the vicinity of 10^6 while increasing h/d reverses the unsteady flow transition and restores uniform convective motion. These observations are in qualitative agreement with the results of Müller and co-workers (*2*) and suggest a considerable amount of tunability to accommodate a variety of reaction conditions and reagent volumes.

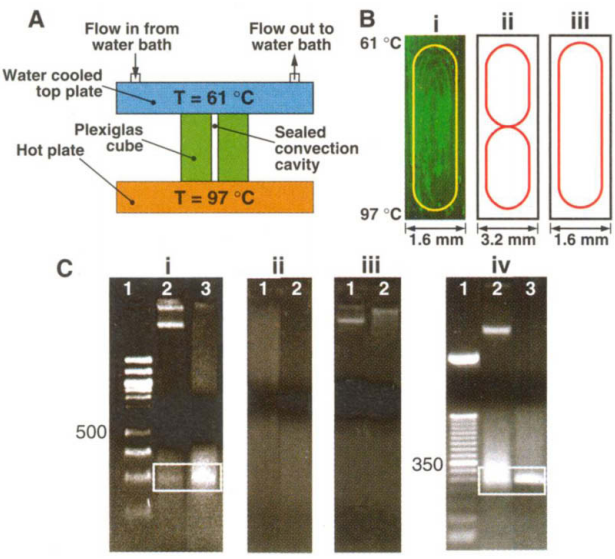

Fig. 1. (**A**) RB-PCR cell schematic. (**B**) Influence of geometry on Rayleigh-Bénard convection (*5*). (i) h/d = 3.3, Ra = 4.6 x 10^5; steady circulatory convective flow between the top and bottom of the cavity. (ii) h/d = 3.3, Ra = 3.7 × 10^6; unsteady "figure-8" convective flow pattern. (iii) h/d = 6.3, Ra = 3.7 × 10^6; steady convective flow is reestablished at the same value of Ra as in (ii). (**C**) DNA amplification in RB-PCR cell. (i) Amplification with 0.1 U/μl Ampli-Taq Polymerase. Lane 1, 100-bp ladder; lane 2, RB-PCR product; lane 3, PCR product generated in a thermocycler using two-temperature cycling (denature: 95°C; anneal: 61°C, 40 cycles). (ii) Negative control with no template. Lane 1, RB-PCR product; lane 2, thermocycler product. (iii) Negative control with no enzyme. Lane 1, RB-PCR product; lane 2, thermocycler product. (iv) Amplification with 0.15 U/μL AmpliTaq Polymerase. Lane 1, 50-bp ladder; lane 2, RB-PCR product; lane 3, thermocycler product. The additional high-migrating band appears in all template-containing reactions and controls (*6*).

On the basis of flow visualization studies in a number of geometries, we selected cavities 1.5 cm in height with $h/d \approx 10$ for PCR experiments. Convective flow was generated with top and bottom surfaces of the cavity maintained at 61°C and 97°C, respectively (*3*). About 9 ng/μl of human DNA template was used, and the target was a 295–base pair (bp) fragment of the single-copy β-Actin gene. As shown in Fig. 1C, the Rayleigh-Bénard PCR (RB-PCR) cell produces an amplification product of the correct size and compares well with the PCR product generated in a thermocycler under similar temperature conditions. In addition to enzyme concentration, the reaction is sensitive to incubation time and the temperature at the top surface of the cell.

The RB-PCR system is exceedingly simple and may be easily assembled in any laboratory. The potential versatility of this system may be further enhanced through improved characterization of convective flow fields in high aspect ratio cavities and studies to optimize PCR in flowing systems.

References and Notes

1. S. Chandrasekhar, *Hydrodynamic and Hydromagnetic Stability* (Clarendon, Oxford, 1961).
2. G. Müller, G. Neumann, W. Weber, *J. Cryst. Growth* **70**, 78 (1984).
3. A 295-bp segment of the human β-Actin gene was amplified. Forward and reverse primer sequences were 5′-TCACCCACAATGTGCCCATCTACGA-3′ and 5′-CAGCGGAACCGCTCATTGCCAATGG-3′. Reactions contained 10 mM tris-HCl (pH 8.3); 50 mM KCl; 4 mM $MgCl_2$; 0.2 mM each dATP, dCTP, dGTP, and dUTP; 9 ng/μl human DNA; and 0.1 U/μl of AmpliTaq Polymerase (PE Applied Biosystems). Reactions were run for about 1.5 hours, aspirated from the reaction chambers, stained with SYBR-Green I (final concentration, 200×), and run on a 1% Agarose gel at 110 V for 1 hour.
4. T. Lindahl, B. Nyberg, *Biochemistry* **11**, 3610 (1972).
5. Motion of a dilute aqueous suspension of fluorescent latex microspheres (6-μm diameter; Polysciences) was observed through a fluorescence stereoscope, imaged using an intensified charge-coupled device camera, and recorded to videotape. Averaging the digitized video stream over a time interval of 1 s produced particle paths representative of microsphere trajectories.
6. This is likely to be an effect of the high temperature at the bottom of the cell (97°C) causing single-stranded scission of the >50 kb template DNA fragments (*4*). A faint band is also observed at the same migration distance in positive reactions and template-containing negative controls generated in the thermocycler but does not appear in the photographs.
7. M.K. and V.M.U. contributed equally to the work and are listed alphabetically in the author list. M.K. and V.M.U. co-conceived the idea and executed the flow studies together. V.M.U. carried out image processing and analysis, and M.K. performed the PCR experiments. We acknowledge funding from the NIH (grant P01 HG01984-01).

Departments of [1]Chemical Engineering and [2]Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109–2136, USA.

*These authors contributed equally to this work.