Microfluidic sterilization

Rui Zhang,^{1,2} Jie Huang,¹ Fei Xie,¹ Baojun Wang,¹ Ming Chu,³
 Yuedan Wang,³ Haichao Li,⁴ Wei Wang,^{1,5,6,a)} Haixia Zhang,^{1,5,6}
 Wengang Wu,^{1,5,6} and Zhihong Li^{1,5,6}

¹*Institute of Microelectronics, Peking University, Beijing 100871,
 People's Republic of China*

²*School of Pharmaceutical Sciences, Peking University, Beijing 100191,
 People's Republic of China*

³*School of Basic Medical Sciences, Peking University, Beijing 100191,
 People's Republic of China*

⁴*Peking University First Hospital, Peking University, Beijing 100034,
 People's Republic of China*

⁵*National Key Laboratory of Science and Technology on Micro/Nano Fabrication,
 Beijing 100871, People's Republic of China*

⁶*Innovation Center for MicroNanoelectronics and Integrated System, Beijing 100871,
 People's Republic of China*

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Nowadays, microfluidics is attracting more and more attentions in the biological society and has provided powerful solutions for various applications. This paper reported a microfluidic strategy for aqueous sample sterilization. A well-designed small microchannel with a high hydrodynamic resistance was used to function as an in-chip pressure regulator. The pressure in the upstream microchannel was thereby elevated which made it possible to maintain a boiling-free high temperature environment for aqueous sample sterilization. A 120 °C temperature along with a pressure of 400 kPa was successfully achieved inside the chip to sterilize aqueous samples with *E. coli* and *Staphylococcus aureus* inside. This technique will find wide applications in portable cell culturing, microsurgery in wild fields, and other related micro total analysis systems.
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Microfluidics, which confines fluid flow at microscale, attracts more and more attentions in the biological society.^{1–4} By scaling the flow domain down to microliter level, microfluidics shows attractive merits of low sample consumption, precise biological objective manipulation, and fast momentum/energy transportation. For example, various cell operations, such as culturing^{5–7} and sorting,^{8–10} have already been demonstrated with microfluidic approaches. In most biological applications, sterilization is a key sample pre-treatment step to avoid contamination. However, as far as the author knew, this important pre-treatment operation is generally achieved in an off-chip way, by using high temperature and high pressure autoclave. Actually, microfluidics has already been utilized to develop new solution for high pressure/temperature reactions. The required high pressure/temperature condition was generated either by combining off-chip back pressure regulator and hot-oil bath,^{11,12} or by integrating pressure regulator, heater, and temperature sensor into a single chip.¹³ This work presented a microfluidic sterilization strategy by implementing the previously developed continuous flowing high pressure/temperature microfluidic reactor.

Figure 1 shows the working principle of the present microfluidic sterilization chip. The chip consists of three zones: sample loading (a microchannel with length of 270 mm and width of 40 μm), sterilization (length of 216 mm and width of 100 μm), and pressure regulating (length of 42 mm and width of 5 μm). Three functional zones were separated by two thermal

^{a)} Author to whom correspondence should be addressed. Electronic mail: w.wang@pku.edu.cn. Tel.: +86-10-6276-9183. Fax: +86-10-6275-1789.

FIG. 1. Working principle of the present microfluidic sterilization. Only microfluidic channel, heater, and temperature sensor were schematically shown. The varied colour of the microchannel represents the pressure and that of the halation stands for the temperature.

isolation trenches. The sample was injected into the chip by a syringe pump and experienced two-step filtrations (feature sizes of $20\ \mu\text{m}$ and $5\ \mu\text{m}$, not shown in Figure 1) at the entrance to avoid the channel clog. All channels had the same depth of $40\ \mu\text{m}$. According to the Hagen–Poiseuille relationship,¹⁵ the pressure regulating channel had a large flow resistance (around $1.09 \times 10^{17}\ \text{Pa}\cdot\text{s}/\text{m}^3$, see supplementary S1 for details¹⁶) because of its small width, thereby generated a high working pressure in the upstream sterilization channel under a given flow rate. The boiling point of the solution will then be raised up by the elevated pressure in the sterilization zone followed by the Antoine equation.¹⁶ By integrating heater/temperature sensors in the pressurized zone, a high temperature environment with temperature higher than 100°C can thereby be realized for aqueous sample sterilization. The sample was collected from the outlet and cultured at 37°C for 12 h. Bacterial colony was counted to evaluate the sterilization performance.

Fabrication of this chip has been introduced elsewhere.¹⁴ The fabricated chip and the experimental system are shown in Figure 2. There were two inlets of the chip. While, in the experiment, only one inlet used and connected to the syringe pump. The backup one was

FIG. 2. The fabricated chip and the experimental system. (a) Two chips with a penny for comparison. The left chip was viewed from the heater/temperature sensor side, while the right one was observed from the microchannel side (through a glass substrate). (b) The experimental system.

FIG. 3. The temperature distribution of the chips (before packaged) with and without thermal isolation trenches (powered at 1 W). The data were extracted from the central lines of infrared images, as shown as inserts.

blocked manually. The sample load zone was arranged in between of the sterilization zone and the pressure regulating zone based on thermal management consideration. A temperature control system (heater/temperature sensor, power source, and multi-meter) was setup to provide the required high temperature. The heater and the temperature sensor were microfabricated Pt resistors. The temperature coefficient of resistance (TCR) was measured as 0.00152 K^{-1} .

Thermal isolation performance of the present chip before packaging with inlet/outlet was shown in Figure 3, to show the thermal interference issue. The results indicated that when the sterilization zone was heated up to 140°C , the pressure regulating zone was about 40°C . At

FIG. 4. Sterilization performance of the present chip with *E. coli* and *S. aureus* as test bacteria. All the original population was $10^6/\text{mL}$. Inserted images showed the images of the culture disk after bacteria incubation.

TABLE I. The *E. coli* cultivation results under different flow rates and sterilization temperatures.^a

	25 °C	70 °C	100 °C	120 °C	25 °C ^b
2 nl/s	1.89/+++	1.38/+	1.16/–	1.04/–	0/+++
4 nl/s	3.78/+++	2.76/+	2.32/–	2.08/–	0/+++

^aData in the table are shear stress (Pa)/population of bacteria, where “+++” indicates a large proliferation, “+” means small but noticeable proliferation, “–” represents no proliferation.

^bOff-chip control group.

this temperature, the viscosity of water decreases to 0.653 mPa·s from 1.00 mPa·s (at 20 °C), which will make the pressure in the sterilization zone reduced from 539 kPa (calculated at 20 °C and flow rate of 4 nl/s) to 387 kPa. The boiling point will then decrease to 142.8 °C, which will guarantee a boiling-free sterilization. In the cases without the thermal isolation trenches, the temperature of the pressure regulating zone reached as high as 75 °C because of the thermal interference from the sterilization zone, as shown in Figure 3. The pressure in the sterilization zone was then reduced to 268 kPa (calculated at flow rate of 4 nl/s) and the boiling temperature was around 130 °C, which was lower than the set sterilization temperature. Detail calculation can be found in supplementary S2.¹⁶

Bacterial sterilization performance of the present chip was tested and the experimental results were shown in Figure 4. *E. coli* with initial concentration of 10⁶/ml was pumped into and flew through the chip with the sterilization temperatures varied from 25 °C to 120 °C at flow rates of 2 nl/s and 4 nl/s. The outflow was collected and inoculated onto the SS agar plate evenly with inoculation loops. The population of bacteria in the outflow was counted based on the bacterial colonies after incubation at 37 °C for 12 h. Typical bacterial colonies were shown in Figure 4. The low flow rate case showed a better sterilization performance because of the longer staying period in the sterilization channel. The population of *E. coli* was around 1.25 × 10⁴/ml after a 432 s-long, 70 °C sterilization (at flow rate of 2 nl/s). While at the flow rate of 4 nl/s, the cultivation result indicated the population was around 3.8 × 10⁴/ml because the sterilization time was shorten to 216 s. A control case, where the solution flew through an un-heated chip at 2 nl/s, was conducted to investigate the effect of the shear stress on the sterilization performance (see the supplementary S3 for details¹⁶). As listed in Table I, the results indicated that the shear stress did not show any noticeable effect on the bacterial sterilization. When the chip was not heated, i.e., the case with the largest shear stress because of the highest viscosity of fluid, the bacterial cultivation was nearly the same as the off-chip results (no stress). The temperature has the most significant effect on the sterilization performance. No noticeable bacteria proliferation was observed in the cases with the sterilization temperature higher than 100 °C, as shown in Figure 4.

Sterilization of another commonly encountered bacterium, *Staphylococcus aureus*, with initial population of 10⁶/ml was also tested in the present chip, as shown in Figure 4. Similarly, no noticeable *S. aureus* proliferation was found when the sterilization temperature was higher than 100 °C.

In short, we demonstrated a microfluidic sterilization strategy by utilizing a continuous flowing high temperature/pressure chip. The population of *E. coli* or *S. aureus* was reduced from 10⁶/ml to an undetectable level when the sterilization temperature of the chip was higher than 100 °C. The chip holds promising potential in developing portable microsystem for biological/clinical applications.

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¹G. M. Whitesides, *Nature* **442**, 368–373 (2006).

²P. S. Dittich, K. Tachikawa, and A. Manz, *Anal. Chem.* **78**, 3887–3907 (2006).

- ³J. West, M. Becker, S. Tombrink, and A. Manz, *Anal. Chem.* **80**, 4403–4419 (2008).
- ⁴D. Mark, S. Haeberle, G. Roth, F. von Stetten, and R. Zengerle, *Chem. Soc. Rev.* **39**, 1153–1182 (2010).
- ⁵I. Barbulovic-Nad, S. H. Au, and A. R. Wheeler, *Lab Chip* **10**, 1536–1542 (2010).
- ⁶S. Srigunapalan, I. A. Eydelnant, C. A. Simmons, and A. R. Wheeler, *Lab Chip* **12**, 369–375 (2012).
- ⁷M. Mehling and S. Tay, *Curr. Opin. Biotechnol.* **25**, 95–102 (2014).
- ⁸T. Franke, S. Braunmuller, L. Schmid, A. Wixforth, and D. A. Weitz, *Lab Chip* **10**, 789–794 (2010).
- ⁹J. Autebert, B. Coudert, F. C. Bidard, J. Y. Pierga, S. Descroix, L. Malaquin, and J. L. Viovy, *Methods* **57**, 297–307 (2012).
- ¹⁰Z. T. F. Yu, K. M. A. Yong, and J. Fu, *Small* **10**, 1687–1703 (2014).
- ¹¹F. Trachsel, C. Hutter, and P. R. von Rohr, *Chem. Eng. J.* **135**, S309–S316 (2008).
- ¹²R. M. Tiggelaar, F. B. Lopez, D. C. Hermes, H. Rathgen, R. J. M. Egberink, F. G. Mugele, D. N. Reinhoubt, A. vanden Berg, W. Verboom, and H. J. G. E. Gardeniers, *Chem. Eng.* **131**, 163–170 (2007).
- ¹³F. Xie, B. Wang, T. Dong, W. Wang, J. Tong, S. Xia, W. Wu, and Z. Li, *Biomicrofluidics* **7**, 034104 (2013).
- ¹⁴R. K. Shah and A. L. London, *Laminar Flow Forced Convection in Ducts* (Academic Press, 1978).
- ¹⁵T. Boublik, V. Fried, and E. Hala, *The Vapour Pressures of Pure Substances* (Elsevier Science Pub. Co., Inc., New York, 2008).
- ¹⁶See supplementary material at <http://dx.doi.org/10.1063/1.4882776> for the flow resistance calculation, the calculation of thermal interference induced pressure regulating performance degradation, and the calculation of shear stress in the present work.