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Technical Note

Integrated cooling system for microfluidic PDMS devices used in biological microscopy studies

Zahra Khaji and Maria Tenje*

Department of Biomedical Engineering, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

E-mail: maria.tenje@angstrom.uu.se

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Abstract

In this work, a two-channel, water-based cooling system was integrated into a polydimethylsiloxane (PDMS)-glass microfluidic device for application in single-cell biological studies. This system is designed to cool living cells to single-digit temperatures *in situ*, without requiring any features of the electron-beam fabricated master mould to be changed, and without interfering either biologically or optically with the cells themselves. The temperature profile inside the device was mapped using multiple thermocouples mounted inside the device, over time. A parametric study including coolant flow rate, distance between the cooling channel and the fluidic channel, and number of active cooling channels was performed to evaluate the performance of the system. By using ice water as the coolant, we have demonstrated stable on-chip cooling reaching an average temperature of 4.9 °C when operated at a coolant flow rate of 23 ml min^{-1} and using two active cooling channels, positioned only 400 μm away from the cell trapping sites. The maximum observed temperature deviation during an 80 min stability test was $\pm$0.2 °C. We have observed that flowing room temperature culture media through the device with active cooling had no influence on the temperature inside the chip, demonstrating its suitability for use in live cell culture experiments. Finally, we have also demonstrated that the active cooling system successfully decreased the cell metabolism of trapped *Escherichia coli* resulting in a decreased growth rate of the bacteria.

Supplementary material for this article is available online

Keywords: integrated cooling, lab-on-a-chip, microsystems, single-cell, microwire moulding

(Some figures may appear in colour only in the online journal)

* Author to whom any correspondence should be addressed.

1. Introduction

The intrinsic properties of microsystems mean they can be constructed on the same scale as typical cells, requiring only a small amount of samples and reagents and provide, short analysis times and high-throughput. This makes them attractive for both basic biological research, such as studying cell–substrate, cell–cell, and cell–medium interactions [1] and clinical applications such as diagnostics, surgery, and therapy [2]. The physical and chemical parameters inside microsystems can often be precisely controlled and therefore such devices can be used to both control and study cell microenvironments. One of the most critical physical parameters for cells is temperature. Precise control of temperature is important not only for cell survival but also when performing temperature-dependent biological protocols such as polymerase chain reaction (PCR).

Several biomedical studies performed on-chip focus on or include temperature regulation systems in order to maintain, create, or study the effect of direct or indirectly generated heat profiles [3–7]. However, most of these studies focus on heating and integrated cooling systems are only used to dissipate excess heat, e.g. when switching between different thermal cycles in PCR [6, 7]. On the other hand, cooling cells down to single-digit positive temperatures, could be useful to e.g. control growth rate for on-chip assays [8]. One requirement for integrated cooling in biomedical microdevices is that the system does not interfere with optical microscopy, which is by far the most commonly used analysis technique. Furthermore, it needs to be small and simple in order to interface with more complicated microdevices comprising several fluidic and/or electrical connections. These conditions make established methods of cooling (e.g. thermoelectric) difficult.

One approach to cooling that has recently gained interest is that of embedding liquid cooling directly inside electronic chips [9]. This has the potential to achieve single-digit positive temperatures inside biomedical microdevices, as it can be implemented in a way that meets the above requirements. Integrated water-based cooling at temperatures around 0 °C in microsystems has been shown in freeze–thaw valves [10, 11], where the cooling/heating rate and therefore the opening/closing speed of the valve is important. However, in these studies, cooling was achieved using an external thermoelectric-based set-up.

This work reports the integration of a two-channel microfluidic-based cooling system in a PDMS-glass microfluidic device for high-throughput single-cell analysis of bacteria. The cooling channels were integrated into the PDMS microfluidic devices during casting without making any modifications to the master mould using a microwire moulding technique, which is a simple and popular technique to manufacture circular PDMS microchannels [12]. The cooling system was characterized with respect to temperature distribution inside the chip under different flow profiles as well as different design parameters of the channels. We also demonstrate the resulting effect on the growth rate of the trapped bacteria when cooling is activated.

2. Material and methods

2.1. Microfluidic device

The PDMS-glass device used in this study was initially developed by Baltekin *et al* [13] for antibiotic susceptibly testing (AST). The PDMS part, which is cast on a silicon-SU8 master mould and closed by bonding to a cover slip, has two rows of cell traps, each containing 4000 channels with width and depth of 1.2 μm and length of 50 μm. Each channel ends with a trapping site where electron beam lithography is used to define an opening of the channel of only 300 nm. The PDMS device has outer dimensions of 12 $\times$ 21 mm^2. Multiple inlets and outlets are incorporated into the device, making it possible to easily and effectively change the perfused medium, but limiting the footprint available for an external cooling system. In addition to using this system for AST, this device is suitable for other single-cell analysis of bacterial cells due to its large number of traps and ease at which medium can be switched and cells washed. However, the device lacks integrated temperature regulation, meaning external temperature regulation systems, such as microscope incubators, need to be used if system shall be operated outside room temperature. In this work, we present an integrated cooling system for even and stable cooling of the device microchannels to single-digit positive temperatures.

2.2. Device fabrication

A fluidic-based cooling system in the form of two straight cylindrical channels for coolant flow was integrated along the chip during the PDMS casting. The channels were designed with a diameter of 0.8 mm and length of 21 mm. The channels were placed with a lateral distance of 1 mm from each of the trapping sites to provide even cooling on all traps. The distance between the trapping sites and the cooling channel (H) was varied between 100, 200, 400, and 1000 μm, figure 1, where 400 μm was identified as the optimal distance.

Stainless steel tubes with an outer diameter of 0.8 mm were cut into the desired lengths and used for making the cooling channels in PDMS. A ring-shaped fixture with an outer diameter of 98.7 mm was milled in FR4 material using a computer numerical control (CNC) machine (S104, LPFK Laser and Electronics AG, Germany). The fixture had trenches for fixing the metal tubes into position during the PDMS casting, figure 2. The metal tubes were fixed in the trenches by uncured PDMS (Sylgard184; Dow Corning) that was cured at 60 °C for 4 h. The fixture was then placed between the master mould and a metal ring defining the outer region of the devices, figure 3, before PDMS was poured into the structure to an approximate thickness of 4 mm and allowed to cure at 60 °C for 4 h.

After curing, the PDMS was released from the metal ring and the master mould. The metal tubes were then carefully removed before the PDMS was diced into 15 individual chips, and finally had inlets and outlets punched. Each chip was then bonded to a cover slip after the surfaces were cleaned with isopropanol and deionized water and activated by corona treatment (D-50E Heavy Duty Generator, Electro-Technic

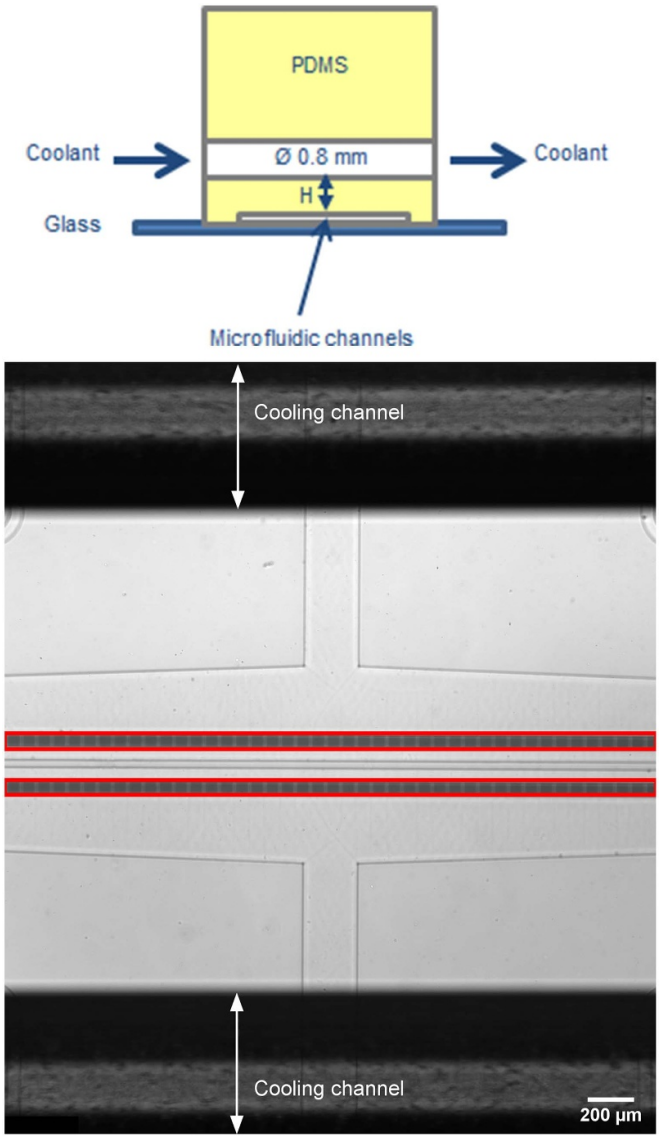

Figure 1. Top: Schematic drawing of the side view of the microfluidic device with the integrated cooling channels. Bottom: Microscopy image of the chip (top view) showing two cooling channels and their lateral distance from the bacteria trapping sites (indicated by red rectangles). White arrows indicate full width of the cooling channels where dark area is reflection from the inserted PEEK tubing.

Products, USA) for 30 s. The devices were placed in an oven at 100 °C immediately after bonding and maintained there for at least 1 h to form a tight bond.

2.3. Experimental set-up

Two pieces of polyether ether ketone (PEEK) tubing (1093294, IDEX Health & Science LLC, USA) with inner and outer diameters of 0.5 and 0.79 mm respectively were cut to an approximate length of 16 cm and placed inside the cooling channels of each chip to stabilize the channels and reduce risk of leakage. The chip was then glued (Rapid epoxy, Bostik SA, France) to a custom-made fixture milled in FR4 using a CNC machine (S104, LPFK Laser and Electronics

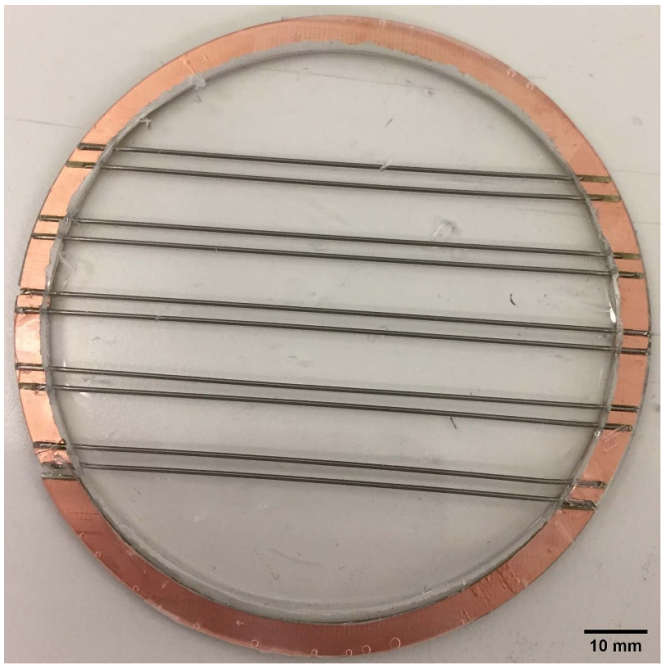

Figure 2. Top view of a ring-shaped fixture accommodating the metal tubes used for making the cooling channels in the PDMS microfluidic devices during casting. The outer and inner diameters are 98.7 and 90.6 mm, respectively.

AG, Germany). The PEEK tubings were connected to silicone (228-0701P, Avantor Inc., USA) and Polytetrafluoroethylene (PTFE) (58696-U, Supelco Inc., USA) tubings with the inner diameter of 1 mm on each side by using standard microfluidic connectors.

For mounting under a microscope, the chip was placed in a 3D printed fixture, figure 4.

Gas-tight glass bottles with gas and liquid connections on the lid were used as reservoirs for the ice-water mixture used as coolant. Flow through the microfluidic chip was controlled by a four-channel pressure-based pump (OB1 MK3+, Elveflow Microfluidics, France).

Temperature measurements were performed using K-type thermocouples connected to a handheld thermometer (TM-947SD, Lutron Electronic, Taiwan) with a sampling frequency of 1 s. The thermocouples were calibrated on ice before use.

2.4. Device characterization

2.4.1. Temperature measurements. To measure the temperature inside the chip, three 0.5 mm diameter holes were punched in the PDMS chip, locations 1–3, figure 4, before bonding. After bonding PDMS to the cover slip and gluing the chip to the FR4 fixture, three thermocouples covered with a non-silicone heat conducting paste (HTCP20S, Electrolube, UK) were placed in the bottom of the holes, figure 4. Hereafter the thermocouples are called TC1, TC2, and TC3, where the numbering order follows the flow direction of the coolant and culture medium inside the chip. Using this placement, the evenness of the cooling system could be investigated.

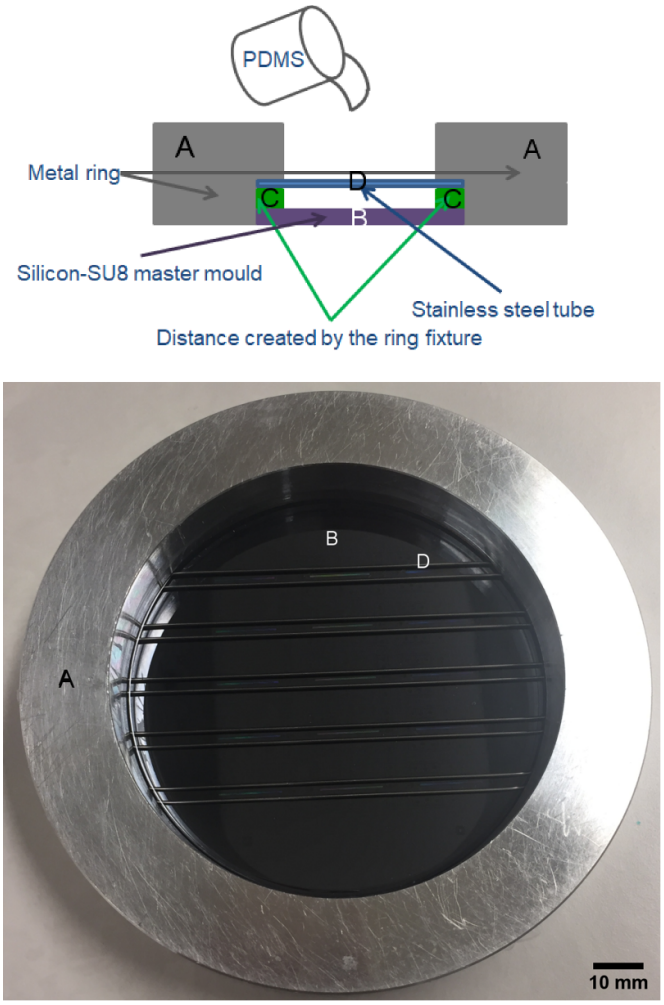

Figure 3. Top: Schematic drawing of the set-up used to form the cooling channels in the PDMS device. Bottom: Photograph showing the metal tubes used for making the cooling channels fixed in place on top of the Si-SU8 master mould by using the custom-made fixture and the metal ring.

A parametric study was performed to monitor the temperature inside the devices with respect to coolant flow rate, distance of the cooling channels from the fluidic channels, and number of active cooling channels. To control the flow, a pressure-driven system was used, and pressures of 100, 400, 800, and 1000 mbar (corresponding to flow rates of 3, 11, 18 and 23 ml min^{-1}, respectively) were chosen for this study. For each experiment, read-outs of the three TCs were logged over 15 min after reaching a steady state, and average values were calculated.

To study the stability of the cooling system, a temperature measurement experiment was performed using two active cooling channels located at a distance of 500 μm above the bacterial traps with a coolant flow rate of 18 ml min^{-1} for 80 min after reaching steady state. Here, a 500 μm distance was chosen as a good compromise between 100 μm (min. distance) and 1000 μm (max. distance).

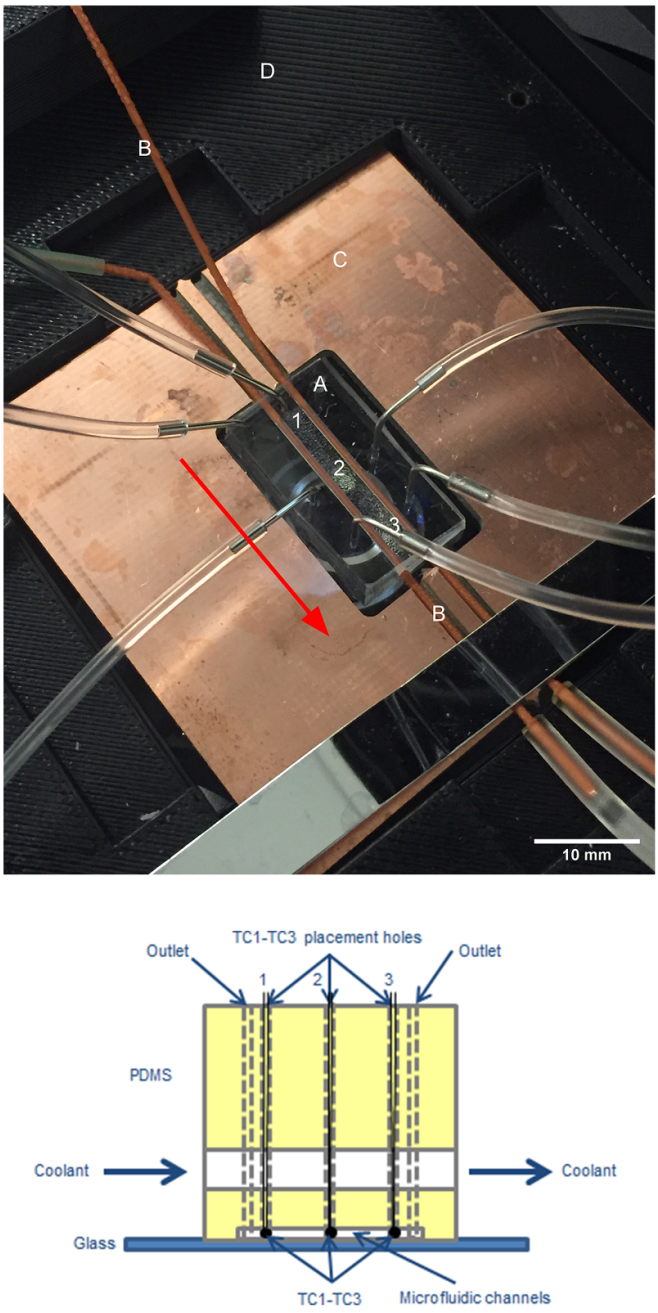

Figure 4. Top: Photograph showing microfluidic device mounted in the experimental set-up. The microfluidic device (A) with PEEK tubings placed inside the integrated cooling channels (B) is glued from the glass side to the back of the custom-made FR4 fixture (C) and then placed in the 3D printed fixture (D), which fits in the stage of the inverted microscope used. The microfluidic inlets and outlets are connected via 90° bent stainless steel tubings connected to Tygon flexible tubing. The bacterial trapping sites are located between the two cooling channels. Numbers 1–3 show the placement of the thermocouples 1–3 used for temperature measurements. The arrow indicates flow direction of the coolant. Bottom: Schematic drawing of the side view of a microfluidic device showing the placement of thermocouples 1–3 during temperature measurements. The thermocouples, covered with a heat conducting paste, were placed at the bottom of holes 1–3.

To evaluate the effect of medium perfusion through the device, room temperature medium was perfused in a device with cooling channels at a distance of 400 μm from the bacterial traps, and the temperature was monitored for 5 min. For this experiment and in order not to disturb the microfluidic channels by punching extra holes, only two thermocouples were used. These were placed in the bottom of two of the chip outlets located in line but approximately 1.5 mm closer to the edges of the chip than positions 1 and 3, figure 4.

2.4.2. Cell experiment. A microfluidic device with cooling channels at a distance of 400 μm was loaded with an MG1655 *Escherichia coli* strain and room temperature super optimal broth medium was perfused for continuous medium supply. Cell growth was observed under an inverted confocal microscope (DMI8, Leica Microsystems, USA) both at room temperature and with applied cooling using both cooling channels and a coolant flow rate of 18 ml min^{-1}.

3. Results and discussion

3.1. Device fabrication

Figure 5 shows the side view of a PDMS device with a cooling channel defined at a distance of 400 μm from the bacterial trapping sites. The cooling channels were intact with perfect cylindrical shape, showing how both channel dimensions and placement can be controlled with the procedure developed. The average measured separation was 493 μm with a standard deviation of 62 μm for a total of 15 chips manufactured during each PDMS casting, (and hence 30 cooling channels). It should be noted that multiple use of metal tubes may result in their partial deformation and hence deviations from the design values.

3.2. Temperature measurements

The results of the temperature measurements are shown in figures 6 and 7. Figure 8 shows the average read-out of each of the three thermocouples for a device with a channel separation of 400 μm under different flow rates and different numbers of channels used.

In general, the temperature decreases with decreasing distance between the cooling channel and traps, increasing coolant flow rate, and increasing number of active cooling channels, figures 6 and 7. For instance, for the flow rate of 3 ml min^{-1}, decreasing the height of one cooling channel from 1000 to 100 μm decreased the average temperature from 15.1 °C to 11.3 °C. By changing the flow rate from 3 to 18 ml min^{-1} when using one cooling channel at a distance of 1000 μm away from the trapping sites, the average temperature changed from 15.1 °C to 11.9 °C. However, a further increase of the flow rate from 18 to 23 ml min^{-1} resulted in only a very small additional temperature decrease, i.e. only a few decimals for all the conditions.

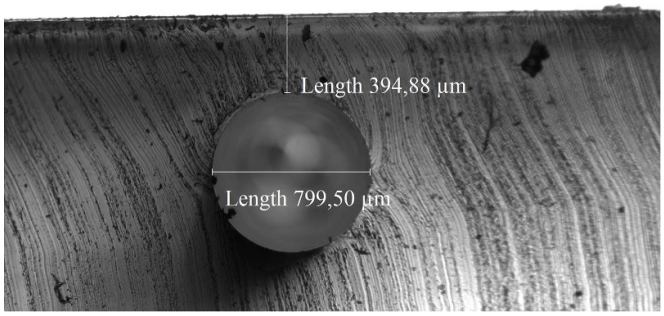

Figure 5. Side view of a cooling channel defined in the PDMS bulk. Design parameters were: channel diameter 800 μm and separation 400 μm.

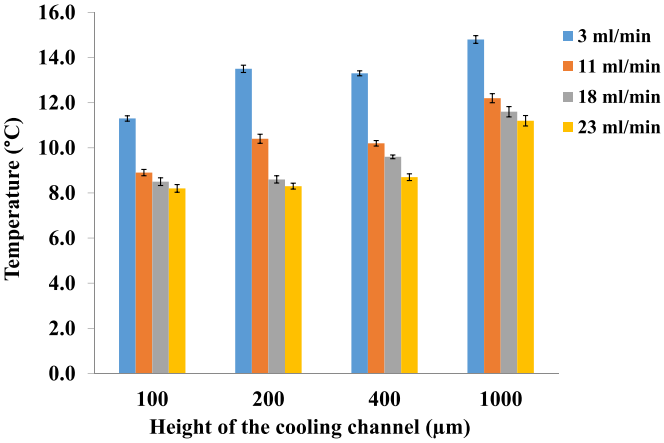

Figure 6. The value of the read-outs of TC1-TC3 averaged over a time period of 15 min after reaching steady state using one active cooling channel, with differing cooling channel distances and coolant flow rate. Error bars indicate one standard deviation ($n = 2700$).

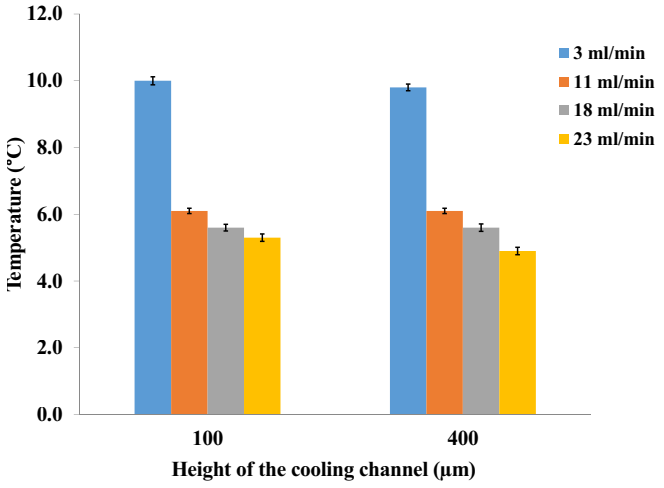

Figure 7. The value of the read-outs of TC1-TC3 averaged over a time period of 15 min after reaching steady state using two active cooling channels, with differing cooling channel distances and coolant flow rate. Error bars indicate one standard deviation ($n = 2700$).

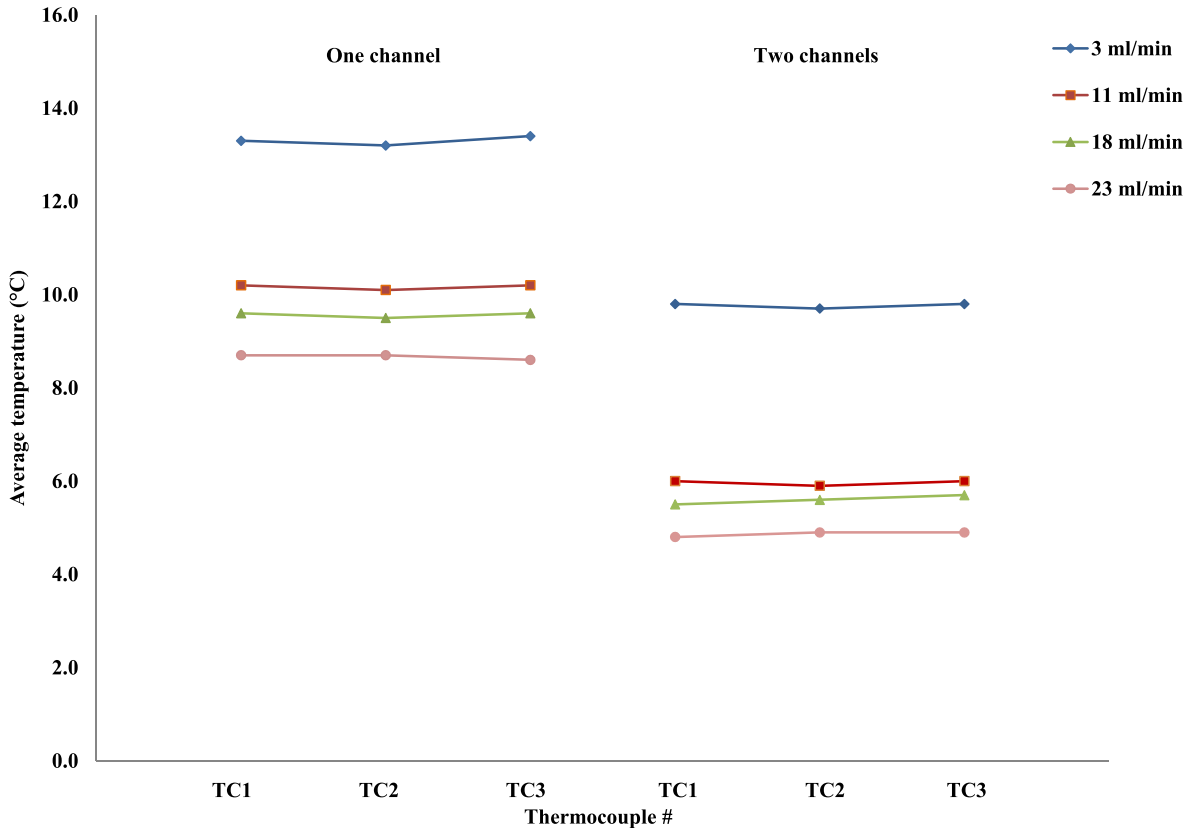

Figure 8. The average value of the read-outs for each of the thermocouples over 15 min under different flow rates and number of active cooling channels, for a chip with a cooling channel separation of 400 μm.

For the same channel height and same increase of flowrate, e.g. from 3 to 11 ml min^{-1}, a larger change in temperature was observed when two cooling channels were used compared to only one, figure 8. We can also see that when using two channels, an increase in the distance between the cooling channel and bacteria traps from 100 to 400 μm had almost no temperature effect. However, we observed several challenges with placing the large cooling channels at a distance of $\leqslant$200 μm from the bacteria trapping sites, for example in the form channel collapse, meaning that the operational risks outweigh the benefits. We therefore chose a distance of 400 μm between the cooling channels and bacteria traps in this work.

With coolant at 0 °C running through two cooling channels placed at a distance of 400 μm (flow rate 23 ml min^{-1}), the minimum achievable average temperature was 4.9 °C. A further feature of this design is that the moulded cooling channels can be lined with tubing made from different materials, such as glass or PEEK, inserted into the channels. In this way, the choice of coolant is not limited to water, and solvent-based coolants can be used when further cooling, (i.e. reaching temperatures below 5 °C) is needed. Additionally, the system design equally well lends itself to heating, by perfusing liquid at elevated temperatures.

For all conditions, the differences between the average read-outs of TC1-TC3 varied between 0.0 and 0.4 °C without any correlation with the cooling profile used.

Perfusing room temperature culture media through a device had no influence on the temperature inside the chip when two cooling channels at a distance of 400 μm were perfused with coolant at a flow rate of 18 ml min^{-1}.

Stability tests showed a maximum temperature deviation of ±0.2 °C from the average value of 6.0 °C during an 80 min measurement.

Finally, figure 9 shows the average temperature read-out of TC1-TC3 over time when using two active cooling channels located 500 μm away from the trapping sites and a coolant flow rate of 18 ml min^{-1}. The device was cooled from room temperature to an average steady-state temperature of 6.0 °C in ∼12 min. The chip returned to 21.0 °C in ∼13 min when cooling was stopped.

3.3. Cell experiment

Cell growth and division inside the chip could clearly be observed in time lapse videos collected with the system at room temperature (supplementary data, video 1). To demonstrate efficient cooling and the effect it has on cellular growth rate, similar data was collected at 6 °C. For this condition, cell growth and division was almost stopped (supplementary data, video 2), before resuming after bringing the chip back up to room temperature.

We have compared the average number of cell divisions per bacterial cell during time lapse imaging of 50 min at room

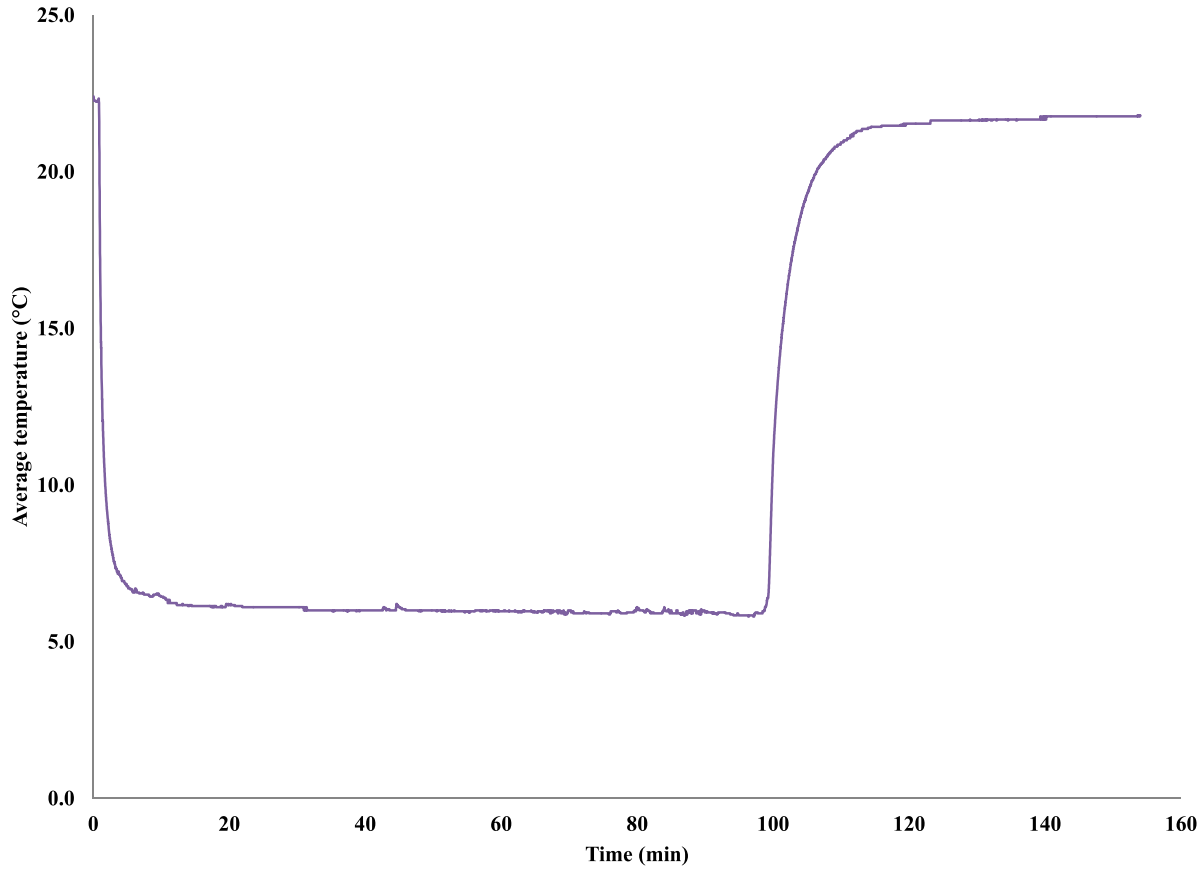

Figure 9. Temperature, average value of the read-outs of three thermocouples, verses time during cooling and warming for a device with a cooling channel with the distance of 500 μm and coolant flow rate of 18 ml min^{-1}.

temperature and at 6 °C. On average, one division per bacterial cell was observed at room temperature, while no divisions were observed when running the cooling system at 6 °C. We note that no cell division occurred even after extending the observation time to 100 min at 6 °C. This demonstrates how the temperature control system can be used to discontinue and re-activate cell divisions *in situ* in a facile way.

4. Conclusions

In this work, a water-based cooling system was integrated in a PDMS microfluidic device for application in single-cell biological studies, without making changes in the PDMS master mould. We have shown that by using ice water as a coolant, efficient cooling of the chip could be achieved by decreasing the distance between the cooling channels and bacteria trapping sites, increasing the number of active cooling channels used, and increasing the coolant flow rate. However, by further increasing the flow rate (>23 ml min^{-1}) or decreasing cooling channel height (<400 μm), when using two channels, no further decrease of the resulting on-chip temperature could be observed.

The manufacturing process presented here can be easily used to create cooling channels in other PDMS devices without any need to change the master mould.

The cooling system is stable over time, cools evenly across the chip with a maximum temperature difference of 0.4 °C, and is not affected by the flow of room-temperature medium through the chip. This makes it fully compatible with biological assays in which conventional bulk protocols are often applied at temperatures between 0 and 4 °C. As an example of such protocols, we successfully demonstrated the ability of the system to decrease cell metabolism and therefore significantly decrease the growth rate of *E. coli*.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

Acknowledgments

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ORCID iDs

Zahra Khaji https://orcid.org/0000-0002-5452-7831
Maria Tenje https://orcid.org/0000-0002-1264-1337

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