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We report for the first time water-based evaporative cooling integrated into a microfluidic chip for temperature control and freezing of biological solution. We opt for water as a nontoxic, effective refrigerant. Aqueous solutions are atomized in our device and evaporation of microdroplets under vacuum removes heat effectively. We achieve rapid cooling ($-5.1\,°C/s$) and a low freezing temperature ($-14.1\,°C$). Using this approach, we demonstrate freezing of deionized water and protein solution. Our simple, yet effective cooling device may improve many microfluidic applications currently relying on external power-hungry instruments for cooling and freezing. © 2015 AIP Publishing LLC. [http://dx.doi.org/10.1063/1.4905184]

Since the first introduction, microfluidics has been employed in a wide range of scientific disciplines. Precise control of temperature is critical for numerous chemical and biological applications.[1] Polymerase chain reaction[2] requires fast and accurate temperature cycling. A stable temperature gradient is essential for thermophoretic fluid manipulation,[3] solute enrichment,[4] and microfluidic screening of organic-compound solubility[5] and protein-crystallographic conditions.[6]

Freezing, cooling below freezing points, is of theoretical interest and practical concern. In life science, freezing of biological systems has been an active research field (i.e., cryobiology).[7] Cell viability under different cryotreatment conditions was studied using microfluidic cooling.[8] Ice interaction with antifreeze protein and ice-nucleation protein is an active research area owing to its application in food science and agriculture.[9] Investigation of ice-nucleation process can be useful for atmospheric science and aerospace engineering.[10,11]

While heating is relatively easy to incorporate into a microfluidic device (i.e., Joule heating), integrated cooling has not been straightforward.[1] Previous microfluidic cooling approaches, including a Peltier element,[8] cooled water/air circulation[4] and pressurized-air impingement[2] rely on external cooling instruments which generally require much power, occupy large space and thus are not amenable for on-chip integration. Guijt *et al.*[12] and Maltezos *et al.*[13] took integrated temperature-control approaches based on evaporative cooling. Streams of refrigerant (e.g., acetone, ethanol, isopropyl alcohol, ethyl ether) and gas (e.g., air, nitrogen) are mixed in a Y-junction microchannel, and evaporation of the refrigerants removes heat by endothermic reaction. Only moderate cooling temperature of $-4\,°C$ and refrigeration rate of $-1\,°C/s$ were attained (air-acetone).[12] The critical limitation of the previous work was the use of flammable, toxic refrigerants, and pressurized gas tanks.[13] Moreover, the Y-junction microchannel mixer could be designed simpler for a higher degree of device integration.

We, for the first time, report an integrated, water-based microfluidic evaporative cooling device. The marked advantage is simplicity; aqueous solution, a straight-microchannel chip and a laboratory vacuum pump are required to achieve a temperature drop from 24 to $0\,°C$ in 4.7 s and even go beyond freezing points. Water is an interesting refrigerant owing to large latent heat of evaporation (2257 kJ/kg vs. 353 kJ/kg for ethyl ether), inflammability, and nontoxicity unlike chlorofluorocarbons or organic solvents used in the previous work.[14] Notably, our method distinguishes itself from other cooling approaches for *ice generation* and *freezing of biological solution* by using aqueous solution as refrigerant. Ice-generating microfluidic instruments were devised but relied on external cooling elements.[10,11] Here, we, for the first time, demonstrate integrated microfluidic ice generation and freezing of protein solution without an external cooling element.

Figure 1(a) shows the experimental setup. A disposable pipet (4 mm diameter) was cut to form a 3 cm-long tube, and two of them are bonded to a PMMA straight-channel chip (Microfluidic ChipShop, Germany). The tubes are centered around two through holes located at both ends of the microfluidic channel. Refrigerant is stored in one tube (i.e., reservoir), exposed to ambient air. The other tube is connected to a 1/2 hp vacuum pump, forming a microfluidic vacuum chamber. A suction flask and a moisture trap filled with desiccant (Drierite, USA) were connected between the vacuum pump and the chamber, preventing moisture from entering the vacuum pump. A square orifice (0.76×0.08 mm^2) was cut out of the tube bottom (Fig. 1(b)). A digital pressure gauge (Autonics, Korea) was used to monitor pressure. Four microthermocouples of $130\text{-}\mu m$ diameter (Omega, USA) were used to measure liquid temperature in the reservoir and measure vapor temperature in the vacuum chamber. Temperature and pressure were recorded using data acquisition boards and a PC. A digital camera was used
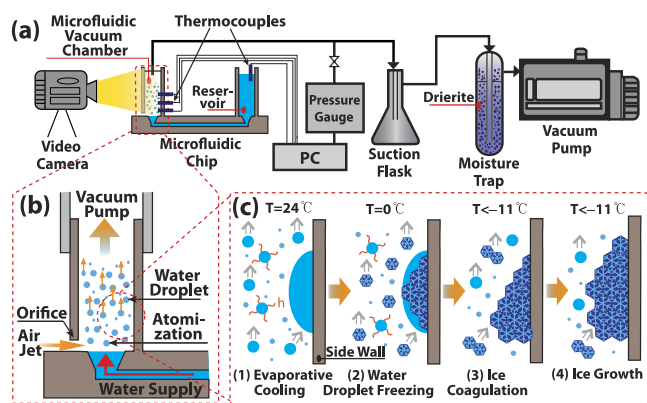
FIG. 1. (a) Experimental setup for the microfluidic evaporative freezer. A vacuum pump is used to atomize aqueous solution, evaporate droplets, and remove vapors from the vacuum chamber. (b) Solution is drawn from the through hole and impinged by air jet through the orifice, resulting in atomization. (c) Aqueous droplets are readily evaporated under vacuum, reducing temperature and freezing the solution.
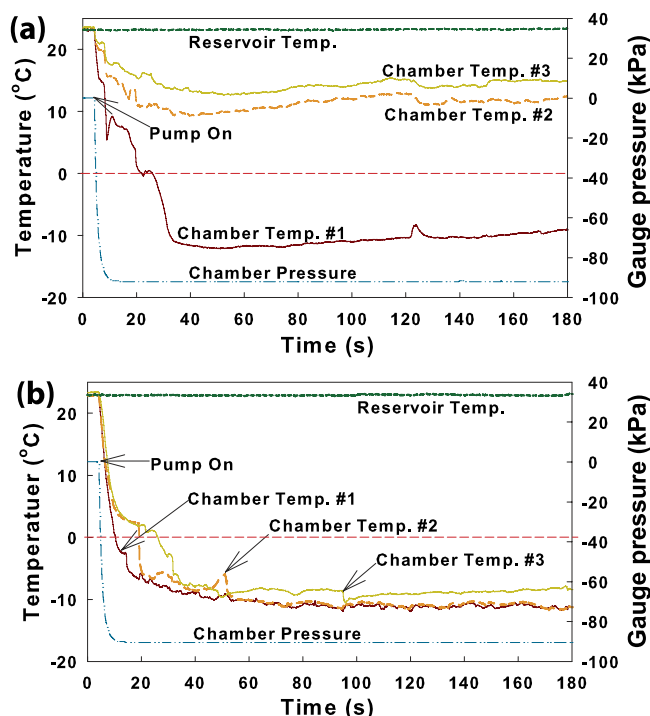

FIG. 2. Temperature and pressure inside the vacuum chamber and the reservoir, when (a) DI water and (b) EG solution were used. The chamber temperatures dropped rapidly after applying vacuum. Temp.#1, 2, 3 were measured using thermocouples, located 2, 7, 12 mm from the chamber bottom, respectively.

to video-capture freezing process in the vacuum chamber. While in conventional evaporative cooling water vaporizes under atmospheric pressure,[15] our cooling device removes heat under vacuum (Fig. 1(b)). Upon applying vacuum, aqueous solution is drawn from the through hole (diameter = 1.4 mm), subsequently impinged by the air jet generated by negative pressure across the orifice and atomized into microdroplets. The estimated diameter of droplet is 9.2 μm for DI (deionized) water and 8.1 μm for 20% v/v ethylene-glycol (EG) solution (Samchun Chemical, Korea).[16] By using the orifice (air) and through hole (water) in the microfluidic chip, in essence, a two-fluid spray nozzle for droplet generation was easily prepared and integrated without complex fabrication process. Owing to vacuum and a high surface-area-to-volume ratio (~35× (water) and ~40× (EG solution) larger than that of the refrigerant stream in the previous Y-junction chip[12]), the droplets vaporize rapidly. During the evaporation, latent heat is removed from the droplets and absorbed by vapor.[14] The water vapor is continuously removed from the vacuum chamber along with the droplets by vacuum suction. As in Fig. 1(c), (1) temperatures of the water droplets as well as the surrounding air decrease because the heat is carried away with the vapor; (2) temperature of the vacuum chamber continuously drops until solution freezes; (3) once the ice forms on the inner surface of the tube, (4) addition of water droplets grows the ice until it blocks the air/solution flow.

We performed experiments to evaluate the water-based evaporative freezer. Two refrigerants, DI water and 20% EG solution were tested. Figure 2(a) shows temperature and pressure changes in the vacuum chamber when DI water is used. While the reservoir temperature was uniform (~24 °C), the chamber temperatures dropped rapidly after vacuum (~9.2 kPa) was applied (flow rate = 1.77 μl/s). We noted the significant difference between temperatures measured at the lowest position (Temp. #1) and the higher positions (Temp. #2, 3). Previous study on evaporative cooling indicates that farther from the droplet-generating nozzle, lower is the temperature because droplet size decreases as moving downstream in air and thus evapora-

tion improves.[14,15] In our case, the ice growing inside the vacuum chamber partially blocks droplet/air flow. The lowest thermocouple contacts the ice, registering the lowest temperature. For the higher positions, however, the reduced droplet/air flow may have decreased evaporation rates, leading to higher temperatures. A portion of the ice was haphazardly sucked from the vacuum chamber, resulting in sudden change in the droplet/air flow and temperature fluctuation (Fig. 2(a)). Temp. #1 reached 0 °C in 11.6 s on average ($n = 7$, cooling rate = −2.1 °C/s). The average lowest temperature was −11.7 °C. Owing to a lower freezing point than that of water and possible undercooling,[17] ice did not form with EG solution. Without flow blockage (flow rate = 1.16 μl/s), heat removal was steady. Therefore, the three thermocouples showed similar temperature (Fig. 2(b)), a stark contrast to the DI water case. Since the distance between the lowest and highest thermocouples was mere 10 mm, neither significant droplet-size nor temperature difference was expected. Temp. #1 reached 0 °C in 7.1 s on average ($n = 5$, cooling rate = −3.4 °C/s). The average lowest temperature was −11.4 °C. In summary, using mild vacuum, we achieved promising cooling performance: cooling temperature of −14.1 °C and cooling rate of −5.1 °C/s (the best result). For temperature control, EG solution would be better because of more stable cooling temperature. Lowering pressure further (using a better pump) and thus improving evaporation, cooling can be improved as was predicted by a theoretical model.[14]

Ice (DI water) was *in-situ* produced and protein solution (10% w/v BSA) was frozen using our device. Fig. 3(a) (Multimedia view) shows sequential image of ice generation.

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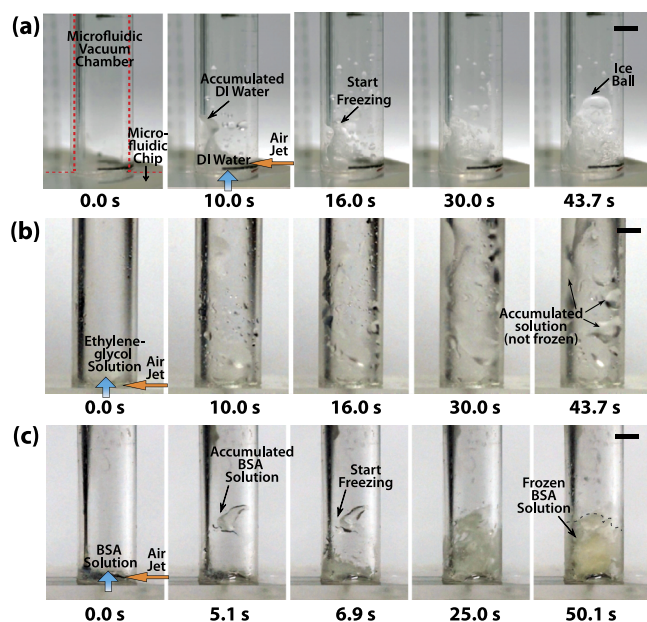
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FIG. 3. Image sequences of on-chip ice generation and freezing of biological solution. (a) DI water turned into an ice ball (ambient temperature of 18.8 °C and vacuum pressure of 6.3 kPa). (b) EG solution did not freeze (24 °C, 9.2 kPa). (c) BSA solution was frozen (24 °C, 5.7 kPa). Thermocouples were not installed for unobstructed view (scale bar = 3 mm). (Multimedia view) [URL: http://dx.doi.org/10.1063/1.4905184.1] [URL: http://dx.doi.org/10.1063/1.4905184.2]

Being pushed by the air jet, water droplets were accumulated on the side wall (10.0 s). As temperature dropped below the freezing point, the accumulated water turned into ice (16.0 s). Adding water, ice continuously grew to an ice ball of 4 mm diameter (43.7 s). This is the first demonstration of on-chip ice generation without any external cooling instrument. As noted from Fig. 3(b), EG solution did not freeze for the aforementioned reasons. The BSA solution (freezing point = −0.05 °C[18]) was tested for freezing of biological solution. The BSA solution started freezing in 6.9 s as in Fig. 3(c) (Multimedia view). The frozen BSA solution almost blocked the vacuum chamber at 50.1 s. We observed yellowish protein precipitation owing to reduced solubility. This intriguing result could support a simple and integrated microfluidic freezing of biological samples for subsequent off-chip analysis or for long-term storage (e.g., cryopreservation).

In conclusion, we successfully demonstrated effective yet "green" microfluidic refrigeration using evaporation of aqueous microdroplets under vacuum. Our microfluidic freezer deemed simple, without an external cooling instrument. Furthermore, a mild vacuum (∼9.2 kPa) used here may be achieved using a portable pump for instrument miniaturization. The vacuum chamber can be easily microfabricated on chip[19] with similar channel geometry and substrate material of the microfluidic chip used here. By tuning nozzle geometry, channel size, chamber volume, and vacuum pressure, one can control refrigeration temperature and cooling rate. An array of evaporative coolers could be incorporated into a single microfluidic device for multivariate temperature control. Using this approach, microfluidic methods requiring cooling or freezing could be performed in a high throughput and a multiplexed manner (e.g., multiplexed PCR, protein crystallization screening), contributing immensely to various fields including chemistry, biological science, food science, and atmospheric science.

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