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Thermoelectric Manipulation of Aqueous Droplets in Microfluidic Devices

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Abstract

This paper describes a method for manipulating the temperature inside aqueous droplets, utilizing a thermoelectric cooler to control the temperature of select portions of a microfluidic chip. To illustrate the adaptability of this approach, we have generated an “ice valve” to stop fluid flow in a microchannel. By taking advantage of the vastly different freezing points for aqueous solutions and immiscible oils, we froze a stream of aqueous droplets that were formed on-chip. By integrating this technique with cell encapsulation into aqueous droplets, we were also able to freeze single cells encased in flowing droplets. Using a live-dead stain, we confirmed the viability of cells was not adversely affected by the process of freezing in aqueous droplets provided cryoprotectants were utilized. When combined with current droplet methodologies, this technology has the potential to both selectively heat and cool portions of a chip for a variety of droplet-related applications, such as freezing, temperature cycling, sample archiving, and controlling reaction kinetics.

INTRODUCTION

The ability to manipulate the contents of channels in microfluidic devices is critical for the success of chemically and biologically oriented lab-on-a-chip technologies. Droplets in microfluidic channels are of particular interest as they enable researchers to work with small sample volumes with a high degree of control over the experimental conditions. Current techniques enable the formation of both streams and individual droplets of varying diameters in a microfluidic device.^{1–9} Droplets offer a number of experimental advantages, namely their ability to function as small reaction vessels where a high concentration of a small amount of reagent can be maintained and the ease of controlling their chemical and physical environment.¹⁰ In combination with other microscale methods, droplets can be finely manipulated, transported, and used for a number of diverse applications. These include reagent concentration^{3, 11} and screening¹², protein crystallization², particle synthesis^{13, 14}, capillary electrophoresis¹⁵, and biological assays.⁴

To date, while droplets have been heated in a microfluidic chip^{14, 16}, cooling and freezing have remained elusive. Heating portions of a microfluidic system, either inadvertently via Joule heating¹⁷ or by design for applications such as particle synthesis¹⁴ and PCR (polymerase-chain reaction)¹⁸, is fairly simple. Both droplet streams and discrete droplets have been heated previously, in nanoliter- and microliter- volumes, respectively.^{14, 16} Cooling or freezing an aqueous solution in a microfluidic chip, however, is more challenging.¹⁹

An attractive device for temperature manipulation of microfluidic systems is the thermoelectric cooler (TEC). TECs are solid-state devices, composed of n- and p-type semiconductors sandwiched between thin sheets of ceramic or another rigid electrically insulating yet thermally

conductive material. When direct current is applied to the array of n- and p-type elements, one side of the device cools due to the Peltier effect and absorbs heat from the surrounding environment. The heat is transported through the device to the opposite side and released, creating a temperature gradient between the two sides of the TEC.²⁰ Microscale TECs, which can be embedded in microfluidic chips, have been developed¹⁹⁻²² and both these and larger TECs have enabled highly localized cooling of nanoliter-sized volumes.^{19, 22-25} While thermally manipulating such small areas has been shown to be sufficient for creating a thermally actuated valve^{19, 23-26}, it is unattractive for droplet manipulation as a sufficiently large area needs to be cooled such that droplets moving through a section of the chip will have sufficient time to experience the change in temperature. An additional complication is that for multiple operations to take place on one chip, a single temperature across the entire device is undesirable, and different portions of the chip may need to have their own set temperatures. Thus, a method that can target select areas of a device larger than the nanoliter volumes previously demonstrated but still sufficiently small for setting different temperatures for different sections of the chip would be ideal for the manipulation of droplet temperature.

This paper reports a microfluidic device that incorporates a 1.5 cm² TEC to manipulate the temperature of droplets flowing in microchannels. Here, we take advantage of the vastly different freezing points for aqueous solutions and immiscible oils so we can selectively freeze or otherwise manipulate the temperature of aqueous droplets while still maintaining flow in the immiscible phase. By combining the TEC cooler with pre-existing techniques that enable the formation of streams of droplets^{1, 2, 8} and selective encapsulation of biological cells⁴, we demonstrate the ability to freeze streams of picoliter-volume droplets as they flow through the microchannel system as well as to freeze single biological cells contained within the droplets. We have confirmed that under optimized conditions, these cells do not experience a significant drop in viability (as determined by a live-dead stain) from either encapsulation in droplets or the freeze-thaw process. Additionally, the temperature of the droplets or other channel contents can be controlled precisely over a large temperature range with this technique, because the temperature difference between the top and bottom of the TEC is directly proportional to the current applied to the device. While we focused on the freezing of droplets in this paper, by reversing the voltage on the TEC and thus reversing the hot and cold sides of the TEC, droplets can be heated in addition to cooled.

MATERIALS AND METHODS

Fabrication of PDMS microfluidic chips

Silicon masters that had features composed of SU-8 negative photoresist were fabricated using standard photolithography techniques.²⁷ The T-channel configuration we used for droplet formation required two different feature heights as the input fluids are restricted to smaller channels before they intersect at the T. This geometry encouraged the formation of droplets with diameters similar to the height of the smaller features. For these devices, we used two-step photolithography. Specifically, a thin (40 μ m) layer of photoresist was spin coated and developed, after which a second 90 m thick layer of photoresist was spun on top of the first layer. The features on the first layer were carefully aligned with the mask for the second layer under a mask aligner before exposure. Following exposure, the second layer was developed and the master was exposed to tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane vapor overnight to silanize the features.

The microchannels were formed out of poly(dimethylsiloxane) (PDMS) via replica moulding, where a 10:1 ratio of prepolymer to catalyst was poured on top of the silicon master, baked for 1 hour at 60 °C, and then cut out and removed from the master. The thin layers of PDMS were obtained by using small amounts of the prepolymer-catalyst mixture and allowing time for the liquid polymer to spread out over the features before baking. Access holes were punched in

the resulting PDMS slabs using a 16-gauge needle. The slabs were then plasma bonded to glass coverslips. Chips that were intended for droplet formation were then baked at 110 °C for two days following plasma bonding to restore the PDMS's hydrophobic surface so that the aqueous phase would not wet the surface of the channels.

PDMS wells used for the cell viability studies were formed using PDMS slabs prepared as before, diced into 10 mm² pieces and punched through the center with a 5 mm diameter cork punch. These PDMS pieces were plasma bonded to a glass coverslip and baked to restore the chip's hydrophobic surface as described above. During experiments, the well opening was covered with a second coverslip to minimize contamination and evaporation.

Fluorescence and bright-field imaging

A solid-state laser (488 nm, Sapphire Coherent, Santa Clara, CA) was used to excite fluorescence, which was imaged with a sensitive camera (Cohu 4910, Cohu, San Diego, CA). Bright-field high-speed imaging was carried out with a fast camera (Fast Camera 13, FastVision, Nashua, NH) and bright-field images during cell viability studies were captured with a digital SLR (E-300, Olympus, Center Valley, PA). High-speed, fluorescence, and bright-field cell viability images were taken on an inverted microscope (Nikon TE300, Nikon, Tokyo, Japan). All other bright-field images were captured on a color camera (Cohu 2222, Cohu, San Diego, CA) using a stereoscope (Nikon SMZ1500, Nikon, Tokyo, Japan). Figure 1 illustrates the setup configuration we used on both a stereo microscope (Fig 1a) and an inverted microscope (Fig 1b).

Droplet generation and temperature manipulation

To generate continuous streams of droplets, polyethylene tubings (PE100, BD, Franklin Lakes, NJ) were inserted into the access holes, then attached to 3 mL syringes so fluids could be introduced into the channels. Fluid flows were driven and controlled by syringe pumps (KD Scientific, Holliston, MA). A T-channel configuration was used for droplet generation as previously described.^{2-4, 8, 12, 15} With the channel design used here, streams of monodispersed droplets formed optimally when the flow rate of the silicone oil was approximately three times that of the aqueous solution. Optimal flow rates for the oil phase were between 0.1 and 1 μ L/min. To stabilize the droplets with surfactant, we added 0.01% w/w sorbitan monooleate (Span 80, Fluka, Buchs, Switzerland) to the silicone oil (AS 4, Fluka, Buchs, Switzerland). While silicone oil can cause PDMS to swell slightly⁶, we found that the effect was negligible in these experiments.

For cell viability studies, an emulsion of cells in either cell culture media or media supplemented with 5% dimethyl sulfoxide (DMSO) (see "Preparation of Cells") was made using a 1:500 ratio of cells in media to oil. Both the AS 4 and mineral oil (M5310, Sigma, St. Louis, MO) contained 0.05% w/w sorbitan monooleate. The cell media and oil mixture was shaken by hand for 5s to generate the emulsion, which was immediately transferred by micropipette to vials for freezing.

We placed the TEC (NL1023T, Marlow Industries, Dallas, TX) either on the PDMS side or the glass side of the microfluidic chip depending on the microscope we used (Fig 1), and secured the TEC with a small amount of thermal compound (Thermal Compound, Part No. 1202, Wakefield Engineering Inc., Wakefield, MA) to ensure complete thermal contact. Optimal cooling was achieved when a potential of 1.1 V was applied to the TEC.

Determination of freezing points

The range of freezing points for common oils used in microfluidics were approximated by freezing the oil in question and then adding a small amount of oil close to the freezing point.

Once the system was close to equilibrium, the temperature of the just-melted oil was measured using a low temperature thermometer. Oils characterized included soybean oil (S7381, Sigma, St. Louis, MO), light mineral oils from Sigma (M3516, Sigma, St. Louis, MO) and Fisher Scientific (Light Mineral Oil NF/FCC, Fisher Scientific, Pittsburgh, PA), and silicone oils from Fluka (AR 20 and AS 4, Fluka, Buchs, Switzerland).

Preparation of cells

Mouse B lymphocytes (ATCC, Manassas, VA) were cultured at 37 °C in cell culture media, which was composed of RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (Invitrogen, Carlsbad, CA) and 1% penicillin-streptomycin solution (Sigma, St. Louis, MO). Cells were either stained with Methylene Blue (Sigma, St. Louis, MO) or the membrane dye DiOC6 (3,3'-dihexyloxacarbocyanine iodide) (Molecular Probes, Eugene, OR). For freezing, the cell media was supplemented with 5% DMSO (BD, Franklin Lakes, NJ) to act as a cryoprotectant. Trypan Blue solution (Sigma, St. Louis, MO) was added to the cell media in a 1:1 ratio as a live-dead stain for cell viability studies just prior to droplet formation.

Simulation parameters

Temperature simulations were performed using COMSOL Multiphysics 3.3 (COMSOL AB, Stockholm, Sweden). We carried out a two-dimensional (2D) simulation, in which we took the plane that cuts across the microchannel (from top to bottom) and through the center of the aqueous droplet. This approximation reduces the spherical droplet to an infinitely long cylinder, but in the plane where we conducted the 2D simulation the solution for a sphere and a cylinder is identical. Because the thermal gradient along the length of the channel was small, the convective thermal flux along the channel length from other droplets was insignificant and thus was not included in our simulation. The thermal diffusivities used in the simulations were $6.72 \times 10^{-7} \text{ m}^2/\text{s}$ for borosilicate glass, $1.43 \times 10^{-7} \text{ m}^2/\text{s}$ for water, $3.27 \times 10^{-7} \text{ m}^2/\text{s}$ for silicone oil, $1.59 \times 10^{-7} \text{ m}^2/\text{s}$ for PDMS, and $1.99 \times 10^{-5} \text{ m}^2/\text{s}$ for air. The physical constants for borosilicate glass, water, and air were obtained from COMSOL Multiphysics 3.3 materials/coefficients library. COMSOL Multiphysics uses constant values for borosilicate glass and air, but varies the properties of water according to temperature. The values displayed for water are at 20 °C²⁸. All values for the physical properties of silicone oil were obtained from Dow Corning and were measured at 25 °C. Values for the thermal properties of PDMS were also obtained from Dow Corning at 25 °C and confirmed by Erickson, *et al.*²⁹

Cell viability studies

Cells encapsulated in cell media-based droplets in AS 4 and in mineral oil were frozen for 24 hours, 48 hours, and 1 week in a conventional freezer maintained at −5 °C in a plastic cryofreezing vial. The aqueous phase was 50% cell media and 50% PBS (phosphate buffered saline) containing Trypan Blue (at 0.4% w/v), which stained cells blue when the cell membrane was compromised due to cell death. Samples were thawed at room temperature. Once thawed, the emulsions were transferred to PDMS wells with a micropipette for observation. Cells that appeared to have taken up Trypan Blue were considered dead while cells that excluded Trypan Blue were considered alive. Normalized cell viability was computed as the ratio of the number of cells viable at the time point in question to the number of cells determined to be alive in the sample prior to encapsulation in droplets. The number of cells examined per set of conditions ranged from 57 to 561 cells, depending on how many droplets settled into the focal plane during the observation period.

RESULTS AND DISCUSSION

Operation of the device and freezing of aqueous solutions in elastomeric microchannels

Our approach to on-chip temperature manipulation of droplets requires a single TEC placed on the chip and secured with a zinc oxide-based thermal compound. We used PDMS based microfluidic systems because PDMS has a natively hydrophobic surface that is required for the generation of aqueous droplets. PDMS-glass devices are also inexpensive and easy to fabricate. Through PDMS as thick as 1 mm, temperatures as low as -20°C could be achieved using this method, which we confirmed by measuring the temperature with a thermocouple. To remove heat from the TEC, we used a dry ice-cooled heat sink affixed to the hot side of the TEC. The underside of the chip was sufficiently cooled under these conditions to cause atmospheric water to condense onto the surface and freeze. To avoid this, an anhydrous sheeting gas composed of tetrafluoroethane was used to perfuse the chip and to prevent condensation so select portions of the microfluidic channels could be viewed.

Upon applying electric current to the TEC, the proximal region of the channel was cooled below the freezing point of water, thereby causing a 'plug' of ice to form in the channel, effectively closing the channel. Figure 2 shows the effect of the formation of this "ice valve", in which we used a Y-shaped channel with two inlets and one outlet (Fig 2a). While ice valves have been demonstrated previously in microfluidic systems²³⁻²⁵, the materials used in these devices were rigid, whereas PDMS is elastic and the successful formation of this valve confirms that PDMS does not stretch and allow fluid to leak past the valve despite the pressure build-up behind the plug. Flow from the top inlet contained 6 μm beads but flow from the bottom inlet did not. As a result, laminar flow was set up in the main channel where the TEC was placed (Fig 2b). Because the pressure applied to the top inlet was slightly greater than that applied to bottom inlet, the laminar stream that contained the beads was slightly wider than the stream that did not. When water froze in the main channel, flow was stopped at the main channel, which led to a flow reversal in which the stream that contained beads began to flow from the top inlet channel towards the bottom inlet (Fig 2c); we also did not observe any fluid flow behind the ice valve.

From the time the TEC was first activated, the formation of a solid ice plug in the channels occurred within 10 seconds under these conditions. This time delay was mostly caused by the time it took to cool the TEC element down from room temperature, and was not limited by the thermal conduction of heat away from the microfluidic chip to the TEC. Our simulations (see section below) showed that when a pre-cooled TEC was applied to the glass side of a microfluidic chip, the temperature in the channel can be cooled to below 0°C within tens of milliseconds. The ice plug formed can be sustained with the TEC, left to thaw, or actively melted by reversing the voltage on the TEC. When the chip was heated with the TEC to remove the ice plug, we observed flow to be restored within 5 seconds or less.

Selective freezing of droplet streams and biological cells in droplets

To easily view the freezing of droplets and droplet-encapsulated cells, we used a T-channel configuration to form streams of aqueous droplets in silicone oil (AS 4). Both the aqueous and oil phases were connected to programmable syringe pumps so fluids could be delivered into the channels at a controlled rate. Depending on the backpressure already built up in the channels, using the above mentioned parameters (see experimental section) droplets formed at a rate of about 1–5/second. With faster flows, however, droplets can be generated at frequencies into the kilohertz range.⁷ Figure 3a shows the design of our channel and the placement of the TEC.

Prior to freezing, the droplets appeared dark in our image because the incident light (Fig 1a) did not allow for sufficient contrast between the cell media and oil (Fig 3c); when frozen, these droplets appeared white because of scattering of the incident light (Fig 3d). After the droplets were frozen, the silicone oil remained liquid and continued to carry the now-frozen droplets through the channel, owing to the difference in freezing points between the aqueous and immiscible phases. Common immiscible phases have freezing points spanning a wide range of temperatures. Acetophenone freezes at 20.5 °C, while perfluorodecalin freezes at -10 °C, and nonane freezes at -53.46 °C.²⁸ Common oils used as immiscible phases also span a large range of freezing points with soybean oil freezing at about -16 °C, mineral oils freezing in the -25 °C to -45 °C range, and silicone oils such as AR 20 and AS 4 freezing at about -70 °C and -100 °C respectively. While water freezes around 0 °C, AS 4 remains liquid with negligible changes in viscosity and minimal clouding until temperatures lower than -60 °C are reached. Surfactants such as the Span 80 used here can affect the temperature stabilities of these fluids, but this effect was negligible in these experiments. As AS 4 is thermostable between -60 °C and 200 °C, a wide temperature range is available for manipulations of droplets in this system.

While cells can be individually manipulated and encapsulated in droplets using an optical trap⁴, we used a bulk average approach so we could easily encapsulate many cells into droplets, which is important for high-throughput cell archiving applications. To ensure that the majority of droplets had one cell in them, we suspended cells in their media at a density that corresponded on average to one cell per droplet volume. For example, cells at a concentration of approximately 8.8×10^6 cells/mL resulted in one cell per 1.13×10^{-7} mL on average, or one cell per 30 μ m-diameter droplet. Figure 4a-d shows cells could be easily encapsulated into droplets using this approach; figure 4e-f shows this droplet-encapsulated cell prior to freezing (Fig 4e) and when frozen (Fig 4f).

Thermal transport

To better understand the process of droplet freezing and to obtain approximate freezing rates for our droplet-freezing method, we modelled our TEC and microfluidic chip with a 2D simulation in the plane that cuts across the microchannel (from top to bottom) and through the center of the aqueous droplet. Our simulations show that the temperature gradient induced by the TEC is steep, and the droplet reached a temperature below 0 °C within five seconds regardless of the side of the chip the TEC was placed (see Figure 5). This simulation result agrees qualitatively with our observations of rapid freezing, and our simulations also support the observation that freezing is not rapid enough to ensure vitrification of the aqueous droplets. At rates of cooling greater than 5000 °C/s, the intracellular water vitrifies before any water flows out of the cell, which prevents dehydration and thus increases cell viability.³⁰ While our simulations estimate that the temperature within the channels and throughout the droplet should drop below freezing within 60 ms of the TEC reaching -20 °C, indicating a cooling rate of 333 °C/s, this rate assumes perfect thermal contact and is clearly an ideal situation. The droplets in figures 3 and 4 changed their opacity and reflectivity when frozen, indicating that there may be degassing and/or the formation of irregular ice crystals; the refractive index change alone (from 1.33 for liquid water to 1.31 for frozen water²⁸) is not sufficient to account for a discernable change in visual contrast between the media. Placing the TEC on the PDMS side of the chip severely retards the cooling process (Figure 5c-d). The glass coverslip not only has a thermal diffusivity that is four times that of PDMS, but the PDMS layer is also an order of magnitude thicker, which explains the two orders of magnitude increase in freezing rate when the TEC was in contact with glass rather than PDMS. Although rapid freezing and vitrification is an important consideration for preserving cells, the rate of freezing is less critical for other droplet-related applications, such as temperature cycling for carrying out single-cell PCR, controlling reaction kinetics in droplets, or inducing colloidal aggregation and protein crystallization.

Cell viability after freezing in droplets

One promising application of our technique is the sorting and archiving of individual cells encapsulated in frozen droplets. The utility of this application depends on the viability of cells after being frozen in droplets. To determine whether cell viability is compromised by droplet encapsulation and freezing, we characterized cell survival post-freezing in droplets for a range of freezing times in two different media and for two different immiscible phases. The aqueous medium used was phosphate buffered saline (PBS) containing 0.4% (w/v) Trypan Blue that was either mixed in equal volume with normal cell media or with cell media supplemented with 5% DMSO (which acts as a cryoprotectant). The immiscible phase was either AS 4 (a silicone oil) or sterile-filtered mineral oil, both containing 0.05% Span 80, a surfactant that facilitated droplet formation and stabilized the formed droplets. Encapsulated cells were observed for one hour after droplet formation in the case of unfrozen droplets, or for one hour after removal from the freezer if the droplets had been frozen immediately after their formation. Using Trypan Blue as a live-dead stain, cells could be visually identified in droplets as either alive or dead (Fig 6a-b).

Figure 7 shows the result of our cell-viability study. All cells contained within droplets experienced a decrease in viability over time, most likely because of the confined volume that contained limited amount of nutrients and oxygen. A more important factor, however, appears to be the immiscible phase we used, with AS 4 being significantly less compatible with cells than mineral oil. This difference may be attributed to the fact that AS 4 and other silicone oils are generally manufactured for industrial use and as a result are not sterile and may contain a number of impurities. In fact, after freezing and thawing, we observed aqueous droplets in AS 4 often had a matrix that formed around them (Fig 6c), which may be impurities that came out of solution after freezing. In contrast, droplets in mineral oil did not have this matrix and were visually indistinguishable after freezing and thawing from those left at room temperature (Fig 6d).

The use of DMSO as a cryoprotectant increased the ability of cells to survive the process of droplet freezing. After a week of freezing, for example, only 28.5% (at one hour post thawing) of cells encapsulated in normal media-based droplets in AS 4 survived the procedure, but this percentage increased to 48.1% when the cell media was supplemented with DMSO. Similarly, when mineral oil was used only 64.4% of cells were alive one hour post thawing when frozen for one week without DMSO, and this percentage of viable cells increased to 81.5% with DMSO.

CONCLUSION

We have demonstrated a simple method for the thermal manipulation of aqueous droplets in microfluidic channels, and utilized this technique to arrest flow in water-filled channels and to freeze aqueous droplet streams in oil. Both the heating and cooling features of the TEC could be used for on-chip PCR, crystallization, droplet shrinkage and expansion, controlling reaction kinetics, and the reversible valving of fluid flow. The potential to rapidly cool individual droplets containing cells or other biological particles for assays or storage allows for microfluidics to be applied to samples that would otherwise degrade in transit through the microfluidic system or before extraction from the microfluidic system for use or storage elsewhere. As this technique does not significantly compromise the viability of cells encapsulated in the frozen droplets when cell-compatible oils and cryoprotectants are utilized, an exciting possibility exists for integration of this method with cell-archiving systems to build libraries of individually encapsulated and frozen cells in aqueous droplets. In combination with other droplet-based technologies, we anticipate our method will become an important part of the microfluidic tool box.

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Figure 1. Schematics of setup

a) An illustration showing the setup used for taking low-magnification images under a stereoscope, where the TEC and the accompanying heat sink were mounted on the glass-side of the chip. An optically transparent acrylic platform supported the chip and held the TEC in place so it was directly in contact with the glass. Illumination light could be introduced both from the bottom of the stereoscope or at an angle from the top (with a fiber-optic light guide). A close-up of the TEC and PDMS microfluidic chip is shown to the right. b) A schematic of the setup used for fluorescence and high-magnification bright-field imaging. Here the TEC and heat sink were mounted on the PDMS side of the chip. Light for bright-field imaging was directed from the top and laser illumination (488nm) for epifluorescence imaging was from the bottom of the chip. A close-up of the microfluidic chip is shown to the right.

Figure 2. Ice valve

a) A schematic showing the simple Y-channel we used in our experiment, which was 100 μm wide and 40 μm high, and the placement of the TEC with respect to the channel. b) Laminar flow was set up in the main channel; 6 μm beads were spiked in the top stream but not in the bottom stream. Pressure applied to the top inlet was slightly higher than to the bottom inlet, which resulted in a slightly wider top stream in comparison with the bottom stream. c) Formation of the ice plug in the main channel arrested flow in the channel and caused the solution from the top inlet to flow towards the bottom inlet. d) Appearance of the channel that was directly in contact with the TEC when the TEC was off, with the beads now fluorescently illuminated, and e) when the TEC was on and the fluorescence now scattered off the ice crystals which had formed in the channel, increasing the background fluorescence in the channel.

Figure 3. Freezing of droplet stream

a) A schematic showing the T-channel geometry we used for droplet generation; the serpentine channel downstream of the T-channel and under which the TEC was placed was made long to ensure the transit time of droplets across the TEC was sufficiently long so the droplets would freeze and remain fully frozen. b) Bright-field image showing a stream of unfrozen droplets. c & d) Image of a stream of droplets over the TEC before (c) and after (d) freezing. Labels in panel (a) show the positions along the channel where the images shown in (b-d) were taken. The main channel in this experiment was 100 μm wide and 90 μm high.

Figure 4. Cell encapsulation and freezing

a-d) A sequence of images showing a B lymphoma cell in a cell-freezing solution composed of cell media with 5% DMSO being encapsulated in an aqueous droplet. e & f) A cell in an aqueous droplet of cell freezing solution prior to freezing (e) and after being frozen (f). The grey circle outlines the droplet. Scale bars represent 30 μm .

Figure 5. Simulations of cooling

a & b) The two-dimensional geometries used to simulate the temperature inside the chip when the TEC was placed on the bottom (glass coverslip) side of the chip (a) and when the TEC was placed on the top (PDMS) side of the chip (b). The grey lines depict the line where the temperature was sampled at various times after we set the TEC temperature at -20°C . c) A plot of the simulated temperature as a function of distance away from the TEC at seven different time points (0.05ms, 10ms, 20ms, 30ms, 40ms, 50ms, 60ms); simulation was performed using the geometry in (a). The center of the droplet is at 0.19 mm away from the TEC. In this simulation the droplet froze within 60ms of the TEC reaching -20°C . d) A plot of the temperature as a function of distance away from the TEC at eleven different time points (0.01s, 0.5s, 1.0s, 1.5s, 2.0s, 2.5s, 3.0s, 3.5s, 4.0s, 4.5s, 5.0s) after the TEC reached -20°C . The geometry shown in (b) was used in this simulation, which showed the droplet froze within 5s. A small bump in the plots can be observed just after 1.0 mm, which is due to the difference in thermal diffusivities between the droplet and AS 4.

Figure 6. Behavior of cells and droplets after freezing

a & b) A cell in a droplet composed of 50% cell media and 50% PBS (phosphate-buffered saline) containing Trypan Blue (at 0.4% w/v in PBS) when (a) alive and (b) dead. c) A thawed droplet in AS 4, and d) in mineral oil. Note the matrix surrounding the droplet in (c). Scale bars in (a & b) both represent 10 μm and the one in (d) is 40 μm .

Figure 7. Cell viability after being frozen in aqueous droplets

Left column shows data from cells in normal media (50/50 cell media and PBS containing Trypan Blue (at 0.4% w/v in PBS)) while right column shows results for cells in freezing media (which contained an additional 5% DMSO supplemented to the cell media). Top row is data for cells frozen in droplets in AS 4, and bottom row shows data for cells frozen in droplets in mineral oil. Normalized cell viability was calculated by dividing the number of cells viable at the various time points in question by the number of viable cells in the original sample.