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Droplet freezing, docking, and the exchange of immiscible phase and surfactant around frozen droplets[†]

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Abstract

This paper describes a platform for cooling microfluidic chips so as to freeze aqueous droplets flowing in oil. Using a whole-chip cooling chamber, we can control the ambient temperature surrounding a microfluidic chip and induce cooling and freezing inside the channels. When combined with a droplet generation and droplet docking chip, this platform allows for the facile freezing of droplets immobilized in resistance-based docks. Depending on the design and shape of the docks, the frozen droplets can either be trapped stably in the docks or be released because deformed non-frozen aqueous droplets turn spherical when frozen, and thus can become dislodged from the docks. Additionally, using this chamber and chip combination we are able to exchange immiscible phases and surfactants surrounding the frozen droplets. The materials and methods are inexpensive and easily accessible to microfluidics researchers, making this a simple addition to an existing microfluidic platform.

Introduction

Droplet microfluidics has attracted much interest from the lab-on-a-chip community in recent years because of the many advantages offered by the ability to compartmentalize samples inside a large number of discrete aqueous droplets.^{1–10} One particularly interesting feature we found could be exploited with droplet microfluidics is that water and oil can have very different freezing and boiling points.⁷ As a result, we were able to freeze a stream of aqueous droplets containing single cells, because while the droplets and cells are frozen, the physical properties of the immiscible phase, including viscosity, do not change significantly from ambient temperature to well below the freezing point of water. This characteristic allowed us to use microfluidics to manipulate a flowing stream of discrete and frozen samples.⁷ Since our first report on freezing aqueous droplets,⁷ several papers have described other attractive features of freezing droplets,^{11–13} including the ability to vitrify cell-containing aqueous droplets in liquid nitrogen¹¹ and the study of ice nucleation.^{12,13}

Despite the interesting capabilities offered by frozen droplets, the experimental setups used for freezing droplets can either be somewhat complex (*e.g.* integrated thermoelectric coolers in our previous report⁷ or a specialized setup for studying ice nucleation¹³), or are

[†]Electronic supplementary information (ESI) available: Supplementary Figure S1, which gives detailed measurements of the cooling chamber.

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incompatible with on-line microscopy imaging (*e.g.* the vitrification of droplets in liquid nitrogen¹¹), or are simply expensive and thus not very accessible to the general microfluidics community (*e.g.* the use of a commercial cryostage¹²). Additionally, commercial cryostages are not easily scalable to larger chip sizes and do not permit tubings to remain connected to microfluidic channels while chips are in the chamber. To address these practical concerns, this paper describes a simple home-built platform for freezing and viewing frozen droplets that allows for transmitted light microscopy and any existing chip design to be used. We used this platform to demonstrate a number of manipulations on droplets, such as docking, release, and the exchange of immiscible phase around frozen droplets.

The ability to exchange immiscible phases and surfactants around aqueous droplets is particularly interesting to us. The majority of microdroplet applications utilize aqueous droplets in an immiscible phase for chemical and biological analysis, and the droplet contents usually attract the most interest. However, the composition of the immiscible phase and surfactants is also critical to success. The immiscible phase and surfactants dictate a number of system properties: the ease of droplet formation,^{14,15} droplet fusion,^{16,17} heat transfer,⁷ and gas solubility.¹⁸ For example, silicone oils that are cytotoxic but good for droplet generation (such as AS 4⁷) can be replaced with biologically compatible mineral oils. Alternately, immiscible phases with refractive indices higher than that of the aqueous solution can be replaced with a lower refractive index immiscible phase to allow conventional optical tweezers to be used for droplet manipulation.¹⁹ Current droplet methodologies make moving from one immiscible phase/surfactant composition for droplet formation to another immiscible phase/surfactant composition for analysis difficult. Changes in immiscible-phase viscosity, surfactant content, and the flow profile in the system can easily distort liquid droplets and perturb the system; droplets can come into contact with and wet channel walls, or change spacing between droplets, which can lead to fusion. A method that allows for immiscible-phase and surfactant exchange after droplet formation but prior to other experimental analysis would allow researchers to use immiscible phase/surfactant composition with the desired properties for each step of the experiment, rather than picking one immiscible phase/surfactant composition that is compatible but not ideal for all steps. Here we demonstrate immiscible-phase exchange using frozen aqueous droplets immobilized in resistance-based docks^{3,9,10,20} and demonstrate how these docks can be used at varying droplet sizes, flow rates, and with solid (frozen) or liquid droplets.

Experimental

Fabrication of microfluidic chips

Silicon wafers with features composed of SU-8 negative photoresist were used as masters and fabricated using established two-step photolithography techniques.²¹ First, a thin (55 μm) layer of SU-8-3050 resist was spin coated, exposed, and developed. Second, a 75 μm layer of SU-8-3050 resist was spun on top of the first layer of features. The exposure mask for the second layer was carefully aligned with the first layer of features under a mask aligner prior to exposure. After development of the second layer, the master was exposed overnight to tridecafluoro-1,1,2,2-tetra-hydrooctyl-1-trichlorosilane vapour to aid in PDMS removal from the master.

All microchannels were formed out of poly(dimethylsiloxane) (PDMS) utilizing replica moulding. A 10 : 1 ratio of prepolymer to catalyst was degassed and poured in a thin (2–3 mm) layer on top of the master and baked at 65 °C for 4 hours before being cut out and removed from the master. A 4–6 mm thick slab of PDMS was plasma bonded to the area above the access holes on the thinner PDMS slab to create longer input wells for the polyethylene tubings. Access holes were punched in this area using a 16-gauge needle and the slab was plasma sealed to glass coverslips to complete the chip. Chips were baked at 110 °C for 2 days following plasma sealing to restore the PDMS's hydrophobic surface.

Imaging

All imaging used a colour camera (Cohu 2222, Cohu, San Diego, CA), VirtualDub capture software, and a stereoscope (Nikon SMZ1500, Nikon, Tokyo, Japan).

Droplet generation

Microfluidic chips were plumbed using polyethylene tubings (PE100) inserted into the access holes. Syringe pumps (Genie Plus, Kent Scientific, Torrington, CT) with 3 mL syringes of silicone oils were inserted into the oil access holes. The first immiscible phase was AR 20 silicone oil (Fluka, Buchs, Switzerland) with 0.05% sorbitan monooleate (Span-80, Fluka, Buchs, Switzerland) and the second immiscible phase was AS 4 silicone oil (Fluka, Buchs, Switzerland) with 0.08% Oil Red O (Sigma, St Louis, MO). A flow-focusing configuration was used for droplet generation as previously described.^{22,23}

Deionized water was used as the aqueous phase and was connected using PE tubing to a hand-controlled micrometre to deliver precise amounts of water to generate droplets.

Cooling chamber construction and use

The cooling chamber (Fig. 1) was constructed out of three aluminium cylinders with plexiglass viewing windows. Helium gas was cooled below 0 °C by passing the gas through 10 feet of copper tubing dipped in N₂(l) prior to flowing into the chamber. Temperature could be controlled by changing the gas flow rate with a regulator on the compressed helium tank. Chamber temperature was continuously monitored using a thermocouple. See Fig. S1[†] for detailed chamber measurements.

Results and discussion

Chamber operation and chip cooling

Our approach to droplet freezing allows for an entire microfluidic chip to be cooled without any major modifications to a microscope or chip design. As shown in Fig. 1a, the entire chip is placed within the cooling chamber during operation and the tubing pass-throughs allow for pressure-driven flow. These pass-throughs also can accommodate other objects that connect the microfluidic chip inside the chamber to instruments outside the chamber, such as wires and leads for resistive heaters and thermocouples. The presence of on-chip resistive

[†]Electronic supplementary information (ESI) available: Supplementary Figure S1, which gives detailed measurements of the cooling chamber.

heaters fabricated as part of the microfluidic chip would impart the ability to address and selectively and independently heat various regions of the chip, potentially even down to the level of individual droplets. For larger areas, resistive heating pads can be placed in contact with the bottom surface of a thin coverslip that forms the floor of the microchannel, and this would allow for heaters to be easily added to any chip design. Both the on-chip and off-chip heaters can be located at different locations in different experiments, allowing for multiple chip configurations to be used within the same cooling chamber.

The materials for the chamber were chosen due to their low cost and ready availability. Any cooling liquid with low temperature and high thermal conductivity can be used for chilling the cooling gas, provided it is colder than the desired temperature and its temperature is not below the condensation temperature of the chosen cooling gas, as this can lead to liquid clogging the cooling-system tubing. As the chamber itself also cools due to the internal cooling gas, additional dry, cooled gas is blown across the viewing windows to remove condensation for imaging.

Specifically, to operate the chamber, the preferred cooling gas is flushed through the cooling-system tubing to remove any air. As the cooling system is flushing out, the chip is placed in the chamber and any tubings or thermocouples are connected. It is recommended that the entire experiment be conducted while the chip is inside the chamber to prevent the removal and reattachment of tubings from perturbing the contents of the channels. The top of the chamber is then screwed into place, the chamber is placed under the microscope, and the copper section of the cooling-system tubing is placed in the cooling liquid. From this point, chamber temperature is controlled by the adjusting the flow rate of the cooling gas based on the temperature read out from a thermocouple placed inside the chamber.

Droplet generation

When using any temperature manipulation system in combination with a droplet-based microfluidic device, care must be taken to ensure all droplets are generated at a uniform temperature. Some immiscible and aqueous phases change viscosity as temperature changes. This can cause the size and spacing of generated droplets to change, perturbing the system and often leading to droplet coagulation and plug formation. In this setup, droplets can be generated prior to any temperature manipulation and in the work shown here we generated all droplets prior to cooling. Alternately, a resistive heater and thermocouple combination that maintains the droplet generation area at a constant temperature would permit droplets to be generated after the chamber has been cooled below freezing. These droplets would then freeze upon flowing into the section of the chip not in contact with the heater. However, this approach also requires a resistive heater to warm the aqueous-phase tubing to keep the contents from freezing during droplet generation. Thus, we opted to generate all droplets prior to cooling the chamber.

A schematic of our droplet generation and docking chip is shown in Fig. 2a. We chose to use a thin (2–3 mm thick) piece of PDMS to form the droplet docking region as PDMS's low thermal conductivity can delay the cooling of channel contents to the chamber's ambient temperature. When generating droplets at one temperature and then freezing the entire chip as demonstrated here, the aqueous interface must be pulled back from the droplet generation

area. If left at the flow-focusing region, the expansion of the aqueous phase as it freezes will disturb the balance between the immiscible and aqueous flows and cause the aqueous phase to expand and fill the oil channels.

Droplet docking and release

Resistance-based docks take advantage of fluid following the path of least resistance.^{3,9,10} The fluidic resistance of the path flowing through the dock and small channel connecting it to the main channel downstream is smaller than that of the main channel itself (see Fig. 2a). However, once an object enters the dock it plugs the small connection channel and fluid is forced to flow around it using the main channel. At relatively low immiscible-phase flow rates, such as at the $0.5 \mu\text{L min}^{-1}$ used in Fig. 2b and c, the interfacial tension of the aqueous droplet was able to withstand the work performed by the immiscible phase to deform the droplet. Thus, the liquid droplets will stay in the resistance-based docks because the work applied to the droplets is not sufficient to deform them to the extent that they can flow through the small constrictions at the docking site (Fig. 2b). These droplets can then be frozen while docked by cooling the chamber below 0°C (Fig. 2c).

At higher flow rates, the work applied to the droplet by the immiscible-phase flow is sufficient to deform the droplet to allow it to flow through the resistance dock (Fig. 3a–f). Should higher flow rates be desired prior to docking, droplets can be frozen after generation but prior to docking by cooling the entire chamber and warming the droplet generation area so that droplets freeze as they enter the colder region of the chip as previously demonstrated.⁷ This is more desirable for those interested in higher droplet generation rates and high-throughput applications. Slower immiscible-phase flow rates limit the droplet generation rate and retard the formation of large numbers of microdroplets.

Further system manipulations can be made by combining smaller docks with larger droplets. Liquid droplets of a slightly larger diameter than a dock will deform slightly to fill the dock (Fig. 2b). As the temperature decreases, water's interfacial tension increases and density decreases,²⁴ so the droplet will begin to round and expand upon freezing (Fig. 2c). Should this sphere be too large for the dock, the droplet can be dislodged from the dock (Fig. 3g), allowing for its removal from the dock. This expansion of the droplets needs to be taken into consideration when designing microfluidic chips for use with any freezing platform and when generating droplets during each experiment.

These frozen droplets offer additional advantages over liquid droplets. Regardless of the amount of surfactant used to stabilize the droplets, frozen droplets will not fuse when they come into contact. Additionally, miscibility between the oil and aqueous phases is no longer a concern after freezing. This allows droplet contents to stay at a constant concentration and remain uncontaminated by any unwanted molecules in the immiscible phase. Finally, freezing permits any reactions or other time-sensitive experiment contained within the droplets to be suspended. The whole microfluidic chip might even be stored to resume an experiment at a later date.

Exchange of immiscible phase

A potential application for docking and freezing droplets is the exchange of the surrounding immiscible phase. Docking alone may be sufficient to allow for the exchange of some immiscible phases, however, the immiscible-phase composition affects the interfacial tension of the droplet and if the exchange involves an immiscible phase that lowers the interfacial tension, droplets may deform and push through the small connecting channels between the docks and main channels as shown in Fig. 3a–f. When droplets are frozen, perturbing the balance between interfacial tension and immiscible-phase flow rates is no longer a consideration. Therefore, a different immiscible phase can replace the one used for droplet generation. Freezing the droplets also allows higher immiscible-phase flow rates to be used during the oil exchange than would be possible with most liquid droplets, reducing the time required for the exchange. Fig. 4 shows the process of exchanging the immiscible phase, where AS 4 with 0.08% Oil Red O replaces AR 20 with 0.05% Span-80. In this experiment, the syringe pump controlling the AR 20 was turned off and the one for AS 4 was turned on with a flow rate of $7.0 \mu\text{L min}^{-1}$, resulting in the AS 4 replacing the AR 20 in the microfluidic channels. As seen in Fig. 4e, this process can be completed without disturbing the docked droplets. The generation and manipulation of droplets are often controlled using surfactants that are soluble in the immiscible oil phase. Therefore, it is also straightforward to exchange the surfactant composition using this method.

Conclusions

This paper described a simple and inexpensive method for freezing docked droplets and for exchanging immiscible phases and surfactants around the frozen droplets. Although not demonstrated here, when this chamber is combined with resistive heaters that can be individually addressed and monitored, this platform offers a wide range of temperature control for microfluidic chips. We anticipate that the droplet methodologies and cooling chamber demonstrated here will become a useful part of the microfluidic tool box.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Chiu DT, Lorenz RM. *Acc Chem Res.* 2009; 42:649–658. [PubMed: 19260732]
2. Chiu DT, Lorenz RM, Jeffries GDM. *Anal Chem.* 2009; 81:5111–5118. [PubMed: 19507850]
3. Edgar JS, Milne G, Zhao Y, Pabbati CP, Lim DSW, Chiu DT. *Angew Chem, Int Ed.* 2009; 48:2719–2722.
4. He M, Edgar JS, Jeffries GDM, Lorenz RM, Shelby JP, Chiu DT. *Anal Chem.* 2005; 77:1539–1544. [PubMed: 15762555]
5. Song H, Chen DL, Ismagilov RF. *Angew Chem, Int Ed.* 2006; 45:7336–7356.

6. Huebner A, Srisa-Art M, Holt D, Abell C, Hollfelder F, deMello AJ, Edel JB. *Chem Commun.* 2007;1218–1220.
7. Sgro AE, Allen PB, Chiu DT. *Anal Chem.* 2007; 79:4845–4851. [PubMed: 17542555]
8. Teh SY, Lin R, Hung LH, Lee AP. *Lab Chip.* 2008; 8:198–220. [PubMed: 18231657]
9. Shi W, Qin J, Ye N, Lin B. *Lab Chip.* 2008; 8:1432–1435. [PubMed: 18818795]
10. Boukellal H, Selimovic S, Jia Y, Cristobal G, Fraden S. *Lab Chip.* 2009; 9:331–338. [PubMed: 19107293]
11. Demirci U, Montesano G. *Lab Chip.* 2007; 7:1428–1433. [PubMed: 17960267]
12. Edd JF, Humphry KJ, Irimia D, Weitz DA, Toner M. *Lab Chip.* 2009; 9:1859–1865. [PubMed: 19532960]
13. Stan CA, Schneider GF, Shevkoplyas SS, Hashimoto M, Ibanescu M, Wiley BJ, Whitesides GM. *Lab Chip.* 2009; 9:2293–2305. [PubMed: 19636459]
14. Tice JD, Lyon AD, Ismagilov RF. *Anal Chim Acta.* 2004; 507:73–77.
15. Link DR, Anna SL, Weitz DA, Stone HA. *Phys Rev Lett.* 2004; 92:054503. [PubMed: 14995311]
16. Lorenz RM, Fiorini GS, Jeffries GDM, Lim DSW, He M, Chiu DT. *Anal Chim Acta.* 2008; 630:124–130. [PubMed: 19012823]
17. Bremond N, Thiam AR, Bibette J. *Phys Rev Lett.* 2008; 100:024501. [PubMed: 18232876]
18. Lowe KC, Davey MR, Power JB. *Trends Biotechnol.* 1998; 16:272–277. [PubMed: 9652139]
19. Lorenz RM, Edgar JS, Jeffries GDM, Zhao Y, McGloin D, Chiu DT. *Anal Chem.* 2007; 79:224–228. [PubMed: 17194143]
20. Tan WH, Takeuchi S. *Proc Natl Acad Sci U S A.* 2007; 104:1146–1151. [PubMed: 17227861]
21. Anderson JR, Chiu DT, Jackman RJ, Cherniavskaya O, McDonald JC, Wu H, Whitesides SH, Whitesides GM. *Anal Chem.* 2000; 72:3158–3164. [PubMed: 10939381]
22. Anna SL, Bontoux N, Stone HA. *Appl Phys Lett.* 2003; 82:364–366.
23. Edgar JS, Milne G, Zhao Y, Pabbati CP, Lim DSW, Chiu DT. *Angew Chem, Int Ed.* 2009; 48:2719–2722.
24. Lide, DR., editor. *CRC Handbook of Chemistry and Physics.* CRC Press; Boca Raton, FL: 2010.

Fig. 1. Cooling chamber. (a) 3-D rendering of the cooling chamber for microfluidic chips. (b) Photograph of the cooling chamber. For details and dimensions, see Fig. S1[†].

Fig. 2.

Droplet docking and freezing. (a) Schematic of droplet docking chip. Large channels are 100 μm wide and 75 μm high. The smaller constriction channels connecting the docks to the main channel are 25 μm wide and 55 μm high. (b) Docked and non-frozen water droplets. (c) Docked and frozen water droplets in AR 20 with 0.05% Span-80. Scale bar for (b) and (c) is 100 μm .

Fig. 3.

Release of docked droplets. (a–f) At higher velocities, non-frozen water droplets can deform and pass through resistance docks as shown in this series of images. (g) When the non-frozen water droplet has to deform to fit in the docks, then subsequent freezing of the droplet will turn the droplet spherical and thus cause the frozen droplet to dislodge from the dock (arrow). Both scale bars are 100 μm .

Fig. 4. Exchange of immiscible phase around docked frozen droplets. (a–e) Docked frozen droplets are switched from being in contact with one immiscible oil phase to another immiscible oil phase. The light-colored oil is AR 20 silicone oil with 0.05% Span-80 and the darker-colored oil is AS 4 silicone oil with 0.08% Oil Red O. Both scale bars are 100 μm .