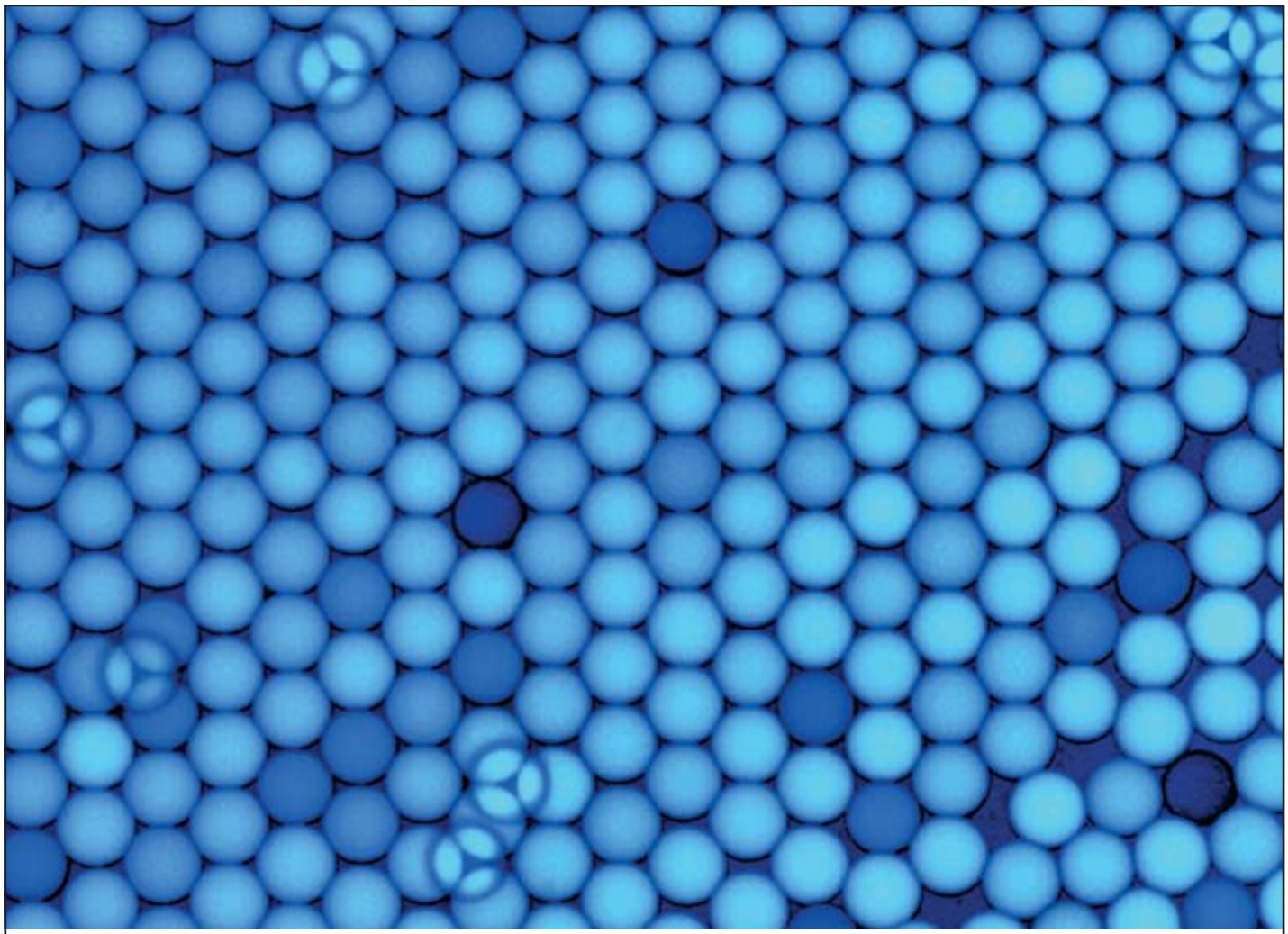

Featuring research on bioassays using drop-based microfluidics from the Weitz lab at the School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA.

Title: Biocompatible surfactants for water-in-fluorocarbon emulsions

Drops of a water-in-oil emulsion are stabilized by a novel non-ionic fluorosurfactant that allows for successful *in-vitro* transcription and translation of genes into enzymes as shown by the accumulation of fluorescent product inside these 20-micron sized drops. The surfactant is necessary to stabilize the drops and make the droplet interface biocompatible.

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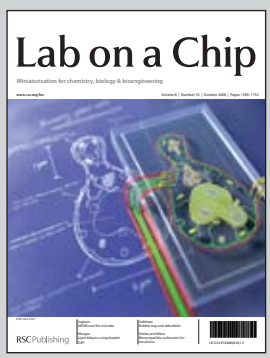

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Biocompatible surfactants for water-in-fluorocarbon emulsions†

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Drops of water-in-fluorocarbon emulsions have great potential for compartmentalizing both *in vitro* and *in vivo* biological systems; however, surfactants to stabilize such emulsions are scarce. Here we present a novel class of fluorosurfactants that we synthesize by coupling oligomeric perfluorinated polyethers (PFPE) with polyethyleneglycol (PEG). We demonstrate that these block copolymer surfactants stabilize water-in-fluorocarbon oil emulsions during all necessary steps of a drop-based experiment including drop formation, incubation, and reinjection into a second microfluidic device. Furthermore, we show that aqueous drops stabilized with these surfactants can be used for *in vitro* translation (IVT), as well as encapsulation and incubation of single cells. The compatability of this emulsion system with both biological systems and polydimethylsiloxane (PDMS) microfluidic devices makes these surfactants ideal for a broad range of high-throughput, drop-based applications.

Introduction

Microfluidic devices have the potential to revolutionize high-throughput screening as they enable assays to be performed with volumes of liquids as low as picoliters. In order to conduct millions of individual reactions with small volumes, it is necessary to compartmentalize reactants. This can be achieved by fabricating microfluidic devices with nanoliter-scale chambers,[1,2] however, such devices are rather complex, they are limited in the number of compartments that can be used simultaneously, and they cannot be reused without an intermediate cleaning step. A much easier and more robust alternative is to encapsulate reactants in drops of a water-in-oil emulsion. Drops are especially useful for biological assays as the individual, picoliter-scale microvessels can contain small numbers of molecules or cells which can nevertheless be at biologically relevant concentrations. Moreover, their small size greatly reduces the volumes of reagents, and thus expense, of screening libraries containing millions of compounds.[3–7] For drops to be truly functional microvessels, however, there must not be any cross-contamination between the drops. For this, it is attractive to use a fluorocarbon oil as the continuous phase, as these oils are both hydrophobic and lipophobic, hence they have low solubility for the biological reagents of the aqueous phase[10–14] and are well suited for inhibiting molecular diffusion between drops.[8] In addition, as compared to hydrocarbon oils, fluorocarbon oils result in less swelling of polydimethylsiloxane (PDMS), a commonly used material for fabricating microfluidic channels.[9] Finally, fluorocarbon oils have good solubility for gases,[7,15,16] which is necessary for the viability of encapsulated cells. However, drops are prone to coalesce; thus, for any drop-based application, surfactants are critical for ensuring that drops are stable. Moreover, surfactants must ensure that biomolecules do not adsorb to the interface.

The surfactants must meet stringent requirements: they must provide stability to the drops, preventing coalescence. In addition, they must produce a biologically inert interior surface for the water drops. These requirements are particularly challenging as the choice of commercially available fluorosurfactants that stabilize water-in-fluorocarbon oil emulsions is limited. Surfactants with short fluorotelomer-tails (typically perfluorinated C_6 to C_{10}) have been used, but do not provide sufficient long-term emulsion stability.[17–24] Fluorosurfactants with longer fluorocarbon tails, such as perfluorinated polyethers (PFPE), offer long-term stabilization even for larger droplets. However, the only available PFPE-based surfactants have ionic headgroups, *e.g.* poly(perfluoropropylene glycol)-carboxylates sold as "Krytox" by DuPont. Their charged headgroups may interact with oppositely charged biomolecules, such as DNA, RNA, and proteins, resulting in the unfolding of their higher-order structure at the drop interface. In many cases, this causes the encapsulated biomolecules to lose their activity.[15] Biological assays thus demand fluorosurfactants with non-ionic headgroups; however, there are currently no such surfactants available.

Here we describe the development of non-ionic fluorosurfactants that address two of the major challenges in performing biological, drop-based assays: these surfactants stabilize

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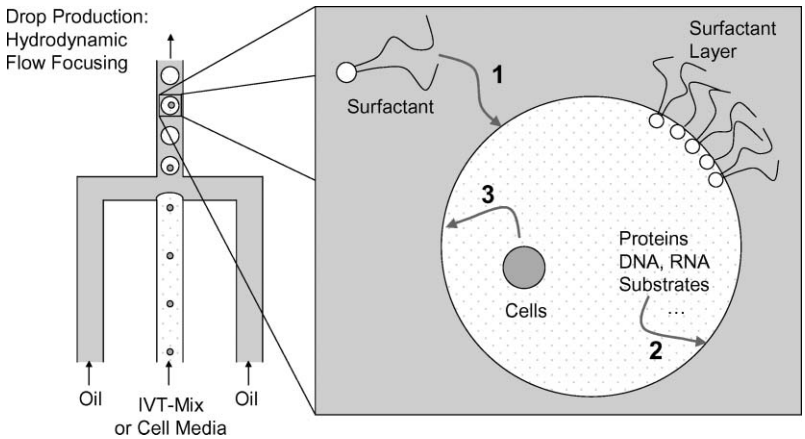

Fig. 1 Drops of a water-in-oil emulsion are used for encapsulation of biological molecules (DNA, RNA, proteins), and/or cells. Left: Drops are generated using a flow focusing geometry in microfluidic devices. Right: Surfactants adsorb to the interface (arrow 1), forming an interfacial surfactant layer. The surfactant layer stabilizes the emulsion and prevents the adsorption of biomolecules and cells to the interface (arrows 2 and 3). Tuning the molecular structure and composition of the surfactant is critical for functional drop-based biological assays. This schematic representation is not to scale.

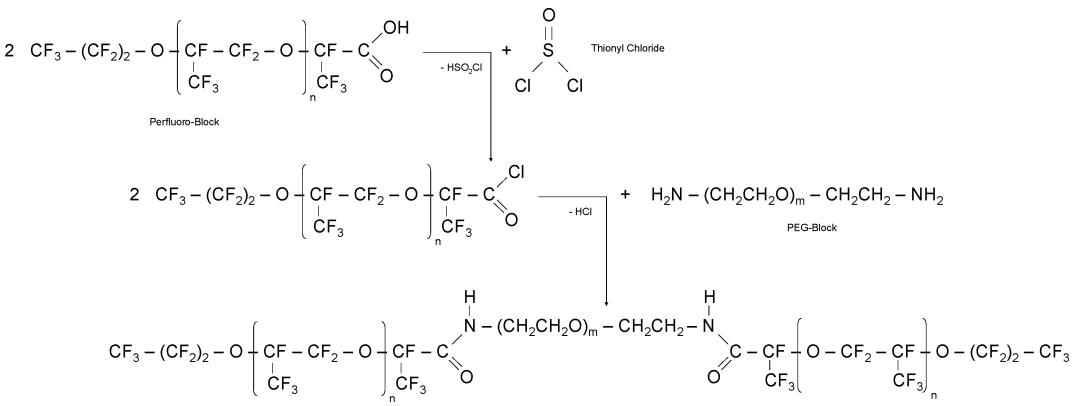

Fig. 2 Synthesis of PFPE-PEG block-copolymer surfactants is performed in a two step process: first PFPE-carboxylic acid is converted to the PFPE acid chloride. This then reacts with a primary amine yielding a peptide bond. Using technical grade reagents, the products contain diblock and triblock copolymers.

aqueous droplets in fluorocarbon oils and make the droplets compatible with biological molecules and cells. The PFPE fluorocarbon chosen for the tail (outer block) provides good stabilization of a water-in-fluorocarbon emulsion while the polyethylene glycol (PEG) moieties chosen for the headgroup (inner block) prevent adsorption of biological materials. We show that these surfactants maintain drop stability as well as biocompatability. We demonstrate the utility of these surfactants by performing *in vitro* translation (IVT) and by showing the growth of encapsulated yeast cells. Our results provide evidence that this new class of surfactant enables the use of aqueous drops in fluorocarbon oils for biological assays.

Surfactant design strategy for emulsion stability and biocompatability

The self-assembled layer of surfactant molecules at the interface between fluorocarbon oil and aqueous phases (Fig. 1) largely determines the performance of a water-in-fluorocarbon oil emulsion. In addition to their critical role in emulsion stability, surfactants can prevent the adsorption of biomolecules at the drop interface. For compatibility with both fluorocarbon oils and biological materials, we chose to make PFPE-PEG block-copolymers (Fig. 2). Oligomeric PFPEs are soluble in fluorocarbon oils and are sufficiently large to provide good steric stabilization of the emulsion. The PEG moieties are soluble in water and prevent the adsorption of biological compounds to interfaces.[25–28] For best emulsion stability *and* biocompatability, it is desirable to have a dense PFPE brush on the outside,[20–23] and a dense PEG brush on the inner side of the interface[26]; this can be achieved by tuning surfactant size and geometry.¶ First, the absolute and relative molecular weight of the surfactant blocks can be adjusted. Second, the surfactant morphology can be changed from a diblock to a triblock copolymer structure for improved surface coverage: PFPE-PEG-PFPE triblock copolymers contain two PFPE-tails and thus cover a larger area on the fluorocarbon side of the interface than a single-tail surfactant. After the formation of a densely packed interfacial layer of diblock and triblock copolymers, small amounts of

¶ Surfactant geometry can be described by the hydrophilic–fluorophilic balance.[45]

PEG-ammonium PFPE-carboxylate ion pairs contained in the product may dissociate and diffuse apart. The use of mixed surfactant systems is known to improve the colloidal stability of emulsions.[20] In addition to these criteria, our experimental requirements guide the design of effective surfactants.

Experimental requirements for surfactant design

Drop formation

Drops of water-in-fluorocarbon oil are typically formed at rates of 1–10 kHz. Their stability requires that the surfactants reach the drop interface rapidly enough to prevent coalescence upon drop collision. Therefore, both the surfactant mobility and the number of mobile surfactant molecules must be sufficient to stabilize newly formed drops. Surfactants must be mobile enough in the oil phase to diffuse to the drop interface on the timescale of drop formation (Fig. 1, arrow 1). The absolute size of the surfactant molecule determines its diffusivity, therefore its molecular weight must not be too large. An additional limitation is imposed by the critical micelle concentration (CMC) which determines the number of mobile surfactant molecules:[20,21] below CMC, all surfactant molecules are freely dissolved in the continuous phase as unimers which are mobile and can diffuse to the drop interface. Upon increasing the surfactant concentration above the CMC, however, the additional surfactant molecules self-assemble into micellar aggregates, while the concentration of free surfactant unimers remains roughly that of the CMC. Appreciable diffusion to the droplet interface is possible only for surfactant unimers; above the CMC, surfactant micelles have to dissolve into unimers in order to be transported to the interface, and this becomes the rate-limiting step. Thus, increasing the concentration of surfactant above the CMC does not necessarily increase the number of mobile surfactant molecules. For sufficiently fast adsorption of block-copolymer surfactants it is thus desirable to have a CMC that is on the order of 10^{-4} mol L^{-1} or greater.

In order to tune the CMC a number of factors are important: one is the choice of oil. More important are the absolute molecular weight of the PEG-moiety,[29] and the surfactant geometry which is determined by both the relative molecular weight of the surfactant moieties and its morphology. A higher CMC can be achieved with shorter PEG-moieties, longer PFPE-moieties, and triblock morphologies. However, if a surfactant lowers the interfacial tension too much, droplet formation in microfluidic devices may not be possible.[15,30]

Incubation

During many biological drop-based experiments, drops must be incubated for the length of time required for the assay, which can be on the order of hours or days. For incubation, drops can either be collected off chip or stored in a PDMS channel. Drop storage can pose a challenge to emulsion stability as the drops remain close packed during the incubation period. Drops of water in fluorocarbon cream due to the large density mismatch between the water and the oil, $\rho_{oil}/\rho_{aq} \sim 1.8$. The volume fraction of the emulsion thus progressively increases during incubation as the fluorocarbon oil is drained from the droplets. In the creamed state, emulsion stability against coalescence depends on the stability of the film of fluorocarbon oil separating neighboring droplets. Surfactants that pack well at the interface and have large, high molecular weight PFPE-moieties[20–23] can maintain a sufficiently thick fluorocarbon oil layer (on the order of 10 nm) between the drops that helps stabilize the oil film. Fluorocarbon oils in which the PFPE-blocks[21] are well dissolved provide the best stability against coalescence.

Reinjection

One of the most severe tests of emulsion stability occurs when the drops are processed after they are formed. In particular, after incubation droplets are often reinjected into a second microfluidic device for further analysis or processing. During reinjection compressed emulsions, with only a thin layer of oil separating the droplets, are flowed into a microchannel, and are thereby subject to large shear forces. Additional shear stress may be exerted on the drop interface by oil flowing in from side channels to space out the drops.[31] Droplet stability under shear depends on the surfactant layer's ability to resist lateral displacement, as this can lead to bare patches at the droplet interface and thus to coalescence.[21,22] Surfactants that form a densely packed interfacial layer will perform best under these conditions.[21,22] Moreover, the surfactant molecules must be sufficiently anchored to the interface so that they do not desorb from the interface under shear and convection. Triblock copolymers have a greater interfacial anchoring strength than diblock copolymers of the same geometry; the presence of even small amounts of such double-tail morphologies in a surfactant system is thus a promising route to improved emulsion stability. Moreover, surfactants with high molecular weight PFPE blocks are favorable as their thick sterically stabilizing brushes are likely to cover any arising bare patches on the outer interface.[22] Due to their relatively large molecular weights, block copolymers are more likely to meet the criteria for emulsion stability than low molecular weight surfactants.

Results and discussion

I. Surfactant synthesis

To determine a reasonable initial molecular weight ratio for the surfactant, we estimate the molar volumes of PFPE and PEG assuming they are dissolved in fluorocarbon oil and water respectively. Additional constraints on the lower and uppper bounds of the absolute molecular weights are imposed by the requirements for a dense outer brush as well as surfactant mobility and geometry. The PFPE blocks must be sufficiently large for good steric stabilization, but must still be able to diffuse to the surfactant interface on the required timescales of milliseconds. By comparison to analogous hydrocarbon-based surfactants,[20] we choose the PFPE blocks to be no smaller than about 10 ether units, and no larger than 60 units [1500–10 000 g mol^{-1}]. Note that the largest blocks we tested are sold as 8000 g mol^{-1}, however, ^{19}F-NMR-analysis confirms a molecular weight of only 6000 g mol^{-1} (see ESI†). While the PEG moieties function to make the surfactant biocompatible, they also help to anchor the surfactant to the interface. The molecular weight of the PEG-moiety must therefore be large enough to provide sufficient anchoring strength. but must nonetheless remain slightly smaller

than the outer PFPE block to maintain a surfactant geometry that opposes neck formation which can lead to coalescence. For these reasons, we choose to use PEG oligomers of at least four units and no more than 20 ethyleneoxide units [200–1000 g mol^{-1}]. Using commercially available reagents, we synthesize block-copolymer surfactants of various absolute and relative molecular weights (PEG 200, 400, 600, 1000 g mol^{-1} and PFPE 1890, 4000, 6000 g mol^{-1}) by coupling PFPE moieties to the biocompatible PEG with a stable peptide bond. The synthesis yields a surfactant mixture containing triblock and diblock copolymers as well as ion pairs of PFPE carboxylate and difunctional PEG-ammonium ions. The application of mixed surfactants is advantageous for interfacial packing[20] and is likely to be essential for the performance of the surfactant system.

To assess the ability of the synthesized surfactants to stabilize emulsions, we dissolve equal weights of surfactant product in fluorinated oil and prepare water-in-fluorocarbon oil emulsions by shaking at room temperature. We find that emulsions of pure water or buffer solution in fluorocarbon oil are sufficiently stabilized by most of the tested surfactants, even at elevated temperature. However, as the combination of BSA and increased temperature can destabilize emulsions, we also perform a more rigorous test using aqueous solutions of 3% BSA as the dispersed phase and incubating the emulsions at an elevated temperature of 37 °C. We observe this destabilization in particular for surfactants with small PEG moieties of 200 g mol^{-1}. The addition of a PEG-rich fluorosurfactant with a short perfluoroalkyl chain, such as Zonyl FSO or Zonyl FSN by DuPont, counteracts this destabilization. Surfactants with larger PEG blocks stabilize these emulsions alone. This suggests that BSA competes for the interface with the fluorosurfactant and will displace it if the aqueous side of the interface is not sufficiently covered with PEG. We investigate emulsion stability over the course of 24 h. The formation of droplets visible by eye indicates that the emulsion is not stable. In contrast, stable emulsions show a dense creamed layer even after a 24 h incubation regardless of the temperature. We choose the most suitable surfactant system for further investigation: the coupling product of 600 g mol^{-1} PEG and 6000 g mol^{-1} PFPE (abbreviated as E2K0660). We find that emulsions stabilized with this surfactants remain stable for weeks.

II. Drop stability

We first test the utility of the surfactant to provide stability upon drop formation. Using hydrodynamic flow focusing,[32] we generate monodisperse drops in PDMS microfluidic devices at rates of 1–30 kHz (Fig. 3a and c, upper chart). Surfactants are necessary to prepare stable emulsions, since drops formed without surfactant coalesce in the microfluidic device and cannot even be collected. Using the E2K0660 surfactant, drops remain stable, even when drops collide immediately after their formation (Fig. 3a and b). By altering the relative flow rates of the oil to the aqueous phases, we can adjust the volume fraction of the aqueous phase, achieving drop formation for volume fractions up to 80% (Fig. 3b(i)). Despite these high volume fractions and the resultant close packing of the drops, we observe no coalescence. This shows that the disjoining pressure[33–35] exerted by the surfactant on the droplet interfaces is sufficient to maintain colloidal stability even shortly after drop formation. Increasing the oil flow rate decreases drop size as illustrated in Fig. 3c, lower chart. Monodisperse drops can be generated and stabilized at rates of up to 30 kHz (Fig. 3c, upper chart).

As a second stringent test of the surfactant, we check the stability of the drops when they are highly compressed. After drops are generated, they are often stored for a length of time, as required for the assay. For many biological assays, the drops are taken off chip and incubated in a vial. Due to the density mismatch of water and fluorocarbon oil of 1/1.8 the droplets cream, resulting in their compression. Assuming a height of the creamed emulsion is 0.03 m, the maximum gravitational compression pressure at the top is about 250 Pa. Alternatively the drops can be stored in PDMS channels since PDMS is permeable to gases[36] and such devices are easily transported to and from an incubator. In this case, to test as many drops as possible, they are typically compressed by draining the oil, presenting a challenging test for the stabilizing ability of the surfactant. After a three-day incubation on-chip in the channel of a PDMS microfluidic device, compressed drops remain stable and do not exhibit coalescence, as shown in Fig. 4. Despite the drops being close packed, they remain monodisperse, as shown by their hexagonal packing. These observations confirm good steric stabilization of the drops by the surfactant. Drops incubated off-chip also remain stable and retain their monodisperse size distribution (Fig. 5 and 6).

As a third stringent test for stability we reinject droplets that were stored off chip into a microfluidic device. For reinjection, we flow the drops through a flow-focusing device and they become more widely spaced as fluorocarbon oil flows through feed-channels on either side of the reinjection channel (Fig. 5). The shear associated with the acceleration of the droplets will deform them, given a typical droplet size of 25–30 μm and an interfacial tension of about 3 mN/m. Deformation can result in coalescence, if the droplets are not sufficiently stabilized by surfactants.[31] Nevertheless, the drops again remain intact and monodisperse. This confirms that the surfactant also provides stability under conditions of shear.

III. Biocompatibility

Biocompatibility of the inner interface of the water drop is an additional challenging requirement for the surfactant. To test for biocompatability of the drop interface, we perform *in vitro* translation (IVT) of plasmid DNA encoding the enzyme β-galactosidase in drops. This assay is sensitive to surfactant biocompatability since a fluorescent product is formed when the encapsulated DNA, the molecules involved in transcription and translation, and the translated protein do not adsorb to the drop interface and the higher-order structure of the protein remains intact. To test this, we encapsulate plasmid DNA encoding the beta-galactosidase enzyme in drops together with an IVT extract, which contains the components required for transcription and translation into the protein, as well as a fluorogenic substrate, fluorescein di-β-D-galactopyranoside (FDG), which itself is not fluorescent. The resulting active beta-galactosidase hydrolyzes FDG and yields a fluorescent product, fluorescein. We generate IVT drops, collect, and store them off-chip at 37 °C for 2 h. After this time, we image the drops by fluorescence

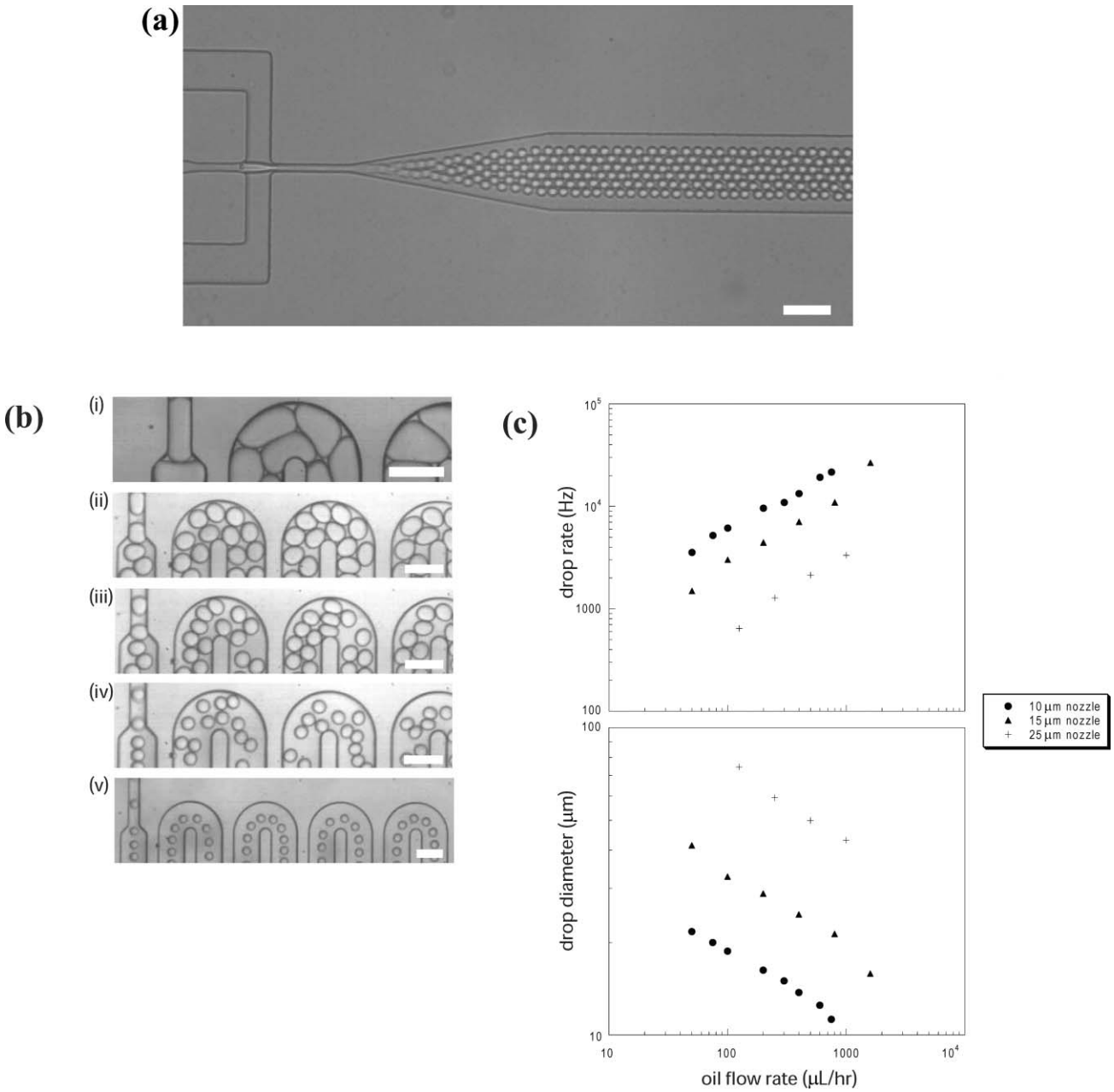

Fig. 3 Drop stability during formation. (a) Drops are generated by flow-focusing from a 25 μm nozzle. Aqueous phase is water, oil phase is R with 1.8% E2K0660 surfactant. Scale, 100 μm. (b) By changing the relative flow rates of oil and aqueous phases, we generate emulsions having different volume fractions of aqueous phase. Even with high volume fractions of up to 80% or at high production frequency up to 30 kHz, drops remain stable despite interfacial contact immediately after drop formation. Volume fractions of (i) 80%, Scale, 25 μm; (ii) 67%, Scale, 15 μm; (iii) 50%, Scale, 15 μm; (iv) 33%, Scale, 15 μm; and (v) 20%, Scale, 10 μm. (c) Drop monodispersity is maintained for a range of drop-making frequencies and sizes, as shown here in the plots of drop production frequency and drop diameter as a function of oil flow rate at constant flow rate of the water phase.

microscopy and observe monodisperse bright green fluorescent drops in the presence of DNA template (Fig. 6). The differences in fluorescence intensity are attributed to concentration fluctuations in the droplet composition during their formation. Using different microfluidic geometries and better pumps can considerably reduce these fluctuations. Drops without DNA template are not fluorescent (not shown). Similarly, IVT is not possible when using ionic fluorosurfactants for emulsion stabilization; no fluorescent products evolve due to protein adsorption at the drop interfaces (data not shown).[15] These results demonstrate that our surfactants stabilize emulsions for storage off-chip and that they can be used for sensitive biochemical assays such as IVT. This biocompatability may be attributed to the PEG-block of our non-ionic fluorosurfactants that provides sufficient protection against the adsorption of biomolecules.

To test for compatibility with cells, we encapsulate an aqueous dispersion of yeast cells in growth medium in drops. Then we incubate the emulsion overnight. Cells remain mobile in the center of the drop, and do not adhere to the interface. Moreover,

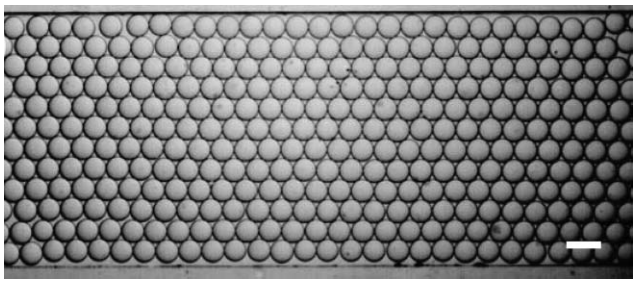

Fig. 4 Drops remain stable and do not coalesce throughout incubation. After incubation for 3 days in the channel of a microfluidic device, drops remain monodisperse. Aqueous phase is cell media, oil phase is FC40 with 1.8% E2K0660 surfactant (w/w). Scale, 100 μm.

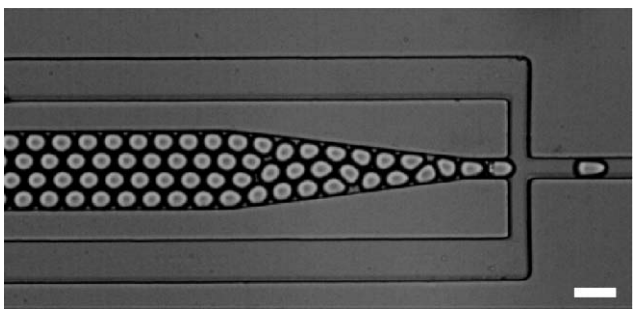

Fig. 5 Drops remain stable during reinjection. After off chip incubation the droplets are reinjected into a second microfluidic device for further manipulation. The compressed emulsion remains stable against drop coalescence and breakup. Scale, 100 μm.

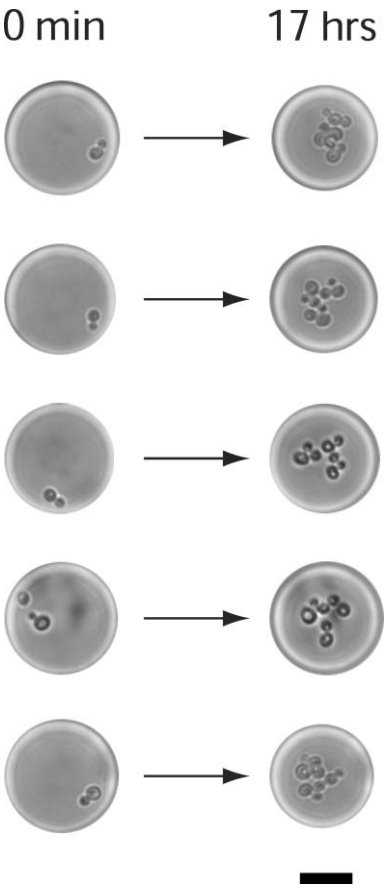

Fig. 7 Surfactants are compatible with cells. The images on the left show yeast cells immediately after encapsulation in 30 μm droplets. During incubation, cells divide and proliferate inside the drops. The images on the right show the same droplets after an incubation time of 17 h. Scale, 25 μm.

encapsulated cells proliferate inside the drops as shown in Fig. 7. Initial doubling times range from 150 to 250 min.[37] These division times are longer than the ~90 min doubling time characteristic of exponential phase growth in bulk, and likely reflect the high density, and slower growth rates, of cells prior to encapsulation.[37] We also use these surfactants to stabilize emulsions for encapsulation and incubation of mammalian hybridoma cells.[38] These cells are more sensitive than yeast to culture conditions, and also remain viable even after storage.

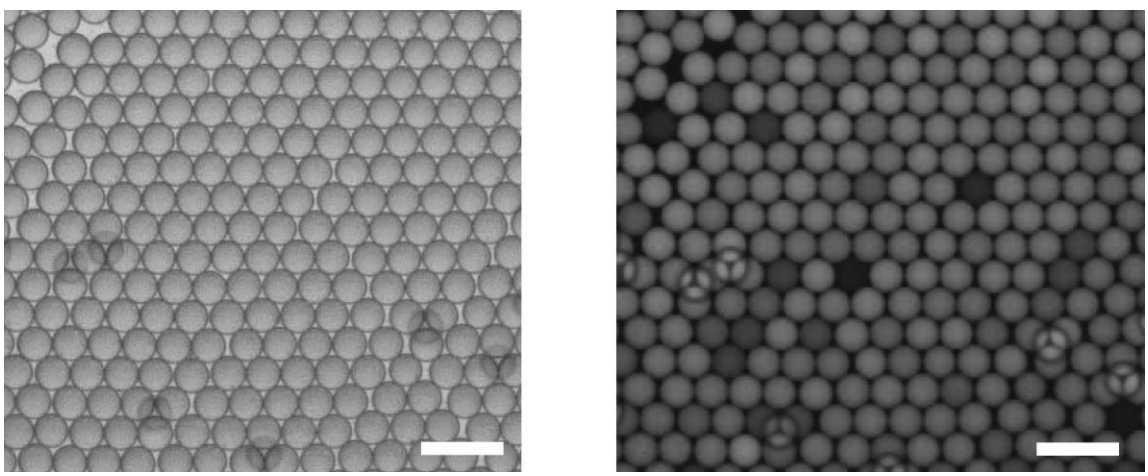

Fig. 6 An emulsion stabilized with non-ionic surfactants remains stable and monodisperse throughout incubation off chip, as shown by the brightfield image (left). It facilitates *in vitro* translation (IVT) as shown by the fluorescence image of these drops (right). We perform IVT on plasmid DNA encoding the enzyme beta-galactosidase encapsulated in droplets together with all the machinery required for transcription and translation into the enzymatically active β-galactosidase protein, as well as the fluorogenic substrate, FDG. Scale, 75 μm.

Conclusions

We have developed a class of novel, non-ionic fluorosurfactants that stabilize aqueous droplets in fluorocarbon oils while maintaining compatibility with biological systems. These surfactants enable picoliter-scale drops of a water-in-oil emulsion to be used as individual compartments for IVT as well as for assays with living cells. In combination with microfluidic tools, water-in-fluorocarbon oil drops offer unprecedented speed and control for high-throughput analysis ranging from *in vitro* biochemistry to single cell studies.

Experimental

Surfactant synthesis

We purchase the Krytox with carboxylic acid functionality from Miller-Stephenson (Danbury, CT). The brand name Krytox refers to poly(perfluoropropylene glycol) (see Fig. 2). Tomah generously provided the PEG reactants with molecular weights of 200, 400, and 1000 g mol^{-1}, which contain 75% diamine and 25% monoamine. We synthesize PEG-diamine with a molecular weight of 600 g mol^{-1} from PEG-diol by a three-step procedure. Briefly, we activate PEG-diol with tosyl chloride. Then we convert the tosylated PEG to phthalimide-terminated PEG. Finally, addition of hydrazine forms the PEG-diamine with 95% or higher conversion of alcohol to amine.[39] We confirm the PEG-diamine product and purity by ^{1}H NMR (results not shown). Bifunctional PEG-amines yield a PFPE-PEG-PFPE triblock copolymer, while monofunctional amines yield diblocks.

We synthesize the fluorosurfactants in a two step process. In the first step, we convert Krytox-carboxylic acid to the acid chloride, as described previously.[40,41] In the second step, we react a small excess of PEG-diamine with Krytox-acid chloride to form the triblock copolymer surfactant with amide bonds between Krytox and PEG. The synthesis is analogous to esterification or amidation procedures that others have described previously.[40,41] ^{19}F and ^{1}H NMR analysis of the final product indicates about 80% conversion to the non-ionic surfactant (see ESI†). We use the surfactant without further purification. We refer to the coupling product of 600 g mol^{-1} PEG and 6000 g mol^{-1} PFPE as E2K0660.

Microfluidic device fabrication

We generate designs for the microfluidic devices in AutoCad and print them at high-resolution (20 000 dpi) on transparencies (Cad/ART Services, Bandon, OR). We use soft lithography to create polydimethylsiloxane (PDMS) devices.[42,43] In brief, we spincoat SU8 2025 photoresist (MicroChem, Newton, MA) onto a silicon wafer (rinsed with methanol and prebaked for 10 min at 210 °C) to a final thickness of 25 μm following the protocol described by the manufacturer. We place the mask on top of the wafer and expose it to UV light (200–250 mJ, OAI, San Jose, CA) to crosslink the exposed pattern; we then remove the non-exposed photoresist using propylene glycol monomethyl ether acetate (PGMEA). We mix PDMS with crosslinker at a ratio of 10:1 (Sylgard 184 Silicone Elastomer, Dow Corning, Midland, MI), and pour it onto the master. We place the devices in a vacuum to remove air bubbles for at least 5 min before baking at 65 °C for 1 h. After baking, we use a biopsy punch (0.75 mm diameter, Harris Uni-Core, Ted Pella Inc., Redding, CA) to punch entry and exit holes in the PDMS. We then treat the PDMS with oxygen plasma and bind it to a glass slide (1.2 mm thickness). Before use, we treat the channels with Aquapel (PPG Industries Inc., Pittsburgh, PA) followed by a flush with air to ensure that the continuous oil phase, and not the aqueous phase, wets the surface.

Device operation

As the fluorinated oil we use either FC-40 (Sigma), a mix of perfluoro tri-n-butyl amine with di(perfluoro(n-butyl))perfluoromethyl amine, or R (RainDance Technologies, Lexington, MA), an oil of proprietary composition. In the oil we dissolve 1.8% (wt/wt) E2K0660 surfactant. We load the oil phase and the aqueous phase (cell suspension or IVT mix) into separate Hamilton glass or 1 mL plastic syringes equipped with $27\frac{1}{2}$ gauge Luer-lok needles (Becton Dickinson, Sparks, MD). We invert the syringes to remove all air bubbles. We then connect needles to polyethylene tubing (Intramedic PE-20, Becton Dickinson, Sparks, MD). For collection and reinjection experiments, we use PEEK tubing and connectors (Vici, Baton Rouge, LA). We generate drops of 25–30 μm diameter using a flow focusing geometry with a 20 μm nozzle width. We use syringe pumps (New Era Pump Systems, Wantagh, NY) to control the fluid flow. To generate drops of water in oil emulsions, flow rates are typically 300–500 μL h^{-1} for the oil and 100 μL h^{-1} for the water. The aqueous phase is either IVT mix, or cell media [Dulbecco's Modified Eagle Medium (DMEM)] with 4.5 g L^{-1} glucose, L-glutamine, and sodium pyruvate (Mediatec, Inc. Herndon, VA) supplemented with 10% (v/v) fetal bovine serum (FBS, SAFC Biosciences, Lenexa KS) and 1% (v/v) Penicillin/Streptomycin], yeast cells and yeast-peptone-dextrose (YPD) media.

Yeast cell culture

We culture yeast cells (*S. cerevisiae*) in YPD at 30°C to a density of OD 600–0.188 (1 mm path length, NanoDrop ND-1000, Wilmington, DE). Gentle agitation provided by a small magnetic stirbar placed inside the yeast syringe prevents the yeast cells from settling to the bottom of the syringe during the injection period; we place a magnetic stirplate beside the syringe pump and the syringe containing the yeast cells.

In vitro translation

The template DNA, plasmid pIVEX2.2EM-LacZ,[44] is a gift of Andrew Griffiths. BSA and lambda-BstEII act as blocking agents to prevent non-specific adsorption of the extremely low concentration of template DNA and other components to the test-tube, tubing, and PDMS channel walls. The final reaction mixture in the droplets contains a 0.7 × dilution of EcoProT7 IVT extract (Novagen), 0.2 mM Methionine, 0.5% BSA, 5 ng μL^{-1} lambda-BstEII digest (New England Biolabs, Ipswich, MA), 0.2 mM FDG (Marker Gene Technologies, Inc., Eugene, OR), 60 pM plasmid DNA (~300 genes per 8.2 pL droplet). We mix all the components of the reaction mixture

except the extract and load them into one syringe, and we load the extract into a second syringe. We inject the two aqueous streams into separate inlets of the device in a 3:7 ratio to give the proper final concentration in the drops. The reaction starts as the two solutions are mixed at the point of drop formation. We incubate the collected drops at ambient pressure and at 37 °C for 2 h and then image them to assay the production of fluorescein from FDG hydrolysis.

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