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A biocompatible surfactant film for stable microfluidic droplets

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Droplets serve as practical compartments for the analysis of individual biological species like nucleic acids and single cells due to the small size and ease of production of droplets. However, coalescence among droplets is a persistent challenge that often precludes the application of droplet-based techniques, particularly in cases when droplets are subject to harsh conditions or must remain stable for extended periods of time. Here, we introduce a versatile film-forming surfactant that forms robustly stable droplets. The film is formed at the droplet interface through covalent interactions between a custom polymer in a fluorinated phase and a diol-containing macromolecule in an aqueous phase. The film can stabilize droplets during polymerase chain reaction (PCR) and is biocompatible. The surfactant provides an archetype for new surfactant chemistries employing random copolymers and interfacial association.

1. Introduction

Aqueous droplets dispersed in fluorinated oils and stabilized by surfactants can serve as inert and versatile compartments for the isolation and analysis of individual nucleic acid molecules and single cells.[1] Compartmentalization in droplets can enable applications such as disease diagnosis from trace samples in blood,[2] analysis of products secreted by individual cells,[3] directed evolution of enzymes,[4] and investigations of differences in gene expression among individual members of cell populations.[5] Many of these applications rely on the polymerase chain reaction (PCR) for the amplification of nucleic acid molecules in droplets. In droplet-based PCR, single nucleic acid molecules contained within droplets are amplified, enabling highly sensitive and quantitative detection of DNA.[6] However, the elevated temperatures and repetitive cycles of heating and cooling required in PCR can destabilize the droplet interface and lead to the coalescence of droplets, which limits the reproducibility and resolution of droplet-based methods due to the uncontrolled mixing of contents previously isolated within individual droplets.

Fluorinated oils commonly serve as the continuous phase in droplet-based biological experiments because they are poor solvents of hydrophilic and lipophilic molecules and thereby reduce the transfer of biological contents among droplets.[7] Fluorinated oils also allow for the diffusion of gases required for the viability of encapsulated cells and are compatible with the polydimethylsiloxane (PDMS) microfluidic devices commonly used to generate droplets.[8,9] Partially fluorinated surfactants that assemble at the interface between aqueous droplets and surrounding fluorinated oils can provide a steric barrier to droplet coalescence. These so-called fluorosurfactants, in polymeric or small-molecule form, are often used to stabilize droplets in droplet-based microfluidics.

Substantial effort has been devoted to the design of surfactants that both stabilize droplets in fluorinated oils and are PCR- and cell-compatible. Tri-block copolymer fluorosurfactants, like FSH_2-Jeffamine,[10] poly(methyl glycerol)-perfluoropolyether,[11] and commercially available $PEG(PFPE)_2$,[3,4] as well as di-block copolymers featuring low molecular weight PFPE chains and a dendritic tri-glycerol moiety,[12] have been reported to offer improved droplet stability during PCR; however the ability of the surfactants to limit coalescence needs to be further improved.[13] Fluorosurfactants with charged headgroups, like Krytox, can stabilize droplets but are known to interfere with enzymatic and biological processes.[14] Though polymer species can be included in the aqueous phase to passivate the charged headgroups,[15] Krytox remains little used as a surfactant in biological applications, and surfactants produced by replacing the charged carboxylate group with a neutral

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moiety[16] generate droplets that can nonetheless be susceptible to coalescence during thermal cycling. As an alternative to surfactants, solid nanoparticles that assemble at the water–oil interface to form Pickering emulsions have been employed to stabilize droplets. Such droplets can be used for cell growth and enzymatic reactions in droplets,[17–19] but slow nanoparticle diffusion can limit the droplet generation rate,[20] it can be difficult to release the contents of droplets,[21] reductions in leakage and cross-talk are limited to specific situations,[22] and the emulsion remains thermodynamically susceptible to coalescence. A promising alternative approach to stabilize droplets is the formation of an interfacial elastic film.[15,23,24] Films are formed through strong interactions, such as chemical bonds, among molecules at the surface of a droplet. These chemical bonds in the interfacial film must be disrupted for droplets to merge and thus present a more substantial barrier to droplet coalescence than the supramolecular interactions of traditional adsorbed surfactants. While examples of film-stabilized systems for aqueous droplets in non-fluorinated oils are abundant,[25] film-forming systems in fluorinated oils are rare, despite their promise for improving biological analysis in droplets. Thus, there is a need for a versatile, biocompatible film-forming system capable of reliably stabilizing droplets without inhibiting their microfluidic formation due to fast precipitation and device clogging. Such a surfactant would extend the application of droplet-based techniques and make droplet-based biological assays more reliable and reproducible.

Here we report a versatile film-forming surfactant system for fluorinated oils that is biocompatible, with little adverse effect on both enzymatic and cellular processes over relevant time periods of hours to days, and can be used to produce exceptionally stable droplets for a wide range of droplet-based biological applications. The film results from covalent interactions between a custom polymer in the fluorinated phase and a diol-containing macromolecule in the aqueous phase. We demonstrate the capabilities of the surfactant system in PCR, where no droplet merging is observed, and in cell incubation, where encapsulated cells exhibit comparable viability to those in droplets stabilized by commercially available fluorosurfactant.

2. Results and discussion

2.1 Design of the fluorophilic copolymer for interfacial association

We form an interfacial film at the surface of droplets through covalent interactions, specifically the formation of a boronic ester, between a custom copolymer in a fluorinated continuous phase and a diol-containing polymer or poly-ol in an aqueous drop phase. We synthesize the custom polymer by the widely accessible free-radical copolymerization of boronic-acid-appended acrylic monomers at a minority fraction and a fluorinated acrylic monomer at a majority fraction, which enables the dissolution of the polymer in fluorinated oils. Specifically, the monomers *N*-[3-(4,4,5,5-tetramethyl-1,3,2,-dioxaborolan-2-yl)phenyl]acrylamide and 1*H*,1*H*,2*H*,2*H*-heptadecafluorodecyl acrylate are copolymerized in

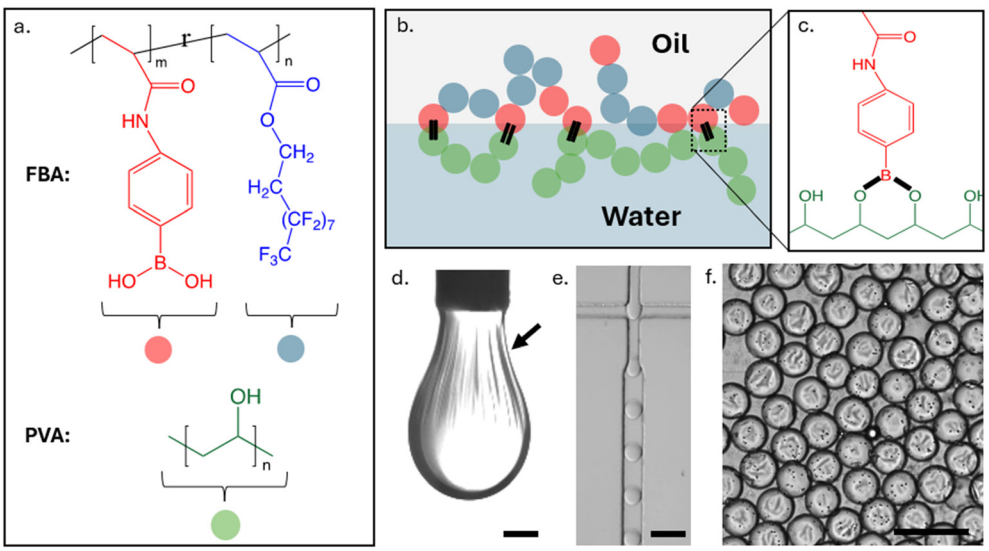

Fig. 1 a) Chemical structure of the fluorinated boronic acid (FBA) copolymer and polyvinyl alcohol (PVA). b) Schematic of the interaction between FBA polymer dispersed in the fluorinated phase and PVA dissolved in the aqueous phase. The association between polymers forms a film at the interface through c) boronic ester bonds. d) A film can be observed when a dispersion of FBA polymer in fluorinated oil is injected into an aqueous solution of PVA. Slight retraction of the fluorinated phase produces visible wrinkling of the film on the surface of the drop. The scale bar is 500 micrometers. e) The film-forming surfactant can be used to produce stabilized droplets in a microfluidic dropmaker. The scale bar is 100 micrometers. f) Evaporation of water reduces the volume of droplets and leads to visible wrinkling on their surfaces, demonstrating the formation and stability of the elastic FBA–PVA interfacial film. The scale bar is 250 micrometers.

dimethylformamide with azobisisobutyronitrile (AIBN) as a thermal radical initiator. Subsequently, the pinacol ester that protects the boronic acid during polymerization is hydrolyzed in hydrochloric acid. The result is a fluorinated boronic acid (FBA) random copolymer, shown in Fig. 1a and S1. The ratio of boronic acid monomer to fluorinated acrylate monomer in the copolymer can be controlled by varying the stoichiometric ratio of the two monomers in the initial reaction. We find that to achieve robust film formation while ensuring good dispersibility of the polymer in the fluorinated solvent, a 1 : 4 molar ratio of boronic acid co-monomer to fluorinated co-monomer in the copolymer is adequate.

2.2 Characterization of the interfacial film

To prepare the FBA copolymer for use as a film-forming surfactant, we disperse the polymer in HFE-7500, a common fluorinated oil, and dissolve polyvinyl alcohol (PVA), the diol-containing component of the film forming system, in water. These polymers react to form covalent bonds at the interface between the oil and aqueous phases. Specifically, the boronic acid of the FBA copolymer bonds with two neighboring hydroxyls of PVA (1,3-diol) to create boronic ester cross-links between the polymers.[26] The FBA copolymers have multiple boronic acid functional groups on a single chain that can interact with multiple 1,3-diols from distinct PVA polymers in the aqueous phase to form a crosslinked polymer network on the surface of droplets, as shown schematically in Fig. 1b and c. This interaction generates an amphiphilic interfacial film between the water and fluorinated oil with a hydrophilic and a fluorophilic side. Once the interfacial polymer network is sufficiently dense, the access of the two polymers to each other at the interface is blocked and film growth stops.

To characterize the film that results from the interaction between the FBA copolymer and PVA at the oil–water interface, we use a pendant drop setup to image a drop formed by injecting a dispersion of FBA copolymer in HFE-7500 into an aqueous solution of PVA.[27] We form a drop at the tip of the blunt needle and wait several minutes for a film to form. Subsequent retraction of the FBA solution back into the needle results in visible wrinkling at the surface of the drop, demonstrating the formation of an elastic interfacial film (Fig. 1d). We note that the formation of an initial film occurs within seconds, which is further supported by transient interfacial rheological measurements that also prove the conclusion that the interfacial film is elastic (Fig. S2).

The curvature of the drop in a pendant drop setup is determined by the balance between gravitational force and surface tension. Thus, by measuring the curvature of the interface, pendant drop is commonly used to quantify the surface tension between liquids of various compositions. We find that the surface tension is already lowered when only the FBA or the PVA are present, with a more substantial decrease in surface tension due to the PVA. In the presence of both polymers in their respective phases, the surface energy is lowered more than four-fold. We note that the surface tension measured upon FBA–PVA film formation has to be regarded as an effective surface tension, since it is calculated from the curvature of the drop while the interfacial film grows. The results of surface tension measurements are shown in Table 1.

2.3 Generating droplets with the FBA–PVA surfactant system

We use the interfacial association between the FBA copolymer and PVA and the resulting amphiphilic film to stabilize droplets during generation in a microfluidic device. We use a co-flow dropmaker in the dripping regime where the perpendicular oil flow pinches off the aqueous stream to form droplets,[28] as depicted in Fig. 1e. When using an aqueous stream of 2 wt% PVA and an oil stream of 3 wt% FBA copolymer, homogeneous droplets are formed that do not coalesce during collection, indicating that stabilization with the interfacial film is achieved within a second or less (residence time in the microfluidic dropmaker). Importantly, when we omit the FBA copolymer, droplets quickly merge into a single separated water phase upon collection despite the low surface tension afforded by PVA, indicating the importance of the copolymer in the oil phase to droplet stability. This observation is in stark contrast to emulsions of water and non-fluorinated oils that can be stabilized with PVA alone. We speculate that for water/fluorinated oil interfaces, the presence of PVA is restricted to the inner water side of the droplet interface and does not provide steric hinderance towards the coalescence of droplets. In fact, we find that water-in-fluorinated oil drops are stable for at least 24 hours of incubation at PVA and FBA concentrations as low as 0.5 wt% and 1.2 wt%, respectively, even after cleaning of the continuous phase with pure HFE-7500 following drop making (Fig. S4). The droplets remain intact during both pipetting and washing. To further confirm the presence of an elastic film around the aqueous droplets formed using PVA and FBA copolymer, we allow water to evaporate from the droplets, decreasing the droplet volume. The observed

Table 1 Surface tension between various water/HFE-7500 compositions

Interface	Surface tension (mN m^{-1})
2 wt% PVA in water/3 wt% FBA copolymer in HFE-7500	10
2 wt% PVA in water/HFE-7500	14
Water/3 wt% FBA copolymer in HFE-7500	29
Water/HFE-7500	42

wrinkling of the interface, as shown in Fig. 1f, reaffirms the presence of the elastic film. Histograms of droplet diameters under various production and processing conditions are provided in Fig. S3. Our FBA-PVA associative surfactant system can also be used to generate oil-in-water droplets, as shown in Fig. S5.

We note that for an interfacial film to form, both FBA and PVA polymers must diffuse to the oil–water interface. In the dripping regime, a new interface is formed with every drop and we can operate devices for over an hour without clogging or adverse wetting of channels. However, when we increase the aqueous flow rate to operate the device in a jetting regime, the resulting fluid jet creates a persistent interface between the oil and aqueous phases. The persistence of this interface leads to the formation of a film that stabilizes the jet. Jetting then proceeds further downstream, ultimately progressing until a film-stabilized tube extends to the outlet of the device. Operating devices in the dripping regime is thus critical to the effective production of droplets. Once droplets form, the FBA copolymer system rapidly stabilizes the interface. We observe no merging of droplets in the outlets of devices, and droplets can resist coalescence shortly after formation even under substantial deformations, as depicted in the outlet channels of Fig. S8. While we do not observe merging as a result of contact between droplets, we do find that droplets can weakly adhere to one another when they are in close contact for several hours. We hypothesize that this interaction is due to polymer chains that span droplets. Gentle pipetting is sufficient to disrupt this interaction, without compromising the integrity of the individual droplets.

2.4 Performing PCR in droplets

To demonstrate the suitability of our surfactant system for biomolecular processes, we perform PCR in droplets using an aqueous mixture containing PCR reagents, PVA, and a fluorescent TaqMan probe that reports the amplification of the DNA template. The fluorescent probe is a modified DNA oligo that generates a fluorescent signal in proportion to the extent of DNA amplification. We prepare two samples of droplets stabilized by our surfactant system. In one sample, we include 137 base pair (bp) DNA template and in the other we omit the template to serve as a control. We thermally cycle the droplets to perform PCR, performing 35 cycles with a maximum temperature of 95 °C. Droplets stabilized using traditional block copolymer surfactant and subjected to the same cycling conditions merge extensively during this process. We characterize the film-stabilized droplets using fluorescence microscopy. Droplets prepared without template exhibit only low background fluorescence, while droplets prepared with DNA template are highly fluorescent, as shown in Fig. 2a and b respectively. Droplets from a sample containing an equal mixture of droplets with and without template exhibit a binary distribution of either high or low fluorescence after cycling, as shown in Fig. 2c. We postulate that the elastic crosslinked interfacial film and the electrostatic repulsion with the negatively charged boronic ester group ensures that polyanionic genetic material remains isolated within individual droplets. In contrast, rhodamine B, a positively charged dye, can migrate out of and between droplets, as shown in Fig. S6, likely due to their association with FBA. This feature might provide a path to selective transport of molecular reagents to and between droplets in the future.

We perform agarose gel electrophoresis to confirm that the desired PCR product is generated in droplets. We use a commercial DNA purification kit to recover the DNA from the film-stabilized droplets. The initial centrifugation through the silica-based matrix of the purification column shears the droplets and the released DNA binds to the matrix. We elute the sample, electrophorese the collected DNA on an agarose gel, and visualize the result using ultraviolet illumination. We observe a single band, shown in Fig. 2d, that is consistent with the expected length of the amplicon.

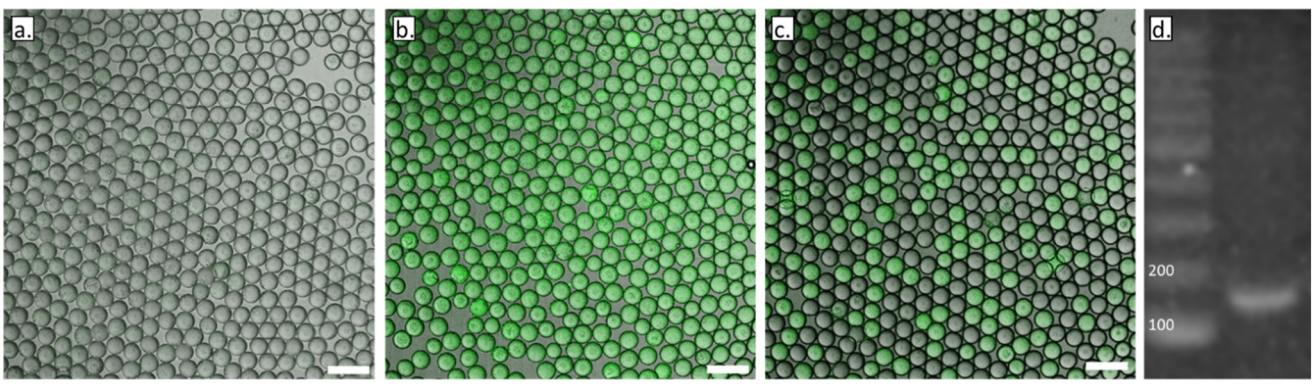

Fig. 2 PCR cycling of droplets a) without DNA template and b) with DNA template. A fluorescent probe indicates the amplification of DNA in the overlaid brightfield and confocal fluorescent micrographs. c) Combining droplets with and without template and cycling indicates selective amplification without observable transfer of material among droplets. d) Gel electrophoresis of amplification product isolated from film-stabilized droplets shows amplification of the targeted DNA (right) next to a DNA ladder (left) with numbers indicating base pairs. Scale bars are 200 micrometers.

2.5 Growing cells in FBA–PVA stabilized droplets

To assess the compatibility of the film-forming surfactant with cells, we compare the viability of mammalian cells in droplets formed using the FBA copolymer system with that of cells encapsulated in droplets using commercially available triblock copolymer surfactant, which is commonly used for cell experiments. We stain mammalian K562 cells with a commercially available live-dead kit, which produces a maximum fluorescent signal at a wavelength of 515 nm when the cell is alive and at 635 nm when the cell is dead, and load the cell into droplets, adjusting flow rates to achieve matching volumes. We incubate the collected droplets at 37 °C, taking samples every 24 hours to image the droplets with a confocal fluorescence microscope (Fig. 3a). For each set of imaged droplets, we count the number of intact live and dead cells in each of 200 droplets to determine the percentage of viable cells. We find that the FBA copolymer surfactant has no immediate cytotoxic effects on encapsulated cells, with 97% of cells viable over the first 24 hours (Fig. 3b). This matches the performance of the commercial surfactant. In both sets of droplets, we see only 50% viability after 48 hours. All cells are dead within 72 hours, suggesting the depletion of nutrient resources in the droplets (Fig. 3b). The stability conferred by the surfactant film also allows for bacterial cells to be incubated, as shown in Fig. S7. The droplets encapsulating the bacterial cells have diameters of over 250 microns. We find that droplets of the same size stabilized by $PEG(PFPE)_2$ surfactant merge during incubation to produce a continuous aqueous phase atop the denser fluorinated phase. Coalescence can be driven by buoyancy forces that press droplets against one another, causing the fluid between them to drain and disrupting stabilizing surfactant layers at their interface. Large droplets are more easily deformed than small droplets, which can increase the contact area between adjacent droplets and consequently, the probability of coalescence. We hypothesize that by maintaining surfactant at the interface between droplets, the cross-linked surfactant film preserves a steric barrier against coalescence.

3. Conclusions

We report an associative polymeric surfactant system that produces remarkably stable water-in-fluorinated oil droplets through the formation of an elastic interfacial film. The film surfactant reliably inhibits droplet coalescence, particularly among droplets exposed to numerous thermal cycles of PCR and prolonged incubations of large microfluidic droplets with diameters over 250 microns. The compatibility of the surfactant with enzymes and cells makes it well suited for a wide variety of droplet-based biological applications that are traditionally difficult to perform because of droplet coalescence. For instance, the stabilization of large droplets, which can be less stable than small drops, can have application in the amplification of RNA by RT-PCR, where cells are typically lysed in droplets and the resulting cellular debris can have inhibitory effects at the high concentrations encountered when using small droplets.[29] The ability to culture cells may also facilitate economical low-volume drug screening based on cell proliferation.[30]

The boronic ester bonds formed by the FBA surfactant system are reversible and could potentially be used to create interfacial film-forming surfactant systems that are stimuli-responsive. The film's mechanical properties, for instance, can be tailored by adjusting the molecular weight of the diol-containing species to achieve capsules whose contents may be released under specified shear stresses. Similarly, the mechanical and chemical properties of the film may be adjustable by changing pH. We perform our experiments with aqueous solutions with pH values in the range of 7 to 8; however, changes in pH can alter the number of boronic ester bonds formed by boronic acid functional groups in equilibrium. This might provide an avenue by which to control film formation and dissolution, and the corresponding capture or release of droplets

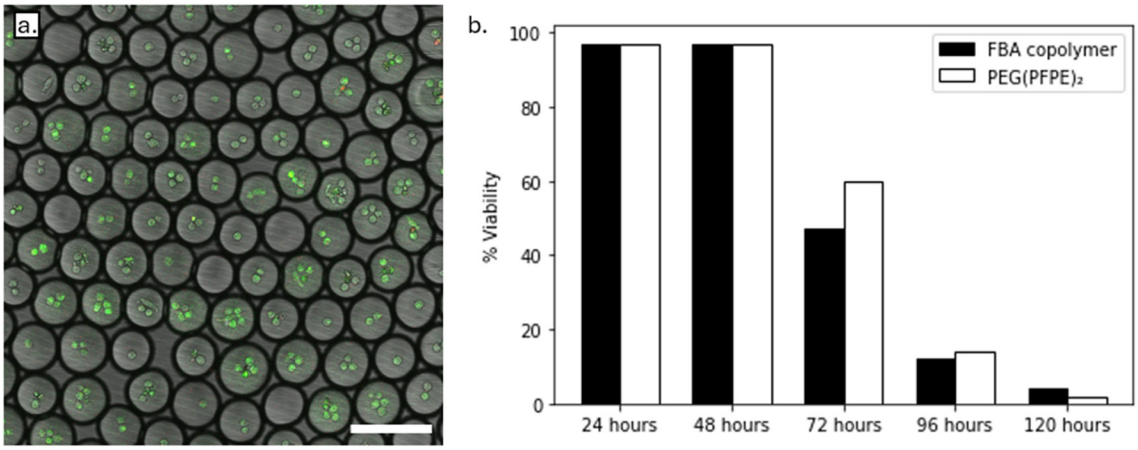

Fig. 3 a) Overlaid brightfield and confocal fluorescence micrograph of stained mammalian cells encapsulated in FBA–PVA film-stabilized droplets. The scale bar is 200 micrometers. b) Cells exhibit similar viability in FBA–PVA-film-stabilized droplets and droplets of the same size stabilized by traditional $PEG(PFPE)_2$ surfactant.

contents. For example, in strong alkaline conditions at pH > 12, boronic esters hydrolyze which would result in uncrosslinking of our amphiphilic interfacial film and potentially controlled release of its cargo.[31] The polymers in the aqueous phase of the interfacial film can also be tailored. Different diol-containing species, including fetal bovine serum (FBS) and dextran can be used in place of PVA, as shown in Fig. S8, and other molecules that interact with boronic acid can also be considered. At sufficiently high concentrations, small molecular weight carbohydrates may also compete with diol-containing polymers for boronic ester bonds formed at the droplet surface, thus offering a possible route to concentration-dependent dissolution of the film.

In general, both the negative charge and boronic-acid functionality of the FBA copolymer, which respectively contribute to the abstraction of positive dyes from droplets and the formation of films with a variety of di- and poly-ol-containing species, are critical to determining the particular implementations and applications for which the polymer is best suited. Fluorescence-based assays in droplets, for instance, are likely to benefit from the use of neutral or negatively-charged dyes. The interaction between FBA copoylmers and polysaccharides, many of which contain multiple tightly spaced hydroxy groups, offers other potential applications. Antibodies, for instance, may be able to be attach to the surface of droplets through the boronic ester bonds formed between the FBA copolymer and the carbohydrates present on antibodies after post-translational glycosylation. Cells expressing high levels of polysaccharides on their surface, like yeast and other fungi, could moreover interact with FBA, possibly offering a means to concentrate or embed cells near the surface of droplets.

Surfactants employing random copolymers like the FBA copolymer may give access to new chemistries with unique characteristics. For example, new chemistries may enable sensing capabilities in complex emulsions, where interactions between analyte molecules and surfactants can alter interfacial geometries to produce optical signatures.[32,33] We expect the FBA copolymer reported here to disperse in a wide variety of fluorinated solvents, including many of the substitutes currently being offered as replacements for HFE-7500. Copolymers with boronic functional groups and co-monomeric sidechains tailored to specific solvent environments such as non-fluorinated oils can further extend our approach and enable the stabilization of droplets in a variety of emulsion systems.[34]

Our FBA copolymer and PVA film-forming surfactant system forms droplets that are extraordinarily stable in PCR and will be of immediate interest to researchers using droplet-based techniques. Further, our work demonstrates that random copolymers can be used for the facile production of functional surfactant films that can be tailored for specific industrial and research applications.

4. Methods and materials

4.1 Copolymer surfactant synthesis

A mixture of azobisisobutyronitrile (AIBN; 0.0301 g, 0.183 mmol, MilliporeSigma), *N*-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]acrylamide (0.2 g, 0.7 mmol, Tokyo Chemical Industry) and 1*H*,1*H*,2*H*,2*H*-heptadecafluorodecyl acrylate (0.927 mL, 2.9 mmol, Tokyo Chemical Industry) in dimethylformamide (3.44 mL, Sigma-Aldrich) is de-aerated by bubbling with nitrogen. The mixture is stirred under inert atmosphere at 70 °C for 48 hours. The solution typically turns turbid within an hour. We wash the product repeatedly in an excess of methanol (MilliporeSigma), centrifuging and decanting the supernatant after each wash. After the final decanting step, we dry the polymer in a vacuum oven at 70 °C overnight. After the polymer has dried, we add it to a 1 M solution of hydrochloric acid (15 mL, MilliporeSigma) and stir overnight at 100 °C under reflux to remove the pinacol protecting group of the boronic acid component of the polymer. Following deprotection, we again wash the polymer in an excess of methanol. After this purification, we dry the polymer overnight in a vacuum oven at 70 °C. Proton-NMR spectra of the protected and deprotected products in deuterated hexafluoroisopropanol taken on a Bruker AVANCE NEO 400 at 400 MHz are shown in Fig. S1 and are used to quantify the composition of the copolymer and successful deprotection.

4.2 Preparation of film components for microfluidics

We disperse the FBA polymer in HFE-7500 (3M, discontinued) at a concentration of 50 mg mL^{-1} by stirring the dried polymer in HFE-7500 in a sealed vial at 55 °C on a hot plate overnight. The next day, we remove the vial from the hot plate to allow the dispersion within the vial to cool and filter the dispersion through a two-micron syringe filter (VWR) and into a collection vial. We measure the concentration of the filtrate by aliquoting 1 mL of the solution to a tared vial, removing the HFE-7500 by evaporation at 60 °C in a vacuum oven, and weighing the solid polymer left behind. We find the concentration of polymer in the dispersion after filtering is ~30 mg mL^{-1}. For the aqueous phase, we dissolve PVA (13–23k, Sigma Aldrich) in water at 2 wt%, stirring until the polymer is fully dissolved.

4.3 Device preparation and operation

We fabricate and operate PDMS microfluidic devices according to methods detailed elsewhere.[3] Briefly, we spincoat SU-8 photoresist (MicroChem) onto a silicon wafer (University Water) and selectively cross-link regions of the photoresist with UV light, using a photomask to template the features. After washing and drying the wafer, we affix it to the bottom of a petri dish (BD Falcon) with Scotch tape and pour mixed PDMS and curing agent (Dow, Sylgard 184) into the dish. We remove air bubbles under vacuum and cure the

mixture overnight. We cut out the resulting PDMS elastomer and peel the slab from the silicon wafer. With a biopsy punch (Ted Pella), we punch holes in the PDMS slab at each fluid inlet or outlet. We then plasma treat the PDMS and a glass substrate and bond the two by pressing them together. We inject Aquapel (Aquapel) into the channels of the device to render them hydrophobic, clear the channels by blowing nitrogen through them, and dry the device in an oven at 65 °C. To form droplets with the device, we attach PE-2 tubing (Intramedic) to syringes containing the aqueous phase or phases and the fluorinated phase and connect the tubing to the appropriate inlets of the device. We attach PE-2 tubing to the outlet of the device for the collection of droplets in an Eppendorf tube. We control the rate at which fluid is injected into the device using syringe pumps (Harvard Apparatus) to avoid jetting.

4.4 PCR in droplets

We generate droplets containing 2 wt% PVA (molecular weight 13–23k, MilliporeSigma), 1× PrimeTIME Gene Expression Master Mix (Integrated DNA Technologies (IDT)), 1× mouse β-actin PCR primers and probe (IDT), and β-actin DNA template at ~1 ng μL^{-1}, in a continuous phase of HFE-7500 with 3 wt% fluorophilic boronic acid copolymer using a microfluidic device and collect the drops in a PCR tube. We produce a second set of drops containing all but the DNA template and collect them in a second PCR tube to serve as a negative control. We take an equal portion of the drops from each of the PCR tubes and combine them in a third PCR tube. We overlay the droplets in each of the tubes with mineral oil (MilliporeSigma) to prevent evaporation and subject the drops to thermocycling at 95 °C for 3 minutes; then 35 cycles of: 95 °C for 10 s, 54 °C for 15 s, and extension at 72 °C for 30 seconds; then hold at 4 °C. After cycling, we add a fraction of each droplet sample to a disposable hemocytometer (Bulldog Bio) and image using fluorescence microscopy.

4.5 Cell preparation

We culture K562 cells in cRPMI cell media (MilliporeSigma) and prepare cells for cell viability monitoring in droplets using a variation of the protocol from Thermo Fisher Scientific's LIVE/DEAD viability/cytotoxicity kit for mammalian cells. Briefly, we pellet cells from culture and stain the cells by resuspending the cells in a solution of calcein AM dye (Thermo Fisher) followed by washing with phosphate buffered saline (PBS; MilliporeSigma) to remove dye that has not been taken up by the cells. We split the cells equally into two volumes and disperse the cells in a solution of Optiprep (MilliporeSigma), ethidium homodimer-1 (Thermo Fisher), and cRPMI, for cells to be loaded into droplets stabilized by $PEG(PFPE)_2$ surfactant (RAN Biotechnologies), or Optiprep, ethidium homodimer-1, and cRPMI with 1 wt% dissolved PVA, for cells to be loaded into droplets stabilized by the interfacial film formed through the interaction of the FBA copolymer and PVA. Optiprep hinders settling of cells during cell loading in the microfluidic device to ensure even loading of droplets, while the ethidium homodimer-1 stains cells red upon cell death. We load cells into droplets using a microfluidic dropmaker. We collect droplets in an Eppendorf tube and add mineral oil to both suppress evaporation of the fluorinated oil and allow oxygen diffusion during incubation. We then incubate the collected dropletsat 37 °C in a cell culture incubator at 95% relative humidity and 5% CO_2. We take a sample of droplets by pipette every 24 hours, add droplets to a disposable hemocytometer (Bulldog Bio) and image using fluorescence microscopy.

Author contributions

JGW and BTD conceptualized the material and emulsion system and devised the methodology. BTD, JAH, and DAW developed the biotechnological methodology. BTD performed most experiments and acquired, analyzed, and curated the data. JGW and DAW administered and supervised the research. RGR performed the bacterial cell experiments. DAW acquired funding. BTD wrote the original draft, JGW and BTD reviewed and edited the final draft of the manuscript. All authors gave approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare. Harvard University filed a patent that is in part based on the results presented here.

Data availability

Supplementary information: Proton-NMR spectra of the FBA copolymer; interfacial rheology data; optical and confocal fluorescent micrographs of droplets with various compositions, aqueous poly-ols, in-drop bacteria growth, and cationic dye migration. See DOI: **https://doi.org/10.1039/D5LC00456J**.

The data supporting this article have been included as part of the SI.

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