Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Surfactant and Oil Formulations for Monodisperse Droplet Emulsion PCR

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Experimental Section

Oil-surfactant mixture preparation

Oil-surfactant mixtures were prepared as follows: A, 4.5% (v/v) Span 80, 0.4% (v/v) Tween 80, and 0.05% (v/v) Triton X-100 in mineral oil. B, 2% (v/v) Abil EM 90 and 0.05% (v/v) Triton X-100 in mineral oil. C, 4% (v/v) Abil EM 90 and 0.05% (v/v) Triton X-100 in mineral oil. D, 3% (w/w) Abil EM 90 and 0.1% (w/w) Triton X-100 in heavy white mineral oil. E, 7% (wt/vol) Abil WE 09, 20% (vol/vol) mineral oil and 73% (vol/vol) diethylhexyl carbonate. F, 7% (v/v) Abil EM 180 in isopropyl palmitate. Span 80, Tween 80, Triton X-100, mineral oil, and heavy white mineral oil were bought from Sigma. Abil EM 90, Abil WE 09, and diethylhexyl carbonate were purchased from Evonik and isopropyl palmitate was purchased from Sigma-Aldrich.

Device setup and droplet generation

The MiCA plate for our experiments was designed with 7 holes. Initially, 7 acid-soluble glass fibers and other insoluble glass fibers were tightly aligned to form a bundle in hexagonal lattice symmetry. The bundle was then melted at high temperature and pulled into a long glass rod with a diameter of 4–5 mm. The glass rod was sliced into thin plates and polished. Each MiCA plate was round with a diameter of 4.5 mm and was 1.0 mm thick.

The fiber-arrayed MiCA plates were then etched in 0.5 M HNO_3 (Sigma-Aldrich) with ultrasonication for 10 h to dissolve the acid-soluble glass fibers leaving 7 through-holes. The plates were then washed with piranha solution (H_2SO_4 and H_2O_2 from Sigma-Aldrich) for 30 min and then water several times until the residual solutions were flushed away. A vacuum drying oven was used to dry the plates. The dried MiCA plates then underwent hydrophobic treatment when exposed to 1H,1H,2H,2H-perfluoro-octyltrichlorosilane (PFOTCS, Sigma-Aldrich) vapor for 1 h. The treated MiCA plates were placed on a hot-plate set to 120 °C overnight.

The MiCA plate and a PTFE gasket were placed in a holder made of PEEK and screwed in tightly to avoid leakage, giving the MiCA emulsifier. When aqueous solution (20 µL PCR buffer, NEB) was transferred to the sample reservoir of the MiCA and centrifugal force was applied (13,000 g) (centrifuge 5430 R, Eppendorf), 52 µm-diameter droplets were formed in 8 min with zero dead volume and collected into 1.5 mL-microcentrifuge tubes (Eppendorf) containing 1 mL of oil-surfactant mixture. After collection, the droplets were thermo-cycled with a normal PCR machine (Biometra) as follows: 10 min at 25 °C – surfactant encapsulation, 2 min at 95 °C – hotstart initiation, 30× or 40× (15 s at 94 °C, 30 s at 57 °C, 30 s at 70 °C) – amplification. After PCR, the droplets were dispensed onto a culture dish using a pipette and observed using a microscope (Nikon).

Digital PCR

The template was a 280 bp double-stranded DNA fragment from the prfA gene in *L. monocytogenes*, which was first synthesized and amplified for sequential digital PCR. All of the oligonucleotide sequences are listed in Table S2.

The diluted template was mixed with reaction buffer (final concentration of 1×polymerase reaction buffer (Invitrogen), 5 mM $MgCl_2$, 0.4 mM each dNTP, 1 µM forward primer, 1 µM reverse primer, 300 nM TaqMan Probe, and 0.1 U/Rxn Platinum™ Taq Polymerase). After thorough vortexing, the reaction mixture was converted into droplets with MiCA. The emulsion droplets then underwent 40 cycles of PCR (10 min at 25 °C for surfactant encapsulation, 2 min at 95 °C for enzyme activation, 40 cycles (15 s at 94 °C and 30 s at 60 ° C) for amplification.

After PCR amplification, the emulsion droplets were transferred to a culture dish using a pipette and observed under a inverted fluorescence microscope (Nikon Ti-E).

Table S1. Oil-surfactant formulas based on aliphatic oil in different emulsion PCR protocols

Base oil	Surfactants	Method	Formula	HLB
Mineral oil	Span 80, Tween 80, Triton X-100	Dropwise addition and constant stirring[1,2]	A, 4.5% (v/v) Span 80, 0.4% (v/v) Tween 80, and 0.05% (v/v) Triton X-100 in mineral oil	5.3
		On chip (T-junction)[3]		
	silicone copolymer (SCP) of Cetyl PEG/PPG-10/1 dimethicone	Dropwise addition and constant stirring[2]	B, 2% (v/v) Abil EM 90 and 0.05% (v/v) Triton X-100 in mineral oil	5.2
		On chip (T-junction)[3]		
		On chip	C, 4% (v/v) Abil EM 90 and 0.05% (v/v) Triton X-100 in mineral oil	5.1
		On chip (flow focusing)[4]	D, 3% (w/w) Abil EM 90 and 0.1% (w/w) Triton X-100 in heavy white mineral oil	5.3
Mineral oil and diethylhexyl carbonate (DEC)	silicone copolymer (SCP) of Cetyl PEG/PPG-10/1 dimethicone, polyglyceryl-4 Isostearate, hexyl Laurate	Mixing[5]	E, 7% (wt/vol) Abil WE 09, 20% (vol/vol) mineral oil and 73% (vol/vol) diethylhexyl carbonate	approx. 5
		Vortex mixing[6]		
Isopropyl Palmitate (IPP)	silicone copolymer (SCP) of cetyl PEG/PPG-10/1 Dimethicone	MiCA (this work)	F, 7% (v/v) Abil EM 180 in isopropyl Palmitate	approx. 5

Table S2. Oligo DNA sequences for digital PCR

Oligo DNAs	Sequence
280-bp fragment (template)	CCGCAAATAGAGCCAAGCTTCCCGTTAATCGAAAAATCA TTAAATTTAGCTAGACTGTATGAAACTTGTTTTTGTAGGG TTTGGAAAACATAGAAAAAGTGCGTAAGATTCTTGCTCA GTAGTTCTTTTAGTTCGTTTATTTTGATAACGTATGCGGT AGCCTGTTCGCTAATGACTTCTAAATTATAATAGCCAACC GATGTTTCTGTATCAATAAAGCCAGACATTATAACGAAAG CACCTTTGTAGTATTGTAAATTCATGATGGTCCCGTTC TCAC
Forward primer for template production	5'-CCGCAAATAGAGCCAAGCTT-3
Reverse primer for template production	5'-GTGAGAACGGGACCATCATG-3'
Forward primer for TaqMan assay	5'-GCCTGTTCGCTAATGACTTCTAAAT-3'
Reverse primer for TaqMan assay	5'-GTGCTTTCGTTATAATGTCTGGCTTT-3'
TaqMan probe	FAM-5'-TAATAGCCAACCGATGTTT-3'-MGB

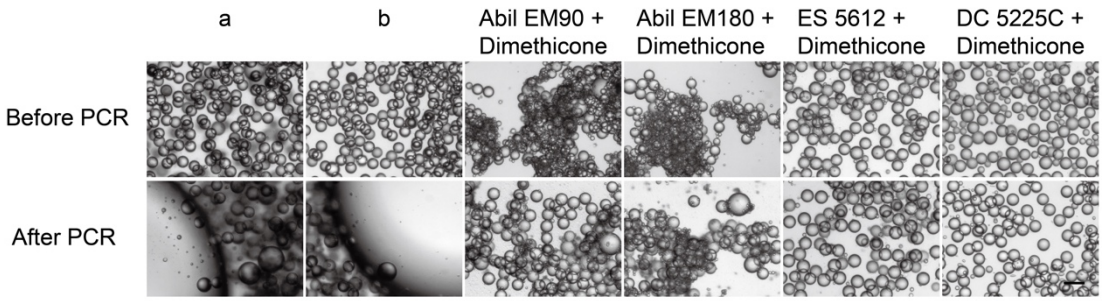

Figure S1. Droplets generated with PCR buffer (NEB) and reported silicone oil–surfactant mixtures (a, 39.8 % (w/w) DC 5225C Formulation Aid, 30 % (w/w) DC 749 Fluid, 30 % (w/w) AR20 Silicone Oil, and 0.2 % (w/w) Triton X- 100 surfactant; b, 40 % (w/w) DC 5225C Formulation Aid, 30 % (w/w) DC 749 Fluid, and 30 % (w/w) AR20 Silicone Oil) or dimethicone (viscosity of 5 cSt at 25 °C, Sigma-Aldrich) supplemented with 7 % (v/v) Abil EM90, 7 % (v/v) Abil EM180, 5 % (w/w) ES-5612 Formulation Aid (PEG-10 Dimethicone) or 40 % (w/w) DC 5225C Formulation Aid by MiCA before and after 40 PCR cycles. Scale bars: 100 μm.

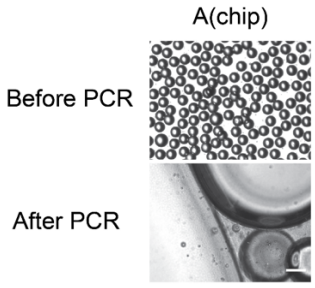

Figure S2. Droplets generated with PCR buffer (NEB) and oil-surfactant mixture of 4.5% (v/v) Span 80, 0.4% (v/v) Tween 80, and 0.05% (v/v) Triton X-100 in mineral oil by microfluidic chip (Droplet Junction Chip with 100 μm etch depth, Dolomite) before and after 40 PCR cycles. Coalescence was obviously observed. Similar results got from chip and MiCA shows MiCA as a method for producing droplets does not change inherent properties of emulsion. Scale bar: 100 μm.

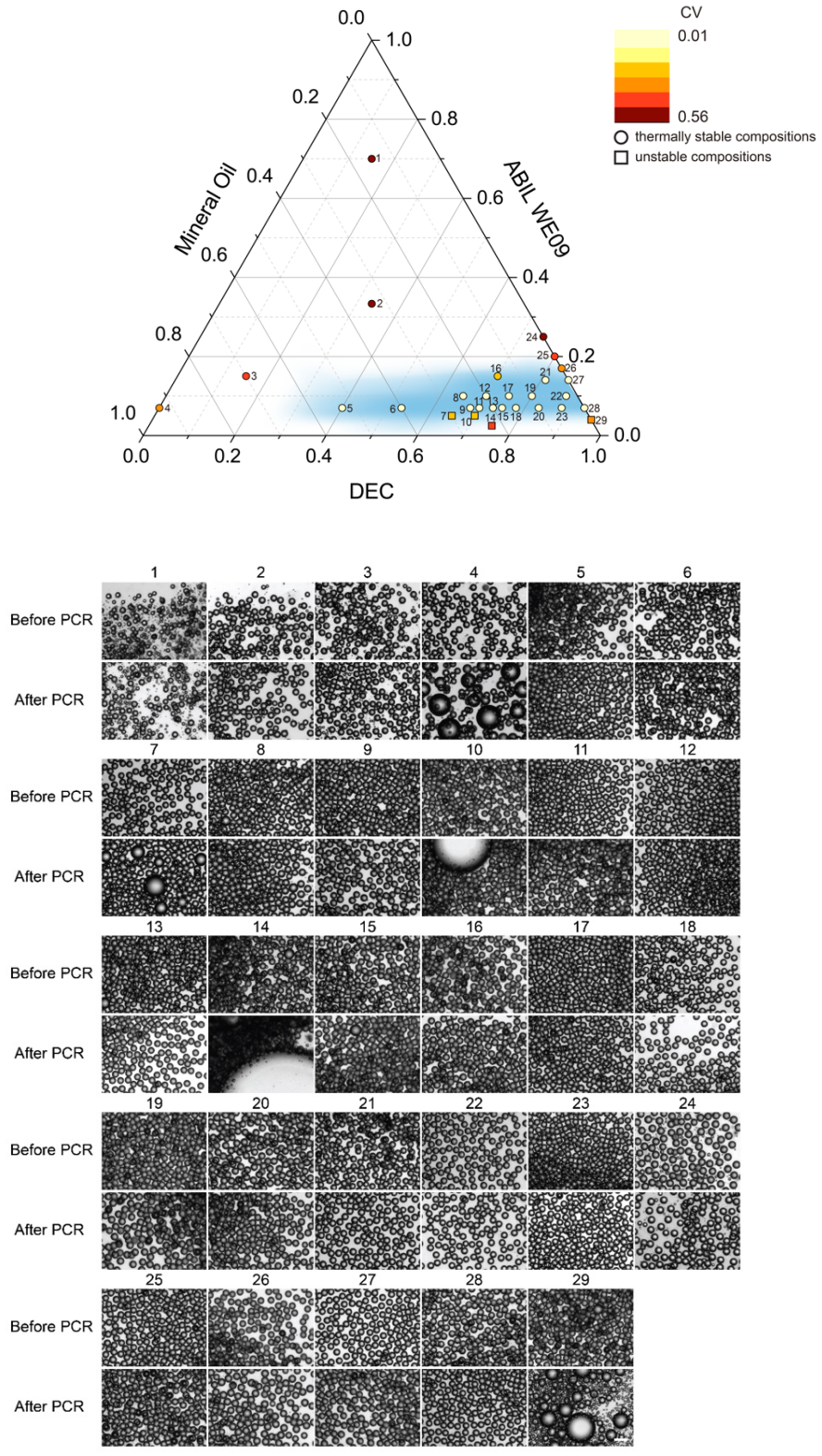

Figure S3. Droplets generated with PCR buffer (NEB) and all oil-surfactant mixtures using MiCA, observed before and after 40 PCR cycles. Scale bar: 100 µm.

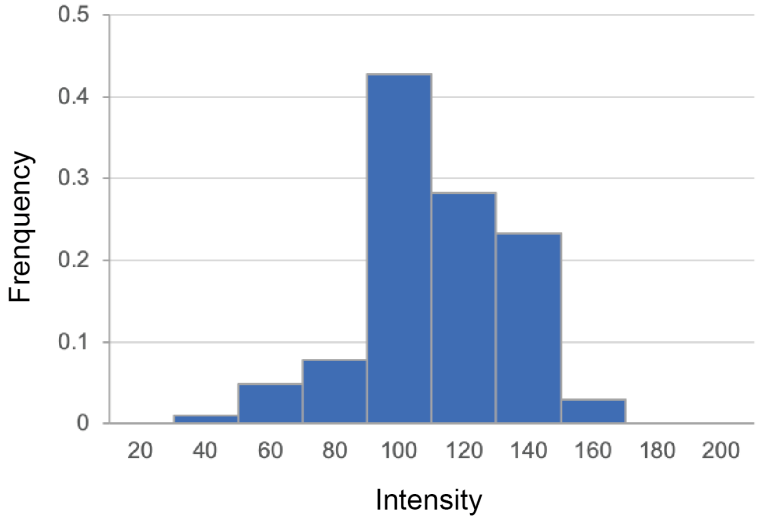

Figure S4. The intensity distribution of droplets with fluorescence signals after 40 cycles of thermal ramping.

Reference

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2. Williams, R. *et al.* Amplification of complex gene libraries by emulsion PCR. *Nature Methods* **3,** 545–550 (2006).
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