Electronic Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2013

Probing Cellular Heterogeneity in cytokine-secreting immune cells using droplet-based microfluidics

Venkatachalam Chokkalingam[a]*, Jurjen Tel[b]*, Florian Wimmers[b], Xin Liu[c], Sergey Semenov[a], Julian Thiele[a], Carl G. Figdor[b], Wilhelm T.S. Huck[a¥]

Supplementary Information

Figure S1

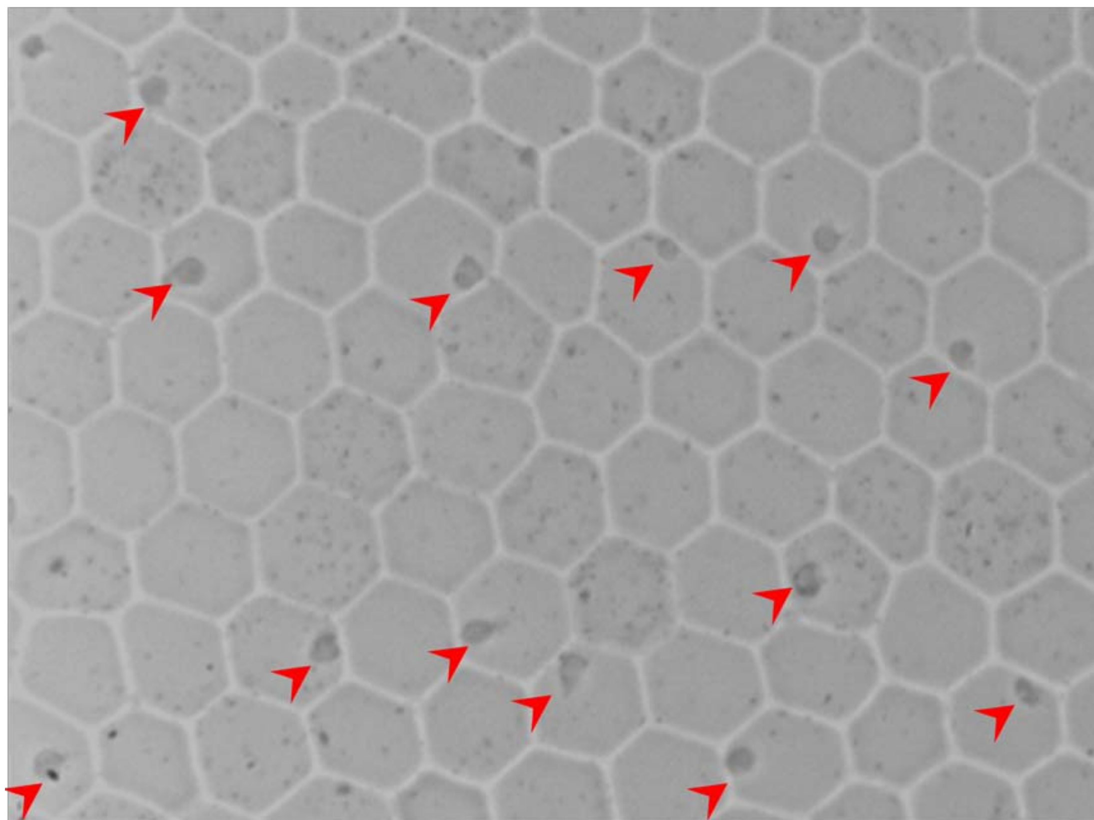
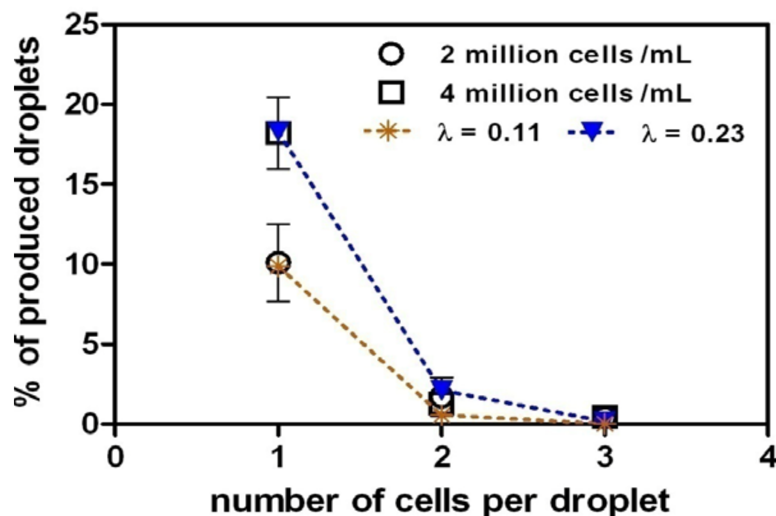


Figure S1. Monodispersity of the produced droplets containing single cells and capture beads. The focus is maintained at the single cells and so, the capture beads (500nm) may look a little out-of-focus

Figure S2

(A)



(B)

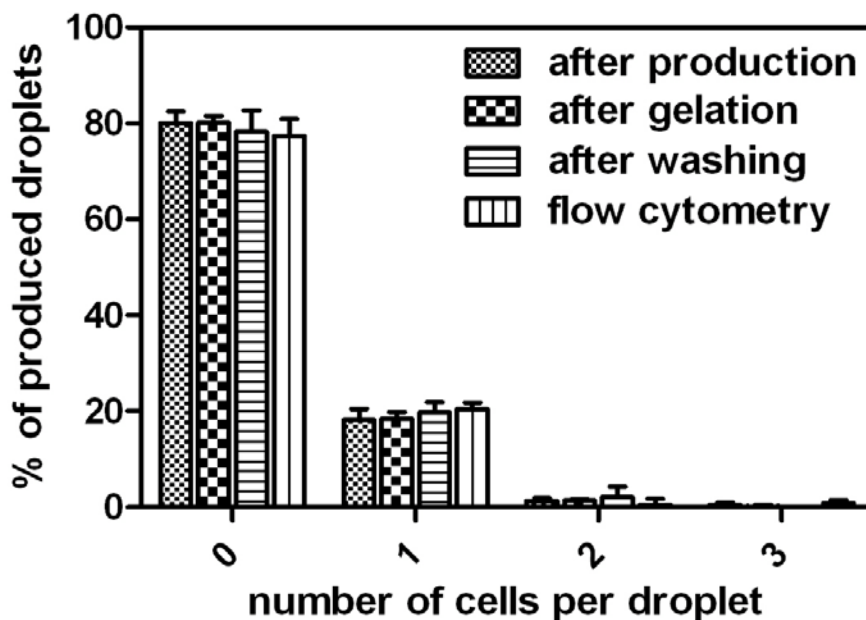


Figure S2. (A) Cell encapsulation efficiency for $2{\cdot}10^6$ and $4{\cdot}10^6$ cells per ml is approximately 11 and 19% (Poisson: λ=0.11 and 0.23), respectively. (B) Analysis of all steps from production up to detection reveals no specific loss of cell containing droplets.

This journal is © The Royal Society of Chemistry 2013

1. Protocols

1.1 Breaking the emulsion (removing oil)

- Centrifuge the gelled agarose beads collected in a Costar® Spin-X® centrifuge tube filter (Corning) for 5 minutes at 1800RPM
- Discard the supernatant in the tube
- Add 0.5 mL of PBS
- Resuspend rigorously by pipetting up and down
- Centrifuge the resuspended agarose beads for 7 minutes at 2700RPM
- Discard the supernatant in the tube
- Repeat the above steps 3 times to get rid of all the oil from the agarose beads

1.2 Incubation of agarose beads with detection antibodies and washing

- Add 50 µL of PBA and appropriate amount of detection antibody solutions, resuspend rigorously and incubate at 4°C for 30 minutes
- Centrifuge for 7 minutes at 2700 RPM and discard the supernatant
- Add 0.5 mL of PBA, resuspend rigorously and incubate at 4°C for 20 min
- Centrifuge for 7 minutes at 2700 RPM and discard the supernatant
- Add 0.5 mL of PBA, resuspend rigorously and incubate at 4°C for 5 minutes
- Centrifuge for 7 minutes at 2700 RPM and discard the supernatant

1.3 Sample for FACS analysis

- Add 200 µL of PBA
- Resuspend rigorously
- Pipette 200 µL from the filter tube into the FACS sample tube

This journal is © The Royal Society of Chemistry 2013

2. Synthesis of tri-block surfactant

In a Schlenk tube, 15.47 g of Krytox® 157 FSH (DuPont™) are degassed under stirring. 15 mL of HFE 7100 (3M™ Novec™) are added forming a two-phase mixture with Krytox. The Schlenk flask is manually shaken until the solution can be stirred with a stir bar. Under nitrogen flow, approximately 2 mL of oxalyl chloride are added. After adding a drop of dimethyl formamide, gas vigorously evolves from the reaction mixture which is removed through the gas bubbler of the Schlenk line. The solution is stirred over night at RT.

The solvent is removed and the residue is taken up in 15 mL of dry HFE 7100. 1.12 g of Jeffamine® ED-900 dissolved in 1 mL dry pyridine is added drop-wise.[1] The turbid reaction mixture is stirred overnight. To determine the conversion of acyl chloride using IR, 100 µL of the reaction mixture are added to 1 mL of methanol giving the methyl ester of unreacted acyl chloride. Supernatant methanol is decanted and 1 mL of HFE 7100 is added to the sample. HFE 7100 and residues of methanol are removed in a rotary evaporator. After adding another 250 µL of HFE 7100, the solution is filtered through a small glass fiber filter. Conversion is determined using the ratio of methyl ester and amide bands at 1788 cm^{-1} and 1720 cm^{-1}, respectively. A calculated amount of Jeffamine is added and the reaction mixture is stirred overnight. The conversion is checked by IR again.

The reaction mixture is poured into 50 mL methanol. After vigorous shaking, methanol is decanted and the residue is washed with another 30 mL of methanol. The residue is dissolved in 10 mL of HFE 7100. These steps are repeated with acetone, THF and methanol. The residue is dried at 2 mbar at 35 °C overnight using a rotary evaporator. The residue is dissolved in 15 mL HFE 7100 and filtered through a syringe filter (0.45 µm, cellulose). Again, the solvent is removed at 2 mbar overnight and finally dried in vacuum yielding a viscous clear oil with yellow-brown color.

[1] Jeffamine is stirred with small pieces of CaH_2 overnight under static nitrogen pressure. The mixture is divided into aliquots and centrifugated to remove CaH_2 and $Ca(OH)_2$. Dried Jeffamine is stored in a glove box prior to use.

3. Microfluidic chip fabrication

Photolithographic fabrication was employed to fabricate microchannels. SU-8 negative photoresist (Micro Resist Technology GmbH, Germany) was used for fabrication purpose. The device fabrication started with creating a design in an AutoCAD program. A high resolution commercial image setter then printed this design on a transparency (JD Photo tools, UK). This transparency served as the photomask in contact photolithography to produce a negative relief in photoresist on a silicon wafer substrate. SU8-2025 photoresist is spin-coated on a round silicon wafer with 50 mm diameter (Simat silicon materials, Germany). Spin coating parameters were optimized to achieve the desired film thickness. Subsequently, the sample was soft-baked for 1 min at 65°C, 3 mins at 95°C and 1 min at 65°C in order to evaporate the solvent and densify the film. Then, the samples were exposed to UV light ($\lambda = 365$ nm) in the mask aligner (Karl Suss MJB 3 UV 300/400) for 21 s through the photomask. After exposure, the sample was post-baked for a time which depended on the thickness of the photoresist (1 min at 65°C, 2 min at 95°C and 1 min at 65°C). The samples were rinsed with developer solution (mr-Dev600, Micro Resist Technology GmbH, Germany) to remove the non-crosslinked regions. The resulting height of the channels were 25 μm. Thus, depending on the design used in the mask, fabrication of SU-8 masters for droplet production was accomplished on a silicon wafer (Fig. SI 3). Following the below mentioned protocol (Table SI 1) resulted in the production of the PDMS microfluidic chips.

PDMS layer with channels	- Pour 10:1 ration PDMS on a SU-8 master with channels (~ 5mm thickness) - Bake at 65 °C in oven for at least 3 hours - Peel off the PDMS - Punch holes with 1.0 mm diameter biopsy punch
Plasma bonding for closed channels	- Treat the PDMS slab and glass cover slip with plasma cleaner (15 sec, 85% power) - Bond PDMS slab with glass coverslip by pressing gently - Bake at 100 °C for 20 minutes

Table S1. PDMS fabrication steps

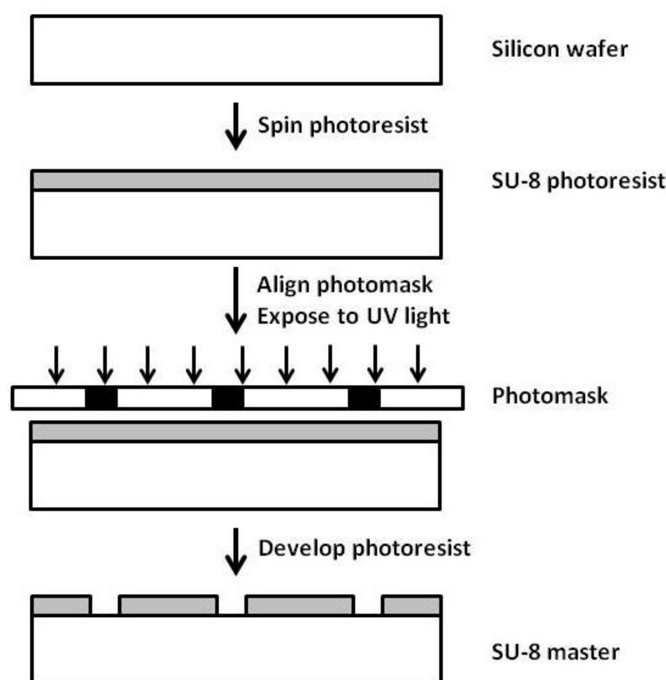


Figure S3. Schematic overview of the microfluidic chip fabrication by photolithography.

4. Cell culture and stimulation

Jurkat cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM; Life Technologies, Grand Island, NY, USA) supplemented with 5% fetal calf serum (FCS; Greiner Bio-One, Alphen a/d Rijn, Netherlands) and 1% Antibiotic-Antimycotic (Life Technologies). In some experiments these steps were followed by a CFSE (Life Technologies) staining. For this purpose, the cells were re-suspended in 1 mL of phosphate buffered saline (PBS) with 0.001 µmol/mL CFSE staining solution followed by seven minutes of incubation at 37°C. Subsequently, 5 mL of FCS were added to stop the reaction. Thereafter the cells were washed and re-suspended in PBS or IMDM at a concentration of 1 to $4{\cdot}10^6$ cells per mL. To study cytokine secretion, Jurkat cells ($4{\cdot}10^6$/mL) were stimulated and incubated for five to six hours at 37°C and 5% CO_2 with ionomycine (1 µg/mL) (Sigma) and 12-O-tetradecanoylphorbol-13-acetate (PMA; 0.1 µg/mL) (Sigma) to mimic the intracellular signaling events following an activation of the T cell receptor (TCR). This TCR-independent stimulation was used to probe and study a broad range of responses.

5. Preparation of microspheres

Microspheres were washed three times with PBS containing $5{\cdot}10^{-5}$ % v/v Tween 20. Spin speed was set at 14.000 rcf and the centrifugation time was 5 minutes. Subsequently, the

microspheres were incubated for 30 minutes at room temperature in PBS containing biotinylated mAbs specific for IL-2 (BD Pharmingen, San Jose, CA, USA) TNF-α or IFN-γ (both Biolegend, San Diego, CA, USA), while being gently shaken. For the coupling 30 mg of IgG where used to saturate 1 g of microspheres. After three washing steps, the microspheres were resuspended at a concentration of $2.422 \cdot 10^{10}$ microspheres per mL.

6. Cell surface/Cytokine staining assay in droplets

The solidified drops were transferred to a Costar Spin-X cellulose acetate spin column (pore size 0.22 µm; Coring Inc., Corning, USA). Subsequently, the oil was removed and droplets were washed three times. Afterwards, surface markers and secreted cytokines inside the droplets were stained using the following antibodies: anti-CD3-PE/FITC, anti-CD69-PerCP (both BD Pharmingen), anti-IL-2-Alexa Fluor® 488, anti-TNF-α-PE and anti-IFN-γ-Alexa Fluor 647® (all three Biolegend). Finally, the droplets were washed and analyzed by flow cytometry using FACScalibur (BD Bioscience).

7. Bright field microscopy

To acquire images of the cells at various stages of the assay, 10 µL of droplet solution in oil was given on a microscope slide. Subsequently, bright field images were taken using an Olympus CKX41 at different magnification.