Microarrays for the study of compartmentalized microorganisms in alginate microbeads and (W/O/W) double emulsions

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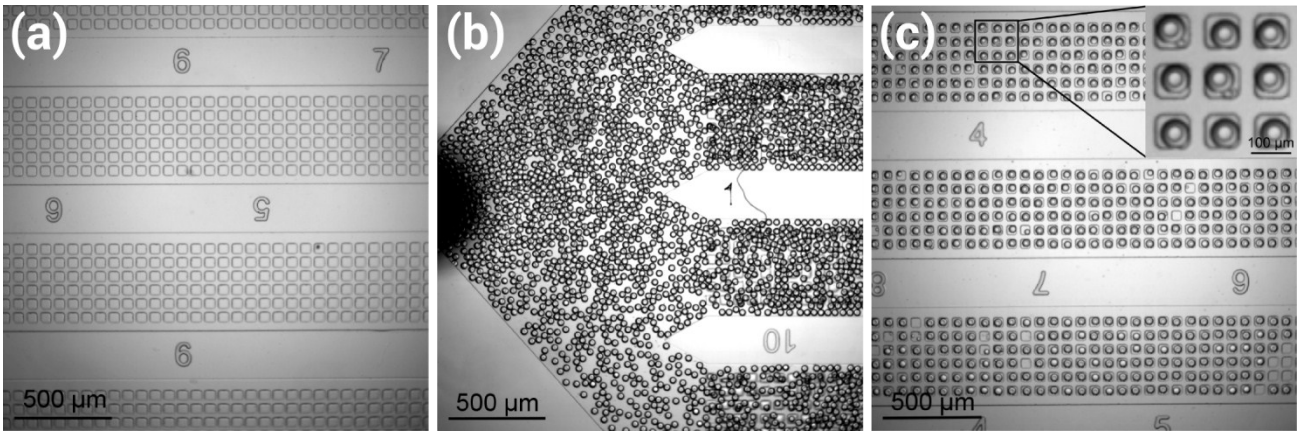
Electronic Supplementary Information



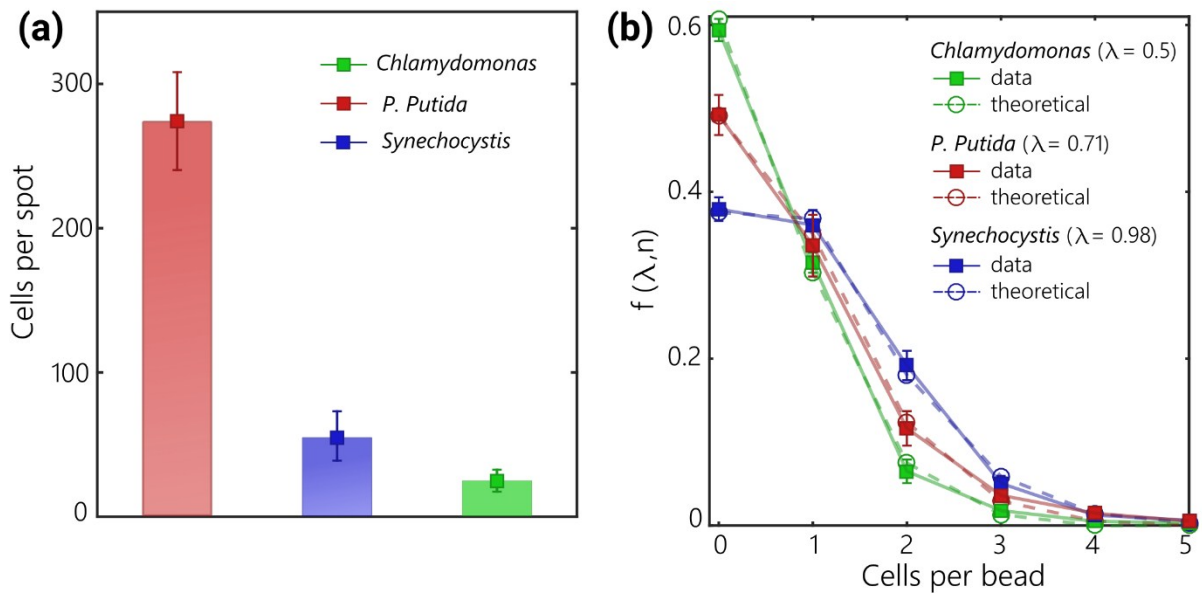
ESI Fig. 1 Collected *P. putida* KT2440-laden double emulsions were injected into a 3D PDMS based microarray device. *(a)* Micrograph of the 3D PDMS microarray device prior to injection of double emulsions. *(b)* Double emulsions were left for 2 min after injection to allow for sedimentation into the 90 x 90 x 80 µm (xyz) microwells and flushed with the same outer aqueous phase used to produce the emulsions (i.e. containing PVA and sucrose dissolved in LB-medium) with 0.5 mM m-Toluic acid to initiate GFP production in the *P. putida*. *(c)* The double emulsions with an outer diameter of 76.1 ± 1.7 µm sediment into the wells, which can only house one double emulsion.

Poisson statistics of encapsulated microorganisms in alginate beads

Microfluidic encapsulation of cells is governed by Poisson statistics[2]. The distribution of the number of cells per bead is given by

$$f(\lambda,n) = \frac{\lambda^n e^{-\lambda}}{n!} \quad (1)$$

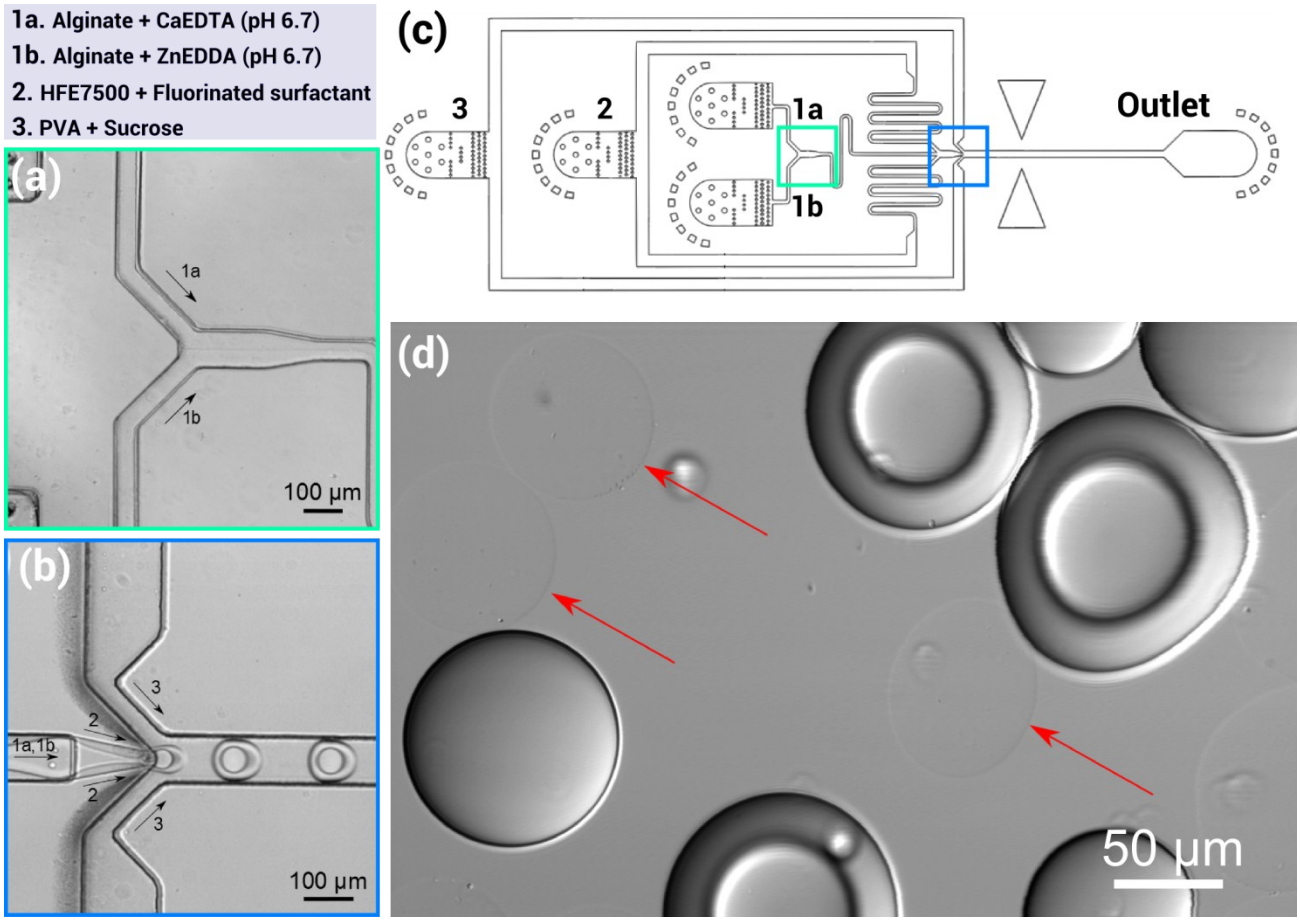
where λ is the average number of cells per bead and n is the number of cells in a particular bead. To determine the distribution of amount of Chlamydomonas, P.putida and Synechocystis in the microfluidics produced alginate beads, we analyzed multiple images (3-5) of hydrogel encapsulated microorganisms for each type of microorganism. The images used for the analysis were populated with 150-350 beads and only small variations in the distribution of amount of cells per bead were found between the images for the same microorganism, which is reflected by the small error bars in ESI Fig. 2b. Images of P.putida were, however, more difficult to analyze due to the small size of the microorganisms and the limitation in image resolution. Hence, the larger deviation between the experimentally determined number of cells per bead between each analyzed image were observed for the P.Putida compared to the other microorganisms. This is reflected by larger error bars (red) in ESI Fig. 2b for this microorganism.



ESI Fig. 2 *(a)* µCP was utilized to pattern glass substrates with 50 x 50 µm square shaped PEI coated spots whereby the microorganisms *Chlamydomonas, P.putida* and *Synechocystis* were immobilized onto the spots with patterning efficiencies of 25.0 ± 6.8, 274.2 ± 34.6 and 56.0 ± 17.8 cells per spot, respectively. These estimates are based on counts from the nine PEI spots displayed in Fig.2 for each microorganism *(b)* Poisson distribution for the density of *Chlamydomonas, P.putida* and *Synechocystis* in alginate beads with average cells per bead (λ) of 0.5, 0.71 and 0.98, respectively. For each microorganism, the calculations were based on multiple images of hydrogel encapsulated microorganisms with a population of 150-350 beads per image. The measured distributions are in good agreement with theoretically predicted Poisson distributions. The slightly bigger error bars for the *P.putida* is attributed to the difficulty in counting these bacteria due to their small size and limited image resolution.

CLEX in (W/O/W) double emulsion cores

A recently proposed PDMS-based microfluidic double emulsion device[1], which was used for the encapsulation of *P. putida* KT2440 (Fig. 7 and Fig. 8), was modified to allow for adaptation of a gelation technique exploiting competitive ligand exchange (CLEX)[3, 4] for crosslinking of alginate in the double emulsion cores (ESI Fig. 3a). Two alginate inner phase solutions were used: (1) 0.6% (wt/vol) alginate with 36 mM CaEDTA and 36 mM MOPS at pH 6.7 and (2) 0.6% (wt/vol) alginate with 36 mM ZnEDDA and 36 mM MOPS at pH 6.7. These were injected into separate inlets in the modified double emulsion device at equal flow rates of 50µL hr^{-1} (ESI Fig. 3b). Hydrofluoroether (HFE7500, 3M®) with 1 wt% fluorosurfactant was used as the middle phase and injected with a flow rate of 400 µL hr^{-1}, whereby a co-flow between this fluid and the alginate solutions was established (ESI Fig. 2c). 10% (wt/vol) PVA with 100 g L^{-1} sucrose was used as the outer aqueous phase to facilitate droplet break-up and was injected with a flow rate of 1200 µL hr^{-1} (ESI Fig. 3c). The collected double emulsions were re-suspended in DIW in which a decrease in osmotic pressure from the surroundings enabled double emulsion bursting, rendering the gelled alginate cores free in solution (red arrow ESI Fig. 3d).



ESI Fig. 3 A recently proposed double-emulsion device[1] was modified to enable double emulsion alginate core gelation with competitive ligand exchange crosslinking (CLEX).[3 4] *(a)* Two alginate solutions; one containing CaEDTA and the other containing ZnEDDA were injected into the device and meet in a co-flow region prior to *(b)* hydrodynamic flow focusing by a fluorinated middle phase containing fluorosurfactants, and droplet breakup by a PVA and sucrose containing outer aqueous phase. *(c)* CAD design of the first layer of the microfluidic device displaying the 4 inlets used to carry out gelation of double emulsion cores with CLEX. The green and blue boxes represent regions displayed in *(a)* and *(b)*, respectively. *(d)* Collected double emulsions were re-suspended in DIW without PVA or sucrose and burst upon change in osmotic pressure between the core and the surrounding. The red arrows indicate alginate microbeads formed in the double emulsion cores.

ESI References

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