

## Electronic Supplementary Information (ESI)

### Transitioning from multi-phase to single-phase microfluidics for long-term culture and treatment of multicellular spheroids

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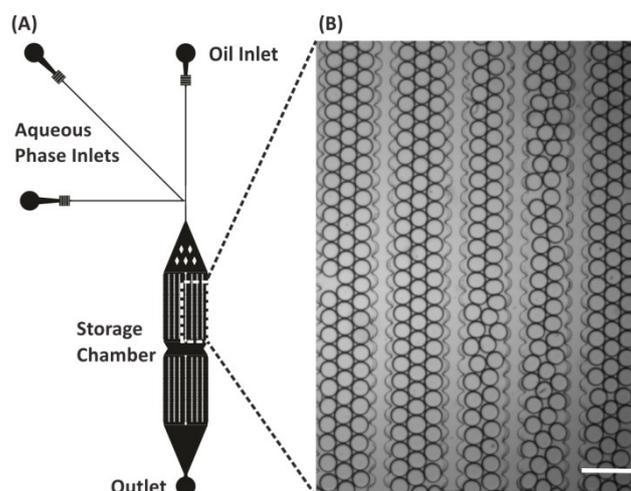
Movie S1-S3.

**Movie S1.** *Emulsion trapping mechanism. The video shows how emulsions are trapped into a round chamber within the serial array of trapping sites. Once a trap has been occupied, the following water-in-oil/surfactant plug bypasses the site and becomes trapped in the next available round chamber. The scale bar is 400  $\mu\text{m}$ .*

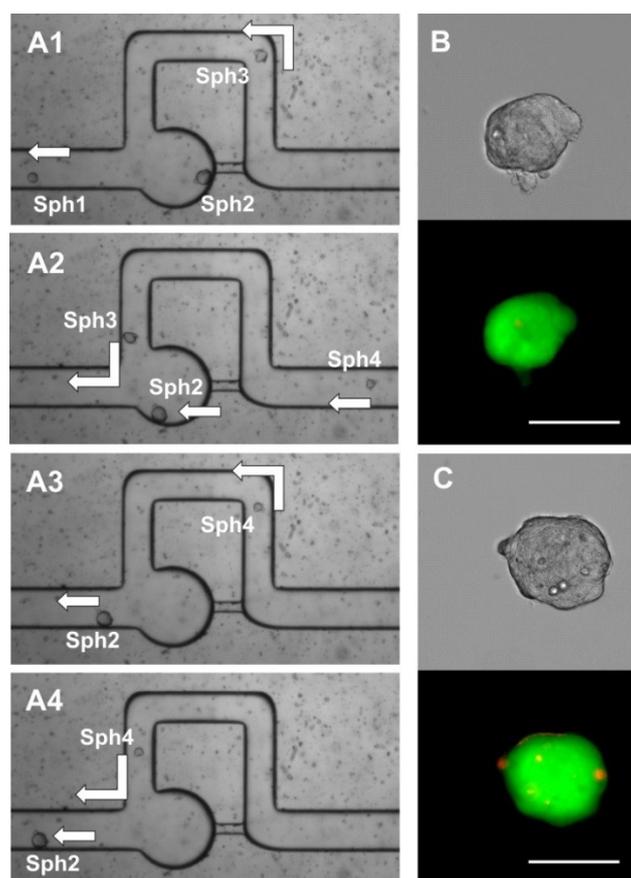
**Movie S2.** *Emulsion destabilisation within the round chambers in the microfluidic device of Figure 2. The video shows a long plug containing cell medium and calcein entering the array of chambers and coming into contact with the trapped emulsions which only contained medium. Subsequently, the coalescence of the long plug with the trapped droplets was evidenced by rupture of the interface and resulted in the diffusion of the calcein from the long plug into the droplets. The scale bar is 400  $\mu\text{m}$ .*

**Movie S3.** *Representative spheroid formation within the microfluidic device. The video shows brightfield time-lapse microscopy (~17 hours) starting from an initial cell suspension encapsulated within droplets that aggregated over time and resulted in compact spheroids. The scale bar is 400  $\mu\text{m}$ .*

Figures S1-S2



**Figure S1.** Microfluidic device used for quantification of emulsion coalescence. (A) Layout of the device with a storage chamber containing up to 1300 emulsions. (B) Representative brightfield image of emulsions stored within the chamber. The scale bar is 350  $\mu\text{m}$ .



**Figure S2.** Spheroid retrieval protocol. (A) Spheroids grown and cultured in the microfluidic devices could be retrieved by reversing the flow direction (from 4A in Figure 2) as show in the time lapse image sequence (A1-A4). (B&C) Examples of brightfield and fluorescent images of spheroids, collected via the auxiliary inlet (3A in Figure 2) following this procedure, and stained off-chip for viability with FDA and PI. Results show that the procedure did not damage or produce disaggregation of the spheroid. The scale bar for (B) and (C) is 80  $\mu\text{m}$ .