Supplementary Information (SI) for Lab on a Chip.

Supplementary Information for Sacrificial Oil Shell Method for the Generation of Alginate Microbeads Adapted to Multicellular Spheroid Culture

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Alginate bead fracture due to yeast growth

In the Figure 2A of the article, yeast growth appears to cause a dramatic fracture of the alginate leading to the outburst of the yeast from the bead. To rationalize this observation, we estimate the order of magnitude of the stress applied by the yeast growth and compare it to the critical stress expected to cause fracture of the the alginate hydrogel.

Just before rupture, the relative increase of the bead volume is $\Delta V_b/V_b^0 \approx 0.1$. This corresponds to an internal pressure of about $\Delta P = K\Delta V_b/V_b^0 \approx 10$ kPa, where $K \approx 100$ kPa is the bulk modulus of the alginate. At this stage the yeast cells are separated from the external medium by an alginate membrane of thickness $e \approx 10$ µm (see Fig. 2A at $t = 36$ h). Assuming that locally - in the region between the yeast population and the bead edge - the bead behave mechanically as a capsule with an elastic shell of thickness s, the resulting orthoradial stress can be estimated as:

$$\sigma_{\theta\theta} = \frac{\Delta P R}{2e}, \tag{1}$$

where R is the bead radius (typically 100 µm). Substituting the measured values yields $\sigma_{\theta\theta} \approx 50$ kPa.

On the other hand, a scaling argument based on Griffith's criterion allows us to estimate the orthoradial stress at which fracture occurs:

$$\sigma_{\theta\theta}^{\mathrm{c}} = [\Gamma_{\mathrm{c}} E/\ell]^{1/2}, \tag{2}$$

where Γ_{c}, E, and ℓ, denote the fracture energy per unit surface, the Young's modulus of the alginate, and the characteristic size of the crack, respectively.

In our case, taking $\Gamma_{\mathrm{c}} \approx 10$ J/m^2 [1], $E \approx 10$ kPa, and $\ell \approx 100$ µm (estimated from Fig. 2A just before fracture), we obtain a critical stress of approximately $\sigma_{\theta\theta}^{\mathrm{c}} \approx 40$ kPa.

The applied orthoradial stress therefore exceeds the estimated critical value, indicating that the pressure generated by yeast growth is sufficient to fracture the alginate bead. The agreement in order of magnitude between the calculated critical and applied stresses supports both the scaling analysis and the proposed fracture mechanism.

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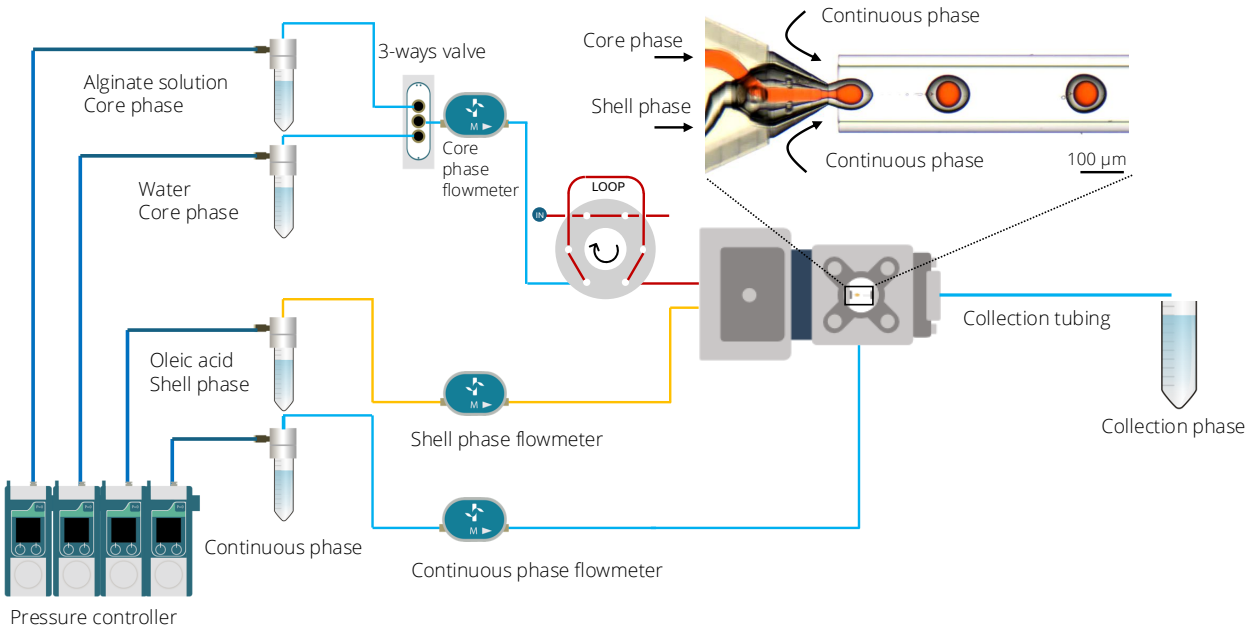
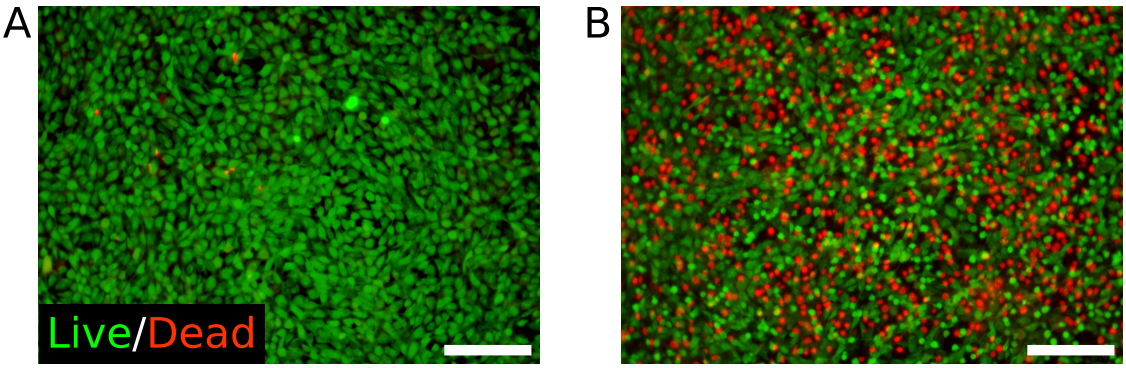
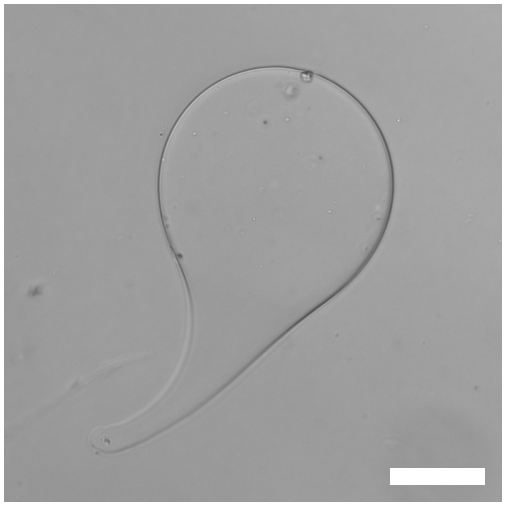
[1] R. Long and C.-Y. Hui, Fracture toughness of hydrogels: measurement and interpretation, Soft Matter **12**, 8069 (2016).



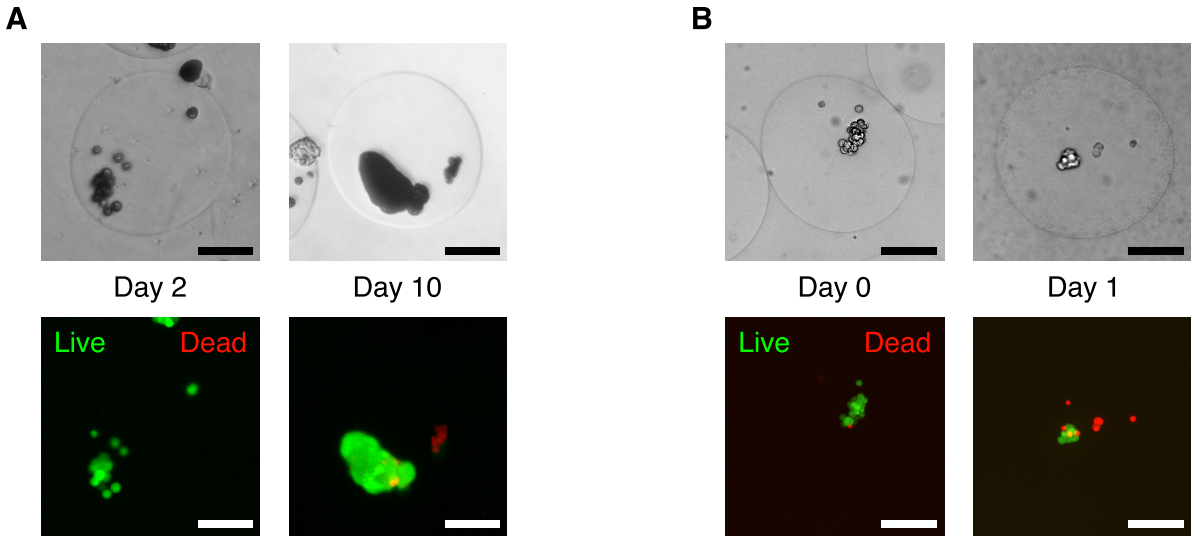
Supplementary Figure 1. **Experimental setup**. Detailed schematic of the experimental setup for double emulsion fabrication, showing pressure controllers, flow sensors, tubing, the sample injector with a sample loop, and the microfluidic device. The system can be primed with water before switching to the alginate solution, thanks to two reservoirs for the core phase: one containing deionized water and the other the alginate solution. The cell solution is injected into the sample-injector loop (loading mode), and then pushed into the microfluidic device by the alginate solution (injection mode). At the end of the experimental campaign, the core phase is switched back to the water reservoir in order to clean the core pathway and prevent any alginate or cell residues from building up.



Supplementary Figure 2. **Cell viability upon exposure to the continuous phase.** Epifluorescence microscopy images of NIH 3T3 cells stained with Calcein AM (Live) and Propidium Iodide (Dead). A: Cells in control conditions (normal culture medium). B: Cells after being exposed to the continuous phase (1% PVA at pH 4.4) for 1 minute and thorough rinsing. This short exposure is sufficient for a majority of cells to die. Scale bar: 100 µm



Supplementary Figure 3. **Tear-shape bead.** Scale bar: 100 µm



Supplementary Figure 4. **Encapsulation of various cell types with the chelate-free approach.** Epifluorescence microscopy images of cells stained with Calcein AM (Live) and Propidium Iodide (Dead) A: MDCK cells are highly viable and are able to form spheroids within the alginate microbeads. B: hiPSC cells are viable right after encapsulation, but viability quickly decreases. No proliferation was observed. Scale bars: 100 μm.