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

Supplementary information

Title of supplementary videos

Video S1 – Growth of a multicellular spheroid in the microfluidic chemostat

Video S2 – Loading of a spheroid in the confining chambers through the sliding element

Video S3 – Confined growth of a spheroid and deformation of the suspended membrane with mechanical growth-induced pressure

Video S4 – Motion of the nematode *C. elegans* in the microfluidic chemostat

Video S5 – Development of a drosophila leg in the microfluidic chemostat

Supplementary figures

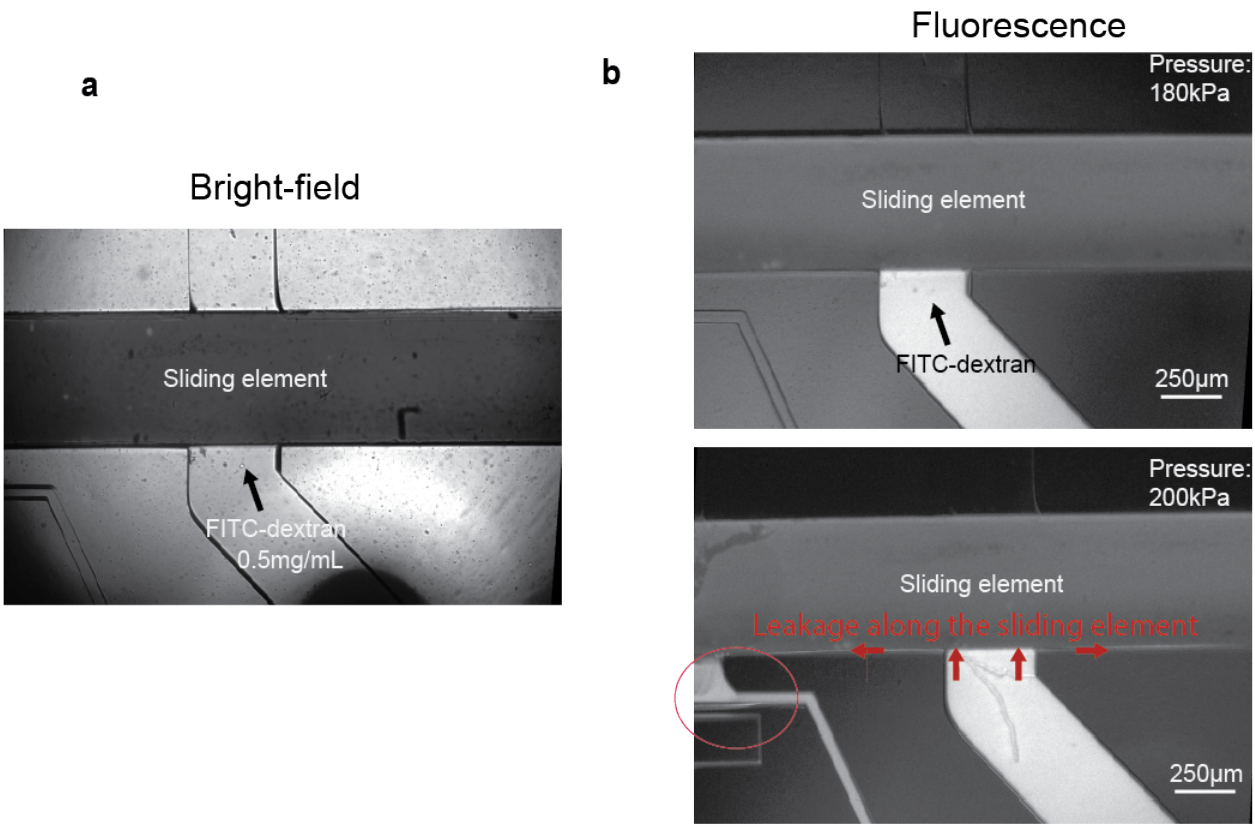

Figure S1: Increased inlet pressure can lead to leakage in the device, through the sliding element.

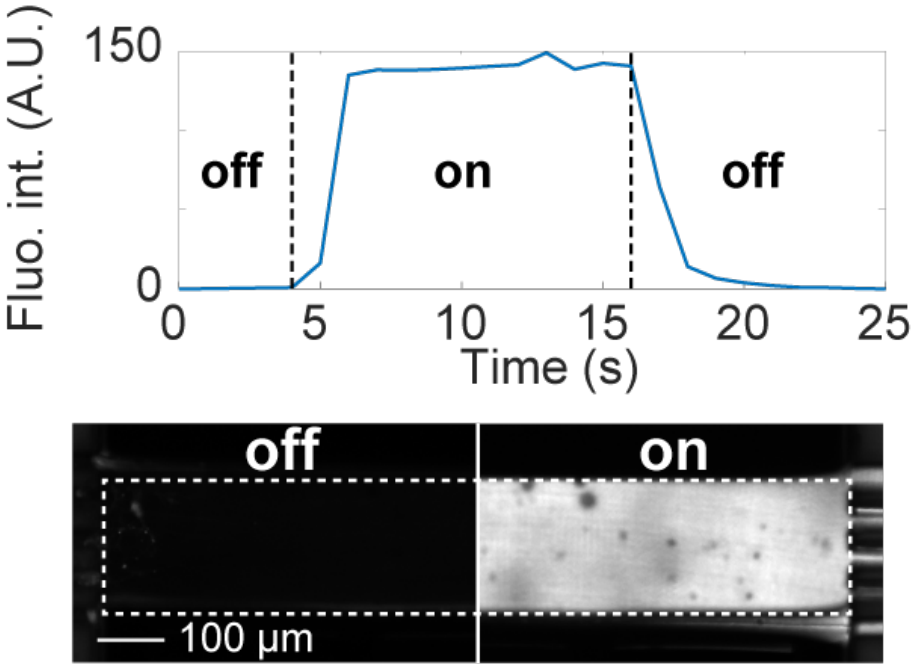

Figure S2: Changing of culture medium inside the device can be achieved within seconds.

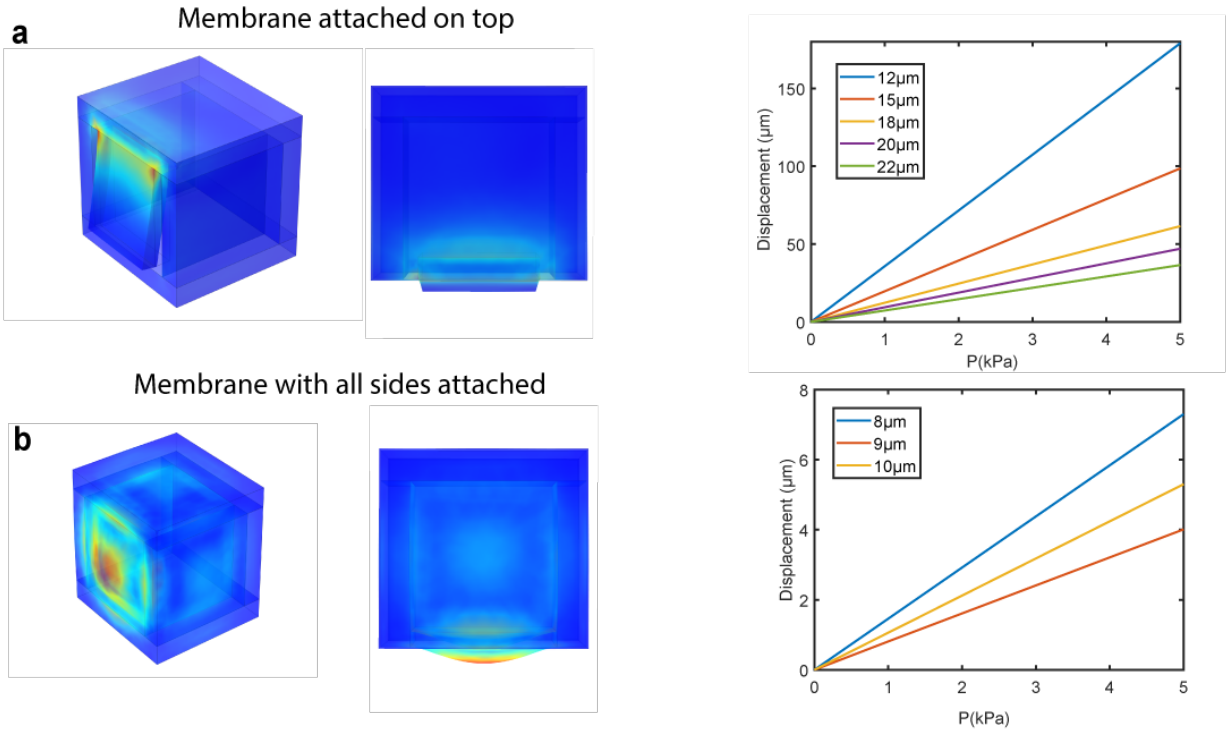

Figure S3. Finite element simulation of different membrane configurations to measure growth-induced pressure. a*. Membrane only attached at the top, and* ***b.*** *membrane attached to the four sides. We notice the much higher deformability of the membrane only attached at the top.*

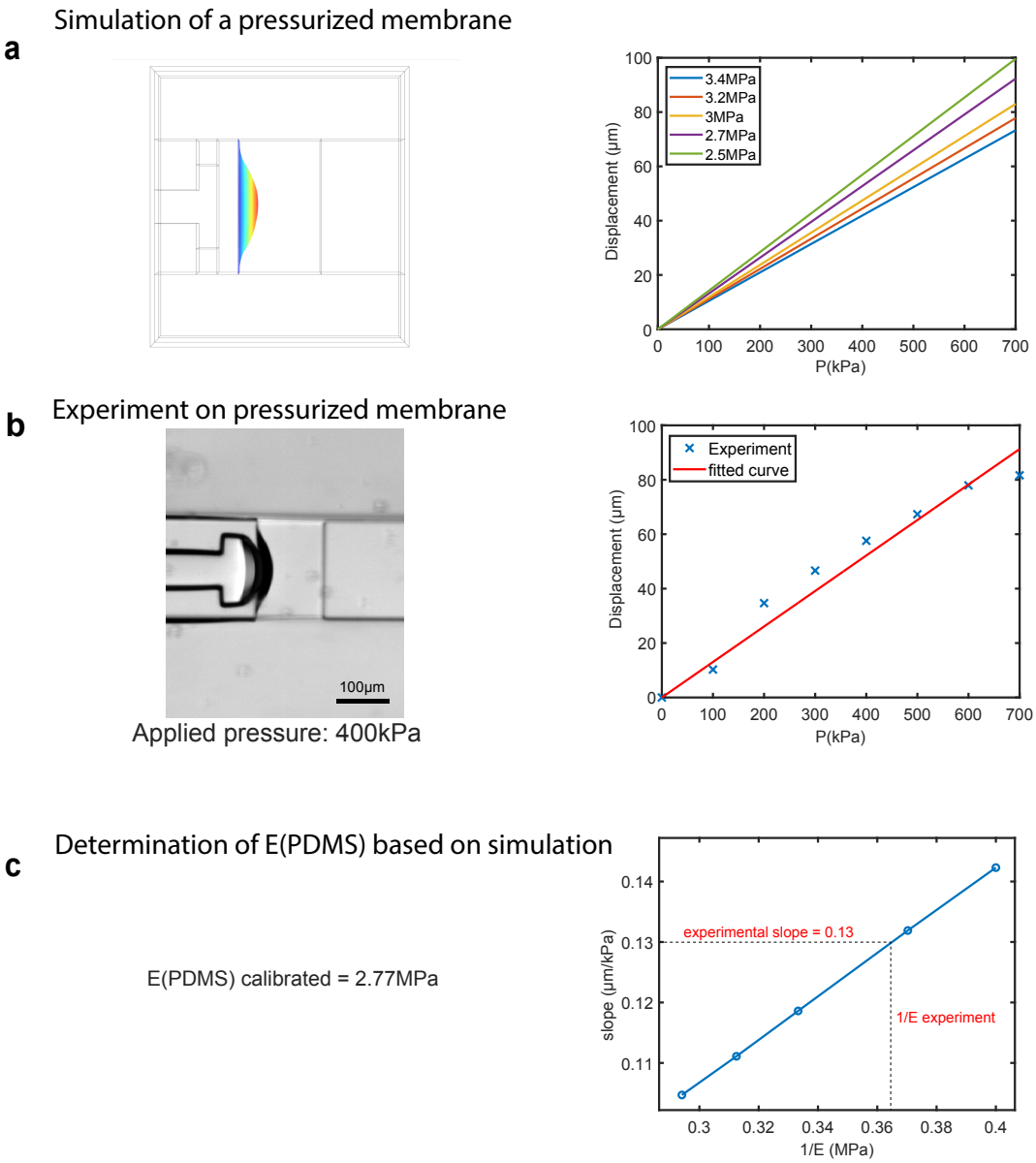

Figure S4: Calibration of the mechanical properties of the PDMS to use the pressure sensor. a. *Simulation and displacement of membrane attached to its four sides as a function of the pressure for different Young's moduli of the material.* ***b.*** *Experiment using a membrane attached to its four sides, and its deformation as a function of imposed pressure.* ***c.*** *The slope of the deformation of the simulated membrane is inversely proportional to the Young's modulus. We use the simulation to infer the experimental Young's modulus, and use this information together with Fig. S1 to measure growth-induced pressure.*

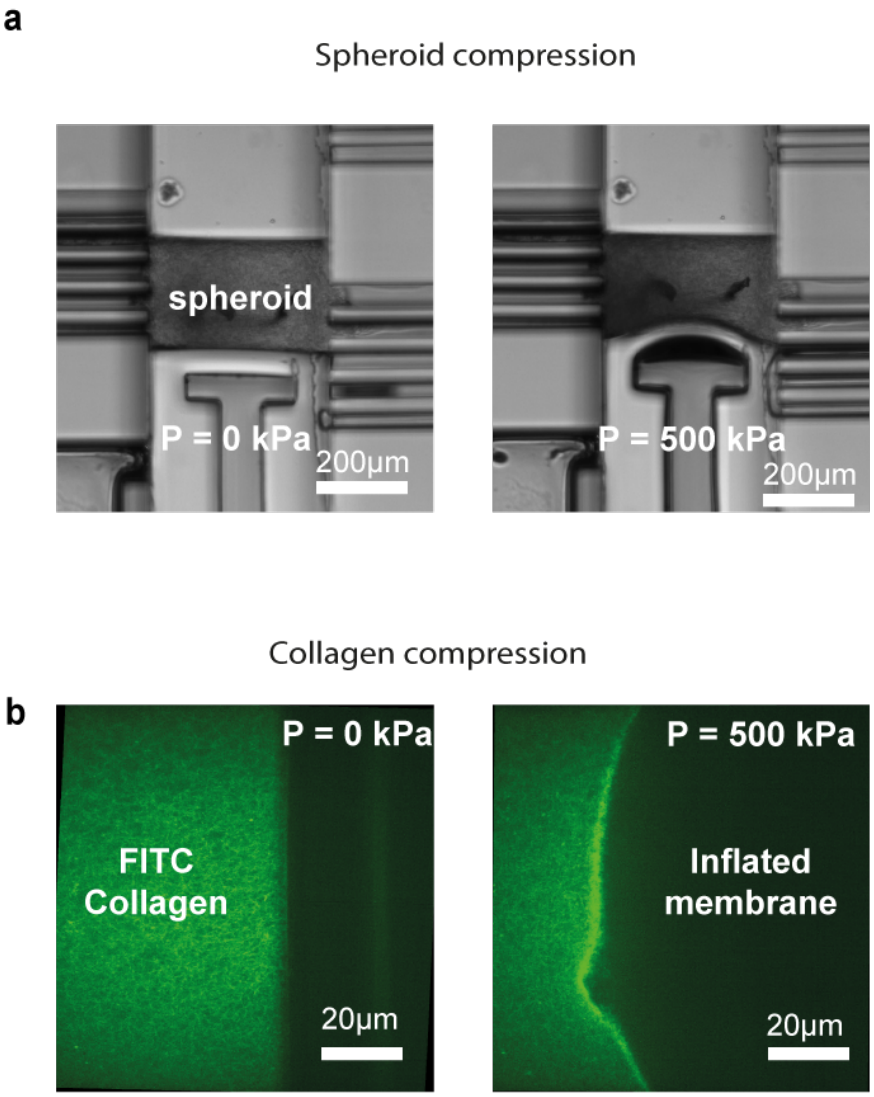

Figure S5: Spheroid and hydrogel compression. *Using the membrane attached to every sides, we can impose a give compression onto a loaded sample, either a spheroid (**a.**) or a collagen hydrogel (**b.**).*

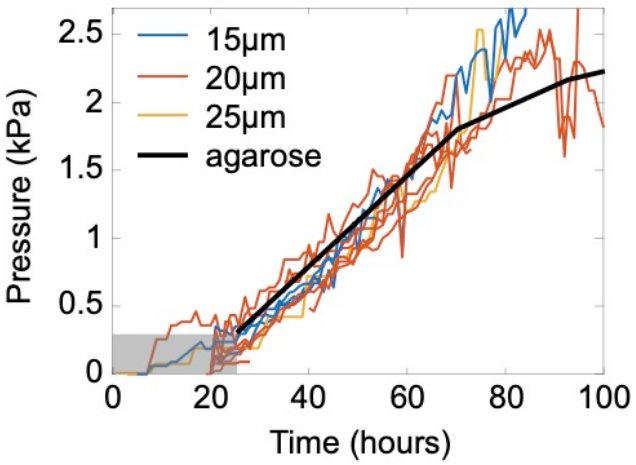

Figure S6: Agarose confined growth vs. microfluidic confinement. *After the deformation of the spheroid to contact the whole surface of the microfluidic chamber, the spheroid is fully confined. This situation is then comparable to the case where the spheroid is fully embedded as a sphere in agarose. We thus shifted in time (24h) and in pressure (250 Pa) the agarose curve to compare the dynamics of growth-induced pressure buildup with the microfluidic confinement, and observe a similar dynamic. A potential decrease for later points inside agarose is observed, and could potentially be attributed to lesser feeding, the spheroid in agarose also being larger than in the chamber.*

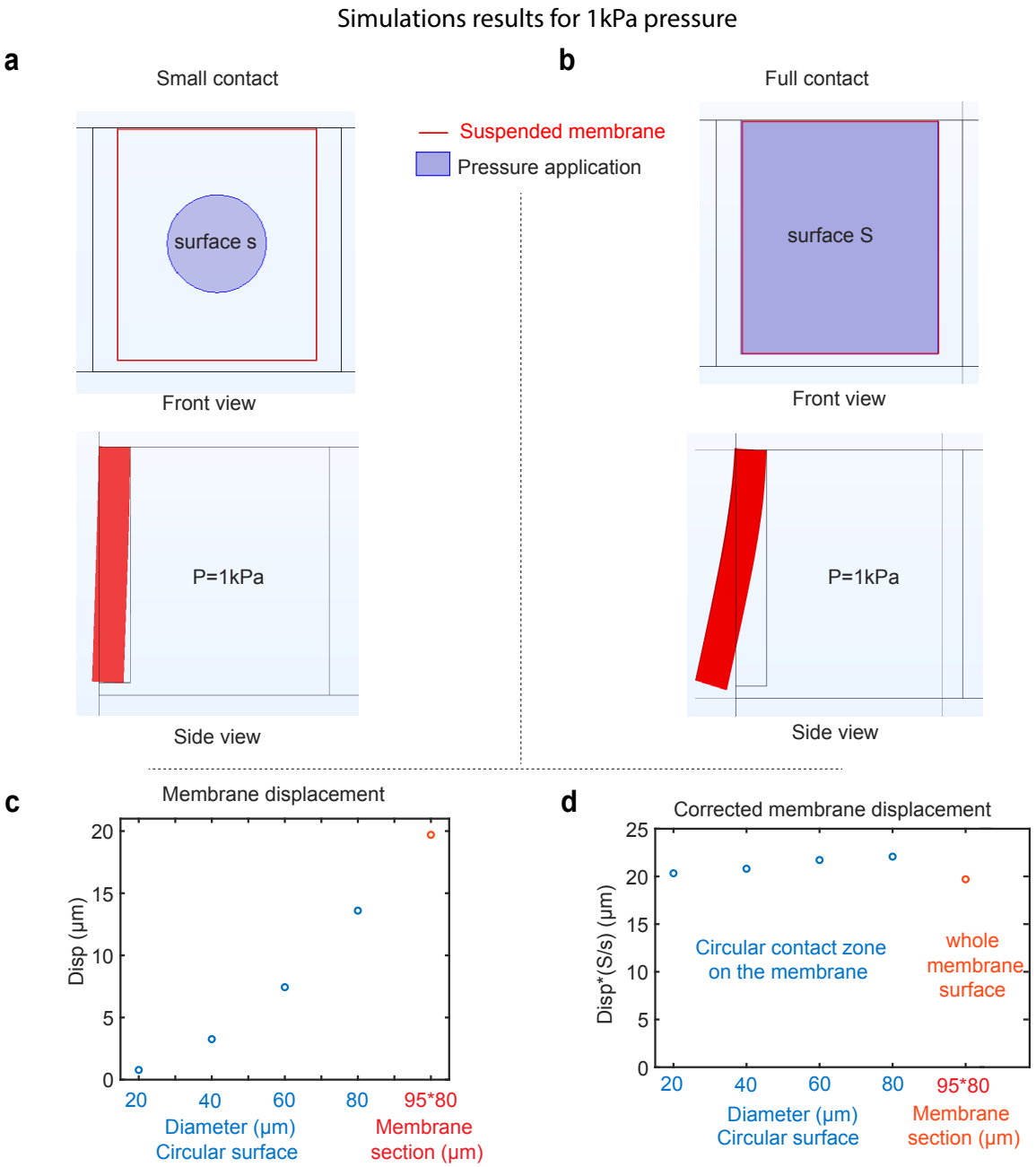

Figure S7: Correction factor when the spheroid does not fully contact the membrane. *When the aggregate does not fully contact the surface, the pressure is applied on a smaller surface. We performed Finite Element simulations where the contact surface is either a small circle (at early time points, **a**) or fully contact the surface (at confluency, **b**). We observed that displacement increased with surface contact diameter (**c**). We showed that a correction factor of the ratio of the membrane surface to the contact surface needs to be applied (**d**). However, because the membrane does not deform uniformly, this correction factor is not exactly the ratio of the surfaces, and tends to decrease with increased contact surface.*

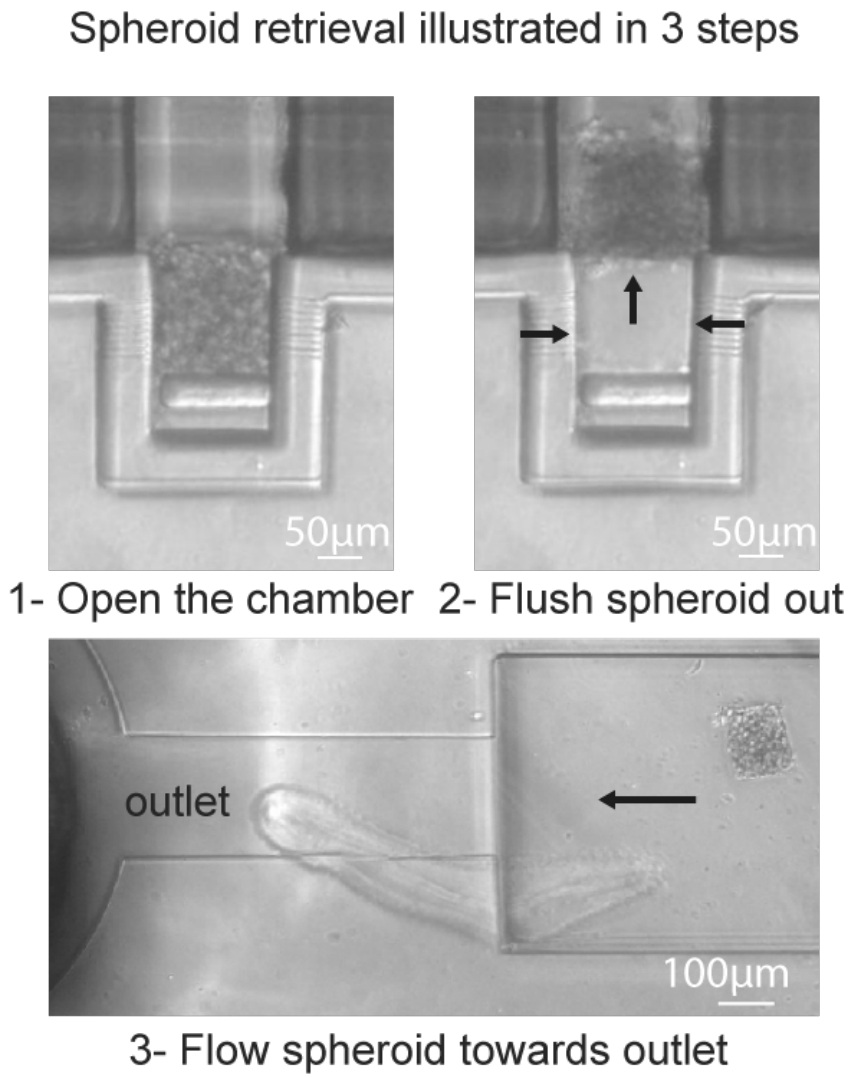

Figure S8 - Procedure to retrieve the spheroid

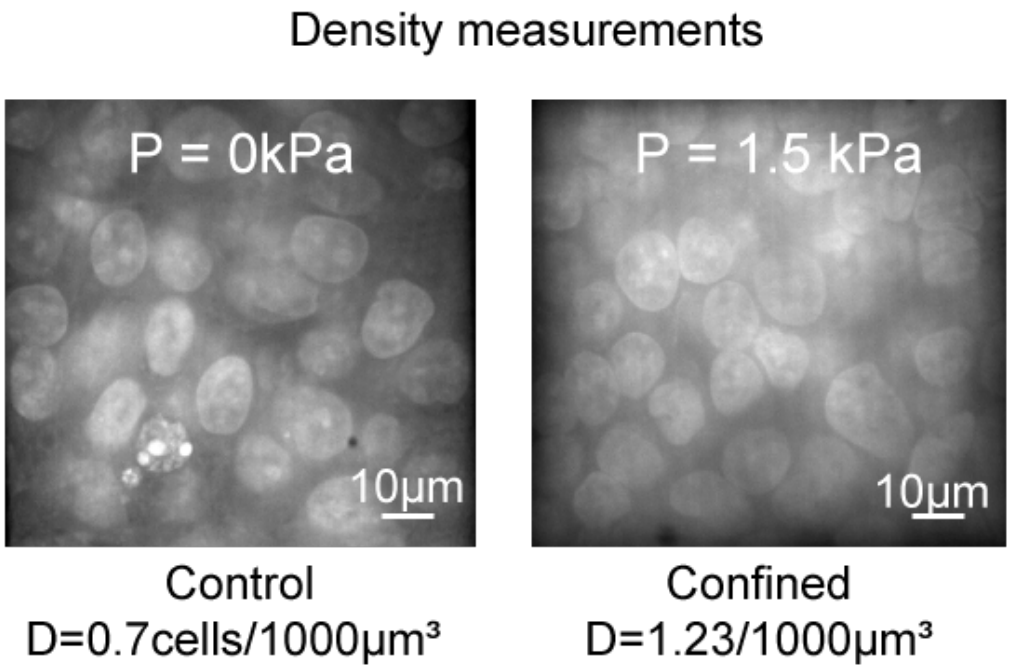

Figure S9: Cell density increases under confined growth. *At the end of an experiment, cells were fixed and nuclei stained with DAPI. 3D stacks were taken and cell density was measured. We observe an almost doubling of cell density under an increase in growth-induced pressure.*