Supplementary information

Materials and methods

PAA beads preparation

Polyacrylamide microbeads used in experiments were fabricated using a water-in-oil emulsion approach. Polymerization of 1 mL of a mix of acrylamide and bisacrylamide, at the ratio (x_a, x_b) has been initiated by catalytic activity of TEMED (Sigma, 0.75 uL) with an oxidizing agent APS (Sigma, 10 µL ammonium persulfate, at 10% w/v dissolved in PBS). For imagining purposes FITC-dextran 500 kDa has been introduced to the PAA mixture prior to polymerization. We have additionally added 10µL of acrylic acid (Sigma Aldrich) to promote EDC functionalization. To form an emulsion of acrylamide/bisacrylamide mix in oil, we used HFE 7500 perfluorinated oil (3M) with PFPE-PEG surfactant (kindly provided by Prof. Garstecki). All solutions were degassed for 15 minutes in the vacuum chamber prior use. The mix of polymerizing acrylamide and oil with surfactant has been vigorously shaken in a glass centrifuge tube by vortexing to achieve droplets with diameter ranging from few micrometres up to ~100µm. We kept emulsion under argon atmosphere for 5 minutes and and later during incubation at 60°C for 1h50 . To separate emulsion and transfer beads into PBS, we used 1H, 1H, 2H, 2H-perfluorooctanol (PFO, Sigma Aldrich). Briefly on the top of the emulsion, 500 µL of PBS were gently added with a subsequent addition of 400 µL of PFO. After incubation for 5 minutes, by gentle pipetting the PBS phase, droplets were transferred into the water phase. Fabricated beads were washed thoroughly with PBS to remove excess of non-crosslinked acrylamide monomers. Beads were filtered on 40 µm cell drainer for incorporation within spheroids.

For experiments with spheroids PAA beads were functionalized with fibronectin by EDC protein coupling. First beads were centrifuged in an ultra-low adhesive eppendorfs for 7 minutes at 2000 rpm and washed 3 times with MES buffer (ThermoFisher). Subsequently, beads were incubated with EDC (26 mg/ml) in a MES buffer for 2 hours under gentle agitation. After, beads were washed 3 times with MES buffer and incubated over night with fibronectin (Sigma Aldrich) at the concentration of 40 µg/ml. To remove excess of fibronectin beads were washed 6 times with PBS.

Flat PAA gels preparation

Flat gels of different compositions were prepared by covering a 55 µL drop of acrylamide/bisacrylamide mix residing on a non-treated coverslip (32 mm diam.) with a pre-silanized coverslip (to promote PAA crosslinking, GE Healthcare Life Sciences, Plusone Bind-Silane) (25 mm diam.). PAA mix has been let to polymerize during 30 minutes at room temperature. Non-treated coverslips were gently detached from a PAA gel that rested attached to the silanized coverslip in the presence of water. Gels were immersed in PBS during 3 days. The thickness of the gel was approximately of 60 µm. One night before experiments, the gels were immersed in a SRB solution. This ensured that the SRB concentration in the gel was homogeneous.

In order to determine the volume fraction of PAA, 1 ml samples of gel were prepared in centrifugal tubes. Subsequently gels were removed from the tube and immersed in PBS for 3 days to reach equilibrium. To remove excess of water gels were wiped with a non-absorbing paper and its weight m_{wet} was measured. Afterwards, gels were dried for 3 days in an oven at 70 °C and the new weight m_{dry} was measured. The volume fraction of polymer for the gels at swelling equilibrium has been calculated using $\phi = m_{dry}/m_{wet}$, neglecting the difference in density between polymer and water.

We denote ϕ_{mix} the initial volume fraction of monomer in the mixture before polymerization and ϕ_0 the one at equilibrium after swelling of the gel in PBS. When plotting ϕ_0, the polymer volume fraction after swelling as a function of ϕ_{mix} (see Figure S3), we observe that except for the gel (8, 0.48), all the gels are swelling after the preparation. Indeed the polymer volume fraction is smaller than the volume fraction of the monomer. The (8, 0.48) is obviously releasing water during the polymerisation, since the polymer volume fraction measured after swelling is higher than the monomer fraction in the solution prepared by mixing the compounds. Figure S3 also shows that increasing the acrylamide

concentration of the mixture gives a gel which swells more if the cross-linker concentration is kept constant.

Cell culture and spheroid formation

Mouse embryonic fibroblasts (were kindly provided by Dr. Destaing) were maintained in DMEM complete medium enriched with 10% fetal bovine serum (Life Technologies 61965-026) under 95% air and 5% CO2 atmosphere.

Spheroids were prepared using agarose cushion method. Briefly 100 µL of agarose (ultrapure agarose, Invitrogen) was dispensed in wells of 96-well plate and let to polymerize at 4'C for 10 minutes. Subsequently 300 cells were dispensed in each well in 150µL of culture medium. Plates were centrifuged at 900 rpm for 5 minutes to accelerate the process of aggregation. After 48 hours of incubation spheroids were ready to use.

To internalize microbeads within the volume of spheroids, beads were added with the single cells solution at the stage of spheroids preparation at the concentration of approximately 15 beads per well.

For pressure measurements using FCS, spheroids were transferred into 8 well Labtek II plates and let to spread partially for 12 hours. The culture medium is supplemented with Sulforhodamin B (SRB, Radiant Dyes Laser) molecules, at concentration ≈ 20 nM. Before running experiments, the culture medium is replaced by a modified version of DMEM without $NaCO_3$, pyruvate, red phenol and supplemented with 10% FBS and 10mM HEPES (PAA Laboratories GmbHed).

Image acquisition

We used a Leica inverted confocal microscope (DM-IRB; Leica Microsystems, Bannockburn, IL) with 40X oil objective (numerical aperture 1.3) to observe 3D organization of cells within spheroids and around incorporated beads. For bulk modulus measurements we acquired images using an inverted Nikon Eclipse microscope equipped with a 20X dry objective (NA 0.5) and an Andor NEO camera.

FCS measurements

The fluorescence correlation spectroscopy (FCS) measurements were performed on a homemade confocal microscope based on an Olympus IX 71 platform, using a 63X, NA 1.2 water immersion Zeiss objective. Excitation was performed with a diode pumped solid state laser (Cobalt, Solna, Sweden) at 561 nm with a power of a few tens of µW in the sample. Fluorescence was first filtered, using a long pass dichroic mirror at 600 nm (Chroma) and then focused on a 50 µm multimode fibre that acts as a pinhole to be detected with an avalanche photodiode. Optical adjustments were performed in the solution surrounding the beads, the multicellular aggregates or the gel, as the observation was done just above the cover slide. The autocorrelation curves of hydrophilic molecules sulforhodamine B (SRB) at concentrations ≈ 20 nM in PAA gel and beads, were obtained by averaging 2 to 4 acquisitions, lasting 20 s each. They looked like normal diffusion, but slower than in solution, because of hydrodynamic interactions between the molecules and the polymer chains[12], [11]. The averaged autocorrelation functions, weighted with SEM, were fitted for times longer than 2 µs, with a one component FCS model and a fixed triplet time of 2 µs, in order to estimate the number of molecules N and especially the diffusion time τ[13, 14]. In case of beads embedded in multicellular aggregates, it was necessary to correct for photobleaching before autocorrelating, to avoid a bias of the diffusion time[15].

Compression measurements – osmotic stress on beads and spheroids

To determine mechanical properties of PAA microbeads, beads were exposed to a number of osmotic pressures controlled by the concentration of dextran (MW 1.5-2 MDa, Sigma) in the solution. Given the hydrodynamic radius = 27 nm of these dextran molecules and the pore size of the hydrogels (less than 17 nm [16]), the molecules are large enough not to penetrate within the polyacrylamide beads. For each pressure condition, deformation of more than 10 beads was measured. Briefly, focus has

been adjusted at the equatorial plane and the diameter has been measured, either manually for non-fluorescent beads, or automatically for FITC-dextran containing beads (IsoData threshold, ImageJ). For simplicity, we define the strain as positive by $(V_0 – V)/V_0$.

Similarly to beads, spheroids were exposed to an isotropic compressive stress by supplementing the medium with a concentration of the same large biocompatible polymer than for beads[7].

Compression measurements – mechanical deformation with a steel bead

To deform gel locally in a controlled manner we used steel beads of mass 55.2 mg and diameter 2.38 mm. Briefly, steel beads were gently deposited on the top of flat PAA gels and the vertical deformation (Δh) has been measured by recording confocal images on a vertical plane with an exposure time of 2 s. The upper and lower surfaces are detected by using a threshold method. Moreover, the characteristic diffusion time has been measured in control region (no deformation) and beneath the bead where the gel was compressed. In this case, the strain was calculated as $(h_0 – h)/h_0$ assuming only a vertical deformation

Supplementary figures

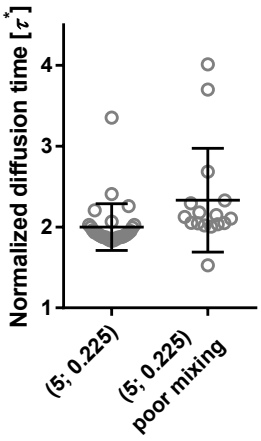

Figure S1 Graph illustrates the importance of the PAA beads fabrication protocol. For optimized conditions the characteristic diffusion time varies little, as compared for the batch were mixing was insufficient. N=20, Error bars S.E.M

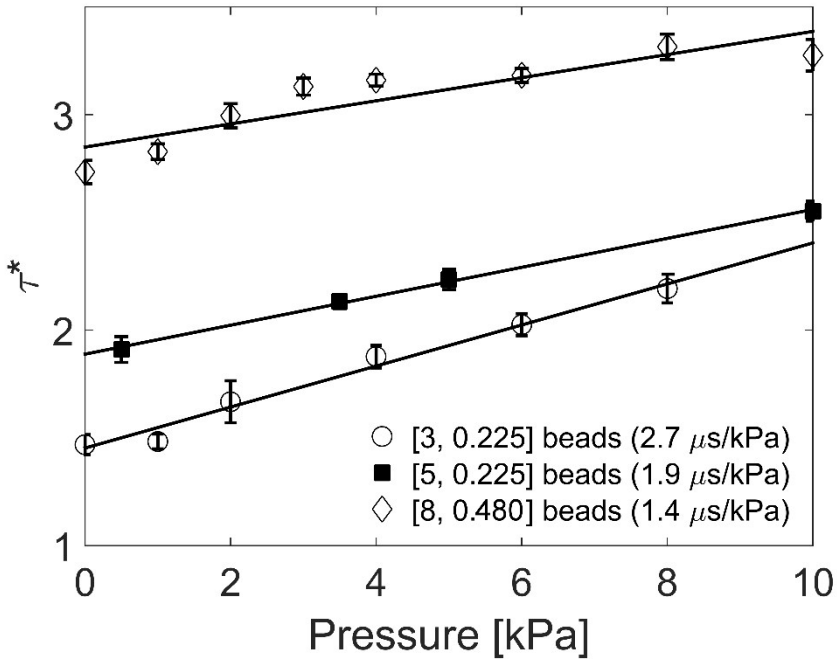

Figure S2 Normalized diffusion time calibration curves for different PAA microbeads compositions: (Acrylamide %, Bisacrylamide %) (3, 0.225), (5, 0.225), and (8, 0.264). Error bars are S.D. The linear fits (τ=A Pressure + B) are as follows: gel (3, 0.225): A = 0.095 ± 0.006, B = 1.45 ± 0.03, R^2 = 0.98; gel (5, 0.225): A = 0.067 ± 0.002, B = 1.89 ± 0.01, R^2 = 0.99; gel (8, 0.480): A = 0.05 ± 0.01, B = 2.85 ± 0.06, R^2 = 0.81

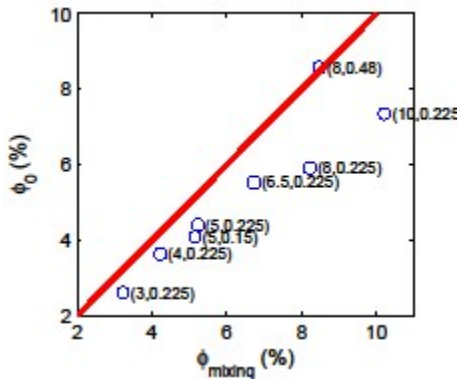

Figure S3: plot of the measured polymer mass fraction ϕ_0 after swelling, as a function of the initial mass fraction ϕ_{mix} of the gel before swelling. The acrylamide and bis-acrylamide initial compositions are noted in percent. The red line corresponds to the case where both volume fractions are the same.