Electronic Supplementary Material (ESI) for Soft Matter.

Cellular Micromasonry: Biofabrication with Single Cell Precision

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Supplementary Text

Rheology of Jammed Microgels

The methacrylic acid microgel particles are synthesized in house, as described in Materials and Methods, and swollen in DMEM supplemented with 5% FBS and 1% penicillin streptomycin, providing the necessary nutrients for cells to function. To determine the material and flow properties of the jammed microgels, we conduct traditional rheological tests including frequency sweeps (1% strain amplitude) and unidirectional shear-rate sweeps (Fig. S1). We find that microgel media prepared within a polymer concentration range of 5 - 6% is well suited for use with the micromasonry technique. Within this range, the microgel media has an elastic shear modulus between 10 and 20 Pa and a yield stress between 1.3 and 2.4 Pa. The stresses measured in shear-rate sweeps are often divided by the shear-rate to determine the viscosity. While the microgel media is dominantly solid-like at low shear-rates, at high shear rates the microgel media becomes a shear-thinning fluid, exhibiting a decreasing viscosity with increasing shear-rate. We use this viscosity curve to estimate the range of Reynold's number the media exhibits during micromasonry procedures.

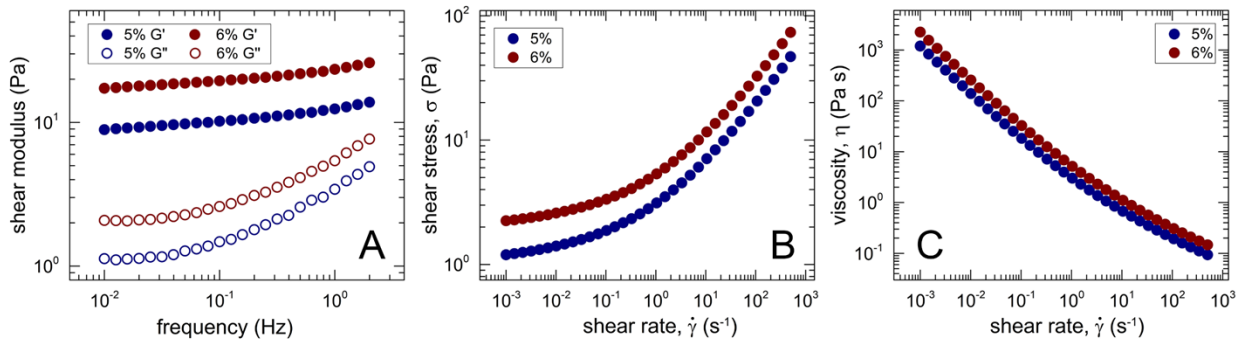

Fig. S1. A) A frequency sweep is performed from 10^{-2} to 2 Hz. *G*' is weakly dependent on frequency and always greater than *G*" for both 5% and 6% MAA microgels. These rheological properties indicate that the microgel media is dominantly solid-like at low levels of strain. B) To determine the yield stress of the microgel media, we perform shear-rate sweeps while measuring shear stress. The plateau in shear stress at low shear-rates corresponds to the yield stress of the microgel media, which we find to be between approximately 1.3 and 2.4 Pa. C) While the viscosity of the microgel media is several orders of magnitude greater than water, cells experience extremely low shear stresses during micromasonry procedures because of the low shear-rates involved.

Microgel Flow and Reversible Cell Displacement Near Translating Capillary Tips

During micromasonry procedures, it is possible that the microgel medium flows excessively and that dispersed cells displace irreversibly because of material's rheological properties. To investigate the degree of microgel flow and irreversible cell displacement during micromasonry procedures, we performed a series of tests in which a microcapillary is translated back and forth near suspended cells. Bright-field and fluorescence videos are collected throughout the tests to monitor the motion of the microcapillary, the cells, and the supporting microgel medium (Figure S2). After one cycle, we see that the cells displace by less than one cell diameter. Since the microgel medium is inherently an optically grainy material, we can use particle image velocimetry (PIV) to measure the entire displacement field after one cycle (Figure S3). We find the root-mean-square displacement, averaged over the entire field of view, to be 4 μm, which is less than a single cell diameter.

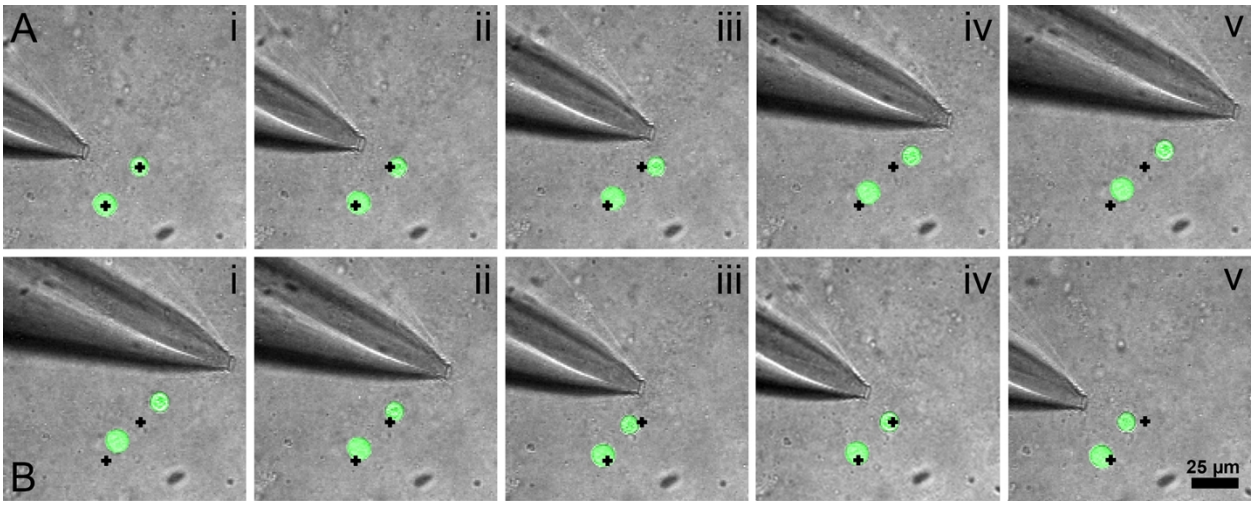

Fig. S2. MDCK cells are dyed with cell tracker green and dispersed in 5% MAA microgel media. (A) A glass microcapillary is translated rightward, passing two cells. The cells' initial positions are marked with a "+". The cell closest to the capillary displaces by more than 1 cell diameter; the cell further from the capillary displaces by less than one diameter. (B) The needle tip is translated leftward, back past the cells. Both cells are moved toward their initial locations, having a final displacement of less than one cell diameter.

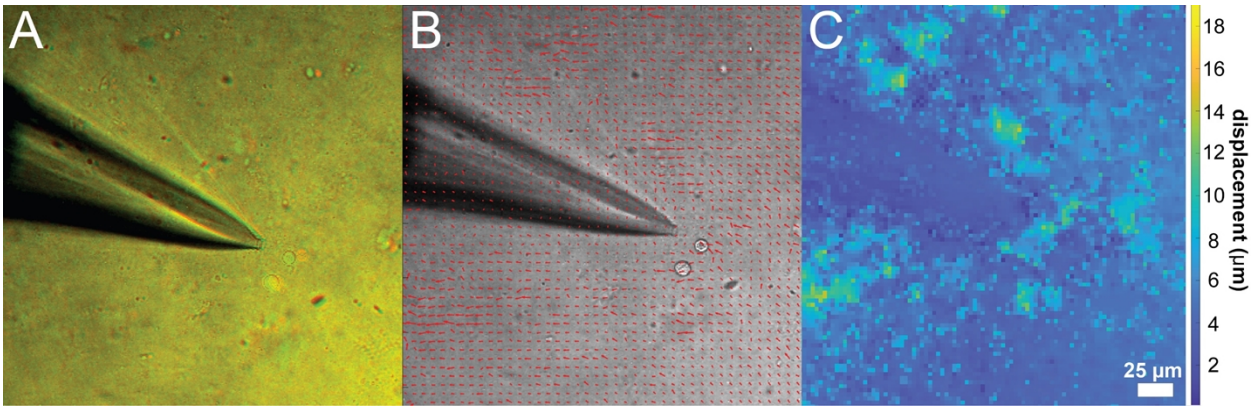

Fig. S3. The microcapillary is translated right and left, past two MDCK cells, through 5% MAA microgel media. (A) False coloring the initial time-point in green and the final time-point in red, an overlay image shows the net displacements through a visible red and green patchiness. (B) We perform PIV to quantify the displacement field throughout the field of view. (C) Creating a displacement map from the absolute values of the displacement vectors, we see that displacements are patchy and largest near the microcapillary. The RMS displacement is 4 μm.

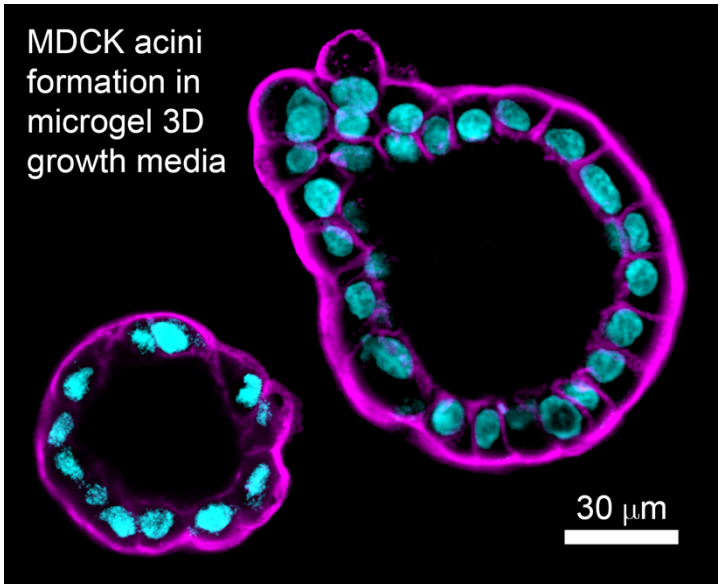

Fig. S4. To test for cell function within the microgel-based 3D media employed in micromasonry, we perform glandular acini culture assays. Isolated cells are dispersed in the microgel media and incubated for 10 days, then fixed and stained with Hoeschst 33342 and Alexa 594 phalloidin. Confocal fluorescence microscopy is performed to test for the emergence of the characteristic monolayer shell structure as well as the polarized cytoskeletal structure typically found in acini. Here we show one acinus not shown in the main manuscript (lower left) and another larger acinus of which a small section is shown in Figure 3E (upper right).

Movie S1. To create structures from singe cells, we dye MDCK cells with cell tracker green and disperse them in 5% MAA microgel media. Using a confocal microscope, we identify a chosen cell, translate the tip of a microcapillary to its surface using the micromanipulator, and lightly aspirate using a CellTram (Eppendorf), applying suction. Once the cell is captured, it is translated to the desired location and deposited next to the previous cell.

Movie S2. To determine whether the micromasonry process leads to irreversible, long ranged, or unpredictable flow patterns in the microgel medium, MDCK cells are dyed with cell tracker green and dispersed in 5% MAA microgel media and a microcapillary is translated through the microgel medium, moving at approximately 0.5 mm/s, which is

the rate we translate the microcapillary during micromasonry procedures. We see that when the microcapillary is reciprocally translated near suspended cells, the net cell displacement is approximately one cell diameter or less.

Movie S3. To observe the behavior of dissociated *Nematostella vectensis* embryos, the cells are dispersed in the microgel medium along with fibronectin-coated polystyrene microspheres. We placed the disassociated embryonic cells around a fibronectin-coated bead. Time-lapse microscopy revealed the cells remained viable and motile, actively spreading on the bead over the course of 13 hours. (false colored; inset: uncolored bright-field image).

Supplementary Methods and Materials

Jammed Microgel Synthesis

To synthesize polyacrylamide microgels with 17 mol% methacrylic acid, a solution of 8% (w/w) acrylamide, 2% (w/w) methacrylic acid, 1% (w/w) poly(ethylene glycol) diacrylate (MW = 700 g mol−1), and 0.1% (w/w) azobisisobutyronitrile in ethanol (490 mL) is prepared. The solution is sparged with nitrogen for 30 min, then placed into a preheated oil bath set at 60 °C. After approximately 30 min, the solution becomes hazy and a white precipitate begins to form. The reaction mixture is heated for an additional 4 hours. At this time, the precipitate is collected by vacuum filtration and rinsed with ethanol on the filter. The microparticles are triturated with 500 mL of ethanol overnight. The solids are again collected by vacuum filtration and dried on the filter for approximately 10 min. The particles are dried completely in a vacuum oven set at 50 °C to yield a loose white powder. The purified microgel powder is dispersed in cell growth media at various concentrations and mixed at 3500 rpm in a centrifugal speed mixer in 5-min intervals until no aggregates are apparent (17, 18). The microgel is then neutralized to a pH of 7.4 with NaOH and 25mM HEPES buffer (Part no. BP299-100) and is left to swell overnight, yielding microgel growth media at concentrations of 5-6% (w/w).

In the case of embryonic experiments, zwitterionic microgels are synthesized in the same method from a solution of acrylamide (AAm), poly(ethylene glycol) diacrylate (PEGda) (MW = 700 g mol^{-1}), azobisisobutyronitrile (AIBN), and carboxybetaine methacrylate (CBMA) as an ionisable comonomer in ethanol (490 mL). The purified microgel powder is dispersed in sea water at 8% polymer concentration and mixed at 3500 rpm in a centrifugal speed mixer in five-minute intervals until no aggregates are apparent. The microgel is then left to swell overnight, yielding microgel the 3D growth media.

For acini assays, an uncharged microgel is synthesized to minimize the potential for non-specific charge interactions to occur with the components of Matrigel. Polyethylene glycol (PEG) is chosen as the microgel polymer species to serve this purpose. To synthesize PEG microgels, a solution of 25% (w/w) poly(ethylene glycol) methyl ether acrylate (M_n = 480 g mol^{-1}, Sigma

Aldrich 454990), 0.4% (w/w) poly(ethylene glycol) diacrylate (M_n = 700 g mol^{-1}, Sigma Aldrich 455008), and 0.15% (w/w) ammonium persulfate (Sigma Aldrich A3678) is prepared in ultrapure water. Separately, a 0.7% (w/w) polyglycerol polyricinoleate (a commercial surfactant, Paalsgaard PGPR-4125) solution is prepared in kerosene. The aqueous and organic solutions are then combined in a beaker, placed on an ice bath, and homogenized at 8000 rpm for 5 minutes to form a milky-white emulsion. Nitrogen is bubbled through the covered solution for 1 hour to displace dissolved oxygen. The deoxygenated reaction mixture is removed from ice and transferred to a 1L round bottomed flask equipped with a magnetic stirring bar. At this time, 0.5% (w/w) of 1,2-Di(dimethylamino)ethane (TEMED, Fisher Scientific BP150) is added dropwise while the solution is stirred in a nitrogen atmosphere for 1 hour. The solution is then exposed to air and stirred for 30 minutes to complete the reaction. To separate the microgels from the kerosene phase, methanol is added to the microgel solution and the mixture is vigorously shaken in 500mL centrifuge tubes followed by centrifugation at 3500 rpm for 5 minutes and removal of supernatant; these washing steps are performed three times to completely remove the surfactant and organic phases. Finally, PBS is added in place of methanol and the washing/centrifugation/supernatant removal steps are repeated twice. The microgel solution is then sterilized by autoclaving for 30 minutes in a liquid cycle at 120 ℃ and 15 psi. The sterile microgel solution is then centrifuged at 2600 rpm for 30 minutes and supernatant is discarded to get 5.7% (w/w) PEG microgels. The microgels are swollen in DMEM and mixed at 3500 rpm in a centrifugal speed mixer for 5 minutes then neutralized to a pH of 7.4 with NaOH. The DMEM-microgel formulation is mixed with Matrigel at final concentrations of 5% (w/w) PEG microgel and 1mg/mL Matrigel.

Cell Culture

MDCK cells (Madin Darby Canine Kidney epithelial cells, NBL-2 ATCC CCL-34) are cultured in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L glucose, L-glutamine, and sodium pyruvate supplemented with 10% FBS and 1% penicillin streptomycin in a 12 well plate. When the cells have reached 70% confluence, a single well is dyed with live cell dye. For control studies, one well is dyed with CellMask orange plasma membrane stain (Thermo-Fisher, part no. C10045) and a separate well with CellTracker green (CMFDA) (Thermo-Fisher, part no. C2925). For gap junction investigations, one well is dyed with CellMask orange and a separate well with CellTraceCalcein Green AM (Thermo-Fisher, part no. C34852), which becomes fluorescent green in live cells. We passage the wells separately by washing with PBS then incubating in 5%

Trypsin—EDTA solution for 5 minutes. The cells are harvested from the plate and placed into separate 15mL centrifuge tubes, where they are centrifuged at 650g for 4 minutes. The supernatant is removed and each pellet is suspended in 1 mL of fresh cell growth media. The cell pellets are dispersed with gentle pipette mixing and 100 μL of each solution is placed in a fresh 15 mL tube and mixed together with light pipetting. About 50 μL of the solution is dispersed in the jammed microgel using a pipet. The microgel 3D culture medium is prepared for each cell type using the corresponding liquid media. 35-mm glass bottom petri dishes are used to facilitate fluorescence imaging. 3 mL of microgel media is loaded into each well. Prior to adding cells, dishes containing microgel media are incubated at 37 °C and 5% CO2.

Acini Culture, Staining, and Imaging

MDCK cells (Madin Darby Canine Kidney epithelial cells, NBL-2 ATCC CCL-34) are cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% penicillin streptomycin in a 6 well plate. At 70% confluence, the cells are passaged from a single well by washing with PBS and incubating in 1mL of 5% Trypsin-EDTA solution for 10 minutes. The trypsin solution is neutralized by adding cell growth media at 90% (v/v) and repetitive pipetting with a 5mL serological pipette is done close to the bottom of the well to create a single cell suspension. The cells are harvested from the plate, transferred to a 15mL centrifuge tube and centrifuged at 180 xg for 5 minutes. The supernatant is removed and the cell pellet is resuspended in cell growth media to create a cell solution at a density of 50,000 cells/mL. In parallel, a 96 well glass-bottom plate are prepared with 200μL of 5% (w/w) PEG microgel / 1mg/mL Matrigel mixture per well and incubated at 37 °C and 5% CO_2. The single cell suspension is then dispersed into the microgel medium at a density of 10,000 cells/mL/well and the cells are cultured for 10 days. To observe acini development, the tissues are fixed at desired timepoints by adding 4% (v/v) paraformaldehyde directly over the microgel medium and the plate is left at room temperature (RT) for 1 hour. Following fixation, the tissue cells are permeabilized for 30 minutes by adding 1% (v/v) Triton X-100 at RT and then rinsed by adding 50% (v/v) PBS directly over the microgel medium for 10 minutes. Tissue cells are then blocked for 2 hours by adding PBS with 5% (w/v) BSA and 0.1% (v/v) Triton X-100 at RT and then rinsed with PBS. Following blocking, an actin staining solution that comprises PBS with 1% (w/v) BSA, 0.01% (v/v) Triton X-100, and ActinGreen 288 ReadyProbes Reagent (Thermofisher R37110) at a final concentration of 2 drops/mL is added directly over the microgel medium. The plate is then covered with aluminum

foil and transferred to 4 °C overnight. The tissues are then rinsed with PBS thrice and a nucleus staining solution that comprises PBS with Hoechst 33342, Trihydrochloride, Trihydrate (Thermofisher H3570) at a final dilution of 1:200 is added directly over the microgel medium. The plate is then covered with aluminum foil and transferred to 4 °C overnight. The tissues are then rinsed with PBS thrice and the acini are imaged using a 63x oil objective on Zeiss LSM 980 confocal microscope.

Embryo Experiments

Nematostella vectensis embryos were obtained from a breeding colony in the Martindale lab at the Whitney Lab for Marine Bioscience (Univ. Florida). Zygotes are deljellied and injected with mRNAs to GFP or mCherry to generate fluorescently labeled blastomeres. Embryos are grown to blastula stages (24-48 hours post fertilization) and dissociated into individual cells using calcium/magnesium free seawater plus 5 mM egtazic acid (EGTA). The embryos are placed in a petri dish and approximately 2 mL of the dissociation solution is added. The embryos are mechanically dissociated using a pipette for 1 minute or until there are no large pieces of tissue visible. The dissociated embryo solution is transferred to a 40 μm cell strainer positioned over a clean petri dish and washed with the dilution solution.

Building Single Cell Structures

Once the cell loaded petri dish is prepared, it is moved to a Nikon C2+ laser scanning confocal microscope. An incubating plate is used to keep the dish warm during the building process. A thin layer of oil is placed over the gel to prevent evaporation. Microcapillary translation is achieved using a Siskiyou MX7600 micromanipulation system and the capture and release of cells is achieved using an Eppendorf CellTram. The microcapillaries are fabricated using a Sutter P-97 micropipette puller and smoothed using a Narshige microforge. To initiate the micromasonry process, the microcapillary is slowly lowered into the gel and centered in the chose field of view using a 4X objective. A ‘layout’ image is taken at 10X magnification to visualize cell locations and colors for specific builds. Structures are built using a 10X objective with an additional 1.5X zoom lens.