Anisotropic material synthesis by capillary flow in a fluid stripe
SUPPLEMENTARY INFORMATION

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Supplementary Figures

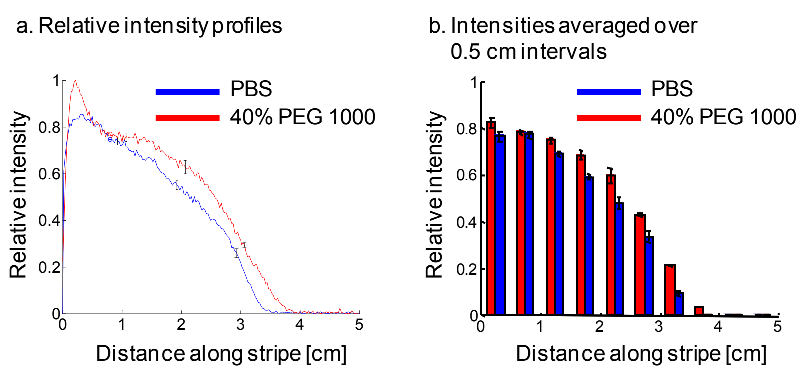

Figure S1. Schematic of averaging relative intensity values over 0.5 cm intervals for later significance testing. (a) Relative intensity profiles along stripe. (b) Discretized profiles obtained by averaging relative intensity profiles in each 0.5 cm interval along stripe.

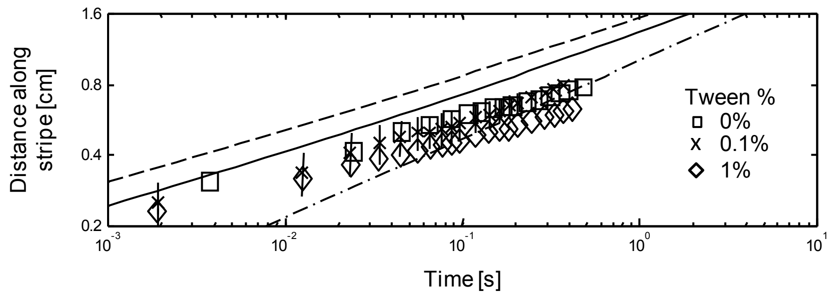

Figure S2. Effec t of surface tension on flow speed. Measured flow speeds following droplet addition onto fluid stripe are plotted in dimensionless coordinates and compared to a previously developed theoretical model.[1] Droplet and pre-wet stripe consist of 20% PEGDM 1000 and the same concentration of the surfactant Tween-20. Standard deviation over three repetitions is less than symbol size except where noted by error bars. Curves (dashed, solid, dash-dot) are the same as those in Figure 2b of the main text, and correspond to a previously derived mathematical model.[1]

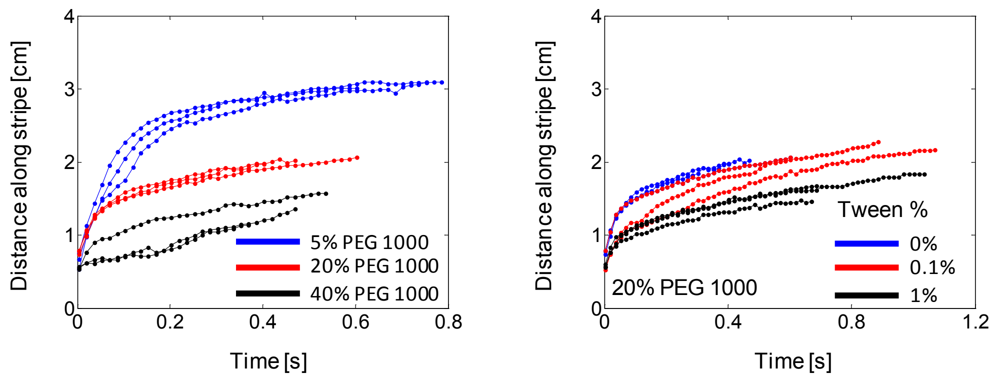

Figure S3. Raw tip position measurements in time during gradient generation, for (a) different concentrations of PEGDM 1000 and (b) 20% PEGDM 1000 with different concentrations of the surfactant Tween-20. Speed decreases with PEG and Tween concentration. Each point is the position of the tip in one frame captured from video. The three lines of each color in (a,b) are replicates of each case. The data in (a,b) corresponds to the scaled averaged positions plotted in Fig. 2a and S2.

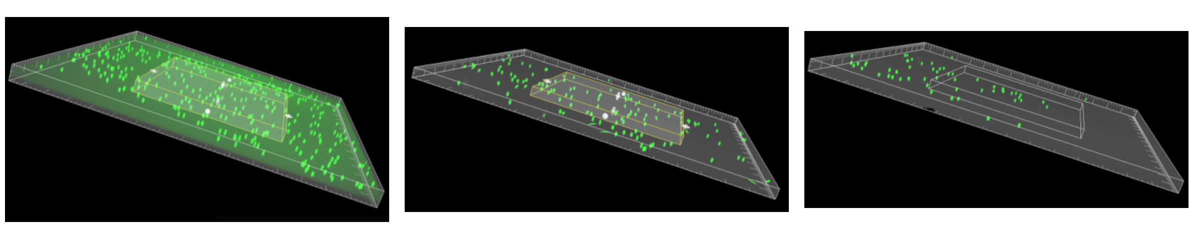

Figure S4. Definition of central regions for confocal images in drop, middle, and tip region, from left to right.

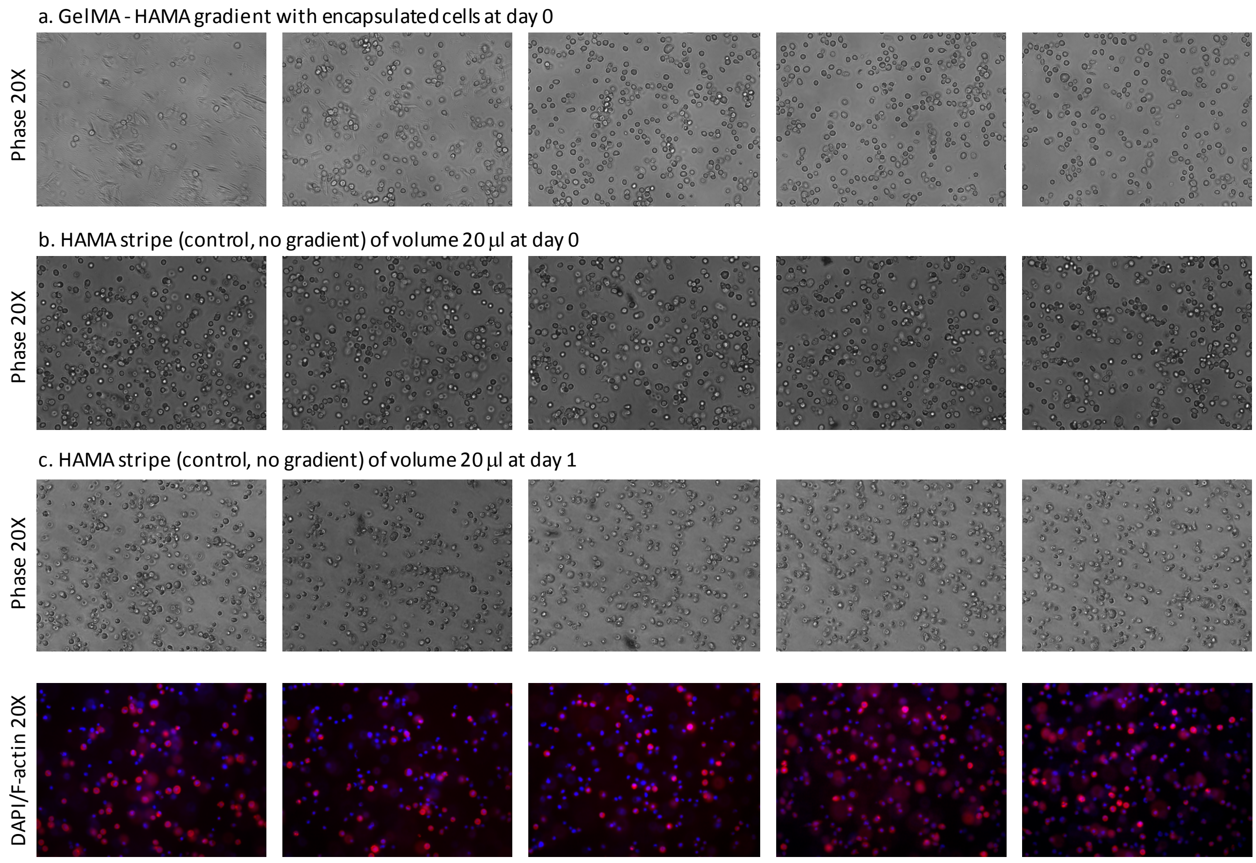

Figure S5. Spreading and concentration on gradients for cells encapsulated in gradient and control hydrogel stripes. Images in each row above were taken near the 0.5, 1.5, 2.5, 3.5, and 4.5 cm locations along the stripe. (a) 20X phase images of encapsulated cells in GelMA-HAMA gradient hydrogel. Cell counts from these images and repetitions were used to create the bar chart in Fig. 5a of main text. (b) 20X phase images of cells on stripe of HAMA at day 0. Cell counts from these images and repetitions used for bar chart in Fig. 5b of main text. (c) Encapsulated cells after 1 day of culture. Both phase and fluorescence images shown. No spreading is observed. Additional caption details may be found in Figure 5 of the main text. All images have the same scale; scale bar = 100 µm.

Table S1. Viscosities of various aqueous polymer solutions found in the literature. For PEG 1000, 2000, 4000, values listed are for PEG dissolved in water (i.e. no salt). We used PEG dissolved in 1X PBS, which had approximately 1% salt content. In the literature, viscosity measurements at this relatively low salt content are approximately the same as those in water,[2] much like the similarity between the viscosities of PBS and water. Values can vary in the literature. For example, ref[3] lists the viscosity of 30% PEG 1000 in water at 298 K as 9.2 cSt, while ref[4] lists the viscosity of 29.5% PEG 1000 at 293 K as 6.3 cSt, which should be higher than the viscosity at 298 K since viscosity decreases with temperature. General fitting formulas have been provided for the viscosity of PEG over different MWs and temperatures.[4] The viscosity of HA varies with shear rate.[5] For 1% HA samples whose average MW is ~50 kDa, the viscosity is approximately constant below the critical shear rate of 164 s^{-1}.[5] Based on the data for Fig. 2b in the main text, the flow rates measured on our fluid stripes for higher viscosity solutions such as 40% PEG 1000 were initially of order 10 cm s^{-1} and decreased to less than 3 cm s^{-1} after approximately 100 ms. Our 15 to 24 μl pre-wet stripes were approximately 200 to 400 μm deep.[1] Thus, the shear rates ranged from 250 to 500 s^{-1} for the initial speed of 10 cm s^{-1} and decreased to less than 150 s^{-1} after approximately 100 ms (when speeds decreased to less than 3 cm s^{-1}). Thus, during the first 100 ms of the flow, the viscosity of HA could have been reduced from its value listed above, but after approximately 100 ms, the shear rates should have been below the critical shear rate.

Aqueous solutions	Viscosity [cm^2 s^{-1}] at 298 K	Viscosity [cSt] at 298 K	Comment/Reference
Water	0.008927	0.8927	Table 14 ref[6]
PBS	0.008997	0.8997	Interp. from Tables 14 & 15 in ref[6]
PEG 1000			
5%	0.0122	1.22	Interp. from table 1, ref[7]
10%	0.0165	1.65	Interp. from table 1, ref[7]
20%	0.034 [293 K]	3.4 [293 K]	Table 1 ref[8] and Table 2 ref[4]
30%	0.092	9.2	Table 2, ref[3]
40%	0.132	13.2	Table 2, ref[3]
40% PEG 2000	0.19378	19.378	Table 1, ref[9]
PEG 4000			
5%	0.0169	1.69	Interp. from table 1, ref[7]
10%	0.0659	6.59	Interp. from table 2, ref[3]
20%	0.111	11.1	Interp. from table 2, ref[3]
30%	0.222	22.2	Interp. from table 2, ref[3]
40%	0.469	46.9	Interp. from table 2, ref[3]
1% HA (avg. MW 50 kDa)	0.5	50	H-4 series in Figure 4, ref[5] (CM=5000 g/mL for MW ~50kDa)
1% Heparin (avg. MW 15.6 kDa)	1.5*0.0089 =0.0134	1.34	Figure 2 in ref[10]
2% Heparin (avg. MW 15.6 kDa)	2*0.0089 = 0.0178	1.78	Extrapolation from Fig 2 in ref[10]
2% Gelatin	0.0251 [303 K]	2.51 [303 K]	Figure 1 ref[11]
5% Gelatin	0.09 0.05 [303 K]	9 5 [303 K]	Figure 3 ref[12]

Table S2. Surface tensions of various aqueous solutions. Values gathered from literature. Surface tension in 1% (w/v) sodium chloride solutions approximately that of distilled water.[13]

Solution	Surface tension [dynes/cm] at 298 K	Comment/Reference
Distilled water	72	"Properties of water" from ref[14]
Tween-20 above CMC (0.04 mM), in water	33 [295K]	Fig. 3 of ref[15]
PEG 1000		
5%	65.8	Interpolated from Fig. 1A of ref[16]
20%	64.6	Interpolated from Fig. 1A of ref[16]
40%	64.0	Interpolated from Fig. 1A of ref[16]

Table S3. Significance testing for Figure 3(a-i). For each gradient profile in Figure 3(a-i), the corresponding relative intensities of the three repeated trials were averaged over 0.5 cm intervals to produce data series consisting of 10 intensity values (Fig. S1). Balanced two-way ANOVA testing in Matlab produced p-values for each pair of data series, listed below. Lines highlighted in gray indicate p-values **above** 0.05.

Case	p-value	Case	p-value
Concentration - PEG 1000		Concentration - PEG 4000	
40% - 20%	0.000277	40% - 20%	1.33E-10
40% - 10%	0.596922	40% - 10%	2.55E-15
40% - 5%	0.189531	40% - 5%	0
40% - PBS	0.000713	40% - PBS	0
40% - 30%	0.077417	40% - 30%	6.73E-05
20% - 10%	0.000203	20% - 10%	1.03E-05
20% - 5%	4.16E-07	20% - 5%	6.55E-08
20% - PBS	9.99E-16	20% - PBS	1.09E-11
20% - 30%	2.27E-09	20% - 30%	0.662963
10% - 5%	0.0459	10% - 5%	0.620671
10% - PBS	7.25E-06	10% - PBS	0.123495
10% - 30%	0.00961	10% - 30%	0.016926
5% - PBS	0.035577	5% - PBS	0.205516
5% - 30%	0.711279	5% - 30%	0.005608
PBS - 30%	0.045853	PBS - 30%	0.000821
Concentration - GelMA		Prewet vol - 40% PEG 1000	
PBS - 3%	3.41E-07	15 ul - 20 ul	0.337104
PBS - 5%	5.77E-15	15 ul - 24 ul	2.29E-07
3% - 5%	0.001877	20 ul - 24 ul	4.16E-09
Prewet vol - 20% PEG 1000		Prewet vol - 5% GelMA	
15 ul - 20 ul	0.487121	15 ul - 20 ul	0.298923
15 ul - 24 ul	0.00716	15 ul - 24 ul	0.003603
20 ul - 24 ul	0.000183	20 ul - 24 ul	0.003314

Vol Ratio - 20% PEG 1000		Vol Ratio - 40% PEG 1000	
1/6 - 1/4	1.81E-12	1/6 - 1/4	1.33E-07
1/6 - 1/3	0	1/6 - 1/3	1.11E-16
1/6 - 1/2	0	1/6 - 1/2	5.30E-09
1/6 - 3/4	0	1/6 - 3/4	0
1/4 - 1/3	5.78E-09	1/4 - 1/3	0.005279
1/4 - 1/2	0	1/4 - 1/2	0.054437
1/4 - 3/4	0	1/4 - 3/4	0
1/3 - 1/2	0	1/3 - 1/2	0.81882
1/3 - 3/4	0	1/3 - 3/4	0
1/2 - 3/4	0	1/2 - 3/4	0
Width - 20% PEG 1000		Width - 40% PEG 1000	
1 mm - 1.5 mm	0.000201	1 mm - 1.5 mm	0.006541
1 mm - 2 mm	1.25E-06	1 mm - 2 mm	0.001128
1.5 mm - 2 mm	0.597803	1.5 mm - 2 mm	0.170409
Profile Class 1		Profile Class 2	
20% P1000 - 10% P1000	0.000203	40% P1000 - 10% P4000	0.193579
20% P1000 - 5% P1000	4.16E-07	40% P1000 - 5% P4000	0.042254
20% P1000 - PBS	9.99E-16	40% P1000 - 30% P1000	0.07733
20% P1000 - 3% Gel	0.720241	40% P1000 - 5% Gel	6.41E-05
20% P1000 - 20% Hep	7.20E-12	10% P4000 - 5% P4000	0.622228
10% P1000 - 5% P1000	0.0459	10% P4000 - 30% P1000	0.884524
10% P1000 - PBS	7.25E-06	10% P4000 - 5% Gel	3.14E-06
10% P1000 - 3% Gel	0.028727	5% P4000 - 30% P1000	0.647624
10% P1000 - 20% Hep	2.50E-13	5% P4000 - 5% Gel	1.71E-08
5% P1000 - PBS	0.035577	30% P1000 - 5% Gel	1.35E-08
5% P1000 - 3% Gel	0.000474	Profile Class 3	
5% P1000 - 20% Hep	1.07E-14	40% P4000 - 40% P2000	1.24E-06
PBS - 3% Gel	2.31E-07	40% P4000 - 30% P4000	6.83E-05
PBS - 20% Hep	0	40% P4000 - 1% HAMA	0.2031
3% Gel - 20% Hep	1.15E-05	40% P2000 - 30% P4000	0.039313
Polymer mix		40% P2000 - 1% HAMA	5.66E-05
HA-Hep - Gel-HA	2.25E-05	30% P4000 - 1% HAMA	0.000313
HA-Hep - Gel-Hep	1.10E-05	Surface tension	
HA-Hep - PEG	0	0% - 0.1%	0.130366
HA-Hep - PEG-Gel3	3.11E-11	0% - 1%	0.164473
Gel-HA - Gel-Hep	0.413298	0.1% - 1%	0.723969
Gel-HA – PEG	0		
Gel-HA - PEG-Gel3	0		
Gel-Hep - PEG	0		
Gel-Hep - PEG-Gel3	0		
PEG - PEG-Gel3	1.23E-13		

Table S4. Significance testing for Figure 4c. Listed below are the p-values from balanced two-way ANOVA testing for each pair of data series in Figure 4c of the main text. Lines highlighted in gray indicate p-values **less** than 0.05.

Case	p-value	Case	p-value
PEG 1000		other solutions	
40p - 20p	0.055249	DMEMwSER - DMEMnoSER	0.142093
40p - 10p	7.73E-06	DMEMwSER - 1pHAMA	0.91115
40p - 5p	0.000157	DMEMwSER - 2pHepMA	0.777841
40p - PBS	0.083409	DMEMwSER - PBS	0.338257
20p - 10p	0.000458	DMEMnoSER - 1pHAMA	0.015074
20p - 5p	0.010375	DMEMnoSER - 2pHepMA	0.048743
20p - PBS	0.815043	DMEMnoSER - PBS	0.329304
10p - 5p	0.235076	1pHAMA - 2pHepMA	0.406792
10p - PBS	0.004977	1pHAMA - PBS	0.037019
5p - PBS	0.056885	2pHepMA - PBS	0.155059
GelMA			
5p-Gel - 3p-Gel	0.370203		
5p-Gel - PBS	0.830359		
3p-Gel - PBS	0.521487		

Supplementary Videos S1, S2. High-definition (HD) videos were captured of the gradient protocol with pre-wet solutions consisting of 20 μl (S1) 5% and (S2) 40% PEGDM 1000. Droplets consisted of 10 μl of the pre-wet solution plus Trypan blue dye. Due to its higher viscosity, the flow speed was noticeably less for the 40% PEGDM solution than for the 5% solution.

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