SUPPORTING INFORMATION

High-throughput combinatorial cell co-culture using microfluidics

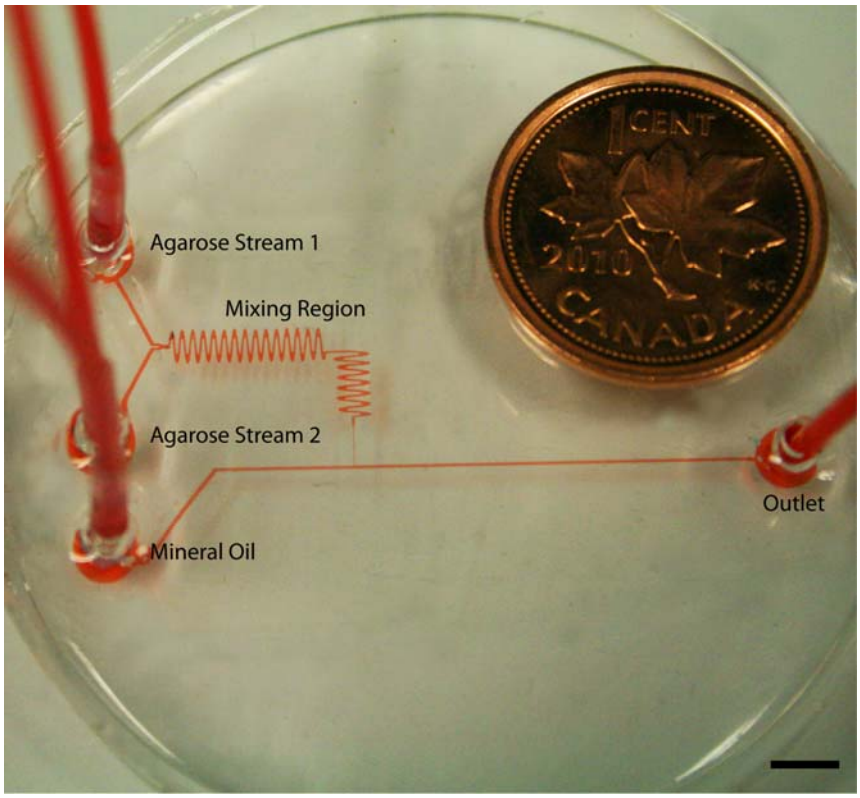

Figure S1. Optical microscope image of the MF device for the generation of cell-laden agarose microgels.

Fig. S1 shows the microfluidic device containing three inlets. At the T-junction, the agarose stream came in contact with the mineral oil. Due to shear forces imposed by the mineral oil, the agarose stream periodically broke into monodisperse droplets. Droplets exited the device at the outlet where they were subsequently collected in HBSS buffer at 4^{o}C. Scale bar is 4 mm. The height of the device is 150 μm. The width of the horizontal channel supplying the mineral oil

phase and the width of the serpentine channel at the point of T-junction are 150 and 20 μm, respectively. The length of the mixing channel is 250 mm.

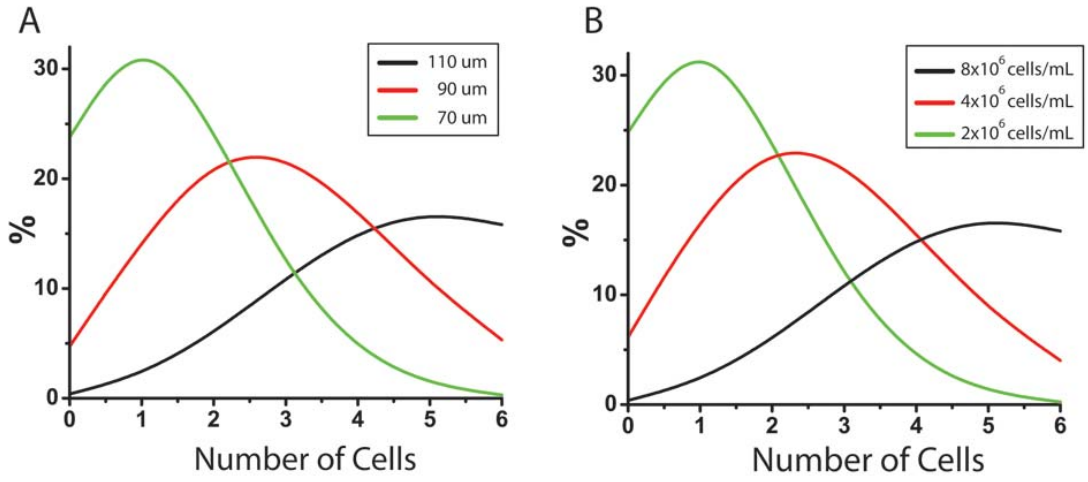

Figure S2. Theoretical Poisson Distribution of Cells in Cell-laden Microgels

Theoretical Poisson distribution curves for the number of cells per microgel were generated by calculating the average number of cells per droplet at a constant cell concentration and droplet volume and subsequently fitting the data to a Poisson distribution by using the Poisson function avaible in Microsoft Excel 2007. Theoretical Poisson distribution of the fraction of microgels containing a particular number of cells **(a)** at a constant cell concentration (8×10^6 cell/mL) and a variable droplet diameter and **(b)** at constant droplet diameter of 110 μm and variable cell concentration.

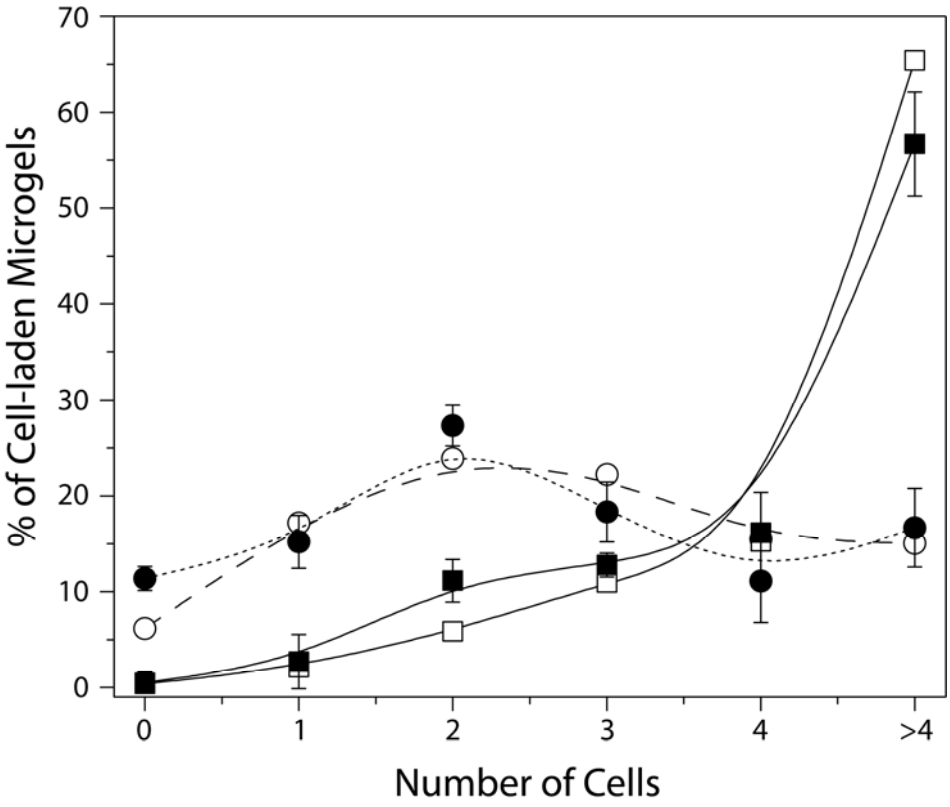

Figure S3. Comparison of the number of encapsulated cells per droplet compared to theoretically expected Poisson Distribution.

Fig. S3 shows the number of cells per microgel determined experimentally (filled symbols) and using Poisson Distribution (empty symbols). The concentration of cells in the feed suspension was $4x10^6$ (dash lines) and $8x10^6$ cells/mL (solid lines). Each experimentally acquired data set was compiled from approximately 300 microgels.

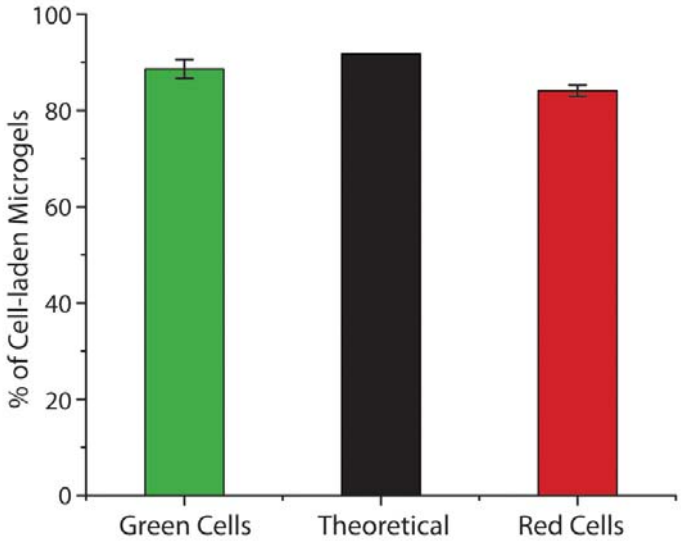

Figure S4. Effect of dilution on encapsulation of individual cell populations

Mixing of two streams of distinct cell suspensions results in the dilution of individual cell populations. For example, for two suspensions, each containing 8 x 10^6 cells/mL, mixing of streams supplied in the MF device at equal flow rates (Q_G:Q_R =1:1) reduced the concentration of individual cell populations to 4 x 10^6 cells/mL. Figure S4 shows good agreement between the theoretical Poisson distribution for the percentage of 110 μm-diameter cell-laden microgels at a cell concentration of 4 x 10^6/mL and the experimental results obtained for the encapsulation of green and red cells in 110 μm-diameter microgels at the flow rate ratio of the corresponding suspensions of 1:1.

Table S1. Calculation of the fraction of droplets, P(*x*), expected to contain *x* cells, for a cell concentration of $2x10^6$ cells/mL at varying droplet diameters. λ is the average number of cells per droplet.

χ / D (μm)	0	1	2	3	4	5	6	λ
	P(χ)							
110	24.81	34.58	24.10	11.20	3.90	1.09	0.25	**1.39**
100	35.09	36.75	19.24	6.72	1.76	0.37	0.06	**1.05**
90	46.61	35.58	13.58	3.46	0.66	0.10	0.01	**0.76**
80	58.50	31.36	8.41	1.50	0.20	0.02	0.00	**0.54**
70	69.82	25.08	4.50	0.54	0.05	0.00	0.00	**0.36**

Table S2. Calculation of the fraction of droplets, P(*x*), expected to contain *x* cells, for a cell concentration of $8x10^6$ cells/mL, at varying droplet diameters. λ is the average number of cells per droplet.

χ / D (μm)	0	1	2	3	4	5	6	λ
	P(χ)							
110	0.38	2.11	5.89	10.95	15.26	17.02	15.81	**5.58**
100	1.52	6.35	13.30	18.58	19.45	16.30	11.38	**4.19**
90	4.72	14.41	22.00	22.39	17.10	10.44	5.31	**3.05**
80	11.71	25.12	26.93	19.25	10.32	4.43	1.58	**2.14**
70	23.77	34.15	24.53	11.75	4.22	1.21	0.29	**1.44**