Artifical Microniches for Probing Mesenthymal Stem Cell Fate in 3D

Supporting Information

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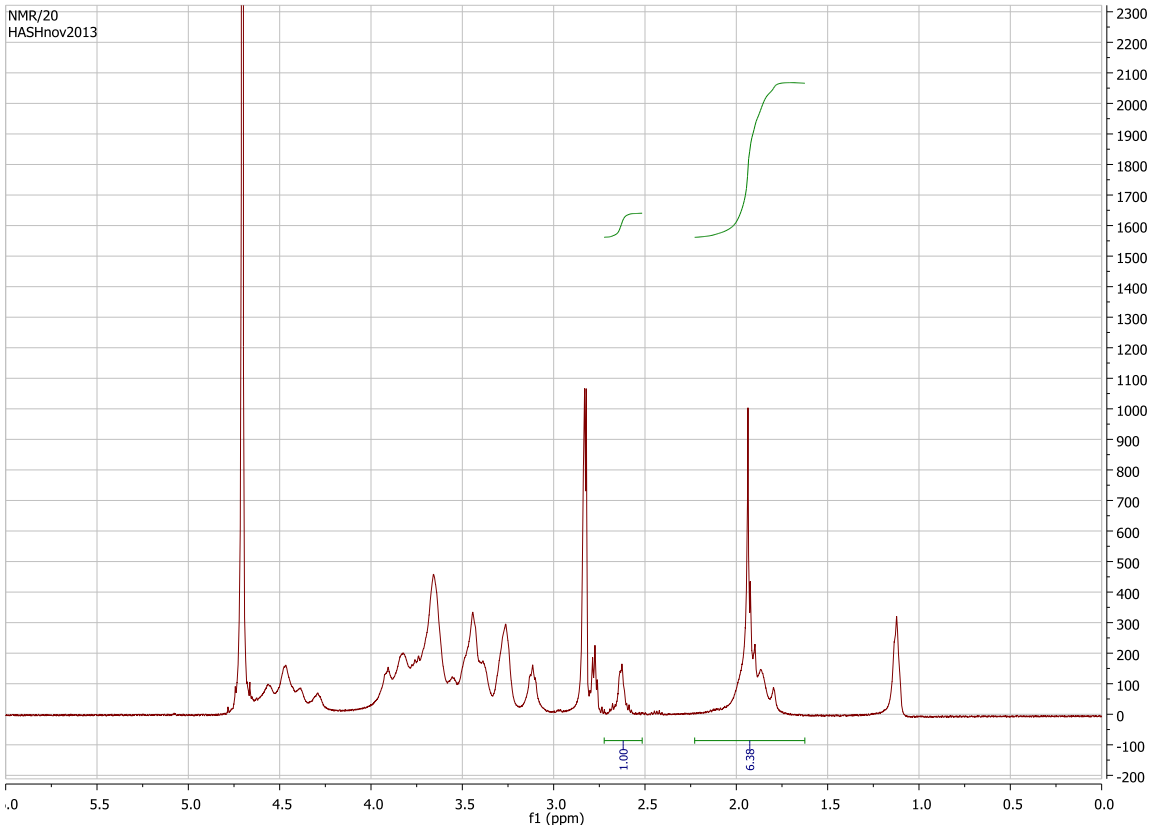

Figure S1 1H NMR spectrum of thiolated hyaluronic acid used in this study, the degree of thiolation is confirmed by Ellman's test to be around 25%.

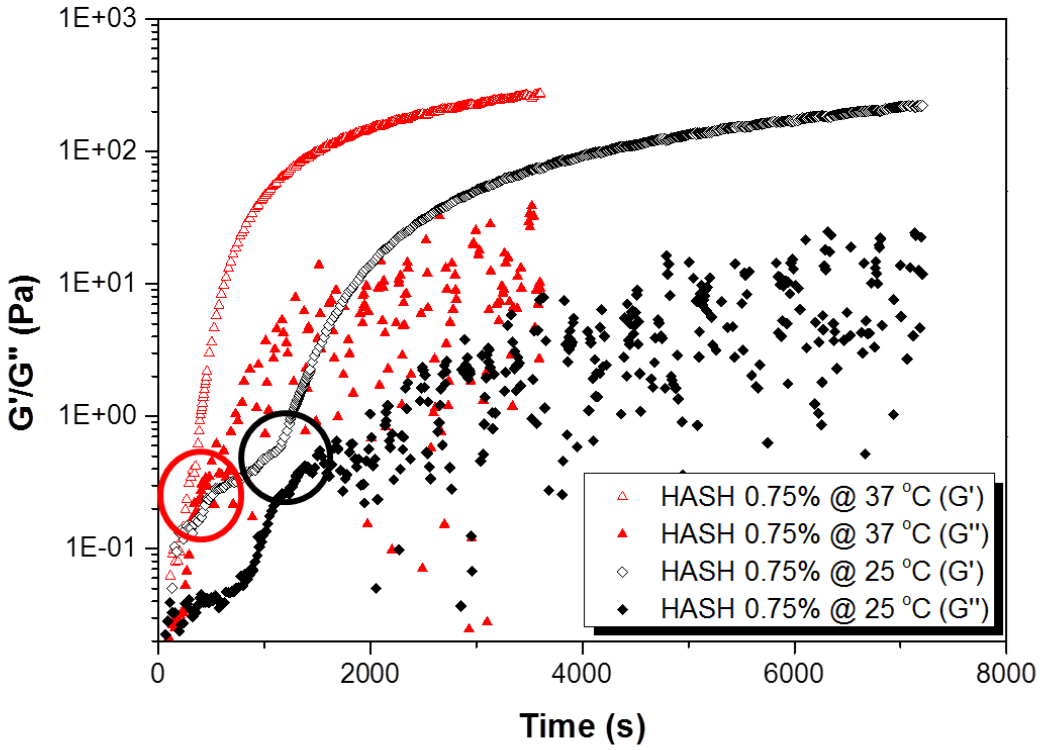

Figure S2 Time-dependent rheological measurements on bulk hydrogels made from thiolated hyaluronic acid (HASH) (0.75%, corresponding PEGDVS and FBNG concentrations used are listed in Table 1) at 25°C and 37°C. It can be seen from the storage (G') and loss (G'') moduli that both the gel point and the time needed to reach the plateau modulus deceases with increasing the temperature. Gel points are marked by circles with corresponding colors showing the transition from liquid-like to solid-like behavior. [1]

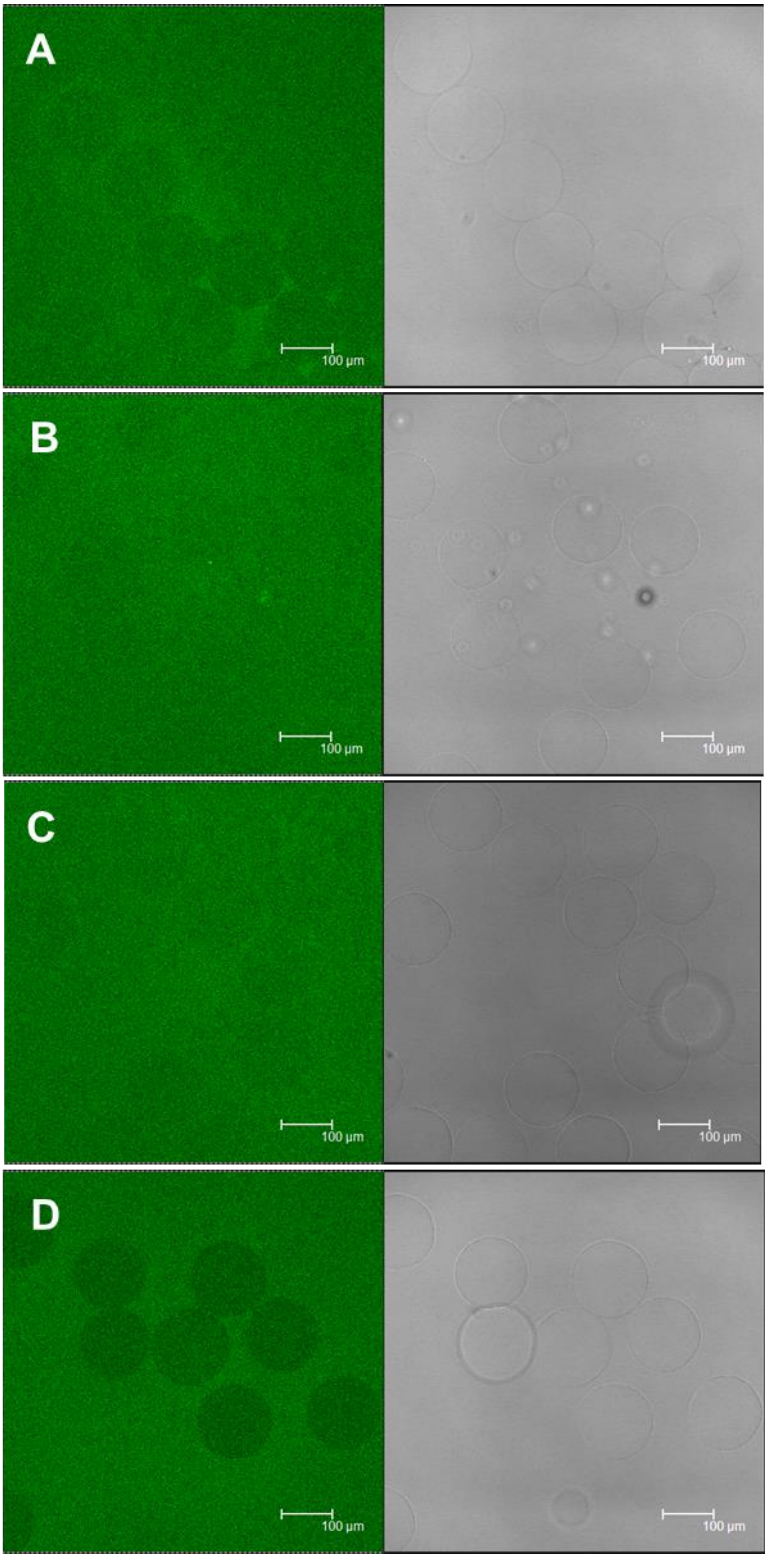

Figure S3 Representative confocal laser scanning micrographs showing that all the obtained hydrogel microbeads with varying HASH concentrations (A: 0.75%; B; 1.0%; C: 1.5%; D: 2.0%) are permeable to fluorescein labelled dextran with a Mw of 2M (corresponding hydrodynamic diameter of 54 nm). Left: fluorescence images; right: DIC images.

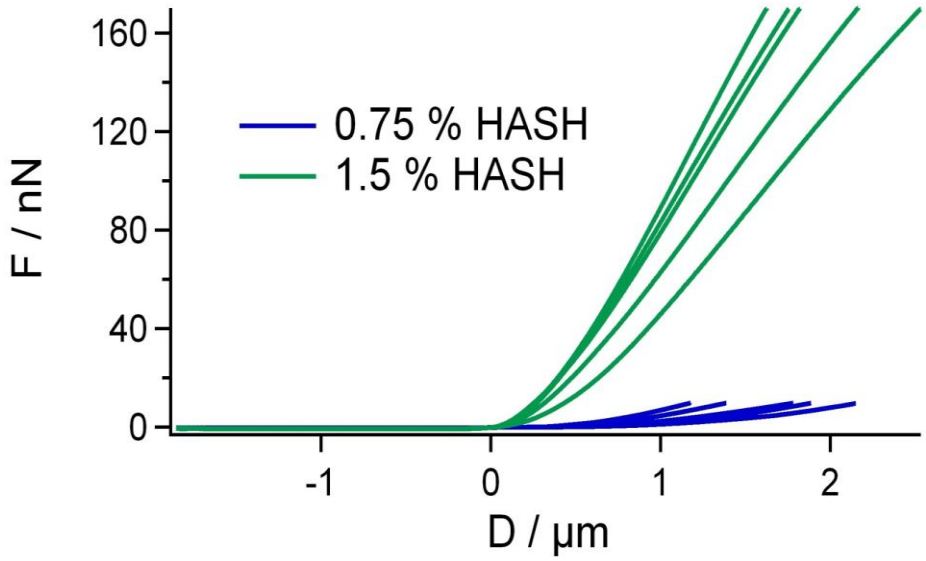

Figure S4 Characteristic force vs. deformation curves of hydrogel microbeads with HASH concentrations of 0.75 % and 1.5 % samples demonstrating the significant differences in their stiffness.

The random encapsulation of cells in microdroplets/microbeads

The encapsulation of cells in microdroplets is random and roughly follows a Poisson distribution. The theoretical probability function (Poisson distribution) can be described as:

$$f(k;\lambda) = \frac{\lambda^k e^{-\lambda}}{k!} \quad (1)$$

Where λ is the average number of cells encapsulated in each droplet. A relatively low cell concentration for the encapsulation experiments of about 1 million cells /mL was used in all the experiments. Aiming at single cell encapsulation, the experimental conditions where the actual λ = 1.38 were chosen (with a total flow rate of the aqueous phase of 400 µL/hr and droplet production rate of 80 droplets/s). The calculated probability values for the percentage of droplets containing k (k = 0-7) number of cells is

plotted in the following figure (**Figure S5**). As shown in the figure, ~ 35% of droplets would contain exactly a single cell, where the other ~ 65% contains either no cells or multiple cells.

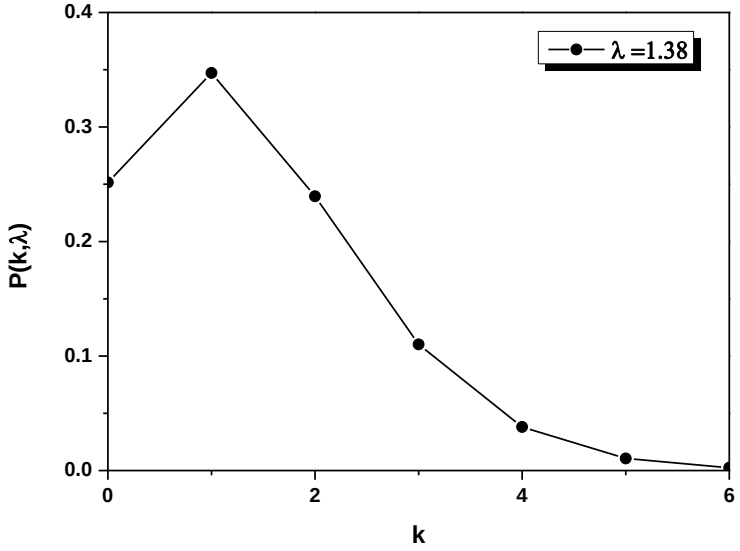

Figure S5 Poisson distribution of cell encapsulation in microdroplets when the average number of encapsulated cells λ = 1.38 under our experimental conditions.

Swelling ratio calculations

The swelling ratios Q of the hydrogel microbeads were obtained by dividing the swollen mass (m_s, μg) of the hydrogel microbeads by their corresponding dry mass (m_d, μg), based on the initial polymer concentrations (c, μg/μL), flow rates of aqueous phase (u_a, μL/hr) droplet generation frequency (f, Hz) and final size/volume (V_s, nL) of the fully swollen beads in MilliQ. The following equations are applied for the calculations:

$$Q = \frac{m_s}{m_d}; \tag{2}$$

$$m_s = m_d + V_s \times \rho; \tag{3}$$

$$m_d = \frac{c \times u_a}{3600 \times f}; \tag{4}$$

$$V_s = 10^{-6} \times \frac{4}{3}\pi\left(\frac{d}{2}\right)^3; \tag{5}$$

Where ρ is the density of water (1 g/mL = 1 μg/nL); d is the diameter of the fully swollen gel beads in MILLIQ (μm). In our experiments the average d measured on all the hydrogel microbeads are between 135 -150 μm, irrespective on the initial concentration of the hydrogel precursors. The c values can be calculated from the listed values in Table 1. f values were obtained from high speed camera videos on droplet production, with actually values between 60-88 when u_a is at 400 μL/hr. The exact values of all the parameters for varying initial HASH concentrations are presented in the following **Table S1**.

Table S1 Swelling ratios of HASH-PEGDVS-FBNG hydrogel microbeads fabricated in our experiment.

sample	HASH concentration (μg/μL)	Total solid concentration (μg/μL)	d (μm)	f (Hz)	V_s (nL)	m_s (μg)	m_d (μg)	Q
1	7.50	21.0	150	60	1.77	1.809	0.039	46
2	10.0	27.7	135	88	1.29	1.324	0.034	39
3	15.0	41.0	135	80	1.29	1.347	0.057	24
4	20.0	54.3	150	84	1.77	1.841	0.071	26

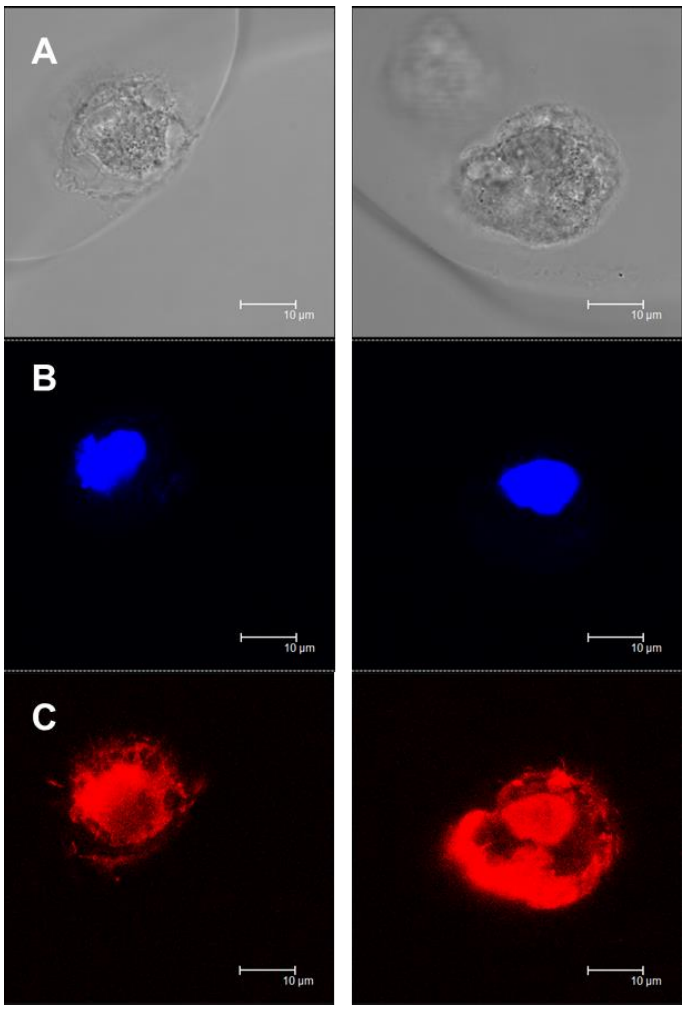

Figure S6 Confocal laser scanning micrographs on cell and nucleus morphologies of encapsulated hMSCs in microniches without FBNG. The HASH concentration used in this case is 2%. DIC images are shown in A. DAPI (B: blue)TRITC-phalloidin (C: red) and were used to stain the F-actin cytoskeleton and the nucleus, respectively. Almost all cells show a rounded morphology and some with micrometer-sized cortical protrusions on the cells towards the surrounding matrix. Scale bars are 10 µm for all images.

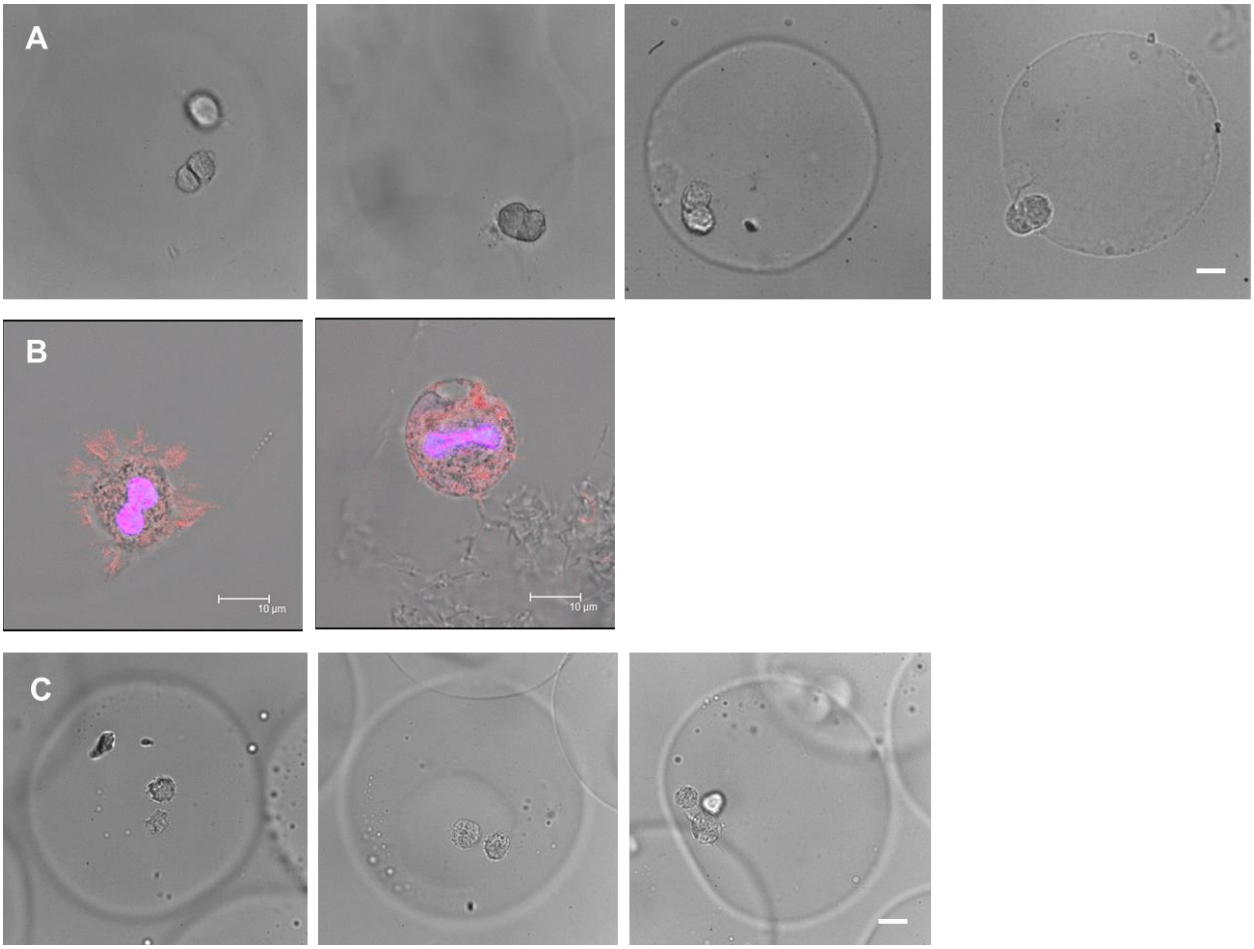

Figure S7 Micrographs showing dividing cells and multiple cells in hydrogel microbeads. (A) Cells in their (late) mitotic phases captured by bright-field microscopy. (B) cells in their (early) mitotic phases captured by confocal microscopy, DAPI (blue) and TRITC-phalloidin (red) were used to stain the the nucleus and the F-actin cytoskeleton, respectively and the overlay image is shown here. (C) Multiple cells encapsulated in one hydrogel microbead. Scale bars are 10 µm for all images.

1. F. Chambon, Z. S. Petrovic, W. J. MacKnight, H. H. Winter, *Macromolecules* 1986, **19**, 2146.