## **DNA-templated assembly of droplet-derived PEG microtissues**

## **Supplementary Information**

**Movie S1.** Microfluidic encapsulation of fibroblasts. Cells are injected as an isopycnic suspension (bottom left), and are combined with a concentrated prepolymer solution (upper left). Perpendicular oil flows (top, bottom) meet at the flow-focusing nozzle and generate droplets of cells in hydrogels. 0.5 ms exposure, 24 fps.



**Fig. S1.** Optimization of acrylate-PEG-streptavidin conjugation. Non-denaturing PAGE gel (top) of purified products from varying molar ratios of reactants. At low ratios, discrete bands of protein with 1-5 modified amines are visible. At higher ratios, streptavidin is overmodified and biotin-binding capacity is significantly reduced. Reaction conditions of interest were further tested by incorporating products into microtissues, binding biotin-DNA, and staining by hybridization with DNA-coated beads (bottom).



Fig. S2. Distribution of cell encapsulation numbers within microtissues. (a) Prior to process modifications, cells that were suspended in prepolymer settled within tubing between the syringe and the device, resulting in oscillating cell density reaching the nozzle and an uneven number of cells per microtissue. (b) When cells are injected in an isopycnic medium, and as a separate stream from concentrated prepolymer, the distribution narrowed to the Poisson limit.



**Fig. S3.** Multi-photon images of fibroblast spreading within RGDS microtissues. (a) Maximum intensity projection and (b) slice images of J2-3T3 fibroblasts spreading on Day 4 post-encapsulation. Red: actin (phalloidin), green: hydrogel (biotin-4-fluorescein), bright-green: nuclei (Hoecht).



Fig. S4. Fibroblast-laden, RGD-decorated microtissues cultured in close contact and in the presence of non-encapsulated fibroblasts. Contiguous microtissue-assembled structures linked by adherent cells formed by D1 post-encapsulation.