Supplementary Information: Hydrogel Optimization

Figures S1(a) and (b) show the minimum exposure conditions required for gelling using UV light in the λ=340±13 nm wavelength band. Based on this analysis, we chose to use a pre-polymer solution consisting of 3.4 kDa PEGDA dissolved at 8% (w/v) in M9 minimal media with 0.1-0.2% (v/v) photo-initiator (2-hydroxy-2-methyl-propriophenone). Array definition was optimized at ~40 μW of UV power for exposure times below 1.8 s. According to Figure S1(b), this combination affords a wide process window for concentrations in the pre-polymer solution and exposure conditions, accommodating changes in the UV lamp intensity with time, etc.

Figure S2 shows a super-array assembled with optical tweezers using the step-and-repeat strategy. This super-array was formed from sixteen homogeneous 4×4 microarrays of G1 *E. coli*. In this case, a 4×4 microarray of *E. coli*, was assembled with optical traps, encapsulated in hydrogel, and then the microscope stage was stepped to a flanking position separated by 30 μm, while maintaining registration with the reference array, and the process was repeated. Figure S2(a) shows a transmission image of the super-array taken at t=0, just prior to IPTG induction via the microfluidic; Figure S2(b) shows the corresponding fluorescence image of the same super-array taken 210 min. after induction. Scoring by the observed fluorescence, only 51% of the cells remain viable after 5 hrs and the viability in the bottom rows is noticeably reduced.

We tentatively attribute the diminished viability in this row to repeated exposure to free radicals formed during photopolymerization. This hypothesis is supported by a trend observed in all super-arrays made this way: the first microarrays assembled tend to show poorer viability. For example, the 3×3 super-array of Figure 5(a) shows a viability of about 70% while the 4×4 super-array (Figure S2) shows a

reduced viability of only 51% and in both cases the first microarrays assembled (lower right) show a poor viability.

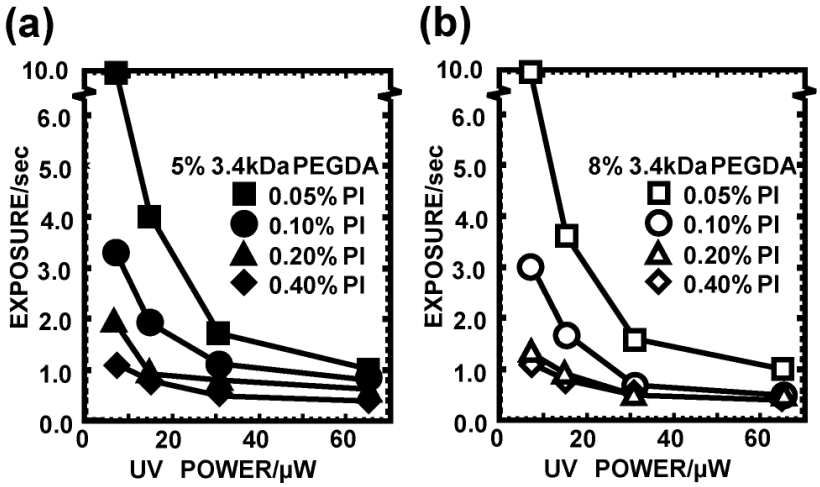

FIGURE S1. UV Exposure conditions for photopolymerizing hydrogel. The formation of the hydrogel is sensitive to the molecular weight and concentration of the PEGDA, the wavelength, power, duration of exposure to UV light, and the concentration of photo-initiator. In these experiments, 3.4kDa PEGDA was exposed to UV light in the λ=340±13 nm wavelength band. **(a)** And **(b)** chart the minimum exposure time required to crosslink the hydrogel pre-polymer versus total power for 5% and 8% (w/v) PEGDA respectively. Power was measured at the back focal plane of the objective with 75% transmission at λ=340 nm.

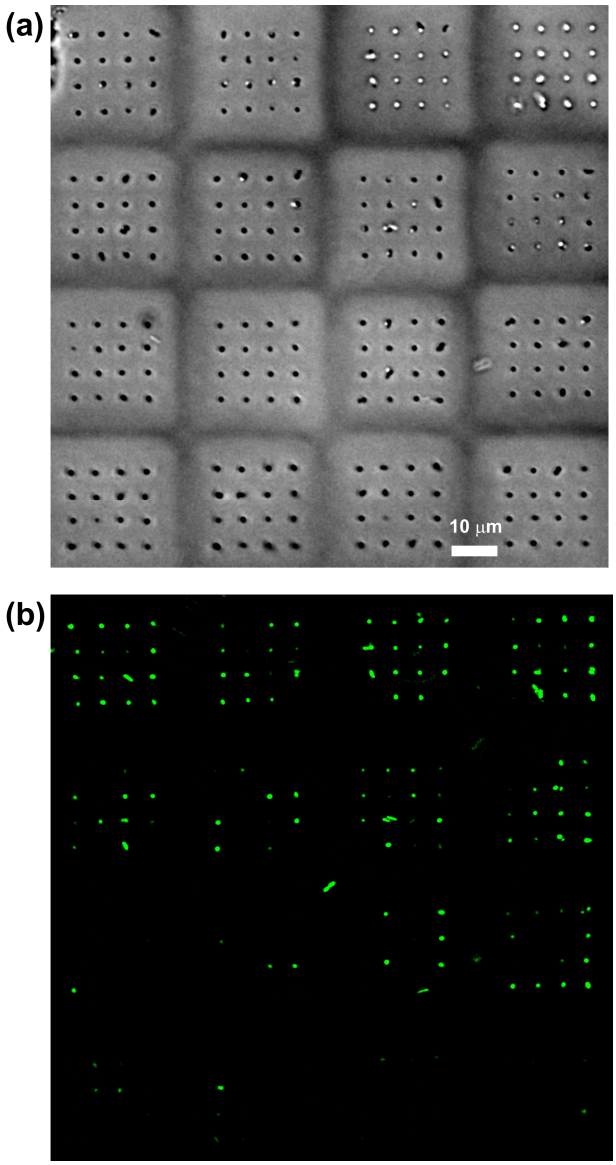

FIGURE S2: Sixteen homogeneous 4×4 microarrays of G1 *E. coli* form a 4×4 super-array. **(a)** Transmission image of the super-array at t = 0, when IPTG is broadcast into the array via the micro fluidic. **(b)** Fluorescence image of the same super-array 210 min. later. Using fluorescence, we find that 51% of the cells remain viable after 5 hrs. The images were taken with a 40X objective with 0.95 NA.