Electronic Supplementary Information for:

Optically Clear Alginate Hydrogels for Cell Entrapment and Culture at Microfluidic Electrode Surfaces

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Detailed Materials and Methods

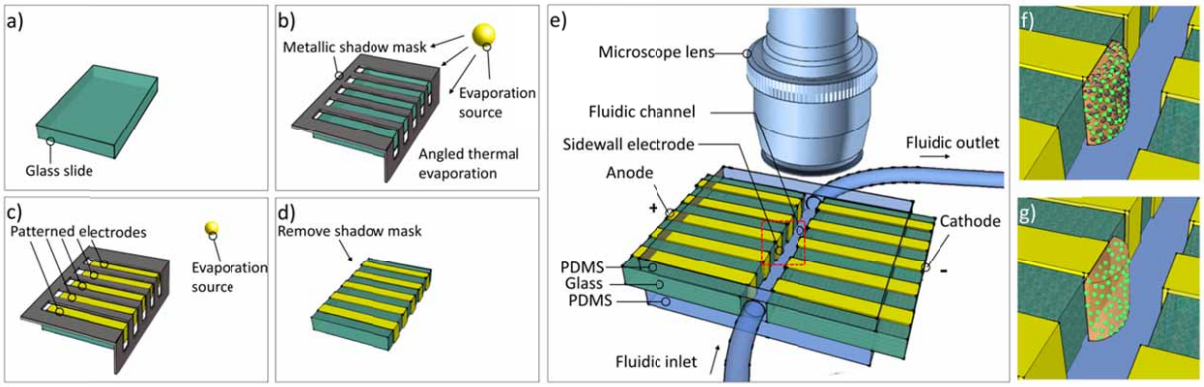

Figure S1. Schematic diagrams illustrating the fabrication procedure of transparent microfluidic device with built-in sidewall electrodes. (a) A glass slide is cleaned with piranha solution and DI water. (b) A bent metallic shadow mask with parallel slits pattern is placed on the glass slide. (c) Multiple parallel electrodes were defined by angled thermal evaporation of Cr and Au. (d) Patterned electrodes on the top and the side of the slide after removal of the shadow mask. (e) Schematic diagram of the microfluidic device with integrated sidewall electrodes. (f) Cell assembly with anodic electrodeposition of 1% alginate and 0.5% $CaCO_3$ particles. (g) Cell assembly with sequential, bilayer anodic electrodeposition of 1% alginate and 0.5% $CaCO_3$ particles.

Microfluidic system fabrication

The fabrication procedure of the microfluidic device with sidewall electrodes can be described as follows: First, glass slides were soaked in piranha solution (H_2SO_4: H_2O_2 = 3:1) for 10 min followed by thorough rinsing with DI water (Fig. S1a). Second, a bent (90º) metallic shadow mask with parallel slits (width: 1 mm) pattern was place onto the glass slide (Fig. S1b). Multiple parallel electrodes were defined by angled thermal evaporation of chromium (Cr) (20 nm) and gold (Au) (100 nm) onto the top and the side of the glass slide (Fig. S1c). Both the electrode width and the separation between electrodes are 1 mm (Fig. S1d). Two glass slides with such patterned electrodes were placed side by side with a separation of ~1 mm (Fig. S1e). They were permanently bonded to two thin layers of cured PDMS (Sylgard 184 Silicone Elastomer Kit, Dow Corning) with oxygen plasma treatment (pressure: 450 mTorr, forward power: 20W, Oxygen flow rate: 20 sccm O_2, plasma treatment time: 30 seconds) to form the ceiling and the floor of the channel (Fig. S1e). The channel height is 1 mm, as defined by the glass slide thickness. The active sidewall electrode areas in the fluidic channel are 1 mm × 1 mm.

Plastic tubes were then inserted into the channel to define the inlet and outlet of the channel. The connections between tubes and channel were sealed with PDMS gel and cured rapidly at 150 ºC.

Cell Culture Conditions

For the initial depositions, *Escherichia coli* cells were cultured overnight in LB medium supplemented with 50 µg/mL ampicillin at 37ºC while shaking at 250 rpm. Overnight cultures were inoculated 1:50 into fresh LB and grown to an OD_{600} of 0.4 before being mixed with an alginate solution.

HCT-8 cells were cultured in RPMI1640 medium (ATCC) with 10% (vol/vol) horse serum (ATCC) at 37ºC in the presence of 5% CO_2 humidified air, according to ATCC specifications, resuspended in DPBS (Invitrogen), and stained with 2 µM calcein-AM (Invitrogen) for 1 hour. Excess dye was removed by centrifuging and resuspending in DPBS (Gibco) before mixing with alginate.

For continuous monitoring of cell growth and behavior, transformed *E. coli* were cultured in LB medium supplemented with 50 µg/mL ampicillin and 10 µM $CaCl_2$ at a constant volumetric flow rate of 2.5 µL/min in a humidified incubator maintained at 37ºC. The devices were removed from the incubator and the media flow was stopped only for the time necessary to image the cells.

Electrodeposition Conditions

The electrodeposition conditions directly impact the hydrogel characteristics. The initial deposition time for the bilayer is critical for ensuring adhesion of the hydrogel to the electrode surface. We found that for initial deposition times less than 30 seconds at a constant current density of 10 A/m^2, the hydrogels would delaminate under flow while culturing cells. 30 seconds of deposition was enough to create a first layer with enough $CaCO_3$ to gel a substantial second layer, nearly 600 µm thick during the 3V deposition. Increasing the deposition voltage above 3V for the second layer results in the formation of bubbles at the electrode surface due to electrolysis of water; this weakens the hydrogel adhesion to the electrode surface. Our empirical results indicate that the given deposition conditions provide a good balance between creating a first layer thin enough to maximize the amount of cells that can be entrapped within the second layer while still retaining enough $CaCO_3$ to gel the thick second layer. While we did not systematically investigate the deposition conditions in this work, we have previously demonstrated that both the electrodeposition current density and $CaCO_3$ concentration impact the resulting dimensions of the hydrogel (Cheng, 2011).

Optical and Fluorescence Microscopy

The microscope objective was located right above the device and was focused on the anode surface in the channel. The optical micrographs were obtained with transmitted light coming from the bottom through the transparent PDMS layers. For fluorescence imaging, the FITC filter set was used for the fluorescein-labeled microspheres and the TRITC filter set was used for the *E. coli* expressing DsRed. ImageJ was to analyze both the bright field and fluorescence images.

Confocal Fluorescence Microscopy and Analysis

A 10× air objective was used for the confocal stack acquisition of Figure 2 in the manuscript. A 40× oil immersion objective was used for confocal stack acquisition of Figure 5 in the manuscript. Figure S2 shows the maximum intensities of the hydrogels created using a single

layer of 1% alginate, 0.5% $CaCO_3$ particles with fluorescent microspheres (S2a) and the bilayer method described in this Technical Innovation (S2b). ImageJ was used for the analysis of fluorescence described in the main text. Each reconstructed confocal stack was converted to a 32-bit grayscale image, and the depth of the hydrogels was divided into 100 µm segments. The average grayscale pixel intensity was determined for each of the segments of each gel. The average grayscale pixel intensity of each of the four gels at each 100 µm depth increment was averaged to produce Figure 3 in the manuscript.

Optical Density (OD) Calculation

An optical density for the cells in the alginate hydrogels was calculated using ImageJ version 1.47c image analysis software. The same four hydrogels were measured for each time point. The region of the gel containing cells was outlined using the polygon selection tool and the grayscale intensity was measured. A blank was created by averaging measurements from 10 images of alginate hydrogels without cells. The optical density was calculated using the following equation:

$$OD = log_{10}(255 - sample) - log_{10}(255 - blank)$$

A value of 255 is the maximum white value for a pixel, representing a complete transmission of light. Subtracting the grayscale value from 255 gives a measure of absorbance. Since the OD_{600} is an absorbance measurement relative to the absorbance of the blank expressed in a logarithmic fashion, the difference of the log value of the hydrogel to the log value of the blank gives a value equivalent to the optical density for a given light source. Using this method allows researchers to track cell growth without needing to place the device in a spectrophotometer, which has its own associated set of complications. One drawback to this method relative to the OD_{600} is that for a given OD_{600}, the approximate cell density (number of cells per unit volume) is known. For this method, more extensive work would be required to determine this value and whether it is stable across different light sources.

Using this method of image analysis, the apparent variability in the hydrogels containing cells decreases over time. This is because a given volume of calcium alginate hydrogel can support only so many bacteria. As the cells grow and approach this upper limit, the optical image becomes uniformly dark, and the fluorescence image becomes uniformly bright. As each gel is measured and averaged, the initial, random differences in population density and protein expression are averaged out, and the variability decreases. There is little variability in the fluorescence image of alginate gels without cells, and this is solely due to system noise. The variability in the optical image of the alginate gels without cells arises from small inclusions of $CaCO_3$ particles in parts of one or two of the gels, but is relatively consistent over time, indicating that the increase in calculated optical density arises solely from the presence and growth of cells in the alginate hydrogel.

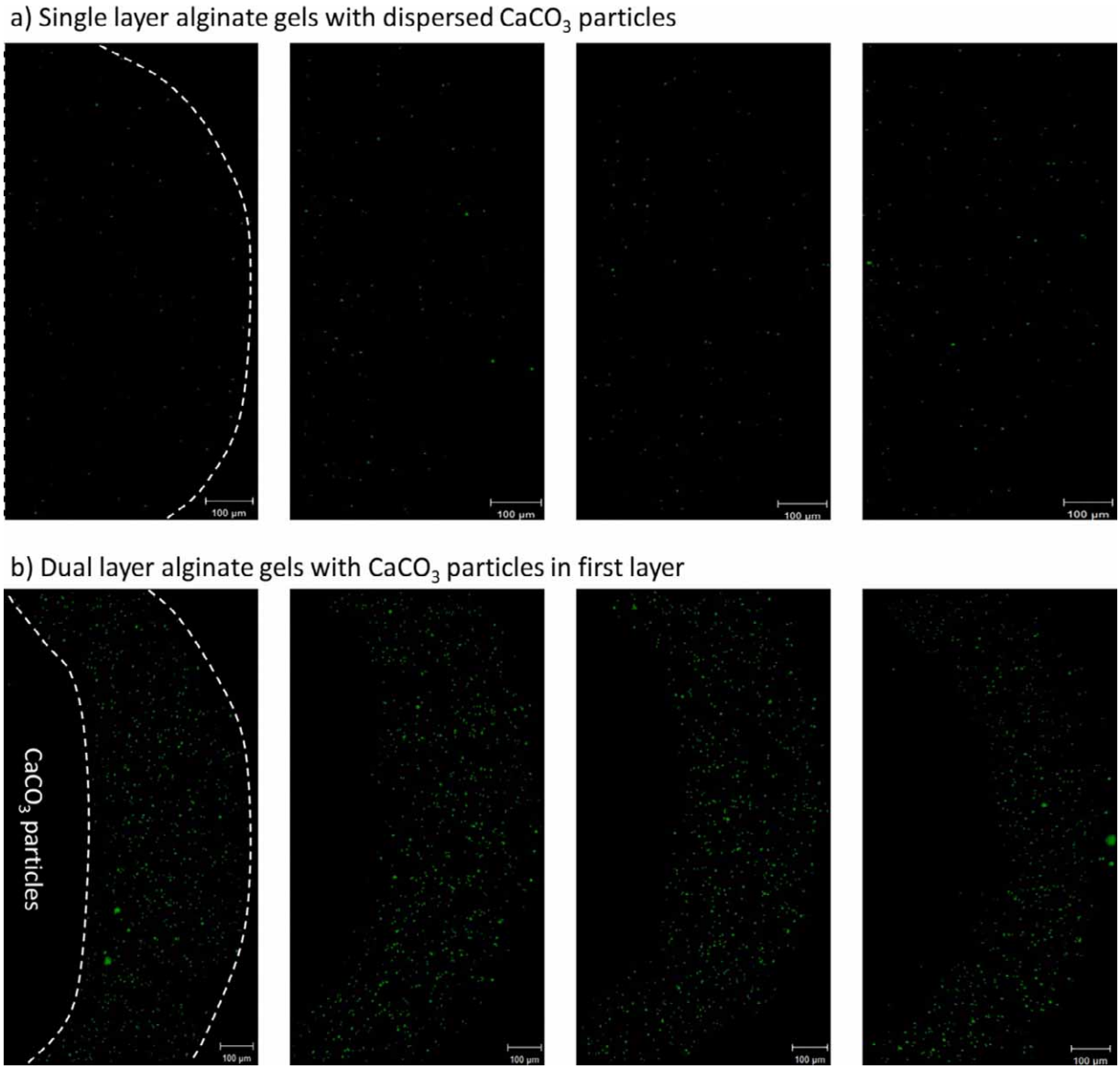

Figure S2. Maximum intensity images constructed from confocal stacks. a) Maximum intensities of the alginate hydrogels created with 1% alginate and 0.5% $CaCO_3$ particles dispersed throughout the hydrogels. The $CaCO_3$ particles block light from the fluorescent microspheres in the hydrogel below. The outline of the hydrogel can be seen faintly, and the first gel is roughly outlined by the dashed white lines. b) Maximum intensities of the alginate created with a 1% alginate and 0.5% $CaCO_3$ particle first layer and a clear 1% alginate second layer. The first layer, devoid of fluorescent microspheres, can be seen as a dark region at the left edge of the images. The edges of the first gel have been outlined by dashed white lines. In all cases, the anode is located on the left hand side of each image.

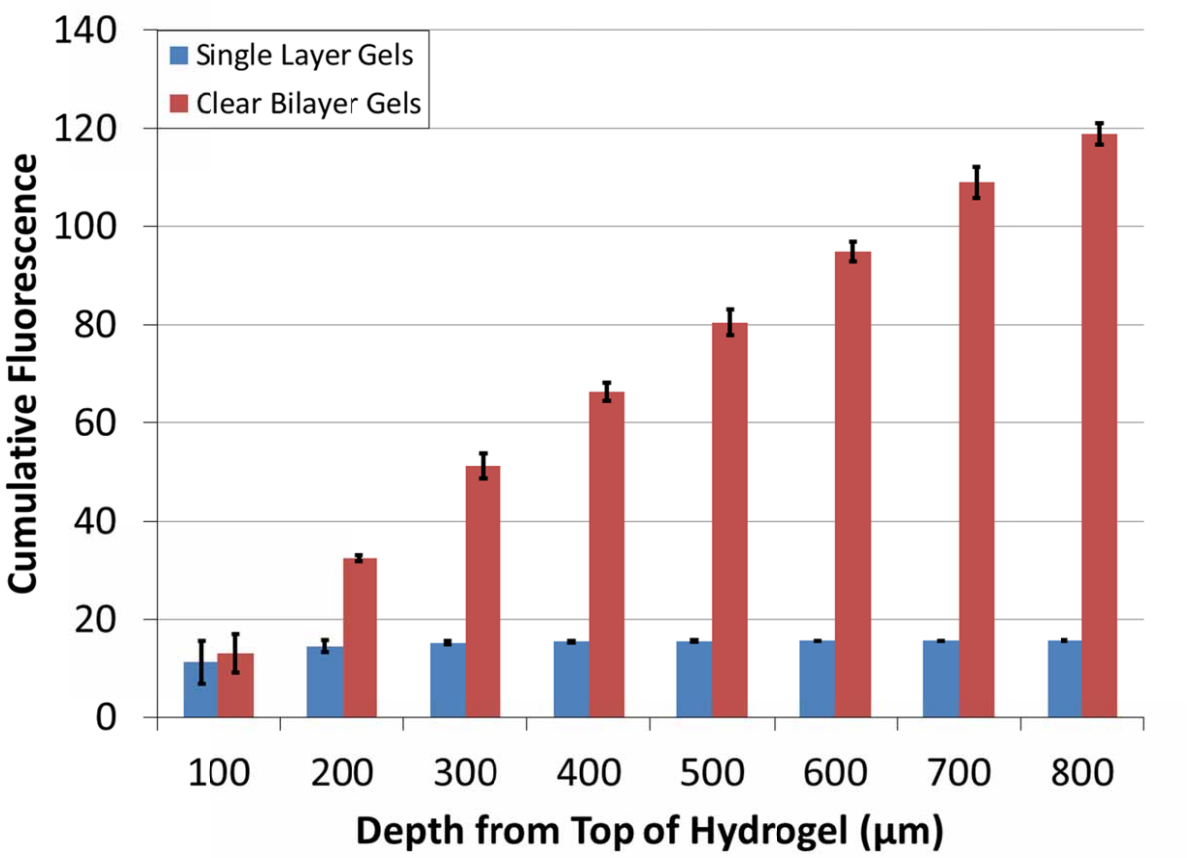

Figure S3. Comparison of the cumulative fluorescence in the single layer and clear bilayer gels. The cumulative fluorescence was measured for each 100 µm of depth of each hydrogel and added to the previous value, giving a measure of the total amount of fluorescence observed by confocal fluorescent microscopy. This figure indicates that the clear bilayer gels allow observation and analysis of hydrogel components at all depths, leading to a more accurate and possibly quantitative approach to observing cells entrapped in calcium alginate hydrogels in microfluidic devices.

References

Y. Cheng, X. Luo, J. Betz, G. F. Payne, W. E. Bentley and G. W. Rubloff, *Soft Matter*, 2011, **7**, 5677-5684.