Supporting Information for

A pH-sensing optode for mapping spatiotemporal gradients in 3D paper-based cell cultures

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Contents: Figures S1 – S5 and Equations 1 – 2

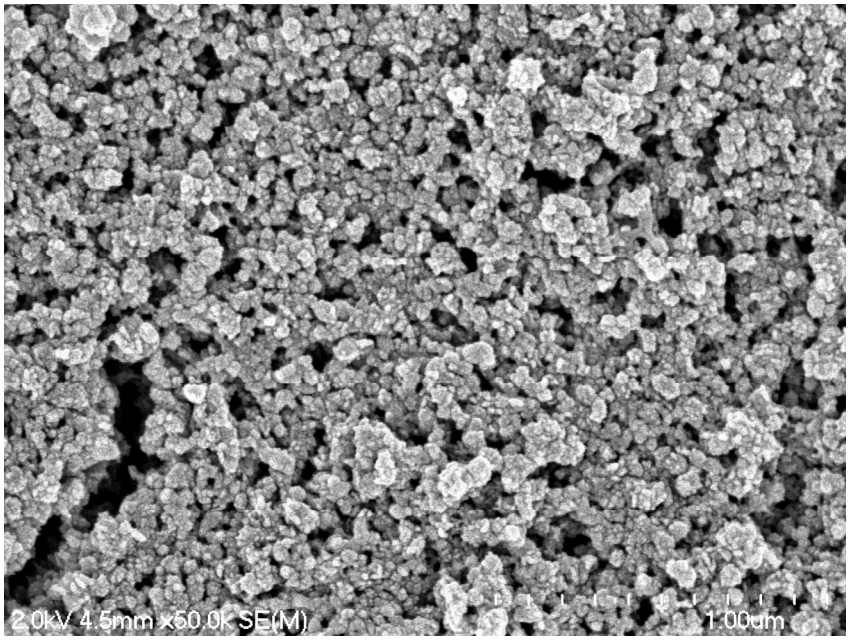

Figure S1. Scanning electron micrograph of diphenylanthracene (DPA) reference particles used in the pH sensing films. These particles were prepared by precipitating a solution containing both polyacrylonitrile and DPA. Particles are approximately 3.3 ± 0.6 nm. Scale bar is 1.00 μm.

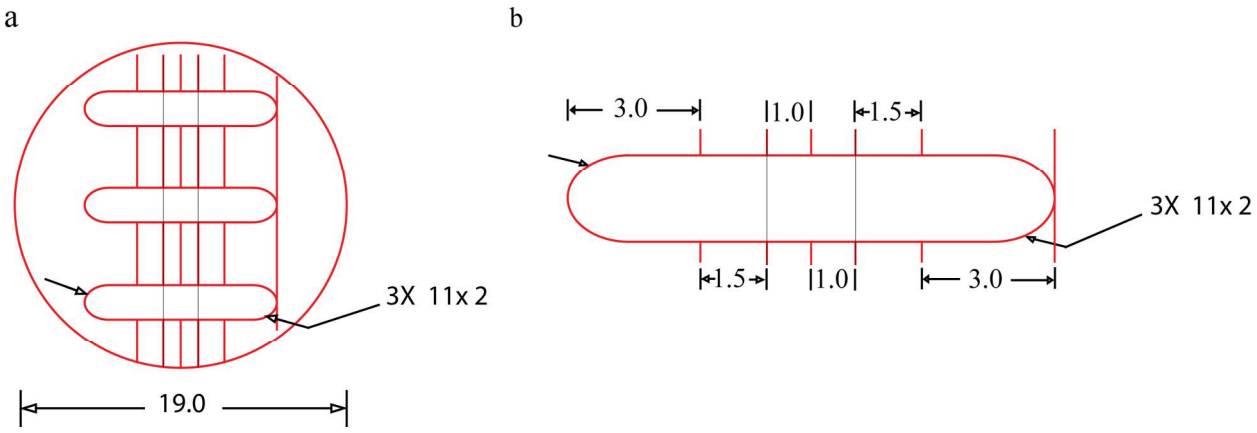

Figure S2. Detailed schematic of the paper-based scaffolds, which were designed in Adobe Illustrator, used in this work: (a) Entire scaffold and (b) a single channel in the scaffold. Each scaffold was prepared by wax-printing the above pattern onto sheets of Whatman 105 paper. The red and gray lines were also printed on the scaffolds to serve as a guide for both seeding and image alignment. All values are in units of millimeters unless otherwise stated.

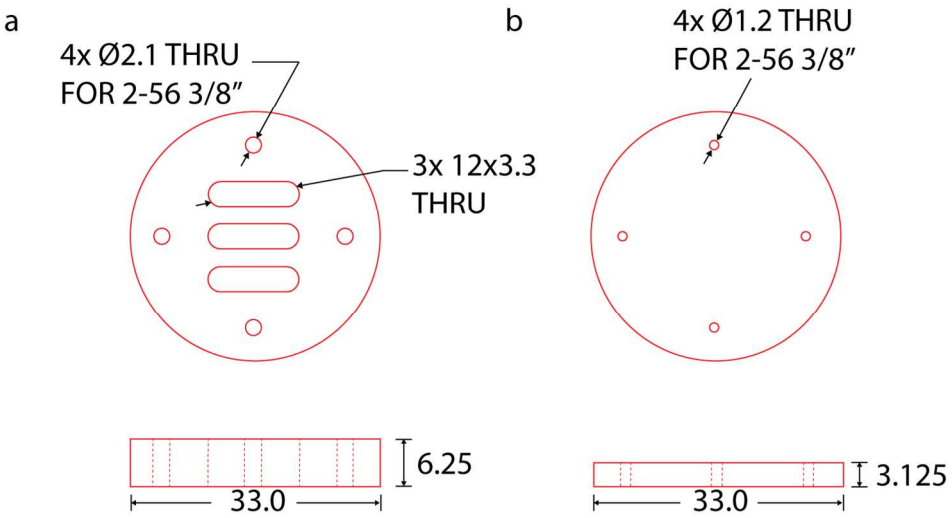

Figure S3. Detailed schematic of the acrylic holders used in this work. The (a) top and (b) bottom portions of the holders were laser cut from 6.25 mm and 3.125 mm sheets of cast acrylic, respectively. The top portion of the holder contained three 12 x 3.3 mm channels, allowing us to deliver culture medium to the cell-containing paper scaffolds. Both the top and bottom portion of the holder contained four holes, which allowed the two halves of the holder to be assembled with 3/8” 2-56 stainless steel screws. The holes in the bottom portion of the holder were threaded with a 2-56 tap. All values listed are in units of millimeters unless otherwise stated.

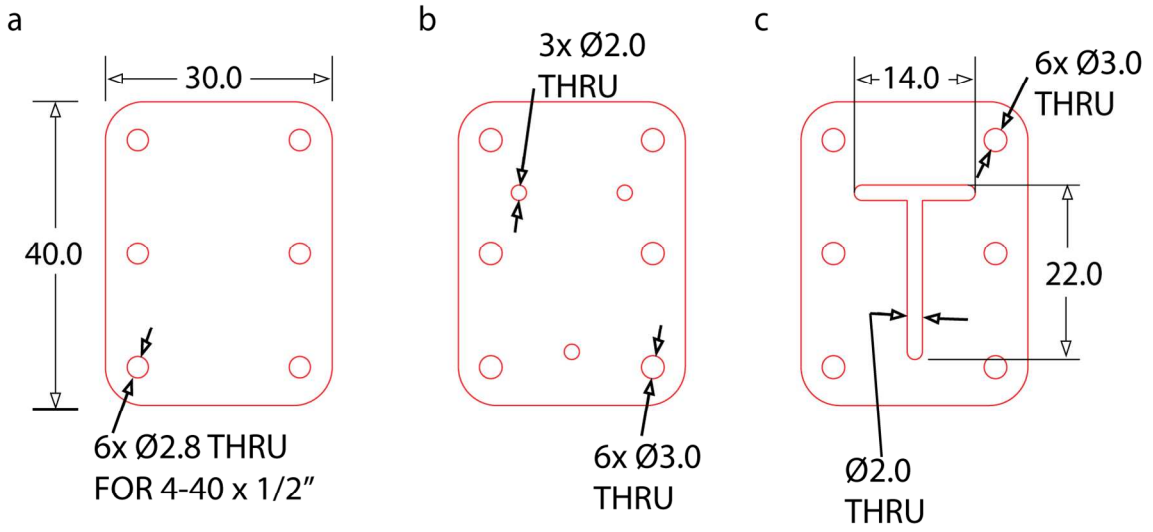

Figure S4. Detailed schematic of the flow cell used to evaluate response and reversibility of the pH-sensing films when exposed to buffered solutions of different pH. (a) The bottom 3.175 mm thick acrylic piece with six holes for screws. (b) The top 3.175 mm thick acrylic piece with six holes for screws and 3 ports, two for delivering the buffered solutions and one for waste collection. (c) A 6.35 mm thick PDMS flow guide designed to deliver phosphate buffered solutions across a defined region of the pH-sensing films film. The two halves of the holder were assembled with 1/2" 4-40 stainless steel screws. All values listed are in units of millimeters unless otherwise stated.

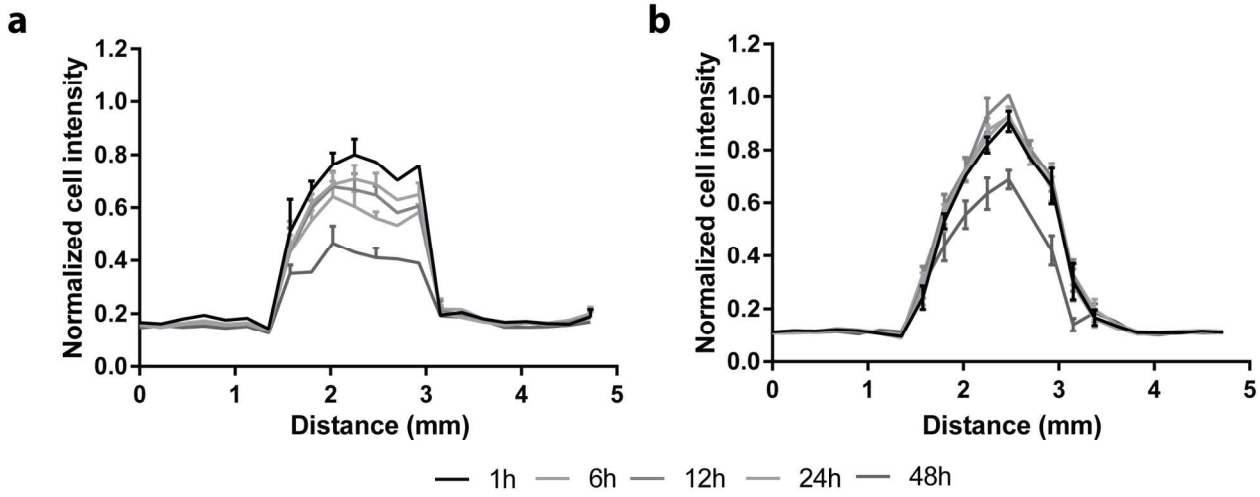

Figure S5. Fluorescence intensity profiles of cell-seeded regions. Each line represents the average intensity across the cell culture after 1, 6, 12, 24, and 28 h of incubation for the (a) open and (b) closed culture configuration. Each plot is the average across three cell cultures (n=3).

(1) Normalization of fluorescence intensity for the pH-sensing films.

(a) $$Normalized\ FLU = \frac{FLU_{tx}}{Control\ FLU_{tx}/Control\ FLU_{t1}}$$

(b) $$Normalized\ DPA = \frac{DPA_{tx}}{DPA_{tx}/DPA_{t1}}$$

(a) Normalization of the fluorescein (FLU) signal from pH sensing thin films. Where FLU_{tx} is the signal of the sample at each time point, $Control\ FLU_{tx}$ is the signal from the control film at each time point and $Control\ FLU_{t1}$ is the intensity of the first time point in the control film. (b) Normalization of the DPA signal from pH sensing thin films. Where DPA_{tx} is the signal from the sample at each time point, $Control\ DPA_{tx}$ is the signal from the control film at each time point and $Control\ DPA_{t1}$ is the intensity of the first time point in the control film.

(2) Boltzmann function used to calculate pH from the fluorescence intensity ratio of fluorescein and DPA.

$$\frac{FLU\ Intensity}{DPA\ Intensity} = I_{minimum} + \frac{I_{maximum} - I_{minimum}}{1 + e^{\left(\frac{pKa - pH}{slope}\right)}}$$

$FITC\ Intensity$ is the intensity signal from the FITC and $DPA\ Intensity$ is the intensity signal from DPA. $I_{minimum}$ is the minimum value that can be obtained, $I_{maximum}$is the maximum value that can be obtained. $Slope$ is the steepness of the curve at the pKa value (i.e., the Hill slope).