Electronic Supplementary Information (ESI) for:

Electrical control of cell density gradients on a conducting polymer surface

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Materials and Methods

I.   Device Fabrication

The PEDOT:TOS devices were fabricated on 1"×1" ITO-coated glass (Kintec). The substrates were cleaned with non-ionic detergent, rinsed with DI water and treated with UV-ozone for 10 minutes. Scotch tape was used to define the geometry of the PEDOT:TOS stripe. A solution of 2-propanol/Fe(III)-p-toluenesulfonate/pyridine in a ratio of 125:25:1 was spin-coated on the substrates at 3000 RPM for 60 seconds, and the substrates were subsequently baked on a hotplate at 80°C for 120 seconds.

Vapour phase polymerization took place in a vacuum chamber made by ReynoldsTech (Syracuse, NY). The substrates were held at 35°C while several drops of 3,4-ethylenedioxythiophene (EDOT) monomer were placed in a heated crucible (80°C) inside the vacuum chamber, and the chamber was evacuated (~100 Torr) using a diaphragm pump. The polymerization was allowed to proceed for approximately 15 minutes. The resulting PEDOT:TOS films were baked at 50°C for 30 minutes, and then gently rinsed in ethanol for 10 minutes (replacing the solvent once) to remove any remaining iron salts in the polymer.

The PDMS reservoirs were made by mixing a 10:1 ratio of base:hardener (Sylgard 184; Dow Corning) and curing at 60°C for 1 hour. The surface of each PDMS reservoir was treated with UV-ozone for 10 minutes prior to attaching to the glass substrates in order to promote adhesion.

II.   Cell growth

The 3T3-L1 cells were grown in Minimum Essential Medium Eagle, Alpha Modification (αMEM), while the MDA-MB-231 cells were grown in Dulbecco's Modified Eagle Medium (DMEM). Both media (Gibco) contained 10% Fetal Bovine Serum (FBS), and 1% Penicillin-Streptomycin.

The applied bias of ±1.5 V was chosen as a trade-off between lower values that would lead to less pronounced redox gradients and higher values that would risk electrolysis.

III.   Quantifying Fibronectin Adsorption

Fibronectin adsorption on the PEDOT:TOS stripe was quantified by performing an immunostaining assay, and comparing the relative fluorescent brightness that was observed on the stripe as a function of position/redox state. One complication is that the optical density of PEDOT:TOS varies with its redox state – the reduced state is nearly clear and transparent, while the oxidized state has a dark purple/blue colour. Since the images were taken on an inverted

microscope in reflectance mode, the optical density of the polymer affects the observed brightness from the immunoassay.

To correct for this artifact, phase-contrast images were used to determine the differences in the transparency of the PEDOT:TOS, so that a correction could be applied to the brightness values measured from the immunoassay. Images were taken in the three locations described in Figure 4, in both fluorescence and phase-contrast mode. The average colour (greyscale) was determined for each image, and a correction factor was applied to the fluorescence values, based on the brightness of a phase-contrast image at the same location on the polymer, as compared to the brightness of a nearby region of bare glass.

Image Analysis with ImageJ

Cell densities were determined through image analysis with ImageJ (NIH). For each device, enough fluorescent images were taken to cover the entire PEDOT:TOS stripe, and the images were stitched together into a single image using the microscope software (AxioVision 4; Zeiss). The images were recorded in greyscale from the microscope, and were first converted to binary (black and white) images to facilitate the image analysis. This was achieved by setting an appropriate value for the Threshold command in ImageJ. There is admittedly no standard or typical value for this threshold – it varies from image to image because of differences in background signal between different devices, as well as across each device (due to autofluorescence of PDMS, etc.).

After thresholding, the Watershed segmentation algorithm was applied to the image, to attempt to separate cells that were touching each other in high-density areas. The success of the watershed algorithm varies substantially from image to image. In particular, it will struggle with very dense regions of cells, where it nearly always fails to segment a large blob of fluorescence into its individual cells. For this reason, we questioned the accuracy of analyzing cell-density by counting the number of "individual" cells produced by the binary/watershed procedure described.

To check how much of an effect the watershed algorithm had, the density analysis was also performed by simply adding up the total fluorescent area produced by cells, in each of the 20 sections of the image reported in the graphs of Figure 3, rather than counting individual "islands" that were supposed to represent individual cells. The results showed very small differences between the two approaches that did not affect the overall shape of the density distribution.

Additional Images of Cell Density Gradients

Some additional images of cell density gradients generated for 3T3-L1 cells are shown in Figure S1 to demonstrate reproducibility.
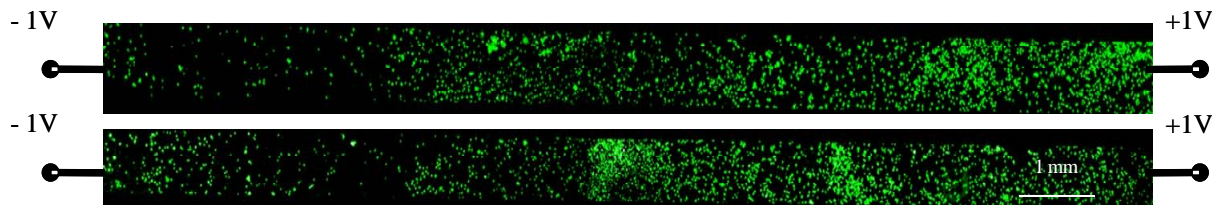

Fig. S1: Two additional fluorescence micrographs of
calcein-green stained 3T3-L1 cells, taken with a 10× objective.