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Supplementary Information

On-Cue Detachment of Cells and Hydrogels from Optically Transparent Electrodes

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Indium tin oxide (ITO)-coated glass slides (75 mm x 25 mm) with sheet resistance of 4-8 Ω, nominal transmittance of >82%, and thickness of 1500-2000 Å were purchased from Delta Technologies (Stillwater, MN, USA). (3-acryloxypropyl) trichlorosilane was purchased from Gelest, Inc. (Morrisville, PA, USA). Heparin (sodium salt, from porcine intestinal mucosa, MW 12 kDa) was purchased from Cellsus Ins. (Cincinnati, IA, USA). Poly (ethylene glycol) diacrylate (PEG-DA, MW 6 kDa, degree of substitution 98 %) was purchased from Sunbio Inc. (Anyang City, Korea). Hydrochloric acid, nitric acid, ethanol, acetone, and anhydrous toluene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Concentrated (10x) Phosphate-buffered saline (PBS) was purchased from Cambrex (Charles City, IA, USA) and diluted before use. Dulbecco's Modified Eagles' Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, and Live/Dead Viability/Cytotoxicity kit were purchased from Invitrogen (Carlsbad, CA, USA). Positive photoresist (AZ 5214-E IR) and its developer solution (AZ 300 MIF) were brought from Mays Chemical (Indianapolis, IN, USA). SU-8 photoresist was from Microchem Corporation (Newton, MA, USA). Silicon wafers were purchased from Wafer World Inc. (West Palm Beach, FL, USA). Polydimethylsiloxane (PDMS) (SYLGARD® 184 silicone elastomer) and its curing agent were purchased from Dow Corning (Midland, MI, USA).

Fabrication of ITO Electrodes.

Arrays of ITO electrodes were fabricated on glass using photolithography and wet etching. Prior to photolithography ITO-coated glass slides were dehydrated at 200 ºC for 24 h. Positive photoresist (AZ 5214-E IR) was spin-coated on the ITO-coated glass slides

at 800 rpm for 10 s followed by 4,000 rpm for 30 s. The photoresist layer was soft-baked at 100 °C for 105 s and exposed to UV light (10 mW/cm^2) through a photomask for 45 s using a Canon PLA-501F mask aligner. The photoresist patterns were then developed in AZ 300 MIF solution for 5 min, briefly washed with DI water, and dried under nitrogen. ITO substrates with patterned photoresist layer were then immersed in an etching solution composed of 20 % (v/v) hydrochloric acid, 5 % (v/v) nitric acid, and 75 % DI water at 55 °C for 5 min. The conductivity of etched areas was measured to verify the removal of ITO. The remaining photoresist was removed by sonicating in acetone for 30 min, followed by washing with DI water and drying with nitrogen.

Silane Modification of ITO Electrodes.

Before silane modification, the micropatterned ITO surfaces were cleaned in an oxygen plasma chamber (YES-R3) at 300 W for 5 min. The substrates were then placed in 2 mM solution of (3-acryloxypropyl) trichlorosilane diluted in anhydrous toluene for 10 min. The reaction was performed in a glove box under nitrogen purge to eliminate atmospheric moisture. After modification, the slides were rinsed with fresh toluene, dried under nitrogen, and dehydrated at 100 °C for 2 h. Contact angle measurements (Rame-Hart goniometer model 190-F1, NJ, USA) were routinely performed to assess the quality of silane modification.

Patterning of Hydrogels and Cells on ITO Electrodes.

Heparin-based hydrogels were prepared by a Michael-type addition reaction between thiolated heparin (Hep-SH) and PEG-DA, as previously reported by us [1]. Briefly, 40 % Hep-SH and 6 kDa PEG-DA (1:1 molar ratio of thiol group of Hep-SH and acrylate group of PEG-DA) were dissolved in cell culture medium (DMEM with 10 %

(v/v) fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin) to make 10 % (w/v) hydrogel. A PDMS stencil (membrane) was employed to pattern heparin hydrogel structures on top of the ITO electrodes. A thin PDMS membrane containing holes (1.3 mm in diameter and 100 μm in thickness) was generated using standard soft lithography protocols.[2] A hydrogel prepolymer solution (adjusted to pH 7.4) was added in the PDMS wells and incubated at 37 °C for 30 min to form gel.

Cultivation and release of murine 3T3 fibroblasts was employed to demonstrate biological applications of our surface manipulation strategy. Fibroblasts were maintained in DMEM supplemented with 10% FBS, 200 U/mL penicillin, 200 μg/mL streptomycin at 37 °C and 5 % CO_2. The cells were collected from culture flasks by trypsinization and resuspended in 1x PBS at a concentration of 0.5 x 10^6 cells/mL. To ensure cell attachment, fibronectin was added at concentration of 500 μg/ml prior to gelation of heparin-containing polymer solution. The cells were seeded onto heparin hydrogel structures through the PDMS stencil described above and were incubated for 1h. This was followed by removal of the stencil, washing with 1x PBS and cultivation of cells on hydrogel structures for 24 h before gel detachment experiments. Both the adherent and detached cell-carrying hydrogels were stained using a Live/Dead Viability/Cytotoxicity kit. Samples were imaged using confocal microscopy (Zeiss LSM 5 Pascal, NJ, USA).

Detachment of Cell-Carrying Heparin Hydrogels

Detachment of hydrogel was carried out in a custom-made Plexiglass electrochemical cell using a three electrode system. A steel wire was connected to contact pads of ITO electrodes using a temperature curable conductive epoxy (EPOTEK, MA, USA). The substrate was then secured in an electrochemical cell and immersed in 500 μL

of 1x PBS serving as an electrolyte solution. Ag/AgCl reference and Pt counter electrodes were positioned in the same electrochemical cell with ITO region serving as a working electrode. To detach the hydrogel, a potentiostat (CH Instruments, TX, USA) was used to apply a voltage of -1.8 V for 60 s to an ITO electrode of interest. Detachment of hydrogel was verified by observation under optical microscopy (Zeiss Axiovert 40, Carl Zeiss, NJ, USA). Hydrogel detachment was also confirmed by cyclic voltammetry, employing 5 mM potassium ferricyanide as a redox reporter molecule, with a potential scan from 0 to 500 mV (vs. Ag/AgCl) at a scan rate of 10 mV/s.

High-resolution images of hydrogels on electrodes with or without cells were obtained by using a scanning electron microscopy (SEM) (Philips XL 30, Eindhmoven, Netherlands) at 10 kV beam voltage and a tilt angle of 30°. Prior to imaging, samples were coated with 6 nm layer of Au-Pd using a sputter coater (Pelco SC7, CA, USA). To visualize cells on top of the hydrogels, the hydrogel patterns were washed with a fresh media followed by washing with PBS twice for 5 min. Cell-carrying hydrogels were exposed to a fixative (4% formalin solution) for 10 min, rinsed with PBS solution, air dried, and then coated with Au-Pd.

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