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Supporting Information

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Rapid Patterning of Cells and Cell Co-Cultures with Spatial and Temporal Control on Surfaces through Centrifugation

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Preparation of Substrates. Preparation of the gold substrates, synthesis of the tetra(ethylene glycol)-terminated alkanethiol, and microcontact printing of hexadecanethiol onto gold surfaces were performed as previously described.[11] Following microcontact printing, substrates were immersed in a solution of 1 mM tetra(ethylene glycol)-terminated alkanethiol in ethanol for 12 hours. After rinsing with ethanol and dried with N_2 gas, substrates were inverted onto a solution of 0.1 mg/mL fibronectin for 2 hours.

PDMS Masks. Using an elastomeric kit (Sylgard®; Dow Corning), PDMS was cured to have a thickness of ~5 mm. A lift-off patterned membrane was generated, creating a well when placed on the SAM.[14]

Cell Culture. Swiss Albino 3T3 fibroblasts (ATCC) were cultured in Dulbecco's Modified Eagle Medium (Gibco) containing 10% calf bovine serum and 1% penicillin/streptomycin. Cells were removed with a solution of 0.05% trypsin in 0.53 mM EDTA and re-suspended in serum-free medium (100,000 cells/mL). The cell-containing solution was then added to the PDMS well. Drosophila Schneider 2 (S2) cells (Invitrogen) and GFP-transfected Drosophila Schneider 2 (S2) cells were cultured in Schneider's Drosophila Medium (GIBCO BRL) containing 10% heat-inactivated fetal bovine serum (FBS), and penicillin/ streptomycin (50 μg/mL). S2 cells were grown in 75 cm^2 flasks containing 10 mL of media for routine maintenance. For passage, cells are resuspended in the same 10 mL of medium that they were growing in, then 3 mL are transferred to 7 mL of fresh medium in a new flask. The cell-containing solution was then added to the substrate in the centrifuge tube for cell patterning.

Cell Staining and Visualization. After allowing fibroblast cells to grow into patterns on substrates for 24 hours, surfaces were rinsed twice in PBS. To 1 mL of PBS, 3 μL of a 10mM stock solution of CellTracker Green CMFDA were added. Substrates were then incubated at 37 °C for 45 minutes with 200 μL of this dye solution. Substrates were rinsed in PBS twice, and then placed in complete medium for at least 1 hour to allow cells to equilibrate.

Centrifugation. To provide a level surface to support the gold substrates during centrifugation, a PDMS cork was created at the bottom of the falcon tubes. After pouring the elastomer mixture (10:1 elastomer:initiator), the tubes were placed in a vacuum chamber to remove any trapped air, incubated at 60 °C for 6 hours, and then at room temperature overnight to allow for adequate cross-linking. The masks were not wetted to create a liquid-proof seal when placed on the SAMs. For every 0.5 cm^2 of substrate surface area, approximately 100 μL of the cell-containing solution was added to the well. Substrates were centrifuged for 1 minute at 2000 rpm in an Eppendorf Centrifuge 5810 (Eppendorf AG; Hamburg, Germany). For this technique, the centrifuge must be equipped with a swinging-bucket rotor. Substrates were then immersed in serum-free medium for two hours, followed by a period in serum-containing medium to allow cell growth. Cells had fully grown into the patterns after approximately 24 hours.