

Electrochemical Desorption of Self-Assembled Monolayers Noninvasively Releases Patterned Cells from Geometrical Confinements

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This report describes a procedure based on the electrochemical desorption of self-assembled monolayers (SAMs) to release patterned mammalian cells from the constraints of these patterns. This procedure uses microcontact printing (μ CP) and readily available thiols—HS(CH₂)₁₁(OCH₂OCH₂)₃OH (C₁₁EG₃) and HS(CH₂)₁₇CH₃ (C₁₈)—to confine cells into patterns; these methods are well established.^{1,2} EG₃-terminated SAMs resist the adsorption of proteins (we call these surfaces “inert”, meaning “inert to the adsorption of proteins”).³ Since mammalian cells attach to and spread on surfaces only if suitable extracellular matrix (ECM) proteins are present, EG₃-terminated SAMs also resist the attachment and spreading of cells.^{1,4} Application of a cathodic potential of < -1.2 V (vs a stainless steel electrode) on gold caused the SAMs to desorb.^{5,6} Removal of EG₃-terminated SAMs from a gold surface allowed the surface to adsorb proteins.⁷ Here we have used μ CP to pattern SAMs and electrochemistry to desorb the confining (EG)₃-terminated SAM; this procedure allows cells to be grown in patterns and then to be released from these patterns. After desorption of the (EG)₃-terminated SAM, cells can attach to, and spread across, previously inert areas (Figure 1).

We used electrochemical desorption of EG₃-terminated SAMs to demonstrate that bovine capillary endothelial (BCE) cells confined to microislands of patterned SAMs can be released from their confinements. Patterning of BCE cells was accomplished by methods previously described,⁸ with C₁₈ as the protein-adsorbing SAM and C₁₁EG₃ as the inert SAM (see Supporting Information for more details).⁹ Cells were confined on these micropatterns in normal growth media for 24 h. After application of a cathodic voltage pulse (< -1.2 V, vs stainless steel, for 30 s), cells began to spread perceptibly from the microislands (Figure 1). We believe that the voltage pulse desorbed some or all of the (EG)₃-terminated SAM and that ECM proteins such as fibronectin (FN) present in the fluid medium or secreted by cells rapidly adsorbed onto regions that had been previously rendered inert by these SAMs. The cells migrated across the entire surface as if they were migrating on “bare” gold (that is, gold with no SAM but with a layer of proteins adsorbed from the medium). They also underwent normal growth and proceeded to cytokinesis on these substrates.

We quantified the amount of proteins adsorbed on the gold surfaces after the voltage pulse by using surface plasmon resonance (SPR).¹⁰ Figure 2 compares the adsorption of serum proteins from the growth medium onto substrates initially coated with C₁₁EG₃ that underwent the cathodic pulses (ranging from -1.0 to -1.8 V, for 30 s). SPR measured the increase in the thickness of the thin organic film (SAMs or proteins) on the gold; this increase was reflected in the change in response units (Δ RU). Δ RU was mainly caused by the adsorption of proteins when the growth medium for

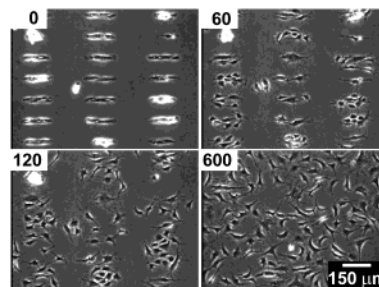


Figure 1. BCE cells were allowed to attach to a surface patterned with C₁₁EG₃ and C₁₈. Application of a cathodic voltage pulse (-1.2 V for 30 s in this case) released the cells from the microislands. The numbers indicate the time elapsed (in minutes) after the voltage pulse.

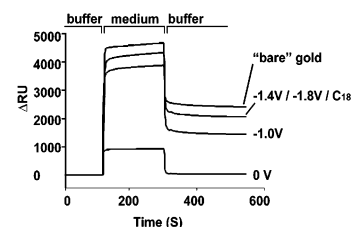


Figure 2. SPR sensorgrams compare the amounts of adsorbed proteins on the SAMs originally coated with C₁₁EG₃ that underwent various cathodic voltages. The sensorgram on the SAM formed by C₁₈ is also shown. “Bare” gold is a gold surface that does not have a SAM.

BCE cells was allowed to flow over the gold substrate.¹¹ The sensorgrams indicate that the gold surface ceased to be inert after a voltage pulse of -1.0 V and became similar to a SAM of C₁₈ in its ability to adsorb proteins after pulses of -1.4 and -1.8 V. By using the Δ RU on the SAM formed by C₁₈ as a complete monolayer of serum proteins, we estimated the coverage of proteins on SAMs-coated gold to be 56% after -1.0 V, and 100% after -1.4 V (or -1.8 V).¹² A bare gold surface seemed to adsorb slightly more proteins than a SAM of C₁₈, but the difference in optical characteristics of these surfaces made a direct comparison difficult.¹³ Cyclic voltammetry also corroborated the desorption of C₁₁EG₃ (see Supporting Information, Figure 1).

Proteins adsorbed on a SAM formed by C₁₈ did not desorb after application of voltage pulses in the range used here. Quantitative fluorescence measurements on rhodamine-labeled FN adsorbed on the C₁₈-covered area of the substrate showed indistinguishable values before and after the voltage pulse. This result agrees with a recent report that similar cathodic potentials did not decrease the quantity of plasma proteins that adsorbed onto gold surfaces.¹⁴

BCE cells appeared to be intact and normal after the voltage pulse. The maximum voltage gradient was 180 mV/mm (1.8 V across a distance of 10 mm between the two electrodes) in our experiment; this voltage gradient is comparable to the voltage

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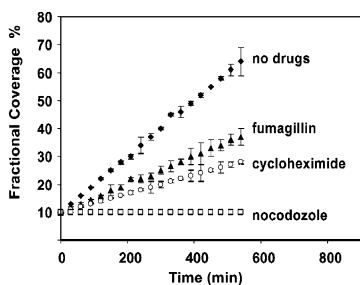


Figure 3. A cell-based screening assay. A summary of the influence of drugs on the motility of BCE cells after application of a voltage pulse (-1.2 V, 30 s). Each datum represents the average of eight fields of cells. Error bars represent one standard deviation from the mean. See Supporting Information for the calculation of the Fractional Coverage.

gradients used in other studies involving mammalian or amphibian cells.¹⁵ Two observations support this claim: (i) Time-lapse video microscopy showed that all cells had normal morphology and migrated normally on the surface after the voltage pulse. Preliminary analysis showed that their average speeds of migration were indistinguishable on the electroactivated substrates from those on a polystyrene Petri dish. (ii) A LIVE/DEAD assay¹⁶ showed that $99.9 \pm 0.05\%$ of the cells had intact membranes and remained viable after a voltage pulse of -1.8 V for 30 s.

We illustrated the value of this method for screening agents whose biological activities are reflected in their influence on cellular motility. When drugs were added to the growth media before the voltage pulse, migration of cells out of the patterns was modified or completely abolished after the voltage pulse (Figure 3). The speed of cell movement out of the patterns provided a simple visual screening for the activities of the drugs on the migratory activities of cells. We tested fumagillin ($100 \mu\text{M}$), a drug that inhibits the motility of capillary cells,¹⁷ cycloheximide ($1 \mu\text{g/mL}$), a general inhibitor of protein synthesis,¹⁸ and nocodazole ($1 \mu\text{g/mL}$), a drug that depolymerizes microtubules.¹⁹ Fumagillin and cycloheximide reduced the rates of cell migration; nocodazole abolished it completely. We believe that this screening assay can evaluate agents that inhibit or promote cell motility, a fundamental process involved in a broad range of biological activities including angiogenesis, inflammation, tissue morphogenesis, cancer, and wound healing.

Similar techniques where cells are first confined and then released have been explored before. Mrksich and co-workers have developed an elegant technique that uses electrochemical oxidation of a hydroquinone-terminated SAM to a quinone-terminated SAM, followed by a Diels–Alder reaction to immobilize a cyclopentadiene-conjugated cell-adhesion peptide sequence to activate inert surfaces for attachment of cells.²⁰ This technique works well and has the advantage that it allows sophisticated control over the molecular composition of the surface; it has the disadvantage that it requires extensive synthesis. We and Toner have developed membrane-based patterning,²¹ where cells are first confined by membranes containing pores and then released by peeling away this membrane. Membrane-based techniques have drawbacks: thin membranes require skills to fabricate, and methods based on them require careful physical manipulation. Many of these processes also require incubation of cells in serum-free media to avoid fouling the inert surfaces; this process damages many types of cells.

The procedure we described here has a number of advantages.

(i) It does not require physical manipulation of thin membranes.

(ii) It does not require extensive organic synthesis. (iii) It does not damage the cells. (iv) It is applicable in normal growth media that contain serum.

In conclusion, we have combined the cell patterning work developed previously from our group² and the electrochemical desorption of SAMs described by Porter, Morin, and others⁶ to modulate the capacity of a surface for the attachment of cells. This technique has the capacity to control the cell–substrate interaction dynamically. It is relatively simple experimentally and can be easily implemented in any biological laboratory. We believe that it has at least two uses: (i) to simplify a class of cell-motility-based assays used in drug discovery and (ii) to provide a new tool for use in fundamental studies of cell biology based on simultaneous spatial and temporal control of cell–substrate interactions.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge at <http://pubs.acs.org>.

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