Video Article

Patterning Cells on Optically Transparent Indium Tin Oxide Electrodes

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URL: http://www.jove.com/video/259
DOI: doi:10.3791/259

Keywords: Cellular Biology, Issue 7, indium tin oxide, surface modification, electrochemistry, cell patterning

Date Published: 8/20/2007

Citation: Shah, S., Revzin, A. Patterning Cells on Optically Transparent Indium Tin Oxide Electrodes. *J. Vis. Exp.* (7), e259, doi:10.3791/259 (2007).

Abstract

The ability to exercise precise spatial and temporal control over cell-surface interactions is an important prerequisite to the assembly of multi-cellular constructs serving as in vitro mimics of native tissues. In this study, photolithography and wet etching techniques were used to fabricate individually addressable indium tin oxide (ITO) electrodes on glass substrates. The glass substrates containing ITO microelectrodes were modified with poly(ethylene glycol) (PEG) silane to make them protein and cell resistive. Presence of insulating PEG molecules on the electrode surface was verified by cyclic voltammetry employing potassium ferricyanide as a redox reporter molecule. Importantly, the application of reductive potential caused desorption of the PEG layer, resulting in regeneration of the conductive electrode surface and appearance of typical ferricyanide redox peaks. Application of reductive potential also corresponded to switching of ITO electrode properties from cell non-adhesive to cell-adhesive. Electrochemical stripping of PEG-silane layer from ITO microelectrodes allowed for cell adhesion to take place in a spatially defined fashion, with cellular patterns corresponding closely to electrode patterns. Micropatterning of several cell types was demonstrated on these substrates. In the future, the control of the biointerfacial properties afforded by this method will allow to engineer cellular microenvironments through the assembly of three or more cell types into a precise geometric configuration on an optically transparent substrate.

Video Link

The video component of this article can be found at http://www.jove.com/video/259/

Protocol

Part I: Patterning of the electrodes

1. Clean and dehydrated ITO coated glass slides were coated with positive photoresist
2. The surface was baked at 100°C to remove solvents from the resist
3. After baking, the surface was exposed to ultraviolet light through a photomask using the Canon Mask Aligner
4. The exposed regions were removed in a developer solution
5. The surface was hard baked to remove any remaining developer solution
6. The ITO regions not protected by photoresist patterning were etched away in an acid etchant
7. Remaining photoresist was removed by sonicating in acetone to form the ITO electrodes

Part II: Surface modification

1. Modified electrodes are cleaned in a plasma chamber and modified with 2% PEG silane in toluene. Incubation is carried out for 2 hours, followed by 2 hours of baking.

Note: This process is carried out in a glove bag filled with nitrogen to avoid presence of moisture, as PEG silane is reactive towards it.

Part III: Electrochemistry

1. Steel wires are attached to electrode pads.
2. The PEG silane modified electrodes are placed in an electrochemical cell to perform electrochemical experiments.
3. PEG silane from interconnected regions is stripped using a three-electrode system in PBS.

Part IV: Cell patterning

1. Fibroblasts are incubated with the recently stripped substrates. Upon incubation, these cells attach to the PEG silane stripped regions; however, the cells will not bind anywhere else on the surface.

2. Patterned cells are incubated in fresh media and visualized using a microscope.

Discussion

In this video, we have demonstrated the cell patterning on optically transparent indium tin oxide electrodes. After fabricating the ITO electrodes using photolithography, they were modified with a cell-resistive monolayer of PEG silane. This monolayer was desorbed using electrochemistry switching the surface from cell-resistive to cell-adhesive. Patterning of 3T3 murine fibroblasts has been shown in his video. We have also patterning hepatocytes, primary cells, and stellate cells using the same technique. In addition, by making multiple individual electrodes, we can extend this technique to assemble multiple cell types. Furthermore, by controlling the geometry of the design, the cells can be spatially and temporally isolated on the surface.

Photolithography and wet etching techniques are standard microfabrication processes. Electrochemistry has been widely studied on gold surfaces but not so much on ITO. Apart from conductivity, ITO also provides advantage of transparency. By using ITO, the samples can be visualized using a simple inverted microscope. The technique presented in this video can be employed to assemble any cell type on the surface in a precise geometric pattern.

References

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