SPATIOTEMPORAL STUDY OF CELL BEHAVIOR ON ENGINEERED EXTRA CELLULAR MATRICES

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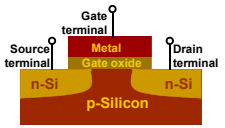
Abstract

Cell behavior is intricately influenced by the extra cellular matrix (ECM). The spatial organization of ECM proteins provides vital cues to the cellular receptors, which dictates the adhesion, spreading and migration of cells. The complex nature of the cell-ECM signaling can be understood better by engineering surfaces with spatial control of ECM proteins that provides controlled environment for studying cell migration. In this work, spatially-defined microarrays of fibronectin (ECM protein) with defined domain size (10-25 μm) have been designed using the Nano eNabler™ (Bioforce Nanosciences, Inc., Ames, IA) and these domains are separated by non-adhesive regions. The adherence and spread of the NIH 3T3 cells cultured on these engineered surfaces have been initially restricted to the patterns created by the ECM proteins. The cell binding to the substrata is dependent on several factors including, time, domain size of fibronectin (FN) and the separation of the non-adhesive region present in-between the domains. These patterned surfaces have been further used for studying directed cell migration. In vivo, a variety of cells respond to endogenous direct current electric fields (dc EF) that occur in the form of transcellular epithelial potential (TEP), which has significant relevance to directed cell migration and wound healing[1]. The application of such small dc EFs in vitro provides electrical signals to cells in order to have a directional migration that strongly depends on the EF gradient (galvanotaxis)[1,2]. The cells on the NeN engineered surfaces have been subjected to electric field to accomplish directed cell movement and study the effect of ECM protein on this directional cell migration.

Introduction and Background



Schematic representation of a Metal Oxide field Effect Transistor (MOSFET)

Novel nanostructures, materials and devices:

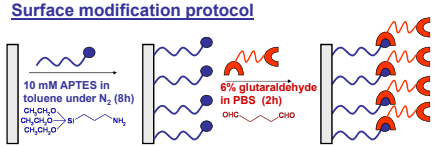
- Improve transistor speeds
- Incorporate molecules within devices (molecular electronics)
- Novel magnetic nanostructures and nanoparticle based devices for memory
- Manipulating and sensing biological processes using tailored surfaces and nanoelectronic devices

In order to have an insight into the intricacies of cell behavior such as adhesion, spreading, and migration, it is essential to understand the relationship between cells and the ECM proteins. A systematic study with substrates having spatially defined regions of ECM proteins (adhesive sites) with defined domain size would shed light on the number of sites required by a cell for a stable adhesion and spreading. Further, in response to an external cue from the environment when the cell gets polarized, it would be interesting to study the ability of the cell to establish spatial, temporal and functional asymmetry on these patterned regions of adhesive and non-adhesive sites.

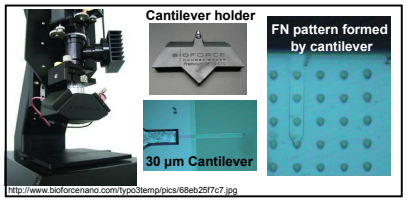
The polarization of cells occurs in response to a variety of external stimuli such as chemotaxis, haptotaxis, galvanotaxis, etc. Endogenous dcEFs occur naturally in vivo as transepithelial potential differences (TEP) and a few millivolts of TEP corresponds to a transcellular dcEFs of 50-500 mV/mm[2,3]. Cells respond to dcEFs with a directional movement towards cathode or anode, which is termed as galvanotaxis[2]. A large number of subcellular mechanisms have been offered to explain galvanotaxis but primarily a change in the intracellular milieu, in particular the intracellular Ca^{2+} concentration results in directed migration[2,3].

Patterning microarrays of fibronectin

Surface modification protocol

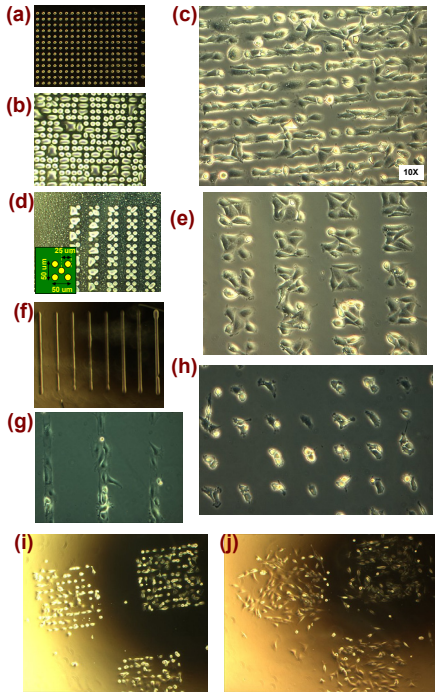


Surface patterning tool – Nano eNabler™



- Spotting solution – 1:1FN:PSB (n-octylglucoside+glycerol in H_2O)
- Size of FN spot in the array varied between 6 μm to 22 μm
- Spot size of the fibronectin depends on (a) size of the cantilever (*10 μm and 30 μm*) and (b) contact speed between cantilever and stage
- Allow overnight reaction of FN followed by hydration for 15 minutes
- Treatment with blocking solution – 2% BSA and subject to cell attachment for 0.5 h

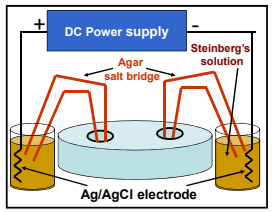
Fibronectin pattern and Patterns of cells



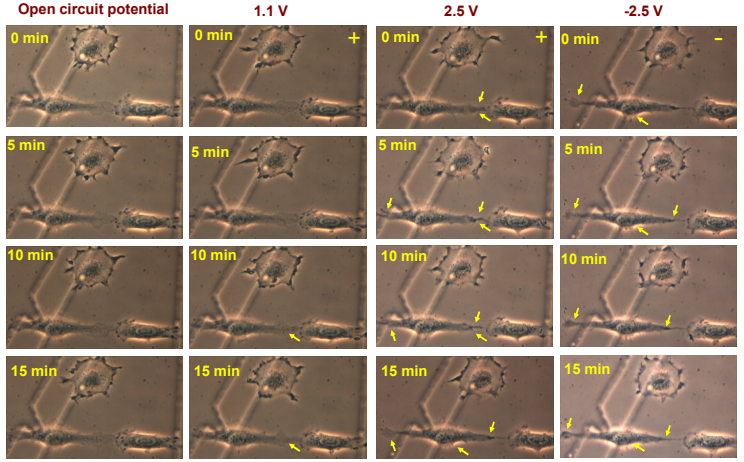
(a)10X10 array of FN spots with 50 μm separation between rows and columns (b) upon hydration and (c) after attachment of cells. Arrays of FN pattern (d) before and (e) after attachment of cells. (f) An array of FN lines (width:25 μm, length:1000 μm) with 100 μm separation between the lines and (g) after attachment of cells. (h) Cell pattern on a 10X10 array of FN with 200 μm separation between rows and columns. (i) A low magnification image (3X) showing three different well-defined 10X10 arrays of cells and (j) boundaries of cell arrays diffuse after 10 hours. Spot size FN with spot size of 22μm.

Galvanotactic response of cell patterns

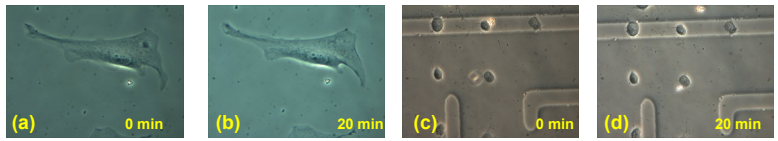
Experimental set-up



- Steinberg's solution[3]: 60 mM NaCl, 0.7 mM KCl, 0.8 mM $MgSO_4.7H_2O$, 0.3 mM $CaNO_3$. $4H_2O$ & 1.4 mM tris base
- 2% agarose gel in Steinberg's solution was used to fill glass tubing to make Agar salt bridge
- DMEM with 10 mM of HEPES used to maintain pH during the study of galvanotactic response
- Microscope stage was maintained at 37 C



Galvanotactic response of cells patterned on a dense array of FN spots. Spot size of FN is 6 μm and the distance of separation between the rows and columns in the array is 10 μm. Cells are in close proximity to each other because of the dense array of fibronectin and spread well on the pattern. No significant polarization is observed for these cells at open circuit potential or at a small applied bias of 1.1 V. At 2.5 V, significant polarization is observed for these cells. New protrusions form towards the cathode and retraction is observed on the anodic end. After reversing the bias to -2.5 V, cells show some amount of retraction away from the anode however, the recovery does not happen to the same extent and the response is slower.



Figs (a, b) cells attached on an unmodified cover slip – non-patterned substrate. Figs (c, d) Cells attached to a 10X10 FN array where the rows and columns are separated by 200 μm. No galvanotactic response is shown by the cells when attached to an unmodified and non-patterned glass cover slip. Also, galvanotactic response is largely impaired by the large distances of non-adhesive regions present between the cells in (c) and (d).

Summary

Nanopatterning of ECM proteins has been demonstrated. Controlled cell attachment and patterning is observed on transparent surfaces. A proof of principle for the galvanotactic movement of cells on patterned substrate has been demonstrated with NIH 3T3 cells. We are now using these substrates to study the effect of nano-patterned ECM matrix proteins on the galvanotactic response of the cells using cancer and other model systems and further couple the patterned nanopatterned substrates with microelectrodes to measure AC impedance of cells.

Acknowledgment: We would like to acknowledge Dr. Susan Bernacki, Department of Biomedical Engineering, NCSU and Dr. James Adams, Bioforce Inc. for their immense help and useful discussions for this project.

References [1]Funk and Monsees, Cells Tissues Organs (2006) 182, 59 [2]Mycielska and Djamgoz, J. Cell Science (2004) 117, 1632 [3]Song, Gu, Pu, Reid, Zhao, Zhao (2007) 2, 1479 [4]Zhao et al. Nature (2006), 442, 45