A Novel Surface Patterning Method for Protein-Cell Interaction Studies

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Abstract

A common technique for studying protein-cell interactions involves patterning an ECM protein or other signaling molecule onto a surface that is suitable for cell growth and subsequent analysis. Current methods of molecular surface patterning include bulk coating, photolithography, microcontact printing, and microarraying. Of these methods, only microarraying can facilitate multiplexed printing of different molecules on the same surface. Features created using pin-tool or ink-jet microarrayers are, however, many times larger than most cells. It was our objective to develop a flexible method of directly patterning multiple molecules onto a surface at the same (or smaller) spatial scale as a single cell. Briefly, we used an instrument (NanoArrayer™) equipped with microfabricated Surface Patterning Tools (SPTs) to draw multiple parallel lines of laminin, poly-D-lysine and a negative control, bovine serum albumin (BSA), onto a funtionalized glass surface. Proteins were loaded into reservoirs on the SPT that connect to microfluidic channels that dispense liquid onto the surface via a process known as FEMTO (Fluidics Enhanced Molecular Transfer Operation). Laminin, poly-D-lysine, and BSA lines were patterned using the NanoArrayer™. Proteins were incubated on the surface overnight, then the surface was blocked and washed to prevent non-specific adhesion of cells. Adherent cells were cultured on the patterned surface and found to adhere specifically to the laminin lines. Subsequent labeling of the proteins with fluorescent antibodies confirmed their presence and identity.

We have concluded that this technique is superior to existing surface patterning methods with respect to multiplexing, flexibility of pattern design, and spatial scale. Future studies using cell signaling molecules to direct differentiation or induce proliferation of individual stem cells hold great potential for human health.

Figure 1. The NanoArrayer system used to create these patterns.

Methods

Surfaces: Prolinker A (Proteogen, Korea), a bifunctional calixcrown-5 derivative was used to modify the amine glass surfaces. Silanized glass slides (Sigma) were cleaned in water for one hour. They were then incubated with Prolinker A suspended in chloroform for 4-5 hours. The Prolinker treated surfaces were finally washed with chloroform (one minute) and acetone (one minute), then air dried and stored at room temperature.

Surface Patterning Tools: SPTs manufactured from silicon dioxide using MEMS techniques served as the print cartridges. The SPTs used here had a single lever 30 µm wide, 300 µm long with a 10 µm wide channel. Prior to loading, the SPTs were treated using the BioForce UV TipCleaner for 30 minutes to make the channels hydrophilic. Samples (1-2µl) were back loaded onto these cartridges, fixed to the SPT holder and mounted onto the NanoArrayer.

Protein Patterning: Laminin, poly-D-lysine and bovine serum albumin were deposited in straight parallel lines and other straight line formations using the NanoArrayer. The lines measured 600 µm long and 10 µm wide, and they were spaced at 40, 60, 80 and 100 µm apart (20 lines each). Following matrix deposition, the samples were incubated overnight at 4ºC. Samples were then washed gently with PBS and air dried. This was followed by blocking with casein for 30 minutes. The slides were washed again with PBS (2 times x 5 minutes each) to remove excess casein, air dried and stored at 4ºC.

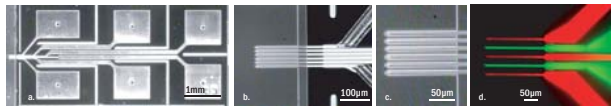


Figure 2. (a-d) Optical images of 6 lever SPT showing reservoirs with microfluidic channels leading down each cantilever. Such SPTs are ideal for simultaneously printing multiple proteins at the micron scale.

Methods

Staining: Samples were stained with rabbit anti-laminin and Alexa 488 anti-rabbit to view the lines/patterns of deposited protein. The cells under consideration were HeLa cervical carcinoma cells, which were allowed varied amounts of time to bind. It was observed that longer incubation times directly correlated with higher non-specific binding. To track cell growth along the protein lines, they were tagged fluorescently with Alexa 594 phalloidin which selectively labels F-actin. The slides were washed with PBST (2 times x 5 minutes each) and images were captured using a Nikon TE 2000-U inverted fluorescence microscope with a Hamamatsu ORCA-ER cooled CCD camera. An overlay of the fluorescent images of the protein domains (arrays/lines) and that from phalloidin labeled cells were performed to study the growth patterns of the HeLa cells.

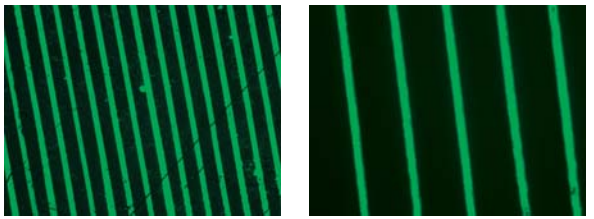
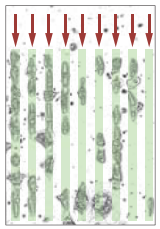


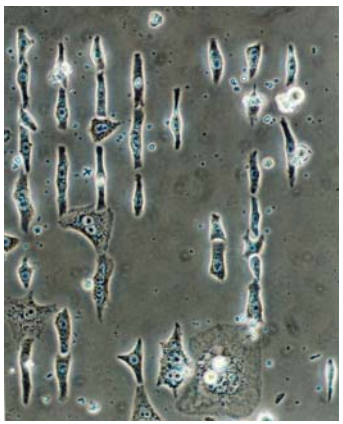
Figure 3. Examples of fluorescently tagged lines of BSA (left) and laminin (right). Not shown are the lines of poly-D-lysine.

Results

HeLa Cells on Laminin Lines

Figure 4. Optical image of HeLa cells generally constrained to vertical lines of laminin. Cells appear elongated with most focal adhesions and mitotic events occurring within the boundaries of the laminin lines. A few cells may be observed bridging two lines or making non-specific contact with the surrounding surface.

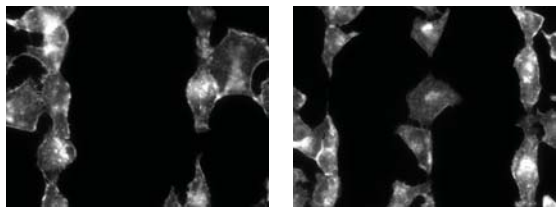




Results

HeLa Cells on Poly-D-lysine Lines

Figure 5. Fluorescent images of Alexa Fluor 594 phalloidin labelled HeLa cells adhering preferentially to lines of poly-D-lysine drawn with the NanoArrayer. As seen in Figure 4, most cells are oriented along the lines with just a few binding non-specifically.



Conclusions

- The NanoArrayer is a useful tool for patterning proteins onto surfaces suitable for cell culture
- Patterns created in this manner may be used to direct or restrict cell growth
- HeLa cells will bind preferentially to laminin or poly-D-lysine features on a blocked surface
- More complex patterns consisting of multiple proteins could be created using the NanoArrayer
- Such patterns could be used to simultaneously anchor cells and induce signal transduction pathways

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