Patterning of extra cellular proteins using the Nano eNabler for single cell studies

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Introduction

Cell patterning at the micron-scale will help elucidate communications, control and differentiation in tissues and may act as a segue to construction of artificial tissues. S e v e r a l microfabrication techniques have been developed to generate controlled patterns of cells to aid the study of attachment and growth of cells.

We have developed a new technology, the Nano eNabler™ System, to pattern surfaces with a variety of proteins. One major advantage of the Nano eNabler (NeN) over conventional microfabrication systems is that it effortlessly prints materials in 3 m-50 m domains in user defined geometric patterns. This cutting-edge technology purveys the flexibility in printing patterns and multiple species with micron-scale spatial resolutions and re-addressing the same domains.

We used the NeN to construct arrays of extracellular matrix proteins onto polystyrene and 3-aminopropyltriethoxysilane (APTES) coated borosilicate glass surfaces that were treated with gluteraldehyde. The arrayed surfaces were incubated with NG 108 and PC12 cells and the binding patterns of cells on these arrays were studied. The number of cells bound to the spot was a function of spot size and could be tuned to a single cell per spot. Dendritic processes from PC-12 cells were directed to adjacent cells in arrays with pitch of up to two cell diameters.

In conclusion, we have demonstrated the application of a novel technology for patterning ECM proteins on surfaces using the NeN to generate single cell arrays.

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Methods

A single lever SPT was incubated in a UV/Ozone chamber to enable fluid flow through the channel. An ECM mixture (Becton-Dickinson, Bedford, MA) was diluted to 0.2mg/ml in PBS buffer containing 5% glycerol and was loaded into the SPT reservoir. The loaded SPT was mounted on the Nano eNabler and incubated for 20 min at 70% RH before the start of the arraying process. The distal end of the SPT was then brought into contact with the substrate to be patterned under precise applied force and environmental control to transfer the ECM mixture from the SPT to the substrate. The amount of liquid transferred is dependent on a variety of factors, including SPT geometry, composition of the material being deposited, environmental conditions, contact time, applied force and chemistry of the surface.

ECM mixtures were printed on polystyrene and aminopropyl triethoxysilane coated glass surfaces that were modified using gluteraldehyde. The printing conditions were moderated to print spots between 7 and 25 microns. Arrays of ECM were constructed in different geometric patterns consisting of tens to several hundreds of spots per array. Following a 2 h incubation at room temperature in sealed humidified Petri dishes, the chip surfaces were blocked using a filter sterilized solution of 4% pleuronic F-127 containing 15 mg/ml BSA in PBS.

Cultures of subconfluent NG 108 cells or PC12 cells were harvested using Trypsin-EDTA. The cells were incubated at 37° C in Tris-EDTA buffer for 15 min, and then triturated to break up clumps. Fresh Culture Media (RPMI-1640 containing 7.5% fetal bovine serum and antibiotics) was added and the cell suspensions collected by centrifugation at low speed. The cells were resuspended in culture media. The chips were removed from the blocking buffer and placed in Petri dish. A suspension of cells (2.5 ml) was placed on the chip and incubated for 7 to 21 min in a CO_2 incubator. Following incubation, the media was removed by aspiration and the surface rinsed twice with complete media to remove the unbound and loosely bound cells. The chips were incubated for 2 to 12 days.

For some studies a differentiation medium (DM) containing RPMI-1640, 0.1% fetal bovine serum and 0.05% horse serum with 100 ng nerve growth factor (NGF) was used. Chips were rinsed 2x in DM and incubated in DM for 2 to 8 days in the CO_2 incubator.

Instrumentation

Fig 1

We have developed a technology for deposition of liquids in femtoliter to attoliter volumes on solid surfaces and can be used to print biological materials in intricate and multiplexed, multicomponent patterns. This technology is embodied in the Nano eNabler™ (NeN) System consisting of the Nano eNabler (Fig 1) instrumentation (the printer) and the surface patterning tools (SPTs) (Fig 2). Components of the Nano eNabler include precision motion control X and Y axis stages, a coarse Z axis stage, a high-precision fine Z axis stage, a video monitoring system, an environmental control system, a force feedback system used to control and maintain printing force, and a graphical user interface for pattern design and instrument control.

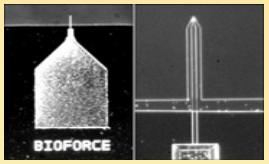
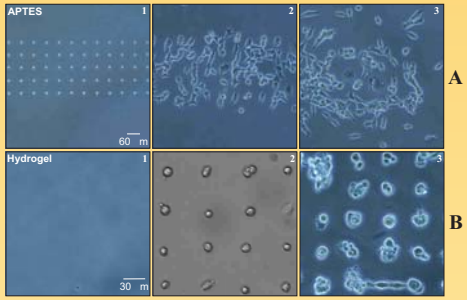


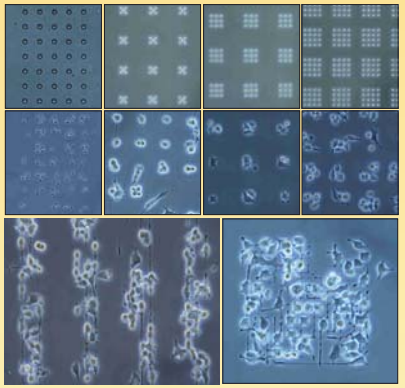
Fig 2

NG108-15 cells



NG108-15 cells on patterned APTES aldehyde glass (AAG)[A] and hydrogel surfaces[B]. A.1 ECM array (5 x 20 , 20 µm spots) on AAG . A.2, 2 h post-plating; A.3, 16 h post plating. B.1, ECM array (5 x 20, 20 µm spots) on acrylamide hydrogel (Perkin-Elmer). B.2, 4 h post-plating; B.3, 72 h post-plating.

PC12 Cells



PC12 cells on patterned APTES aldehyde surfaces [C &D]. C. ECM was printed as either arrays of single spots (20 µm spots, 60 µm pitch) or array of arrays with each array having 5, 9 or 12 spots (7 µm spots, 12 µm pitch). PC12 cells were applied to these arrays and images collected at 48 h (single spot arrays) and 24 h (for array of arrays). D. PC-12 cells bound to ECM arrays were stimulated using NGF (100ng/ml).

Arraying flexibility

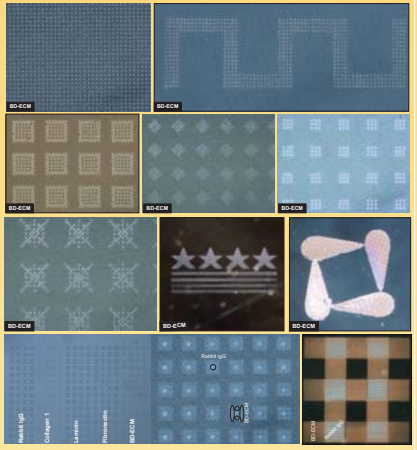


Figure 3. NeN generated patterns of ECM mixtures on unmodified polystyrene

Conclusion

Patterned ECM arrays on hydrogel and aldehyde-modified APTES coated glass were found to support PC12 and NG108-15 cell attachment. Neurite outgrowth was observed for both cell types on APTES aldehyde surfaces. For PC12 cells, neurite outgrowth followed and appeared to interact with the ECM spots forming a rectilinear pattern used in the array. Cell behavior differences were apparent. NG108-15 cells moved from sites of ECM spot capture arrays into blocked areas of the chip. PC12 cells did not. On hydrogel surfaces, NG108-15 cells seldom migrated from ECM spots, did not form neurites or cell extensions, and multiplied to form small aggregates of cells. We expect that this technology will enhance and enable the study of axon and neurite-producing cells. For example, with this technology it may be possible to study ventral surface membrane contact with adherent, differentiation-inducing, signal transductants.