Flexible Patterning of ECM's for Cell Adhesion Studies

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Introduction

Attachment and confinement of living eukaryotic cells to discrete locations on a substrate will promote understanding of tissue development, cell differentiation, response to stimuli, cell-cell communication, healing and regeneration. Microcontact printing and other methods have been employed to pattern cells; however, these methods are limited in terms of flexibility of changes in array design and ease of multiplexing two or more proteins.

Figure 1

We have developed a technology for deposition of liquids in femtoliter to attoliter volumes on solid surfaces. This technology can be used to print biological materials in intricate and multiplexed patterns. This technology is embodied in the Nano eNabler™ (NeN) System consisting of the Nano eNabler (Fig 1) instrumentation (the printer), the surface patterning tools (SPTs): (Fig 2) and the substrates. 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.

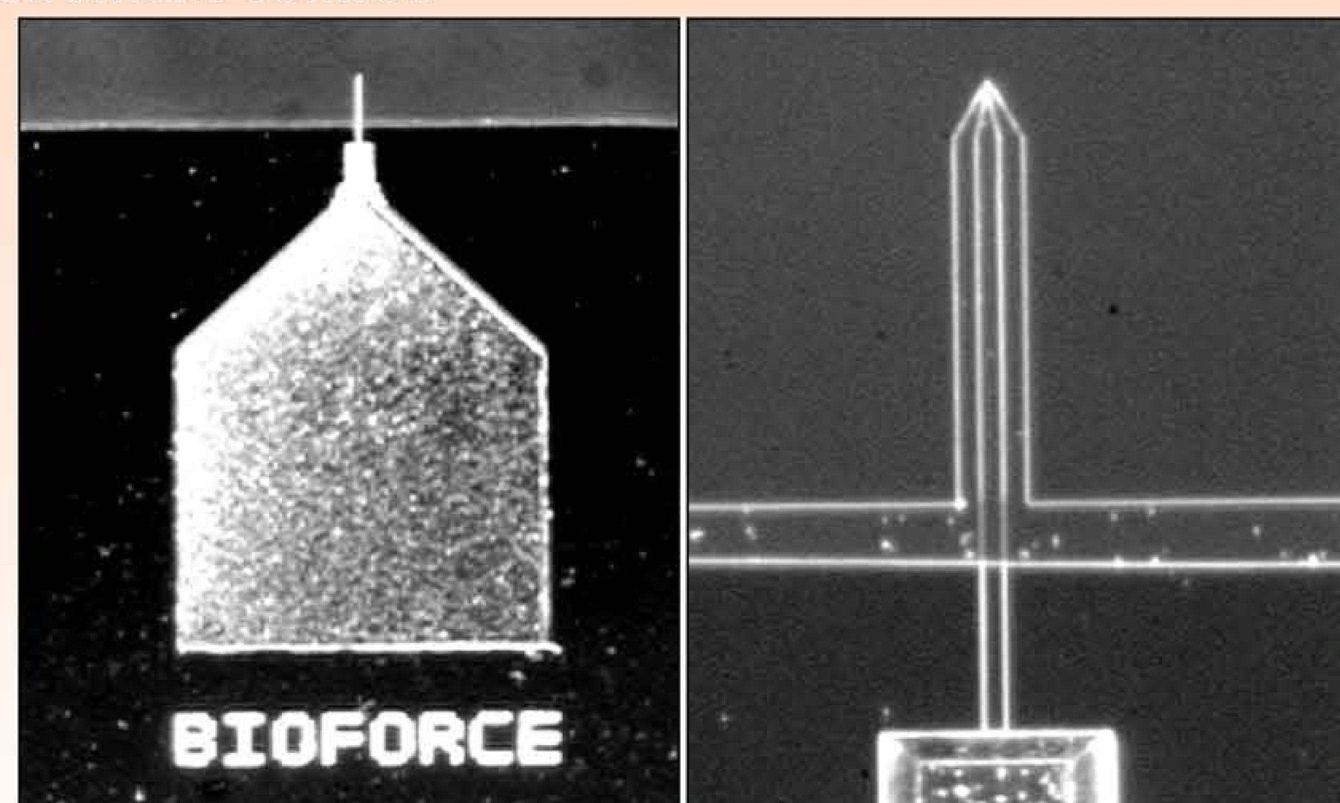

Figure 2

Methods

An extra cellular matrix (ECM) mixture (Becton-Dickinson, Bedford, MA) was diluted to a final concentration of 0.25 mg/mL in Ca^{2+} and Mg^{2+} free PBS buffer containing 5% glycerol. A 10 µm, single lever SPT (BioForce Nanosciences, Ames, IA) was incubated in a UV/ozone environment to impart hydrophilicity to the well and the channel. ECM mixture (0.5 µL) was loaded into the SPT reservoir and transferred to the distal end of the cantilever by capillary flow in the channel. The loaded SPT was placed on the Nano eNabler and brought into contact with the substrate under precise applied force and environmental control (70% relative humidity) to transfer the deposition material 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. Using the same conditions and concentration, rabbit IgG (Biomeda, Foster City, CA) was loaded and arrayed with a separate SPT.

Several arrays were written inside sterile polystyrene Petri dish lids using the ECM and IgG mixtures. Multiplexed arrays consisted of more than 4000 depositions with sizes between 5 to 10 µm and pitches between 7 to 15 µm. The arrayed surfaces were stored desiccated at 4^{0} C. For cell binding, desiccated arrays were rehydrated by incubation in a humid enviornment in the closed dish for 15 min. The unarrayed Petri dish surface was blocked using a 4% pluronic F127 (Sigma, St. Louis, MO) solution in 1 x PBS for an hour.

Murine NIH 3T3 cells were grown in 75 cm^2 T-flasks in RPMI-1640 media containing 7.5% fetal bovine serum and antibiotics (complete media) at 37^{0} C in a humidified CO_2 incubator. Cells in T flasks were released from the surface using 0.25% trypsin-EDTA and collected by centrifugation. Cells were suspended in complete media at 1.2 – 2.0 x 10^6 cells/mL for attachment studies.

Cells sufficient to cover the arrayed areas and controls (~200 µL) were incubated on the arrayed Petri dishes in a CO2 incubator for 20 min with occasional agitation. Following incubation, the media was removed by aspiration and the surface rinsed twice with complete media to remove the unbound and any loosely bound cells. The Petri dishes with arrayed NIH 3T3 cells were incubated for 16 h and images were collected. Cells remained attached to arrays for up to 14 days.

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Results

We observed that NIH 3T3 cells bound specifically and reproducibly to the ECM arrayed regions of the multiplexed arrays and did not bind to rabbit IgG nor to blocked polystyrene surfaces. The cells multiplied and remained attached to the arrayed sections of the dishes eventually filling in the interstices of the ECM domains. We also found that the cells took the shape of the arrayed domains, being guided by the pattern of attachment points if they were not in contact with other cells

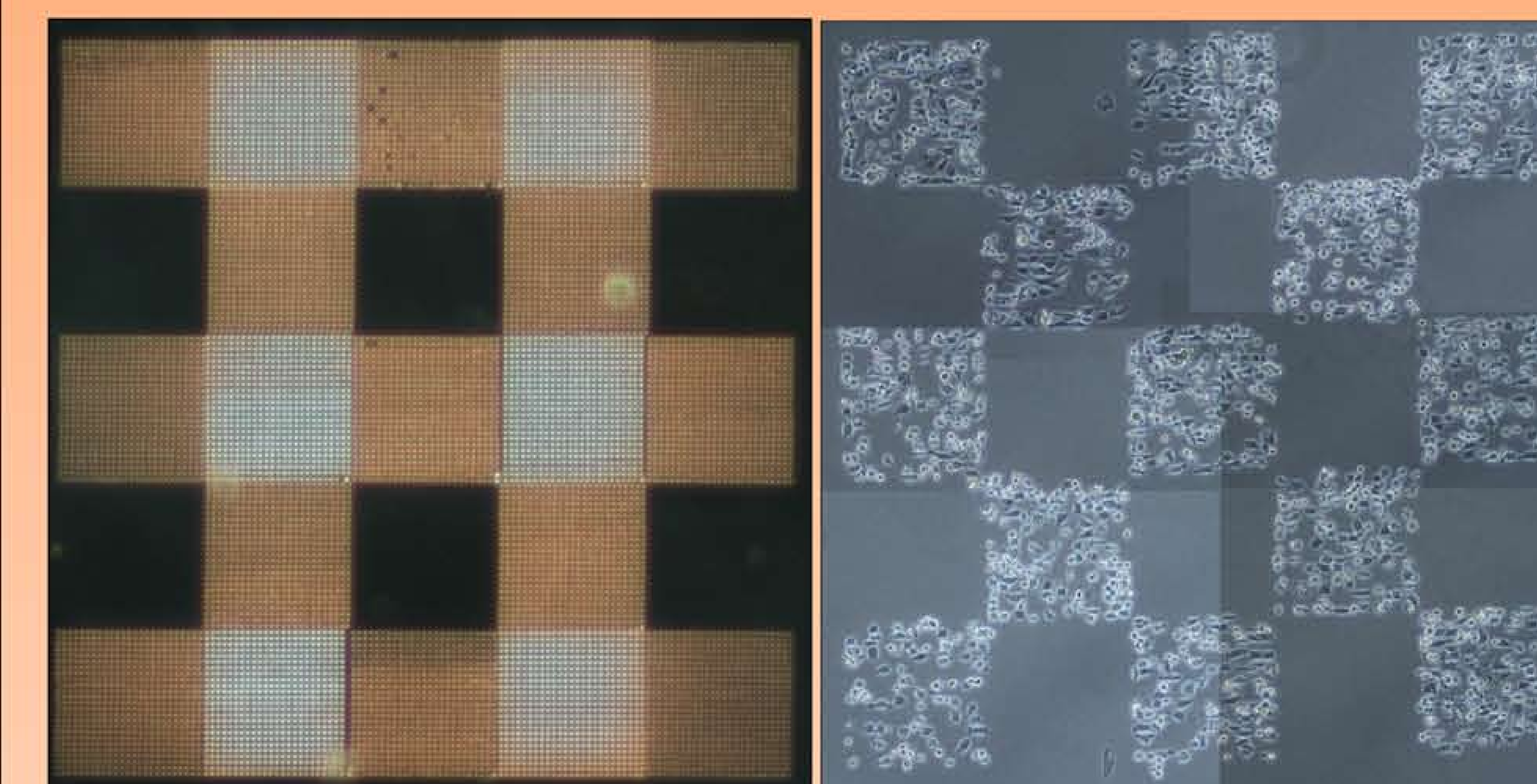

Figure 3

Furthermore, the cells did not migrate off of the arrays when printed on polystyrene. On other surfaces including glass, aldehyde acivated aminopropyltriethoxysilane (APTES) glass, polydimethylsiloxane (PDMS), tissue culture treated polystyrene and UV ozone treated polystyrene, cells bound to ECM domains on arrays remained mobile and would spread out leaving the domains if they were not density inhibited. Because most cells in culture rapidly remodel extracellular matrix proteins after adherence, the printed proteins might be remodeled, degraded or replaced over time. The data suggests, however, that lamellipodia used in locomotion cannot place NIH 3T3 cell extracellular matrix proteins on polystyrene thus restricting cells to arrayed loci. Cumulatively, these data also indicate that the NeN and SPTs can generate large, multiplexed arrays of proteins to polystyrene with protein retention in domains for use in cell binding studies. In addition, multiplexing using rabbit IgG or other protein might be used to compete with binding to elucidate biochemical interactions and requirements for cell attachment.