APPLICATION NOTE

Single Cell Micropatterning

NanoFabrication Systems

Introduction

Cellular micropatterning techniques are widely used to investigate fundamental aspects of cell behavior. Cells reportedly respond to their microenvironment and cell morphology has been shown to be important to function at an individual cell level as well as a tissue and organ level. The long rod shaped myocytes of the heart, the elongated neurons of the brain and nervous system, and the spindle shaped fibroblasts of connective tissue are all believed to play a role in the function of their respective tissues.

The ability to place cells at defined positions on a substrate is critical to the development of cell based sensors, as well as to basic cell biology research and tissue engineering applications. Earlier cell patterning techniques were based on the principles of microcontact printing, which enables either indirect (Chen et al., 1996) or direct (Thery et al., 2009) deposition of proteins. The principle limitations of microcontact printing are that its relatively large feature sizes enable the study of just one protein over a large area and its original mask or stamp restricts the number of potential pattern sizes and shapes. Another drawback is that patterning of multiple materials on the same substrate at sub-cellular scales is very difficult to achieve with microcontact printing.

Dip Pen Nanolithography® (DPN®) is an established method of nanofabrication in which materials are deposited onto a surface using a sharp tip. Unlike microcontact printing, DPN enables controlled deposition of a wide variety of materials onto various substrates with nanoscale registry, all under ambient conditions. Here we demonstrate tip-based, direct placement of multiple proteins onto a substrate, followed by micropatterning of single cells on the deposited protein domains. This methodology enables pattern construction at sub-cellular scales as well as impromptu changes in pattern geometry.

Cell Patterning Principles

NanoInk's desktop nanolithography platform, the NLP 2000 System, was employed to create sub-cellular protein arrays. First, using a custom designed one-dimensional 12-tip M-type cantilever "pen" array and a proprietary carrier solution, multiple proteins were uniformly patterned onto substrate. The NLP 2000 System was capable of printing arbitrary protein shapes at sub-cellular dimensions, as shown in Figure 1. For further details on depositing multiple proteins with the NLP 2000 System, refer to NanoInk's "Multiplexed Protein Arrays Application Note."

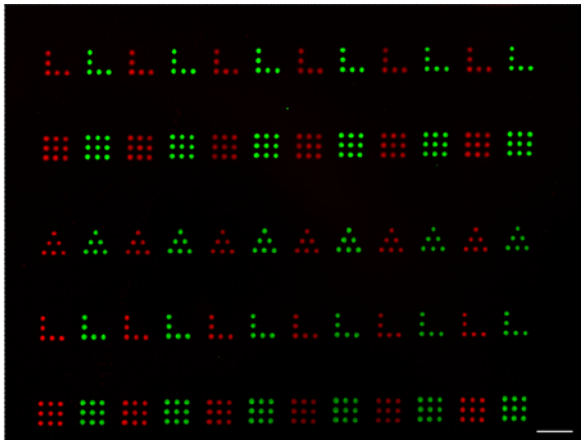

Figure 1. Fluorescent image of a multiplexed pattern of various shapes of laminin (green) and fibronectin (red) created with the NLP 2000 System. Scale bar = 50 µm.

In the second phase of this study, fibronectin was patterned onto an epoxy coated glass surface using the M-type cantilever "pen" array. Various geometric shapes of fibronectin features were constructed on a single slide, then the deposited fibronectin was allowed to couple to the slide surface.

After printing, non-patterned areas of the glass slide were blocked with BSA to prevent subsequent non-specific cell attachment. NIH 3T3 cells were added to the substrate and the slide was incubated to promote cell attachment to fibronectin patterns. Following incubation for a defined period of time, substrates were washed to remove non-specific and loosely bound cells. Specific binding of cells to fibronectin patterns was evident, although cells were still spherical and did not conform to the pattern geometry.

Substrate-bound cells were further incubated for 2 hours in a CO_2 incubator. During the incubation period, cells spread out on the protein patterns to which they had bound and began to resemble the geometric shapes of the arrayed fibronectin domains.

Single Cell Micropatterning

After this second incubation step, cells were fixed and then labeled with nucleus-, actin filament- and fibronectin-specific fluorescent tags. Cell attachment was observed in greater than 80% of the patterned fibronectin domains, as shown in Figure 2. In addition, Figure 3 clearly demonstrates that the shape of the deposited fibronectin pattern clearly directed cellular response following cell attachment.

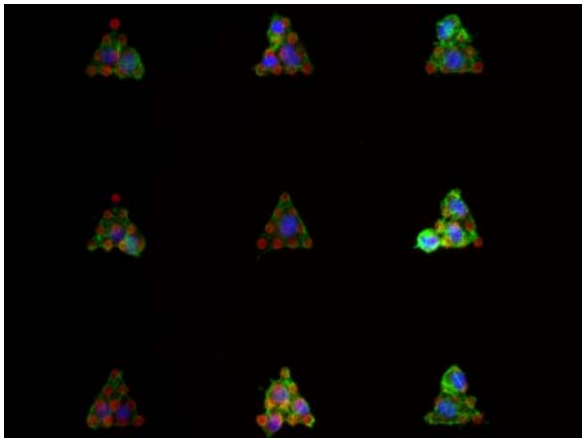

Figure 2. 20x fluorescent images of fibroblasts attached to fibronectin (red) patterned on epoxy-functionalized glass slides, showing actin (green) and nuclei (blue).

Fibronectin pattern layout was easily manipulated within the NLP 2000 System software design window. This allowed for facile control over dot size, dot pitch, pattern size, pattern shape, and pattern separation. Cellular response could be manipulated by varying the underlying surface dot size and pitch, while the number and location of attached cells could be controlled by modifying the overall fibronectin pattern.

Conclusions

We have demonstrated that the NLP 2000 System has the ability to design patterns with nanometer scale resolution and to quickly print large arrays of protein features. These capabilities provide unlimited platform flexibility for investigating cell behavior such as adhesion, migration, differentiation, cell-cell interaction, cell-matrix interaction, wound healing, and metastasis at the single cell level.

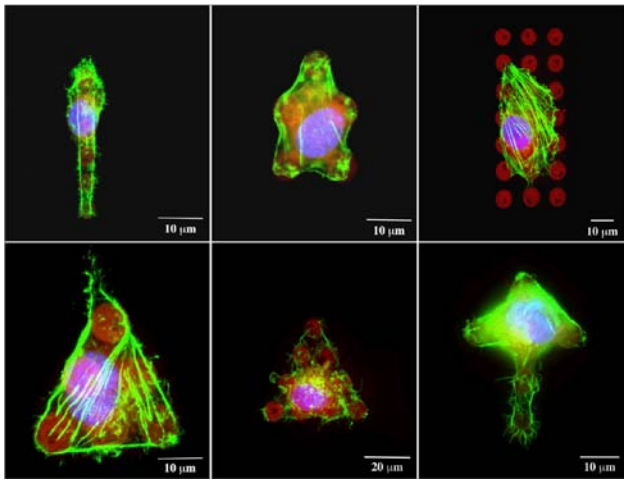

Figure 3. 63x fluorescent images of fibroblasts attached to fibronectin (red) patterned on epoxy-functionalized glass slides, showing actin (green) and nuclei (blue).

References

Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M., Ingber, D.E. Micropatterned Surfaces for Control of Cell Shape, Position, and Function. Biotechnol. Prog. 1998.

Théry, M. and Piel, M. Adhesive Micropatterns for Cells: A Microcontact Printing Protocol, Cold Spring Harb. Protoc. 2009.

NanoInk Products Used

NLP 2000 System
DPN® Pen Arrays: Type M
DPN® Inkwell Arrays: Type M-12MW

Learn more about NanoInk products and services at www.nanoink.net. Or call us at 847-679-NANO (6266).