

## Introduction

Micropatterned substrates are powerful new tools for investigating mechanisms of cell function, and they have made recent and substantial contributions to the understanding of basic cell biology, cancer, and stem cell differentiation. Living cells interact with their environment through signals mediated by proteins and other signaling molecules. Some of these molecules are soluble and can act at large distances, but many signaling molecules are immobilized and require a direct physical contact with the cell, through cell-cell contacts or a cell-extracellular-matrix interaction, for example. Recent experiments using micropatterned surfaces have made clear that the spatial organization of biomolecules is central to a cell's function -- that cells read, interpret, and use the information conveyed by their surroundings<sup>1,2</sup>.

Protein micropatterned substrates can define the size, shape, location of cells on a substrate, reduce the variability in cell-based measurements, and direct cell functions such as adhesion, spreading, DNA synthesis, mitosis, migration, and apoptosis. Some recent examples from the literature are discussed below, highlighting the utility of protein micropatterns for investigating fundamental properties of cells.

## Cell morphology, structure, and function

The spatial organization of molecules at a sub-cellular scale affects the morphology, structure, and functions of cells. For cell-matrix interactions the spatial distribution at both short and long length scales are important. For example, a spacing of 440 nm for RGD peptides has been shown to be sufficient for integrin to mediate fibroblast spreading but not sufficient for the formation of focal adhesions<sup>3</sup>. The latter requires significantly smaller spacing. On a longer length scale, patterns of molecules are involved in migration guidance<sup>4,5</sup>.

Protein micropatterns provide biologists with subcellular-scale control over the local microenvironment of the cell. One particularly notable line of work is that originating from the Ingber and Whitesides groups, where patterns of extracellular matrix (ECM) proteins were shown to be capable of controlling cell shape; for example, a square protein pattern could direct cells to take on a square morphology<sup>6</sup>. The same study showed that the degree of DNA synthesis within the cells and apoptosis of the cells could be modulated along a spectrum by plating the cells on progressively smaller adhesive islands.

More recent efforts have demonstrated that patterns of ECM proteins of varying geometries can modulate the internal organization of cells grown on the patterns. Cell polarity and the distribution of stress fibers, traction forces, the actin network, and microtubules and the positions of organelles such as the nucleus, centrosomes and Golgi apparatus are all governed by the pattern of adhesive ECM molecules on a surface<sup>7</sup>. The axis of cell division is also guided by the shape of the cell and its contacts with the ECM<sup>8</sup>, and the normal function of cell-cell contacts requires proper organization and position of molecules in adhesion complexes<sup>1</sup>.

Two-dimensional patterns of ECM proteins can influence cell growth in the third dimension as well. Growth patterns of dorsal root ganglia explants grown in interface cultures, in which

the cells were plated at the interface of a 2D patterned substrate and a collagen matrix, varied with the type of ECM molecule and the pattern. For example, neurites extended along the surface of the gel and aligned with the pattern when the DRGs were plated on laminin patterns, but they extended into the matrix when the cells were plated on patterns of chondroitin sulfate proteoglycans<sup>9</sup>. The neurites on the laminin patterns were also longer than in other cultures.

## Cancer

The organization and molecular composition of the immediate surroundings of tumor cells are critical to phenomena such as tumor growth, angiogenesis, and metastasis<sup>10,11,12</sup>. Fibronectin micropatterns of cell-adhesive regions demonstrated the ability of focal adhesion kinase to suppress and promote growth of endothelial cells and underscore the multiple mechanisms by which a single molecule can control cell proliferation<sup>13</sup>. A micropatterned co-culture of bone and prostate cancer cells showed that the expression of metastasis-associated genes are regulated in response to heterotypic contact and soluble factors<sup>14</sup>. Human leukemia cells grown on a culture plate patterned with the cytokine erythropoietin (Epo) apoptosed on Epo-free regions but not on Epo-rich regions<sup>15</sup>. The experimental control of the spatial organization of adhesion and ECM components thus provides new opportunities to investigate the fundamental mechanisms of cancer biology<sup>16,17</sup>.

## Stem cells

The local biomolecular microenvironment can control stem cell differentiation via soluble factors, topographic and mechanical cues, and by biochemical cues in the ECM. Experiments using micropatterned surfaces showed that cell shape regulates the commitment of human mesenchymal stem cells (hMSCs) to the adipogenic or to osteogenic lineage by modulating the cytoskeletal tension and RhoA activity<sup>18</sup>. Further work with large micropatterned areas that defined multicellular islands demonstrated that the location of cells in the island, on the periphery versus at the center, and the resulting differing mechanical forces on the cells determined the lineage commitment of hMSCs in the different locations<sup>19</sup>.

Other work with stem cells demonstrated that morphology does not always imply functional phenotype. Cortical neural precursor cells grown on micropatterns of laminin lines had a more mature morphology, less proliferation capacity, and greater decrease of nestin expression than cells grown on clusters of ECM dots, suggesting that the protein lines drove neuronal differentiation<sup>20</sup>. However, whole-cell patch clamp measurements showed no difference in electrophysiological properties between the cells. In this system the development of the electrophysiological functions of the cells on both patterns ceased at an immature stage.

## Cellular migration and dynamics

The direction of cellular migration has historically been controlled using gradients of soluble signaling molecules, but work with micropatterned surfaces has shown that static arrangements of ECM proteins can direct cell migration. Cells plated on geo-

metric patterns of ECM extended microspikes, lamellipodia, and filopodia preferentially from the corners rather than from the edges<sup>21,22</sup>. Cells plated on tear-drop-shaped patterns polarized in the direction of the blunt end of the pattern, and when released by electrochemical desorption, the cells migrated in the direction of the blunt ends<sup>23</sup>. Fibroblasts cultured on patterns of rectangles 1 µm wide and up to 8 µm long spread and moved in directions determined by the aspect ratio and spacing of the rectangles, showing that the defined positioning of focal adhesions spatially controlled Rac activation<sup>24</sup>.

Comparisons of human epidermal keratinocyte migration on one-dimensional (1D) line patterns of fibronectin, 2D patterns, and in 3D fibrillar matrices revealed that the migration phenotype on the 1D lines mimics that of cells in 3D matrices more than that of cells on 2D patterns<sup>25</sup>. In 1D and 3D environments, the cells exhibited rapid, uniaxial migration that was independent of ECM-ligand density and dependent on myosin II contractility and microtubules. The work implies that migration in 3D fibrillar matrices is based on a 1D mechanism.

## Cell-cell interactions

Because protein micropatterns can be designed to define the shapes and distributions of cells on a substrate, they can be used to investigate cell-cell interactions in a systematic manner that minimizes variability due to the random size and orientation of cells and the heterogeneity in the types and geometries of cell-cell contacts. Cell pairs grown in 'bow-tie' patterns of fibronectin, in which each cell of a pair spread in an opposing trapezoid, and contact occurred along the narrowest part of the pattern, were used to quantify the structural and functional interactions between cardiomyocytes and cardiac fibroblasts, skeletal fibroblasts, and bone-derived mesenchymal stem cells. Over 5,000 individual cell pairs were analyzed, and the expression levels and spatial distributions of connexin-43 and N-cadherin, electrical and mechanical coupling proteins, respectively, varied significantly with the size of the contact region and the types of cells in the pair<sup>26</sup>.

## Summary

Over the last one hundred years, the tools for *in vitro* cell culture have evolved from glass Petri dishes to plastic dishes uniformly coated with adhesion-promoting proteins or other ECM mimics. Recently protein micropatterned substrates for cell culture have become powerful new research tools for investigations into cell structure, function, and pathology. Because they allow precise control over the local protein environment of the cell, they can be used to dictate the size, shape, and position of cells in culture, normalize cell responses to stimuli, and direct cellular functions. Using protein micropatterned substrates in combination with standard *in vitro* biochemical, genetic, and measurement techniques, biologists can ask and answer new classes of questions about cellular function and develop a new understanding of how cells interact with their local environment.

<sup>1</sup> Thiery, J. P. Cell adhesion in development: a complex signaling network. *Curr Opin Genet Dev* 13, 365-371 (2003).

<sup>2</sup> Ingber, D. E. Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ Res* 91, 877-887 (2002).

<sup>3</sup> Massia, S. P. & Hubbell, J. A. An RGD spacing of 440 nm is sufficient for integrin alpha V beta 3-mediated fibroblast spreading and 140 nm for focal contact and stress fiber formation. *J Cell Biol* 114, 1089-1100 (1991).

<sup>4</sup> Brown, A. et al. Topographic mapping from the retina to the midbrain is controlled by relative but not absolute levels of EphA receptor signaling. *Cell* 102, 77-88 (2000).

<sup>5</sup> Kiryushko, D., Berezin, V. & Bock, E. Regulators of neurite outgrowth: role of cell adhesion molecules. *Ann N Y Acad Sci* 1014, 140-154 (2004).

<sup>6</sup> 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* 14, 356-363 (1998).

<sup>7</sup> They, M., Pepin, A., Dressaire, E., Chen, Y. & Bornens, M. Cell distribution of stress fibres in response to the geometry of the adhesive environment. *Cell Motil Cytoskeleton* 63, 341-355 (2006).

<sup>8</sup> They, M. et al. The extracellular matrix guides the orientation of the cell division axis. *Nat Cell Biol* 7, 947-953 (2005).

<sup>9</sup> Kofron, C. M., Fong, V. J. & Hoffman-Kim, D. Neurite outgrowth at the interface of 2D and 3D growth environments. *J Neural Eng* 6, 16002 (2009).

<sup>10</sup> Griffith, L. G. & Swartz, M. A. Capturing complex 3D tissue physiology in vitro. *Nat Rev Mol Cell Biol* 7, 211-224 (2006).

<sup>11</sup> Bissell, M. J., Rizki, A. & Mian, I. S. Tissue architecture: the ultimate regulator of breast epithelial function. *Curr Opin Cell Biol* 15, 753-762 (2003).

<sup>12</sup> van Kempen, L. C., van Muijen, G. N. & Ruiter, D. J. Melanoma progression in a changing environment. *Eur J Cell Biol* 86, 65-67 (2007).

<sup>13</sup> Pirone, D. M. et al. An inhibitory role for FAK in regulating proliferation: a link between limited adhesion and RhoA-ROCK signaling. *J Cell Biol* 174, 277-288 (2006).

<sup>14</sup> Satcher, R. L. J., Dvorkin, K., Levenson, A. S., Vandebroek, T. & Stupp, S. I. Gene expression in cancer cells is influenced by contact with bone cells in a novel coculture system that models bone metastasis. *Clin Orthop Relat Res* 54-63 (2004).

<sup>15</sup> Ito, Y., Hasuda, H., Yamauchi, T., Komatsu, N. & Ikebuchi, K. Immobilization of erythropoietin to culture erythropoietin-dependent human leukemia cell line. *Biomaterials* 25, 2293-2298 (2004).

<sup>16</sup> Pirone, D. M. & Chen, C. S. Strategies for engineering the adhesive microenvironment. *J Mammary Gland Biol Neoplasia* 9, 405-417 (2004).

<sup>17</sup> Ingber, D. E. Can cancer be reversed by engineering the tumor microenvironment? *Semin Cancer Biol* 18, 356-364 (2008).

<sup>18</sup> McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriaraju, K. & Chen, C. S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev Cell* 6, 483-495 (2004).

<sup>19</sup> Ruiz, S. A. & Chen, C. S. Emergence of patterned stem cell differentiation within multicellular structures. *Stem Cells* 26, 2921-2927 (2008).

<sup>20</sup> Magnusson, A. K., Linderholm, P., Vieider, C., Ulfendahl, M. & Erlandsson, A. Surface protein patterns govern morphology, proliferation, and expression of cellular markers but have no effect on physiological properties of cortical precursor cells. *J Neurosci Res* 86, 2363-2375 (2008).

<sup>21</sup> Parker, K. K. et al. Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces. *FASEB J* 16, 1195-1204 (2002).

<sup>22</sup> Brock, A. et al. Geometric determinants of directional cell motility revealed using microcontact printing. *Langmuir* 19, 1611-1617 (2003).

<sup>23</sup> Jiang, X., Bruzewicz, D. A., Wong, A. P., Piel, M. & Whitesides, G. M. Directing cell migration with asymmetric micropatterns. *Proc Natl Acad Sci U S A* 102, 975-978 (2005).

<sup>24</sup> Xia, N. et al. Directional control of cell motility through focal adhesion positioning and spatial control of Rac activation. *FASEB J* 22, 1649-1659 (2008).

<sup>25</sup> Doyle, A. D., Wang, F. W., Matsumoto, K. & Yamada, K. M. One-dimensional topography underlies three-dimensional fibrillar cell migration. *J Cell Biol* (2009).

<sup>26</sup> Pedrotty, D. M. et al. Structural coupling of cardiomyocytes and noncardiomyocytes: quantitative comparisons using a novel micropatterned cell pair assay. *Am J Physiol Heart Circ Physiol* 295, H390-400 (2008).