New Tools for Understanding the Role of the Extracellular Matrix in Cell Morphology: A Combined Photopatterning in Nanotopography Study

Hamed Ghazizadeh[1], Elliot Fisher[1], Heejoon Choi[1], Pierre-Olivier Strale[2], Nicholas A. Geisse[1]
[1]Curi Bio, Inc., Seattle, WA, USA; [2]Alvéole, Paris, France

Summary

In this application note, the differential impact of the chemical and structural components of the Extracellular Matrix (ECM) was investigated and compared to the structure and organization of HeLa cells. Morphology was assessed for cells cultured on micropatterned Fibronectin (FN) protein arrays formed on nanotopographical features. Through this approach, the delineation of the role and importance of each of these extracellular cues in directing cell organization was accomplished. Spatial patterning of ECM proteins has been repeatedly demonstrated to closely control cell and tissue shape and intracellular organization[1, 2, 3, 4, 5]. When FN patterns are formed in the presence of nanoscale physical features, cells will preferentially align to the orientation of the mechanical cues, while the specific transmembrane proteins that involved in cell adhesion will organize based on the FN patterns.

Introduction

Cells use structural and chemical cues from their environment to drive a number of important processes. Studies show that the extracellular matrix (ECM) plays a critical role in the differentiation, development, and function of various cell types. In response to this, cell biologists and bioengineers have developed new tools — primarily adapted from the microelectronics industry — to better understand the role that these cues play in controlling and directing cell function and signaling. It has been demonstrated that nanoscale topographical features in the local environment of the cell can influence processes such as morphology, alignment, adhesion, migration, proliferation, and cytoskeleton organization. Additionally, factors such as size, orientation, geometry, and physicochemical properties of the cell microenvironment all contribute to biological function.

Attempts to replicate the geometric structure of the ECM have been accomplished using a variety of techniques. Early strategies utilized mechanical damage or scoring the surfaces of cell culture plates to induce cell and tissue alignment[6]. More sophisticated techniques involved the use of oriented electrospun fibers[7, 8, 9] and direct microcontact printing of ECM proteins. These techniques trade complexity, reproducibility, and precision with ease-of-use and practicality. While some of these techniques offer highly

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precise ECM proteins patterning down to a few nanometers, they often rely on complex micro – and nanofabrication techniques that require the use of expensive equipment in a cleanroom laboratory. Furthermore, the complexity and prolonged timeline of these approaches makes experimental iteration difficult and even economically unfeasible for many researchers.

Findings

Recent innovations in UV-sensitive chemistry and microscopy have enabled a new method which combines the high-precision and reproducibility of lithography techniques with the ease-of-use and accessibility of microscopy approaches to control the geometrical organization of ECM proteins. Based on the LIMAP technology[10], the PRIMO maskless photopatterning system (Fig.1A) from Alvéole (Paris, France) enables researchers to design and fabricate new ECM proteins geometries (micropatterns) in a matter of minutes using a bench-top, optical-microscopy based instrument. Under the control of the LEONARDO software, PRIMO allows one to project any UV images (λ=375 nm) through the objective of an inverted microscope onto the substrate of interest. The combined action of the structured light and a photoactivatable reagent (PLPP) degrades the anti-fouling coating (Fig.1B, top right) allowing for the biomolecule of interest to be adsorbed specifically where UV light was projected (Fig.1B, bottom left) and the subsequent cell adhesion.

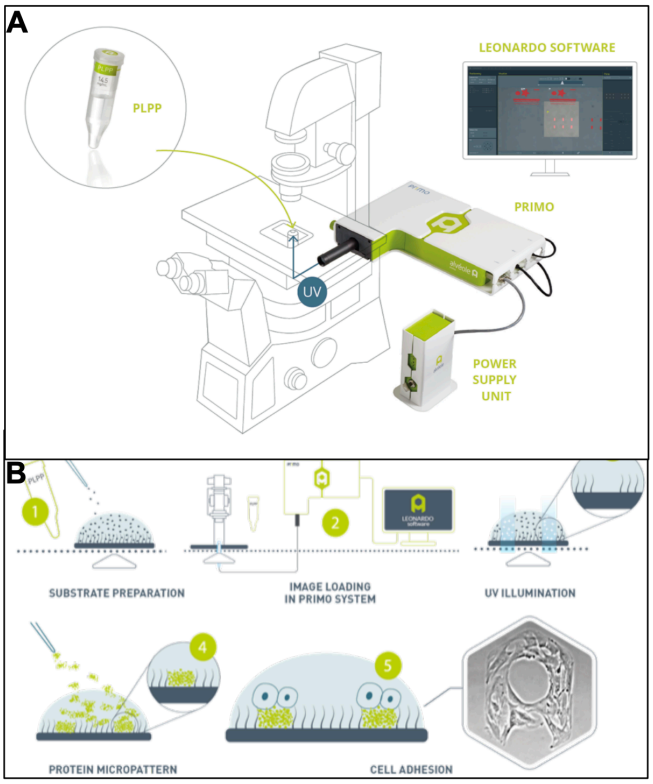

Figure 1: PRIMO versatile maskless photopatterning. *(A) PRIMO system installed on an inverted microscope, and complementary products developed by Alvéole, PLPP and LEONARDO.* ***(B)*** *Key steps of the micropatterning process using PRIMO optical system, LEONARDO software and the PLPP photo-initiator, followed by cell seeding.*

In addition to controlling the spatial pattern of the ECM, the physical shape of the cell's microenvironment plays an important role in directing cell and tissue organization. Many of the previously reported approaches are incompatible with other techniques like optical microscopy. NanoSurface Plates from Curi Bio (Seattle, USA) provides a precise nanotopography while maintaining compatibility with standard laboratory techniques (Fig.2A). The nanopatterned culture surface topography (Fig.2B) closely mimics the fibrillar structure of the ECM (Fig.2C). As adherent cells attach to the surface, small membrane invaginations in the scale of a focal adhesion form on the grooved surface (Fig.2D). This structural patterning drives higher-order cytoskeletal and tissue structure similar to what is seen is native tissue but is typically absent in cells grown on traditional glass and plastic cultureware, (Fig.2E) promoting the development of more physiologically relevant structural and functional phenotypes[11].

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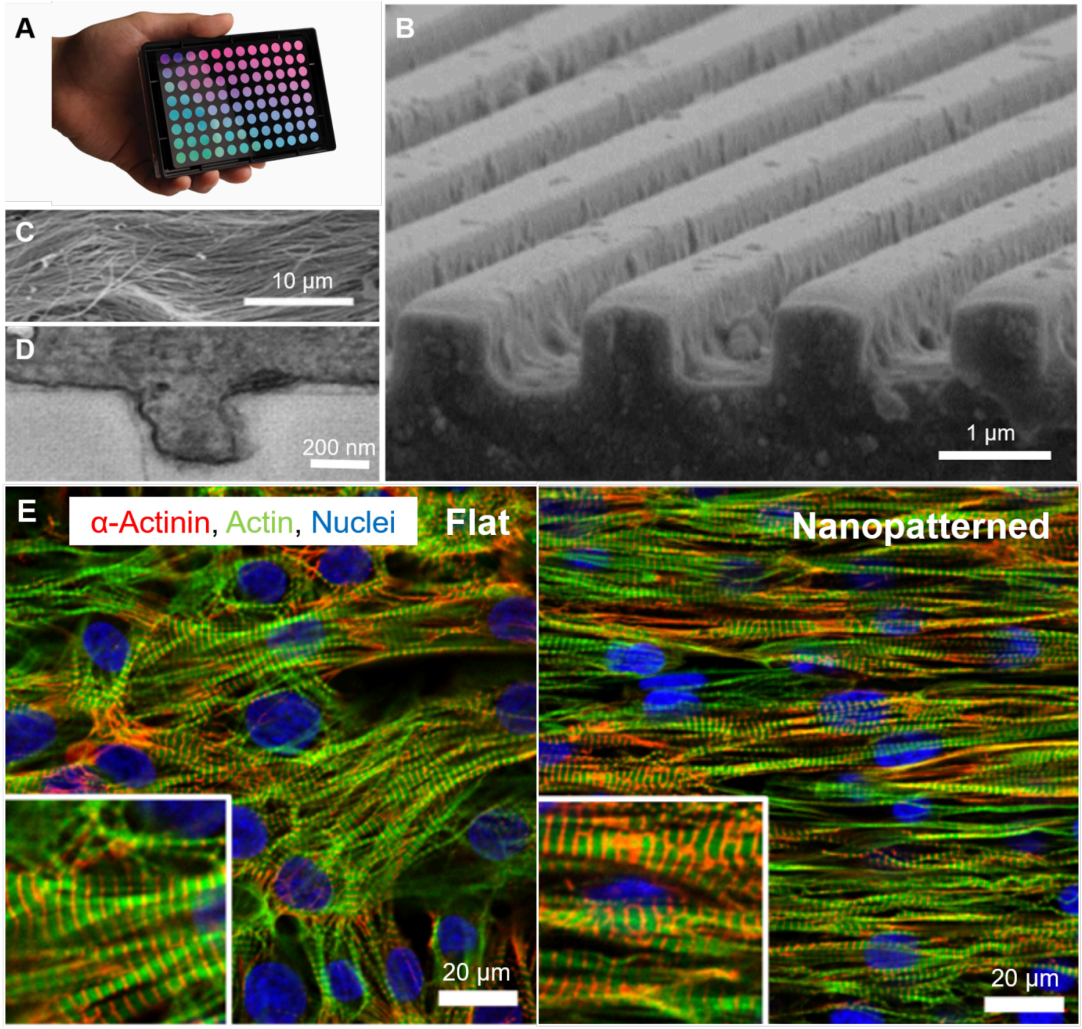

Figure 2: Structural impact of nanotopographical patterned substrates. ***(A)*** *The 96-well plate features a glass bottom with nanopatterned surface topography.* ***(B)*** *Scanning Electron Microscopy image of the culture surface shows the highlyprecise and accurate nanoscale features that are used to mimic the native extracellular matrix.* ***(C)*** *The aligned architecture of the underlying matrix of the native myocardium.* ***(D)*** *Small membrane invaginations in the scale of focal adhesions form on the grooved surface following cell adhesion on the substrate.* ***(E)*** *Heart cells cultured on NanoSurface Plates demonstrate enhanced structural and phenotypic development.*

To assess the relative effects of ECM protein micropatterning and nanopatterned structural surfaces on cell orientation, stripes of Cy3-conjugated FN were micropatterned with the PRIMO system onto NanoSurface Plates. Protein stripes were arrayed both along and perpendicular to the direction of the nanotopography (Fig. 3). In the absence of structural cues, cells cultured on FN patterns preferentially oriented along the length of the ECM stripe and were constrained to areas of patterned proteins with little to no growth on 'bare' areas between ECM-coated areas. Similarly, cells grown on nanopatterned surfaces aligned along the length of the groove pattern. In this experiment, both techniques (ECM protein micropatterning and surface nanopatterning) were combined and their relative contributions to cellular organization were measured.

HeLa cells were seeded onto these specially-prepared surfaces and allowed to adhere for 3 hours. After incubation, cells were fixed in 4% paraformaldehyde with a permeabilizing agent. Actin cytoskeleton was visualized using a fluorescently tagged phalloidin. Focal adhesions were stained using anti-integrin-β1staining

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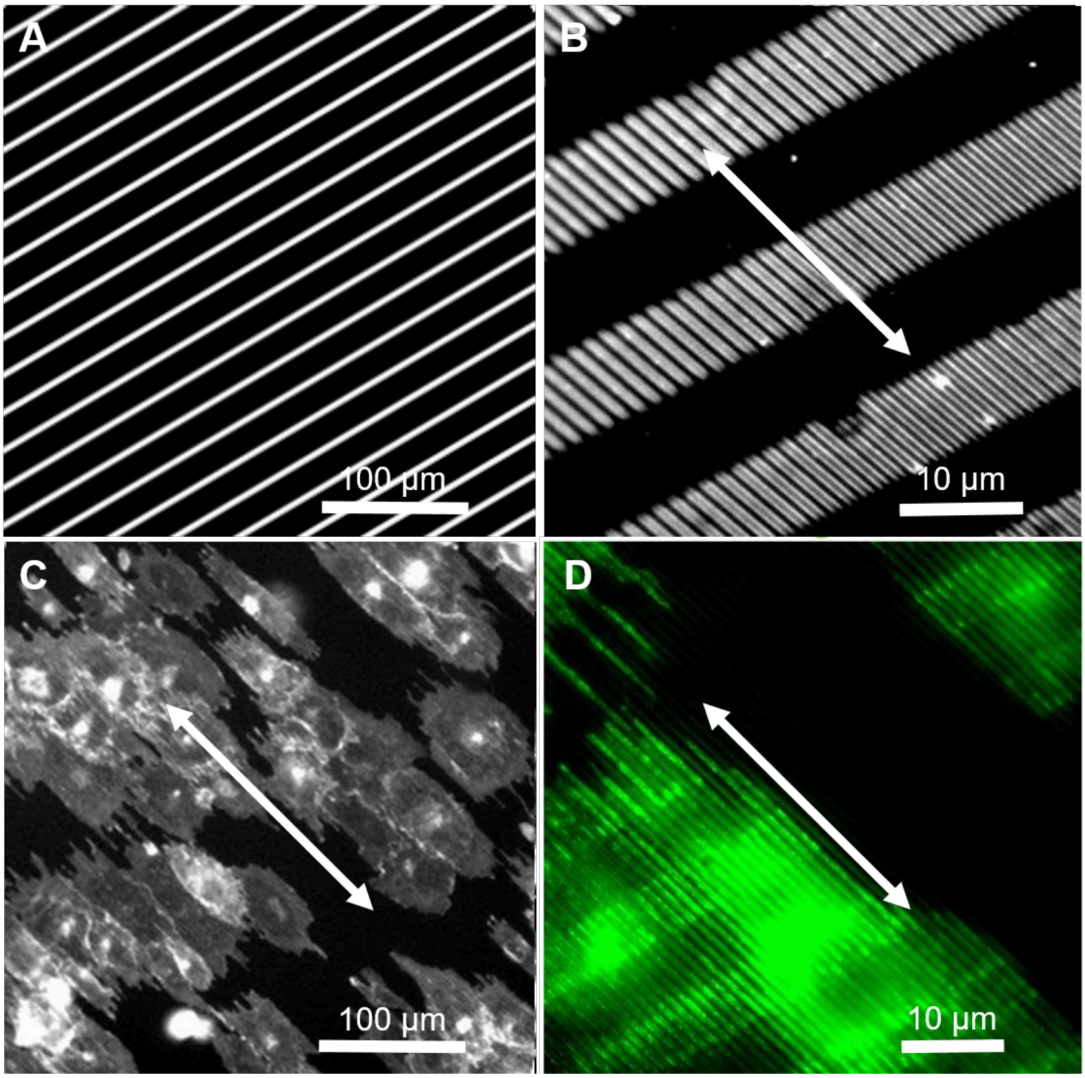

Figure 3: The relative contributions of ECM patterning and surface patterning on cell orientation. *The structure of the FN pattern as* ***(A)*** *designed in the LEONARDO photopatterning software, and* ***(B)*** *visualized on NanoSurface Plates using Scanning Electron Microscopy. Protein stripes were arrayed perpendicular to the direction of the nanotopography (as shown by the arrow).* ***(C)*** *The wide-field images of actin stained cells show that HeLa cells preferentially align along the axis of the NanoSurface Cultureware (as shown by the arrow).* ***(D)*** *The focal-adhesion associated proteins (integrin-β1 staining) are preferentially organized to areas of FN deposition which is in direct contrast to the physical nanostructures on the surface.*

and revealed with a secondary antibody. The structure of the FN pattern as designed (Fig. 3A) in the LEONARDO photopatterning software is clearly seen on the NanoSurface Plates 3B), while regular breaks in the stripes due to the trench-like topography of the culture surface can be observed across the sample surface. Wide-field images of actin stained cells show that HeLa cells preferentially align along the axis of the NanoSurface Plates, which runs diagonally from top left to bottom right in the image 3C). Small invaginations at both the leading and lagging edge of the cells indicate that these membrane processes closely follow the physical structure of the surface. Furthermore, several cell pairs in close proximity had nuclei that were directly adjacent to each other along the axis of the topography, suggesting that cell movement was also influenced by topographical orientation. This phenomenon was observed across various patterns of FN protein (Fig. 3C, D) on the same nanotopographically patterned surface. Intriguingly, despite the overall cell orientation being

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biased along the nanotopography of culture surface, integrin-β1 stains 3D) clearly show that these focal-adhesion associated proteins preferentially organized to areas of FN deposition, which is in direct contrast to the physical nanostructures on the surface.

Conclusions

With the advent of new technologies, precise control of the biochemical and mechanical properties of the *in vitro* cell environment is now accessible to more researchers, opening the door to new approaches aiming to better understand cell-ECM relationships. These new techniques must be compatible with traditional instrumentation and assays to provide a complete picture of how cells interact with their environment. In addition to the geometrical boundaries of protein micropatterns, the physical structure of the cell microenvironment also plays a critical role in directing cell structure and function. As researchers continue to build better biomimetic *in vitro* systems, the tools and techniques represented here will become increasingly vital to the cell biology research community.

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Curi Bio, Inc.
3000 Western Avenue, Suite 400
Seattle, WA 98121 USA
www.curibio.com

Alvéole
30 Rue de Campo Formio
75013 Paris, France
www.alveolelab.com

References

[1] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, and D. E. Ingber, "Geometric control of cell life and death," *Science*, vol. 276, no. 5317, pp. 1425–1428, 1997.

[2] D.-H. Kim, E. A. Lipke, P. Kim, R. Cheong, S. Thompson, M. Delannoy, K.-Y. Suh, L. Tung, and A. Levchenko, "Nanoscale cues regulate the structure and function of macroscopic cardiac tissue constructs," *Proceedings of the National Academy of Sciences*, vol. 107, no. 2, pp. 565–570, 2010.

[3] N. A. Geisse, S. P. Sheehy, and K. K. Parker, "Control of myocyte remodeling in vitro with engineered substrates," *In Vitro Cellular & Developmental Biology-Animal*, vol. 45, no. 7, pp. 343–350, 2009.

[4] K. K. Parker, A. L. Brock, C. Brangwynne, R. J. Mannix, N. Wang, E. Ostuni, N. A. Geisse, J. C. Adams, G. M. Whitesides, and D. E. Ingber, "Directional control of lamellipodia extension by constraining cell shape and orienting cell tractional forces," *The FASEB journal*, vol. 16, no. 10, pp. 1195–1204, 2002.

[5] H. N. Kim, D.-H. Kang, M. S. Kim, A. Jiao, D.-H. Kim, and K.-Y. Suh, "Patterning methods for polymers in cell and tissue engineering," *Annals of biomedical engineering*, vol. 40, no. 6, pp. 1339–1355, 2012.

[6] N. Geisse, A. Feinberg, P. Kuo, S. Sheehy, M. Bray, and K. Parker, "Micropatterning approaches for cardiac biology," *Micro-and Nanoengineering of the Cell Microenvironment*, 2008.

[7] D. Li, Y. Wang, and Y. Xia, "Electrospinning of polymeric and ceramic nanofibers as uniaxially aligned arrays," *Nano letters*, vol. 3, no. 8, pp. 1167–1171, 2003.

[8] C. Guetta-Terrier, P. Monzo, J. Zhu, H. Long, L. Venkatraman, Y. Zhou, P. Wang, S. Y. Chew, A. Mogilner, B. Ladoux, *et al.*, "Protrusive waves guide 3d cell migration along nanofibers," *Journal of Cell Biology*, vol. 211, no. 3, pp. 683–701, 2015.

[9] Y. Liu, J. Wei, J. Lu, D. Lei, S. Yan, and X. Li, "Micropatterned coculture of hepatocytes on electrospun fibers as a potential in vitro model for predictive drug metabolism," *Materials Science and Engineering: C*, vol. 63, pp. 475–484, 2016.

[10] P.-O. Strale, A. Azioune, G. Bugnicourt, Y. Lecomte, M. Chahid, and V. Studer, "Multiprotein printing by light-induced molecular adsorption," *Advanced Materials*, vol. 28, no. 10, pp. 2024–2029, 2016.

[11] A. S. Smith, E. Choi, K. Gray, J. Macadangdang, E. H. Ahn, E. C. Clark, P. Tyler, M. A. Laflamme, L. Tung, J. C. Wu, *et al.*, "Nanomea: a versatile platform for high-throughput analysis of structure-function relationships in human stem cell-derived excitable cells and tissues," *bioRxiv*, p. 453886, 2018.

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