Nanoscale Crater Interfaces Guide Cell Migration and Patterning

W. M. Reese[1], S. Koo[2], H. Jeon[2,4], C.P. Grigoropoulos[2], and K. E. Healy[1,3]

[1]Department of Materials Science and Engineering, [2]Department of Mechanical Engineering, [3]Department of Bioengineering
University of California at Berkeley, Berkeley, CA 94720
wmr@berkeley.edu
[4]Biomedical Research Institute, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea

Surfaces that manipulate cell adhesion, motility, and differentiation are of significant interest for various biomedical applications such as tissue regeneration, cell patterning, and biocompatibility. Here, we employ multiphoton ablation lithography to create gradient and isometric patterns of nanoscale craters (Fig. 1.). The fabricated features are on the order of adhesion sites between cells and the extracellular matrix, thus allowing for the control of focal adhesion formation and thereby the control of cell migration.

Our fabrication technique produces controlled and regular structures by ablating transparent dielectric materials through nonlinear absorption processes using intense femtosecond laser pulses.[1] This process of direct write lithography consistently reproduces craters in quartz without creating thermal stress or collateral damage.[2] We are able to alter the size, aspect ratio, and spacing by varying the pulse energy, numerical aperture of the focusing lens, and the pulse frequency respectively. A schematic of the fabrication process is presented in Fig.2.

Experiments on patterned quartz substrates reveal that nanoscale craters guide fibroblast migration. The substrates used in this study feature arrays of craters with diameters of 600-1000 nm, depths of 110-350 nm, and spacing of 2-10 μm. On these surfaces, sufficient planar surface area is available for nascent focal adhesion complexes (FXs; size ≈ 1 μm^2), which are responsible for creating the traction forces needed to propel migrating fibroblasts.[3] However, the area needed to mature FXs into focal adhesions (FAs; size > 2 μm^2) is limited under certain surface conditions. As illustrated in Fig. 3., there is a statistical difference ($p \leq 0.0001$) in the relative frequency of FXs and FAs between fibroblasts (NIH 3T3) seeded on ablated and unablated fibronectin coated quartz with 4 μm showing the largest disparity. Through the manipulation of the craters spatial arrangement, cells can be guided into various patterns such as lines (Fig. 4.).

These nanopatterned surfaces can serve as tools for mechanobiological studies and for the characterization of physical attributes of surfaces necessary for cell patterning.

---

[1] C. P. Grigoropoulos, *Transport in Laser Microfabrication: Fundamentals and Applications* (Cambridge University Press, 2009)
[2] B. Stuart et al., Phys. Rev. Lett. **74**, 2248 (1995)
[3] K. A. Beningo et al., J. Cell Biol. **153**, 881 (2001)

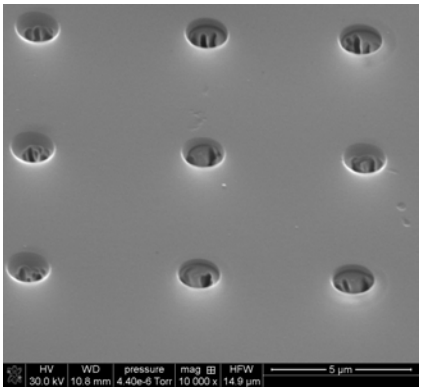

Fig. 2 – Scanning Electron Micrograph of isometrically patterned nanoscale craters taken at 45° tilt. Dimensions: 1µm diameter, 300 nm depth, and 6µm

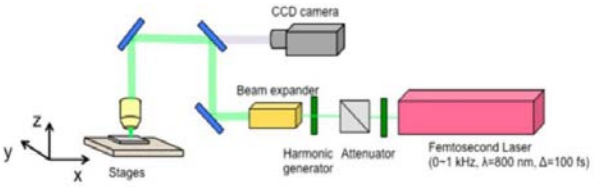

Fig. 1 – Schematic representation of the setup used for laser ablation nanofabrication.

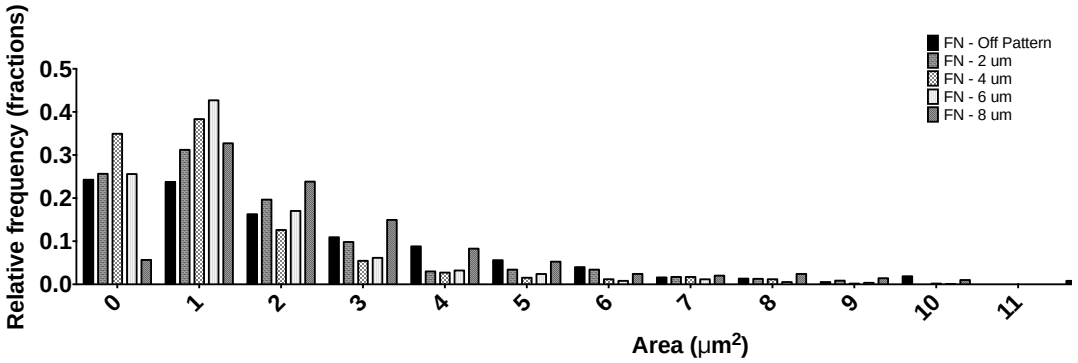

Fig. 3 – Histogram of focal adhesion size distribution of cells off pattern, on 2 µm, 4 µm, 6µm, and 8 µm isometric patterns.

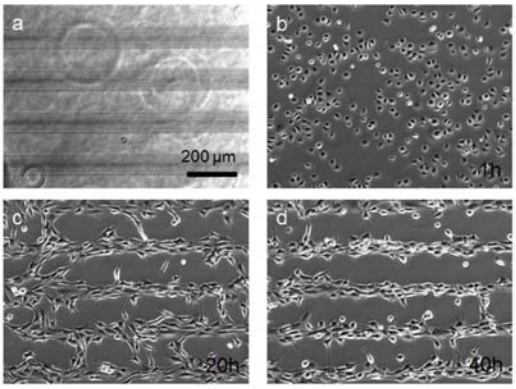

Fig. 4 – Time course images of 3T3 fibroblasts on isometric patterns coated with fibronectin.