Biomimetic Nanopatterns as Enabling Tools for Analysis and Control of Live Cells

By *Deok-Ho Kim, Hyojin Lee, Young Kwang Lee, Jwa-Min Nam,** and *Andre Levchenko**

It is becoming increasingly evident that cell biology research can be considerably advanced through the use of bioengineered tools enabled by nanoscale technologies. Recent advances in nanopatterning techniques pave the way for engineering biomaterial surfaces that control cellular interactions from the nano- to the microscale, allowing more precise quantitative experimentation capturing multi-scale aspects of complex tissue physiology *in vitro*. The spatially and temporally controlled display of extracellular signaling cues on nanopatterned surfaces (e. g., cues in the form of chemical ligands, controlled stiffness, texture, etc.) that can now be achieved on biologically relevant length scales is particularly attractive enabling experimental platform for investigating fundamental mechanisms of adhesion-mediated cell signaling. Here, we present an overview of bio-nanopatterning methods, with the particular focus on the recent advances on the use of nanofabrication techniques as enabling tools for studying the effects of cell adhesion and signaling on cell function. We also highlight the impact of nanoscale engineering in controlling cell-material interfaces, which can have profound implications for future development of tissue engineering and regenerative medicine.

1. Introduction

Cell and tissue function can be profoundly affected by both soluble and insoluble macromolecules that comprise the extracellular matrix (ECM) or mediate extracellular communication via direct or indirect cell-cell communication (**Figure 1**). In particular, living cells are exquisitely sensitive to the local micro- and nanoscale topographic and biomolecular patterns constituting complex and hierarchical adhesive ECM microenvironment in three dimensions.[1] ECM typically consists of a viscoelastic network with nanofibrous proteins that provide biological and chemical moieties as well as physical framework supporting cell attachment and growth. Over the last decade, microfabrication techniques have sufficiently progressed to recapitulate many ECM cues, making them progressively more useful for applications in biology and tissue engineering.[2–4] However, our understanding of how cellular interactions with engineered ECM, especially on nanoscale, coordinate diverse cellular processes is still limited.[5] Studies on the effect of nanopatterning on cell behavior are of great importance because the length scale of such nanopatterns is physiologically relevant due to consistency with the sizes of many functional biomolecules and their complexes, ranging from a few to hundreds nanometers, including the fibers of ECM proteins, the components of basement membrane, and focal adhesions.[1,6]

Recent progress in developing techniques for fabrication of biocompatible materials at nano-scale, the scale of large molecular complexes characteristic of ECM environments, may help address these questions and challenges.[7,8] The resulting synthesis and fabrication of structural and functional materials can thus help mimic both mechanical properties (the elasticity and rigidity) and structural features (molecular composition, fiber- or lattice-like nano-scale organization, etc.) of the extracellular milieu (**Table 1**). These methods and materials therefore offer new tools that allowing for much better approximation and more precise manipulation of the behavior of living cells and tissues, promising new insights into cell biology and its control by the biophysical and chemical features of extracellular environment. For instance, nanolithographically defined cell adhesion substrata can offer unique opportunities for *in vitro* cell culture experimentation, by allowing the spatially controlled presentation of chemical and biophysical stimuli.[16,92] This enables modulation of the cell adhesive signals on a scale commensurate with individual adhesion complexes, which, in turn, define many aspects of cell adhesion and migration.[9,10] Furthermore, the ability to display biofunctional molecules or physical structures on the surface over a large area and on multiple length scales, ranging from nano- to centimeters, can permit synthesis of hierarchically organized experimental platforms that provide multi-scale multi-input spatial control of cell homeostasis and function, while allowing a greatly increased throughput of the analysis and statistical reliability (**Figure 2**). Due to their unprecedented level of control, these techniques are beginning to be adopted by cell biologists and bioengineers to probe cellular processes and engineer cell functions with

[*] Dr. D.-H Kim, Prof. A. Levchenko
Department of Biomedical Engineering
Johns Hopkins University
Baltimore, MD 21218 (USA)
E-mail: alev@hju.edu
H.-J Lee, Y. K. Lee, Prof. J.-M. Nam
Department of Chemistry
Seoul National University
Seoul, 151-747 (South Korea)
E-mail: jmnam@snu.ac.kr

DOI: 10.1002/adma.201000468

desired phenotypic responses. Although the field is still in an almost embryonic but rapidly developing state, it is possible to envisage potential medical devices exhibiting large area nanostructured interfaces, with the features defined on multiple length scales. Some recent harbingers include nanopatterned cardiac tissue scaffold[131] and tubes with internal nanoscale topography.[132]

Here we review bio-nanofabrication techniques for cell biological applications and discuss how nanoengineered adhesive or textured interface can be used to address the fundamental questions in cell biology, e.g., immune cell signaling and stem cell differentiation. In particular, we focus on the recent advances in the use of bio-nanoengineered surfaces in elucidating adhesion and signaling in cellular interactions enabled by employing emerging nanopatterning methods. We also highlight the impact of nanoscale engineering of cell-material interactions on controlling complex cell function, which has implications for functional tissue engineering.

2. Recent Advances of Nanopatterning Techniques for Cell Biological Applications

For the past few years, we have witnessed an explosive development of techniques for bio-nanopatterning of surfaces for cells adhesion, enabling fundamental studies of cell biology (**Table 2**). Therefore, in contrast to several excellent recent reviews on nanofabrication techniques,[11,12] here we focus on four heavily used and promising bio-nanopatterning techniques, particularly useful for cell biological applications.

2.1. Dip-Pen Nanolithography

Scanning probe lithography techniques hold multiple advantages over other conventionally used nano-fabrication techniques, when viewed through the prism of biological applications. For instance, it can be performed under ambient conditions, unlike e.g., electron-beam lithography (EBL) and focused-ion beam (FIB) lithography-based methods (discussed below), which require operation in vacuum, making them relatively much more expensive and potentially incompatible with many applications. Unlike the nanografting techniques that disrupt pre-formed self-assembled monolayer (SAM) to generate a pattern of interest,[13–15] dip-pen nanolithography (DPN) uses a functionalized atomic force microscope (AFM) tip to directly transfer molecules of interest to form SAMs and other patterns on various surfaces via surface meniscus formed between the tip and the surface. Various biomolecular nanopatterns can be directly generated by coating tips with different biomolecules within a humid chamber at room temperature. Moreover, the

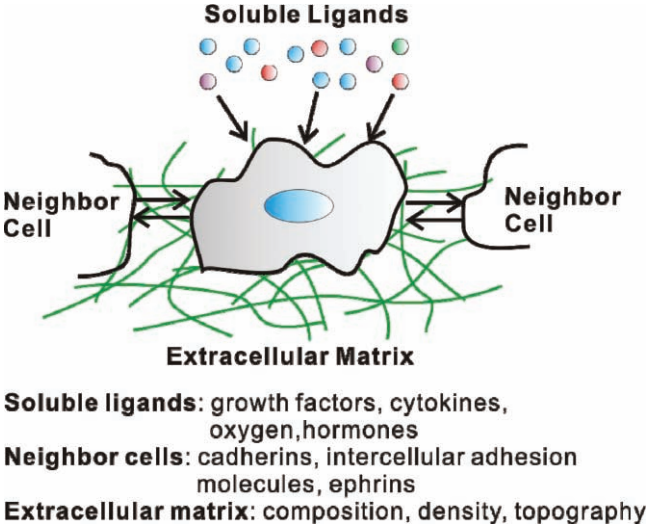
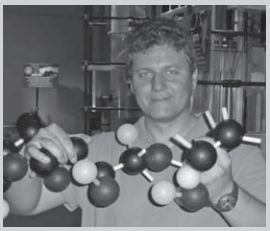


Figure 1. A schematic representation of the complexity of the native cellular microenvironment.



Prof. Levchenko got his B. S. and M.S. degrees at the Moscow Institute of Science and Technology, frequently referred to us as the Russian Caltech. He then crossed the Pond to study at Columbia University, culminating in a PhD in Bioengineering. He simultaneously worked at the Memorial Sloan-Kettering Cancer Center on problems of cancer drug resistance. As a post-doctoral scholar at Caltech, he worked with faculty in Biology and Computer Science on understanding cell signaling in changing environments. Dr. Levchenko was then appointed to a faculty position at Johns Hopkins where he works on Systems Biology of cell signaling and communication.

Prof. Jwa-Min Nam received B.S. degree in chemistry from Hanyang University. He moved to Northwestern University, USA to obtain his Ph.D. in chemistry (advisors: Professors Chad A. Mirkin and Mark A. Ratner). In 2004, he briefly served as a consultant for Nanosphere, Inc. and received the Collegiate Inventors Award from the National Inventors Hall of Fame, USA. He then moved to Professor Jay Groves' lab at UC Berkeley as a postdoctoral fellow. In 2006, he received the Victor K. LaMer award from the Division of Colloid and Surface Chemistry, American Chemical Society. He is currently an associate professor of chemistry at Seoul National University, Seoul, South Korea and on a scientific advisory board for Medifron DBT. Professor Nam's major research areas include the design and synthesis of biologically functional nanomaterials and nanoplatforms and their use in biomolecular detection, disease diagnostics, cell assays and gene delivery.

Table 1. The fabrication examples of natural cellular nanostructures using nanotechnology.

Type	Nanometer-sized Cellular Parts	Nanometer-sized Artificial Counterparts	Nanofeature Mimicking Methods
Channel or Pore	Ion Channel Nuclear Pore	Nanoporous Carbon Capsule	-Carbon Materials Synthesis[121]
		Nanoporous Organic/Inorganic Shell	-Self-Assembly Polymer[122]-Core-Shell Method[123]
Vesicle	Lysosome Endocytic Vesicle Neurotransmitter	Polymer Vesicles	-Polymer Crosslinking[124]-Polymeric Micelles[125]
		Lipid Vesicles	-Nanosized Liposome[126]
		Charged Polypeptide	-Peptide Crosslinking[127]
		Inorganic Vesicles	-Inorganic Synthesis[128]
Receptor	T/B Cell ReceptorNK Cell ReceptorImmune Signaling ComplexNeurotransmitter ReceptorIntegrin	Peptide Receptor Patterning	-Supported Lipid Bilayer[96–99]-E-beam Lithography[56,96]-Dip-Pen Nanolithography[18,35]
		Nanoscale Ligand Patterning	
		Receptor-Functionalized Nanoparticles	-Covalent or Electrostatic Conjugation of Protein with Nanoparticles[89,129]
Physical or Chemical Cue	Nanostructure and Surface Morphology of ECM-Matrix Nuclear Laminar Fiber ECM Proteins (e.g vitronectin, fibronectin and laminin)	Synthetic Polymer Nanofibrous Scaffold	-Electrospinning[64,65,109]-Polymer Crosslinking[61]
		ECM Protein-Containing Nanofiber	
		Ordered/Disordered Surface Texturing	-Self-Assembly Block Copolymer[89]-Patterned Nanotube[27]-Bio-MEMS[130]
		Nanopatterning of Functional Peptides /Proteins	-Dip-Pen Nanolithography[19-21,30,35,36]-Nanocontact Printing[42]-Nanoimprinting[47]

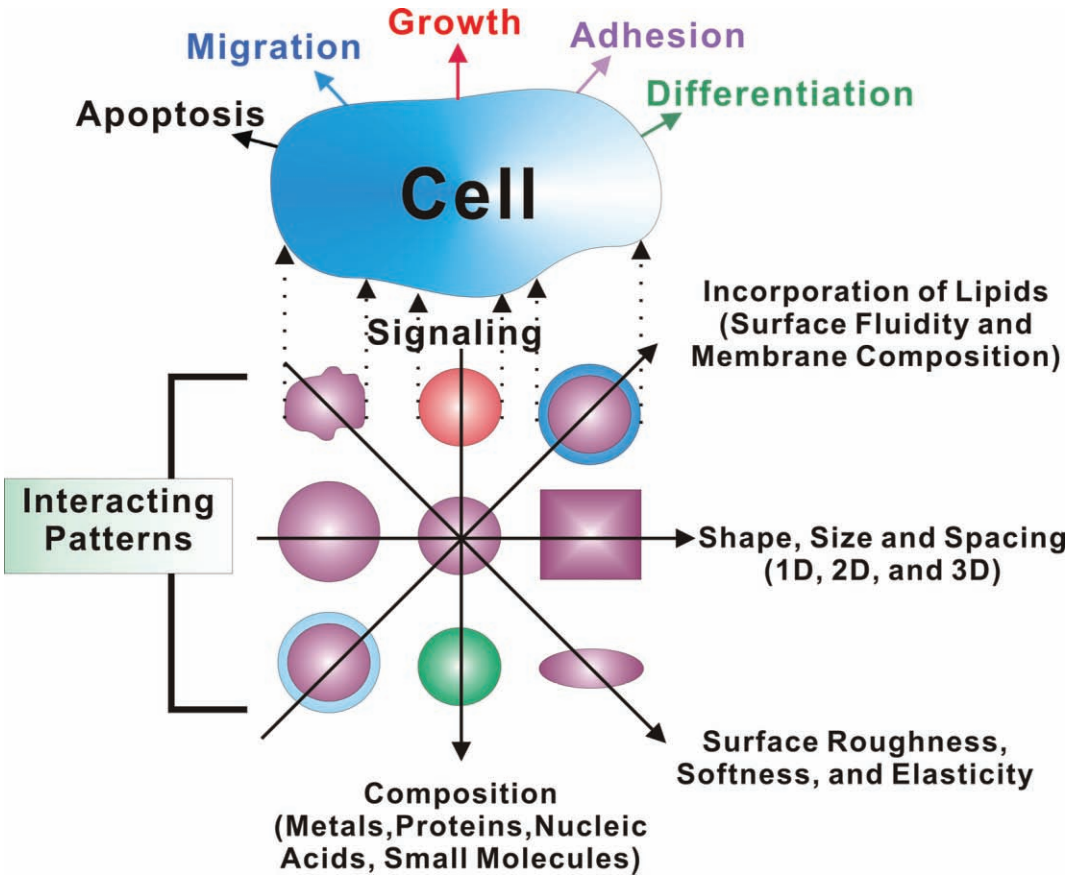


Figure 2. A schematic representation of bio-functional surface array system patterned with chemical and biomechanical signaling and adhesion molecules. The precise nano-scale control of cell function can allow for simultaneous presentation of multiple signals to single cells with tight spatio-temporal control, for example, by incorporating fluidic lipid membrane systems, controlling shape, size and spacing of molecular and topographic patterns, modulating substrate mechanics, and diversifying the composition of patterned ECM proteins. These capabilities enabled by bio-nanopatterning techniques can eventually allow one to better understand adhesion-receptor mediated signal transduction processes at the molecular and cellular level in a high-throughput, systematic way.

Table 2. Summary of nanofabrication methods for cell biological applications.

Fabrication Methods	Advantages	Limitations	Highest resolution and patterned biomaterials	Applications
Dip-pen nanolithography	Can create precise geometries and patterns. Direct-write capability	Low throughput. Substantial time and effort in optimization process especially for generating a large array of non-SAM molecules	30 nm collagen-like peptide lines,[29] 200 nm retronectin dots,[16] enzymes,[35] lipid[18]	Cell adhesion,[16] Cell infectivity,[34] T-cell activation[18]
Micro- and nanocontact printing	Easier to pattern larger areas than with DPN. Simple, fast and inexpensive.	Not available for multicomponent biomolecule nanopatterning.	70 nm titin multimer protein lines,[42] 150 nm Streptavidin,[117] 42nm dendrimer lines[42]	Cell spreading,[84] intracellular signaling[85]
Electron beam lithography	Precise geometries and patterns can be created. No mask needed.	Expensive equipment. Time consuming, small surface coverage. Increasing area, lowers resolution. Negative resists have even lower resolution.	1 μm fibronectin,[55] 1 μm poly(ethylene glycol)[118]	T-cell activation,[96,97] Growth cone guidance[118]
Electrospinning	Can create aligned fibrous meshes of biological polymers such as collagen	Low accuracy. Can only create fibers.	30 nm collagen protein,[52] glactose ligand-coated poly(ε-caprolactone-co-ethyl ethane phosphate) nanofiber[64]	Cell migration,[69] tissue engineering,[119] neurite growth,[120] stem cell differentiation[109]
Self-assembly block-copolymer	Simple, fast, no special equipment needed. Higher order structures can be fabricated.	Not easy to control. Requires engineering of molecules that will self-assemble.	Gold nanodots (<8nm) coated with cyclic RGDfk[90]	Cell adhesion and spreading[89,92]

capability of DPN to "directly write" biologically relevant molecules, preserving in the process their structure and functionality, provides tremendous flexibility in the fabrication of protein-based nanostructures, thus constituting a powerful strategy to study the important hierarchical assembly processes of biological systems, especially to pattern multiple biological components on the same surface for multiplexing different molecules of interest for parallel comparison.[16–18]

To date, many direct-write DPN methods for the generation of protein nanoarrays on nickel oxide, silicon oxide, and gold surfaces have been reported.[19–21] However, this technique still faces several important challenges. Substantial time and effort required for optimization of these processes, especially for generating a large array of non-SAM molecules including proteins, have been major obstacles to practical use of DPN in cell biological applications. Moreover, the fabrication throughput remains a substantial limitation of the widespread use of DPN technology, requiring large area parallelization capabilities to be developed to realize its full potential. To address this problem, a sub-100 nm, centimeter-scale, parallel DPN method using multiple-probes has been recently reported, demonstrating a marked increase in the DPN throughput.[22] A novel 55000-pen two-dimensional (2D) array was used to pattern gold substrata with sub-100 nm resolution over square centimeter areas.[23] More recently, polymer pen lithography, which uses a soft elastomeric tip array rather than hard silicon or silicon nitride tips mounted on individual cantilevers, has also emerged as a possible solution.[24] Using the commercially available DPN systems equipped with thermally actuated cantilevers can help address potential "user-unfriendliness" of the DPN technique, allowing users to generate multicomponent, complex patterns in an automated, high-throughput way.[25]

The above challenges notwithstanding, DPN power as a patterning tool has been amply demonstrated. DPN-generated chemical templates with tailored chemical composition, size and patterned shape/geometry provide a great opportunity to integrate various materials into functional nanostructures over a large area. For example, spin-coating of polymer blends of poly-3-hexylthiphenene/polystyrene (P3HT/PS) on mercaptohexadecanoic acid (MHA) dot-patterned Au substratum results in the spontaneous formation of nanoscale polymer structure that is attributed to heterogeneous nucleation of P3HT on MHA patterns.[26] Single-walled carbon nanotube (SWCNT) and other building-blocks were also used to form two-dimensional arrays using DPN-synthesized nano-chemical contrast.[23,27] Furthermore, DPN can be used to fabricate nanohole arrays and lithographic masters.[28] In this approach, patterns of MHA on gold substrate were generated by DPN, and surrounding areas were passivated by octadecanethiol. The exposed gold can be used as an electrode to plate silver from solution, generating raised features and structures that can be transferred to polydimethylsiloxane (PDMS) to make a lithographic master, or alternatively, they can be etched to make arrays of nanoholes.[28]

In a powerful recent example of its applicability to biologically relevant experimentation, DPN has been used to print collagens and collagen-derived peptides (30–50 nm line width) without disrupting the triple-helical structures and biological activities of these complex polymers.[29] The DPN-enabled nanoarrays have also been used for nano-based assays. For example, Lee *et al.* used nanoarrays of the anti-p24 antibody to screen for the human immunodeficiency HIV-1 virus (HIV-1) p24 antigen in serum samples.[30] Moreover, DPN serves as an ideal tool to investigate single virus particle control,[31–33] which allows one to examine single-cell infectivity with well-defined parameters determining a rate of viral infection, such as density and spatial distribution of virus particles,[34] as well as a patterning method to demonstrate molecular interactions between various bio-molecules including integrins and enzymes.[35,36]

Recently, fluid phospholipids [1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)] were successfully employed as a versatile ink for DPN under humidity-controlled conditions with lateral resolution down to 100 nm.[37] It was used as a carrier ink for patterning of functional lipophilic materials such as head group-modified lipids for the semisynthetic fabrication of model peripheral membrane-bound proteins.[18] Massively parallel delivery of multi-component lipids and lipid analogues to the surface with sub-micrometer resolution could closely imitate heterogeneous microdomains of native cell membranes known as lipid rafts.

2.2. Micro- and Nanocontact Printing

Soft lithography refers to a set of fabrication techniques that involve molding, imprinting, or embossing with a template made of soft polymers, such as the elastomer PDMS.[38] These soft lithography techniques can be used to robustly and straightforwardly create micro- and nanopatterns at low cost and high efficiency compared to conventional photo and electron beam lithography techniques. A number of soft lithography-based techniques including microcontact printing (μCP),[39] capillary force lithography,[135] molding in capillaries,[136] microtransfer molding,[137] and replica molding[138] have been developed to pattern chemically modified surfaces or structured biomaterials interfaces. Among them, μCP has become a convenient and widely-used technique for the patterning of biological molecules for cell biological applications.[39] In μCP process, the microstructured elastomer stamps are used to transfer biochemical 'ink' composed of biologically interesting molecules onto substrata routinely used in cell biology studies (e.g. plastic and glass). μCP allows simultaneous patterning over the entire substratum, without relying on the use of an expensive stepper or writing tools. These mostly positive characteristics inherent in the straightforward stamp-dependent method also give rise to certain limitations and disadvantages. In the sub-micrometer range, for instance, the PDMS mold can lose its mechanical integrity and deform in unintended ways, resulting in inherently limited spatial resolution.[40] Overall, generating patterns in the sub-micrometer range using soft lithography is closely associated with the structural fidelity of the elastomeric stamps. The use of a thin film stamp bonded to a rigid glass support in conjunction with the aligner significantly improved the runout, eliminated contact of recessed regions of the stamp with the substratum, and led to sub-micrometer patterns produced over a 3 inch diameter substratum with an accuracy of greater than or equal to 40 nm.[41]

Nanocontact printing (nCP), an extension of μCP, is a highly parallel, manufacturable, and additive process, which enables printing of 100-nm structures frequently using a stiffer elastomeric stamp and high molecular weight inks to limit diffusion.[42] Sylgard 184 PDMS (soft PDMS), the most commonly used polymer stamp, is characterized by the low elastic modulus that makes it susceptible to the structural collapse and the rounding of sharp corners due to surface tension after stamp is peeled off from the master. Due to the mechanical defects of the soft PDMS, there has been a need for a new mold material that can provide a rigidity high enough for fine and dense features with a high aspect ratio and yet a degree of flexibility for a conformal contact over a nonflat large area. For example, alternative siloxane polymers consisting of vinyl and hydrosilane end-link polymers (hard PDMS) that exhibit superior mechanical properties over soft PDMS were developed for the application in high resolution microcontact printing.[43] In spite of its high fidelity, working with hard PDMS is difficult owing to a higher propensity to be broken easily during stamp fabrication. Composite stamps, combining a thin layer of hard PDMS and a thick slab of soft PDMS can improve various soft lithographic performances, ensuring high-fidelity of stamps and conformal contact on a substratum.[44] Sub-50-nm dendrimer line patterns over 3×3 mm^2 were created by nanocontact printing that employed V-shaped composite stamps.[42] The use of other elastomers has also been explored. Ultraviolet (UV)-curable polyurethane acrylate (PUA) molds with tunable modulus were also successfully used to enable microcontact printing of 250-nm lines.[40] The use of poly(ethylene) laminated on poly(methylmethacrylate) sheet has been proposed as the soft template for the nanocontact printing.[45] Nakarnatsu *et al.* reported that the hydrogen silsesquioxane (HSQ) transferred pattern with 35 nm line width was obtained onto Si surface.[45] Nanocontact printing process has also been used to fabricate dot arrays of different size features. A micromachined elastomeric PDMS stamp with two dimensional arrays of pyramidal tips were used to array dot pattern with variable dot size and density.[46] Variable-dot-size printing was achieved by applying different contact pressure to induce variable mechanical deformation and thus the variable contact area of PDMS tips.[46] Using stamps generated by UV nanoimprint lithography, the dense nanopattern was printed down to feature sizes of 30 nm and periods of 100 nm (1×10^{10} features/cm^2).[47] Sub-500 nm alignment accuracy for multilayer printing was also obtained using a traditional contact mask aligner, which can be further improved by modifications of stage and alignment metrology.[47]

2.3. Electron Beam Lithography

EBL was first developed by the semiconductor and integrated circuits industry to generate photomasks used in photolithography and electronic features in the nanometer regime. It has been rapidly adopted by biological researchers who want to explore cellular responses to spatially controlled stimuli generated by nano- or micro-engineered surfaces. In a typical EBL process, pre-designed areas of electron-sensitive resist are serially exposed to magnetically manipulated electron beams, making the exposed areas soluble or insoluble in particular solvents (depending on the properties of the resist used). After careful development of the resist, approximately 30 ~ 40 nm resolution for arrayed features can be achieved.[48] Due to the fact that EBL does not require a physical mask for a desired pattern and has a capability to precisely control the size and arrangement of patterned components on the nanometer scale, allowing even with a defined degree of disordering, it has been used as a tool for studies of how the topographical parameters of the cell adhesion substratum can control such cell properties as cell adhesion, alignment and differentiation of stem cells.[49,50]

The EBL-generated topographies in the resist can be transferred to desired materials that lie beneath the resist via reactive ion etching. Metal deposition on a patterned resist followed by removing a resist with a proper organic solvent leads to the formation of submicron-scale metal patterns on a substratum that exactly duplicate a negative image of a pre-patterned resist, the technique referred to as 'lift-off'.

Rather than creating relatively simple nanostructures by subsequent dry etching and metal evaporation process, various selective surface modification strategies associated with EBL to display biologically relevant molecules in an arrayed format have recently been developed. When a lithographically generated architecture is comprised of chemically distinctive materials, their surfaces can be selectively modified with different molecules for the absorption of biomolecules on a specific region.[51] For example, the surface of pre-patterned SiO_2 structure and an exposed area of underlying indium tin oxide (ITO) layer was transformed into a protein-adhesive/protein-repellent contrast, where dodecyl phosphate and poly(L-lysine)-*graft*-poly(ethylene glycol) are used to render ITO surface hydrophobic and to passivate the SiO_2 regions against nonspecific protein adsorption, respectively.[51] Dodecyltrichlorosilane monolayer was grafted on the exposed Si surface resulting from EBL by gas-phase reaction, which was followed by the removal of poly(methyl methacrylate) (PMMA) mask and subsequent liquid phase back filling of oligo(ethylene glycol)trimethoxysilane.[52] The use of this method led to formation of anisotropic, flat, chemical nanopatterns composed of hydrophobic tracks embedded in a protein repellent matrix, guiding preferential absorption of collagen proteins and promoting their self-assembly into aligned nanofibrous structure.

Energy that is applied to the surface by electron beam irradiation is sufficient to ablate SAMs from the surface and induce change in chemical composition of the exposed materials by breaking covalent bonds.[53,54] The resulting surface patterns exhibiting chemical contrast to protein absorption can be employed as a template to spatially organize biomolecules in two-dimension. The functional properties of ECM-coated surfaces can be patterned based on a direct writing approach that uses highly focused electron beams to locally denature fibronectin thin film.[55] In a variation of the technique, polystrene-b-poly[2-vinylpyridine($HAuCl_4$)] diblock copolymer micellar monolayer was employed as a negative resist in EBL.[56] The exposed area of the polymer was resistant to lift-off process with toluene or dimethylformamide.[56] Subsequent reduction of encapsulated gold ions by hydrogen plasma treatment and surface functionalization realized deposition of c(RGDfK)-thiol peptide-modified 6-nm gold nanoparticles that activate individual integrins on cells in predefined architecture.[56] The appropriate dose of electron beams induces site-specific chemical reactions such as formation of covalent bonding. With this approach, submicron-sized cell-repulsive poly(ethylene glycol) hydrogels were cross linked to silanized Si wafer.[57] Recently, the technique that tackles multiple protein patterning with EBL was reported. Eight-arm PEG polymers of which ends are modified with one of four orthogonal protein-reactive moieties, biotin, maleimide, aminooxy, or nickel (II) nitrilotriacetic acid, were serially cross-linked to the Si wafer with high registrability using EBL.[58] Proteins with an appropriate conjugating moiety were also specifically immobilized onto the corresponding PEG surface through simple incubation without additional reagents.[58] However, the fabrication areas in EBL are usually limited to several microns and this technique comes at a relatively high cost, barring investigations of many types of cell responses to complex nanopatterns. In addition, contrary to other techniques highlighted in this paper, unavoidable substrate exposure to a high vacuum environment seriously limits the use of this method for the direct patterning of proteins and lipids which could be damaged in a dried environment.

2.4. Electrospinning Technique

Many cell types can organize their extracellular matrices with nanofibrous structural units, the diameter of which can range from a few tens of nanometers to about a hundred of nanometers. The physicochemical and biological aspects of such a hierarchically structured cellular environment can be mimicked by electrospun polymer nanofibers prepared by relatively simple processes. When an external electric field is introduced to a polymer solution forced to be extruded through the spinneret, a so-called Taylor cone is formed, which results from equilibrium of surface tension and an applied electric filed. Above a threshold voltage, a tiny solution jet comes out from the surface of the Taylor cone and extends toward a grounded collector. As the solvent partially evaporates during the extension, the jet is solidified and subjected to extensional stress due to electrostatic repulsion, resulting in the deposition of long and thin nonwoven polymer fibers on a collector usually in a random fashion.

High surface area to volume ratio originating from the fibrous structure of electrospun nanofibers is similar to that of native ECM, where it might be responsible for a great degree of interactions between resident cells and artificial ECM in contact with the cell membrane. It allows cells to highly integrate the complex mechanical and chemical stimuli from an extracellular biological context and provide a corresponding feedback on them. Mesh-like topography of electrospun fibers consisting of interconnected nano- or micro-sized pores is particularly beneficial to the diffusion and delivery of biochemical cues such as growth factors and cytokines. It also makes it possible for cells to communicate each other through direct cell-cell contact. So, electrospinning is considered to be one of the most promising methods to fabricate synthetic cellular supports for the applications in tissue engineering and regenerative medicine.

For the best performance, the factors affecting interaction between the engineered ECM and cells residing on it, including the resultant material's Young's modulus, mechanical strength, hydrophilicity, degradation kinetics and biocompatibility, can be precisely tailored by varying the chemical composition of materials used.[59–61] These could be used for the development of various cell assays including cell phenotypic change-based disease diagnostic assay.[61] In addition to synthetic and natural homopolymers, copolymers and synthetic-synthetic or synthetic-natural polymer blends have been employed to render artificial cellular microenvironment suitable for maintaining and inducing certain cell fates and functions.[62,63] Surface modification of electrospun fibers with ligands for specific cell

receptors and ECM proteins can further enhance the biologically relevant features and thus applicability of electrospinning in a biomedical research fields. For instance, the hepatocyte-specific galactose ligand-coated poly(ε-caprolactone-co-ethyl ethane phosphate) nanofiber cell scaffold can promote the formation of mechanically stable hepatocyte-nanofiber constructs, while maintaining other hepatocyte cellular functions.[64] Nonwoven poly(ε-caprolactone) mats, the surface of which is modified with a layer-by-layer assembled gelatin film and subsequently functionalized through mineralization of bonelike calcium phosphate, can provide an efficient bone tissue engineering platform.[65] The use of core-shelled nanofibers is an alternative approach to realizing the valuable material properties that cannot be obtained by using only simple fibrous structures.[66,67] Core-shell-structured nanofibers have great merit not only in controlled release of encapsulated biological molecules but also in protection of inner fragile polymers during the electrospinning process.[68]

Besides chemical composition and structure of individual fibers, topographic aspects, including fiber orientation, porosity and spatial dimensionality, play an important role in guiding particular cellular responses.[69,70] Considerable efforts have been dedicated to the development of techniques to align fibers in a controlled manner. To a certain degree, a high-speed rotating collector defines a direction of electrospun nanofibers.[71] Application of the electrically conductive substrata separated by a gap with a width between hundreds of micrometers and several centimeters can successfully prepare uniaxially aligned fiber arrays suspended over the gap.[72] It was demonstrated that when electroconductive templates are employed as collectors, electrospun nanofibers can also be organized into complex microscale patterns.[73,74] The resulting architecture of the deposited fibers was closely associated with that of conductive templates used. With this technique, interestingly, fibers could also be collected as woven fibrous mats by time-dependent arrangement of the conductive protrusions.[74] But the simplest and most practical method to give a directional preference to electrospun scaffolds could be mechanical stretching of coated fibers.[69]

Electrospinning-deposited nanofiber meshes are mostly developed and used as two-dimensional entities, since fibers are deposited in a form of sheets, and the forming pores can be too small to allow cell migration into the inner regions of fiber meshes. Some modifications, including the incorporation of macropores or the reduction of fiber density, have been used to allow the colonization of cells among the three-dimensional (3D) environment of the nanofibers.[86] Furthermore, there have been a host of attempts to fabricate artificial nanofiber ECM in a controlled 3D fashion.[70,75,76] In fabricating and controlling natural-like ECM structures, hybrid 3D architectures, formed by alternate stacking of two building units (a few hundred-micrometer-sized fibers as structural supports and electrospun nanofibers as cell attachment scaffolds), were recently proposed.[77] Specifically designed 3D macroscopic tubular structures with microscopic patterns were fabricated using 3D collecting templates.[78] As mentioned above, by alternating architecture of 3D collecting templates and introduction of conductive protrusions on the surface of those, electrospun fibers could be deposited in a variety of geometric configurations along with well-defined microscopic fiber orientations. Despite of the above-mentioned advances in electrospinning techniques, it is still challenging to pattern the electrospun fibers on multiple length scales, ranging from nm to μm, with highly accurate registration for the ultimate realization of *in-vivo*-like 3D ECM platforms.

3. Analysis and Engineering of Cell Signaling and Function with Nanopatterned Surfaces

3.1. Nanopatternings for Cell-Matrix Interactions

The dynamic interface between the ECM and cells is crucial for regulating important cellular processes such as signal transduction, growth, differentiation, motility and apoptosis.[79,80] The key role of cell-ECM interactions belongs to members of the integrin superfamily. Integrins are heterodimeric adhesion receptors which interact noncovalently with the arginine-glycine-aspartate (RGD) and other motifs on the ECM proteins, such as fibronectin and vitronectin. The major function of the integrin family of receptors is to provide a physical connection between the ECM adhesion proteins and the intracellular cytoskeletal and signaling molecules.[81] The conversion of physical signals, such as contractile forces or external mechanical perturbations, into chemical signaling events is a fundamental cellular process that occurs at the cell–ECM interfaces, known as focal adhesions (FAs).[82] The assembly of integrins along the cell membrane is one of the first events observed during the formation of focal adhesions. Integrin-mediated adhesion modulates the activity of Rho GTPases and signaling molecules at FAs such as FAK, Src, integrin-linked kinase (ILK), and Shc, thus regulating cellular processes.[83]

Cell adhesion, spreading and migration require the dynamic formation and dispersal of contacts with ECM. Micro- and nanocontact printing techniques allow for the systematic reduction of ECM island size to pursue the lower limits of cell-ECM contact areas necessary for cell spreading. Lehnert *et al*. found that the extent of cell spreading is directly correlated with the total substratum coverage with ECM proteins, independently of the ECM geometrical pattern.[84] These findings are valuable for designing artificial surfaces that optimally interact with living cells and tissues. Combined with real-time live cell imaging techniques, micro- and nanocontact-printed ECM proteins can be utilized to control and analyze adhesive signaling events triggered by spatial control of cell-matrix interactions. For example, Rac-FRET analysis of NIH3T3 fibroblast cells cultured on circular or linear ECM islands with 1 μm width and various lengths (1 to 8 μm) and 1 to 4.5 μm wide-separation by nonadhesive barrier regions revealed that Rac becomes activated locally within these nascent focal adhesions within minutes after ECM binding and that it triggers lamellipodia extension from adjacent sites.[85] These findings suggested Rac1 GTPase-based sensory mechanism that cells use to detect their local microenvironment and guide directional cell spreading. Patterning nanoscale ECM islands on surfaces provides numerous opportunities for future advancement in our understanding of cell-matrix interactions. DPN was used to construct arrays of ECM proteins with 100- to 350-nanometer

features.[16] These nanoarrays exhibit almost no detectable nonspecific binding of proteins to their passivated portions, thereby providing suitable tools for studying surface-mediated biological recognition processes such as cellular adhesion at the nanoscale in an array format. Recently, the multicomponent micro- and nanostructured supported lipid membranes were also used as cell culture substrates for the selective adhesion and activation of T-cells (**Figure 3**).[18] These biomimetic membrane patterns were produced by selective adsorption of functionalized or recombinant proteins based on streptavidin or histidine-tag coupling.

Cell adhesion, gene transfection and cell viability might also be affected by the nanoparticles immobilized on the cell substratum. For example, Nam *et al.* demonstrated that cell adhesion was strongly affected by microarrayed nanoparticle density.[87] This study used a noncontact microarrayer for the straightforward, large-scale modification of cellular nanoenvironments. The study also revealed an dramatic change in the F-actin bundle and network formation on nanoparticle-modified substrata compared to control glass surfaces.

The number, availability and distribution of ECM binding sites dictate cell shape and motility. The spacing between integrin ligands might be also important in regulating cell behavior and function. Cell-adhesive gold nanodots coated with cyclic RGDfK have been used to test the possibility that living cells can sense the differences in spacing of nanopattern features, which in turn might strongly affect cell adhesion, polarization, and spreading. By varying the nonsocial organization of RGD in alginate gel, for instance, it was found that changing the spacing between the RGD coated areas alters cell spreading and proliferation.[30] Using controlled modulation of the self-assembly of diblock copolymer micelles, Spatz and coworkers demonstrated that the spacing of gold nanodots (< 8 nm) coated with cyclic RGDfK peptide had a strong influence on the formation and localization of the focal adhesion of cells.[88] Furthermore, the strongest polarization of cell bodies occurred in the synthetic nanoenvironments with the spacing range of gold dots between 60 and 70 nm, suggesting that the universal length scale for integrin clustering and activation might exist and limit cell adhesion and polarization (**Figure 4**).[89] Ligand-integrin interaction can also affect the number of spread cells as a function of the spacing.[90] This number reduced when the distance between integrin ligands exceeds 58 nm; and cells formed stable adhesions only if this distance was maintained.[91] The nanodots thus constitute a very valuable tool allowing unique access to an important length scale for cell adhesion studies and enabling control of the assembly of single integrins during the formation of focal adhesion clusters. This technology will likely benefit from the introduction of more complex patterning, as demonstrated through creation of the spacing gradient surfaces of cyclic RGDfK peptide patches. For instance, Hirschfeld-Warneken *et al.* demonstrated that the gradients of RGDfK peptides induced the orientation of MC3T3 osteoblast cells towards higher particle densities, when the values of the gradients was at least 15 nm/mm.[92]

3.2. Nanopatternings for Cell-Cell Interactions

Cell-cell communication plays a central role in determining cell behavior and function, but this type of interaction is much

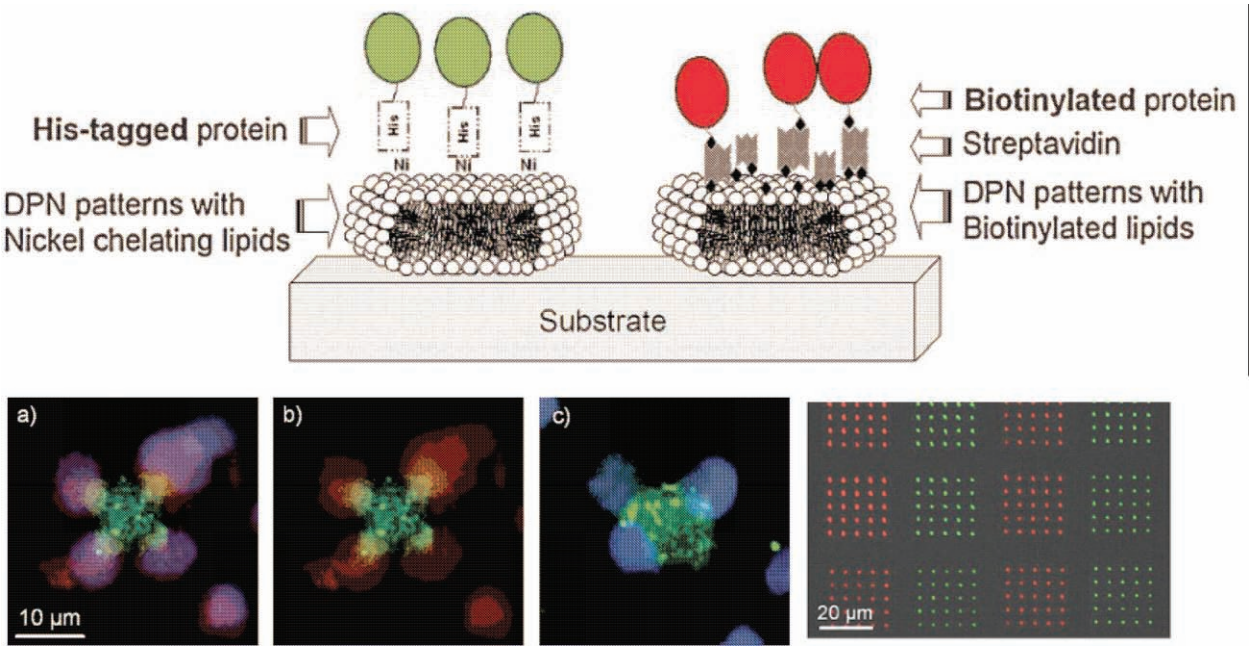


Figure 3. A schematic representation of protein-coupling strategies to spatially patterned lipid support and chemical structure of the lipid using dip-pen nanolithography (top panel). Fluorescence microscopy images of T-cell selectively adhered to and activated by functional proteins (bottom panel). Reprinted with permission from ref. [18].

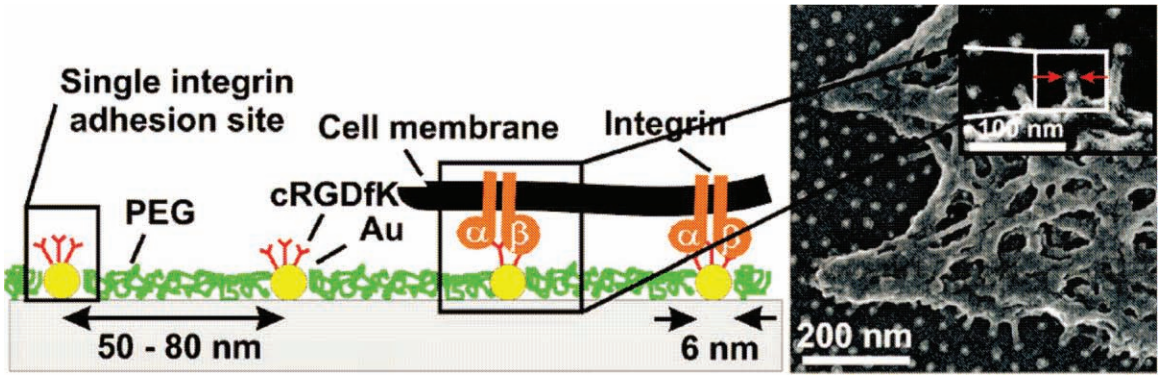


Figure 4. A schematic of a biofunctionalized gold particle substrate in contact with a cell membrane (left panel) and a scanning electron micrograph of MC3T3 osteoblast that is adhering to a gold particle (right panel). Reprinted with permission ref. [89].

less explored using micro- and nanopatterned surfaces than the cell-matrix interaction. However, as some of the recent studies suggest, there is a considerable potential for novel techniques described here to enhance our understanding of the underlying cell signaling biology in cell-cell communication. For instance, Mannix *et al.* recently demonstrated that superparamagnetic 30 nm beads, conjugated to approximately 30 DNP-Lys ligands per bead and bound to cell surface IgE-Fc1RI receptor complexes, magnetize when exposed to magnetic field, and aggregate owing to bead-bead attraction in the plane of the membrane.[93] The resulting clustering of the bound receptors acts as a nanomagnetic cellular switch that directly transduces magnetic inputs into physiological cellular outputs, with rapid system responsiveness and non-invasive dynamic control.[93]

An important aspect of the plasma membrane is that its main component is a mixture of lipids, which are fluid and adjustable to environment. Thus, to mimic cell-cell interaction, it may be also important to pattern and observe cell membrane on a platform tailored to systematically study the dependence of cell-cell communication on lipid bilayer properties. Cell-membrane-like SLB could help address this challenge. SLB generates a fluid lipid bilayer on a glass surface and allows exploration of various cell-cell communication systems in a controlled manner.[94,95] It provides a systematic research platform to have better understanding of direct cell-cell contact signaling especially related to a coordinated recognition process, such as immunological synapse formation and functions. Chromium patterns in supported lipid bilayer (SLB) system restrict diffusive lateral movement of membrane-associated components within a certain region.[96] Most recently, it was shown that T cell receptor signaling can be altered by SLB and patterned protein surface that modulate immunological synapse structure formation.[96,97] Reconstitution of the immunological synapse using cell-biolayer systems enabled visualization of the molecular interactions responsible for cell-cell recognition and signaling, leading to interesting insights into the physical forces that they exert.[94] In order to make ECM hybrid array using supported bilayer membranes, the layer was further fabricated onto the underlying substratum to impose geometric constraints on immunological synapse formation (**Figure 5**).[96] Analysis of the resulting alternatively nanopatterned synapses revealed a causal relation between the radial position of T cell receptors (TCRs) and signaling activity, with prolonged signaling from TCR microclusters that had been mechanically trapped in the peripheral regions of the synapse. These experiments provide insight into how signaling is extinguished in individual TCR clusters in the immunological synapse, which may be attributed to temporal or spatial processes such as recruitment of inhibitors or changes in the actin cytoskeleton that feed back on signaling. Similar benefits are to be expected for the study of the formation and function of neuronal synapses. Using supported bilayers containing neuronal adhesion proteins, Pautot *et al.* reconstituted a neuronal synapse with a living cell and found that presynaptic beta-neurexin (Nrx) and postsynaptic neuroligin-1 (Nlg) interaction rapidly establishes a weak, specific adhesion between pre- and post-synaptic processes.[98]

The resulting model membrane bilayer can be co-patterned with ECM and soluble ligands for mimicking more complex, *in-vivo*-like extracellular environments for live cell assays.[96,99] Modeling of interaction between the epidermal growth factor (EGF) and the EGF-receptors (EGFR) tyrosine kinase presents an example of a prototypical signaling system amenable to this analysis.[99] The system allowed fast local enrichment of EGF induced by the EGF-EGFR interactions, facile *in situ* monitoring of fluorescently labeled EGF, and temporal analysis of cellular phenotypes in a surface-assay format.

4. Emerging Applications in Stem Cells and Tissue Engineering

Nanofabrication techniques pave the way for engineering novel biomaterials for stem cell and tissue engineering, allowing one to extend the control of the engineered tissues to the molecular level, with a strong potential for dramatic enhancement of tissue regeneration methodologies. In particular, recent advances in nanofabrication techniques demonstrate a considerable potential to generate nanostructured scaffolds for functional tissue engineering. Nanostructured biomaterials could be used as instructive extracellular microenvironments for morphogenesis in tissue engineering.[100] Arguably, for

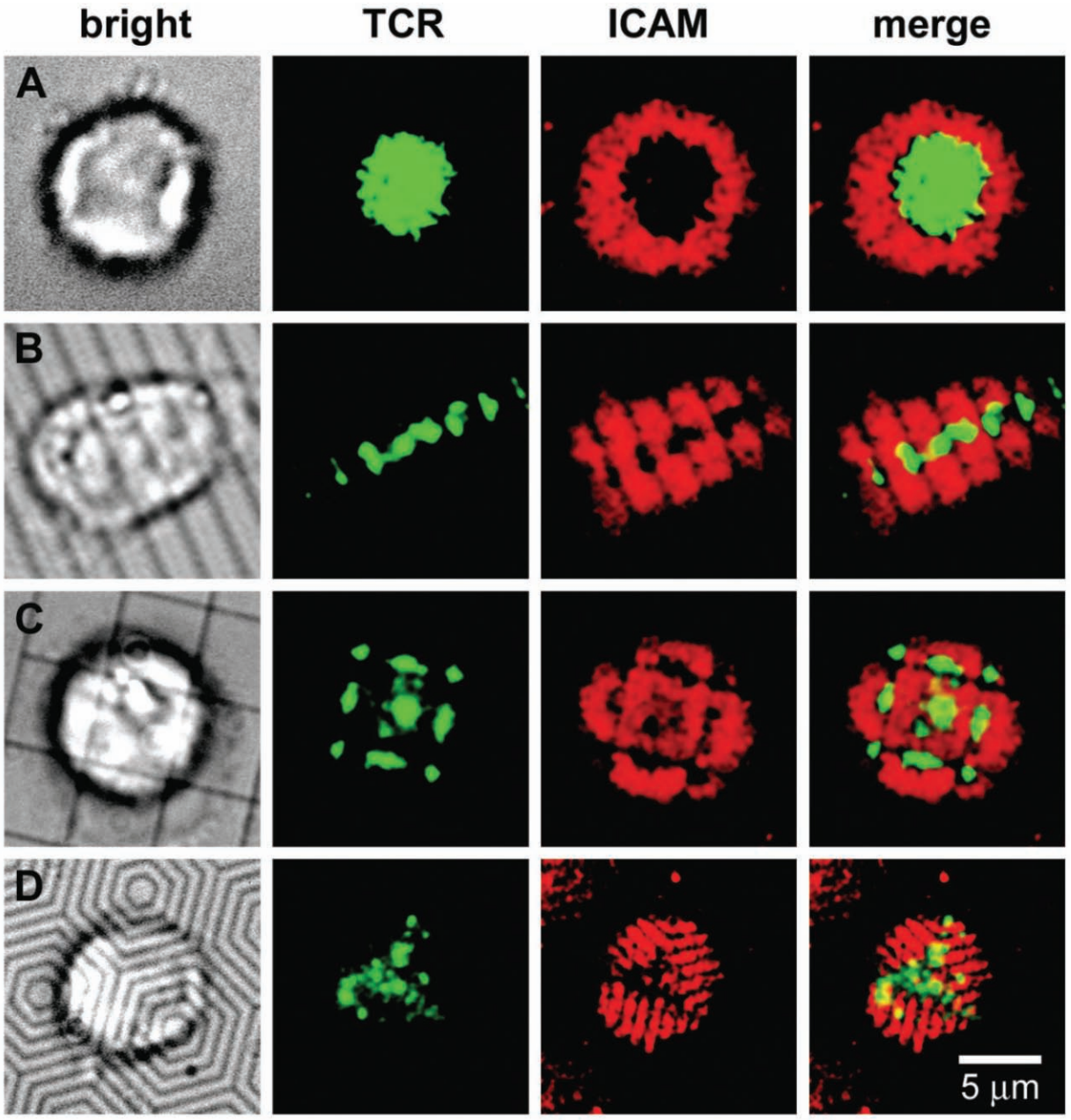


Figure 5. The immunological synapse formation is altered by geometrical constraints of the substratum. T cells were incubated with fluorescently labeled anti-TCR H57 Fab (green) before being introduced to supported bilayers containing GPI-linked pMHC (unlabeled) and ICAM-1 (red). Chromium lines are visible in brightfield imaging, although they are only 100 nm across, as verified by electron microscopy. Images are at 10 min after cells were introduced. Immunological synapse on unpatterned substratum (A), 2-μm parallel lines (B), 5-μm square grid (C), and concentric hexagonal barriers (spacing 1 μm) (D). Reprinted with permission from ref. [96].

tissue engineering scaffolds, a simple and highly reproducible method to fabricate well-defined nanostructures with aligned nanoembosses or nanofibers of controllable aspect ratios on a large area is required to mimic ECM environment with a high degree of structural and mechanical similarity. As a step in this direction, 3D collagen fibers comparable in size with those found in ECM were created through electrospinning technique, which has been extensively used for fabricating tissue scaffolds.[101–103] Nanofibrous polymers fabricated by electrospinning were employed to regulate myogenesis, by way of cell and cytoskeleton alignment, myotube assembly, myotube striation, and myoblast proliferation.[104,105]

Incorporation of common integrin adhesive peptide sequences (e.g. RGD) into nanostructured biomaterial scaffolds could also be beneficial for more precise analysis of the role of ECM in progenitor cell biology and tissue engineering. For example, conjugation

of biotinylated RGD to biotinylated poly(ethylene glyocol) (PEG) and poly-lactic acid copolymers promoted endothelial cell spreading in contact the polymer.[106] Furthermore, self-assembling nanofiber scaffold enabled selective differentiation of neural progenitor cells by incorporating the pentapeptide epitope isolucine-lysine-valine-alanine-valine (IKVAV), known to promote neurite sprouting and to direct neurite growth.[107] Biological activity of surface topography, in the form of oriented nanofibrous scaffolds was highlighted in recent publications.[69,71,108,109] Such surfaces, capable of providing topographic cues can be used to create structurally organized stem cell sheets, which in turn appears to promote differentiation to a specific lineages, providing a powerful tool in engineering of tissue constructs for regenerative medicine applications. For example, this methodology was used to not only enhance the differentiation of mouse embryonic stem cells into neural lineages but also facilitated neurite outgrowth (**Figure 6**).[109] Thermosensitive hydroxybutyl chitosan (HBC) was also electrospun into aligned nanofiber meshes, again resulting in highly aligned orientation of stem cell sheets through the anisotropic substratum topography.[115] hMSCs cultured on the surface of chitosan nanofiber scaffolds showed cytoskeletal alignment and nucleus elongation and underwent myogenic differentiation evidenced by an upregulation of the myogenic genes collagen IV, desmin, Pax-3, Pax-7 and myogenin compared with hMSCs on HBC films.[115] The differentiated cells could be recovered in sheet form by thermally induced dissolution of the substratum. Multiple such cell sheets can conceivably be stacked to produce the thicker polymer-free tissue suitable for implantation, which might be used in engineering of tissue constructs, particularly for cardiac and muscle regeneration.

It is postulated that through interactions with nanopatterns of different size and geometry, one might significantly influence

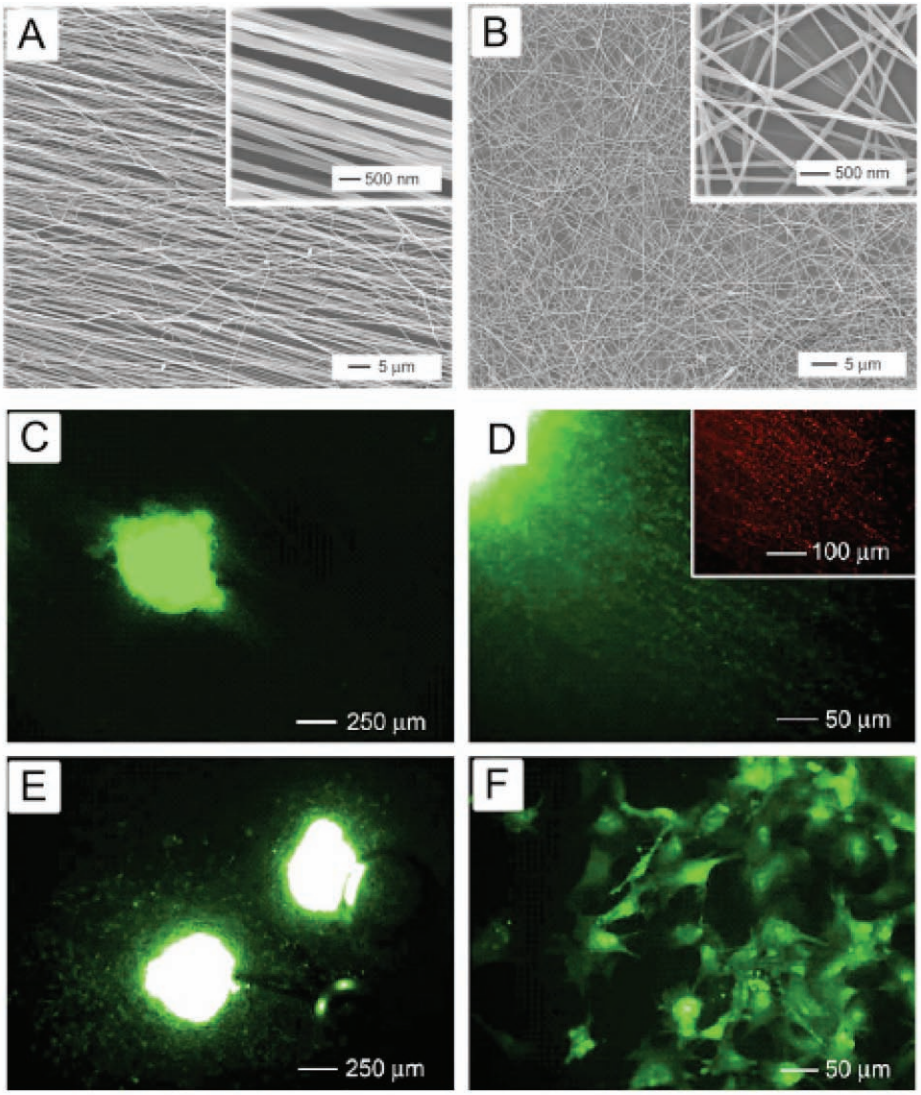


Figure 6. Scanning electron microscopy images of (A) aligned PCL nanofibers prepared by electrospinning and (B) randomly oriented PCL nanofibers. Fluorescence microscopy images of CE3 embryoid bodies after seeding onto (C, E) aligned and (D, F) randomly oriented PCL nanofibers for 14 days. Reprinted with permission from ref. [109].

stem cell spreading, migration, proliferation and differentiation. For example, there is indeed growing support for the nanoscale-dependent differentiation of mesenchymal stem cells (MSCs) to osteoblasts. In one study, Park *et al*. reported that adhesion, spreading, growth, and differentiation of rat bone marrow MSCs were critically dependent on the TiO_2 nanotube diameter in osteogenic induction media (**Figure 7A**).[112] They found that not only adhesion, proliferation, and migration, but also osteogenic differentiation of rat bone marrow MSCs were highest on 15-nm nanotubes and decreased dramatically on 70- and 100-nm nanotubes. Analysis of phosphorylation of the focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK) confirmed high extent of focal contact formation on nanotubes smaller than 30 nm as compared to 100 nm nanotubes and a flat TiO_2 surface (control) (Figure 7B).[112] Very interestingly, Oh *et al*. recently reported that stem cell behavior on TiO_2 nanotubes can be controlled solely by altering nanotube diameter without using osteogenic induction media.[116] Culturing human MSCs on a range of nanotubes with diameters between 30 and 100 nm, cell stretching and expression of osteogenic differentiation markers was highest on 100-nm nanotubes, whereas cell-adhesion rates increased with decreasing tube diameter, with a maximum at 30 nm. In contrast to the small diameter of ~15 nm in Park *et al*.'s study,[112] Oh *et al* illustrated that the optimum length scale for cell differentiation was shown to be the large-diameter nanotubes (100 nm). The findings of opposite effects of nanotube diameter on osteogenesis of MSCs showed the striking variabilities and opportunities to choose different substrate topography for the purpose of influencing and controlling stem cell fate and motivate more systematic studies on the effects of TiO_2 nanotube geometry, materials, processing parameters, surface chemistry, crystal structure, and other differentiation approaches on behaviors of different types of stem cells. This will help us achieve the ultimate goal of establishing the optimum microenvironment, including that presented by nanotubes, for the control of stem cell fate.

A recent study examined the effect of a nanoscale disordered pattern on human MSC differentiation (Figure 7C).[113]

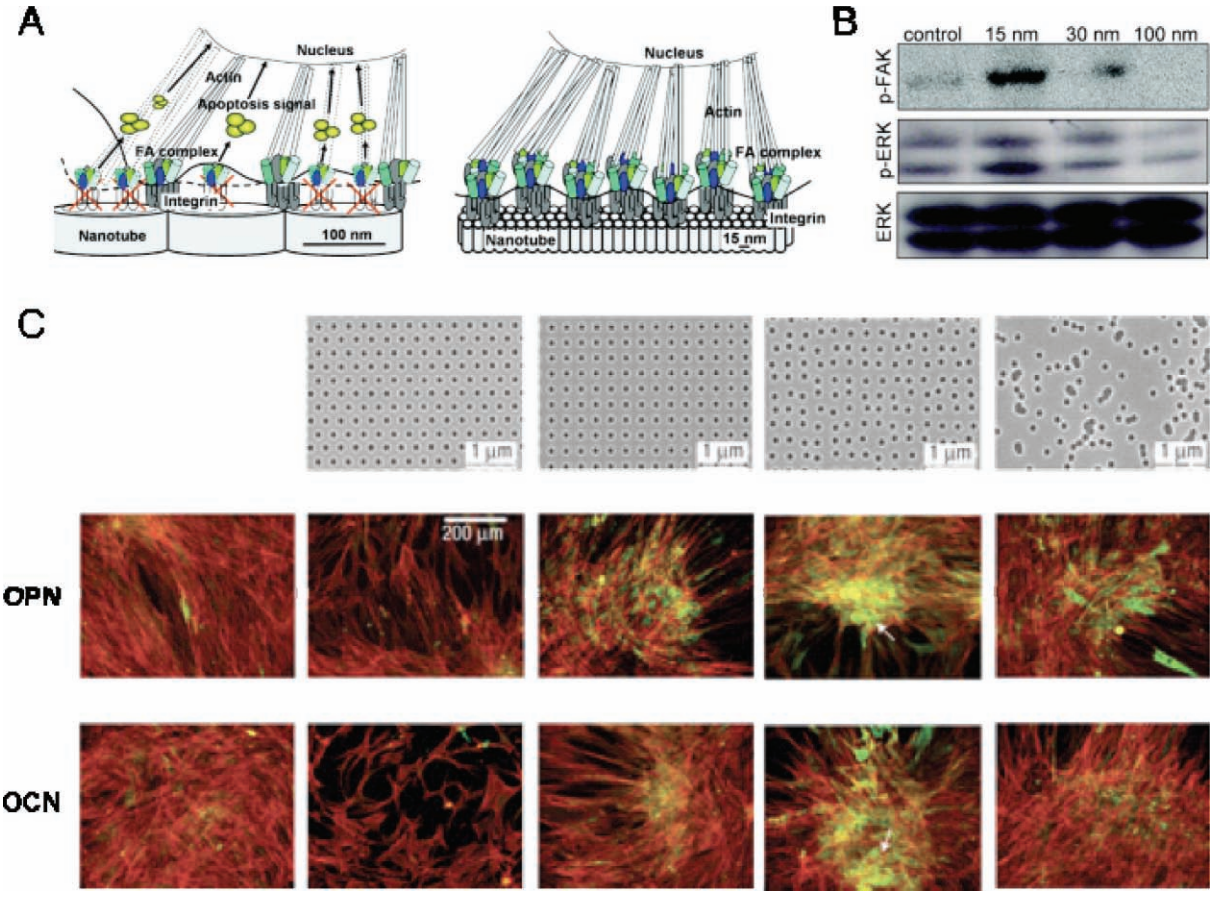


Figure 7. Nanotopographic control of stem cell differentiation. (A) Hypothetical model showing the lateral spacing of focal contacts on nanotubes of different diameters. A spacing of 15 nm seems optimal for integrin assembly into focal contacts, thus inducing assembly of actin filaments and signaling to the nucleus. Nanotubes larger than 70 nm diameter do not support focal contact formation and cell signaling, thus leading to apoptosis. Reprinted with permission from ref. [112]. (B) Phospho-FAK (Tyr526) and phospho-ERK proteins show a maximum of phosphorylation on 15 nm nanotubes. Reprinted by permission from (ref.112). (C) Scanning electron micrographs of highly ordered symmetric, disordered and random symmetric surfaces fabricated by electron beam lithography (top panel). All have 120-nm-diameter pits (100 nm deep, absolute or average 300 nm centre–centre spacing) with hexagonal, square, displaced square 50 (± 50 nm from true centre) and random placements. OPN and OCN staining images of human mesenchymal stem cells (bottom panel). Actin=red, OPN/OCN=green. Reprinted by permission from (ref. 113).

The PMMA substratum was embossed with 120 nm diameter, 100 nm deep nanopits over 1 cm^2 from an original pattern defined using electron beam lithography. Nano-displaced topography significantly increased osteospecific differentiation, even in the absence of specific chemical osteogenic stimuli, demonstrating that topographical strategies might provide novel ways to potential therapeutic applications.[113] In a combinatorial cell stimulation by complex nanopatterned polymeric surfaces, with variable, minutely corrugated and porous templates as well as nanopatterned ECM proteins, stem cell proliferation and differentiation might be guided in a much more controlled fashion.

5. Conclusion and Perspectives

It is widely acknowledged that the highly rigid and flat surfaces with poorly defined surface chemistry present in the commonly used Petri dishes and flasks do not present cells with bio-mimetic mechanical or chemical micro-environments. What is less appreciated is that there is now an emergent toolset of highly precise biocompatible methodologies allowing to circumvent this limitation of cell biological analysis and take cell biology into the new technologically empowered and precisely controlled research age.

Recent advances in development of bio-nanoengineered surfaces are opening new opportunities to investigate fundamental mechanism of regulating cellular interactions on multiple length scales in a more systematically controlled way. We anticipate that the future research will progress in the direction of more detailed analysis of complex biological function with expanding sets of complex nano-scale tools. Many available nanopatterning methods will likely find an ever expanding use complementing each other to generate various functional and structural libraries of 1D, 2D, and 3D bio-nanoarrays (arrays of proteins, lipids, nucleic acids, etc) with controlled feature sizes, shapes, pitches, compositions, and mechanical properties (**Figure 8**). Importantly, the development

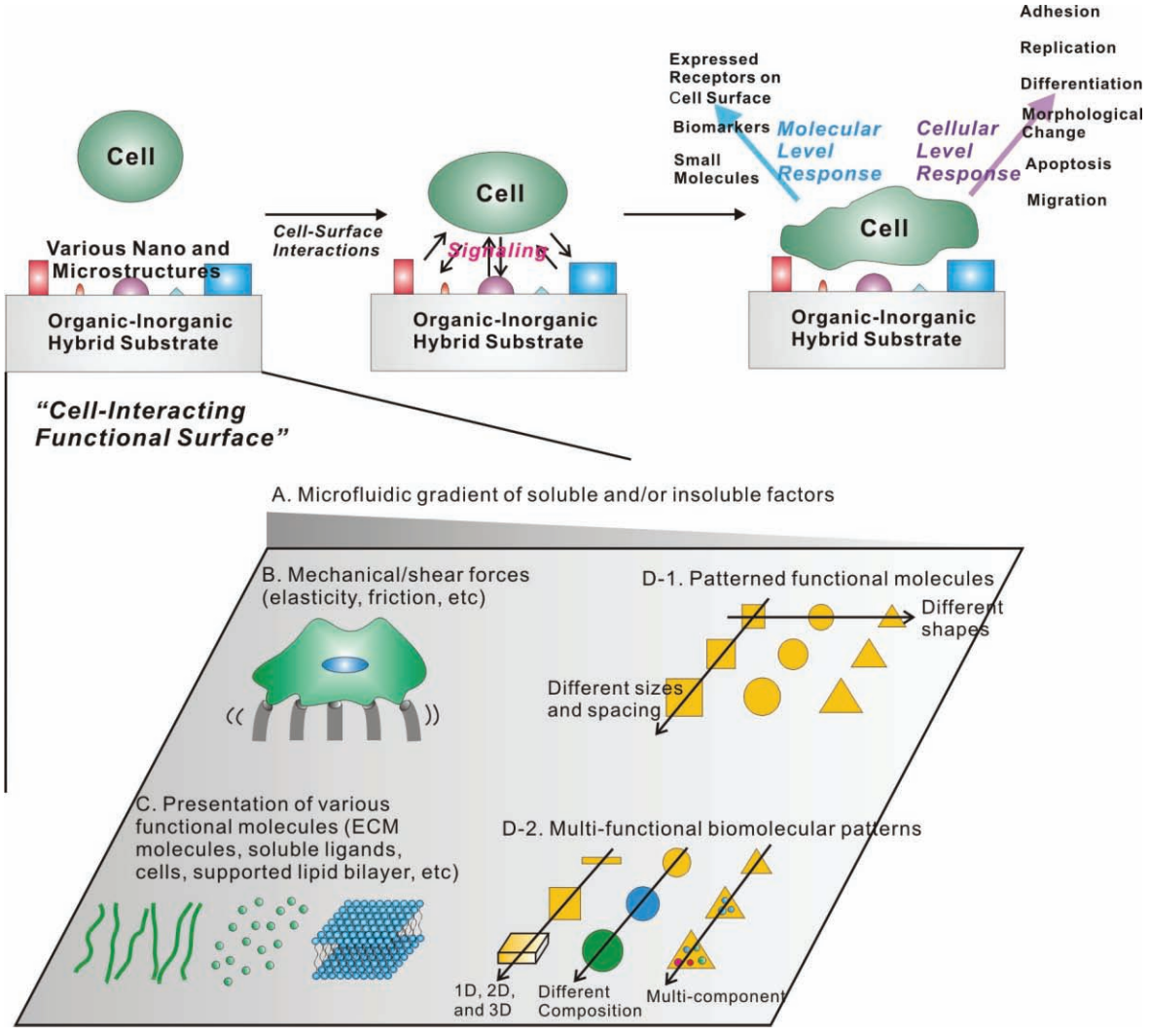


Figure 8. A schematic illustration of combinatorial cellular microenvironment integrated with bio-nanoengineered functional surface interfaces. Many available nanopatterning methods will likely find an every expanding use complementing each other to generate various functional and structural libraries of 1D, 2D, and 3D bio-nanoarrays (arrays of proteins, lipids, nucleic acids, etc) with controlled feature sizes, shapes, pitches, and compositions.

of various, functionally supported lipid bilayer-ECM hybrid arrays could allow for studying complex cellular processes, such as cell-cell communications within an ECM environment in a more biomimetic way. Furthermore, it is still challenging to generate nanopatterned surfaces for cell biological applications in a cost-effective, reproducible, biocompatible, massively parallel, and high-throughput manner.

The future developments might concentrate on developing more complex, variable matrix-like structures[111] and materials and advancing their biomedical applications, e.g. for tissue regeneration and repair. Developing tissue scaffolds may also benefit if, in addition to varying the geometrical design of nanostructures in tissue scaffolds, mechanical properties of these structures, such as their rigidity, can be modulated. This could be simply achieved by fabricating nanostructured scaffolds using UV-curable hydrogels with different amount of curing agent. The rigidity can be increased using larger amounts of the curing agent. Alternatively, polymers of different molecular weight can be used. This approach is especially promising in light of the recent finding that human MSCs specify lineage and commit to phenotypes with extreme sensitivity to tissue-level elasticity.[110] The results have significant implications for understanding physical effects of the *in vivo* microenvironment and also for therapeutic uses of nanostructured polymeric scaffolds with tissue-like rigidity.[110]

Signaling and other cellular functions can be very different in 2D cell culture system, compared to engineered 3D systems that re-iterate cell-matrix interactions in living organisms. Most of bio-nanopatterning techniques currently available should therefore be further developed to pattern biomaterials on non-flat surfaces and in 3D. The techniques for fabricating complex 3D nanostructures required for cell biological applications furthermore need to be simple and easy to reproduce. For tissue engineering applications in particular, currently available 3D micropatterning techniques enabling one to pattern various 3D features into constructs incorporating, live cells such as in hydrogels[133,134] can be further improved so as to provide cells with precisely localized molecular cues with a sub-micron resolution.

Better understanding of adhesion-mediated signal transduction through the nanoscale control of cell-matrix and cell-cell interactions could open up novel strategies to manipulate cell locomotion, matrix assembly, or cell interactions in tissue engineering applications. Since many powerful nanopatterning methods have been developed and some of them are commercially available, exploring and discovering new cell biology and medicine based on biologically functional nanopatterned platforms loom on the horizon.

Acknowledgements

Authors D.-H. K. and H. Lee. and contribute equally to this work. This work was supported by the National Institutes of Health (1R21EB008562-01A1) and the American Heart Association (0815104E). This work was also supported by the National Research Foundation of Korea (20090077361), the 21C Frontier Functional Proteomics Project (FPR08-A2-150) and the Nano R&D Program (2008-02890) through the Korea Science and Engineering Foundation from Ministry of Education, Science and Technology. H. Lee and Y. K. Lee were partially supported from the Seoul Science Fellowship. We also acknowledge K.-J. Jang for helpful discussions.

Received: February 8, 2010
Published online: August 27, 2010

[1] M. Stevens, J. George, *Science* **2005**, *310*, 1135.
[2] N. Li, A. Tourovskaia, A. Folch, *Crit. Rev. Biomed. Eng.* **2003**, *31*, 423.
[3] A. Khademhosseini, R. Langer, J. Borenstein, J. Vacanti, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2480.
[4] D. H. Kim, P. K. Wong, J. Park, A. Levchenko, Y. Sun, *Annu. Rev. Biomed. Eng.* **2009**, *11*, 203.
[5] B. Geiger, J. P. Spatz, A. D. Bershadsky, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 21.
[6] B. Geiger, A. Bershadsky, R. Pankov, K. M. Yamada, *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 793.
[7] G. A. Silva, *Nat. Rev. Neurosci.* **2006**, *7*, 65.
[8] L. Ferreira, J. M. Karp, L. Nobre, R. Langer, *Cell Stem Cell* **2008**, *3*, 136.
[9] X. Zhu, K. L. Mills, P. R. Peters, J. H. Bahng, E. H. Liu, J. Shim, K. Naruse, M. E. Csete, M. D. Thouless, S. Takayama, *Nat. Mater.* **2005**, *4*, 403.
[10] C. S. Chen, J. Tan, J. Tien, *Annu. Rev. Biomed. Eng.* **2004**, *6*, 275.
[11] Y. Chen, A. Pepin, *Electrophoresis* **2001**, *22*, 187.
[12] B. D. Gates, Q. Xu, M. Stewart, D. Ryan, C. G. Willson, G. M. Whitesides, *Chem. Rev.* **2005**, *105*, 1171.
[13] G. Liu, S. Xu, Y. Qian, *Acc. Chem. Res.* **2000**, *33*, 457.
[14] J. Liu, J. Von Ehr, C. Baur, R. Stallcup, J. Randall, K. Bray, *Appl. Phys. Lett.* **2004**, *84*, 1359.
[15] M. Liu, N. Amro, C. Chow, G. Liu, *Nano Lett.* **2002**, *2*, 863.
[16] K. Lee, S. Park, C. Mirkin, J. Smith, M. Mrksich, *Science* **2002**, *295*, 1702.
[17] K. Wadu-Mesthrige, N. A. Amro, J. C. Garno, S. Xu, G. Liu, *Biophys. J.* **2001**, *80*, 1891.
[18] S. Sekula, J. Fuchs, S. Weg-Remers, P. Nagel, S. Schuppler, J. Fragala, N. Theilacker, M. Franzreb, C. Wingren, P. Ellmark, C. A. Borrebaeck, C. A. Mirkin, H. Fuchs, S. Lenhert, *Small* **2008**, *4*, 1785.
[19] K. B. Lee, J. H. Lim, C. A. Mirkin, *J. Am. Chem. Soc.* **2003**, *125*, 5588.
[20] J.-M.Nam, S. W. Han, K. B. Lee, X. Liu, M. A. Ratner, C. A. Mirkin, *Angew. Chem. Int. Ed.* **2004**, *43*, 1246.
[21] J. Lim, D. Ginger, K. Lee, J. Heo, J.-M. Nam, C. Mirkin, *Angew. Chem. Int. Ed.* **2003**, *42*, 2309.
[22] K. Salaita, S. Lee, X. Wang, L. Huang, T. Dellinger, C. Liu, C. Mirkin, *Small* **2005**, *1*, 940.
[23] K. Salaita, Y. Wang, J. Fragala, R. A. Vega, C. Liu, C. A. Mirkin, *Angew. Chem. Int. Ed.* **2006**, *45*, 7220.
[24] F. Huo, Z. Zheng, G. Zheng, L. R. Giam, H. Zhang, C. A. Mirkin, *Science* **2008**, *321*, 1658.
[25] X. F. Wang, D. A. Bullen, J. Zou, C. Liu, C. A. Mirkin, *J. Vac. Sci. Technol. B* **2004**, *22*, 2563.
[26] D. C. Coffey, D. S. Ginger, *J. Am. Chem. Soc.* **2005**, *127*, 4564.
[27] S. Myung, M. Lee, G. T. Kim, J. S. Ha, S. Hong, *Adv. Mater.* **2005**, *17*, 2361.
[28] K. S. Salaita, S. W. Lee, D. S. Ginger, C. A. Mirkin, *Nano Lett.* **2006**, *6*, 2493.
[29] D. L. Wilson, R. Martin, S. Hong, M. Cronin-Golomb, C. A. Mirkin, D. L. Kaplan, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13660.
[30] K. Lee, E. Alsberg, S. Hsiong, W. Comisar, J. Linderman, R. Ziff, D. Mooney, *Nano Lett.* **2004**, *4*, 1501.
[31] C. L. Cheung, J. A. Camarero, B. W. Woods, T. Lin, J. E. Johnson, J. J. De Yoreo, *J. Am. Chem. Soc.* **2003**, *125*, 6848.

[32] J. C. Smith, K. B. Lee, Q. Wang, M. G. Finn, J. E. Johnson, M. Mrksich, C. A. Mirkin, *Nano Lett.* **2003**, *3*, 883.
[33] R. A. Vega, D. Maspoch, K. Salaita, C. A. Mirkin, *Angew. Chem. Int. Ed.* **2005**, *44*, 6013.
[34] R. A. Vega, C. K. Shen, D. Maspoch, J. G. Robach, R. A. Lamb, C. A. Mirkin, *Small* **2007**, *3*, 1482.
[35] J. Hyun, J. Kim, S. L. Craig, A. Chilkoti., *J. Am. Chem. Soc.* **2004**, *126*, 4770.
[36] M. Lee, D. K. Kang, H. K. Yang, K. H. Park, S. Y. Choe, C. Kang, S. I. Chang, M. H. Han, I. C. Kang, *Proteomics* **2006**, *6*, 1094.
[37] S. Lenhert, P. Sun, Y. Wang, H. Fuchs, C. A. Mirkin, *Small* **2007**, *3*, 71.
[38] A. J. Torres, M. Wu, D. Holowka, B. Baird, *Annu. Rev. Biophys.* **2008**, *37*, 265.
[39] S. A. Ruiz, C. S. Chen, *Soft Matter* **2007**, *3*, 168.
[40] P. J. Yoo, S. J. Choi, J. H. Kim, D. Suh, S. J. Baek, T. W. Kim, H. H. Lee, *Chem. Mater.* **2004**, *16*, 5000.
[41] T. Burgin, V. E. Choong, G. Maracas, *Langmuir* **2000**, *16*, 5371.
[42] H. W. Li, B. V. O. Muir, G. Fichet, W. T. S. Huck, *Langmuir* **2003**, *19*, 1963.
[43] H. Schmid, B. Michel, *Macromolecules* **2000**, *33*, 3042.
[44] T. W. Odom, J. C. Love, D. B. Wolfe, K. E. Paul, G. M. Whitesides, *Langmuir* **2002**, *18*, 5314.
[45] K. Nakarnatsu, K. Tone, H. Namatsu, S. Matsui, *J. Vac. Sci. Technol. B* **2006**, *24*, 195.
[46] J. M. Hong, F. M. Ozkeskin, J. Zou, *J. Micromech. Microeng.* **2008**, *18*, 015003 [6pp].
[47] J. Gu, X. Y. Xiao, B. R. Takulapalli, M. E. Morrison, P. Zhang, F. Zenhausern, *J. Vac. Sci. Technol. B* **2008**, *26*, 1860.
[48] C. Vieu, F. Carcenac, A. Pe'in, Y. Chen, M. Mejias, A. Lebib, L. Manin-Ferlazzo, L. Couraud, H. Launois, *Appl. Surf. Sci.* **2000**, *164*, 111.
[49] W. A. Loesberg, J. te Riet, F. C. van Delft, P. Schon, C. G. Figdor, S. Speller, J. J. van Loon, X. F. Walboomers, J. A. Jansen, *Biomaterials* **2007**, *28*, 3944.
[50] M. J. Dalby, N. Gadegaard, R. Tare, A. Andar, M. O. Riehle, P. Herzyk, C. D. Wilkinson, R. O. Oreffo, *Nat. Mater.* **2007**, *6*, 997.
[51] J. W. Lussi, C. Tang, P. A. Kuenzi, U. Staufer, G. Csucs, J. Voros, G. Danuser, J. A. Hubbell, M. Textor, *Nanotechnology* **2005**, *16*, 1781.
[52] F. A. Denis, A. Pallandre, B. Nysten, A. M. Jonas, C. C. Dupont-Gillain, *Small* **2005**, *1*, 984.
[53] J. Rundqvist, J. H. Hoh, D. B. Haviland, *Langmuir* **2006**, *22*, 5100.
[54] D. Pesen, W. F. Heinz, J. L. Werbin, J. H. Hoh, D. B. Haviland, *Soft Matter* **2007**, *3*, 1280.
[55] J. Rundqvist, B. Mendoza, J. L. Werbin, W. F. Heinz, C. Lemmon, L. H. Romer, D. B. Haviland, J. H. Hoh, *J. Am. Chem. Soc.* **2007**, *129*, 59.
[56] M. Arnold, M. Schwieder, J. Blummel, E. A. Cavalcanti-Adam, M. Lopez-Garcia, H. Kessler, B. Geiger, J. P. Spatz, *Soft Matter* **2009**, *5*, 72.
[57] P. Krsko, T. E. McCann, T.-T. Thach, T. L. Laabs, H. M. Geller, M. R. Libera, *Biomaterials* **2009**, *30*, 721.
[58] K. L. Christman, E. Schopf, R. M. Broyer, R. C. Li, Y. Chen, H. D. Maynard, *J. Am. Chem. Soc.* **2009**, *131*, 521.
[59] E. D. Boland, T. A. Telemeco, D. G. Simpson, G. E. Wnek, G. L. Bowlin, *J. Biomed. Mater. Res. B.* **2004**, *71*, 144.
[60] M. C. McManus, E. D. Boland, D. G. Simpson, C. P. Barnes, G. L. Bowlin, *J. Biomed. Mater. Res. A* **2007**, *81*, 299.
[61] J. Seo, H. Lee, K. Char, J.-M. Nam, *Biomacromolecules* **2009**, *10*, 2254.
[62] D. Rabuka, R. Parthasarathy, G. S. Lee, X. Chen, J. T. Groves, C. R. Bertozzi, *J. Am. Chem. Soc.* **2007**, *129*, 5462.
[63] Y. Zhang, H. Ouyang, C. T. Lim, S. Ramakrishna, Z. M. Huang, *J. Biomed. Mater. Res. B* **2005**, *72*, 156.
[64] K. N. Chua, W. S. Lim, P. C. Zhang, H. F. Lu, J. Wen, S. Ramakrishna, K. W. Leong, H. Q. Mao, *Biomaterials* **2005**, *26*, 2537.
[65] X. Li, J. Xie, X. Yuan, Y. Xia, *Langmuir* **2008**, *24*, 14145.
[66] Y. Z. Zhang, Z. M. Huang, X. J. Xu, C. T. Lim, S. Ramakrishna, *Chem. Mater.* **2004**, *16*, 3406.
[67] A. V. Bazilevsky, A. L. Yarin, C. M. Megaridis, *Langmuir* **2007**, *23*, 2311.
[68] H. L. Jiang, Y. Q. Hu, P. C. Zhao, Y. Li, K. J. Zhu, *J. Biomed. Mater. Res. B* **2006**, *79B*, 50.
[69] S. Patel, K. Kurpinski, R. Quigley, H. Gao, B. S. Hsiao, M. M. Poo, S. Li, *Nano Lett.* **2007**, *7*, 2122.
[70] Y. Ji, K. Ghosh, X. Z. Shu, B. Q. Li, J. C. Sokolov, G. D. Prestwich, R. A. F. Clark, M. H. Rafailovich, *Biomaterials* **2006**, *27*, 3782.
[71] B. M. Baker, R. L. Mauck, *Biomaterials* **2007**, *28*, 1967.
[72] D. Li, Y. L. Wang, Y. N. Xia, *Nano Lett.* **2003**, *3*, 1167.
[73] D. Li, G. Ouyang, J. T. McCann, Y. N. Xia, *Nano Lett.* **2005**, *5*, 913.
[74] D. M. Zhang, J. Chang, *Adv. Mater.* **2007**, *19*, 3664.
[75] W. J. Li, R. Tuli, C. Okafor, A. Derfoul, K. G. Danielson, D. J. Hall, R. S. Tuan, *Biomaterials* **2005**, *26*, 599.
[76] L. Buttafoco, N. G. Kolkman, P. Engbers-Buijtenhuijs, A. A. Poot, P. J. Dijkstra, I. Vermes, J. Feijen, *Biomaterials* **2006**, *27*, 724.
[77] L. Moroni, R. Schotel, D. Hamann, J. R. de Wijn, C. A. van Blitterswijk, *Adv. Funct. Mater.* **2008**, *18*, 53.
[78] D. Zhang, J. Chang, *Nano Lett.* **2008**, *8*, 3283.
[79] F. G. Giancotti, E. Ruoslahti, *Science* **1999**, *285*, 1028.
[80] B. Geiger, A. Bershadsky, *Curr. Opin. Cell Biol.* **2001**, *13*, 584.
[81] M. J. Humphries, *Curr. Opin. Cell Biol.* **1996**, *8*, 632.
[82] A. D. Bershadsky, N. Q. Balaban, B. Geiger, *Annu. Rev. Cell Dev. Biol.* **2003**, *19*, 677.
[83] S. Li, J. L. Guan, S. Chien, *Annu. Rev. Biomed. Eng.* **2005**, *7*, 105.
[84] D. Lehnert, B. Wehrle-Haller, C. David, U. Weiland, C. Ballestrem, B. A. Imhof, M. Bastmeyer, *J. Cell Sci.* **2004**, *117*, 41.
[85] N. Xia, C. K. Thodeti, T. P. Hunt, Q. Xu, M. Ho, G. M. Whitesides, R. Westervelt, D. E. Ingber, *Faseb J.* **2008**, *22*, 1649.
[86] X. Liu, P. X. Ma, *Biomaterials* **2009**, *30*, 4094.
[87] K.-J. Jang, J.-M. Nam, *Small* **2008**, *4*, 1930.
[88] M. Arnold, E. A. Cavalcanti-Adam, R. Glass, J. Blummel, W. Eck, M. Kantlehner, H. Kessler, J. P. Spatz, *ChemPhysChem* **2004**, *5*, 383.
[89] M. Arnold, V. C. Hirschfeld-Warneken, T. Lohmuller, P. Heil, J. Blummel, E. A. Cavalcanti-Adam, M. Lopez-Garcia, P. Walther, H. Kessler, B. Geiger, J. P. Spatz, *Nano Lett.* **2008**, *8*, 2063.
[90] E. A. Cavalcanti-Adam, T. Volberg, A. Micoulet, H. Kessler, B. Geiger, J. P. Spatz, *Biophys. J.* **2007**, *92*, 2964.
[91] E. A. Cavalcanti-Adam, A. Micoulet, J. Blummel, J. Auernheimer, H. Kessler, J. P. Spatz, *Eur. J. Cell Biol.* **2006**, *85*, 219.
[92] V. C. Hirschfeld-Warneken, M. Arnold, A. Cavalcanti-Adam, M. Lopez-Garcia, H. Kessler, J. P. Spatz, *Eur. J. Cell Biol.* **2008**, *87*, 743.
[93] R. J. Mannix, S. Kumar, F. Cassiola, M. Montoya-Zavala, E. Feinstein, M. Prentiss, D. E. Ingber, *Nat. Nanotechnol.* **2008**, *3*, 36.
[94] J. T. Groves, *Sci STKE* **2005**, *2005*, pe45.
[95] J. T. Groves, N. Ulman, S. G. Boxer, *Science* **1997**, *275*, 651.
[96] K. D. Mossman, G. Campi, J. T. Groves, M. L. Dustin, *Science* **2005**, *310*, 1191.
[97] J. Doh, D. J. Irvine, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5700.
[98] S. Pautot, H. Lee, E. Y. Isacoff, J. T. Groves, *Nat. Chem. Biol.* **2005**, *1*, 283.
[99] J.-M. Nam, P. Nair, R. Neve, J. Gray, J. Groves, *ChemBioChem* **2006**, *7*, 436.
[100] M. P. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **2005**, *23*, 47.
[101] J. A. Matthews, G. E. Wnek, D. G. Simpson, G. L. Bowlin, *Biomacromolecules* **2002**, *3*, 232.
[102] K. S. Rho, L. Jeong, G. Lee, B. M. Seo, Y. J. Park, S. D. Hong, S. Roh, J. J. Cho, W. H. Park, B. M. Min, *Biomaterials* **2006**, *27*, 1452.

[103] K. Chua, C. Chai, P. Lee, Y. Tang, S. Ramakrishna, K. Leong, H. Mao, *Biomaterials* **2006**, *27*, 6043.
[104] J. Deutsch, D. Motlagh, B. Russell, T. A. Desai, *J. Biomed. Mater. Res.* **2000**, *53*, 267.
[105] N. F. Huang, S. Patel, R. G. Thakar, J. Wu, B. S. Hsiao, B. Chu, R. J. Lee, S. Li, *Nano Lett.* **2006**, *6*, 537.
[106] S. M. Cannizzaro, R. F. Padera, R. Langer, R. A. Rogers, F. E. Black, M. C. Davies, S. J. Tendler, K. M. Shakesheff, *Biotechnol. Bioeng.* **1998**, *58*, 529.
[107] G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler, S. I. Stupp, *Science* **2004**, *303*, 1352.
[108] S. Y. Chew, R. Mi, A. Hoke, K. W. Leong, *Biomaterials* **2008**, *29*, 653.
[109] J. W. Xie, S. M. Willerth, X. R. Li, M. R. Macewan, A. Rader, S. E. Sakiyama-Elbert, Y. N. Xia, *Biomaterials* **2009**, *30*, 354.
[110] A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, *Cell* **2006**, *126*, 677.
[111] D. H. Kim, C. H. Seo, K. Han, K. W. Kwon, A. Levchenko, K. Y. Suh, *Adv. Funct. Mater.* **2009**, *19*, 1579.
[112] J. Park, S. Bauer, K. von der Mark, P. Schmuki, *Nano Lett* **2007**, *7*, 1686.
[113] M. J. Dalby, N. Gadegaard, R. Tare, A. Andar, M. O. Riehle, P. Herzyk, C. D. Wilkinson, R. O. Oreffo, *Nat. Mater.* **2007**.
[114] A. Tourovskaia, X. Figueroa-Masot, A. Folch, *Lab Chip* **2005**, *5*, 14.
[115] J. M. Dang, K. W. Leong, *Adv. Mater.* **2007**, *19*, 2775.
[116] R. McBeath, D. M. Pirone, C. M. Nelson, K. Bhadriraju, C. S. Chen, *Dev. Cell* **2004**, *6*, 483.
[117] M. Pla-Roca, J. G. Fernandez, C. A. Mills, E. Martinez, J. Samitier, *Langmuir* **2007**.
[118] P. Krsko, T. E. McCann, T. T. Thach, T. L. Laabs, H. M. Geller, M. R. Libera, *Biomaterials* **2009**, *30*, 721.
[119] A. Martins, J. V. Araujo, R. L. Reis, N. M. Neves, *Nanomedicine* **2007**, *2*, 929.
[120] L. Yao, N. O'Brien, A. Windebank, A. Pandit, *J. Biomed. Mater. Res. B: Appl. Biomater.* **2009**.
[121] J. K. Holt, H. G. Park, Y. Wang, M. Stadermann, A. B. Artyukhin, C. P. Grigoropoulos, A. Noy, O. Bakajin, *Science* **2006**, *312*, 1034.
[122] E. Jeoung, T. H. Galow, J. Schotter, M. Bal, A. Ursache, M. T. Tuominen, C. M. Stafford, T. P. Russell, V. M. Rotello, *Langmuir* **2001**, *17*, 6396.
[123] I. Slowing, B. G. Trewyn, S. Giri, V. S.-Y. Lin, *Adv. Mater* **2007**, *17*, 1225.
[124] N. Ben-Haim, P. Broz, S. Marsch, W. Meier, P. Hunziker, *Nano Lett* **2008**, *8*, 1368.
[125] H. Kukula, H. Schlaad, M. Antonietti, S. Forster, *J. Am. Chem.Soc* **2002**, *124*, 1658.
[126] S. V. Boddapati, G. G. M. D'Souza, S. Erdogan, V. P. Torchilin, V. Weissig, *Nano Lett* **2008**, *8*, 2559.
[127] V. Marchi-Artzner, B. Lorz, C. Gosse, L. Jullien, R. Merkel, H. Kessler, E. Sackmann, *Langmuir* **2002**, *19*, 835.
[128]J.-S. Jan, S. Lee, C. S. Carr, D. F. Shantz, *Chem. Mater.* **2005**, *17*, 4310.
[129] J.-M. Nam, C. S. Thaxton, C. A. Mirkin, *Science* **2003**, *301*, 1884.
[130] B. Bhushan, *Microelectronic Engin.* **2007**, *84*, 387.
[131] D. H. Kim, E. Lipke, P. Kim, R. Cheong, S. Edmonds, M. Delannoy, K. Y. Suh, L.Tung, A. Levchenko, *Proc. Natl. Acad. Sci. USA* **2009**, *107*, 565.
[132] N. Gadegaard, M. J. Dalby, M. O. Riehle, A. S. G. Curtis, S. Affrossman, *Adv. Mater.* **2004**, *16*, 1857.
[133] S. H. Lee, J. J. Moon, J. L. West, *Biomaterials* **2008**, *29*, 2962.
[134] C. A. DeForest, B. D. Polizzotti, K. S. Anseth, *Nat. Mater.* **2009**, *8*, 659.
[135] K. Y. Suh, M. C. Park, P. Kim, *Adv. Funct. Mater.* **2009**, *19*, 2699.
[136] E. Kim, Y. Xia, G. M. Whitesides, *Nature* **1995**, *376*, 581.
[137] Y. M. Zhao, Y. Xia, G. M. Whitesides, *Adv. Mater.* **1996**, *98*, 837.
[138] Y. Xia, E. Kim, Y. M. Zhao, J. A. Prentis, G. M. Whitesides, *Science* **1996**, *273*, 345.