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REVIEW

Nanocombinatorics with Cantilever-Free Scanning Probe Arrays

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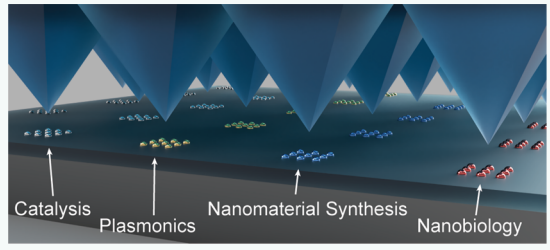
ABSTRACT: **The effectiveness of combinatorial experiments is determined by the rate at which distinct experimental conditions can be prepared and interrogated. This has been particularly limiting at the intersection of nanotechnology and soft materials research, where structures are difficult to reliably prepare and materials are incompatible with conventional lithographic techniques. For example, studying nanoparticle-based heterogeneous catalysis or the interaction between biological cells and abiotic surfaces requires precise tuning of materials composition on the nanometer scale. Scanning probe techniques are poised to be major players in the combinatorial nanoscience arena because they allow one to directly deposit materials at high resolution without any harsh processing steps that limit material compatibility. The chief limitation of scanning probe techniques is throughput, as patterning with single probes is prohibitively slow in the context of large-scale combinatorial experiments. A recent paradigm shift circumvents this problem by fundamentally altering the architecture of scanning probes by replacing the conventionally used cantilever with a soft compliant film on a rigid substrate, a substitution that allows a densely packed array of probes to function in parallel in an inexpensive format. This is a major lithographic advance in terms of scalability, throughput, and versatility that, when combined with the development of approaches to actuate individual probes in cantilever-free arrays, sets the stage for scanning-probe-based tools to address scientific questions through nanocombinatorial studies in biology and materials science. In this review, we outline the development of cantilever-free scanning probe lithography and prospects for nanocombinatorial studies enabled by these tools.**



KEYWORDS: *combinatorial synthesis, scanning probe lithography, hard and soft material deposition, nanolithography, nanocombinatorics, nanoscience, polymer pen lithography, cantilever-free, catalytic screening, nanofabrication, molecular printing*

One of the cornerstone advantages inherent to nanotechnology is that miniaturized systems occupy less space, and therefore extraordinarily large numbers of components can occupy small areas.[1−3] The semiconductor industry has taken full advantage of this fact by continually increasing the areal density of electrical devices in microelectronics to the point where each processor now contains billions of transistors.[4] From a research perspective, this scaling presents major opportunities because it, in principle, allows one to multiplex experimental conditions to perform classes of experiments that involve too many permutations to be undertaken at the benchtop scale. Although miniaturization is important from an efficiency perspective, materials that are nanometer scale can exhibit properties that differ from those observed at the bulk scale. For example, nanometer-scale metallic nanoparticles exhibit enhanced activity as heterogeneous catalysts, and nanoscale domains of biomolecules can cooperatively interact with biological cells to dictate the formation of focal adhesions, which in turn can affect important processes like differentiation.[5,6] This field that involves the massive parallelization of experimental conditions at the nanoscopic scale where properties emerge is known as nanocombinatorics or combinatorial nanoscience.[7] Due to the wide range of applications of nanotechnology, this field is ideally suited to address questions that require screening enormous numbers of experimental conditions in fields including biotechnology, catalysis, and nanoelectronics.

The success of combinatorial nanoscience experiments, and consequently the major challenges in this field, are dictated by how effectively nanomaterials can be *synthesized* and *screened*. Two areas where this challenge is abundantly clear are catalysis and tissue engineering. In heterogeneous catalysis, it has been shown that nanoparticles can exhibit higher activity than one would expect based upon their surface area.[5,8] This synergistic effect has been hypothesized to arise from a number of sources including the curvature of the surface or high densities of grain

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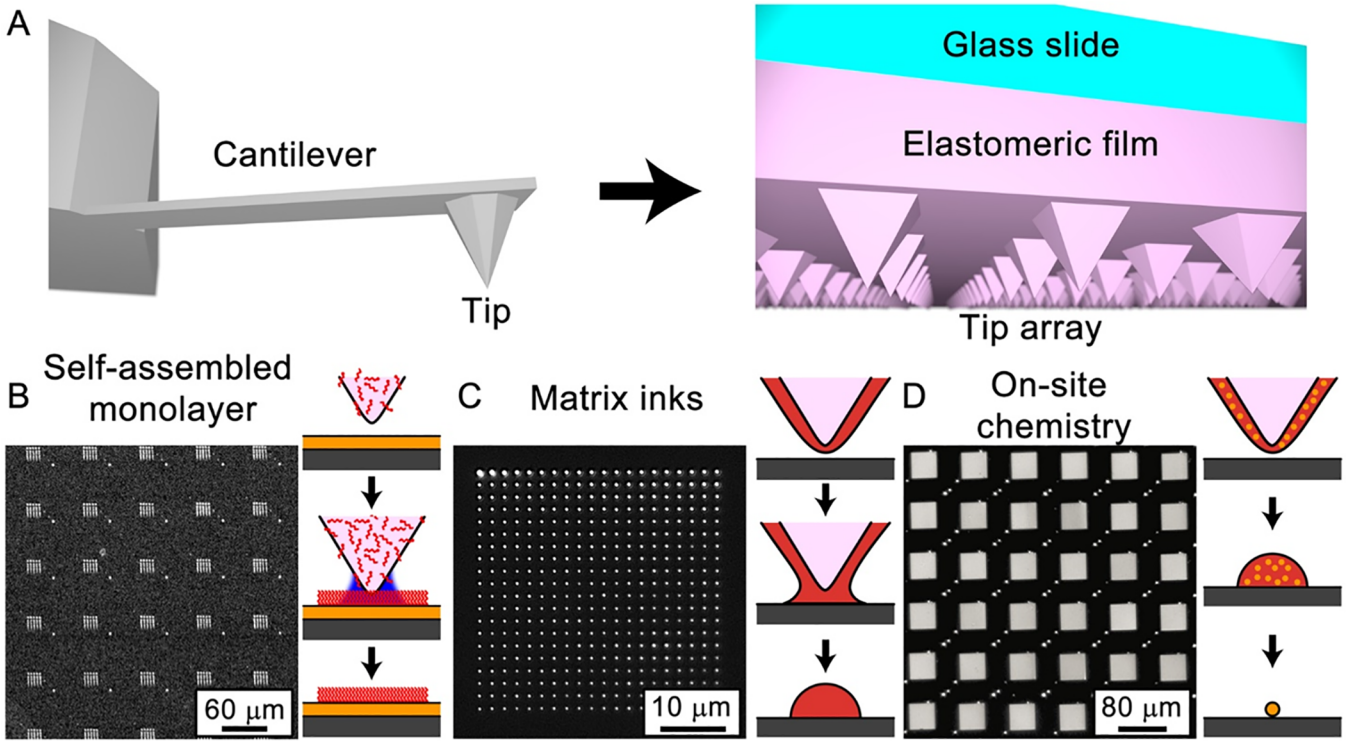

Figure 1. Cantilever-free scanning probe lithography (CF-SPL). (A) Schematic showing the evolution from a cantilever-based scanning probe with a single tip to an array of probes resting on an elastomeric film on a glass slide. (B) Schematic and electron micrograph depicting self-assembled monolayers printed using CF-SPL. Reproduced with permission from ref 25. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Schematic and fluorescence image depicting matrix-ink features printed using CF-SPL. Reprinted from ref 26. Copyright 2013 American Chemical Society. (D) Schematic and electron micrograph depicting on-site chemistry being performed in features printed by CF-SPL, specifically, the synthesis of single metal nanoparticles in polymer nanoreactors. Reprinted with permission from ref 27. Copyright 2010 National Academy of Sciences.

boundaries. However, understanding the origins of this enhancement, or simply finding the optimal nanoparticle to catalyze a given reaction, has been a major challenge due to the difficulty inherent in preparing large numbers of particles that only differ by a single parameter (*e.g.*, composition or size). In the case of tissue engineering, it is known that interactions between cells and the extracellular matrix can dictate the phenotype and even differentiation pathway of those cells.[6,7,9] Although myriad experiments have explored the behavior of cells on a variety of surfaces,[10,11] culture-to-culture variations complicate interpretation of these experiments. Furthermore, patterning multiple biological materials on the scale of individual focal adhesions is difficult because conventional patterning techniques used in microelectronics processing are not biocompatible due to their reliance on harsh acids, bases, and organic solvents. These two fields, catalysis and tissue engineering, represent major candidates for nanocombinatorics as they (1) exhibit important features at the nanoscale and (2) have enough experimental parameters that they require massive numbers of experiments to fully elucidate phenomena.

In this review, we overview cantilever-free scanning probe lithography (CF-SPL) as a collection of synthetic processes that enable nanocombinatoric experiments by addressing the challenge of making large-scale arrays of nanoscale soft materials. Conceptually, CF-SPL represents the convergence of soft lithography[12] and scanning probe lithographic techniques such as dip-pen nanolithography (DPN) where, instead of a cantilever supporting a single pen, an elastomeric film on a rigid backing layer supports a massive array of pens (Figure 1A).[13−15] Initially, CF-SPL was developed as a process for parallelizing DPN in a manner that addressed the throughput challenge associated with serial scanning techniques. Although DPN was parallelized through the development of cantilever arrays,[16−18] the widespread applicability of these approaches was limited by the reliance on delicate structures made through complex micromachining. The first CF-SPL approach is known as polymer pen lithography (PPL) and utilizes a massive array (11 million pens were initially demonstrated) of elastomeric pens to define patterns using a material transfer process analogous to DPN.[15] Based upon this architectural advance, a host of experiments and protocols have been made possible, including those that achieve nanoscale feature resolution across centimeter-scales—patterns that represent control over *7 orders of magnitude in length or 14 orders of magnitude in area*.[19−21] This advance is particularly relevant for nanocombinatorics as CF-SPL allows one to rapidly and arbitrarily pattern hard and soft materials with nanometer-scale features over large spatial scales. Previous reviews touched on how material transport in CF-SPL differs from transport in DPN[22,23] or the evolution of CF-SPL.[19−21,24] Herein, we review recent progress and future directions of this discovery platform with a specific focus on the scientific questions that can be addressed using CF-SPL to perform nanocombinatorial experiments. As case studies, we discuss recent advances in understanding nanoparticle-based heterogeneous catalysis and directing the differentiation of stem cells with nanopatterned surfaces.

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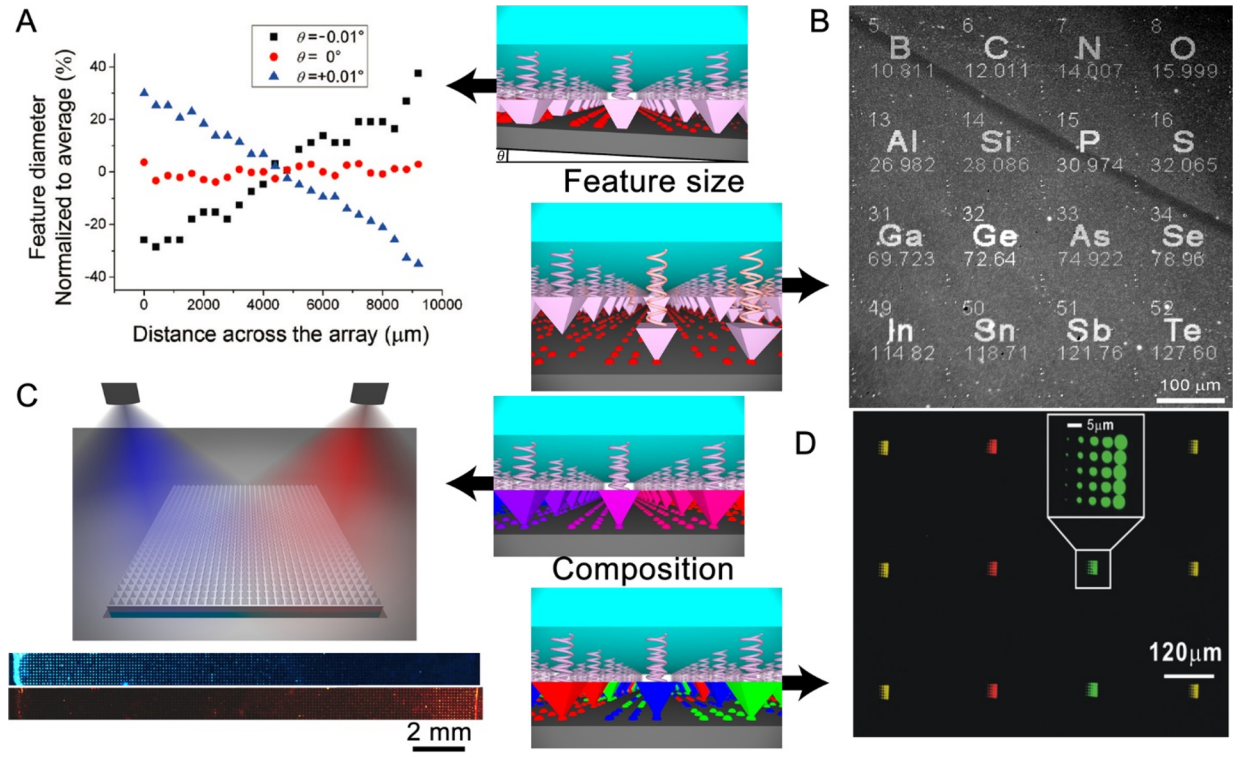

Figure 2. Printing features that vary in size and composition with CF-SPL. (A) Tilting the pen array with respect to the surface allows one to synthesize features whose sizes vary across the sample. Reproduced with permission from ref 25. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (B) Thermally actuating individual tips allows for printing arbitrary features and stitching together patterns written by multiple pens. Reprinted with permission from ref 62. Copyright 2013 National Academy of Sciences. (C) Spray-coating the pen array with different inks from multiple nozzles allows for a gradient of ink compositions to be written using a single pen array. Reprinted with permission from ref 66. Copyright 2018 National Academy of Sciences. (D) Inkjet printing can be used to ink individual pens such that patterns with arbitrary compositions can be written. Reprinted with permission from ref 67. Copyright 2009 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

DIRECTLY WRITING MATERIALS WITH CF-SPL

The principal reason that CF-SPL probes are enabling tools for combinatorial nanoscience is that they can be used to directly write patterns of a specified "ink" on a surface. In this arena, CF-SPL benefits from a large body of work wherein DPN has been used to pattern myriad materials including biological molecules such as DNA or proteins, magnetic nanostructures, nanoparticles, viruses, and polymers.[28−37] Much like the invention of DPN,[38] material transport using CF-SPL was first demonstrated through writing self-assembled monolayers (SAMs) composed of the small molecule 16-mercaptohexadecanoic acid (MHA) onto gold surfaces using PPL (Figure 1B).[15,39] The initial focus on SAM-forming molecules was due to the fact that small molecules are an important class of inks that represent a modular way of defining surface chemistry. Specifically, PPL-patterned SAMs have been used for applications such as immobilizing proteins[7] and nanoparticles,[40] directing the surface-initiated growth of polymers to produce microelectrode arrays,[41] and preparing antifouling surfaces.[42]

Although SAM-forming molecules are a modular way of dictating surface chemistry, it is often advantageous to directly write more complex or diverse materials. An alternate paradigm for direct writing utilizes inks containing water-soluble polymers such as polyethylene glycol (PEG) with typical molecular weights of ∼2 kDa.[23,43] Such polymers are an important class of patterning material because they can be used as a vehicle for the transport (or "matrix") of larger materials such as nanoparticles (Figure 1C).[43,44] Phospholipids and cholesterol have also been used as a matrix for the deposition of nanomaterials.[45] Furthermore, matrix materials can be engineered to bind to the material of interest, a principle which was demonstrated by using the block copolymer poly(ethylene oxide)-*b*-poly(vinylpyridine) to transport metal salts onto surfaces in a process known as scanning probe block copolymer lithography (SPBCL).[27,46] Interestingly, SPBCL highlights a use of polymers beyond acting as a matrix for transport as the deposited polymer features can also serve as nanoreactors that facilitate chemical reactions such as the synthesis of inorganic nanoparticles[27] or Staudinger ligation (Figure 1D).[47]

It is important to note that the soft material composition of PPL pen arrays also makes them capable of patterning materials that cannot be patterned using conventional DPN. Specifically, it has been found that nonpolar solvents can be absorbed into the pen array to allow the patterning of water-insoluble materials such as poly(styrene)-*b*-poly(vinylpyridine).[26] In this way, CF-SPL provides material diversity greater than that of DPN.

CONTROL OVER FEATURE SIZE WITH CF-SPL

With any discussion of a patterning technique, it is important to address the ability to control the size of the patterned features. Due to the fact that depositing small molecules from a scanning probe is understood to be a diffusion process,[48] the principle experimental factor used to control feature size is

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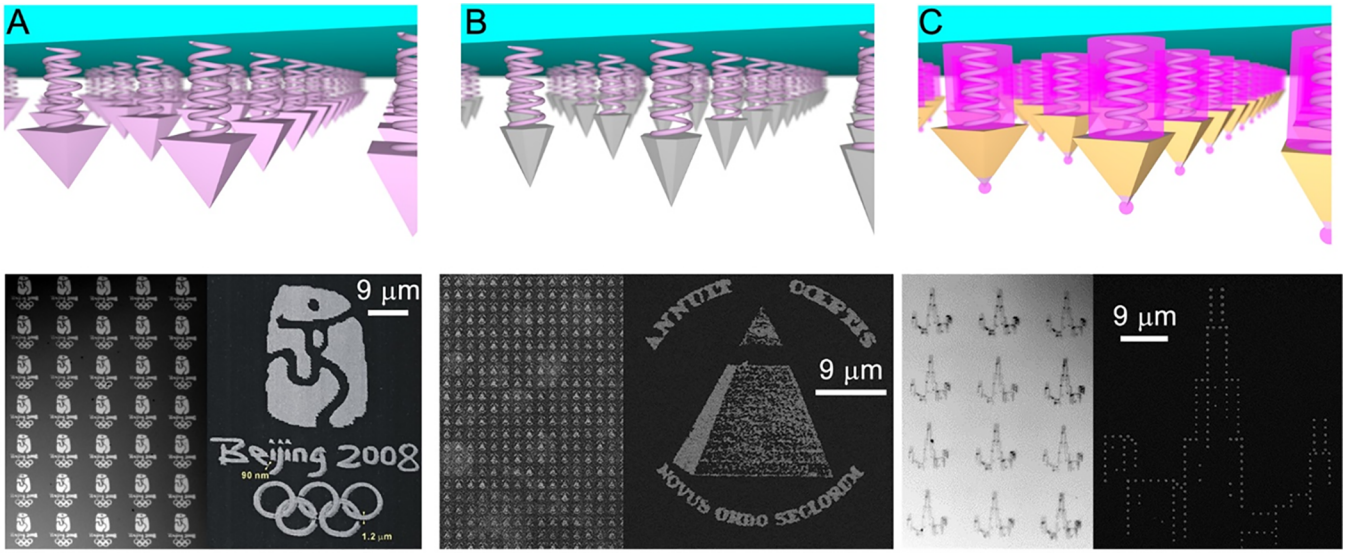

Figure 3. CF-SPL pen array architectures. (A) Elastomeric pens are used for polymer pen lithography (PPL). This technique has the advantage that tips can be intentionally deformed to adjust feature size without changing writing speed. Reprinted with permission from ref 15. Copyright 2008 AAAS. (B) Hard silicon pens are used for hard-tip, soft-spring lithography (HSL), which achieves consistently high resolution across centimeter scales. Reprinted with permission from ref 80. Copyright 2011 Springer Nature. (C) Elastomeric pen arrays that are coated with an opaque layer can be used for diffraction-unlimited near-field photolithography provided that subwavelength apertures are constructed at the apex of each pen. Reprinted with permission from ref 81. Copyright 2010 Springer Nature.

dwell time.[38,48] In the case of patterning polymeric or liquid inks, transport can be understood to be a fluid flow[34,49] and retraction speed can be used as an additional parameter to dictate feature size.[35] Elastomeric pens, as used in PPL, add an additional factor for controlling feature size as the pens themselves may deform, resulting in a force-dependent contact area between the pen and surface.[25,50] Furthermore, the ability to locally control pressure allows the exploration of tip-directed chemistry: for example, force has been used to control the kinetics of the Huisgen reaction[51] as well as the Diels–Alder reaction.[52] Although a force-dependent feature size indicates that pen arrays must be level with respect to the patterning surface in order to write uniform arrays of features, the force–distance relationship is predictable, thus providing a way for algorithmically leveling the pen array with respect to the surface.[53−55]

Although leveling a pen array with respect to a surface is important for uniform patterning, one advantage of PPL in the context of nanocombinatorics is that deliberately tilting an array with respect to a surface will generate gradients in feature size (Figure 2A).[7] This capability was later verified to also allow a gradient of lipid features to be patterned in a single process.[56] The ability for a uniform array of pens to generate a predictably nonuniform array of features is a critical aspect for performing nanocombinatorics. Beyond gradients of feature sizes, ideally, one would be able to generate arbitrary features with each pen independently. Borrowing techniques from the microfluidics community,[57,58] pressurized microchannels can actuate individual pens by moving them in and out of contact with a surface.[59,60] However, the scalability of this method is poor as separate pneumatic controls are required for each pen. In contrast, local thermal actuation can be used to reliably actuate pens by taking advantage of the high coefficient of thermal expansion inherent to elastomers (Figure 2B).[61,62] Strikingly, this method can be used to move pens at speeds over 300 μm/s through distances of several micrometers using powers commensurate with those required to operate cantilever-based probes that are thermally actuated,[63,64] thereby making it an appealing method for facile large-scale pen actuation. Recently, a method was demonstrated that further improved on the scalability of actuation, wherein the pen array was rendered partially opaque through the inclusion of carbon nanotubes into the elastomeric support and local light absorption was used to actuate pens.[65] This photothermal method was shown to be able to move pens up to 3 μm out of plane using modest illumination intensities, providing a path toward massively parallel actuation.

CONTROL OVER FEATURE COMPOSITION WITH CF-SPL

In addition to controlling feature size, it is necessary to control feature composition. Both drop-casting and spin-coating have been successfully used to introduce a uniform quantity of ink across arrays for patterning single-material patterns.[15,39] Although patterns of a single material are important for some applications, intentionally synthesizing patterns with spatially heterogeneous compositions is more powerful from a combinatorial perspective. Toward this end, recent work has shown that by spraying different ink solutions from multiple nozzles onto a pen array, pens can be inked with a continuous linear compositional gradient in a manner that allows compositional gradients to be synthesized using PPL (Figure 2C).

Typically, the pen-to-pen spacing in CF-SPL is tens of micrometers, allowing direct inking via low resolution additive methods. For example, inkjet printing can be used to deposit inks into a periodic array of wells. Subsequent dipping of a PPL pen array into these wells allows for independent control over the ink coating each pen in the array, a process that has been used to pattern multicomponent protein arrays (Figure 2D).[67] Integrated microfluidics can also be used to automatically fill such ink wells.[68,69] Additionally, one can microcontact print as well as pipet inks onto large regions of the array to specify the ink composition for predetermined blocks of pens.[70,71] These

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approaches have allowed PPL-based experiments to pattern chemically inhomogeneous patterns to serve as stamp pads for subsequent functionalization[72] and for direct writing onto microdevices.[73] It is worth emphasizing that the ability to directly write dissimilar soft materials in coordinated nanoscale regions is a key advantage of CF-SPL as it is difficult, if not impossible, to achieve by other techniques, especially those that require sequential exposure and development processes.

PEN ARRAY ARCHITECTURES

An important advantage of the cantilever-free architecture is that pen arrays themselves are modular: pen composition can be chosen to obtain desired printing properties. Originally, the pens and elastomeric backing film were composed of either commercially available polydimethylsiloxane (PDMS) or a stiffer formulation known as hard PDMS (Figure 3A).[15,39] Pens composed of PDMS have also been modified to include PEG to allow them to better absorb water for humidity-independent patterning.[26] Other polymers besides PDMS have been explored, and stiffer polymers result in a slightly decreased sensitivity to applied force in terms of feature size.[74] Building on this realization, by making a polymer pen array composed of two elastomers of different moduli (*i.e.*, the pens are stiffer than the backing layer), it is possible to reduce the sensitivity to tip−sample extension and more reliably print small features.[75] More drastic alterations to pen array architecture have been explored including making the pens different heights to encode a distribution of feature sizes[76] or using close-packed spherical microparticles as pens.[77] Finally, the planar architecture of these pen arrays allows them to be easily coated with graphene, a process which renders them conductive and reduces wear during tip−sample contact.[78] Interestingly, the idea of coating pen arrays has translated to the biological realm where enzyme-coated pen arrays have been used to drive chemical transformations in a contact-dependent manner.[79]

A key observation in CF-SPL was the realization that by making the pens rigid but retaining the compliant backing layer, one can achieve force-independent patterning. Specifically, in hard-tip, soft-spring lithography (HSL), arrays of pyramidal silicon pens on a PDMS backing layer are used to reliably print features as small as 40 nm over centimeters (Figure 3B).[80] The mechanical properties of these pens are well understood in that they exhibit linear force−distance relationships, and their spring constants can be tuned in the 7 to 200 N/m range, a range consistent with intermediate to high stiffness cantilever-based systems.[82] Interestingly, due to the linear dependence of the spring constant on the geometric properties of the pen array, the spring constant in HSL can, in principle, be specified with greater precision than is commonly obtained in cantilever-based systems in which the spring constant is dependent on geometric parameters such as cantilever length and thickness to the third powers.[83] Importantly, more easily synthesized PPL pen arrays can be modified to have the same force-independent patterning capability of HSL—PPL arrays coated with silica using a low-temperature plasma-enhanced chemical vapor disposition step were found to have the force independence inherent to HSL, while at the same time retaining the optical transparency of PPL.[84]

PATTERNING WITH ENERGY

Although many of the enabling capabilities of CF-SPL stem from directly depositing materials, the ability to direct the transfer of energy bears extreme promise for realizing synthetic capabilities for nanocombinatorics. An early example of transferring energy with CF-SPL was the use of HSL to mechanically deform polymeric surfaces.[80] Since this observation, graphene-coated HSL pen arrays have been used to pattern with electrical energy or heat with sub-100 nm resolution.[78]

In general, the most widely used lithographic methods use light to define patterns,[85] and CF-SPL has been extensively explored in this context. The transmission of light through elastomeric pyramidal pens has been studied from the perspective of photolithography[86] and adaptive optics,[87] but the attainable resolution is not better than the far-field diffraction limit when unmodified elastomeric pyramids are used.[88] In contrast, by depositing opaque layers on the pyramidal pen arrays that have subwavelength apertures at the tip of each pen, the pen arrays can be used for diffraction-unlimited lithography (Figure 3C).[81] This approach, known as beam pen lithography (BPL), has been used to pattern 100 nm scale features over centimeters. In this context, light is an especially useful form of energy for patterning because it can be massively multiplexed with relative ease using technologies such as digital micromirror devices. Such multiplexing capabilities grant the ability to pattern arbitrary nanoscale features with each pen in a centimeter-wide array.[89] This technology has been utilized as a lithographic tool to define nanoreactors to synthesize oxide nanostructures in high throughput.[90]

Due to the widespread interest in patterning with light, many cantilever-free pen array architectures for optical lithography have been explored. For example, pen arrays coated with metal films featuring apertures at the tips of each pen have been made serially by focused ion beam milling[81] or in parallel using either reactive ion etching or electrochemical processes.[89,91−93] It has also been shown that pens without apertures (*i.e.*, those that feature continuous metal films across the entire pen array) still allow one to pattern, but with lower resolution.[94−96] The degradation in resolution associated with having no apertures is somewhat mitigated if the pens are composed of a high refractive index material.[97] Alternatively, carbon black can be placed in the space between the tip array and the surface,[98] as was originally reported in the context of light valves.[87] Importantly, a detailed study of the optics of BPL has shown that an opaque film on the backing film is very important for reducing unwanted illumination in all cases.[88]

A recent advance that has great significance for the prospects of performing nanocombinatorial studies using CF-SPL is the ability synthesize materials using BPL in a fluid environment. This concept was first proved by synthesizing nucleotide nanoarrays in an aqueous environment.[99] Significantly, these first explorations showed that, much like immersion lithography techniques used by integrated circuit manufacturers,[100] operation in a high refractive index environment can increase the resolution of BPL.[99] Furthermore, by encompassing the BPL pen array inside a microfluidic channel with inlet and outlet ports, it is possible to introduce a series of chemical reagents without removing the pen array, thus allowing for the synthesis of multicomponent structures such as multicolor brush polymers.[101] This advance is particularly enabling

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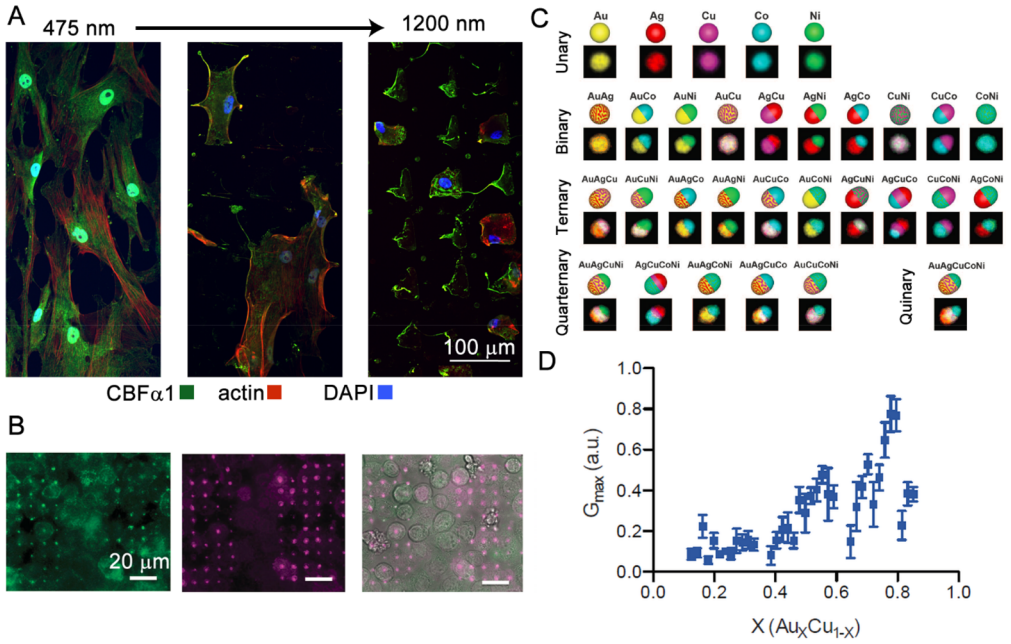

Figure 4. Nanocombinatorial studies using CF-SPL. (A) Tilted polymer pen array experiments were used to pattern a surface with a gradient of fibronectin features between 475 and 1200 nm in size. The behavior of mesenchymal stem cells was observed to be dramatically different using epifluorescence microscopy. Reprinted with permission from ref 7. Copyright 2012 National Academy of Sciences. (B) Patterned allergens were used to study the activation of mast cells. The green activation signal (left) and purple colocalization signal (middle) are merged with a bright-field image (right). Reproduced with permission from ref 107. Copyright 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Up to five-component nanoparticles synthesized using a combination of CF-SPL and scanning probe block copolymer lithography (SPBCL). Reprinted with permission from ref 109. Copyright 2016 AAAS. (D) First example of combinatorial screening with a single CF-SPL-patterned array to discover an optimal catalysis for the synthesis of nanomaterials. Reprinted with permission from ref 66. Copyright 2018 National Academy of Sciences.

because it means that BPL could be used to synthesize highly complex biomolecular arrays with higher resolution than is possible using far-field optics.[102] Additionally, BPL is well suited for use with the myriad photochemistries that have been explored to define functional surfaces, of which applications in biology are particularly promising.[103]

COMBINING PATTERNING WITH ENERGY AND MATERIALS

Ultimately, there are many ways of patterning using energy, but a truly enabling capability afforded by CF-SPL is the ability to simultaneously deliver materials and energy with the same pen array. Indeed, the local delivery of energy can be used to control both feature size and composition, and the combination of the two can yield distinct structures. For example, a BPL pen array has been used to directly write polymer features containing carbohydrate precursors.[104] The pen array was subsequently used to illuminate each individual feature for a prescribed amount of time, thereby controlling the degree of carbohydrate polymerization. Thus, two independent variables were controlled: (1) the area of the pattern (dictated by material transfer) and (2) the chain length of the carbohydrate (dictated by illumination).[104] Since then, several other synergistic approaches have been explored, including sequentially printing chemicals and modifying them with light,[88] or modulating the ink itself through photopolymerization or photoisomerization reactions to change patterning dynamics *in situ*.[105] Because of the multiple degrees of freedom, and the ease of multiplexing the delivery of light, these approaches exhibit considerable promise for realizing multicomponent libraries for nanocombinatorics.

NANOCOMBINATORIAL STUDIES WITH CF-SPL

Based on these synthetic capabilities, reports of nanocombinatoric experiments performed using CF-SPL have begun to emerge. The first example was a study of the differentiation of mesenchymal stem cells in response to their local extracellular environment. Specifically, by utilizing a tilted-PPL patterning experiment to generate a gradient of features composed of extracellular matrix (ECM) proteins, it was possible to ascertain the ECM protein density that allowed cells to adhere and differentiate down osteogenic pathways (Figure 4A).[7] Cell biology experiments such as this are an ideal use of CF-SPL due to the connection between the length scales of the experiments: cells tens of microns in size are commensurate with typical pen-to-pen pitches, whereas the focal adhesions are tens to hundreds of nanometers, which is the scale of individual features written by CF-SPL.[7] It was also recently shown that multicomponent patterns composed of up to three independently patterned biomolecules can be used to interact with cells, as was shown in experiments exploring the interactions between MCF7 cells and combinatorial sets of patterns of proteins.[71] Interestingly, multicomponent patterning was achieved in this case by using different oligonucleotide strands that were subsequently used to orthogonally immobilize different peptides.[71] Such complex multicomponent patterns of biomolecules can also be used to capture rare circulating tumor cells.[106] In another promising example of combinatorial studies of cell-surface interactions enabled by CF-SPL, arrays of allergens were patterned using PPL and used to study the activation of mast cells (Figure 4B).[107] Finally, by combining PPL with self-assembled monolayer laser desorption−ionization (TCAL-SAMDI) mass spectrometry, it is

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possible to quantify the enzymatic activity of individual cells in massively parallel arrays, in a post-cell-lysing format.[108]

A second major example of nanocombinatoric studies relying on CF-SPL is the study of nanoparticle-based heterogeneous catalysis. Together with SPBCL, CF-SPL represents a way of reliably patterning single-nanoparticle features with controllable sizes and compositions in desired locations. Indeed, it was recently shown that, in addition to two-component alloys,[110] as many as five-component alloys can be robustly synthesized with predictable composition and structure (Figure 4C).[109] Because synthesizing these structures is difficult through other means, this methodology allows one to synthesize and study particles from a vast size and compositional parameter space to identify structures that are worth investigating at a larger scale. As the first example of this, the catalytic reduction of 4-nitrophenol using CF-SPL-patterned nanoparticles revealed that AuPd alloy nanoparticles have enhanced catalytic activity compared to their monometallic counterparts.[110] In the field of electrochemistry, the combination of CF-SPL-based experiment and computational predictions prompted the discovery of AuCuPt nanoparticles as high performing catalysts for the hydrogen evolution reaction.[111] Recently, CF-SPL was used to generate combinatorial megalibraries that were compatible with high-throughput automated experimentation[112] to allow nanoparticles chosen from the AuCu parameter space to be rapidly screened for the growth of single-walled carbon nanotubes.[110] Importantly, these experiments led to the discovery of Au_3Cu_1 as a previously unidentified optimal composition (Figure 4D). These examples have set the stage for CF-SPL to provide a rapid means of determining the structure−property relationships that govern nanoparticle heterogeneous catalysts in a nanocombinatorial setting.

These two nanocombinatorial studies serve as examples that CF-SPL can be used to realize arrays of nanoscale structures with sufficient variety and complexity to enable diverse experiments and that there is knowledge to be gained by performing these types of experiments in diverse fields ranging from biology to catalysis. In the coming years, it is expected that many more examples will come to light. This trend will become increasingly apparent as advanced materials and devices are realized through nontraditional patterning approaches. For instance, electron beam lithography was used to pattern DNA molecules which templated the assembly of optical metasurfaces.[113] CF-SPL is ideally suited for these type of patterning tasks where soft or bioactive materials are patterned with spatial control over their composition.

CONCLUSIONS AND OUTLOOK

Collectively, the studies described above represent not only the power of CF-SPL as an enabling synthetic tool for nanocombinatoric studies but also the potential of CF-SPL as a discovery tool. However, synthesis is only half of the nanocombinatorics challenge—screening the products rapidly becomes a rate-limiting step as the scale of the experiments continues to increase. Given the success of CF-SPL for nanocombinatorial synthesis of millions of discrete features, the need in nanocombinatorics shifts from improving CF-SPL resolution and throughput to the development of methods that can analyze these patterned systems at a rate comparable to their production. With the development of this versatile platform, it is possible to expand the materials genome by utilizing the nanoscale in a way that was not previously possible.

In closing, CF-SPL brings capabilities to the table in terms of throughput, materials versatility, and pattern complexity, all in a low cost—and therefore widely adoptable—format. The ability to positionally encode nanoscale domains of soft and hard materials across centimeters has ramifications that span chemistry, biotechnology, and materials science; thus, CF-SPL may be regarded as an "experimental sandbox" that enables a diverse class of new experiments.

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Notes

The authors declare the following competing financial interest(s): We have financial interests in TERA-print, LLC which could potentially benefit from the outcomes of this research.

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VOCABULARY

nanocombinatorics, the study of materials *via* massive parallelization of experimental conditions at the nanoscopic scale where distinct properties emerge; **scanning probe lithography**, a patterning technique that utilizes an array or probe connected to a piezo system to deposit nanomaterials in a user-controlled manner onto a substrate; **cantilever-free lithography**, a collection of lithography techniques that have nanoscale resolution across the macroscale; **soft materials**, materials such as proteins, polymers, and DNA which are traditionally hard or impossible to pattern by other techniques; **multiplex**, the parallelization of components often resulting in increased capabilities and throughput

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