[Frontiers in Bioscience S1, 406-419, June 1, 2009]

Fabrication of biological arrays by unconventional lithographic methods

Sun Min Kim[1], Kahp Yang Suh [2]

[1] Department of Mechanical Engineering, Inha University, 253 Young Hyun-dong, Nam-gu, Incheon, 402-751, Republic of Korea, [2] School of Mechanical and Aerospace engineering and the Institute of Advanced Machinery and Design, Seoul National University, San 56-1, Shinlim-dong, Kwanak-gu, Seoul, 151-742, Republic of Korea

TABLE OF CONTENTS

1. ABSTRACT

Biological arrays with dimensions less than 100 nm are emerging as a new tool for biological research in the fields of genomics, proteomics, cell analysis, and tissue engineering. In this review, unconventional lithographic methods for the fabrication of biological arrays are summarized and compared in terms of spot size, density, resolution and ease in fabrication. The methods are classified into three categories: a) direct transfer of biological species on the substrate, b) selective deposition on the patterned surface using an adhesion-controlled template material, and c) stencil or membrane-based deposition on specific regions of a substrate. This review would be useful to related researchers in bioscience and bioengineering.

2. INTRODUCTION

Biological arrays with biomolecules (e.g., DNA and proteins), cells, or tissues on solid substrates provide valuable information for biological research in the fields of genomics, proteomics, cell analysis, and tissue engineering (1). DNA arrays are used for measuring gene expression levels and genotyping or detecting subtle variation in gene sequences, which are useful for disease diagnosis, evaluation, and drug development (2-5). Protein arrays enable screening protein-protein or protein-ligand (e.g., DNA, lipid, and drugs) interactions, which can be utilized for evaluating/diagnosing disease susceptibility, and for finding potential therapeutic targets (6-12). Cell arrays are useful tools for high-throughput cellular investigation in drug screening, toxicology testing, and functional genomic

studies (13-16). In addition, tissue arrays of embedded biopsies of donor block are used for phenotypic analyses to examine the stage and progression of the disease by monitoring the expression of molecular markers in these arrays (17-19).

Key issues in biological array systems include uniformity, high resolution of patterned arrays, and high-throughput analysis with these arrays. Miniaturization of biological arrays can enhance the sensitivity of biomolecular analysis and achieve single molecule detection and rapid molecular analysis by reducing the spot size (20). For example, if one can reduce the diameter of the spots in an array to the order of 10^3, then a 10^6- fold gain in sensitivity is possible for reading the array with single molecule sensitivity (21). The array of molecular size spots is also required for single molecule detection and rapid, high-throughput molecular analysis because biological molecules are in the range of 1-100 nm (e.g. albumin is in the range of 1-10 nm and most gene and DNA are in the range of 1-100 nm) (22).

A variety of fabrication techniques have been developed to achieve tiny size of biomolecular spots as small as single molecule in array systems. Fabrication techniques for miniaturization used in biological research have emerged with developments in the electronics industry, evolving into a smaller, faster, and cheaper fabrication technology with respect to semiconductor market demand (23). As a result, biological arrays containing features with dimensions of less than 100 nm, more similar to the dimensions of single biomolecule, are now emerging in biological applications. The motivation for these improvements is to increase the density of arrays, lower their cost, and increase their performance per array system (24).

Fabrication techniques for biomolecular arrays are typically categorized into top-down and bottom-up approaches (24). The top-down approach creates micro- or nanoscale structures from a bulk starting material, while the bottom-up approach utilizes the interactions of molecules and colloidal particles to self-assemble into structures of interest (25). The conventional fabrication techniques are based on various light sources in the top-down approach. These top-down approaches include photolithography (26-28), electron beam lithography (29-34) and focused ion beam lithography (35-37). These conventional techniques are commercially available and widely implemented in manufacturing, but they are often limited by their high cost, difficult multiple-step processes, and poor accessibility to the clean room facilities necessary for processing. Conventional techniques are also restricted to planar fabrication of semiconductor materials and expose the substrates to corrosive chemicals, high-energy radiation, and high temperature (24). These limitations motivate the development of new, unconventional methods which appear to be more flexible for nanofabrication, such as nano-imprint lithography (38-46), soft lithography (47-54), scanning probe nanolithography (including dip-pen nanolithography) (55-64). Unconventional fabrication techniques are in research stage and tools for nano-pattern molding are just entering commercial production. They are favorably applicable to biological materials and to sensitive organic material without deterioration. These novel unconventional methods have the potential to be future low-cost and simple process for nanoscale pattern formation and replication.

Although there are several reviews for biological arrays in micro-scale (65-67), we focus on the unconventional fabrication of nanoscale biological arrays (< 100 nm) in this review. It is difficult to summarize all of the fabrication techniques being developed at present because the field is continually changing and the boundaries constantly being challenged and modified. However, the objectives of the fabrication techniques are the same: patterning of uniform, dense arrays of tiny spots of biomolecules with low costs.

In this review the state-of-the-art fabrication methods for biological arrays, especially unconventional approaches, are outlined to provide the reader with an overview of current fabrication techniques. Each method can be categorized into one of three categories; a) direct patterning: biological species are directly transferred and patterned on the substrate, b) selective deposition on patterned surface: biological species selectively adhered to the patterned surfaces, and c) stencil-based patterning: patterned stencils can be used to pattern biological species to specific region of a substrate (*See* Table 1 and Figure 1). However, it should be noted that in many instances, more than one fabrication process can be implemented to achieve the final production of a device.

3. METHODS FOR FABRICATING BIOLOGICAL ARRAYS

3.1. Direct transfer of biological species

Direct patterning methods are used to form micro- or nanoarrays by means of the direct deposition of biological molecules on the substrate. This approach includes dip-pen nanolithography (DPN) based on AFM (atomic force microscopy) (60-64, 68-73), direct contact printing (stamping) based on soft lithography (12, 74-76), and droplet dispensing technique based on inkjet printing (77-80).

3.1.1. Dip-pen nanolithography (DPN)

DPN employs an AFM tip to print spots at the nanoscale. AFM offers ultra-high resolution and *in situ* imaging capability which can be applied for nanofabrication (64). AFM has two modes of operation: scanning and tapping modes. The ink-coated AFM tip transfers proteins or other biomolecules to the substrate with high resolution (sub-50-nm) using the tapping mode. The solution with biological molecules flows from an AFM tip to a substrate by capillary action when the AFM tip is brought into contact with the substrate (1). Figure 2 shows an example of protein patterning using DPN. An AFM tip transfers biomolecules that bind with target proteins to the substrate creating nano-patterns. Then, the rest of substrate is covered with the bio-blocking molecules which do not bind with biomolecules. Subsequently, the substrate is

Table 1. Comparison of unconventional nanofabrication methods for biological arrays

Category	Technique	Resolution	Applications	Advantages	Disadvantages
Direct patterning	Dip-pen nanolithography	~50 nm	Patterning organic molecules, polymers, DNA, proteins, peptides, and colloidal particles	- Deposit multiple compounds with high precision. - No damage from severe conditions (UV, ion or electron-beam irradiation, non-polar solvents) - Various choice of patterning biomolecules	- Slow patterning speed - Patterning resolution depends on the environmental conditions (ambient humidity and temperature)
	Direct contact printing	~100 nm	Patterning solvents, metals, polymers, DNA, proteins and cells	- Easy replication of stamp - Fast printing by parallelization - Low-cost batch production - Minimize the problems of sample carry-over and cross contamination	- Increase in the pattern size due to the swelling of stamp - Distorted patterns due to the deformation of stamp
	Droplet dispensing	~100 nm	- Patterning proteins & DNA - Stand-alone liquid dispenser - Tool for liquid handling robots	- Inexpensive robotic system for positioning printing heads - Non-contact printing - Reproducibility - Scalability of sample volume	- Contamination by undesirable satellite droplets - Difficult to clean printing nozzles to exchange the biological solutions - High shear rate
Selective deposition on patterned surfaces	Nanoimprint Lithography	~10 nm	- Patterning proteins & DNA - Patterning substrates coated with polymers	- Mass production over a large area with low-cost, high throughput, and high reproducibility - Simplicity, high density and resolution	- Breakage of the substrate due to very high pressure - Bubble-like defects
	Capillary Force Lithography	~100 nm	Patterning of polymeric materials, proteins and cells	- Patterning a broad range of materials - Simplicity, low cost	- Non-uniform wettability over large areas
	Indirect contact printing	~100 nm	- Patterning SAM - Patterning substrates with selective adhesive molecules	Same with direct contact printing	Same with direct contact printing
Stencil-based patterning	PDMS stencil	~ 50 μm	Patterning chemically and thermally sensitive compounds, including cells, proteins, wax, and sol.	- Convenient way for patterning cells on surfaces - A broad range of substrates can be used - Replicated many times from the same master mold	Problems in large-area application by membrane detachment
	Parylene membranes	~30 μm	Patterning chemically and thermally sensitive compounds, including cells, proteins, wax, and sol.	- Easily removed or attached to a surface due to its mechanical robustness (compared to PDMS) - Small size, high performance, low cost, and low power	Many steps and special equipments (compared to those for PDMS membrane)

exposed to the biomolecule solutions and target proteins specifically adhere to the patterned biomolecules. The resolution of DPN depends on a variety of parameters including tip size, writing speed, ambient humidity, substrate roughness, and nature of the sample solution (21). DPN can deposit multiple compounds, sequentially, precisely, and exclusively on the substrate. The patterning substrates for DPN do not need to be exposed to severe conditions such as UV, ion or electron-beam irradiation, or non-polar solvents so that cross-contamination or damage to the substrate can be avoided.

DPN has been developed to pattern a variety of inks including organic molecules, polymers, DNA, proteins, peptides, and colloidal particles. Also, substrates have been extended to many insulating, semiconducting, and metallic substrates (64). These capabilities of DPN enable a variety of biomolecules to be patterned in the form of nanoarrays on the substrates. Lee *et al.* fabricated nanoarrays of mecaptohexadecanoic acid (MHA) dots for anti-p24 antibody immobilization to screen for the human immunodeficiency HIV-1 virus p24 antigen in serum samples. This nanoarray-based immunoassay system can overcome the detection limit of conventional ELISA (enzyme-linked immunosorbent assays) by several orders of magnitude (81). Kang *et al.* directly constructed integrin $\alpha_v\beta_3$ nanoarrays using DPN and studied the interaction between integrin and vitronectin cell adhesion protein (82). This study demonstrated that the patterned nanoarrays of integrin proteins retain their biological selectivity after surface immobilization. DPN was also used to pattern viruses which are in the range of 20-200nm length scale (83). This work showed the capability of DPN which can position individual biological structures on the substrate to investigate biorecognition and virus-cell infectivity processes. DPN also can fabricate nanoarrays of extracellular–matrix components which can mimic the environment encountered by cells. These patterning approaches can be a useful tool for the study of cell-cell adhesion and the mechanism of cell migration (64).

DPN can pattern nano-scale structures on the substrate but the speed of printing is slow because DPN is a serial printing method. Slow printing reduces the efficiency of patterning and limits the functionality because the small volume of samples tends to dry out quickly. Recently,

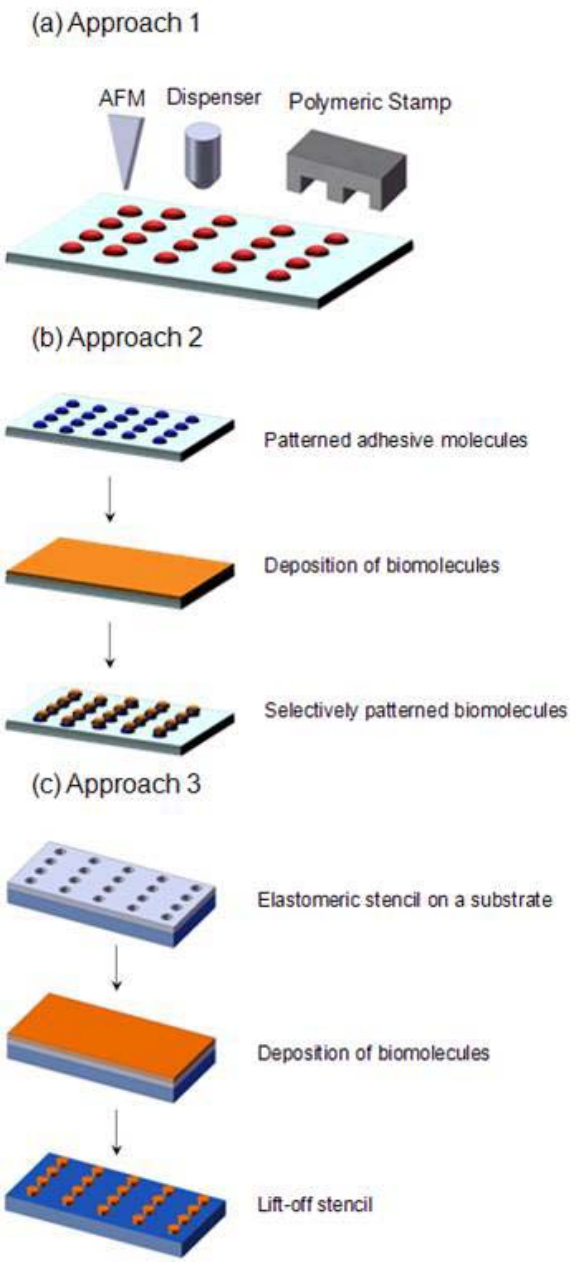

Figure 1. Classification of fabrication methods for biomolecular arrays. (a) Direct transfer of biomolecules (using AFM tip, dispenser, and polymeric stamp), (b) selective deposition of biological species on patterned surfaces, and (c) stencil based patterning with lift-off.

multi-tip DPN techniques have been developed for parallel printing to improve the printing speed and printing different types of biological molecules simultaneously (84, 85).

3.1.2. Direct contact printing

Contact printing is a direct patterning method using an elastomeric stamp fabricated by soft lithography (75, 76). Soft lithography was first introduced by G.M. Whitesides and co-workers (47, 50) and includes a family of techniques involving a soft polymeric mold such as polydimethylsiloxane (PDMS) replicated from an original hard master. The mold masters are typically fabricated by photolithography to define a pattern of the stamp. Stamps are made by curing a prepolymer of PDMS on the mold master. After curing, PDMS stamps are soaked in the molecular "ink" and brought into the conformal contact with a substrate to transfer the ink to the substrate surface (Figure 3).

Contact printing was initially developed for patterning self-assembled alkylthiol monolayers on gold surfaces (86) in micro-scale but its flexibility makes it possible to pattern various molecules including solvents, metals, polymers, DNA, proteins and cells in a much higher resolution (76). The resolution of the contact printing is dictated by the resolution of the fabrication process of the mold masters. To achieve nanoscale resolution, mold masters for nanocontact printing are prepared by electron beam lithography (EBL), scanning probe lithography (SPL) or LIGA process (20). Renault *et al.* used EBL to fabricate a polymer stamp for patterning single or several protein molecules (12). They achieved protein nanoarrays with a resolution less than 100 nm using nanocontact printing (nCP) technique. The capability of patterning single protein can improve the study of molecular biophysics and individual protein-protein interactions (76). Contact printing was also used to pattern extracellular matrix (ECM) proteins to study cell behavior. Lehnert *et al.* fabricated PDMS stamps with nanoarrays (~300nm×~300nm squares) using silicon mold master prepared by EBL (87). They showed that the extent of cell spreading directly depends on the total substratum coverage with ECM-proteins, rather than on the geometrical pattern.

Contact printing enables easy replication of stamp, fast printing by parallelization, and low-cost batch production. The polymer stamps also minimize the problems of sample carry-over and cross contamination. However, contact printing has some limitations which are mainly caused by the use of soft polymer stamp (75). The swelling of stamp during inking often results in an increase in the pattern size by diffusion of the excessive printed molecules on the substrate. The deformation of stamp also poses a challenge that results in distorted patterns (88). The nature of elastomeric stamps shows pairing, buckling, or roof sagging during contact with the surfaces. Several techniques have been developed to reduce or overcome these problems. Submicron thick stamp on a rigid support can eliminate sagging problem (89) and the stamps formed from a stiff layer of hard PDMS and a flexible layer of conventional PDMS can reduce the deformation problem and achieve high resolution patterns (49, 51). The development of cost effective master fabrication techniques and reducing stamp deformation and related ink-transfer problems will allow the contact printing to become more applicable to biological fields in the future.

3.1.3. Droplet dispensing

Droplet dispensing is an alternative method which directly transfers biomolecules on the substrate. Several droplet dispensing approaches have been developed

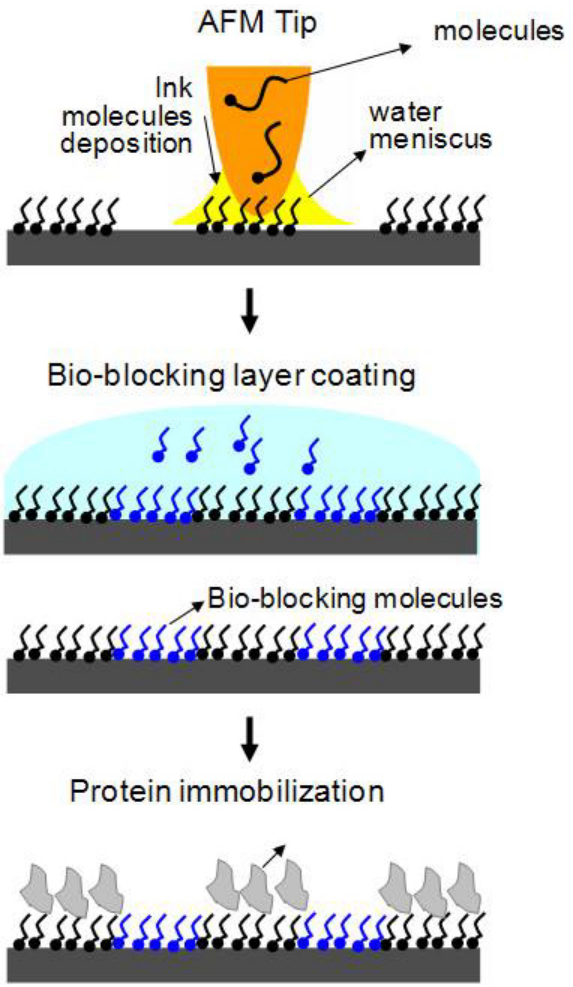

Figure 2. Schematic drawing of dip-pen nanolithography (DPN) for biological nanoarrays.

but of these the technique based on inkjet printing technology is most popular due to low cost and rapid implementation (90). Commercially available inkjet printers are modified to dispense biomolecule solutions instead of inks (1). The advantages of using commercially available inkjet printers for biological array fabrication are: i) inexpensive robotic system for positioning printing heads; ii) non-contact printing for minimizing the possibilities of sample contamination; iii) small sample delivery (8-95 pL per droplet); and reproducibility and scalability of sample volume (90-92).

The two main types of inkjet printers available in the market today are bubble-jet (or thermal inkjet) printer and piezoelectric printer. Bubble-jet printer uses resistive heater to evaporate a small volume which drives the dispensing of an ink droplet through a nozzle. The printing head assembled in the ink cartridge is composed of a nozzle plate with several ink ejection orifices connected to resistive heaters and contact with an ink reservoir. Droplet sizes depend on the temperature gradient applied, frequency, and ink viscosity (92). In the bubble-jet printing system, the local temperature gradient produced by resistive heaters can reach 200^{o}C and this high temperature may induce denaturization of biological materials (90). Allain *et al.* fabricated microarrays containing PCR-amplified genomic DNA extracts from mice tumors on a Zetaprobe membrane using a modified bubble-jet printer (91). This system enables the rapid and reliable deposition of DNA onto the membrane and then these membranes were used in a hybridization assay with fluorescent probes for detection in a biochip. Okamoto *et al.* have fabricated DNA microarrays using bubble-printer to eject 5'-teminal-thiolated oligonucleotides to a glass surface (90). It was noted that the denaturation of biological molecules would come into play by the heat transfer associated with bubble generation and demonstrated ejecting DNA solutions ranging from 10 to 300 bases in strand length, and concentrations ranging from 0.02 to 1.6 mg/mL without any damage.

Piezoelectric printer uses piezoelectric actuators to dispense droplets. Ink droplets are formed by the mechanical deformation of capillary nozzles using a ceramic piezoelectric layer. There is no temperature increase in the droplets dispensing process with piezoelectric actuators, and hence thermal denaturation of biological molecules is not a concern. Blanchard *et al.* produced the high-density arrays of oligonucleotides (93). Piezoelectric printer heads dispense small drop of reagents to a chemically modified silicon oxide surface, where they react to synthesize DNA. Budach *et al.* also used piezoelectric printer mechanism to deliver oligonucleotide capture probes to binding membranes for hybridization assays (94).

Inkjet printing is an attractive method for fabrication of biological arrays due to low cost and reliable drop dispensing but this technique has some drawbacks: i) inkjet has a tendency to produce undesirable satellite droplets which possibly contaminate surrounding spots, ii) it is difficult to clean printing nozzles to exchange the biological solutions. This problem is more important in piezoelectric printing because the nozzles are separated from the ink reservoirs and all connecting channels must be cleaned, and iii) droplets experience high shear rate while passing through the nozzle and smashing with substrate surface. There is a risk of denaturing biomolecules in the solution under this high shear rate (1). Several techniques have been suggested to solve these problems. For example, Tseng *et al.* introduced an innovative bubble-jet printer nozzle that has an extra thermal resistive heater to eliminate the satellite droplets by forming secondary vapor bubble, which pinches off or trims the exiting droplet at the nozzle (95).

3.2. Selective deposition of biological species on patterned surfaces

Selective deposition methods use different adhesiveness of biological molecules on the patterned surface by using variations of surface properties such as surface charge, chemistry, hydrophilicity, and topology (14, 96-98). Based on these differences it is possible to selectively localize biomolecules to pre-defined regions on a substrate. This approach includes nanoimprint

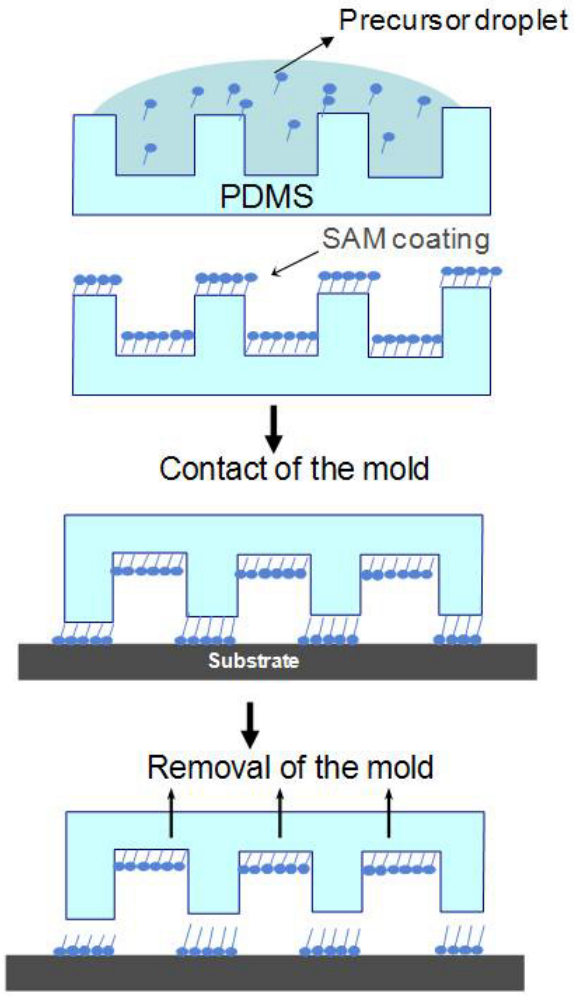

Figure 3. Schematic illustration of contact printing.

lithography (NIL) (38-46), capillary force lithography (CFL) (99-107), and indirect contact printing (47-53).

3.2.1. Nanoimprint Lithography (NIL)

NIL is a forming process in which a master with nanoscale features is pressed into a polymer substrate resulting in a relief replica of the master on the substrate (38). The basic principle of NIL is that a polymer substrate such as polystyrene is first heated above its softening temperature and then a mold (or master) is physically pressed against the polymer substrate, rather than by modifying the resist's chemical structure by irradiation. After an enough contact time between the mold and the substrate, the system is cooled down below the softening temperature, followed by de-molding from the substrate (20). The advantage of NIL is mass production of polymeric nanostructures as small as 10 nm over a large area with low-cost, high throughput, and high reproducibility (39). NIL can imprint fine structure directly in polymer materials and generate patterned features using polymers with certain desirable physical and chemical functionalities such as polycarbonate (PC), polystyrene (PS) and polymethyl methacrylate (PMMA). (40)

NIL has been used in nanoscale protein patterning (41, 42) due to its simplicity. Hoff *et al.* presented a flexible technique for selective patterning of bioactive proteins with nanoscale resolution (sub-100nm features) using surface passivation with a fluoropolymer and then studied the specificity of the biotin/streptavidin linkage (41). After the nanopatterning of the substrate using NIL, the patterned substrate was modified sequentially with an aminosilane, biotin, streptavidin, and finally biotinylated target protein to immobilize proteins (Figure 4). It was reported that the biomolecules were attached to the desired regions ("patterned regions") with a high density, not being attached to the other regions ("unpatterned regions"). They further demonstrated that the functionality of patterned antibodies was retained by patterning the target protein and observing its binding using fluorescence detection. Falconnet *et al.* also showed patterning of proteins with nanoscale resolution using NIL (42). They produced streptavidin patterns with feature sizes in the order of 100 nm and the streptavidin patterns were used as a platform for subsequent immobilization of biotin-tagged proteins or vesicles at controlled surface densities. This process combines NIL and molecular assembly patterning by lift-off (MAPL). Briefly, PMMA polymer layer is spin-coated on the niobium oxide (Nb_2O_5) sputter-coated silicon wafer or Pyrex plate. The PMMA layer was then imprinted using a mold followed by a dry etching step, converting the substrate into a PMMA/Nb_2O_5 contrast. A biotin functionalized copolymer, poly (L-lysine)-graft-poly (ethylene glycol)-biotin (PLL-g-PEG/PEG-biotin), spontaneously adsorbs on the oxide surfaces. After PMMA lift-off, the background was filled with protein-resistant PLL-g-PEG. Finally, streptavidin selectively adsorbed on the biotin areas and thus can be used as a universal platform for immobilization of biotin-tagged molecules.

Since NIL allows for features as small as 10nm (39), the placement of individual biomolecules would be possible. NIL also enables the patterning of multiple proteins on a single substrate because of high contrast and resolution (protein densities more than an order of magnitude higher than those currently available). These characteristics have the potential to improve sensitivity, reduce required analyte volumes, and increase the number of proteins that can be screened against on a single chip (41).

3.2.2. Capillary Force Lithography (CFL)

A new lithographic technique called capillary force lithography (CFL) has been developed by simply combining NIL and soft lithography to overcome several shortcomings of NIL (99, 100). NIL requires a very high pressure, typically on the order of 10^9 Pascal (N/m^2), which could cause breakage of the substrate. A recent study reported that material transport limits the performance of NIL in general, especially when the mold is negative or has recessed features within a large elevated surface level. The pattern transfer turned out to be unsatisfactory and bubble-like defects were observed, possibly due to lack of conformal contact (108)

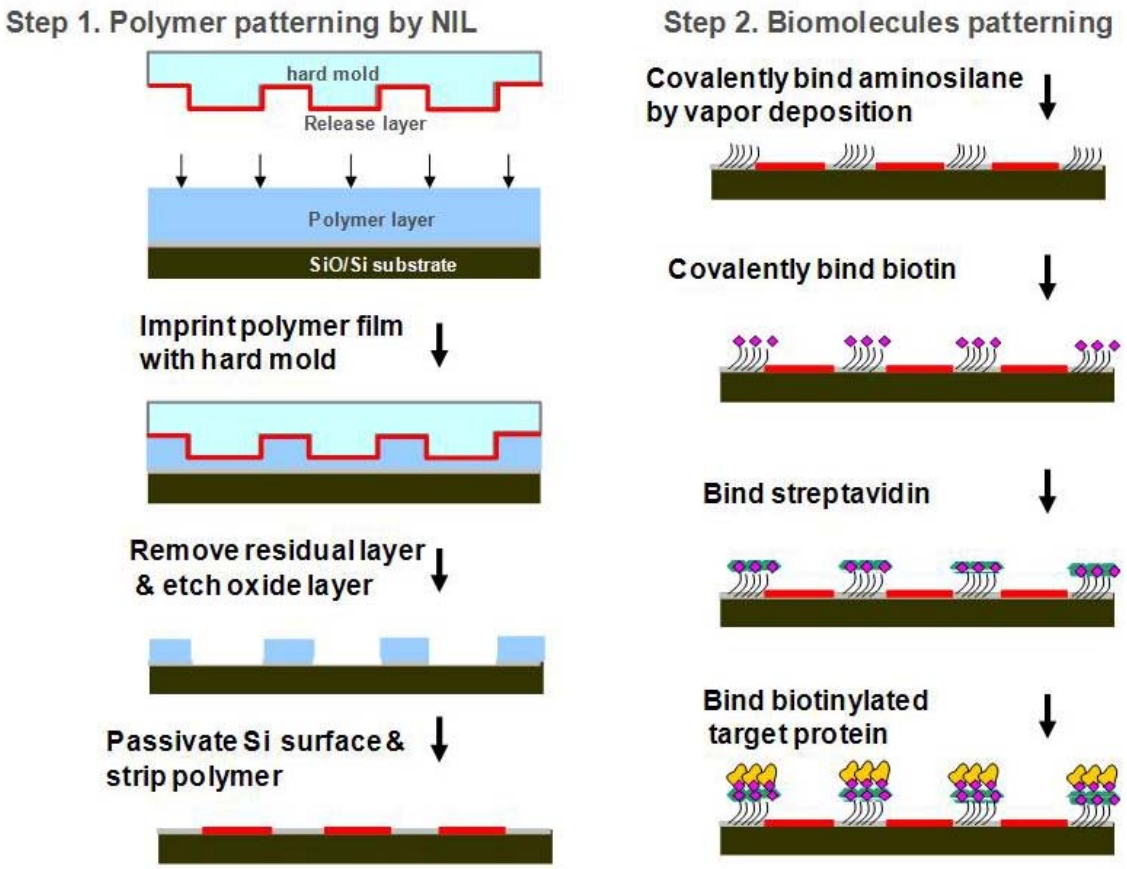

Figure 4. Schematic illustration of nanoimprint lithography for protein patterning.

CFL is based on capillary rise of polymer melt into void spaces engraved in a patterned mold (99). The fabrication procedure is similar to NIL except that a hard mold is replaced by a soft mold (typically PDMS mold) and thus no pressure is applied. When a patterned (positive or negative) mold is placed on a polymer surface and heated above the polymer's glass transition temperature (T_g), the polymer melt rises into the void space of the channels formed between the mold and the polymer by capillary force, thereby generating a negative replica of the mold (Figure 5). The pattern formation is also possible with a solvent-laden polymer or a UV-curable resin followed by solvent evaporation or exposure to UV light (103-106). In addition, a UV curable mold made from polyurethane functionalized with acrylate groups has been introduced to replace PDMS mold for sub-100nm lithography, thus expanding the use of CFL to studies of cell biology (103).

Suh *et al.* demonstrated the fabrication of poly(ethylene glycol) (PEG) microstructures for protein patterning using CFL (101). A uniform PEG film spin coated onto silicon or glass substrate was molded with a patterned PDMS stamp by means of capillary force. The patterned PEG structures act as a physical and biological barrier for the adhesion of proteins and cells. The patterned substrates consist of two regions: the molded PEG surface that acts as a resistant layer and the exposed substrate surface that promotes protein and cell adsorption. As seen in Figure 5, fibronectin molecules which mediate cell adsorption only adhere to the non-PEG region and fibroblast cells in the sample solution selectively adhere to the patterned fibronectin molecules. A notable finding was that the substrate surface can be directly exposed during the molding process due to the ability to control the wetting properties of the polymer on the stamp, which is a key factor to patterning proteins and cells. Khademhosseini *et al.* also developed a method for patterning cells using layer-by-layer deposition of ionic biomolecules (102, 107). Hyaluronic acid (HA), which is a biocompatible and biodegradable material (109, 110), were patterned on glass slides by CFL, followed by fibronectin adsorption onto the non-HA patterned region because HA is repellent to protein adsorption. Then, cells were seeded and only attached to the fibronectin coated region. Subsequent ionic adsorption of poly-L-lysine (PLL) to HA pattern was used to change HA surfaces from cell repulsive to cell adhesive. Finally, the different type cells were seeded and attached to the PLL pattern. Co-culturing of ES cells with fibroblasts and hepatocytes with fibroblasts were successfully performed by utilizing this method (102). Both HA and PLL are commercially available and do not require any chemistry or complex techniques for immobilization.

CFL provides a general platform for patterning a broad range of materials since it can be applied to substrates such as glass, silicon, silicon dioxide and

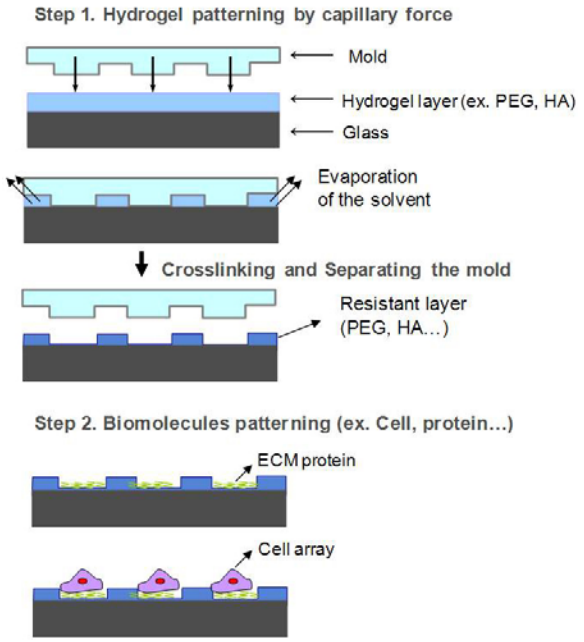

Figure 5. Schematic of protein or cell arrays using capillary force lithography (CFL).

polymers. As a result, it could be a valuable tool for protein chips and high-throughput cell screening devices involving nanoarrays of various biomolecules (~500nm to ~500 μm) on a large area.

3.2.3. Indirect contact printing

It has been discussed earlier in section 0 that the micro-nano contact printing enables direct patterning of proteins and DNA on a substrate. However, contact printing also enables indirect pattering of biological molecules by patterning the surface with SAMs (self-assembled monolayers) which extensively control the adsorption of proteins and cells on the substrate (14, 111, 112). Lopez *et al.* presented a method for controlling both the concentration and spatial distribution of proteins adsorbed onto patterned SAMs (111). Patterned SAMs were formed by the serial chemisorptions of two or more functionalized alkanethiols on gold substrate using contact printing. The spatially-defined areas that resist protein adsorption were formed from oligo(ethylene glycol)-terminated thiols and other areas that allow protein adsorption were formed from thiols terminated by nonpolar and ionic groups. Contact printing also has been used to control the interaction between cells and substrates. Singhvi *et al.* patterned islands of SAMs that promote adsorption of cell-adhesive ECM proteins by using contact printing technique (14). These islands have the same length scale with individual cells; when cells were seeded onto the substrates they preferentially bound to the patterned islands and spread to conform to their size and shape.

Contact printing usually involves PDMS molds for micro-scale patterns but this mold has many limitations to high-resolution nanopatterning because of its low modulus. To solve this challenge, researchers have used a number of alternative molds, such as composite PDMS (49), bilayer PDMS (51), photocurable PDMS (45), and a photocurable polyurethane acrylate (PUA) (113). These stamps have been used to form sub-100nm patterns and further developed to obtain features as small as 30 nm (114).

3.3. Stencil-based patterning

Elastomeric stencil can be used to pattern biomolecules to specific region of a substrate in array patterns (115-119). Elastomeric stencils with microengineered holes typically fabricated by soft lithography can be reversibly sealed on a substrate to promote patterned deposition of biomolecules or cells directly on a substrate.

Ostuni *et al.* presented a PDMS stencil based *lift-off* approach to generate patterned cell-arrays (117). For fabrication of a stencil membrane, PDMS polymer was spin coated on a microstructured silicon wafer containing photoresist posts to generate a PDMS membrane which has circular or square holes less than 50μm. Subsequently, the cured membrane was brought into conformal contact with a substrate. Cell adhesion promoting molecules such as fibronectin or gelatin were absorbed to the patterned holes in the membranes and then membrane was removed from the substrate to generate a pattern of adhesion promoting molecules. After coating the rest of the substrate with cell repulsive molecules to inhibit cell adhesion, cells were only patterned on the adhesive regions (Figure 6).

This membrane-based patterning offers a more convenient way for patterning cells on surfaces and for studying cell spreading. PDMS stencils are applicable to a broad range of substrates which can absorb adhesion promoting proteins and can make conformal contact with PDMS membranes (117). PDMS membranes also can be replicated many times from the same master mold because replication procedure does not break the mold (118). However, the attachment of PDMS membranes over the substrates and the peeling off membranes after patterning can be a trouble in large-area applications (120).

Recently, microfabricated parylene membranes have been used as stencils for patterning various biological molecules (121-123). Pal *et al.* presented a photolithographically patterned parylene-SU-8 bilayer microstencil that can be used for micropatterning (121). The parylene layer enables mechanical peeling of the hybrid film from a hydrophilic substrate. SU-8 layer provides a mask for parylene etching as well as height to the microstencil for controlling the amount of material patterned. The amount of material patterned can be controlled using a variety of techniques, including spin coating and thin film deposition. The parylene stencils can have a conformal contact with silicon, glass, and polymer substrates, and then be used for patterning chemically and thermally sensitive compounds, including cells, proteins, wax, sol, and cyclized-perfluoropolymer (CYTOP).

Parylene membranes have several advantages over PDMS membranes. Parylene membranes can be easily

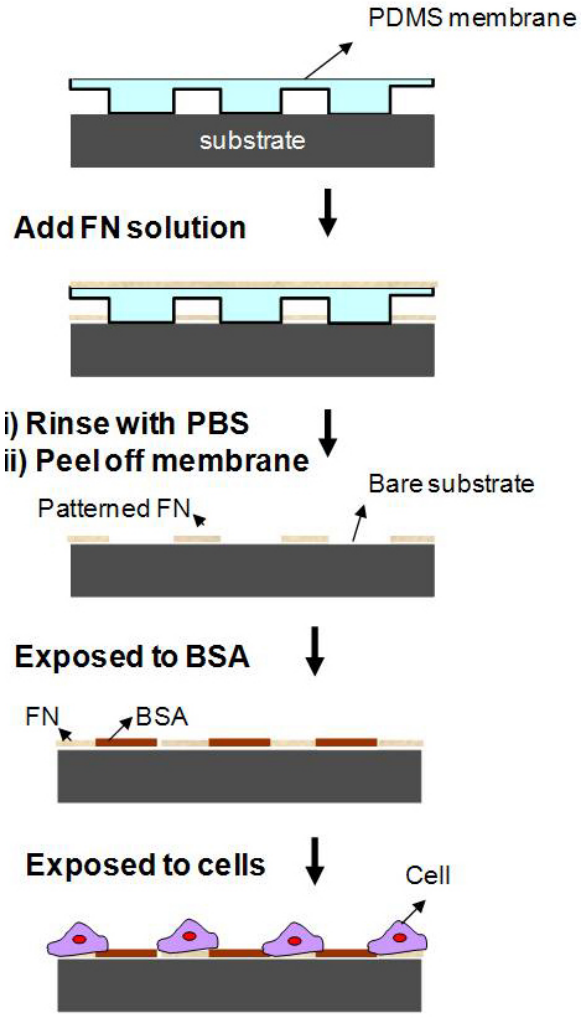

Figure 6. Schematic of micropatterning of proteins or cells using an elastomeric membrane stencil.

removed or attached to a surface without tearing due to its mechanical robustness compared to PDMS (124, 125) and can form a reversible binding with hydrophobic surfaces. However, fabricating a parylene membrane requires many steps and special equipments compared to those for PDMS membrane (123).

4. CONCLUSIONS

Biomolecule arrays provide valuable information and high-throughput analysis for biological research including genomics, proteomics, cell analysis, and tissue engineering. Uniformity, high resolution, and high-throughput analysis are key factors in biological array systems. A variety of fabrication techniques based on conventional microfabrication process were used for micro to nano array systems. However, the limitations of the conventional fabrication techniques including high cost, difficult multiple-step processes, and poor accessibility to the facilities motivate the development of new, unconventional methods which are easily accessible and economically viable for micro- to nanofabrication. It appears that the combination of conventional and unconventional methods can cover a broad range of length scale and can be the effective way for mass production of biological arrays in nanoscale. In recent years, various biocompatible materials have been studied in conjunction with unconventional nanopatterning methods to reduce the processing steps and contamination of patterned molecules. With increasing demands in biological research, biological array systems would continuously evolve into a more flexible platform integrated with smart, functional molecules.

5. ACKNOWLEDGEMENT

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund, KRF-2007-331-D00064) for Sun Min Kim, and the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea Government (MOST) (R01-2007-000-20675-0), the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-003-D00040), and the Grant-in-Aid for Strategy Technology Development Programs from the Korea Ministry of Knowledge Economy (No.10030046) for Kahp Yang Suh.

6. REFERENCES

1. Barbulovic-Nad, I., M. Lucente, Y. Sun, M. J. Zhang, A. R. Wheeler & M. Bussmann: Bio-microarray fabrication techniques - A review. *Critical Reviews in Biotechnology*, 26, 237-259 (2006)

2. Seeman, N. C.: DNA engineering and its application to nanotechnology. *Trends in Biotechnology*, 17, 437-443 (1999)

3. Jiang, X. H., W. Q. Liu, J. J. Chen & X. Q. Lin: Application of DNA nanotechnology. *Progress in Chemistry*, 19, 608-613 (2007)

4. Schena, M., D. Shalon, R. Heller, A. Chai, P. O. Brown & R. W. Davis: Parallel human genome analysis: Microarray-based expression monitoring of 1000 genes. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 10614-10619 (1996)

5. Shalon, D., S. J. Smith & P. O. Brown: A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Research*, 6, 639-645 (1996)

6. Martinsky, T.: Protein microarray manufacturing. *PharmaGenomics*, 2, 42-46 (2004)

7. Martinsky, T.: Enabling Tools for Protein Microarrays. In: Protein Microarrays. Ed: M. Schena. Jones and Bartlett Publishers, Inc., Sudbury, MA (2005)

8. Bernard, A., E. Delamarche, H. Schmid, B. Michel, H. R.

Bosshard & H. Biebuyck: Printing patterns of proteins. *Langmuir*, 14, 2225-2229 (1998)

9. Blawas, A. S. & W. M. Reichert: Protein patterning. *Biomaterials*, 19, 595-609 (1998)

10. Wadu-Mesthrige, K., S. Xu, N. A. Amro & G. Y. Liu: Fabrication and imaging of nanometer-sized protein patterns. *Langmuir*, 15, 8580-8583 (1999)

11. Moerman, R., J. Frank, J. C. M. Marijnissen, T. G. M. Schalkhammer & G. W. K. van Dedem: Miniaturized electrospraying as a technique for the production of microarrays of reproducible micrometer-sized protein spots. *Analytical Chemistry*, 73, 2183-2189 (2001)

12. Renaultt, J. P., A. Bernard, A. Bietsch, B. Michel, H. R. Bosshard, E. Delamarche, M. Kreiter, B. Hecht & U. P. Wild: Fabricating arrays of single protein molecules on glass using microcontact printing. *Journal of Physical Chemistry B*, 107, 703-711 (2003)

13. Flaim, C. J., S. Chien & S. N. Bhatia: An extracellular matrix microarray for probing cellular differentiation. *Nature Methods*, 2, 119-125 (2005)

14. Singhvi, R., A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. C. Wang, G. M. Whitesides & D. E. Ingber: Engineering Cell-Shape and Function. *Science*, 264, 696-698 (1994)

15. Wu, R. Z., S. N. Bailey & D. M. Sabatini: Cell-biological applications of transfected-cell microarrays. *Trends in Cell Biology*, 12, 485-488 (2002)

16. Kim, S. M., S. H. Lee & K. Y. Suh: Cell research with physically modified microfluidic channels: A review. *Lab on a Chip*, 8, 1015-1023 (2008)

17. Kononen, J., L. Bubendorf, A. Kallioniemi, M. Barlund, P. Schraml, S. Leighton, J. Torhorst, M. J. Mihatsch, G. Sauter & O. P. Kallioniemi: Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nature Medicine*, 4, 844-847 (1998)

18. Nocito, A., J. Kononen, O. P. Kallioniemi & G. Sauter: Tissue microarrays (TMAs) for high-throughput molecular pathology research. *International Journal of Cancer*, 94, 1-5 (2001)

19. Hasirci, V. & H. Kenar: Novel surface patterning approaches for tissue engineering and their effect on cell behavior. *Nanomedicine*, 1, 73-89 (2006)

20. Lee, L. J.: Polymer nanoengineering for biomedical applications. *Annals of Biomedical Engineering*, 34, 75-88 (2006)

21. Leggett, G. J.: Biological nanostructures: platforms for analytical chemistry at the sub-zeptomolar level. *Analyst*, 130, 259-264 (2005)

22. Langer, R.: Drug delivery and targeting. *Nature*, 392, 5-10 (1998)

23. Wood, M. A.: Colloidal lithography and current fabrication techniques producing in-plane nanotopography for biological applications. *Journal of the Royal Society Interface*, 4, 1-17 (2007)

24. Gates, B. D., Q. B. Xu, M. Stewart, D. Ryan, C. G. Willson & G. M. Whitesides: New approaches to nanofabrication: Molding, printing, and other techniques. *Chemical Reviews*, 105, 1171-1196 (2005)

25. Xie, X. N., H. J. Chung, C. H. Sow & A. T. S. Wee: Nanoscale materials patterning and engineering by atomic force microscopy nanolithography. *Materials Science & Engineering R-Reports*, 54, 1-48 (2006)

26. Goodberlet, J. G.: Patterning 100 nm features using deep-ultraviolet contact photolithography. *Applied Physics Letters*, 76, 667-669 (2000)

27. Klauk, H., D. J. Gundlach, M. Bonse, C. C. Kuo & T. N. Jackson: A reduced complexity process for organic thin film transistors. *Applied Physics Letters*, 76, 1692-1694 (2000)

28. Geissler, M. & Y. N. Xia: Patterning: Principles and some new developments. *Advanced Materials*, 16, 1249-1269 (2004)

29. Harnett, C. K., K. M. Satyalakshmi & H. G. Craighead: Bioactive templates fabricated by low-energy electron beam lithography of self-assembled monolayers. *Langmuir*, 17, 178-182 (2001)

30. Zhang, G. J., T. Tanii, T. Funatsu & I. Ohdomari: Patterning of DNA nanostructures on silicon surface by electron beam lithography of self-assembled monolayer. *Chemical Communications*786-787 (2004)

31. Chen, K. S., I. K. Lin & F. H. Ko: Fabrication of 3D polymer microstructures using electron beam lithography and nanoimprinting technologies. *Journal of Micromechanics and Microengineering*, 15, 1894-1903 (2005)

32. Zhang, G. J., T. Tanii, T. Zako, T. Hosaka, T. Miyake, Y. Kanari, T. W. Funatsu & I. Ohdomari: Nanoscale patterning of protein using electron beam lithography of organosilane self-assembled monolayers. *Small*, 1, 833-837 (2005)

33. Fujita, J., Y. Ohnishi, Y. Ochiai & S. Matsui: Ultrahigh resolution of calixarene negative resist in electron beam lithography. *Applied Physics Letters*, 68, 1297-1299 (1996)

34. Robinson, A. P. G., R. E. Palmer, T. Tada, T. Kanayama & J. A. Preece: A Fullerene derivative as an electron beam resist for nanolithography. *Applied Physics Letters*, 72, 1302-1304 (1998)

35. Bergman, A. A., J. Buijs, J. Herbig, D. T. Mathes, J. J. Demarest, C. D. Wilson, C. T. Reimann, R. A. Baragiola, R. Hu & S. O. Oscarsson: Nanometer-scale arrangement of human serum albumin by adsorption on defect arrays created with a finely focused ion beam. *Langmuir*, 14, 6785-6788 (1998)

36. Rennon, S., L. Bach, J. P. Reithmaier, A. Forchel, J. L. Gentner & L. Goldstein: High-frequency properties of 1.55 mu m laterally complex coupled distributed feedback lasers fabricated by focused-ion-beam lithography. *Applied Physics Letters*, 77, 325-327 (2000)

37. Bach, L., I. P. Reithmaier, A. Forchel, J. L. Gentner & L. Goldstein: Multiwavelength laterally complex coupled distributed feedback laser arrays with monolithically integrated combiner fabricated by focused-ion-beam lithography. *Applied Physics Letters*, 79, 2324-2326 (2001)

38. Chou, S. Y., P. R. Krauss & P. J. Renstrom: Imprint lithography with 25-nanometer resolution. *Science*, 272, 85-87 (1996)

39. Chou, S. Y., P. R. Krauss, W. Zhang, L. J. Guo & L. Zhuang: Sub-10 nm imprint lithography and applications. *Journal of Vacuum Science & Technology B*, 15, 2897-2904 (1997)

40. Bao, L. R., X. Cheng, X. D. Huang, L. J. Guo, S. W. Pang & A. F. Yee: Nanoimprinting over topography and multilayer three-dimensional printing. *Journal of Vacuum Science & Technology B*, 20, 2881-2886 (2002)

41. Hoff, J. D., L. J. Cheng, E. Meyhofer, L. J. Guo & A. J. Hunt: Nanoscale protein patterning by imprint lithography. *Nano Letters*, 4, 853-857 (2004)

42. Falconnet, D., D. Pasqui, S. Park, R. Eckert, H. Schift, J. Gobrecht, R. Barbucci & M. Textor: A novel approach to produce protein nanopatterns by combining nanoimprint lithography and molecular self-assembly. *Nano Letters*, 4, 1909-1914 (2004)

43. Park, S., S. Saxer, C. Padeste, H. H. Solak, J. Gobrecht & H. Schift: Chemical patterning of sub-50-nm half pitches via nanoimprint lithography. *Microelectronic Engineering*, 78-79, 682-688 (2005)

44. Truskett, V. N. & M. P. C. Watts: Trends in imprint lithography for biological applications. *Trends in Biotechnology*, 24, 312-317 (2006)

45. Lee, H., S. Hong, K. Yang & K. Choi: Fabrication of nano-sized resist patterns on flexible plastic film using thermal curing nano-imprint lithography. *Microelectronic Engineering*, 83, 323-327 (2006)

46. Park, I., J. Cheng, A. P. Pisano, E. S. Lee & J. H. Jeong: Low temperature, low pressure nanoimprinting of chitosan as a biomaterial for bionanotechnology applications. *Applied Physics Letters*, 90, (2007)

47. Zhao, X. M., Y. N. Xia & G. M. Whitesides: Soft lithographic methods for nano-fabrication. *Journal of Materials Chemistry*, 7, 1069-1074 (1997)

48. Kane, R. S., S. Takayama, E. Ostuni, D. E. Ingber & G. M. Whitesides: Patterning proteins and cells using soft lithography. *Biomaterials*, 20, 2363-2376 (1999)

49. Schmid, H. & B. Michel: Siloxane polymers for high-resolution, high-accuracy soft lithography. *Macromolecules*, 33, 3042-3049 (2000)

50. Whitesides, G. M., E. Ostuni, S. Takayama, X. Y. Jiang & D. E. Ingber: Soft lithography in biology and biochemistry. *Annual Review of Biomedical Engineering*, 3, 335-373 (2001)

51. Odom, T. W., J. C. Love, D. B. Wolfe, K. E. Paul & G. M. Whitesides: Improved pattern transfer in soft lithography using composite stamps. *Langmuir*, 18, 5314-5320 (2002)

52. Gates, B. D. & G. M. Whitesides: Replication of vertical features smaller than 2 nm by soft lithography. *Journal of the American Chemical Society*, 125, 14986-14987 (2003)

53. Hung, A. M. & S. I. Stupp: Simultaneous self-assembly, orientation, and patterning of peptide-amphiphile nanofibers by soft lithography. *Nano Letters*, 7, 1165-1171 (2007)

54. Kim, P., K. W. Kwon, M. C. Park, S. H. Lee, S. M. Kim & K. Y. Suh: Soft lithography for microfluidics: a review. *Biochip Journal*, 2, 1-11 (2008)

55. Jiang, F. Z., K. Khairy, K. Poole, J. Howard & D. J. Muller: Creating nanoscopic collagen matrices using atomic force microscopy. *Microscopy Research and Technique*, 64, 435-440 (2004)

56. Fernandez-Cuesta, I., X. Borrise & F. Perez-Murano: Atomic force microscopy local anodic oxidation of thin Si3N4 ayers for robust prototyping of nanostructures. *Journal of Vacuum Science & Technology B*, 24, 2988-2992 (2006)

57. Liu, Z. G., Z. Li, G. Wei, Y. H. Song, L. Wang & L. L. Sun: Manipulation, dissection, and lithography using modified tapping mode atomic force microscope. *Microscopy Research and Technique*, 69, 998-1004 (2006)

58. Quate, C. F.: Scanning probes as a lithography tool for nanostructures. *Surface Science*, 386, 259-264 (1997)

59. Snow, E. S. & P. M. Campbell: FABRICATION OF SI NANOSTRUCTURES WITH AN ATOMIC-FORCE MICROSCOPE. *Applied Physics Letters*, 64, 1932-1934 (1994)

60. Piner, R. D., J. Zhu, F. Xu, S. H. Hong & C. A. Mirkin: "Dip-pen" nanolithography. *Science*, 283, 661-663 (1999)

61. Demers, L. M., S. J. Park, T. A. Taton, Z. Li & C. A. Mirkin: Orthogonal assembly of nanoparticle building blocks on dip-pen nanolithographically generated templates of DNA. *Angewandte Chemie-International Edition*, 40, 3071-3073 (2001)

62. Lee, K. B., S. J. Park, C. A. Mirkin, J. C. Smith & M. Mrksich: Protein nanoarrays generated by dip-pen nanolithography. *Science*, 295, 1702-1705 (2002)

63. Lim, J. H., D. S. Ginger, K. B. Lee, J. Heo, J. M. Nam & C. A. Mirkin: Direct-write dip-pen nanolithography of proteins on modified silicon oxide surfaces. *Angewandte Chemie-International Edition*, 42, 2309-2312 (2003)

64. Salaita, K., Y. H. Wang & C. A. Mirkin: Applications of dip-pen nanolithography. *Nature Nanotechnology*, 2, 145-155 (2007)

65. Pirrung, M. C.: How to make a DNA chip. *Angewandte Chemie-International Edition*, 41, 1276-1289 (2002)

66. Choudhuri, S.: Microarrays in biology and medicine. *Journal of Biochemical and Molecular Toxicology*, 18, 171-179 (2004)

67. Heller, M. J.: DNA microarray technology: Devices, systems, and applications. *Annual Review of Biomedical Engineering*, 4, 129-153 (2002)

68. Wilson, D. L., R. Martin, S. Hong, M. Cronin-Golomb, C. A. Mirkin & D. L. Kaplan: Surface organization and nanopatterning of collagen by dip-pen nanolithography. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 13660-13664 (2001)

69. Demers, L. M., D. S. Ginger, S. J. Park, Z. Li, S. W. Chung & C. A. Mirkin: Direct patterning of modified oligonucleotides on metals and insulators by dip-pen nanolithography. *Science*, 296, 1836-1838 (2002)

70. Zhang, H., K. B. Lee, Z. Li & C. A. Mirkin: Biofunctionalized nanoarrays of inorganic structures prepared by dip-pen nanolithography. *Nanotechnology*, 14, 1113-1117 (2003)

71. Lee, K. B., J. H. Lim & C. A. Mirkin: Protein nanostructures formed via direct-write dip-pen nanolithography. *Journal of the American Chemical Society*, 125, 5588-5589 (2003)

72. Nam, J. M., S. W. Han, K. B. Lee, X. G. Liu, M. A. Ratner & C. A. Mirkin: Bioactive protein nanoarrays on nickel oxide surfaces formed by dip-pen nanolithography. *Angewandte Chemie-International Edition*, 43, 1246-1249 (2004)

73. Ginger, D. S., H. Zhang & C. A. Mirkin: The evolution of dip-pen nanolithography. *Angewandte Chemie-International Edition*, 43, 30-45 (2004)

74. Lange, S. A., V. Benes, D. P. Kern, J. K. H. Horber & A. Bernard: Microcontact printing of DNA molecules. *Analytical Chemistry*, 76, 1641-1647 (2004)

75. Quist, A. P., E. Pavlovic & S. Oscarsson: Recent advances in microcontact printing. *Analytical and Bioanalytical Chemistry*, 381, 591-600 (2005)

76. Ruiz, S. A. & C. S. Chen: Microcontact printing: A tool to pattern. *Soft Matter*, 3, 168-177 (2007)

77. Owen, J. I., T. L. Niederhauser, B. A. Wacaser, M. P. Christenson, R. C. Davis & M. R. Linford: Automated, controlled deposition of nanoparticles on polyelectrolyte-coated silicon from chemomechanically patterned droplet arrays. *Lab on a Chip*, 4, 553-557 (2004)

78. Gutmann, O., R. Niekrawietz, R. Kuehlewein, C. P. Steinert, B. de Heij, R. Zengerle & M. Daub: Impact of medium properties on droplet release in a highly parallel nanoliter dispenser. *Sensors and Actuators a-Physical*, 116, 187-194 (2004)

79. Saya, D., T. Leichle, J. B. Pourciel, C. Bergaud & L. Nicu: Collective fabrication of an in-plane silicon nanotip for parallel femtoliter droplet deposition. *Journal of Micromechanics and Microengineering*, 17, N1-N5 (2007)

80. Gutmann, O., R. Kuehlewein, S. Reinbold, R. Niekrawietz, C. P. Steinert, B. de Heij, R. Zengerle & M. Daub: A highly parallel nanoliter dispenser for microarray fabrication. *Biomedical Microdevices*, 6, 131-137 (2004)

81. Lee, K. B., E. Y. Kim, C. A. Mirkin & S. M. Wolinsky: The use of nanoarrays for highly sensitive and selective detection of human immunodeficiency virus type 1 in plasma. *Nano Letters*, 4, 1869-1872 (2004)

82. Lee, M., D. K. Kang, H. K. Yang, K. H. Park, S. Y. Choe, C. Kang, S. I. Chang, M. H. Han & I. C. Kang: Protein nanoarray on Prolinker (TM) surface constructed by atomic force microscopy dip-pen nanolithography for analysis of protein interaction. *Proteomics*, 6, 1094-1103 (2006)

83. Vega, R. A., D. Maspoch, K. Salaita & C. A. Mirkin: Nanoarrays of single virus particles. *Angewandte Chemie-International Edition*, 44, 6013-6015 (2005)

84. Haynes, C. L. & R. P. Van Duyne: Nanosphere lithography: A versatile nanofabrication tool for studies of size-dependent nanoparticle optics. *Journal of Physical Chemistry B*, 105, 5599-5611 (2001)

85. Lutwyche, M., C. Andreoli, G. Binnig, J. Brugger, U. Drechsler, W. Haberle, H. Rohrer, H. Rothuizen, P. Vettiger, G. Yaralioglu & C. Quate: 5X5 2D AFM cantilever arrays a first step towards a Terabit storage device. *Sensors and Actuators a-Physical*, 73, 89-94 (1999)

86. Kumar, A. & G. M. Whitesides: Features Of Gold Having Micrometer To Centimeter Dimensions Can Be Formed Through A Combination Of Stamping With An

Elastomeric Stamp And An Alkanethiol Ink Followed By Chemical Etching. *Applied Physics Letters*, 63, 2002-2004 (1993)

87. Lehnert, D., B. Wehrle-Haller, C. David, U. Weiland, C. Ballestrem, B. A. Imhof & M. Bastmeyer: Cell behaviour on micropatterned substrata: limits of extracellular matrix geometry for spreading and adhesion. *Journal of Cell Science*, 117, 41-52 (2004)

88. Rogers, J. A., K. E. Paul & G. M. Whitesides: Quantifying distortions in soft lithography. *Journal of Vacuum Science & Technology B*, 16, 88-97 (1998)

89. Tormen, M., T. Borzenko, B. Steffen, G. Schmidt & L. W. Molenkamp: Sub-mu m thick rubber-elastic stamp on rigid support for high reliability microcontact printing. *Microelectronic Engineering*, 61-2, 469-473 (2002)

90. Okamoto, T., T. Suzuki & N. Yamamoto: Microarray fabrication with covalent attachment of DNA using Bubble Jet technology. *Nature Biotechnology*, 18, 438-441 (2000)

91. Allain, L. R., M. Askari, D. L. Stokes & T. Vo-Dinh: Microarray sampling-platform fabrication using bubble-jet technology for a biochip system. *Fresenius Journal of Analytical Chemistry*, 371, 146-150 (2001)

92. Allain, L. R., D. N. Stratis-Cullum & T. Vo-Dinh: Investigation of microfabrication of biological sample arrays using piezoelectric and bubble-jet printing technologies. *Analytica Chimica Acta*, 518, 77-85 (2004)

93. Blanchard, A. P., R. J. Kaiser & L. E. Hood: High-density oligonucleotide arrays. *Biosensors & Bioelectronics*, 11, 687-690 (1996)

94. Budach, W., A. P. Abel, A. E. Bruno & D. Neuschafer: Planar waveguides as high performance sensing platforms for fluorescence-based multiplexed oligonucleotide hybridization assays. *Analytical Chemistry*, 71, 3347-3355 (1999)

95. Tseng, F. G., C. J. Kim & C. M. Ho: A high-resolution high-frequency monolithic top-shooting microinjector free of satellite drops - Part I: Concept, design, and model. *Journal of Microelectromechanical Systems*, 11, 427-436 (2002)

96. Lee, J.-S., M. Kaibara, M. Iwaki, H. Sasabe, Y. Suzuki & M. Kusakabe: Selective adhesion and proliferation of cells on ion-implanted polymer domains. *Biomaterials*, 14, 958-960 (1993)

97. Singhvi, R., G. Stephanopoulos & D. I. C. Wang: Effects of Substratum Morphology on Cell Physiology - Review. *Biotechnology and Bioengineering*, 43, 764-771 (1994)

98. Oakley, C. & D. M. Brunette: Topographic Compensation - Guidance and Directed Locomotion of Fibroblasts on Grooved Micromachined Substrata in the Absence of Microtubules. *Cell Motility and the Cytoskeleton*, 31, 45-58 (1995)

99. Suh, K. Y., Y. S. Kim & H. H. Lee: Capillary force lithography. *Advanced Materials*, 13, 1386-1389 (2001)

100. Suh, K. Y. & H. H. Lee: Capillary force lithography: Large-area patterning, self-organization, and anisotropic dewetting. *Advanced Functional Materials*, 12, 405-413 (2002)

101. Suh, K. Y., J. Seong, A. Khademhosseini, P. E. Laibinis & R. Langer: A simple soft lithographic route to fabrication of poly (ethylene glycol) microstructures for protein and cell patterning. *Biomaterials*, 25, 557-563 (2004)

102. Khademhosseini, A., K. Y. Suh, J. M. Yang, G. Eng, J. Yeh, S. Levenberg & R. Langer: Layer-by-layer deposition of hyaluronic acid and poly-L-lysine for patterned cell co-cultures. *Biomaterials*, 25, 3583-3592 (2004)

103. Kim, P., D. H. Kim, B. Kim, S. K. Choi, S. H. Lee, A. Khademhosseini, R. Langer & K. Y. Suh: Fabrication of nanostructures of polyethylene glycol for applications to protein adsorption and cell adhesion. *Nanotechnology*, 16, 2420-2426 (2005)

104. Kim, D. H., P. Kim, I. Song, J. M. Cha, S. H. Lee, B. Kim & K. Y. Suh: Guided three-dimensional growth of functional cardiomyocytes on polyethylene glycol nanostructures. *Langmuir*, 22, 5419-5426 (2006)

105. Kim, P., H. E. Jeong, A. Khademhosseini & K. Y. Suh: Fabrication of non-biofouling polyethylene glycol micro- and nanochannels by ultraviolet-assisted irreversible sealing. *Lab on a Chip*, 6, 1432-1437 (2006)

106. Suh, K. Y., A. Khademhosseini, S. Jon & R. Langer: Direct confinement of individual viruses within polyethylene glycol (PEG) nanowells. *Nano Letters*, 6, 1196-1201 (2006)

107. Fukuda, J., A. Khademhosseini, J. Yeh, G. Eng, J. J. Cheng, O. C. Farokhzad & R. Langer: Micropatterned cell co-cultures using layer-by-layer deposition of extracellular matrix components. *Biomaterials*, 27, 1479-1486 (2006)

108. Scheer, H., H. Schulz, T. Hoffmann & C. Torres: Problems of the nanoimprinting technique for nanometer scale pattern definition. *Journal of Vacuum Sceince & Technology B*, 16, 3917-3921 (1998)

109. Vercruysse, K. P. & G. D. Prestwich: Hyaluronate derivatives in drug delivery. *Critical Reviews in Therapeutic Drug Carrier Systems*, 15, 513-555 (1998)

110. Luo, Y., K. R. Kirker & G. D. Prestwich: Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery. *Journal of Controlled Release*, 69, 169-184 (2000)

111. Lopez, G. P., H. A. Biebuyck, R. Harter, A. Kumar & G. M. Whitesides: FABRICATION AND IMAGING OF

2-DIMENSIONAL PATTERNS OF PROTEINS ADSORBED ON SELF-ASSEMBLED MONOLAYERS BY SCANNING ELECTRON-MICROSCOPY. *Journal of the American Chemical Society*, 115, 10774-10781 (1993)

112. Mrksich, M., L. E. Dike, J. Tien, D. E. Ingber & G. M. Whitesides: Using microcontact printing to pattern the attachment of mammalian cells to self-assembled monolayers of alkanethiolates on transparent films of gold and silver. *Experimental Cell Research*, 235, 305-313 (1997)

113. Choi, K. M. & J. A. Rogers: A photocurable poly (dimethylsiloxane) chemistry designed for soft lithographic molding and printing in the nanometer regime. *Journal of the American Chemical Society*, 125, 4060-4061 (2003)

114. Hasirci, V., E. Vrana, P. Zorlutuna, A. Ndreu, P. Yilgor, F. B. Basmanav & E. Aydin: Nanobiomaterials: a review of the existing science and technology, and new approaches. *Journal of Biomaterials Science-Polymer Edition*, 17, 1241-1268 (2006)

115. Jackman, R. J., D. C. Duffy, O. Cherniavskaya & G. M. Whitesides: Using elastomeric membranes as dry resists and for dry lift-off. *Langmuir*, 15, 2973-2984 (1999)

116. Duffy, D. C., R. J. Jackman, K. M. Vaeth, K. F. Jensen & G. M. Whitesides: Patterning electroluminescent materials with feature sizes as small as 5 mu m using elastomeric membranes as masks for dry lift-off. *Advanced Materials*, 11, 546-552 (1999)

117. Ostuni, E., R. Kane, C. S. Chen, D. E. Ingber & G. M. Whitesides: Patterning mammalian cells using elastomeric membranes. *Langmuir*, 16, 7811-7819 (2000)

118. Folch, A., B. H. Jo, O. Hurtado, D. J. Beebe & M. Toner: Microfabricated elastomeric stencils for micropatterning cell cultures. *Journal of Biomedical Materials Research*, 52, 346-353 (2000)

119. Folch, A. & M. Toner: Microengineering of cellular interactions. *Annual Review of Biomedical Engineering*, 2, 227-256 (2000)

120. Yamato, M., C. Konno, M. Utsumi, A. Kikuchi & T. Okano: Thermally responsive polymer-grafted surfaces facilitate patterned cell seeding and co-culture. *Biomaterials*, 23, 561-567 (2002)

121. Pal, R., K. E. Sung & M. A. Burns: Microstencils for the patterning of nontraditional materials. *Langmuir*, 22, 5392-5397 (2006)

122. Wright, D., B. Rajalingam, S. Selvarasah, M. R. Dokmeci & A. Khademhosseini: Generation of Static and Dynamic Patterned Co-cultures using Microfabricated Parylene-C Stencils. *Lab on a Chip*, 7, 1272-1279 (2007)

123. Wright, D., B. Rajalingam, J. M. Karp, S. Selvarasah, Y. Ling, J. Yeh, R. Langer, M. R. Dokmeci & A. Khademhosseini: Reusuable, reversibly sealable parylene membranes for cell and protein patterning. *Journal of biomedical materials research Part A*, 85A, 530-538 (2008)

124. Noh, H. S., K. S. Moon, A. Cannon, P. J. Hesketh & C. P. Wong: Wafer bonding using microwave heating of parylene intermediate layers. *Journal of Micromechanics and Microengineering*, 14, 625-631 (2004)

125. Lotters, J. C., W. Olthuis, P. H. Veltink & P. Bergveld: The mechanical properties of the rubber elastic polymer polydimethylsiloxane for sensor applications. *Journal of Micromechanics and Microengineering*, 7, 145-147 (1997)

Key Words Biological arrays, Unconventional lithography, Dip-pen nanolithography, Contact printing, Droplet dispensing, Nanoimprint lithography, Capillary force lithography, Stencil-based patterning

Send correspondence to: Kahp Yang Suh, Address: School of Mechanical and Aerospace engineering and the Institute of Advanced Machinery and Design, Seoul National University, San 56-1, Shinlim-dong, Kwanak-gu, Seoul, 151-742, Republic of Korea, Tel: 822-880-9103, Fax: 822-883-0179, E-mail: sky4u@snu.ac.kr

http://www.bioscience.org/current/vol1S.htm