Microcontact printing: A tool to pattern

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Microcontact printing has proven to be a useful technique in the patterned functionalization of certain chemicals onto surfaces. It has been particularly valuable in the patterning of biological materials. In this review, we describe the basic principles of the technology as well as its use in several applications, with an emphasis on biological ones. We also discuss the limitations and future directions of this method.

1 Introduction

The need to fabricate patterns of materials onto surfaces at ever smaller length scales first emerged in the middle of the last century, when electrical circuit designs rapidly increased in size and complexity. Even though transistors promised improvements over vacuum tubes, the problem of scaling up simple circuits to carry out more complex functions became a practical impossibility.[1] Because the components had to be soldered and connected manually, a certain frequency of faulty connections could not be avoided, inevitably leading to system failure in large circuits. Furthermore, the power needed to drive these massive systems was also problematic. In the early 1960s, the first integrated circuits containing several transistors and resistors on millimeter-scale dies were produced by fabricating these circuits out of a single slab of semiconductor material.[1–3] By depositing and etching different materials onto these chips, all of the components of a system could be constructed simultaneously, in the correct positions within the circuits. Ultimately, the need for placing components individually was eliminated altogether, and these new fabrication processes precipitated the miniaturization and reliability of modern electronics.

Photolithography, the technology used to generate the initial template of patterns in electronic circuits, has been extended to pattern other materials. The technique uses light and a photomask to generate a pattern of photosensitive resist layer lying on top of a substrate.[4] The photoresist pattern in turn is used to control the spatial distribution of other materials. This technology can also be applied to generate patterns of different surface chemistries.[5] Even though this method has proven useful in patterning a variety of materials, it is not compatible with compounds that are sensitive to light or etchants, such as certain biological agents, or that cannot be deposited onto photoresists or metals. A technique that could afford rapid preparation of substrates as well as patterning of a wider range of materials was needed.

Microcontact printing (μCP), described by the Whitesides group in 1993, has met some of these needs. In this method, an elastomeric stamp with bas relief features is used to transfer an 'inked' material onto a substrate. The general concept of printing is simple, used by kindergarten students to stamp paint figures on paper. Even though μCP was originally used as a method to pattern gold,[6] its value in patterning surfaces for other applications quickly became apparent.[7] Since then, μCP has been used by researchers in widely differing fields to

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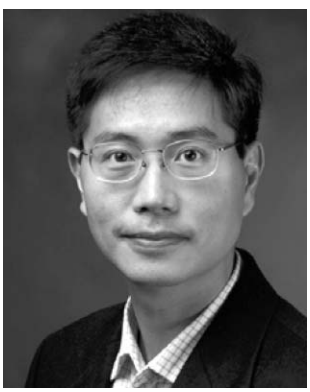

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pattern water,[8,9] salt,[9] organic solvents,[8–10] metals,[6,8,11,12] polymers,[13,14] DNA,[15,16] proteins,[17,18] and cells.[19,20]

Here, we will review the emergence of μCP as the technique of choice to pattern some materials, especially for biological applications. We will present the principles of μCP as well as applications where it has been used to pattern molecules with nanometer resolution. We will also discuss the limitations of the technique and the potential future developments that will make it even more versatile.

2 Principles of microcontact printing

2.1 PDMS and molding

Baked clay tubes with relief designs were used by the Olmecs in Mexico around 1000–800 BC to print repeat patterns, possibly on their bodies or onto cloth.[21] In the 3rd century BC, the Chinese imperial seal was pressed onto clay to authenticate documents, and was later used to transfer ink onto paper, which was invented *ca.* 105 AD.[22] Metals, wood and stone were used as stamps[22] but were eventually replaced by rubber in 1866.[23] Although technologically different, μCP shares the same fundamentals as these older techniques.

The stamps used for μCP are made of a silicone elastomer – polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning) – that molds with very high fidelity to a patterned template. PDMS is a liquid prepolymer at room temperature due to its low melting point (about −50 °C) and glass transition temperature (about −120 °C).[24] To fabricate PDMS stamps with bas relief features, the prepolymer is mixed with a curing agent, poured onto a template, and cured to crosslink the polymer (Fig. 1). To date, patterns with features just under 50 nm have been faithfully reproduced using μCP with PDMS stamps.[25] In addition to forming a physical stamp with such

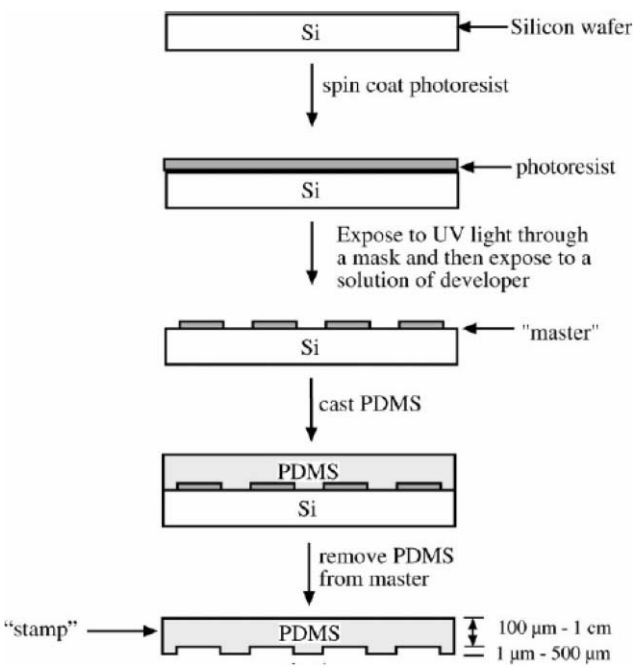

Fig. 1 Diagram illustrating the fabrication of a PDMS stamp. (Reprinted with permission from ref. 26. Copyright 1999 Elsevier.)

small features, there are several other properties of PDMS stamps that support efficient transfer of the ink to the substrate. Because PDMS is elastomeric, the stamp deforms macroscopically to allow the raised features to conform to the substrate over large areas (a few cm^2).[26] A deformable stamp also allows the stamp to be lifted off the substrate without smearing the patterned ink. The low surface energy of PDMS allows it to be easily separated from the template during fabrication, to bind reversibly to the substance to be transferred during printing, and to facilitate peeling of the stamp from the substrate after printing.[27] The low surface energy is due to the flexibility of the siloxane chain and the low intermolecular forces between the methyl groups.[28] The surface energy of the template can be lowered further to enhance peeling of the stamp by binding fluorinated silanes to it. Lastly, PDMS is relatively inert and does not react with many chemicals. However, it does swell in organic solvents, limiting its use in patterning chemicals dissolved in them.[29]

2.2 Fabrication of templates

UV photolithography is the most common technique for fabrication of the templates used in the molding of stamps for μCP. First, a thin layer of photoresist – an organic polymer sensitive to ultraviolet light – is spun onto a silicon wafer using a spin coater. The photoresist thickness is determined by the speed and duration of the spin coating. After soft baking the wafer to remove some solvent, the photoresist is exposed to ultraviolet light through a photomask. The mask's function is to allow light to pass in certain areas and to impede it in others, thereby transferring the pattern of the photomask onto the underlying resist. The soluble photoresist is then washed off using a developer, leaving behind a protective pattern of cross-linked resist on the silicon. At this point, the resist is usually kept on the wafer to be used as the topographic template for molding the stamp. Alternatively, the unprotected silicon regions can be etched, and the photoresist stripped, leaving behind a wafer with patterned silicon making for a more stable template. The lower limit of the features on the stamp is dictated by the resolution of the fabrication process used to create the template. This resolution is determined by the diffraction of light at the edge of the opaque areas of the mask and the thickness of the photoresist.[4] Smaller features can be achieved with extremely short wavelength UV light (~200 nm). Templates can also be produced by micromachining, or they can be prefabricated, such as diffraction gratings.[30]

2.3 Printing

After peeling the PDMS from the template and cutting them to size, the stamps are loaded or 'inked' with the material that is to be printed. For the molecules to transfer onto the substrate, binding to the new surface must be more energetically favorable than staying on the stamp. The surface chemistries of the stamp and substrate, therefore, are important in determining transfer efficiency. Because the stamp is deformable, there are limits on the aspect ratios of the features (Fig. 2A). If the height (h) is much greater than the width (w) of the feature, the structures end up collapsing together while peeling off the template or during the inking process due to

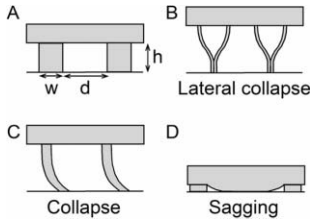

Fig. 2 Diagram illustrating different μCP failure events. **A**. PDMS stamp with features of height *h*, width *w*, and gap distance *d*. **B**. Lateral collapse occurs when adjacent structures make contact and remain adherent. **C**. Collapse happens when the features buckle under the weight of the stamp. **D**. Sagging occurs when the roof of the stamp collapses against the substrate.

capillary action (Fig. 2B),[31,32] or they collapse against the substrate such that a wall of the feature comes into contact with the substrate (Fig. 2C).[32] On the other hand, if the height is much less than the distance (*d*) between features, the roof of the stamp sags, causing contact between the stamp and the substrate in regions where it is not desired (Fig. 2D).[32,33] Sagging can be eliminated by fabricating a submicron thick stamp on a rigid support.[34]

The stamp can be physically deformed to print features with dimensions different from the features originally on the stamp.[35] Mechanically compressing or stretching the stamp horizontally resulted in the deformation of both the raised and recessed features.[35] The deformed stamp was then used to stamp patterns containing these modified features. Applying vertical pressure on the stamp during printing caused the raised relief features to flatten against the substrate resulting in larger printed features and smaller gaps in between them.[35] Swelling the stamp with an organic solvent also led to larger pattern features and smaller gaps in between because the swelling increased the dimensions of the raised PDMS.[35] The application of compression, stretch and vertical pressure on the stamp can yield submicron features even though the undistorted stamp is of lower resolution.

3 Applications of μCP

3.1 Patterning self-assembled monolayers

One class of material that has been widely used as an ink for μCP is molecules that form self-assembled monolayers, or SAMs.[27,36] μCPed SAMs have been used to pattern surface properties at the molecular level.[37] The small molecules used to make these two-dimensional semi-crystalline molecular films have head domains that bind the substrate and tails that form highly ordered structures oriented away from the surface.[27,36] The tails are typically alkyl chains with different functional groups at the ends. These functional groups are key in regulating the surface properties of the substrate, such as wettability, roughness, and reactivity, and have thus afforded scientists a simple method to modify the surface chemistry of a substrate.[27,36] Alkanethiols ($HS(CH_2)_nX$) are perhaps the most commonly used SAM-forming molecules. The sulfur atom in the head coordinates with gold or silver such that the tail is directed away from the surface with a specific orientation to the substrate (Fig. 3).[38] The result is a film of the functional

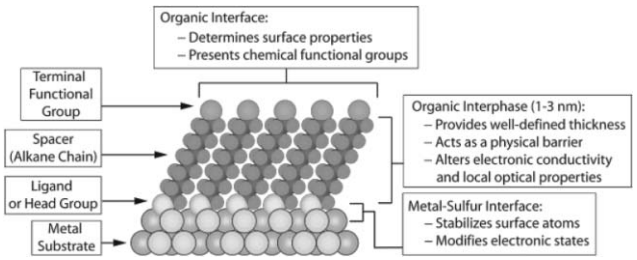

Fig. 3 Diagram showing the binding of the thiol group in alkanethiols to a metal substrate to form crystalline self-assembled monolayers presenting a terminal functional group. (Reprinted with permission from ref. 27. Copyright 2005 American Chemical Society.)

group at the end of the tail being presented at the surface. Siloxanes comprise another common SAM (reviewed in ref. 36). The trichlorosilane head of alkyltrichlorosilane condenses with hydroxylated surfaces as well as with neighboring siloxane molecules forming a highly ordered, closely packed monolayer.[39] Similar to alkanethiols, the tail consists of an alkyl chain with a functional group. An advantage of using silanes over alkanethiols is that they can be μCPed directly onto hydroxylated glass or silicon and do not require a layer of evaporated metal such as gold. On the other hand, their functional groups cannot be readily altered because the trichlorosilane group is easily hydrolyzed[37] and the monolayers they form are not as ordered as alkanethiol SAMs.[36] The thiol groups of alkanethiols are relatively inert, permitting easy modification of the terminal groups and easy handling for routine use. Stamps are loaded with SAM-forming molecules by inking the stamp and then drying it gently with air. The favorable interactions of alkanethiols with gold[38] and silanes with hydroxylated surfaces[36] drive the transfer of these molecules from the stamp to the surface.

One application of alkanethiols is as a protective layer to generate patterns of gold on a surface.[40] SAMs of alkanethiols can function as a nanometer-thick resist, protecting the underlying gold from chemical etchants. Micromachining and microwriting were used first to pattern the SAM and thus control where the gold was removed.[7] However, micromachining and microwriting are slow, serial processes. To address this shortcoming, Kumar and co-workers developed the method to print SAMs onto gold using PDMS stamps, and coined it μCP.[6] By using this simple method they created complex features of gold with a resolution of 1 μm and later demonstrated this approach for microelectronic applications.[11]

The μCP of SAMs has also been used to generate patterns of different surface chemistries juxtaposed on a surface. First, a stamp is used to pattern the first alkanethiol onto a substrate. Then, the bare, unstamped regions are filled in by immersing the substrate in a solution of a second alkanethiol. By using two alkanethiols with terminal groups that confer different hydrophobicities, the wettability of the surface can be patterned.[7] Hexadecane and other organic materials were patterned with micrometer resolution by patterning islands of methyl-terminated alkanethiols (hydrophobic) surrounded by carboxylic acid-terminated SAMs (hydrophilic).[10] By patterning polyurethane prepolymer using this technique and then polymerizing it, an array of microlenses was created.[10] By

reversing the alkanethiols, *i.e.* generating carboxylic acid-terminated islands surrounded by methyl-terminated SAMs, islands of water were created.[9] Patterning salt solutions and allowing them to evaporate led to the formation of salt crystal arrays.[9]

SAMs have been used extensively to control the adsorption of proteins.[7,19,41] Even though proteins adsorb onto many surfaces, it has been difficult to understand what properties of the surface control protein adsorption, because the irregularities and chemical functionalities of most surfaces have been difficult to control. SAMs provided a model substrate where well-defined changes in surface properties could be easily rendered,[42] and led to a more complete understanding of the chemical basis for controlling protein adsorption.[41,43] Methyl-terminated SAMs promoted protein adsorption whereas hydrophilic ones were less effective.[41] Oligo(ethylene glycol)-terminated alkanethiols were able to resist protein adsorption.[43] Substrates with patterned regions of adsorbed protein were generated by printing islands of methyl-terminated alkanethiol surrounded by oligo(ethylene glycol)-terminated SAMs.[7]

The limit of resolution for patterning alkanethiols is now known to be partly determined by the fabrication of the stamp, and partly by the mechanism of transfer. Alkanethiols transfer to gold in seconds to form SAMs.[27] In addition, the alkanethiols can spread laterally from the regions of direct contact of the stamp to noncontacted areas, analogous to bleeding of inks on paper.[10,44,45] Molecules within the stamp and on the walls of the raised features diffuse to the areas of contact with the substrate, and from there spread to the non-contacted regions of the substrate.[44,46] Higher inking concentrations led to more alkanethiol diffusing into the PDMS stamp; this reservoir in turn increases the extent of spreading of the alkanethiol during contact.[44] Increasing the contact time between the stamp and substrate also resulted in increased spreading.[44] Spreading is also dependent on temperature and humidity.[46] Fortunately, because the speed of spreading is low even at high inking concentrations (less than 0.03 μm s^{-1}[44]), and initial stamp transfer occurs in seconds, this effect can largely be limited.

The spreading of SAMs can be used to control the size and shape of the patterned features.[35,45] If μCP is performed under water, the water acts as a barrier to the diffusion of the molecules from the recessed regions of the stamp to the substrate.[35] The molecules then diffuse away from the contacted areas on the substrate in a regular fashion such that the front remains intact and not blurred, and the regions with bare substrate shrink. Longer contact times and higher loading concentrations result in greater spreading and smaller bare areas.[45] This technique makes it possible to have bare regions with nanometer lengthscale even though the stamp itself does not contain sub-micron features.[35] The same method can be used to pattern rectangles of bare substrate by using a single stamp with lines to stamp once, and then rotating it 90° and stamping the same substrate a second time but with a different contact time.[45]

3.2 Patterning proteins

The patterning of proteins has helped the advancement of biosensors,[37,47] cell biology research,[20,48] and tissue engineering.[49] Even though SAMs were instrumental in patterning proteins for a variety of applications, several factors have hampered their widespread adoption for use in biological settings. SAM printing requires access to an evaporator to coat substrates with a layer of gold, and some alkanethiols need to be synthesized in house. To address these shortcomings, James *et al.* pioneered the direct μCP of proteins, a technique that permitted the patterning of proteins while eliminating the need for SAMs.[17] By using polylysine solution as the ink, millimeter scale dots of protein were transferred onto glass. The challenge of adsorbing the positively-charged polylysine onto the non-polar surface of the stamp was overcome by plasma oxidizing the stamp to make it more hydrophilic and therefore more favorable for the polylysine to reversibly bind the PDMS surface. Bernard *et al.* used μCP to pattern a variety of proteins with a resolution of 1 μm.[18] They found that a monolayer of immunoglobulin antibody adsorbed to an unmodified hydrophobic PDMS stamp during the inking process, and that it transferred fully after only a few seconds of contact with glass, polystyrene, or hydrophobic silicon. The stamped immunoglobulins were specifically recognized by secondary antibodies. Stamped enzymes such as horseradish peroxidase and bovine chymotrypsin retained at least 50% of their activity.[18]

During the inking step, protein molecules undergo conformational changes to adsorb onto the PDMS surface.[50,51] For efficient μCP to occur, the receiving surface needs to have properties that make it more favorable for the protein to transfer than to remain on the stamp. To characterize and understand what factors contribute to the quality of μCP of proteins, Tan *et al.* studied the transfer efficiency of proteins printed onto model substrates composed of SAMs with different functionalities. They mixed different quantities of methyl-terminated alkanethiol with the hydrophilic hydroxyl, carboxylic acid, or poly(ethylene glycol)-terminated alkanethiols as substrates for printing proteins.[52] Interestingly, hydrophobic methyl-terminated thiol resisted protein transfer while the hydrophilic alkanethiols increased it, an inversion of their ability to mediate protein adsorption. A threshold percentage of hydrophilic alkanethiol in the mixture was necessary for transfer to occur (Fig. 4). As the percentage was increased, the transfer of the protein improved. Besides making the substrate more hydrophilic, increasing the hydrophobicity of the stamp appeared to improve μCP. When the stamp was functionalized by the addition of $-CF_3$ groups, some protein transfer was observed even onto substrates with 100% $-CH_3$ coating, and complete transfer occurred onto 20% COOH-terminated thiols.[52] These results showed that the relative hydrophobicities of the substrate and stamp are key parameters in protein μCP.

Another protein patterning method exploits the strong interaction between biotin and avidin rather than relying on nonspecific polar and nonpolar interactions. An avidin pattern was printed onto a polymeric substrate containing biotin (PLA-PEG-biotin).[53] Avidin binds the biotin moiety with a very high affinity, and because of its multivalency has sites available for binding biotin after printing. Addition of a solution of biotin-conjugated protein leads to the capture of that protein by the available biotin-binding sites on avidin and

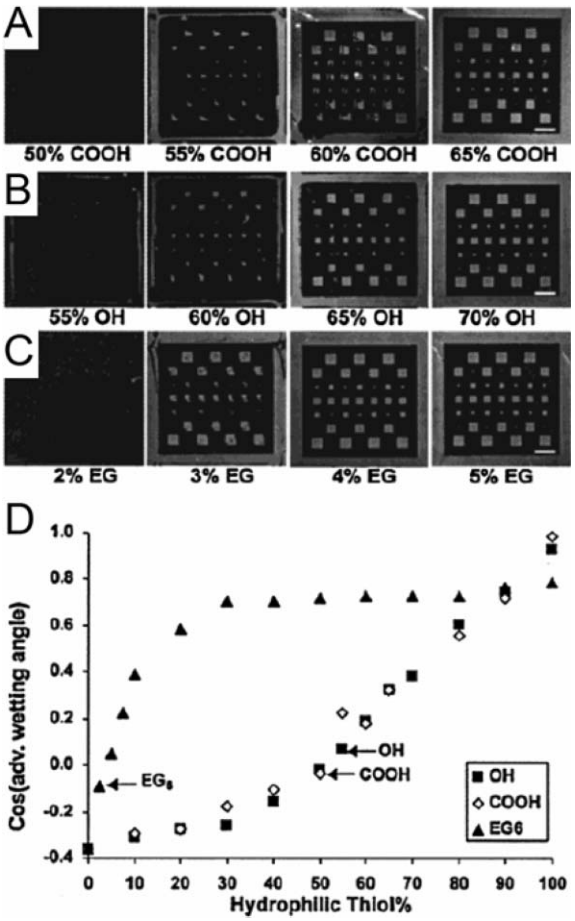

Fig. 4 Protein printing on SAMs with different alkanethiol proportions. **A–C**. Images of fluorescently-labeled protein printed on SAMs made by mixing CH_3-terminated thiols with COOH–, OH–, and poly(ethylene glycol)-terminated thiols. **D**. Graph of the cosine of the contact angle made by a drop of water on SAMs against percentage of hydrophilic thiol. The arrows indicate the minimum percentage hydrophilic thiol at which protein transfer was observed. (Reprinted with permission from ref. 52. Copyright 2002 American Chemical Society.)

consequently the patterning of that protein. This system was used to pattern cells by using biotinylated RGD, a moiety recognized by integrins, the adhesion receptors of cells. A variation of this technique entails patterning the biotinylation of the substrate rather than the avidin.[54] After carboxylic acid groups were introduced on the polymer surface and activated using pentafluorophenol, a stamp inked with biotin-amine was brought into contact with the surface. The amine reacted with the surface thereby biotinylating the polymer at the contact sites. Because the biotinylation reaction was being patterned, this technique facilitated the patterning of protein on other polymeric substrates such as polyethylene, polystyrene, poly(methyl methacrylate), and poly(ethylene terephthalate).[55] When added in solution, streptavidin, an avidin analogue, bound to the patterned biotin, thus patterning the polymer with protein.

The advantage of patterning protein indirectly by using the avidin–biotin system to capture proteins from solution is that the protein is more likely to retain its native conformation than when it is printed directly, where it has to undergo some structural rearrangement to adsorb to the stamp and/or transfer to the substrate. A stamped monolayer of Azurin metalloprotein, for example, had a thickness less than the native size of the protein.[51] However, because different proteins will undergo dissimilar conformational changes and because extent of rearrangement does not necessarily correlate with loss of function, the effects of μCP on protein conformation are not well understood. Interestingly, printed laminin, an extracellular matrix (ECM) protein, underwent supramolecular self-assembly upon μCP to form physiologic mesh-like polygonal networks.[56] One significant disadvantage of using the avidin–biotin technique for patterning is that the biotinylation of the protein that is to be patterned requires an extra synthesis reaction, which adds to the cost and duration of the technique. It is also possible that the biotinylation reaction may result in the loss of function of some proteins.

3.3 Patterning cells

μCP has been used to advance our understanding of how cells interact with substrates. Singhvi *et al.* printed islands of SAMs that promoted adsorption of cell-adhesive ECM protein surrounded by regions that prevented it. These islands were of the same length scale as individual cells such that when cells were seeded onto the substrates they preferentially bound to the printed islands, and spread to conform to their size and shape. Proliferation increased with island size, but albumin production, an indicator of liver function, decreased.[20] Chen *et al.* showed that cell area not only regulated proliferation, but also apoptosis, or programmed cell death (Fig. 5A).[48] By patterning cells such that they spread over multiple dots of ECM instead of a single island, they showed that the projected area of the cell rather than the ECM area dictated proliferation. Thery *et al.* used μCP to study the effect of cell shape on cell division axis orientation.[57] They were able to decouple adhesion from overall cell shape by printing ECM in different geometries. Cell division axis orientation was determined by the adhesion pattern, rather than the shape of the cell (Fig. 5B). Recently, μCP was used to study growth in endothelial and epithelial monolayers.[58] Proliferation was highest in cells at the monolayer edges (Fig. 5C) and was dependent on the tractional stress within the sheet. These studies demonstrate that the patterning of cells can lead to new findings not possible with traditional cell culture techniques.

3.4 Patterning DNA

μCP has also been used to pattern DNA.[15,16] Stamps inked with DNA ranging from 20-bp oligonucleotides to 1600-bp PCR fragments transferred onto aminated glass with micrometer resolution.[15] Microarrays with different oligonucleotides were printed by using a spotter to load the stamp. Because the DNA molecules adsorbed in a layered, entangled meshwork, the first print resulted in partial transfer of the DNA. This allowed for the same stamp to be used to print multiple substrates without reloading, although the amount transferred decayed exponentially.[15,16] This decay was compensated by the increased sensitivity of the stamped arrays to detect complimentary DNA in the analyte solution. Even an array printed

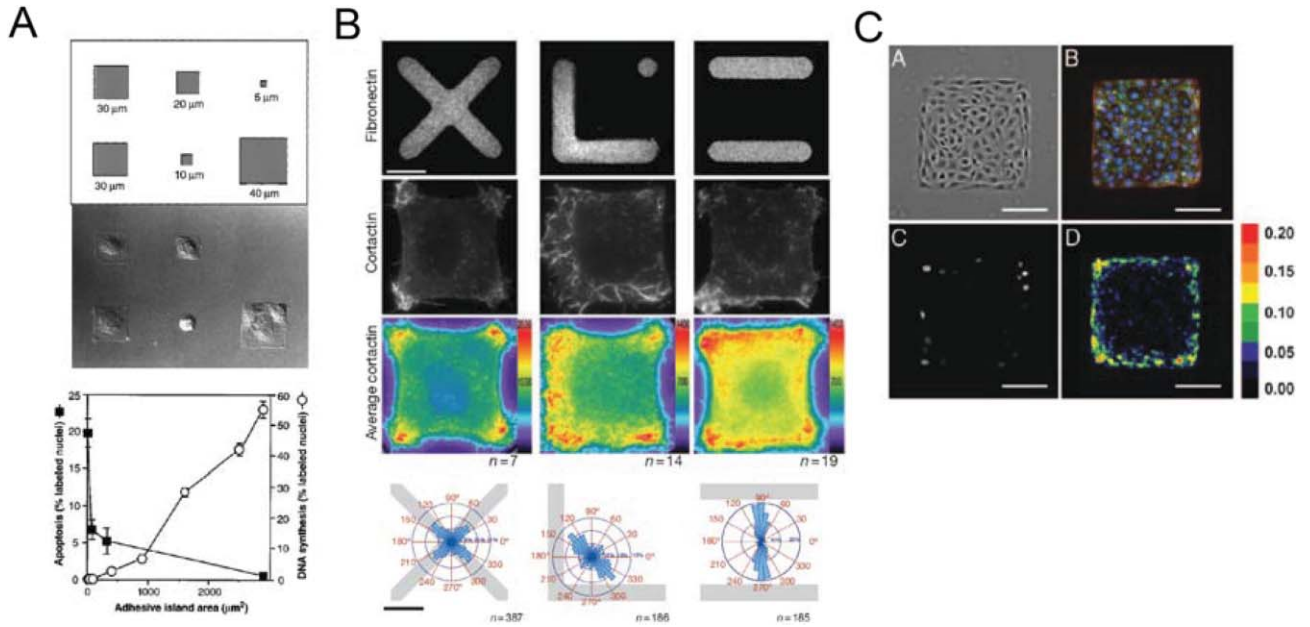

Fig. 5 Patterning cells by μCP of ECM for the study of cell biology. **A**. Micrograph of bovine adrenal capillary endothelial cells on square islands of different sizes. The graph shows apoptosis (dark squares) and DNA synthesis (open circles) against island size. (Reprinted with permission from ref. 48. Copyright 1997 AAAS.) **B**. The first two rows are fluorescence images of different patterns of fibronectin and the distribution of HeLa cell membrane ruffles visualized by staining for cortactin, a cytoskeleton-binding protein. The third row is a heat map generated by averaging a number of cortactin images. The fourth row shows the distribution of cell division axis orientation for the different shapes. Scale bars are 10 μm. (Adapted by permission from Macmillan Publishers Ltd: Nature Cell Biology from ref. 57. Copyright 2005.) **C**. A monolayer of bovine pulmonary artery endothelial cells cultured on 250 μm squares of fibronectin. The heat map in the bottom right panel represents average proliferation localization in 50 monolayers. Scale bars are 100 μm. (Reprinted from ref. 58. Copyright 2005 National Academy of Sciences.)

with a stamp that had already been used twice was two orders of magnitude more sensitive than a spotted one (Fig. 6). The increased binding efficiency between the probe and stamped DNA was due to increased accessibility on the stamped spots as compared to adsorbed spots. Another method for developing arrays with different fragments involved loading flat stamps using a multi-channel microfluidic network.[15] The binding of complementary nucleotides was found to be specific. The reduction in time and DNA material are the major advantages of using μCP to pattern DNA arrays since a spotted stamp can be used to print multiple arrays, as well as more homogeneous and sensitive dots.

4 Comparison to other techniques

Numerous techniques besides μCP have been used to pattern these types of materials. Micromachining has been used to pattern SAMs.[59] First, alkanethiols, which form SAMs on gold, are added to a substrate. Then a scalpel or carbon fiber is used to scratch a 1 μm groove across the substrate, exposing bare gold. The substrate is then immersed in a second alkanethiol which forms SAMs in the scratched regions. The limitations of this simple method are that it is too crude to allow for the precise control of pattern shapes and sizes, and that more sensitive materials cannot be patterned by physical deformation. Microwriting is also used to pattern SAMs.[60] In this procedure, micropens are employed to write monolayers of alkanethiols or alkylsiloxanes on gold or glass respectively, and then the remaining areas are filled in by adding a different alkanethiol or silane to the substrate. Dip-Pen Nanolithography (DPN) affords higher resolution patterning than microwriting. By exploiting the capillary action between an atomic force microscope (AFM) tip and a substrate, patterns with 50 nm features can be written directly (Fig. 7A).[61,62] DPN is also more effective than μCP at producing multi-molecule patterns, although because it is a serial process it is slower than stamping, where the whole

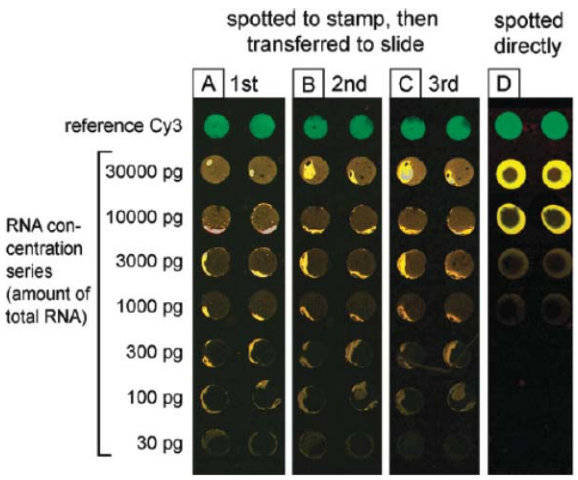

Fig. 6 DNA arrays made by μCP a spotted PDMS stamp three in times in succession or by spotting directly. The arrays show hybridizations using different concentrations of RNA starting material. (Reprinted with permission from ref. 15. Copyright 2004 American Chemical Society.)

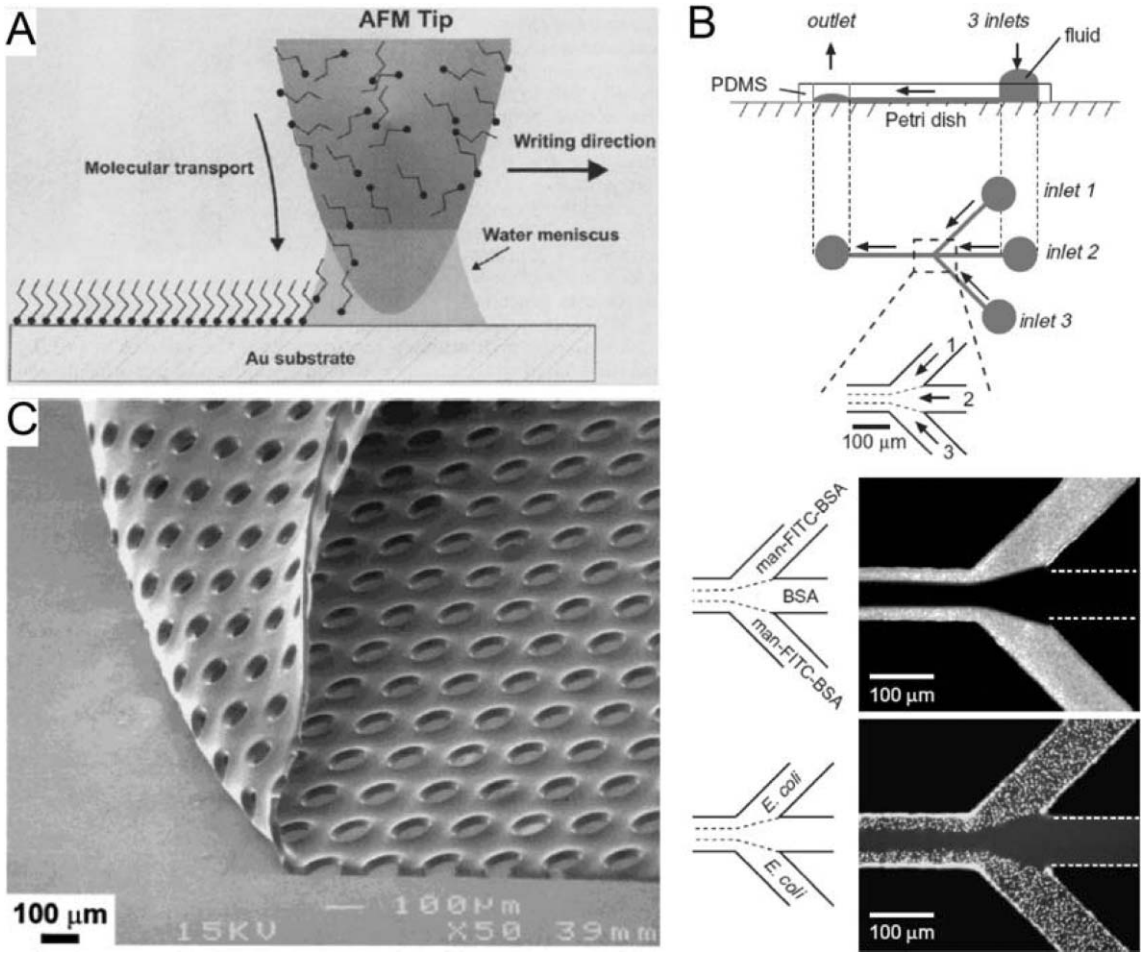

Fig. 7 Techniques other than μCP used for patterning. **A**. Schematic of DPN showing how an AFM tip is used to write alkanethiols on a gold substrate. (Reprinted with permission from ref. 61. Copyright 1999 AAAS.) **B**. Laminar flow patterning using microfluidic channels. Side and top view schematics of a PDMS microfluidic device used to pattern man-FITC-BSA and *E. coli*. (Reprinted from ref. 66. Copyright 1999 National Academy of Sciences.) **C**. SEM of a 50 μm thick PDMS membrane with 100 μm holes. (Reprinted with permission from ref. 67. Copyright 2000 American Chemical Society.)

pattern is transferred as one. Photolithography is another option for the fabrication of complex patterns. While it is normally used to give resist layers a three-dimensional topography, it can also be used to spatially regulate surface chemistry modification. By exposing certain surfaces to UV light through a photomask, the regions that are exposed can be selectively modified.[5,63,64] By varying exposure time, the density of the chemical modification can be modulated. In μCP, on the other hand, the density of the stamped material is not easy to control. The main disadvantage of photolithography is the need for specialized equipment. While only a fraction of chemicals can presently be patterned by light, just like with μCP, future efforts will focus on increasing the materials that can be patterned using this technology.

Like μCP, some of the alternative technologies used in patterning are based in soft lithography, or the use of elastomers for patterning materials. Microfluidic channels made of PDMS can be used to flow solutions of proteins along specific paths on glass.[65] Using parallel laminar streams makes it possible to pattern lines of different materials without any gap in between them (Fig. 7B).[66] Because the volume of the channels is extremely small (<1 μl), very little reagent is needed. One major drawback of patterning using microfluidics is that elaborate patterns with features more complex than parallel stripes, such as arrays, are hard to achieve without additional steps. Elastomeric membranes with holes are a better option for such patterns (Fig. 7C). They are made by spinning PDMS onto a template with raised features until the membrane thickness is less than the resist height, leaving holes of defined geometry in the membrane.[67] After curing and peeling it off the template, the membrane is placed on a substrate and used as a stencil to pattern proteins or cells. While this system is easier to use than microfluidic channels, the membranes can be fragile and can tear when they are handled.

Three-dimensional hydrogels patterned using PDMS stamps have been used to spatially organize cells.[68] Collagen gels molded with stamps were used to pattern two different cell populations,[69] or to form perfused microvascular tubes *in vitro*.[70] Microfluidic channels within alginate gels were used to control the concentrations of fluorescein and dextran within the gel.[71] Whereas μCP has proven useful in patterning cells on

flat substrates, these hydrogel-based techniques offer a way to pattern cells in 3D while using the same PDMS stamps used in μCP.

5 Future directions

It is evident that μCP has become a useful, convenient, and widely-used technique for the patterning of many materials. Most studies have focused on understanding its mechanism as well as defining applications for it in the fields of surface chemistry, microelectronics, and cell biology. We expect future developments in μCP to further improve its spatial resolution and versatility.

The μCP of nanoscale elements confers several advantages over the printing of micrometer features. The elements of an array can be more closely packed, allowing for higher density grids, the etching of integrated circuits bearing smaller and more modules becomes possible, and the molecular-level facets of cell adhesion can be examined. Because PDMS stamps adopt the features of the template that they are cast from, the key to printing smaller features lies in using higher resolution lithography to make the templates. Using extreme ultraviolet light (10 to 14 nm) instead of the traditional 365 nm light used in photolithography is one way to create templates with ~100 nm features. Electron-beam lithography, where electron-sensitive resists are 'written' onto with a focused beam of electrons, can achieve a resolution of 10–100 nm.[4] In X-ray lithography, X-rays are used to pattern the resist through a mask with 1–10 nm features. Since e-beam and X-ray lithography can only be used to pattern thin resists, they need to be used in conjunction with deep reactive ion etching (DRIE) if micrometer heights are needed to avoid stamp roof sagging. Narrow ion beams can be used for direct removal of metal with single nanometer resolution in focused ion beam (FIB) lithography. Because these processes require more sophisticated equipment than simple UV photolithography, the printing of nanoscale features is currently less widespread.

In recent years, methods for the fabrication of templates that do not require the use of a clean room have been reported. Solventless polymerization was used to make a template with raised features made by ring-opening metathesis polymerization.[72,73] By spatially restricting the catalyst onto a substrate using μCPed SAMs or PDMS channels and then exposing the surface to volatile monomers, the polymerization is patterned. Chemical vapor deposition polymerization, which also occurs at the gas–solid interface, has been used to make relief features.[74] PDMS channels were used to control where paracyclophane monomers deposited to form poly(*p*-xylylenes). Capillary Force Lithography is another technique used for making templates.[75] When a PDMS stamp was brought into contact with a thin PMMA layer, the PMMA melt rose along the side walls of the stamp by capillary forces without filling in the features. After cooling, the second-generation template was used to make stamps with submicrometer features. Even though these methods require the use of a prefabricated stamp to make the template, they are useful in reproducing templates without utilizing photolithography.

Researchers have already ventured into Nanocontact Printing (NCP) as a method to pattern materials. Stamps cast from V-shaped gratings used for AFM tip characterization (thereby avoiding the use of complex lithography) were used to print lines of dendrimer and protein with widths less than 50 nm.[25] Nanoprinted dendrimers also functioned as a resist in the patterning of palladium.[76] Nanoprinted ECM was used to study the effect of adhesion size and separation on cell spreading.[77] PDMS stamps with arrays of squares with sides as small as 300 nm were cast from templates made by electron beam lithography and used to print ECM protein. The extent of cell spreading over the arrays of ECM squares was dependent on the percentage of substrate covered by ECM rather than on the size of the adhesions. Electron beam lithography was also used to make stamps for printing nanoscale protein aggregates.[78] Single protein printing was attained by using low inking concentrations. The capacity to pattern single proteins may enhance the study of molecular biophysics and individual protein–protein interactions. As we continue to develop a better understanding of the limits and capabilities of NCP, this technology is likely to find many more uses in the near future.

μCP has the potential to facilitate the patterning of multiple different molecules on a single substrate. Traditionally, multiple stamps are each inked with a different material and aligned to the substrate using fiduciary marks. Alignment, however, is laborious and prone to inaccuracies over centimeter scale areas.[79] Because the alignment is performed using a microscope on one region of the substrate, areas far from that region may not have the features aligned. To overcome this, methods that load distinct inks on different regions of a stamp such that a single printing transfers all of the inks in registration have been developed.[80] One way this was accomplished was by using multilevel stamps where loading the different levels with distinct inks was possible by collapsing the stamp using different pressures.[80] Combining stamp collapse and microfluidics also yielded multiprotein patterns.[80] "Multicolor printing" was achieved with DNA by using DPN to ink the features on a stamp with different inks.[81] Even though these advancements are significant, new technologies are needed to make the printing of the hundreds of molecules necessary to make protein and DNA chips easier.

Reactive μCP has been used in recent studies to improve the binding of the transferred molecules to the substrate. Slides functionalized with aldehyde groups supported more transfer of Bovine Serum Albumin (BSA) in the presence of a film of water in between the stamp and slide than controls in the absence of water, perhaps due to increased covalent coupling between the BSA and aldehyde groups.[82] Poly(*N*-hydroxysuccinimidyl methacrylate) (PNHSMA) films on oxidized silicon or glass covalently bound printed amino-end functionalized PEG.[83] BSA and DNA did not adsorb onto the PEG-protected areas but made covalent bonds with bare PNHSMA resulting in patterns. Future developments in reactive μCP will help improve the stability of patterned biomolecular substrates.

Polymers other than PDMS have recently been used to fabricate stamps. Even though PDMS possesses most of the qualities desirable in a stamp, it is not suitable for the printing of all materials. Polar compounds don't adsorb well onto the hydrophobic surface of PDMS. A slightly hydrophilic stamp

made of poly(ether-ester) performed much better than PDMS or plasma-oxidized PDMS stamps at transferring lines of a polar ink onto gold.[84] Using the ink as a resist, the gold was etched and the resulting features analyzed. The poly(ether-ester) stamp resulted in structures with clean walls and tops even after the eighth printing with the same stamp, a result not possible with the other two kinds of stamp. The improved performance of poly(ether-ester) over PDMS stamps was due to the ability of the poly(ether-ester) stamp to take up the ink into the hydrophilic polymer bulk.[84] Even though the surfaces of oxidized PDMS stamps are wetted by polar inks, repeated printing was not possible because of poor ink uptake by the hydrophobic bulk. Another approach for the μCP of polar inks is to make PDMS hydrophilic by plasma oxidation followed by binding to poly(ethylene oxide).[85] By patterning the oxidation using contact masks, this technique was extended to flat stamps for the patterning of proteins[85] and hydrophilic inks.[86] PDMS is also incompatible with organic solvents used to dissolve certain hydrophobic molecules because they cause PDMS to swell, distorting the relief structures.[29] To make possible the loading of molecules dissolved in hydrophobic solvents without swelling the stamp, perfluoropolyether – a polymer recently developed to make microfluidic devices compatible with organic solvents[87]– could be used to fabricate stamps for μCP. The continued search for techniques to print as many materials as possible will make μCP even more versatile in the future than it is now.

6 Conclusion

Since its conception more than a decade ago, microcontact printing has proven to be invaluable in the patterning of certain materials. Because the method is easy to use, it has enabled researchers both from, and outside of, the physical sciences to fabricate surfaces with spatially patterned material, from metals and liquids to SAMs and biological molecules, without the need for elaborate equipment or uncommon reagents. Consequently, the technique has opened the door to new innovations and findings. Future efforts will focus on making the technology simpler, while affording complex patterns and single molecule resolution.

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