Trends in imprint lithography for biological applications

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Imprint lithography is emerging as an alternative nanopatterning technology to traditional photolithography that permits the fabrication of 2D and 3D structures with <100 nm resolution, patterning and modification of functional materials other than photoresist and is low cost, with operational ease for use in developing bio-devices. Techniques for imprint lithography, categorized as either ‘molding and embossing’ or ‘transfer printing’, will be discussed in the context of microarrays for genomics, proteomics and tissue engineering. Specifically, fabrication by nanoimprint lithography (NIL), UV-NIL, step and flash imprint lithography (S–FIL), micromolding by elastomeric stamps and micro- and nano-contact printing will be reviewed.

Introduction

Nanometer surface topologies can be fabricated by imprint lithography (~5 nm minimum feature, 30 nm resolution) [1–3]; in addition, it is possible to control the spatial distribution of chemical species on the structured surface. The creation of low-cost devices with <100 nm feature sizes and aspect ratios of 1 to 80 can be achieved at a tenth of the cost of conventional photolithography [4–6]. Imprint lithography, as discussed here, describes the general process of using a mold, template or stamp, populated with structures, to produce a pattern by applying pressure. Techniques for imprint lithography are categorized as ‘molding and embossing’ or ‘transfer printing’. Fabrication by molding and embossing includes techniques such as nanoimprint lithography (NIL), UV-NIL, step and flash imprint lithography (S–FIL), and micromolding by elastomeric stamps, whereas micro- and nano-contact printing are termed transfer printing processes. It is noted that soft lithography broadly refers to molding, embossing and printing methods exclusively using an elastomeric mold and/or stamp such as poly(dimethylsiloxane) (PDMS) [7]. Applications for these techniques, as related to microarrays for genomics, proteomics and tissue engineering, will be discussed.

Conventional patterning by photolithography

Conventional photolithography (Figure 1a) generates patterns by selectively exposing the photoresist-coated surface (see Glossary) with UV light, followed by etch to yield the structures. Because shorter wavelengths are required to produce smaller structures, significant cost increases are necessary to improve the capabilities of the equipment and facilities needed to fabricate photomasks (see Glossary) [8]. Additional challenges include maintaining the chemical integrity of the surface in the presence of developers and etchants to define the final pattern and the use of strictly planar substrates [8].

Molding and embossing processes

Nanoimprint lithography

A spun-on thermoplastic polymer film, such as poly(methyl methacrylate) (PMMA), is heated above its glass transition temperature, Tg (see Glossary), and a rigid mold is pushed into the film (Figure 1b) [9]. Once the polymer has cooled below the Tg, the surface is textured and separated from the mold. This method requires high processing temperatures and pressures to enable the polymer film to flow. Furthermore, filling micrometer-scale recesses within the mold with high viscosity polymers is challenging and requires the film to travel longer lateral distances, as compared with smaller structures. For PMMA, the typical process temperature is >110°C and the applied pressure is between 40 and 130 atm. This process is also known as thermal imprinting or hot embossing.

UV-NIL

UV-NIL is a variation of NIL that uses a spun-on low-viscosity monomer [10] or polymer [11] and occurs at room temperature and low imprinting pressures: a rigid, transparent mold contacts the fluid, and the material is UV cured to lock in the nanostructures of the mold, rather than being cooled.

Glossary

Centipoise (cPs) – a centimeter–gram–second unit of dynamic viscosity, equal to one-hundredth of a poise.
Glass transition temperature (Tg) – the mid-point of a temperature range, below which materials gradually become more viscous as they change from liquid to solid.
MAPL – a patterning technique, whereby a ligand (or ligands) can be immobilized at a controlled surface density inside a defined area to capture molecules in their environment.
Photomask – a high-purity quartz or glass plates used in photolithography that contain precision images of integrated circuits or chips, used as masters for the optical transfer of these images.
Photoresist – a light-sensitive material used to form a patterned coating on a surface. Can be a positive resist – the light-exposed portion is soluble to the developer solution – or a negative resist – the light-exposed portion is insoluble to the developer solution.
Step and flash imprint lithography (S–FIL)

S–FIL uses a low viscosity monomer (<5 cPs; see Glossary) that is dispensed as droplets deposited on the substrate, rather than as a spun-on film (Figure 1c) [12].
substrate when the mold is removed. This process uses the lowest imprint pressures possible (<0.02 atm), given that low viscosity liquids flow readily to fill the recesses of the mold. S–FIL offers the opportunity to modify the material chemistry, drop by drop, in creating the surface pattern and enables printing on non-planar surfaces. The alignment accuracy has been reported to be ±10 nm [13].

**Micromolding**

Solvent-assisted micromolding (SAMIM) [14], micromolding in capillaries (MIMIC) [15] and microtransfer molding (μTM) [16] are techniques related to micromolding by way of an elastomeric stamp. In SAMIM, the surface of the stamp is wetted with a solvent and pressed against the polymer film at ambient conditions. The solvent softens the polymer, and as the solvent evaporates, the polymer conforms to the elastomeric mold. SAMIM enables molding of polymers that cannot be implemented with S–FIL or NIL. However, the solvent can distort the features by swelling the mold. Isolated nanostructures can be formed with MIMIC and μTM: in MIMIC, structures are filled with a thermal or photo-curable, low viscosity liquid by way of capillarity; for μTM, the stamp is filled with a pre-polymer, placed on a substrate and cured. When the stamp is removed, the patterned structure is left on the substrate. These techniques are favorable for non-planar surfaces.

**Transfer printing process**

Microcontact printing (μCP) is widely used to transfer chemical molecules onto a surface using a patterned elastomeric mold or stamp, which is typically fabricated from poly(dimethylsiloxane) [7,17]. The transferred molecules, or ‘inks’, are coated onto the stamp before contact with the substrate (Figure 1d) and deposited in a pattern defined by the raised surfaces of the stamp. This process is attractive because of the ease and low cost of generating the stamp with commercially available precursors. However, the pattern resolution is dependent upon the resulting distortion of the stamp during contact with the substrate and the lateral dimensions of the inks. μCP is limited to micron length scales, owing to the surface diffusion of low molecular weight inks. Nanocontact printing (nCP), an extension of μCP, enables printing of <100 nm structures using a stiffer elastomeric stamp and high molecular weight inks to limit diffusion [18–20].

All of the above mentioned imprinting methods are limited mostly by the reliable production of defect-free molds. These molds are patterned using direct-write electron beam tools that are capable of a minimum feature size of 5 nm [21]; they are subsequently developed, etched and diced to attain the final mold. Variations introduced during these processing steps will affect the pattern fidelity, the resolution and the replication of the patterns. Furthermore, the molds are treated with a fluorinated release agent to assist with separation from the cured monomer or thermally set polymer [22]. The release agent has a limited lifetime, resulting in the imprint material adhering to the mold rather than the substrate, which causes the surface of the mold to foul or can break features on the imprint material.

**Applications**

**Microarrays for genomics and proteomics**

DNA and protein microarrays are crucial tools, offering miniaturized processes that need smaller sample volumes and are executed in parallel with high throughput. However, improvements in microarray manufacturing are necessary to increase the sensitivity and accuracy of these devices. Projections by the Business Communications Company (http://www.bccresearch.com/) anticipate that worldwide market sales for DNA sequencing and proteomics will increase from $7.8 billion in 2004 to $17.5 billion by 2009. This growth is driven mainly by pharmaceutical and biotech companies searching for lower-cost and faster turn-around solutions in the discovery and development of new drugs and diagnostics.

**DNA microarrays**

These arrays enable chemically unique locations to be treated simultaneously with a sample fluid, and sites where reactions occur identify a specific characteristic of the sample. DNA microarrays are fabricated by either direct synthesis at the target site (in-situ synthesis) or targeted transfer of pre-synthesized molecules by direct spotting. In-situ synthesis uses lithographic masks to direct UV light on the substrate, which de-protects localized regions for coupling with biological probes.

High capital costs are incurred with photolithographic processes. Affymetrix, which holds ~50% of the market (http://www.forbes.com/2002/11/06/cx_mh_1106sf.html), with biochips costing between $100 and $500 each, implements a method incorporating selective UV illumination to control the growth of DNA sequences, and photolithographic markers for identifying specific locations for illumination [23]. Direct spotting of microarrays is performed with inkjet printing or contact pins to deliver the probes onto planar substrates or onto microscale structures on those substrates. The latter fabrication method enables customization of the spotting chemical by the end-user. An alternate approach is to apply the probes directly onto the substrate by micro-contact or nanocontact printing to achieve denser and smaller spot sizes for improved detection sensitivity.

Promising academic developments are emerging to demonstrate that molding, embossing and contact printing techniques are viable for fabrication of these arrays. For DNA sequencing purposes, nanochannels (100 nm wide and 200 nm deep), created by imprinting, were used to stretch DNA that was subsequently assayed to identify protein binding sites (Figure 2a) [24] (Morton, K. et al. Quantitative protein/DNA analysis in nanoimprinted protein binding sites (Figure 2a) [24] (Morton, K. et al. Quantitative protein/DNA analysis in nanoimprinted protein binding sites. Third International Conference on Nanoimprint and NanoPrint Technology, 2004). Ohtake et al. reported that DNA directly patterned by NIL retained its activity, showing that these biomolecules can handle the processing conditions [25]. Xiao et al. illustrated the utility of large-scale microcontact printing when used during the coupling step for fabricating oligonucleotide arrays, with no observable differences in detection as compared with standard microarray methods [26]. Research by Moorcroft et al. suggests that the synthesis of oligonucleotide probes directly on PDMS is a reasonable alternative to
glass substrates for engineering microarrays [27]. Li et al. showed that nCP can be used to print polyamidoamine dendrimers for coupling to complementary oligonucleotides [20].

**Protein microarrays**

These biochips enable many proteins to be studied simultaneously, to understand their interactions with each other and with non-proteinaceous molecules (i.e. nucleic acids, lipids and organic compounds). There are two broad categories of protein microarrays: capture biochips and interaction biochips. Capture biochips detect the presence and amount of proteins; they are fabricated with capture agents that are integrated or left bare for the end-user to develop their own capture agents. Interaction biochips measure real-time interactions among immobilized proteins and other proteins or biomolecules. A majority of the current protein microarrays are capture biochips, which are constructed on a planar substrate. Exceptions to these are the biochips produced by Zyomyx (http://www.zyomyx.com), which are microfabricated from silicon substrates containing a surface with 3D pillars. The top of each pillar is coated, using dip pens or inkjet, with specific capture agents to prevent non-specific binding. Another approach to generate these surface structures is to mold or emboss the pattern directly on the substrate using NIL or S–FIL, followed by microcontact printing to chemically functionalize the structures, thus circumventing the need to use a photomask, developers and etch, as required with photolithography.

Several groups are working towards demonstrating the utility of imprint lithography for protein patterning. Hoff et al. showed that selective patterning of protein with high throughput, using NIL, is possible for structures with a minimum size of 75 nm [28]. In addition, increases in the sensitivity and detection levels of protein were observed when using a combination of NIL and molecular assembly patterning by lift-off (MAPL; see Glossary) [29] for fabricating large-area, high-density protein patterns.
with 25 nm feature sizes (Park, S. et al.). Chemical patterning of sub 50 nm half pitches via Nanoimprint lithography and its application to protein patterning. Third International Conference on Nanoimprint and Nanoprint Technology, 2004). Using μCP, Bénard et al. showed that multi-component patterning is feasible for a system employing sixteen different proteins stamped onto a polystyrene surface [30], and Li et al. reported that nCP can print functional protein lines of 40 nm (Figure 2b) [31]. Furthermore, patterning of antibodies onto glass substrates can be achieved with their antigen-binding selectivity intact [32]. In addition to stamps made of PDMS, stamps of agarose gel enabled printing of protein gradients that needed only sub-nanomolar quantities of protein [33].

Tissue engineering

Patterned surfaces and their chemical landscape provide cues for cells to attach, migrate and assemble into functioning tissue. Photolithography is typically used to generate the surface structure but is limited in applications where bio-specific adsorption and functionalities are required to mimic the microenvironments for cellular development. Thus, imprint methods are useful because they permit direct construction of a variety of shapes with varying physico–chemical properties and are accessible methods for academic research. Recently, Vozzi et al. provided a simple approach for forming 2D and 3D biomaterial scaffolds by taking individual layers of poly(D-,L-lactide–coglycolide), made by micromolding, and thermally laminating the layers together to form scaffolds with feature sizes of between 10 and 30 μm [34].

In vivo cells encounter patterned surfaces of varying shapes and ranging in length from micrometers to nanometers. μCP and nCP methods are enabling researchers to explore the connection between cell behavior and surface geometry and size. Grids and lines of 2 μm [35], honeycomb networks of ~25 μm [36] and dots with a minimum size of 0.3 μm [37], created by μCP as a function of separation spacing between the structures, influence the morphology, spreading and migration of various cell lines (Figure 3). Initial reports from Abrams et al. that state that basement membranes comprise nanometer-sized pores, ridges and fiber structures (~< 70 nm) [38] have spurred investigations into the cellular response to nanometer structures. Recently, Yim et al. observed significant elongation of smooth muscle cells on a functionalized grating formed by a combination of NIL and nCP (350 nm line width, 700 nm pitch and 350 nm depth) compared with non-patterned surfaces [39]. Using NIL, Johansson et al. showed that nerve cells can be guided to grow along the top of, rather than inside, patterned grooves (between 100 and 400 nm width and 300 nm depth) [40]. More detailed reviews of surface patterning related to tissue engineering include Curtis and Wilkinson [41], Ito [42], Kane et al. [43] and Liu and Chen [44].

Conclusions

The future of imprint applications is in the area of biotechnology. As photolithography becomes increasing expensive and complex, it has provided opportunities for using non-conventional techniques in nanofabrication. The imprint lithography techniques discussed in this review have the advantage of permitting fabrication of <100 nm structures on non-planar surfaces, imprinting of 2D and 3D structures, patterning and modification of functional materials other than photoresist and are low cost, with operational ease for academic and industrial research to use in developing devices. We expect imprint lithography to play a significant role in enabling biological applications.

References

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