

Small scale structures: the fabrication of polymeric nanostructures for biomedical applications using pattern replication techniques

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Resum

Els polímers són uns excel·lents candidats per a la producció de dispositius biomèdics que incorporin estructures nanomètriques. Característiques com ara bones propietats òptiques i de segellament, cost de fabricació baix, disseny ràpid i, sobretot, biocompatibilitat són avantatges que poden fer decantar els científics cap a la producció d'aquests dispositius. Aquest article recopilatori vol mostrar alguns dels mètodes i les tècniques que es fan servir per a la fabricació de nanoestructures amb polímers mitjançant tècniques de replicació que poden ser rellevants per a la producció de dispositius biomèdics. Es posa l'èmfasi en la producció de rèpliques polimèriques, per mètodes d'estampació i amb l'ús per a la fabricació de motius de la tecnologia del «focused ion beam» com a mètode senzill per a l'obtenció de manera reproducible de gran quantitat de nanoestructures. Es descriu l'ús d'aquestes estructures en les tècniques d'estampació, juntament amb consideracions de fabricació específiques. La maduresa assolida per la nanotecnologia basada en els polímers, conjuntament amb les primeres aplicacions d'aquests en l'anàlisi de cèl·lules aïllades i del comptatge de molècules d'ADN, ens indica que aquests materials constitueixen una alternativa viable a les nanotecnologies basades en el silici per a aplicacions biomèdiques.

Paraules clau: aplicacions biomèdiques, polímers, estampació en calent, litografia per nanoimpressió

Abstract

Polymers are excellent candidates for the production of biomedical devices incorporating nanometric structures. Good optical transparency and sealing properties, low fabrication costs, fast design realization times, and, crucially, biocompatibility are all advantages that can be exploited by scientists for the production of such devices. Here, we review some of the methods and techniques used in the fabrication of polymeric nanostructures by pattern replication techniques that may be of relevance in the production of biomedical devices. Emphasis is placed on imprint production of polymeric replicas, with master fabrication using focussed ion-beam technology, as a relatively simple method for reproducibly obtaining large numbers of nanostructures. The use of these structures in polymer-casting techniques is also described, together with some specific fabrication considerations. The maturity reached by polymer-based nanotechnologies, together with the first polymer-based applications for single-cell analysis and for counting single DNA molecules, demonstrates that polymers constitute a viable alternative to silicon-based nanotechnologies for biomedical applications.

Keywords: biomedical applications, polymers, hot embossing, nanoimprint lithography

1. Introduction

The possibility of manufacturing miniature laboratory systems that can be used to produce chemical reactions or manipulate single biomolecules within nanoliter volumes of fluids [1] has been one of the main driving forces behind a

multi-disciplinary effort to develop nanometric apparatuses. In the literature, nanodevices are commonly characterized as having an active part with *at least one* dimension ranging in size from a few nanometers to hundreds of nanometers [2]. However, technologies for realizing planar nanostructured devices with one dimension in the nanometer range have been available for some time. Epitaxial and chemical vapor deposition (CVD) techniques can be used to produce thin films of material with nanometer thicknesses [3, 4] which may then be controllably patterned on a micrometer scale using conventional lithographic techniques. In recent

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years, technologies such as energetic beam lithographies, nanoimprint lithography, and microcontact printing (μ CP) have enabled controllable and repeatable fabrication of nanostructures with *more than one* dimension in the nanometer range, opening the door to a number of possible applications in biomedicine [5]. We discuss the latter type of structures in this review.

Nanometric structures can be fabricated using techniques such as optical, imprint, scanning probe, and soft lithographies. Optical lithography techniques are utilized in the production of nanometer-sized features by using exposure radiations in the ultra-violet (UV) region of the electromagnetic spectrum [6]. However, presently, optical lithography is constricted to a minimum feature size of approximately 70 nm [7]; to progress to smaller dimensions, new methods, such as F_2 laser lithography [8] and extreme UV/X-ray lithographies [8, 9], will have to be developed. Unfortunately, the development of techniques such as these is problematic and embodies the technical challenges inherent in using a resist [10].

Polymer nanofabrication, based on pattern replication techniques, consists of making a master stamp or mold (hereafter referred to simply as the master) which is then used to replicate superficial nanostructures onto a polymer. The comparatively low operating costs and low-level complexity of the replication mechanism, the possibility of producing repeatable nanoscale features over a large area, and the fact that a given master can be used several times [11] make polymer nanofabrication appealing with respect to biomedical device applications. In addition, pattern replication techniques are parallel in nature and side-step some of the disadvantages inherent within other forms of lithography [12]. For instance, the resist problems and environmental issues present in optical lithography, such as optical scattering and the disposal of powerful etchant chemicals, are avoided.

Finally, nanostructures can be produced using scanning-probe technologies [13]. This method involves the movement of individual molecules or atoms via scanning-probe microscope cantilever tips [14]. Unfortunately, the linear nature of this technique means that the production of a relatively large structure requires the moving and positioning a large number of building blocks using a single cantilever tip. This takes time, and therefore the replication of large areas of structures using these methods is impractical. However, recent advances towards multiplexing scanning tunneling microscopy (STM) tips may speed up this technique [15].

In the text that follows, we review some of the methods and techniques currently available for the production of polymeric nanostructures using imprint technologies, with a view to their use in biomedical device production. The types of polymers suited for this task will be outlined before reviewing master fabrication methods. Among the different replication techniques already developed, we will concentrate on hot embossing lithography (HEL) and nanoimprint lithography (NIL), and the use of these methods to produce secondary masters for subsequent polymer-casting techniques. Finally, a number of biomedical applications described in the literature, based on polymer nanostructures, will be outlined.

2. Polymers for nanostructure fabrication

Structural materials for biomedical applications, incorporating nanostructures in this case, need to satisfy a minimum set of requirements. Primarily, the materials need to be biocompatible, i.e., they have to be inert towards the bioanalyte present within the device (possibly via surface modification, such as by the use of an anti-fouling layer [16]). Regarding fabrication, the construction materials need to be inexpensive and simple to machine, permitting the production of complex device structures with dimensions ranging from hundreds of microns down to a few tens of nanometers or less. If required, the materials have to be compatible with fluidic applications and provide rigid, smooth surfaces with dimensions relevant to the biological sample (allowing experiments to be performed under near physiological conditions). Finally, the materials should preferably be compatible with metallization technologies, allowing the user to take advantage of non-invasive, electrokinetic manipulation methods and electrical-based analysis techniques.¹

Common structural materials satisfying the above requirements are based on silicon (e.g., pure silicon, glass, or quartz) and on carbon (in the form of polymers and plastics [20], and, recently, in the development of diamond-based substrates [21]). Following initial interest in silicon-based substrates; attention is now shifting towards the use of polymers in an effort to exploit their inherent advantages [22].

Apart from the desirable optical and physical properties of polymers (see below), their advantages include the fact that polymers are simple to use: the simplest fabrication technique is merely to pour the polymer onto the substrate, within a suitable container, and then bake to harden the polymer (e.g., polymers such as epoxy resins [23]). Polymer structures are cheaper to produce than silicon-based fabrication technologies, thereby normally dispensing with the need for a high-energy apparatus or time-consuming, multi-step fabrication techniques. Finally, past experience has enabled scientists to improve on the natural properties of polymers (e.g., flexibility) to produce polymeric structures with properties comparable to their silicon-based counterparts (with respect to aspect ratio, for example [24]).

Fabrication of nanoscale polymeric structures can be achieved using a number of polymer types; most commonly including thermoplastic [25] and elastomeric [26] polymers. Thermoplastic, amorphous polymers are used for imprinting because the viscosity of the polymer is largely dependent on temperature. Near its glass transition temperature (T_g), the polymer softens and can be deformed into the shape of the mold with the help of applied pressure. Room-temperature imprinting can be achieved through

1. This determines that the electrode materials used in the device also need to be inert with respect to the sample, both when passive and upon activation. The electrode materials should also be patternable so that the electrodes can be easily positioned within the fluidic chambers of the device [e.g. 17], or nearby, for applications such as dielectrophoresis [18] and electrorotation [19].

careful choice of a polymer with the appropriate melting point and T_g [27]. Polymers are also now being developed with nanofabrication in mind, displaying properties such as higher T_g [28] that are desirable for some nanoimprinting applications.

The polymer utilized most frequently in imprinting processes is poly(methylmethacrylate) PMMA [29]. PMMA is an amorphous, thermoplastic polymer with a $T_g \sim 105^\circ\text{C}$. It is hard and stiff, with low thermal-expansion and pressure-shrinkage coefficients ($\sim 5 \times 10^{-5}$ per $^\circ\text{C}$ and $\sim 5 \times 10^{-11}$ Pa^{-1} , respectively), making it a perfect candidate for imprinting techniques. PMMA does have the disadvantages of brittleness and notch sensitivity, as well as poor fatigue and solvent resistances. However, this is offset by its optical properties (colorlessness, transparency, and UV resistance), which, together with its excellent optical clarity, make it ideal for use in the production of biomedical apparatuses.

For polymer-casting techniques, the polymers need to be elastomeric, which allows them to conform to the superficial structures in the master. A commonly used example of this type of polymer is poly(dimethylsiloxane) PDMS [30]. PDMS is an elastomeric polymer with good thermal stability and homogeneity, characteristics required during the curing step, and it is non-hygroscopic and isotropic. It is a good candidate for the production of apparatus for biomedical applications due to its chemical inertness, durability, and optical transparency down to 300 nm. Furthermore, it is deformable enough, after curing, to make conformal contact to the surface of a substrate or covering material, greatly facilitating any attempts at bonding to the material. Structures with dimensions greater than 1 μm are easily reproduced, with good resolution using a soft polymer (Young's modulus ~ 3 MPa), such as Sylgard 184 (a PDMS-based product, Dow Corning, USA); however, harder materials (Young's modulus ~ 10 MPa) are required to achieve optimal resolution [31]. Control of the amount of polymer cross-linking means that the Young's modulus of PDMS can be "tuned" to suit the requirements of the application [26].

3. Fabrication methods

3.1. Master fabrication

The first step in all replication techniques using polymers consists of fabrication of the master. Available fabrication technologies with nanometer resolution include optical, scanning-probe, and energetic-beam lithographies. These nanometric lithographic techniques are most often used in conjunction with standard microfabrication technologies for the production of micrometer-sized structures within devices. However, the previously mentioned disadvantages of the optical and scanning-probe methods suggests that, at least for the present time, energetic-beam nanofabrication technologies offer the most efficient method of producing masters for replication technologies.

Nanolithographic methods based on energetic beams, for the production of large or complex structures, usually involve long fabrication times (as only a small volume of material is pat-

terned per second) and high equipment/energy costs (due to the need to form the energetic particle beam under high vacuum conditions), making them impractical for mass production [32]. However, the high-quality nanometric structures (e.g., with aspect ratios of 25 and above [32]) that are produced using these methods make them ideal for the fabrication of masters for replication technologies.

Electron-beam lithography (EBL) is the most common energetic-beam system. The method uses high-energy electrons (100–200 KeV) from a small electron probe (1–10 nm) to write directly onto a photoresist, causing either the breakage or formation of bonds within the resist material. After this patterning, the excess resist is removed using a chemical developer, and the substrate is etched using a chemical etchant. With EBL, trenches down to 30 nm wide (widths down to 7 nm have been reported [33]) can be satisfactorily fabricated, and masters for NIL with comparative dimensions have been produced this way [34]. However, EBL resolution depends heavily on the resist properties, and the resist is often the limiting factor for this technique.

Focused ion beam (FIB) milling can be used to directly remove material from a required substrate [35]. FIB milling is similar to EBL in terms of application; however, there is a fundamental difference between the two techniques. The ions used in FIB consist of charged atomic matter many orders of magnitude more massive than the electrons used in EBL. Thus, the accelerated ion beam can easily be used to dislodge the atoms of the substrate surface and hence mill away unwanted material. This therefore precludes the need for a resist (and its associated chemistry) as required in EBL. In this way, FIB milling has been used to produce trenches 50 nm deep and ~ 8 nm in width, and electrodes with a 30-nm spacing [36]. Deep ion-beam lithography is a new technique that can be used to produce 3-D nanostructures and is particularly adept at creating side walls with almost 90° angles [37] and aspect ratios up to 100 [38]. By using FIB, masters for imprint technologies containing nanometric dimensioned structures can be fabricated out of materials such as silicon (including silicon dioxide and silicon nitride), metals, and polymers.

Although the FIB technique is most useful as a direct method of nanostructure fabrication, it can also be utilized in the production of structures in conjunction with a resist [39] and for patterning surfaces by ion implantation [40]. FIB can also be used to image surfaces and to machine thin sections of a sample for imaging [41], while a commercially available FIB apparatus incorporates an inbuilt scanning-electron microscope (SEM) for real-time process imaging [42]. Finally, as an additional technique, FIB can be used to deposit material onto a substrate surface [41]. A gas precursor is introduced into the path of the ion beam, which is then broken down by energetic secondary electrons and deposited on the surface. This deposition can be performed on conducting and insulating substrates alike, which is particularly valuable for the production of electrodes or the protection of samples containing environmentally sensitive materials. The wide range of materials available for etching and deposition makes FIB one of the more versatile apparatuses for nanotechnology production.

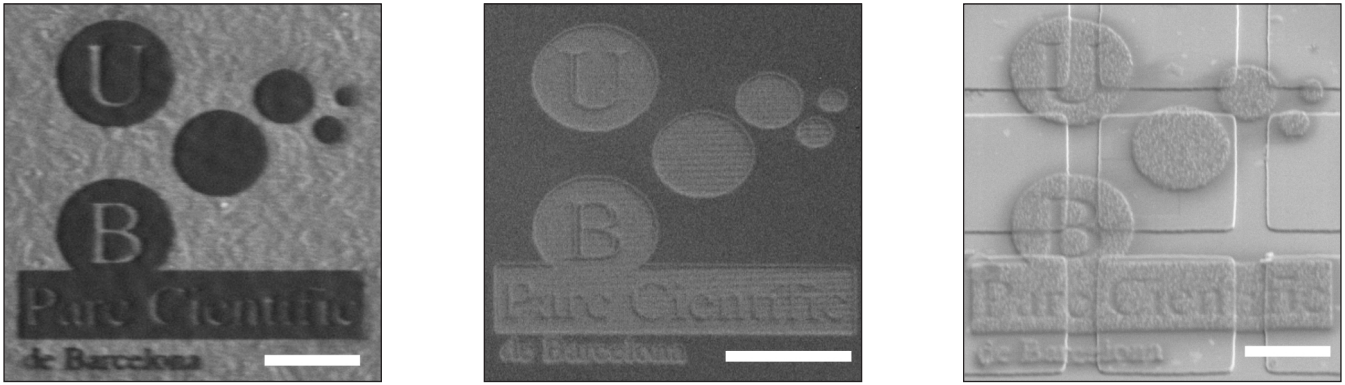


Figure 1. SEM images of (a) a PCB logo focused ion beam (FIB)-etched into polysilicon (bar = 2 μm) displaying features with nanometric dimensions; (b) a 75-nm-deep PCB logo etched in silicon-nitride-coated silicon (bar = 50 μm) used for producing polymer replicas; (c) a PCB logo deposited using tetraethylorthosilicate (TEOS) (bar = 5 μm).

Figure 1 gives an example of the versatility of FIB lithography via the etching of substrate materials, such as polysilicon (which can be used as a sacrificial layer in the production of a silicon-based devices) and silicon-nitride-coated silicon (a common material for the production of imprint lithography stamps), and the deposition of tetraethylorthosilicate (TEOS).

3.2 Fabrication of nanostructures in polymers using replication techniques

3.2.1 Hot embossing lithography

Hot embossing lithography is an imprint technique in which a polymer substrate is imparted with a patterned structure by embossing, using a master, at elevated pressures and temperatures (Fig. 2) [43]. The embossing is performed on a press system within which the pressure and temperature can be controlled. Within the press, one surface holds the master, with the negative of the desired pattern on its surface, and the other surface holds a sheet of polymer, such as PMMA [11]. The temperature of both surfaces is increased under vacuum (which helps prevent the formation of air bubbles in the polymer [12]) after which they are brought into contact, and the polymer is embossed at a controlled force for a specified time (Fig. 2a). To aid in separation of the master and the polymer, while retaining the embossed structure, the temperature is lowered to below that of the T_g of the polymer before removing the embossing force. The master and polymer can then be

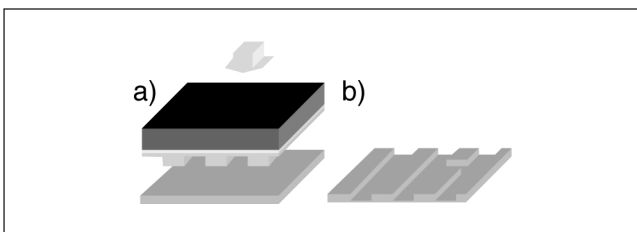


Figure 2. Schematic diagram of hot embossing lithography (HEL). The master containing the superficial structures, previously milled in the surface, is pressed into the polymer under temperatures above that of the glass transition temperature (T_g) of the polymer (a). After a predetermined time period, the temperature is reduced and the pressure is released, allowing the master to be separated from the polymer, revealing the superficial structures replicated in the polymer surface (b).

separated, and a polymer surface containing the required structures, which are the negative of those on the surface of the master, is obtained (Fig. 2b). The lateral accuracy for the HEL technique is approximately $\pm 3 \mu\text{m}$, while height resolution is in the range of tens of nanometers, depending on the applied pressure and temperature.

3.2.2 Nanoimprint lithography

Like HEL, NIL is a method for replicating structures by means of applied pressure and temperature [44]; but, unlike HEL, it allows replication of nanostructures with both a lateral and a vertical resolution that is well-inside the nanometer range. The imprinting process (Fig. 3) is similar to that for hot embossing, the main difference being that the substrate is a thin layer of polymer deposited onto a suitable substrate, rather than a free-standing polymer sheet. Again, the polymer is heated to a temperature above its T_g , and elevated pressures, normally higher than those used for HEL, are applied to replicate the nanostructures of the master in the polymer film (Fig. 3a). A thin layer of polymer remains within the compressed areas of the polymer (Fig. 3b), which helps to avoid contact between the master and the substrate, thereby preserving the master and prolonging its life-time. In this way, a master can be used up to ~ 40 times. An example of the polymers used in NIL is 950k PMMA (PMMA with a molecular weight of 950,000 in anisole solvent),

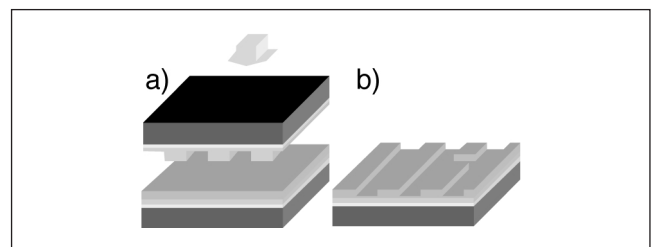


Figure 3. Schematic diagram of nanoimprint lithography (NIL). The technique is similar to that for hot embossing (Fig. 2), with the exception that now the polymer is a thin film that has been spin-coated on a suitable substrate. To avoid thermal expansion problems, the master and the substrate on which the polymer is spun are made of the same material. Again, the master is pressed into the polymer under temperatures above that of the T_g of the polymer for a period of time (a) before the temperature is reduced and the pressure is released, yielding superficial structures replicated in the polymer surface (b).

which can be spun onto a suitable substrate. Both thermally and photochemically cross-linkable polymers with low T_g characteristics [45], and semiconducting polymers [46] have also been evaluated for use in NIL, the latter with a view to the production of organics-based electronics. In NIL, the process time and temperature are dependent on the polymerization rate of the polymer. The substrate and the master tend to be the same material in order to avoid the problem of different temperature-dependent expansion rates between the two pieces during the imprint process.

It is worth mentioning that the imprinting step in NIL is often used as the first step of a pattern-transfer process onto a suitable substrate, or in the fabrication of electrodes. Subsequently, metal deposition, anisotropic etching, and lift-off processes are used to produce the final structure. Note that, in this case, the final structure is not fabricated from polymer, but from materials such as silicon or metal. NIL has been used to produce 10-nm-wide PMMA structures [34], and 100-nm-wide trenches, with a spacing of 300 nm, over a 6-inch silicon-wafer substrate [47].

One of the problems that may occur in HEL and NIL is adhesion between the master and the polymer being imprinted. Avoiding this problem requires prior knowledge of the physics of adhesion [48] in order to guide the choice of suitable material combinations for the master and the polymer. To avoid sticking, the material from which the master is made should be hydrophobic, for example, silicon nitride [49] or nickel [50]. Careful control of the imprinting conditions, such as by releasing the imprinting force at temperatures close to the T_g of the polymer, will also help to eliminate sticking problems. If sticking still occurs between the master and the polymer, anti-sticking layers can be applied, in which materials such as halogenated silanes [51] or PTFE [52] are deposited on the master from the vapor phase via room-temperature adsorption or plasma-deposition techniques. These materials increase the hydrophobicity of the master surface, reducing the possibility of adhesion to the polymer; however, they also increase the complexity of the fabrication technique and thus are used as a last resort.

3.2.3 Polymer casting

Soft lithography techniques [53] have in common the use of an elastomeric polymer and low contact forces, and include μ CP [54] and polymer casting [55]. The latter involves the production of a polymeric master replica by casting the liquid prepolymer against a master that has patterned relief structures on its surface [53] (Fig. 4). The polymer is poured onto the master (Fig. 4a) within a suitable container and allowed to settle into the pattern on the master (Fig. 4b). The entirety is baked to harden the elastomeric polymer and then the polymer is simply peeled off the master (Fig. 4c), breaking the weak physical bonds formed between the polymer and the master during the baking step. The polymer has to be elastic enough to assure conformal contact with the master, but rigid enough to maintain the stability and lateral resolution of any small structures. Examples of the elastomeric polymers used in this technique are poly(styrene) (PS) [56] and, commonly, poly(dimethylsilox-

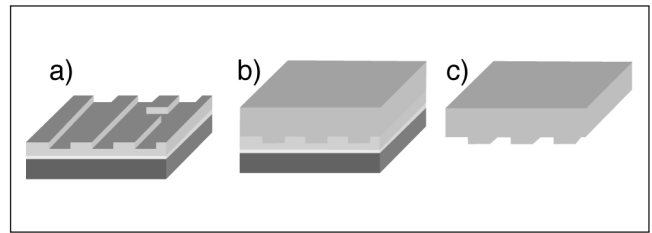


Figure 4. Schematic diagram of polymer casting. Here, the master containing the superficial structures (a) is placed into a suitable container with the structures uppermost. An elastomeric polymer is poured onto the master and cured by heating (b). When the polymer is fully cured, it can be peeled off the master, revealing the replicated superficial structures (c).

ane) (PDMS), which has been used to mold 200-nm-wide electrode structures with a 50-nm gap [57].

Polymer structures fabricated via casting techniques can be used either directly or as a template for the transfer of structures onto other substrates, e.g., in μ CP. In this process, a polymeric master, produced using the above-described method, is coated with an “ink” and brought into contact with the substrate. The ink organizes itself in areas of mutual contact between the master and the substrate, forming a patterned self-assembled monolayer (SAM) on the substrate surface. μ CP has been used for alkane-thiol [58] and protein [59, 60] patterning and can serve as the basis for wet etching of the substrate. Possible applications of this technique include electrode fabrication and selective chemical deposition; for example, 100 nm wide trenches have been fabricated using μ CP [61].

3.3 Device production

After production of the master using lithographic techniques, polymeric devices can be fabricated using a combination of the above-described methods. The master can be formed in one of two ways (Fig. 5): (1) the superficial features can be machined so that they are below the substrate surface (a negative stamp; Fig. 5a), or (2) they can protrude above the surface of the substrate (a positive stamp; Fig. 5b). The former method has the advantage that less material needs to be removed from the master, reducing its fabrication time. Using these masters to perform a single lithography step produces structures in the polymer that are reversed with respect to the primary master. Performing a subsequent step, such as polymer casting replication using the first polymer replica as a secondary master, produces superficial structures with the same orientation as the original, primary master. Therefore the fabrication protocol can be designed, depending on the required replica topography (positive or negative), so as to minimize the time and ex-

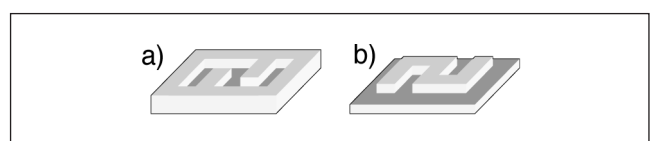


Figure 5. Diagrams of (a) a negative master, in which the superficial features are below the surface of the master material, and (b) a positive master in which the superficial features protrude above the surface of the master.

pense required to produce the primary master (which is usually the most time-consuming/expensive step).

Often, once fabricated, a polymeric structure needs to be sealed, e.g., for fluidic applications, as much to prevent evaporation of the nanoliter amounts of solvents as to keep them confined within the device structure. In this respect, polymers have an advantage over silicon-based materials because they can be thermally annealed at low temperatures, eliminating the need for chemical adhesives or high-temperature bonding [27]. Apart from annealing, other common bonding techniques include lamination, plasma bonding, and solvent-assisted bonding [62]. Lamination techniques involve the bonding of dissimilar polymers at elevated temperature using lamination materials [63]. Plasma bonding uses a beam of ionized gas particles (normally oxygen) to activate the surfaces of the polymer prior to placing them in contact with each other. The plasma forms hydroxyl (-OH) bonds on the polymer surface which, when the two polymer pieces are brought into contact and lightly heated, produces a permanent adhesion via the formation of C-O-C bonds [64]. This treatment also temporarily makes the non-bonded areas hydrophilic, a valuable characteristic when designing fluidic applications as osmotic flow is eased [20]. Similarly, in solvent-assisted bonding, the polymer surface is

activated by immersing the polymer in a solvent for a period of time (e.g., ethanol for PMMA bonding [62]) and then heating the two polymer pieces within a press. In this case, the surface of the polymer is partially dissolved; then, as the solvent evaporates, the polymer at the interface resets, causing the two pieces to adhere to each other. Most of these bonding techniques have been applied to microstructures; however they are also expected to be valid for nanostructures, although requiring a higher degree of precision.

Finally, after sealing the structure, a fluidic device needs to be connected to the outside world using fluidic connections, which can be realized through a micrometer-sized pool (which is interfaced to the nanofluidics) to external pumping systems [65]. A flow representation of a fluidic device fabrication process, including two replication steps, is presented in Fig. 6.

4. Examples of biomedical applications using polymer nanostructures

Devices containing nanostructured elements are expected to have biomedical applications involving the manipulation, characterization, and analysis of single cells and single biomolecules. On-chip devices, designed to perform single-cell analysis (Lab-in-a-Cell) [66] or functions such as trapping, sorting, and analysis of single biomolecules [5], open up a vast field of application whose limits cannot be foreseen at present. The first examples of biomedical applications using silicon-based nanotechnologies have already been developed, and their polymer counterparts are currently starting to appear.

Polymeric nanodevices that may have considerable relevance in biomedical applications are those consisting of non-fluidic open systems specifically designed to study the local chemical, electrical, and mechanical properties of single cells. Non-fluidic based systems are defined as those based on nanostructured open surfaces where a variety of functions can be performed and which do not require fluid or particle flow. These nanoscale structures are in contact with the cell at a number of sites and can provide a variety of experimental options, for example, the success of cell culturing can be investigated on different nanostructures. Studies aimed at investigating the adhesion of fibroblasts to 27-nm-high islands of PS showed that, after initial rapid adhesion and cytoskeletal formation on the polymer surface, compared to a control surface, the cells formed poor contacts [67]. In this case, the PS was patterned via polymer demixing so as to produce randomly ordered columns of polymer. In a similar study, directed cell culturing on polymers that had been chemically modified to produce nanostructured surfaces was investigated through fibroblast adhesion to polymers such as polycaprolactone and polyurethane [68]. The polymers in this case had been briefly treated with a corrosive agent in order to structure their surfaces. Further studies involving fibroblast and collagen cell culturing on polymer substrates with ordered structures [69] have been reported; however, in these cases the structures used to control cell growth were micrometer sized. This highlights that, while a number of investigations studying cell/structure interac-

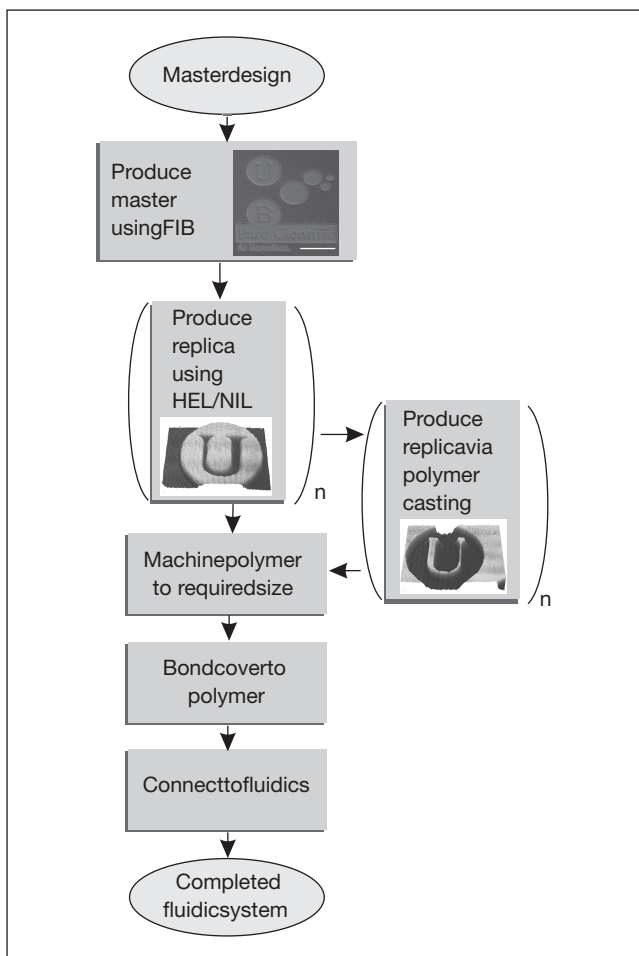


Figure 6. Flow diagram detailing an example of the production of a fluidic device for biomedical applications. As an example of the structures produced, white light interferometric images are presented for HEL/NIL-fabricated and polymer-cast structures using a FIB-etched silicon nitride master (SEM image from Fig. 1b).

tions at the cellular scale are currently underway, the study of these interactions should perhaps be extended to the use of more ordered nanostructures, obtainable with the aforementioned replication techniques.

Different nanoscale sites can be chemically modified to investigate cell interactions under different surface conditions. Nanofabricated polymer structures generated by ion beam lithography have been produced on PMMA for use as biological arrays [29]. The hydrophobicity of the polymer surface can be altered by the implantation of calcium (Ca^+) or phosphorous (P^+) ions onto the nanomachined PMMA. Possible applications for the final ion-implanted devices include osteoblast cell adhesion and cultivation with a view to bone tissue engineering [70]. As an extension of this technique, and utilizing future sensor miniaturization technology, nanostructures could be produced to hold individual sensors, which would allow exploration of the localized chemical and physical conditions on the cell surface.

A second subset of polymeric nanodevices that may be of considerable interest in biomedicine are those consisting of fluidic systems. In fluidic-based applications, the flow of fluids or particles is an essential ingredient in the performance of the device. These devices usually require the fabrication of sealed structures in the form of nanochannels or nanoreservoirs containing nano-obstacles or similar nanostructures. Nanostructures are usually designed to perform a variety of biological functions, such as continuous sorting, sizing, and the analysis of single biomolecules [5].

One example of a biomedical application based on polymer nanotechnology was presented recently [71]. The device consists of a PDMS pore, 3 μm long and 200 nm in diameter, connecting two 5- μm -deep reservoirs. It was constructed by replica molding using a master fabricated by a combination of standard microfabrication techniques and EBL. The PDMS part of the device is sealed onto a glass substrate containing previously defined platinum electrodes. A detection method based on the resistive pulse technique of particle sizing allows the device to detect and identify the size of small particles passing through the nanopore. Applications of the device to the detection and counting of single DNA molecules [71] and to the direct detection of antibody-antigen binding processes [72] have been successfully demonstrated.

In addition to the previous examples, it is worth mentioning a number of biomedical applications using silicon-based nanotechnologies, since these types of systems could alternatively be fabricated using polymer-based nanotechnologies. Examples include: the stretching of single DNA molecules by means of entropic forces located at the interface between regions of different entropies and generated by arrays of nanopillars [73], which gives rise to a sorting device through the application of a pulsed voltage [74]; the sorting of DNA molecules by the rectification of Brownian motion through an array of asymmetric micro/nanopillars [75]; the scanning of the structure of stretched single DNA molecules by near-field optical methods through nano-slits [76]; and the sizing and counting of single DNA molecules on T-shaped nanofluidic structures [77] and on a confined entropic structure [78].

Due to the inherent advantages polymer-based devices have over devices based on silicon, together with previous experience in polymer fabrication at the microscale [30], it is likely that, in the near future, the importance of polymer nanotechnologies will grow considerably within the biomedical field.

5. Conclusion

This review has described some of the methods used in the fabrication of polymeric nanostructures, via pattern replication techniques, for use in biomedical applications. Polymer-based nanodevices have several advantages over silicon-based devices; among others, their low cost, biocompatibility, transparency, and rapid prototyping. The examples given here show that polymeric replication techniques based on nanoimprinting and polymer casting can be used to produce polymeric nanometric structures with high resolution and repeatability. The development of biomedical devices incorporating these types of polymeric nanostructures is currently in progress, aided by the production of both nanopatterned polymer surfaces for single-cell analysis and nanofluidic systems for the sizing and counting of single DNA molecules. Future developments along similar lines should soon allow biomedical experimentation on individual cells and biomolecules using very low cost, all-polymer nanodevices.

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