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Shaping biodegradable polymers as nanostructures: Fabrication and applications

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The interest in micro- and nano-devices based on biodegradable polymers for *in vivo* applications is growing rapidly and the key to these applications lies in the fashioning of features analogous to the size of cells. This paper presents fabrication techniques for leveraging micro- and nanostructures on biodegradable polymers. Innovative approaches such as replication molding, laser interference lithography, nanosphere lithography, and block copolymer lithography are discussed. These techniques demonstrate excellent potential for fabricating biodegradable polymeric devices in both a laboratory and industry scale.

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Introduction

Micro- and nano-devices have had a significant impact on medical technology, greatly enhancing the efficacy of many existing drugs and enabling the construction of entirely new therapeutic modalities. The success of these next-generation medical devices is contingent on the leveraging of features unique to the size of organelles and cells. Conventional fabrication techniques have been successfully employed to create medical devices on silicon [1], glass [2], and thermoplastic elastomers [3]. For example, a silicon-based device incorporates multiple sealed small compartments, which are opened on demand to deliver a dose of drug [1]. A small-scale, free-floating polymeric drug delivery patch adheres to the mucosal membrane in the intestine, shielding the drug from luminal proteolytic enzymes [3]. The limitation with these nondegradable devices is their subsequent surgical removal and the inherent difficulty in retrieving such devices from tissues [4]. Thus, it is advantageous to employ biodegradable polymers, which are designed to break down into various non-toxic products over time [5]. However, the fabrication of micro- and nanostructures on biodegradable polymers remains a challenge [6]. There have been significant efforts devoted to develop fabrication platforms for forming micro- and nanostructures on biodegradable polymers and in the following sections this paper will review the current approaches that have been applied and their applications.

Replication molding techniques

Replication technologies are proven useful for biodegradable polymer microfabrication because the principles behind these processes are straightforward and well known. The underlying principle is the replication of a microfabricated mold tool, which represents the inverse geometry of the desired polymer structure [7]. The expensive microfabrication step is only necessary for the initial fabrication of the master structure, which then can be replicated many times into a polymer substrate. In addition to the cost advantage,

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replication techniques also offer the benefit of freedom of design: the master can be fabricated with a large number of different microfabrication technologies, which allow various geometries to be realized. A drug delivery device that consists of a reservoir-containing poly(lactic acid) substrate and a poly(lactic-co-glycolic acid) membrane is an illustrative example [8]. Various cells recognize three-dimensional geometrical configuration on the surfaces and their growth can be guided and controlled by microfabricated grooves. Examples of applications include orienting cell attachment and controlling subsequent cell proliferation [9].

Microimprinting lithography

Microimprinting lithography, also known as compression molding or hot embossing, is a simple process that is widely used to fabricate microstructures for data storage and microfluidic applications [10,11]. The basic principle of microimprinting lithography is to heat a polymeric substrate near or above its glass transition temperature (T_g) and then stamp it against a bas-relief mold for pattern transfer. After time, the system temperature is gradually lowered to release internal stress from the crystallization and different thermal expansion coefficients of the master and the polymer film. The resulting biodegradable polymer film can be peeled off easily with a surface pattern that is inversed to the one of the quartz mold. As shown in Fig. 1, a periodic array of alternating rectangular holes was generated on poly(ε-caprolactone) (PCL) by pressing a preheated quartz mold (~40 °C) against the PCL substrate.

Microimprinting lithography is a rapid and relatively inexpensive technique capable of generating high-resolution nano-scale features on both planar and nonplanar surfaces without the use of chemical additives. Although the master can be used multiple times, cleaning is occasionally necessary after each use. Additionally, the method requires the thermoplastic elastomer to have a good thermal stability near the glass transition temperature T_g.

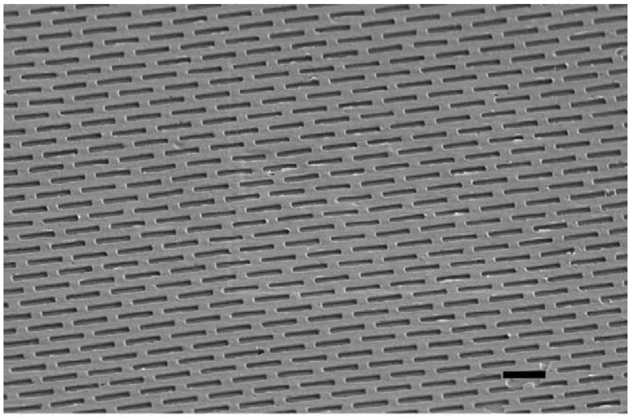

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Figure 1. Hot embossed periodic rectangular hole array on PCL. Holes are 4 μm in length, 1 μm in width, and 0.5 μm in depth. Scale bar indicates 5 μm.

Soft lithography

Soft lithography is a collective name for a set of non-photolithographic techniques that are based on self-assembly and replication molding [12]. Soft lithography uses a bas-relief patterned elastomer as a stamp to generate patterns ranging in size from 30 nm to 100 μm. The primary element of this fundamental technique is the stamp that can be prepared by cast molding a cross-linkable elastomer (e.g., polydimethylsiloxane (PDMS)) over a master with surface relief structures. The casted polymer is then heated and solidified within a few hours via hydrosilylation reaction and can be peeled off easily for subsequent use.

Pattern transfer can be realized by solvent-assisted molding, in which a polymer solution (e.g., PCL in chloroform) is casted between a stamp and a non-stick surface. The patterned polymer film can then be peeled off after the solvent is evaporated. The great potential of this technique lies in its capability of rapidly generating not only micro- and nanotopographic structures but also surface chemical patterns on biomaterials, which facilitates the study of cell reaction to nanostructures [13].

Laser interference lithography

Laser lithography is a minimally invasive, rapid technique for patterning materials on the micro-scale [14]. It is a single step process that emits photons with sufficient energy to break the chemical bonds of the target material directly. The irradiated material is dissociated into its chemical components and no liquid phase transition occurs thereafter. This important feature makes laser micromachining very attractive for biodegradable polymers, because thermal damage to the neighboring areas can be minimized [15].

Laser interference lithography is based on selectively exposing or ablating a photosensitive substrate by the interference of two coherent laser beams. A laser beam is divided using a beam splitter, and the split beams illuminate the substrate from opposite directions, forming an angle 2θ. This procedure creates an interference pattern of sinusoidal intensity. The periodicity, p, of this standing wave is controlled by the beams incident angles and the laser wavelength, λ, $p = \lambda/(2 \sin \theta)$ (Fig. 2). Material is removed where local laser intensity is above the ablation threshold, whereas it remains almost intact where the energy minima lie. This method produces a pattern of parallel lines or dots.

The advantage of using such a simple method is that submicron structures, such as nerve guides, can be obtained over a large field size without solvent or expensive masks involved. However, owing to its periodic nature, this method is less flexible compared to replication techniques in terms of producing arbitrary nanostructures. Another potential

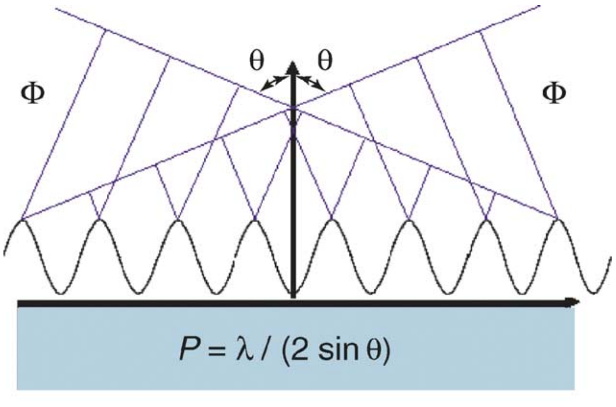

Figure 2. Schematic illustration of laser interference lithography. A laser beam is divided using a beam splitter, and the split beams illuminate the substrate from opposite directions, forming an angle 2θ. The periodicity, p, of this standing wave is determined by the beams incident angles and the laser wavelength, λ, $p = \lambda/(2 \sin \theta)$.

feature of laser ablation is the capability of surface modification of microfabricated structures concurrent with structure formation (e.g., channels) [16]. Many reactive species are formed both at the polymer surface and in the gas phase during the laser ablation process. The incorporation or reaction of these ablation products at the nascent channel walls can result in surface chemical functionality that is significantly different from that in the bulk of the polymer [17]. For example, incorporation of nitrogen or oxygen can give rise to amino, hydroxyl, carboxylic, or phenolic functional groups on the surface [18]. These types of surface functionalities are thought to play an important role in electroosmotic flow, a commonly used means to pump solution through microchannels [19].

Nanosphere lithography

The aforementioned ''top-down'' nanofabrication platforms are useful in surface patterning, however, cost and process considerations limit their applicability. Fabrication of periodic nano-scale structures over large areas using self-organizing systems is of great interest because of the simplicity and low cost. These techniques utilize self-assembly, which uses chemical or physical driving forces. Self-assembly techniques are ''bottom-up'' methods that allow simple control of the pattern size and massive parallel processes that use porous alumina [20], block copolymers [21,22] and colloidal particles [23–25]. Spherical colloids are commonly used as building blocks to create polymeric micro- and nanostructures because of the relative ease of forming long-range ordered structures. One important application of spherical colloidal self-organizing system is nanosphere lithography, which utilizes the unique shadow mask [26,27] or optical enhancement effect [23,24].

Laser-assisted nanosphere lithography

Direct laser patterning provides a micron-scale resolution due to the optical diffraction limit of the laser wavelength. To achieve nano-scale resolution, near-field photolithographic techniques were developed by delivering a laser beam through a hollow near-field tip or illuminating the tip of a scanning probe microscope with a pulsed laser [28,29]. However, these near-field nanolithographic techniques have rarely been used in an industrial setting due to their limited throughput, hollow tip blockage, and difficulty in process control.

It has been found that a spherical particle can act as a lens, and therefore, intensifies the incoming laser beam if the sphere diameter is larger than the laser wavelength. Near-field enhancement can play an important role if the diameter of the spherical particle is equal or smaller than the wavelength. We developed an approach involving the illumination of a nanometer-sized sphere array using a laser beam to pattern a solid surface in a massively parallel fashion [23,24].

To demonstrate this technique on patterning PCL, we dropped a colloid of silica spheres (diameter = 640 nm, in water) onto the PCL substrate. As the solvent evaporated under a controlled humidity, capillary forces drew the nanospheres together, and the nanospheres reorganized themselves in a hexagonally close-packed pattern on the substrate (Fig. 3A). The as-deposited nanosphere array can include a variety of defects that arise as a result of nanosphere polydispersity, site randomness, point defects, and line defects. A variety of methods have been used to minimize the defects, for example, the Langmuir–Blodgett technique [30]. The PLC sample with an array of nanospheres on the surface was irradiated using a single pulse of an UV laser, for instance, second or third harmonic wave of a Nd:YAG laser or an ArF excimer laser. Nanospheres in the laser exposed area were ejected, leaving holes with the same hexagonal pattern as the spheres formed on the PCL surface (Fig. 3B). The rest of the spheres can be washed off by ultrasonic treatment in water.

This laser-assisted nanosphere lithography method is a simple and clean (solvent free) process for producing nano-pit arrays that can improve cell adhesion or serve as drug reservoirs where precisely controlled geometry is not required. Moreover, this method is not limited to flat substrate by its nature.

Nanosphere ''shadow mask'' lithography

A monolayer of close-packed nanospheres can be conveniently used as a ''shadow mask'' (a physical barrier that has through voids) in selective material deposition or etching [26,27]. The patterning process begins by the deposition and formation of monolayers of hexagonally close-packed nanospheres using self-assembly techniques. Metal is then deposited through the monolayer mask, partially filling the gaps between the nanospheres. A lift-off process then removes the monolayer of nanospheres and a periodic array of triangu-

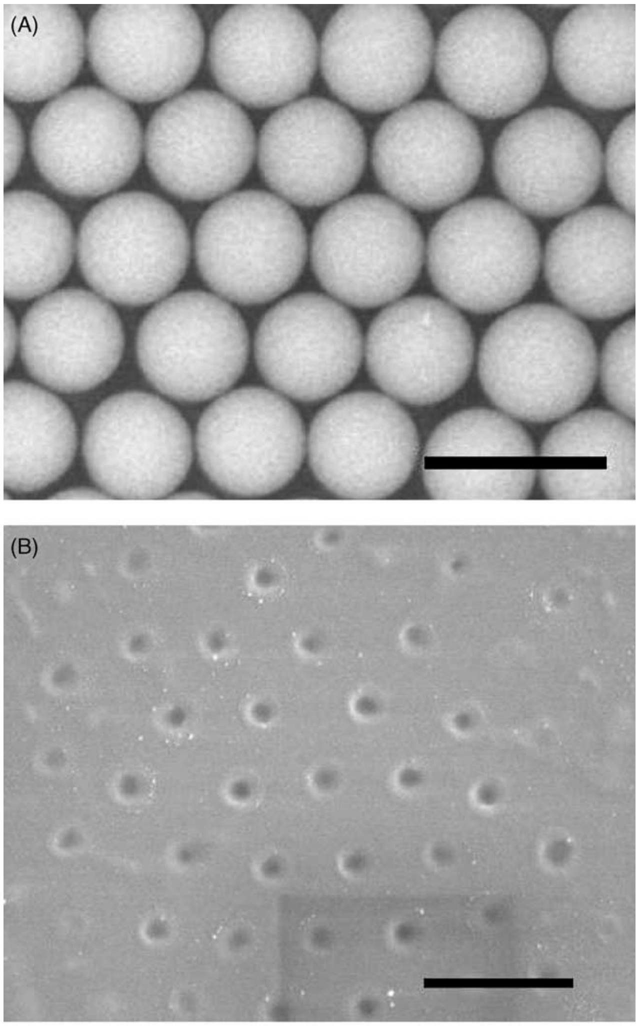

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Figure 3. (A) Hexagonally close-packed silica sphere (640 nm in diameter) array. The size of defect-free grains is ~200 μm^2. (B) Nano-pit array after laser irradiation. The diameter of the pits is about half of that of the spheres and the depth is ~200 nm. Scale bars indicate 1 μm.

larly shaped nanometer-scale particles consisting of the metallic material is left on the substrate. The metallic particles then in turn act as a mask for reactive ion etching (RIE). The part of the substrate that is not covered by the particles is removed. In addition, the nanosphere layers can be used directly as masks for RIE [26]. Although large defect-free areas have been obtained using nanosphere lithography, the range of types of patterns and pattern conditions inherently limits the process. Moreover, the RIE process can significantly alter the surface chemistry by re-depositing various byproducts, which might not be suitable for clinical usage.

Block copolymer lithography

Block copolymers, which microphase-separate into a monolayer of densely packed, periodic, cylindrical or spherical structures on a surface, have been used for patterning or templating various thin film materials. This is a novel technique for nanolithography, which can be an attractive alternative to photolithography using hard (UV or X-ray) radiation. In block copolymer lithography for semiconductors, masks were made from block-copolymer films (i.e., polystyrene-*b*-polyisoprene copolymer), in which one component (i.e., polyisoprene) has selectively been removed [21,22]. The feature density achieved is approximately 10^{11} holes/cm^2. These voids provide an effective thickness variation in the polymer film, which functions as a mask for pattern transfer using RIE (Fig. 4). Many high-density nanostructures, such as posts or holes, have been made by etching, electroplating, or chemical reactions using block copolymer lithographic templates. In these nanostructures, the feature size and feature spacing are primarily controlled by the molar mass and chemistry of the block copolymer, which is well defined by the polymer synthesis route.

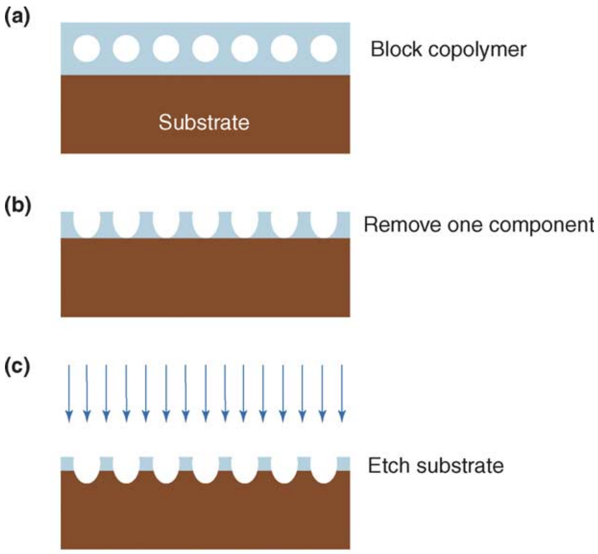

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Figure 4. Schematic illustration of block copolymer lithography: (a) a block copolymer layer is spin-coated and annealed. It phase-separates into close-packed periodic structures. (b) One component is selectively removed. (c) Pattern transfer through selective etching of the substrate material.

A trilayer pattern transfer technique has been introduced to pattern arbitrary surfaces [22]. An organic–inorganic composite mask consists of a top layer of spin-coated block copolymer, a plasma enhanced chemical vapor deposited silicon nitride intermediate layer, and a spin-coated and annealed polyimide bottom layer on a silicon substrate. The addition of intermediate layers enhanced the selectivity in the RIE.

Nanostructures fabricated by block copolymer lithography lack long-range order because the typical ‘‘grain size’’ over which the block copolymer features are ordered is in a submicron range. ‘‘Top-down’’ lithographic techniques can be combined to significantly improve the long-range order. A

Table 1. Comparison summary

Name of technologies	Replication techniques	Interference lithography	Nanosphere lithography	Block copolymer lithography
Pros	• Arbitrary patterns • Multiple use of the mold • Compatible with non-planer surfaces • High fidelity	• No masks needed • Single step process • Solvent free • High uniformity in large area	• Simple process • Massive parallel • Solvent free • Compatible with non-planer surfaces	• Massive parallel • Good size control • Feature size ~ 10 nm
Cons	• Mold contamination • Might need solvent	• Simple periodic structures only	• Limited size, geometry and quality • RIE byproducts	• Multiple steps • Solvent required • High processing temperature • RIE byproducts
Cost	Master might be expensive	Intermediate	Low	Low
References	[7–12]	[13–20]	[24–27,30]	[20,21,31–34]

process that uses topographical patterns on a substrate to orient the growth of a thin film is known as graphoepitaxy. A block copolymer can form a long-range ordered sphere array in the grooves of a silicon oxide topographic relief on a silicon substrate [31]. Similarly, by using advanced extreme ultraviolet interference lithography, a block copolymer laminar periodic (period, approximately 48 nm) thin film mask can be produced with 5 μm defect-free grain [32]. Recently, nanoimprinting lithography was applied to locally control the self-assembly of diblock copolymers and determined the precise positioning of the phase-separated domains via topography of the mold, rather than the substrate [33]. Additionally, micro-contact printing was used to distribute polystyrene-*b*-poly(methylmethacrylate) ''ink'' onto the substrate with a micro-meter resolution [34].

Although this block copolymer lithography technique has not been applied to patterning polymeric materials (i.e., biodegradable polymers) to our knowledge, we believe by carefully choosing the masking material and deposition processes it is feasible to produce nanostructures on a surface of a biodegradable polymer. Similar to nanosphere ''shadow mask'' lithography, where RIE is used to transfer patterns, features with high aspect-ratio (ratio of height/width), such as deep holes or reservoirs, could be produced for drug delivery.

Related articles

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Conclusions

By employing newly developed fabrication techniques, with manufacturing costs and biocompatibility in mind, we have the unique ability to engineer a micro- or nano-scale biomimetic environment. These approaches allow us to study cellular interactions in a nanometer scale and to effectively control drug delivery. Important characteristics of each fabrication technique are summarized in Table 1. Top-down approaches, some of which are reviewed in this article, provide great flexibility and control of the structures on a micron or nanometer scale. The bottom-up approaches, which involve molecular scale self-assembly, are widely believed to have great potential in terms of fabricating nano-scale devices. We anticipate that the combination of top-down and bottom-up approaches will lead to new technologies in the fabrication of novel drug delivery systems.

Outstanding issues

- The combination of top-down and bottom-up techniques will ultimately lead to the fabrication of new therapeutic modalities and novel drug delivery systems.
- Processes such as reactive ion etching (RIE) might present issues with biocompatibility and potentially induce inflammatory responses.
- Micro- and nanostructures will provide further insights for the design of new *in vitro* applications such as diagnostics and array technologies.

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