Micro and nano-fabrication of biodegradable polymers for drug delivery

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Abstract

This paper presents state-of-the-art micro and nano-fabrication techniques for biodegradable polymers. Replication molding, using a rigid or elastic master, can pattern structures on a polymer surface in a submicron resolution at a low cost. Layer-by-layer rapid prototyping methods are promising in producing controlled release units with complicated geometries, release mechanisms and the ability to control microstructure and composition. Special attention is paid to the fast, flexible, and non-invasive laser fabrication techniques that have great potential in the fabrication of biodegradable polymer drug delivery devices in both a laboratory and industry scale.

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Keywords: Microfabrication; Nanofabrication; Biodegradable polymer; Drug delivery

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1. Introduction

The advances in micro- and nano-fabrication technology have enhanced the tools available to create clinically important therapeutic applications. Microfabrication technology has been applied to the successful fabrication of a variety of implantable and oral drug delivery devices based on silicon, glass, silicone elastomer, or plastic materials [1–5]. Such devices would permanently remain in the biological tissue if not removed surgically. Because of the inherent difficulty in retrieving small-scale devices from tissues, it is advantageous to apply biodegradable polymers that would naturally degrade and disappear in tissue over a desired period of time. Biodegradable polymer conduits and tissue engineering scaffolds were produced using extrusion [6], fiber bonding [7], salt leaching, and laminating [8]. However, micro and nano-fabrication of biodegradable polymers with precise control over surface microarchitecture, topography, and size remains an important challenge. Traditionally, silicon-based micro-electromechanical systems (MEMS) are fabricated by the repeated application of unit process steps such as thin-film deposition, photolithography, and etching. Unfortunately, these methods are not suitable for biodegradable polymers. Significant effort has been devoted to develop novel micro and nano-fabrication techniques for biodegradable polymers in the recent years. This paper will review some of the current approaches targeting drug delivery devices.

2. Biodegradable polymers

Over the past decade the use of biodegradable polymers for the administration of pharmaceuticals and biomedical devices has increased dramatically. The most important biomedical applications of biodegradable polymers are in the areas of controlled drug delivery systems [9], in the forms of implants and devices for bone and dental repairs [10,11].

Biodegradable polymers can be either natural or synthetic. In general, synthetic polymers offer greater advantages than natural ones in that they can be tailored to give a wider range of properties [12]. The general criteria for selecting a polymer for use as a degradable biomaterial are to match the mechanical properties and the degradation rate to the needs of the application. Commonly used biodegradable polymers, along with their selected physical and chemical characteristics, are listed in Table 1. Poly(ε-caprolactone) (PDLA) is a biodegradable polymer with extensive medical applications due to its biodegradable property that has been proven harmless to human body cells. PDLA has been used as a substrate material for potential applications in nerve regeneration in the field of tissue engineering. Poly(ε-caprolactone) (PDLA) is a biodegradable polymer with extensive medical applications due to its biodegradable property that has been proven harmless to human body cells. PDLA has been used as a substrate material for potential applications in nerve regeneration in the field of tissue engineering.
lactone) (PCL), an aliphatic polyester, is one of the most important biodegradable polymers in medicine. Some of the applications of PCL are sutures and biocompatible medical devices. Poly(vinyl alcohol) (PVA) is used in a wide range of applications such as adhesives, fibers, textile, paper-sizing, and water-soluble packaging. It is also used to modify the degradation profile of other polymers [13–15]. These polymers have unique features such as controllability of mechanical properties, tailoring of degradation rates, and minimal toxicity and immune response that make them ideal for medical uses. In the following sections, we will present several micro and nano-fabrication techniques developed for biodegradable polymers.

3. Replication techniques

Replication technologies are proven useful for biodegradable polymer microfabrication because the principles behind these processes are straightforward and well known in the macroworld. The underlying principle is the replication of a microfabricated mold tool, which represents the inverse geometry of the desired polymer structure. The expensive microfabrication step is only necessary for the initial fabrication of this master structure, which then can be replicated many times into the polymer substrate. In addition to the cost advantage, replication techniques also offer the benefit of the freedom of design: the master can be fabricated with a large number of different microfabrication technologies, which allow various geometries to be realized.

3.1. Microimprinting lithography

Also known as “hot embossing” or “compression molding”, microimprinting lithography is one of the most widely used processes to fabricate microstructures for data storage, wave gratings, or microfluidic applications [13,16–18]. In hot embossing, a master with a micro-scale relief structure on the surface was first fabricated by standard integrated circuit techniques. A chrome lithographic mask was produced on a quartz wafer with the desired micropatterns using e-beam lithography. The quartz substrate was etched using reactive ion etching (RIE) through the mask, leaving areas covered by chrome. After removing the chrome, the quartz substrate was silanized by exposure to the vapor of CF$_3$(CF$_2$)$_6$(CH$_2$)$_2$SiCl$_3$ for ~ 30 min. We used this mold as a micro die to transfer the geometric micropatterns to the biodegradable polymer.

PCL films were obtained by casting a PCL chloroform solution (80% w/v) onto a glass wafer. They were then placed on the quartz die and heated between two plates just below the melting temperature $T_{m}$, which was 57 °C. A vacuum was applied to prevent the formation of air bubbles due to entrapped air. A 500 psi (3.4 MPa) pressure was placed between two plates for 10 min. Temperature was gradually lowered to release internal stress from the crystallization and different thermal expansion coefficients of the master and the polymer film. The resulting PCL film with a surface pattern was peeled off easily. The geometry was inverted to the one of the quartz mold, while the corners were rounded and the walls were slightly distorted (Fig. 1A). Adhesion between the mold and the polymer was minimized by the carbon fluoride surface coating. The pattern distortion was due to the remaining internal stress built up at the corners and to the lack of robustness of the material itself. This fast, relatively inexpensive technique is capable of patterning nano-scale features on a planar surface. However, it requires the thermoplastic polymer to have a good thermal stability near the glass transition temperature $T_g$.

3.2. Soft lithography

Soft lithography is a general term of several non-photolithographic techniques based on self-assembly and replica molding for micro and nano-fabrication. In soft lithography, an elastomeric stamp with patterned relief structures on its surface is used to generate patterns and structures with feature sizes ranging from 30 nm to 100 μm. This convenient, effective, and low-cost method has been widely used in micro/nano-fabrication, particularly, in biomaterial fabrications [19,20]. Xia and Whitesides [21] had a detailed review on soft lithographic techniques.

The essential element of this technique is the elastomeric stamp prepared by cast molding a cross-linkable elastomer over a master with surface relief structures. A quartz master was fabricated using e-beam lithography and RIE, as introduced in the
previous subject. Alternatively, a master can also be produced using standard photolithographic processes on an SU-8 photoresist. A Poly(dimethylsiloxane) (PDMS) elastomer (Sylgard™ 184) kit including a liquid silicone rubber base and a curing agent was mixed and poured over the quartz master. The liquid was heated and solidified at 70 °C within a few hours via hydrosilylation reaction. The PDMS stamp was ready to use after it was peeled off (Fig. 1B).

We used solvent-assisted molding as a demonstration of soft lithographic techniques. A drop of PCL solution in chloroform (80% w/v) was placed on a glass wafer. The PDMS stamp was then applied to the liquid against the wafer with little force. The solvent was evaporated at room temperature for 24 h before the PCL film formed and was peeled off from the stamp. The PCL film had a surface relief structure conformal to the structure on the quartz master, despite imperfections caused by adhesion between PCL and the PDMS stamp can be seen (Fig. 1C).

4. Rapid prototyping techniques

Rapid prototyping techniques have been applied to manufacturing components with complex geometries beyond the reach of conventional precise machining. The fabrication process is directed by computer-aid design (CAD) of a certain component. Methods including direct deposition [22–24], selective laser sintering [25], three-dimensional printing [26], and stereolithography [27–29] have been developed recently, which build components in a laminated fashion.

4.1. Direct deposition methods

Direct deposition microfabrication techniques, which are essentially micro-scale extrusion, were solely derived from their macrofabrication counterpart [22]. A pressure-assisted microsyringe method was used to create a poly(lactic-co-glycolic) acid (PLGA) scaffold with micro-scale porosity [23]. A solution of PLGA in a volatile solvent was placed inside a syringe and expelled through a 10-µm needle. The syringe was mounted on the z-axis of a three-axis micropositioning system. The lateral resolution of the deposited structure ranged up to 5 µm, depending on the process parameters. Similarly, PCL scaffolds were fabricated using the micro-extrusion of PCL filaments under several MPa, resulting in a resolution of several hundred microns [24]. 3D scaffolds can be made by stacking 2D layers in both techniques. However, the geometry is limited since the upper layer has to be supported by the lower one.
4.2. Three-dimensional printing

Three-dimensional printing has demonstrated the capability of fabricating microstructures and controlling local composition with a high resolution in the interior of the component [26]. From a computer model (CAD) of the desired part, a slicing algorithm draws detailed information for every layer. Each layer begins with a thin distribution of powder spread over the surface of a powder bed. Using a technology similar to ink-jet printing, a binder material selectively joins particles where the object is to be formed. A piston that supports the powder bed and the part-in-progress lowers so that the next powder layer can be spread and selectively joined. This layer-by-layer process repeats until the part is completed (Fig. 2). Unbound powder is removed, leaving the fabricated part.

Devices consisting PCL and polyethylene oxides (PEO) were fabricated to demonstrate control of drug delivery profiles by controlling the position, composition, and microstructure [26]. The top and bottom layers of the device were constructed by binding PCL power into thin solid layers. A cellular-type pattern was printed with PEO, which has a faster degradation rate, in the intermediate layers. Dyes, which represent drugs, were selectively placed within the cells manually. Control over the release mechanism by controlling device wall composition, anisotropy, and microstructure was achieved with this technique [26].

4.3. Laser stereolithography

Similar to three-dimensional printing but working in a liquid environment, laser stereolithography is a method that allows real three-dimensional microfabrication [27–29]. A 3D solid model designed with CAD software is numerically sliced into a series of 2D layers with an equal thickness [30]. The code generated from each sliced 2D file is then executed to control a motorized $x$–$y$–$z$ platform immersed in a liquid photopolymer. The liquid polymer is selectively exposed to a focused laser light, which moves in $x$–$y$ directions. The polymer cures and forms a solid in the focal point only. After the first layer is formed the elevator moves downward and a new layer of polymer is solidified according to the design (Fig. 3). This layer-by-layer micro-manufacturing enables complex internal features such as complex passageways and curved surfaces to be accurately produced. Furthermore, by using different proteins and microparticles containing polymer solutions for each layer (or even for partial layers),
we are able to create a precise spatial distribution of biochemical microenvironments.

PLGA (50:50) microparticles encapsulating fluorescein or rhodamine isothiocyanate were used as a model drug containing microparticles [30]. Polyethylene glycol dimethacrylates (PEGDMA, Mw 1000) was mixed with a cyto-compatible photoinitiator (Darocure 2959) along with different microparticles. This mixture was photo-polymerized, layer-by-layer using a frequency tripled Nd:YAG laser (355 nm) and a micromanipulator stage. Different spatial distributions of the microparticles were created and evaluated using a laser scanning confocal microscope to optimize laser energy, initiator concentration, accuracy of spatial distribution, etc.

A cellular structure is shown in Fig. 4. Square pores were formed by solidifying vertical walls with a 400-μm interline spacing. We can also control the spatial distribution of fluorescein and rhodamine microparticles inside a photo-crosslinked polymer layer. A cellular structure containing fluorescein microparticles (1 μm in diameter) and a quadrant structure, with precisely controlled distribution of fluorescein and rhodamine microparticles (5 μm in

Fig. 4. Cellular-type structure produced by laser stereolithography. Fluorescein microparticles were embedded in PEG-DMA walls. 400 × 400 μm pore size.

Fig. 5. Confocal fluorescent microscope images of (A) cellular-type structure containing fluorescein microparticles; (B) precise spatial distribution of biodegradable microparticles within photo-polymerized scaffold structures; (C) 3D reconstructed confocal image of a micro-fabricated scaffold structure with spatially patterned fluorescein and rhodamine encapsulated microparticles.
diameter), are shown in Fig. 5A and B. A computer reconstructed cross-section of a particle contained two-layer structure is shown in Fig. 5C. As indicated, precise, pre-designed distributions of controlled release biofactors could be engineered within such scaffold structures [30].

Future work will focus on the functionalization of the PEG macromer with extracellular matrix components to increase cell adhesion and prevent factor diffusion after release. Cell attachment and growth of mesenchymal stem cells (MSCs) will be optimized and followed by attempts to grow hybrid mesenchymal tissue structures (e.g., cartilage and bone or bone and ligaments).

5. Laser micromachining

5.1. Lasers for micropatterning

Laser micromachining makes it possible to pattern polymeric materials on the micro-scale, avoiding the difficulties associated with non-photon techniques. Photons of the laser light act as “clean particles,” and the laser irradiation is essentially non-invasive and a single-step process. The most popular lasers used for machining polymers are the UV lasers including excimer, argon-ion, tripled and quadrupled Nd: YAG, fluorine, helium–cadmium, metal vapor, and nitrogen lasers [31]. The small wavelengths allow strong interactions of the beam with a variety of materials. If the incident photon energy is high enough to break the chemical bonds of the target material directly, the material is dissociated into its chemical components and no liquid phase transition occurs. This photochemical process has greatly minimized heat effects compared with the photothermal process involved in visible and infrared lasers. This important feature makes UV laser micromachining very attractive for biodegradable polymer materials, since thermal damage to the non-machined part can be minimized. An alternative tool for micromachining polymers is femtosecond solid-state lasers with a near-infrared (NIR) wavelength, ultrashort pulse and high peak power. In polymers, the extremely high intensities generated with femtosecond pulses can produce very high concentrations of free electrons via multi-photon absorption and avalanche ionization. Such strongly non-linear interaction processes can further enhance the localization of the excitation energy, thus increases the resolution in surface patterning [32].

5.2. Laser micropatterning of polymers

We used a variety of lasers to pattern biodegradable polymers [33,34]. The micromachining setup for biodegradable polymer ablation consists of four main parts: a laser system, a beam delivery system, a micrometer-resolution x–y sample stage, and an online monitoring system (Fig. 6). The beam delivery system consists of a mask, field lens, turning mirrors and an imaging lens to the beam for micromachining. A spherical lens was used to drill microholes, while a cylindrical lens was used to produce micron-sized channel type patterns. A charge-coupled device (CCD) camera coupled with a TV monitor is used to provide online machining information.

A frequency quadrupled Nd:YAG at 266 nm and a solid-state femtosecond laser at 700 nm (with a Gaussian beam profile) were used to fabricate microhole arrays (Fig. 7). A circular mask was used to

![Fig. 6. Schematic setup of a laser micromachining system.](image-url)
Fig. 7. Laser-drilled microholes in (A) PVA by 266 nm laser; (B) PVA by 193 nm laser; (C) PCL by 700 nm femtosecond laser (10 μm in diameter); (D) PDLA by 700 nm femtosecond laser (8 μm in diameter).

Fig. 8. Laser ablated microchannels in (A) PVA by 193 nm laser; (B) PDLA by 193 nm laser; (C) PVA by 308 nm laser; (D) PDLA by 308 nm laser.
shape the beam. A XeCl excimer laser (308 nm) and an ArF excimer laser (193 nm with a uniform beam profile) were used to irradiate the polymers to fabricate microchannels (Fig. 8). Lenses with 25 and 50 mm focal lengths were used to obtain microchannels with different width grooves. The arrays of holes drilled could be used as degradable microfilters or drug reservoirs while the channels could be used for microfluidic delivery systems. All the experiments were conducted in ambient air. Details of the lasers used in this work are listed in Table 2.

No melting or redeposition was noticed in the case of 193 nm laser (Figs. 7 and 8A,B), while resolidified polymer debris were found around the machined holes and channels at wavelengths of 308 and 266 nm (Figs. 7A and 8C,D). This is because at the 308 nm wavelength, the photon energy of light is only 4.02 eV. This energy is not high enough to break the polymer bonds. At 193 nm wavelength, the photon energy of the laser beam reaches about 7.9 eV, which is well above the bond energies of the polymer. The femtosecond laser delivered a clean cut on the edge of the holes (Fig. 7C,D). The irregularities in the hole shape can be attributed to the spherical aberration and non-circularity of the original laser beam. By using advanced beam delivery systems and beam shapers, the irregularity of the hole shape can be corrected.

Another potential feature of laser ablation is the capability of surface modification of microfabricated structures concurrent with structure formation (e.g., channels) [35]. 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 [36]. Incorporation of nitrogen or oxygen can give rise to amino, hydroxyl, carboxylic, or phenolic functional groups at the surface [37]. These types of surface functionalities are thought to play an important role in electroosmotic flow, a commonly used means to pump solution through microchannels [38].

6. Nanosphere lithography

Laser direct patterning provides a resolution on the order of microns due to an optical diffraction limit of the laser wavelength, which is $\lambda/2$ NA, where $\lambda$ is the wavelength and NA is the numerical aperture of the focusing lens. A typical value of NA in air ranges from 0.5 to 1.0. Therefore, the machining resolution is in the order of $\lambda$. In order to achieve nano-scale resolution, near-field photolithographic techniques were developed for nano-structuring by delivering a laser beam through a hollow near-field tip or illuminating the tip of a scanning probe microscope with a pulsed laser [39,40]. A strong local optical field was established between the sample surface and the sharp tip when the surface/tip gap was of a few nanometers. Structures with lateral dimensions below 30 nm, and therefore well below the minimal resolvable feature size of half the wavelength of the light, were produced underneath the tip [41–43]. However, this kind of near-field nanolithographic technique has hardly been used in an industrial setting due to its limited throughput, hollow tip blockage, and difficulty in process control.

We used a new approach involving the illumination of a nanometer-sized sphere array using a laser beam to pattern a solid surface in a mass production fashion [44–46]. A spherical particle may act as a lens and therefore intensifies the incoming laser beam if the sphere diameter is equal or smaller than the wavelength.

6.1. Sample preparation and processing

PCL thin films with a flat surface (surface roughness in the order of 10 nm) were prepared and used as a substrate for better investigation, even though a flat surface is not required for this process. A 1% (w/v) colloid of silica spheres (diameter = 640 nm) was dropped onto the PCL substrate [47]. Water was then evaporated from the solution in a chamber under a
controlled humidity. As the solvent evaporated, capillary forces drew the nanospheres together, and the nanospheres reorganized themselves in a hexagonally close-packed pattern on the substrate (Fig. 9A). The as-deposited nanosphere array may include a variety of defects that arise as a result of nanosphere polydispersity, site randomness, point defects, and line defects.

With a setup similar to that of laser micromachining, samples were irradiated with the second and third harmonic wave of a Nd:YAG laser or an ArF excimer laser. The laser beam was focused by a lens (f = 50.8 mm) onto the sample mounted on a three-dimensional precision stage. All experiments were performed under ambient conditions.

6.2. Nanostructure formation

Most of the spheres in the round shape area exposed to the laser pulse with the diameter of 10 \( \mu \)m disappeared, leaving holes with the same hexagonal pattern as the spheres were formed on the PCL surface (Fig. 9B). A similar phenomenon was observed in Fig. 9C, where spheres at the monolayer edge and adjacent holes were aligned in a hexagonal arrangement, and therefore the spheres located themselves on the holes.

Laser energy was varied from a minimum threshold energy, below which no clear nanostructure was observed, to a maximum energy, beyond which the polymer surface was ablated directly by the laser pulse. In three cases, the diameter of the hole were 430 nm (Fig. 9C), 360 nm (Fig. 9B) and 240 nm (Fig. 9C), corresponding to 355, 266, and 193 nm laser wavelengths, and remained unchanged within the experiment laser energy range. The features were cleaner as the laser wavelength decreased.

The enhanced optical field by nanospheres in the near-field region, which, we believe, produces nano-scale features, can be explained by Mie scattering. When the diameter of the sphere is equal or greater than the wavelength, light is scattered elastically according to the Mie scattering law. The electric field around a Mie sphere (sphere diameter larger than the laser wavelength) is enhanced by several times of the incident light towards the forward area of the sphere [48].

One advantage of this process lies in its simplicity and massively parallel capability for nano-scale sur-

Fig. 9. (A) Self-assembled nanosphere monolayer in close-packed hexagonal form. (B) Nanohole array in PCL by 355 nm laser. Spheres at the monolayer edge and adjacent holes are aligned in a hexagonal arrangement. (C) Nanohole array in PCL by 266 nm laser. (D) Nanohole array in PCL by 193 nm laser.
face patterning. Future work is needed on characterizing changes in surface chemical properties in laser processed areas.

7. Summary and future prospects

We summarize the important characteristics of each fabrication technique in Table 3. 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 molecular interactions at the cellular level and to control drug delivery. Top–down approaches, some of which are reviewed in this article, provide great flexibility and control of the structures on a micron or submicron scale. The bottom–up approaches, which involve the 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.

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Table 3
Characteristics of fabrication techniques

<table>
<thead>
<tr>
<th>Fabrication techniques</th>
<th>Critical dimension</th>
<th>Throughput</th>
<th>Solvent</th>
<th>Feature flexibility</th>
<th>Tool cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>µ-Imprinting lithography</td>
<td>~ 50 nm</td>
<td>high</td>
<td>none</td>
<td>2D structures</td>
<td>master is expensive</td>
</tr>
<tr>
<td>Soft lithography</td>
<td>~ 50 nm</td>
<td>high</td>
<td>organic solvent</td>
<td>3D is possible by laminating</td>
<td>soft master is inexpensive</td>
</tr>
<tr>
<td>Direct deposition</td>
<td>~ 5 µm</td>
<td>low</td>
<td>organic solvent</td>
<td>simple geometry</td>
<td>tools are simple and inexpensive</td>
</tr>
<tr>
<td>Three dimensional printing</td>
<td>~ 50 µm</td>
<td>low</td>
<td>organic solvent</td>
<td>any 3D structures</td>
<td>high-resolution ink-jet system required</td>
</tr>
<tr>
<td>Laser stereolithography</td>
<td>~ 10 µm</td>
<td>low</td>
<td>water as solvent</td>
<td>any 3D structures</td>
<td>high-resolution positioning system required</td>
</tr>
<tr>
<td>Laser micromachining</td>
<td>~ 1 µm</td>
<td>intermediate</td>
<td>none</td>
<td>2D structures</td>
<td>high-resolution positioning system required</td>
</tr>
<tr>
<td>Nanosphere lithography</td>
<td>~ 100 nm</td>
<td>high</td>
<td>none</td>
<td>restricted by self-assembled sphere patterns</td>
<td>cheap</td>
</tr>
</tbody>
</table>

References


