Nanofabrication Techniques for Controlled Drug Release Devices

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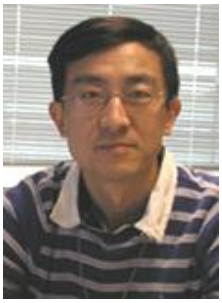

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New drugs and delivery systems have undergone a rapid development in recent years for treating cancer, HIV aids, diseased organs, damaged nerves to relieve pain, preventing disease and restoring health to human beings (1). The application of nanotechnology in nanomedicne for drug delivery is a new field and has raised great attention. Compared to conventional drug delivery mechanisms, nano-scale systems can deliver the drugs to diseased cells directly and minimize damage to healthy cells, and they can also be made small enough to be implantable in the body. Alternatively, by modifying the shells of the drug, shells can release the drug automatically under certain stimuli. Nanoscale controlled-release drug systems provide highly selective and effective therapeutic modalities (2).

Controlled-Release Drug Delivery Vehicles

The first generation nanotechnology-based controlled-released drug products appeared around 2005. The history and projected development timeline was published by the Nanotech Project Organization (3). From its fast growth pace, it is believed that nanotechnology will change our current drug delivery mechanisms in the near future. The research and development of nanotechnology-based drug delivery systems started from the preparation of nanoparticles and then grew with the appearance of other nanomaterials such as nano-rods, nano-tubes, nano-lamellae, nano-vesicles and even more complicated structures. However, most published works have dealt with the development or application of the nanomaterials, and there are only limited reports on the use of nanofabrication techniques to fabricate and control the desired nanostructures for making controlled drug delivery systems.

Nanofabrication refers to modern fabrication technologies which can produce structures with at least one dimension less than 100 nm. Nanofabrication is a new fabrication concept originally proposed by the electronics industry to make ultra high density integrated circuits. However, its basic principle, molding, printing, and writing can be traced to centuries-old lithography concepts and applied to very diverse areas including materials science and engineering, the life sciences, energy, and medicine. Nanofabrication techniques have been used for making 2-dimensional (2D) and 3-dimensional (3D) nanostructures.

For the purpose of controlled drug delivery, fabricated nanostructures should include structural, mechanical, and electronic features. The fabricated nanostructures can dispense drugs in the optimum dosage for long periods, which can reduce the toxicity and improve the efficacy of the

drug. Here we will focus on the fabrication of nanoparticles, micro/nano mechanical electronic systems and micro-needle arrays.

Nanofabricated Particles

Nanoparticles have demonstrated great advantages in comparison to microparticles. In drug delivery, for instance, nanoscale particles can travel through the blood stream without sedimentation and can penetrate tissues such as tumors. Nanoparticle-based encapsulating structures have been used to deliver drugs to target sites for cancer therapeutics (4). The encapsulating particle is used to release the drugs through surface or bulk erosion and diffusion. The release can also be controlled by changing environmental conditions, such as pH, light, temperature or by the presence of analytes. In the past, controlled-release particles were normally made by chemical synthesis. The use of nanofabrication techniques to produce nanoparticles has a very short history but has already attracted great attention. For example, nano-scale particles have been fabricated with well-defined sphere, cube or other shapes by using a process called particle replication in nonwetting templates (PRINT). In the PRINT process, a mold is fabricated by conventional lithography and then filled with a liquid. The liquid in the mold cavities is then converted to a solid by either curing the liquid precursor or evaporating the solvent. Finally, the particles in the cavity can be removed from the mold and released, or transferred, to form a 2D array or free particles (5). PRINT allows for the precise control over particle size, shape, composition and surface properties. This precise control of the size and the shape, which is difficult to achieve when the particles are made by chemical synthesis, enables the study of the impact of these parameters on the mechanism of cellular internalization (6). A number of studies have been carried out to characterize the cellular internalization mechanisms of nontargeted organic nanoparticles as a function of size, shape, composition, and surface charge (cationic, anionic) in human cervical carcinoma epithelial (HeLa) cells (7).

In addition to studying the particle itself, more and more work on particle-based drug delivery is focusing on the targeting of nanoparticles to the desired tissues. A number of methods have been developed such as controlling the size, charge, hydrophobicity of the particles, and adding antibodies and peptides, that recognize specific cell surface proteins and receptors, to the nanoparticle surface (8).

In addition to solid particles, micro/nano fabrication techniques can also produce 3D cubes with designed size, shape and surface patterns to encapsulate and deliver pharmaceutical agents and other molecules in vivo or in vitro (9).

Micro- or nano Electromechanical System

Micro- or nanoscale systems have been built using nanofabrication technologies to deliver drug particles and study the drug particle behavior inside the body. For example, microfluidic devices have been fabricated to mimic the body's vasculature which can be used to test and optimize the interaction of targeted nanoparticles with the cells that line cancer blood vessels (8). By using such microfluidic devices, nanoparticle characteristics such as size and surface properties can be optimized before performing costly animal and clinical experiments.

Micro/nano-fabricated microreservoir devices are being explored extensively. For drug release applications, the microreservoirs are required to be made from biocompatible materials: silicon, gold, silicon dioxide, silicon nitride, and some polymers have been proven as good biocompatible materials. Silicon is the most popular material which is widely used in both micro-electronic and bio-medicine industries. The silicon microreservoirs are normally made by deep Si etching (10). The silicon surface can be further modified to increase biocompatibility and decrease biofouling. The reservoir size can be easily increased by changing the size of the open area and the etching depth. For drug delivery purpose, the etched reservoir needs a membrane to cover the drug after it is loaded. The drug contained in a micro-reservoir is released when the membrane is opened or removed under a certain stimulus. This kind of device can be implanted for local drug delivery. For example, a silicon device was used for the in-vivo release of fluorescent dye and radiolabeled 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), a chemotherapeutic agent. (10). Gold is one of the promising membrane candidates because of its anti-corrosive and good mechanical properties (11). Gold membranes were found to exhibit elastic behavior up to a pressure of 4.14×10^5 Pascal. Other good candidates for making the microreservoir and the membrane are polymers (12). For example, a microreservoir made out of lactic acid and a membrane made of copolymers of poly (lactic acid) and poly (glycolic acid) (PLGA) have been successfully fabricated by using molding and injection molding technologies respectively. The devices range from 480 μm to 600 μm in thickness, and each reservoir has a volume of 120 nL to130 nL. The release was controlled by dissolving the polymer membrane. The degradation time of the reservoir membranes varies with molecular weight and

lactic:glycolic ratio, thus allowing the timing of the release from each reservoir to be engineered. Other researchers are pursuing similar polymer-based microreservoir devices (13).

In addition to the membrane-controlled drug release itself, Micro/Nano Electro-Mechanical Systems (MEMS/NEMS) technology is also emerging as the natural choice to fabricate micropumps to dose and deliver the drug. Several extensive reviews have described the state of the art up to 2009 (14, 15). Self-actuated pumps have been proposed which respond to levels in blood pressure, lipid or glucose to treat hypertension, atherosclerosis and diabetes, using flexible membranes as nano-sensor, nano-actuator and nano-pump [16]. Electrokinetic pumps that rely on direct current are very promising to deliver pure water, pure organic solvents, inorganic buffers, biomacromolecules and hydrogels (17, 18, 19). Another means of moving the fluid has been proposed that also relies on a small electric current propagating along a nano-channel and locally increases the wall thickness, thereby "pushing" the fluid along (20). Shape memory actuators are also a promising technique to deliver the drugs (21). Implantable piezoelectric valve-based pumps have been fabricated for insulin delivery (22) using a 12-level silicon micromachining technology. Additional references on the latest development on programmable flow pumps, typical nanofabrication sequences and embedded piezoelectric actuators can be found in (21).

In summary, micro/nano engineered drug delivery systems can be used to maintain biological activity of the drugs and facilitate the local, accurate and controlled release of potentially complex drug-release profiles. (23).

Microfabricated Needles

Through-skin is another way to deliver drugs into the body. Traditional needles are big and cause pain in the drug injection process. Micro/Nano fabrication techniques enable to shrink the needle sizes down to micrometer or even nanometer scales (24). Because of the shrinkage of needle size, micro-needles can be used to deliver compounds to cells in cultures or into localized regions of tissues. The micro-needles can penetrate deeply enough for the therapeutic compounds to enter the systemic circulation. (25). Micro-needles can also be fabricated as an array to increase the delivery dosage. Besides improving drug delivery efficiency, these microfabricated needles can reduce the pain for the patient. The size of the microneedles is small enough to avoid activating sensory nerves in the tissue (26). Currently, microneedles are being fabricated from silicon, glass (silicon dioxide), and metal.

Micro/Nanofabrication Techniques

Conventional lithography processes

Most nanoparticles, micro/nano electromechanical systems and micro/nano needles are fabricated by conventional nanofabrication techniques which are based on radiation beams (optical, laser, ion and electron) and radiation sensitive materials to define the patterns on the surface.

Ultraviolet (UV) is the most popular beam for nanofabrication which is called photolithography. In photolithography, a photomask is required (27). The photomask is an opaque material on a transparent background which contains features of desired shapes and sizes. UV light passes through the transparent area to expose a light-sensitive material (photoresist) which is then "developed" in solvents to dissolve the exposed (positive photoresist) or unexposed (negative photoresist) regions and provide a patterned surface. The resolution of optical lithography is light wavelength dependent (28). Although the resolution of optical lithography has been pushed down to sub 50 nm (29), the cost of the tools, mask fabrication and photo-resists is extremely high and has become a roadblock for most researchers and small businesses.

Electron is another popular beam for making even smaller structures. Unlike photolithography, electron beam lithography (EBL) is a maskless direct writing technology. The designed nanopatterns are generated by scanning a focused electron beam across an electron-sensitive resist coated on a substrate. The e-beam-exposed resist is then developed to remove the exposed area (or un-exposed area, depending on the tone of the resist). Finally, the surface pattern can be further transferred into the underlying substrate by plasma or ion etching. Compared to photolithography, the advantage of EBL is its high resolution: 3 nm to 5 nm isolated lines in poly(methylmethacrylate) (PMMA) (30) and 1 nm to 2 nm resolution in metal halide resists have been demonstrated (31). The drawback of EBL is its low throughput because of the low sensitivity of the resist and sequential writing nature of the process. Similar to EBL, a laser beam (32) can also used for direct pattern writing.

Unlike the above radiation beams, an ion beam does not need a radiation sensitive media to generate the pattern. A focused ion beam (FIB) can write patterns on a substrate directly (such as semiconductors, metals or ceramics) without major forward- and backscattering. Ion sources can focus on spots of the order of 10 nm. (33). 3 nm to 6 nm features have been demonstrated. (34).

Non-conventional lithography processes

In addition to the above radiation-based nanofabrication techniques, a number of non-radiation technologies have been proposed recently to improve the pattern resolution and/or reduce the cost. Many newly developed techniques, such as stamping, molding, scan printing and self-assembly, are already or potentially good candidates for fabricating nano-medicine devices.

Stamping-based nanofabrication is called Soft lithography which uses topographically-patterned flexible poly(dimethylsiloxane) (PDMS) as a stamp to print chemical normally self-assembled monolayers (SAMs) on a substrate. Those SAMs then react with the desired chemical or bio-chemical molecules to form micropatterns of various materials (35). Different procedures such as Micro Contact Printing (μCP), replica molding (REM), microtransfer molding (μTM), micromolding in capillaries (MIMIC), solvent-assisted micromolding (SAMIM), and patterning by etching at the nanoscale (PENs) have been developed (36).

Molding-based nanofabrication is called nanoimprint lithography (NIL) (37). NIL uses a hard mold to form nano-structures by pressing into a deformable polymer layer deposited on the substrate surface. 5 nm or smaller features, (38) over large area (39) have been demonstrated. Pressure and temperature are critical for pressing the mold into the polymer layer, and a mold-release layer coating is needed for separating the mold from the polymer layer after the imprint (40). The patterned polymer layer can be an inert pattern transfer layer or a bio-active material. Not only solid polymer thin films, but also liquid precursors can be printed and then cured by UV exposure (41, 42). In addition to replicate nanostructures on a surface, NIL can also be used to replicate nanoscale particles by using a process called PRINT (43) as mentioned before in the nanofabricated particle section.

Scan printing-based nanofabrication is another useful technique for the fabrication of bio-medical devices. Dip pen nanolithography (DPN) is one of the well known techniques (44) in this category. In DPN, an atomic force microscopy (AFM) tip is dipped into the ink and then scanned across the surface. Currently, DPN is able to write patterns as small as 15 nm (45). Self-assembled monolayers, small organic molecules, macromolecules, nanoparticles and metal ions can be used as ink materials to write on a variety of surfaces (46). A massively parallel tip scanning approach (called 2D DPN) has been developed to improve the throughput (47). E-jet printing is another high resolution surface pattern writing technology. It uses glass microcapillary nozzles to deliver liquid “inks” on a surface. Sol-gels, DNA and other liquid

molecules can be printed (48). Another “pen”-based technology called polymer pen lithography, (PPL) combines the scanning probe contact printing concept and microcontact printing process to create a massively parallel elastomeric array of pyramids to write a surface pattern over large areas (49). The above scan printing nanofabrication technologies enable direct writing of organic, polymer, biomolecules and sol-gel materials on surfaces and have been used to fabricate nanoarrays of proteins, nucleic acids, and other soft-materials for bio-medicine research (50).

Self-assembly-based nanofabrication is a low-cost process for making regular patterns over a large area. The colloidal surface self-assembly process was first explored. Physical interactions can align colloids into regular patterns on the surface (51). These patterned colloids can be functionalized directly to produce a device, or used as a mask to pattern the underlying substrate (52) to achieve desired charge, roughness and chemistry on different substrates for biomedicine and other applications (53). Phase separation is a molecular level self-assembly which is being extensively studied to pattern nano-scale features. The phase structures exhibit interesting morphologies, such as sphere, rods, lamellae and bi-continuous structures (54, 55). In addition to form surface nano-patterns, polymer phase separation structures can also be used to produce a wide range of sponge-like scaffolds by selectively removing one phase from the film. The fabricated nano-porous structures are very useful in Nanomedicine especially for controlled drug delivery (56). Templated self-assembly is a process which uses the topographical and/or chemical templates to guide the self-assembly of colloidal particles or molecular phase-separation (58, 58) in a more regular shape. Both patterned or blank templates can guide a regular pattern formation. Lithographically induced self-assembly (LISA) is a newly discovered polymer thin film self-assembly pattern formation process which uses a blank template on top (59). A variety of self-assembly patterns, e.g., concentric rings, rods, and pillars, in polymer thin films has been achieved (60). In addition to surface patterning, the self-assembly concept has also been applied to produce 3D structures. This new process uses surface forces to fold a precisely fabricated 2D surface pattern into a 3D structure (9). Since 2D lithographic patterning is very well developed, it is very useful to transform the patterned 2D templates into 3D objects. 3D structures such as rings, tubes and polyhedrons have been demonstrated. It is clear that components with complex surface patterns can result in more complex assemblies (61). The fabricated 3D cage will be very useful to encapsulate the drugs for controlled-release applications.

Conclusion

The convergence of three major advances in technology (biomaterials and conventional lithography in the last 40 years, and emerging lithography in the last 15 years) has enabled the development of completely new drug delivery schemes and devices. As these three technologies continue to "push the envelope", along with the other associated nanofabrication processes, and as economies of scale come into play, we can be confident that nanorobots, and nanomedicine in general, will become a mainstay in human healthcare.

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