MICROFABRICATED PARTICULATE DEVICES FOR DRUG DELIVERY

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in the
Graduate School of The Ohio State University

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The Ohio State University
2005

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ABSTRACT

Microfabrication technology, with many technologies originally developed for producing computer chips, is being used to fabricate particulate drug delivery microdevices with uniform dimensions, well-defined and asymmetrical structures, multiple components, and multiple functions. For this application, conventional silicon-based microfabrication techniques have some drawbacks, such as the use of expensive cleanroom-based facilities and limited ability for processing polymers. In this dissertation, polymer-based microfabrication technology, in particular soft lithography, is used to produce particulate polymeric microstructures for drug delivery. Plate-like particulate polymeric microstructures made of various biomedical polymers with different sizes and shapes have been created. They may be used as drug-carrying vehicles in a number of applications such as ocular drug delivery. Microstructures with single reservoir have been produced by polymerizing liquid resin filled in ring-shaped recessed microfeatures. Microstructures containing multiple reservoirs have also been fabricated by combining polymer printing and embossing. These reservoir-containing microstructures may be used for oral delivery or macromolecular drugs. Capsule-like
microstructures with model drug encapsulated by a biodegradable polymer have been fabricated. This type of microstructures may be used as injectable system for sustained drug delivery. By combining two polymer layers with different swelling ratios together, we have produced self-foldable microstructures that may be used as a novel strategy to enhance mucoadhesion and drug permeation in transmucosal drug delivery. In conclusion, the polymeric microstructures produced in this dissertation have potential to be used in a variety of drug delivery applications and polymer-based microfabrication technology hold promise to produce the highly engineered, multi-functional, and “intelligent” next generation drug delivery microdevices.
ACKNOWLEDGMENTS

I wish to thank my parents for their constant support on my study.

I thank Dr. Derek J. Hansford and Dr. L. James Lee, for their guiding on my research.

I am also grateful for the help from Nick Ferrell, Hongyan He, and all the other members in Dr. Hansford’s group and Dr. Lee’s group for their help on my research.
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Anytime a drug is taken, there exists a matter of delivery. Although the therapeutic effect of a drug is usually of paramount importance, the way to deliver it can also be critical. To elucidate this point, we can use chemotherapy for cancer as an example. Cancer is a neoplastic disease extensively affecting public health. One strategy of cancer treatment is to eliminate cancerous cells while sparing normal cells. Many drugs can kill cancerous cells efficiently but they kill normal cells as well. As a result, the outcome of the chemotherapy is significantly limited. If there is a delivery method able to sequester the drugs only to the cancer cells and spare the normal cells, the therapeutic effect would be drastically improved. With thousands of drugs used nowadays for treating numerous diseases, how to deliver them to achieve the maximum benefits has become a very important issue.

Drug in this dissertation is a term covering all therapeutically active compounds including not only conventional small-molecular-weight therapeutics, but also
biomacromolecules such as proteins and nucleic acids. A drug delivery system or device is loosely defined as a system composed of therapeutics and entities that by themselves are not therapeutically effective but enable and/or facilitate the therapeutics to exert their effects. This chapter will first give an overview of drug delivery, including its classification, challenges, and current status of development. Available microfabrication techniques in drug delivery will then be reviewed in detail, with an emphasis on microfabricated particulate drug delivery systems because of their close relationship with the work in this dissertation.

1.2 Classification of Drug Delivery Technologies

Drug delivery technologies are historically classified according to the route through which a drug is administered into the body. For example, oral route means a drug is taken through the mouth into the gastrointestinal (GI) tract and absorbed there. Other important routes include intravenous injection (injected into the vein), intramuscular injection (injected into the muscle), subcutaneous injection (injected under the skin), pulmonary (into the deep lungs), ocular (on the eyes), buccal (through the internal wall of the cheeks), sublingual (under the tongue), nasal (in the nasal cavity), vaginal (in the vaginal tract), rectal (in the rectal tract), transdermal (through the skin), and implanted (inside the body). Therefore, drug delivery as a research discipline conventionally studies how a drug is delivered into the body without considering the fate and the effect of the drug after it is in the body. The fate and the effect have been the subjects of pharmacokinetics and pharmacodynamics, respectively. However, highly sophisticated drug delivery systems are increasingly being developed to exert their effects after they are administered into the body, in particular, into the systemic circulation. They are usually in
the size range of nanometers and have unique combination of capacities such as controlled release and targeting. Although not included in the conventional category of drug delivery methods, this new group of delivery systems is definitely an important component of modern drug delivery technology.

1.3 Challenges in Drug Delivery

Drug delivery is not only a research discipline, but also an industry involving multiple parties including patients, health care workers, manufacturers, and insurers. The success of a drug delivery technology is thus dependent on whether it can benefit all parties involved. More specifically, to be successful, a novel drug delivery technology should be able to deliver drugs in a manner that improves therapeutic effect of the drug, improves patient compliance, lowers the overall cost of the therapy, or has a combination of any of them. With respect to improving therapeutic effects, the challenges include:

- Targeting specific organ, tissue, or cells;
- Enabling localized drug release;
- Reducing systemic toxicity;
- Reducing immunogenicity of non-vaccine drugs;
- Lowering delivery-associated risk;
- Increasing drug bioavailability;
- Optimizing pharmacokinetics.

To improve patient compliance, the challenges include:

- Enabling self-administration;
- Lowering frequency of administration;
- Minimizing discomfort associated with administration;
• Minimizing possible infection associated with administration.

To lower the cost of the therapy, the challenges include:

• Lowering involvement of health care worker;
• Lowering the cost of delivery systems;
• Increasing drug bioavailability.

The list above, which is highly concise but not exhaustive, sketches the major driving forces for the rapid advance in the field of drug delivery during the past several decades and the current blooming status of drug delivery research that will be briefly reviewed in the forthcoming section.

1.4 Current Status of Drug Delivery

A myriad of novel drug delivery technologies are under development and significant advances are being made in nearly all branches of drug delivery. However, due to the limited space, this section will only briefly review the current status of those areas in which most important developments are being made with respect to the impact on public health. The selected technologies are organized based on the conventional classification of the drug delivery, i.e., routes of delivery. Drug delivery systems at the nanometer scale will be included.

1.4.1 Oral

The oral route has long been the most convenient and most widely used drug delivery method worldwide. However, many drugs including the emerging biotechnology drugs such as peptides, proteins, and nucleic acids are not suitable for this route because they are subject to massive degradation in the GI tract and low permeability through the intestinal epithelium. As a result, considerable efforts are being made to develop devices
able to protect these drugs in the GI tract from degradation and enhance drug absorption. Such advanced drug delivery systems include liposomes [1], mucoadhesive patches [2,3], nanoparticles [4], absorption enhancing agents [5], and microfabricated devices [6-12]. Oral vaccination is also attracting significant interest [13]. Although encouraging results have been obtained, none of the advanced systems have reached the market so far.

1.4.2 Injection

Injection is the gold standard for systemic drug delivery and the major delivery method for the drugs that are not suitable for oral delivery. This method is, however, associated with various problems such as pain, burst drug release, needle phobia, risk of infections, and involvement of health care workers. For chronic diseases (e.g. insulin-dependent diabetes) requiring repetitive drug administrations, these problems become more serious. Two strategies are being taken to address these problems. One is to improve the current injection technology, and the other is to develop alternative delivery methods. A successful example of improving the current injection technology is an injectable depot system based on biodegradable microspheres to lower the injection frequency and enable constant drug release [14]. Much more efforts are being making to develop the alternative delivery methods for injections such as oral, pulmonary, and transdermal.

1.4.3 Pulmonary

Pulmonary drug delivery was initially used to treat lung disorders such as asthma. Currently, considerable effort is focused on the systemic delivery of therapeutic peptides and proteins via this route because the lungs have large surface area, rich supply of blood, and alveolar membrane with high permeability. Moreover, pulmonary delivery is
non-invasive in contrast to injection and with less drug degradation than the oral route. Insulin (for diabetes) is the drug receiving the most intense interest for this delivery method as a substitute for injection, because the disease requires long-term management and frequent administration. Some encouraging scientific and engineering developments such as large porous particles for deep lung deposition [15] have been made in this area in recent years and pulmonary delivery of drugs to the systemic circulation will likely become a reality in the near future.

1.4.4 Transdermal

Delivering drugs across the skin is attractive for several reasons: ease of access, applying, and ceasing the delivery; sustained and steady drug release; reduced systemic side effects; avoidance of drug degradation in GI tract and first-pass hepatic metabolism; and absence of pain. However, the skin functions naturally as a barrier to foreign substances, preventing the entrance of the majority of drugs. Therefore, researchers are developing various methods to enhance the drug permeation across the skin. The notable technologies include electroporation, ionophoresis, sonophoresis, jet injection, laser irradiation, and microneedles [16-18]. Delivery of many drugs including biological drugs such as insulin has been successfully demonstrated using these technologies.

1.4.5 Drug Delivery Systems at Nanometer and Submicron Scales

It is generally accepted that “nano” (as in nanotechnology) means a size range from 1 nm to 100 nm. Drug-delivery systems that fall into this range, if prepared with certain surface properties and functions, are likely to have a longer circulation time in the blood stream and a higher chance of uptake by targeted cells than larger particles. The nano-systems for drug delivery are typically constructed on the combination of drug
molecules with non-therapeutic agents in a variety of forms, including liposomes [19], micelles [20], solid nanoparticles [20], drug-polymer conjugates [21,22], and drug-polymer complexes [23]. The non-therapeutic agents can be synthetic polymers [23], lipids [19], antibodies [liposome antibody], peptides [24], and vitamins [19] to serve functions such as targeting, protecting the drug, controlling the size of the system, and as a carrier for other functional groups. Driven by our deeper understanding of human body and diseases, the rapid development of nanotechnology, emergence of new therapeutics, and our everlasting desire for better drug therapy, drug delivery nanosystems are becoming one of the fastest growing areas in drug delivery.

1.5 Microfabricated Drug Delivery Systems

1.5.1 Introduction

Drug delivery can be classified according to not only the route of delivery, but also many other criteria such as the key technology employed in a drug delivery system. Microfabrication is a set of technologies originally developed by the microelectronics industry for manufacturing integrated circuits and it is now being adopted to make advanced drug delivery systems, among other applications.

Microfabrication includes a number of techniques, represented by thin-film deposition, photolithography, and etching, allowing for creating structures at the micrometer and even nanometer scales with high precision and complexity. In recent years, application of this technology to other areas is growing rapidly. In the field of health care, microfabrication has created devices for medical diagnostics [25], drug discovery [26], drug delivery [27], cell immunoprotection [28], tissue engineering [10], and biosensors [29].
Microfabrication for drug delivery is a relatively young but fast advancing field. Numerous devices have been designed, fabricated and tested for this purpose. The following section will give brief introductions on three representative technologies, microneedle array, implantable systems, and particulate systems, with an emphasis on the particulate systems because fabrication of particulate drug delivery systems is the focus of this dissertation.

1.5.2 Microneedle Array for Transdermal Drug Delivery [30,31]

Microneedle array for transdermal drug delivery meets one of the most-pursued goals of drug delivery --- delivering macromolecules with useful bioavailability without causing pain. It works by physically piercing the skin with a plurality of needles that are long enough to penetrate the stratum corneum, the permeability-limiting layer of the skin, but short and small enough to not stimulate the nerves underneath to avoid a sense of pain. Each of the microneedles is significantly smaller than a conventional needle, but its delivery capacity is compensated by the large quantity.

A variety of microneedles have been fabricated with various materials and structures by different technologies. Length is one of the most critical parameters determining the performance of the device. It has been found that a length of 25 to 1000 μm is able to penetrate the surface layer of the skin without causing pain. Silicon was the first material used to make microneedles due to the well-established silicon-based microfabrication technology. Metals are also used for their superior mechanical properties and well-established safety record as used in conventional needles. Polymers, with their low-cost, ease of processing, and biodegradability, are promising materials for making practical microneedle-based devices.
Unlike a conventional hypodermic needle, whose critical part is a hollow tube, microneedles can be either hollow or solid. As a result, several methods, rather than injecting drug through a hollow needle, are used to deliver drugs into the skin. One method is based on producing holes in the skin as microchannels for drug permeation into the body. It is performed by poking solid microneedles into the skin, removing the microneedles, and applying drug-containing patch to the area. A slight different method is to apply drugs without taking the microneedles out of the skin once they are inserted. Drugs can diffuse into the body through the space between the microneedles and the skin. Besides passive diffusion, drugs can also be physically brought into the skin by coating microneedles with a drug and injected into the skin through hollow microneedles. Compared with the methods using solid microneedles, injection through hollow microneedles can achieve a much higher delivery rate, and be more controllable and versatile in terms of the type and amount of drugs, and duration and rate of delivery. However, the fabrication and use of hollow microneedles is more complicated and difficult than the solid ones.

Microneedle-based devices have been demonstrated to deliver a variety of therapeutics including proteins, nucleic acids and even nanoparticles. For example, using this method, insulin has been delivered to diabetic rats and successfully modulated their blood glucose levels. Furthermore, a genetic vaccine was delivered to humans using silicon micro-projections, showing significantly improvement in efficacy and patient compliance compared to conventional injection [32].
1.5.3 Microfabricated Implant Systems

Nanopore Biocapsules

Silicon membranes with highly uniform pores as small as 10 nm have been produced using standard microfabrication technologies. The membranes were incorporated into capsules encapsulating insulin-producing cells for immunoisolation by allowing passage of small molecules such as glucose, oxygen, and carbon dioxide while precluding the entrance of immune components such as immunoglobins. *In vivo* animal study of this microfabricated biocapsule has demonstrated its immunoisolatory effectiveness and the feasibility of using it as an alternative to conventional polymeric capsules as *in vivo* insulin secreting bioreactors [28,33].

Silicon Chips [34]

Standard microfabrication processes have also been employed to create a microdevice for implant drug delivery. The device was a silicon chip consisting of multiple reservoirs at the micrometer scale. Each reservoir was filled with drug and covered by a thin gold membrane. The gold membrane was dissolvable by applying a low electrical voltage, allowing the release of the drug by diffusion. Since multiple drugs can be incorporated into the device and each reservoir can be addressed individually, controllable and complex drug release patterns can be achieved.

Polymer Chips [35]

Similar to the silicon chip, an implantable, reservoir-containing drug delivery system has been fabricated with biodegradable polymers. The drug release was controlled by the degradation of the polymers. Single or multiple drugs can also be incorporated to achieve prolonged and complex release pattern, as demonstrated with human growth
hormone, dextran, and heparin. Although less controllable than its silicon counterpart, this polymer device offers an advantage over silicon chip that surgery is not needed for removing the used device from the body due to the biodegradability of the device material.

Smart Systems [27,36]

One of the envisioned next-generation drug delivery devices is the implanted “smart” drug delivery system capable of monitoring a patient’s physiological condition and reacting by releasing appropriate type and amount of pre-loaded drugs at the right rate when a disease is detected. This type of delivery systems will not only be highly desirable for treating chronic diseases such as diabetes, hypertensions, and cancers, but also revolutionize drug delivery by providing individualized drug therapy. To make the device “smart”, sensing, controlling, and releasing components such as chemical sensors, reactors, electrical circuits, channels, reservoirs, actuators, pumps, and valves, together with multiple therapeutic compounds must be integrated into the system. The device must also be small enough to fit in the limited space in the body. Microfabrication is well-qualified for making such a compact system. However, despite the current advanced microdevice technology and pharmaceutical science, integrating them into autonomous devices working in the body still poses a great challenge due to the high stability and accuracy required for the devices, high complexity in terms of device structure and components, and complicated biological environment encountered by the devices within the body.
1.5.4 Microfabricated Particulate Devices

Conventional particulate drug delivery systems are microparticles (including microspheres and microcapsules), which have been clinically used or are under clinical trials as injectable depot systems for sustained drug release [14], as blood vessel blocking and drug release system in embolization therapy [37], or as drug carrying vehicles in pulmonary and oral drug delivery [15]. Polymers are the major materials used for making drug delivery microparticles by a number of methods such as spray drying, emulsification and solvent evaporation, and phase separation [38]. Although well developed for massively producing various types of microparticles for drug delivery due to their self-assembly nature, these manufacturing methods are incapable of producing particulate microstructures with highly uniform, well-defined, non-spherical, asymmetrical, and complicated structures, which would be potentially advantageous for certain drug delivery applications. For example, a microdevice with flat shape would have relatively larger surface area for tissue binding than a microsphere and less area exposed to the flow of luminal contents such as blood in the blood vessels and food in the GI tract once bound to the internal surfaces of the lumen, making them potentially better for bioadhesion-based drug delivery. Monodispersed device size also enables better control of the distribution of the microdevices in the body and the profile of drug release. Asymmetrical structure has been used in devices at the millimeter scale for achieving unidirectional drug release [2,3].

IMEDD Inc. has fabricated porous devices using silicon and silicon dioxide for both intravenous and gastrointestinal drug delivery [7,8]. The devices were fabricated using a series of techniques, including chemical vapor deposition, photolithography,
plasma etching, pirahna (mixture of \( \text{H}_2\text{SO}_4 \) and \( \text{H}_2\text{O}_2 \)) etch, electroionization, and electropolishing. The devices have precise sizes and shapes, including both flat and curved geometries. The dimensions of the devices range from several microns to several hundreds of microns with a pore distribution of 20 to 100 nanometers. Drug and permeation enhancer were loaded into the porous devices by capillary suction.

IMEDD’s devices offer several advantages over conventional liquid formulations. First, they provide protection of the drugs from attack of surrounding enzymes. Second, they can create a locally high concentration of drugs and permeation enhancer at the site of absorption. Third, their uniform sizes may contribute to better controlled drug release. In vitro study showed that the microfabricated devices induced higher drug permeation across Caco-2 cell monolayer than traditional liquid drug formulations. Particles of sizes 2 \( \mu \text{m} \) and 5 \( \mu \text{m} \) were injected into the vasculature of mice and did not show toxicity.

However, the devices are also associated with some drawbacks. Compared to other advanced drug delivery devices such as mucoadhesive patches [2,3] and lectin-conjugated microspheres [39] for oral drug delivery, iMEDD’s devices do not possess bioadhesivity, thus they are not able to stay at the desired location of the GI tract for sustained drug release. Since bioadhesivity also leads to the intimate contact between the devices and the intestinal wall, the lack of this function offsets the benefits of the high local concentration of the drug and permeation enhancer created by the devices. Moreover, they do not offer unidirectional drug release as mucoadhesive patches. For parenteral drug delivery, silicon and silicon dioxide microparticles are probably not acceptable due to their non-biodegradability in the body. The last concern is the potential high cost associated with the materials, facilities, and manufacturing, which is likely a
limiting factor for this technology unless significant improvement on therapeutic effect and patient compliance can be achieved.

A different strategy was used to make microdevices with well-defined drug reservoirs for containing drugs [9-12]. Silicon dioxide (SiO$_2$) and poly(methyl methacrylate) (PMMA) were the two materials demonstrated to produce the devices. The procedure for the fabrication of SiO$_2$ devices started with growing a thermal silicon oxide on a silicon wafer, followed by the deposition of polysilicon by low-pressure vapor deposition. SiO$_2$ was then deposited as a device layer. Photolithography, consisting of steps of photoresist deposition, UV exposure, and removal of uncrosslinked photoresist, was conducted to form a hard mask defining the lateral size and shape of the devices. Buffered oxide etchant or reactive ion etch (RIE) was used to remove SiO$_2$ at the area that is not covered by photoresist. H$_2$SO$_4$ and H$_2$O$_2$ were used to remove the photoresist. Second photolithography was performed with precise alignment to form a hard mask to define the location, size and shape of the reservoirs. RIE was used to carve reservoirs. After removing the photoresist with H$_2$SO$_4$ and H$_2$O$_2$, the reservoir-containing microdevices were released by etching the underneath polysilicon layer with concentrated KOH solution at elevated temperatures.

PMMA microdevices were fabricated using a similar method. A thin film of PMMA was first deposited to define the thickness of the final microdevices. Photolithography and RIE were then performed twice to carve the device out and make reservoirs subsequently as in the process of making SiO$_2$ devices. Precise alignment was also required to define the location of reservoirs. Solvents were used to develop and remove the photoresist and etching solution was used to release the devices.
The microdevices had the square shape with a width ranging from 50 to 150 μm, and thickness of 2 - 5 μm. One or four reservoirs with widths of 25 - 100 μm, depth of 0.5 - 3 μm were fabricated. Drug loading in the reservoirs was carried out using a micro-injector. The devices were attached with lectins, a group of bioadhesive proteins, on the reservoir side of the devices and enhanced adhesion to Caco-2 cells was observed.

Envisioned by the researchers, the microfabricated device has many features making it attractive for oral delivery of macromolecules. First, the devices have precise sizes, shapes and structures that may lead to better controlled device performance. Second, they are flat and thin, which can maximize their contact area with the intestinal wall and minimize side areas exposed to the flow of food content in the intestinal lumen. Third, the devices can be made small enough to enter the space between the intestinal villi but large enough to avoid being taken up by the intestinal epithelial cells. Fourth, multiple reservoirs can be made in the devices with precise geometry for loading multiple agents such as drugs, permeation enhancers, and protease inhibitors to increase drug absorption. Finally, bioadhesive molecules can be bound only to the reservoir-containing side of the devices, realizing prolonged device retention at the desired GI location, a high local concentration of released agents due to the close contact between the device and the absorption tissue, and unidirectional drug release to the intestinal epithelium.

Although impressive progress has been made, there are some problems with this approach. For example, a number of processing steps during the fabrication involve the use of highly corrosive etching solutions, toxic solvents, ionizing radiation, and elevated temperatures. Furthermore, precise alignment required for reservoir carving and drug loading using microinjection is time-consuming. Selective filling of drug and other agents
into different reservoirs in individual devices and capping would be challenging. Finally, as the IMEDD’s technology, the cost may also be the limiting factor that will hinder this technology from entering the clinical applications.

1.5.5 Silicon versus Polymer in Drug Delivery

The first-generation microfabricated drug delivery devices are largely made of silicon-based materials, owing to the availability of highly-advanced microfabrication technology developed for these materials by the microelectronics industry. Silicon-based materials possess a number of characteristics for drug delivery applications. They are biologically, chemically, and mechanically stable in the body. They can be used to make high-precision structures even down to the nanometer scale. Moreover, when high-performance electronic components are needed in the device, silicon-based materials would be highly desirable.

Unfortunately, the physical and chemical properties of silicon-based materials, including poor impact strength/toughness, lack of optical clarity, non-biodegradability, and high cost, are not appropriate for many biomedical applications. On the contrary, polymeric materials can have a wide variety of properties, including high toughness, optical clarity, good biocompatibility, and biodegradability. Polymers can also be therapeutically active, environment-sensitive, versatile in surface properties, and relatively inexpensive in materials and processing, making them attractive for numerous biomedical applications. Moreover, conducting polymer-based microelectronics are under rapid development and show great promise to be used in certain low-cost, biomedical applications that do not require high-speed performance [40].
Aside from the materials, conventional microfabrication techniques for silicon-based processing, such as low-pressure chemical vapor deposition (LPCVD) of thin film, photolithography, and etching, are also not desirable for many biomedical uses because ionizing radiation, toxic solvents, corrosive enhants, and elevated temperatures are commonly employed in silicon-based processing and they may damage labile biological macromolecules or cells. In addition, the manufacturing cost is relatively high because certain techniques such as photolithography require the use of expensive cleanroom-based facilities to make every device.

Polymer-based microfabrication technology is superior to silicon-based microfabrication in terms of cost and versatility. Many techniques have been developed or are under development for polymer microfabrication. Some of them are the "micro-version" of conventional polymer processing techniques such as solution casting, injection molding, and hot embossing. Some are developed mainly for polymer microfabrication such as soft lithography [41], which uses an elastomeric silicone mold with surface relief features to generate micro- and even nano-structures. All these techniques rely on the use of a master with microfeatures made by micromachining or photolithography. Masters can be used to generate polymer devices directly or to make a silicone mold. The master and mold can be used repeatedly to create many polymer devices in a non-cleanroom environment, lowering the cost of these methods significantly. Although many polymer microfabrication techniques also use ionizing radiation, organic solvents, and elevated temperature and pressure, with the availability of a large variety of polymers and processing techniques, the chance of finding the appropriate materials and
techniques for the fabrication of drug delivery devices that is compatible with the incorporated drugs and clinical applications is relatively high.

1.5.6 Conclusions

Microfabrication technology is beginning to make a significant impact on drug delivery. Although silicon-based microfabrication techniques have produced the first-generation drug delivery microdevices, polymeric materials and related microfabrication technology are likely to become more widely used in drug delivery for their potential to make low-cost, disposable, and biodegradable devices.

One class of the systems produced by conventional microfabrication is the particulate devices mainly for oral drug delivery. They possess many characteristics potentially favoring drug delivery such as highly uniform, well-defined, asymmetrical structures that can not be created by conventional microparticle formation methods. With the aim to further extend this technology and promote it into practical use, we have developed polymer-based microfabrication methods to create a variety of polymeric particulate microstructures for drug delivery applications. The fabrication, characterization, and test of these microstructures form the themes of this dissertation.
2.1 Introduction

This chapter introduces the use of soft lithographic techniques for the fabrication of particulate polymeric microstructures. Soft lithography has been used to generate a wide variety of microstructures [41]. However, it has not, to my knowledge, been used to produce particulate polymeric microstructures for drug delivery applications. Although a functional drug delivery microdevice would probably need more than one component constructed in a relatively complicated structure as reviewed in Chapter 1, this chapter will only describe the production of particulate microstructures with single-layered structure as a starting point. However, control of the lateral shape, thickness, and materials of the particulate microstructures will be included.
2.2 Experimental

2.2.1 Materials

The poly(dimethyl siloxane) (PDMS, Silastic T2) was purchased from Dow-Corning. The acetone, chitosan, poly(ethylene glycol methacrylate) (PEGMA) with an average number molecular weight (Mn) of 526, poly(ethylene glycol dimethacrylate) (PEGDMA) with Mn of 330, cold water soluble poly(vinyl alcohol) (PVA), and glutaraldehyde aqueous solution (50 wt%) were purchased from Sigma-Aldrich. 2, 2-dimethoxy-2-phenylacetophenone (Irgacure 651) was donated by Ciba Specialty Chemicals (Tarrytown, NY, USA). Poly(lactic-co-glycolic acid) (PLGA, lactic acid to glycolic acid ratio = 50:50, Tg = 48.5 °C according to the manufacturer) was purchased from Alkermes (Cincinnati, OH, USA).

2.2.2 PDMS stamp preparation

Silicon masters with designed microfeatures were produced by standard photolithography using either chrome or transparency masks. PDMS stamps were prepared by casting PDMS resin and curing agent at a 10:1 weight ratio against the masters for 48 hours at room temperature.

2.2.3 Fabrication of PLGA Microstructures Using Stamps with Protruding Microfeatures

Figure 2.1 illustrates the process for the fabrication of PLGA particulate microstructures using a stamp with protruding microfeatures. Firstly, the stamp was immersed in a PLGA/acetone solution for 30 s and pulled out at a speed of ~1 cm/s. Depending on the concentration of the PLGA solution, either isolated PLGA microstructures or a continuous PLGA film formed on the stamp as schematically shown.
in Figure 2.1. PDMS stamps with two types of protruding microfeatures, square with round corners and cross-shaped, were used.

For a stamp with the round-cornered square protruding microfeatures that was dipped in PLGA/acetone solutions with concentrations of 1 and 3 wt%, the stamp was dried in air for about 20 s and placed on a glass slide covered with a thin PVA layer, which was prepared by casting a 2 wt% PVA/water solution on the slide. After maintaining the contact for about 20 s at room temperature without external pressure, the stamp was peeled away, leaving the PLGA microstructures on the PVA/slide. Water was finally added to release the microstructures. The same protocol was applied when a stamp with cross-shaped protruding microfeatures and a 2 wt% PLGA solution were used.

For a stamp with the round-cornered square protruding microfeatures that was dipped in PLGA/acetone solutions with concentrations of 5 and 7 wt%, the stamp was dried in air for about 1 min and then placed on a heated glass slide (~100 μm thick) covered with a thin PVA layer on a hotplate (Fisher Isotemp Digital Hotplate, Fisher Scientific) and removed immediately from the slide (contact time: ~5 s), leaving the PLGA microstructures on the PVA/slide. The printing temperatures were 80 °C for a 5 wt% and 120 °C for a 7 wt% solution, respectively. The same protocol was applied when a stamp with cross-shaped protruding microfeatures and 3, 4, 5, and 6 wt% PLGA solutions were used.
Figure 2.1: Schematic illustration of the process for the fabrication of single-layered particulate PLGA microstructures using a stamp with protruding microfeatures covered by (left) isolated PLGA microstructures and (right) continuous PLGA film.

2.2.4 Fabrication of PLGA Microstructures Using a Stamp with Recessed Microfeatures

Figure 2.2 shows the process for the fabrication of thermoplastic particulate microstructures using a stamp with recessed microfeatures, or microwells. The stamp was first immersed in a PLGA/acetone solution for 30 s and pulled out at a speed of ~1 cm/s. Different concentrations produced different degree of coverage of PLGA on the stamp as schematically shown in Figure 2.2. After the stamp was dried in air for about 1 min, it was placed on a glass slide heated on the hotplate and removed immediately (contact
time: ~5 s), leaving a mesh-like PLGA film on the slide. For a stamp dipped in a 2 wt% PLGA/acetone solution, 80 °C was set as the temperature for printing. For 3, 4, and 5 wt% PLGA/acetone solutions, the printing temperature was 120 °C. The stamp was then placed on a PVA-covered glass slide immediately after (< 5 s) the slide was briefly (~5 s) exposed to water vapor generated from a 90 °C water bath. A pressure of ~90 kPa (measured by a balance on which the slide was placed) was added on the stamp manually for 10 s. The stamp was removed with square PLGA microstructures left on the slide. Water was finally added to release the microstructures.
2.2.5 Fabrication of Chitosan Microstructures from Aqueous Polymer Solution

The fabrication process for producing chitosan microstructures is shown in Figure 2.3. An aqueous solution of 3 wt% acetic acid was used as solvent to make a 3 wt% chitosan solution. The chitosan solution was brushed across the stamp surface with an. The solution was trapped in the discrete microwells due to discontinuous dewetting [42]. Water in the solution evaporated immediately, leaving a chitosan film on the bottom of the microwells. Glutaraldehyde aqueous solution (50 wt%) was added on the PDMS
stamp to crosslink the chitosan in the microwells for 10 min. The sample was then rinsed with deionized water and dried in air. To transfer the chitosan microstrips out of the microwells, the stamp was placed on a PVA-covered glass slide immediately after (< 5 s) the slide was briefly (~5 s) exposed to water vapor generated from a 90 °C water bath. A solid weight was set on the PDMS stamp to generate a pressure of ~30 kPa for 5 min. The stamp was then peeled away with the microstrips stuck to the PVA/glass slide. Water was added to release the microstrips by dissolving the underlying PVA film.
Figure 2.3: Schematic illustration of the process for the fabrication of chitosan single-layered particulate microstructures from aqueous polymer solution.

2.2.6 Fabrication of Poly(PEGMA-co-PEGDMA) Microstructures from Liquid Reactive Resin

Microstructures were also made from a reactive resin consisting of PEGMA and PEGDMA with a weight ratio of 1:1 and 3 wt% Irgacure 651 as photoinitiator. Figure 2.4 shows the fabrication process. The PEGMA/PEGDMA resin was brushed across the stamp surface with an applicator. It was trapped in the microwells without wetting the
surrounding areas. The resin was then exposed to UV light with an intensity of 31.4 mW/cm² (EXFO Novacure 2000 Ultraviolet/Visible Spot Curing System) for 2 min under nitrogen environment. The stamp was placed on a PVA-covered glass slide immediately (< 5 s) after the slide was briefly (~5 s) exposed to water vapor generated from a 90 °C water bath. The stamp and the slide were then maintained on the hotplate at 100 °C for 1 min. After they cooled down to room temperature, the stamp was peeled off, leaving the microstructures on the slide. Finally, by adding water, PVA was dissolved and the microstructures were released.
Figure 2.4: Schematic illustration of the process for the fabrication of poly(PEGMA-co-PEGDMA) single-layered particulate microstructures from liquid reactive resin.

2.2.7 Characterization

An optical profilometer (WYKO NT3300, Veeco Instruments, Woodbury, NY, USA) and a Scanning Electron Microscope (SEM, Hitachi S-3000H) were used to characterize the samples. Micrographs of the microstructures were captured using an Olympus BH-2 optical microscope, a Nikon TMS inverted phase contrast optical microscope, and a Fujifilm Finepix 2600 digital camera. The lateral sizes and thickness of the microstructures were characterized using the optical profilometer. Thicknesses at
the centers of ten randomly chosen microstructures were acquired from the optical profilometry data. The lateral sizes of the microstructures were measured from the optical micrographs using an image analysis software: Image Tool (version 3.00, The University of Texas Health Science Center, San Antonio, TX, USA).

2.3 Results

2.3.1 PLGA Microstructures Produced Using Stamps with Protruding Microfeatures

Figure 2.5(A) displays the stamp with round-cornered square protruding microfeatures. The width and height of the micropillars was 24.7 μm (STD = 0.5 μm, n = 10) and 6.5 μm, respectively. The center-to-center distance between the adjacent microfeatures was 40 μm. The stamp with cross-shaped microfeatures is shown in Figure 2.5(B). The microfeatures had an end-to-end width of 197 μm (STD = 2 μm, n = 10), arm width of 40 μm, and height of 6.8 μm.

Figure 2.6 shows the results of the important steps in the fabrication of PLGA microstructures using the stamp with the round-cornered square protruding microfeatures and PLGA/acetone solutions with a series of concentrations. Concentration has a significant effect on the size of the microstructures. PLGA/acetone solutions with relatively low concentrations, 1 and 3 wt%, produced dot-like PLGA microstructures on the surface of the protruding microfeatures as shown in Figure 2.6(A-1) and (B-1), with a positive correlation between the lateral size and the concentration. A 5 wt% PLGA solution created a PLGA film covering almost the entire surface of individual protruding microfeatures with only very small areas at the four corners uncovered as shown in Figure 2.6(C-1). When the concentration reached 7 wt%, the entire surface of a protruding microfeature was covered by PLGA (Figure 2.6(D-1)).
Although not visible under microscope, PLGA usually covered the floor surrounding the protruding microfeatures. For a stamp dipped in a 1 or 3 wt% solution, the PLGA film on the floor can be printed on a heated (e.g. 120 °C) glass slide by pressing the stamp to bring the PLGA on the floor into contact with the substrate. For a stamp dipped in a 5 or 7 wt% solution, the entire PLGA film can be printed off simply by putting the stamp on a heated (e.g., 120 °C) glass slide and peeling it off after the slide cooled down to room temperature.

Figure 2.5: SEM image of the PDMS stamps with (A) round-cornered square and (B) cross-shaped protruding microfeatures. The scale bars are 50 μm (A) and 200 μm (B), respectively.
Figure 2.6: Optical micrographs of PLGA on stamp (A-1, B-1, C-1, and D-1), PLGA on PVA (A-2, B-2, C-2, and D-2), and released PLGA microstructures in water (A-3, B-3, C-3, and D-3) produced from different PLGA concentrations: A: 1 wt%, B: 3 wt%, C: 5 wt%, and D: 7 wt%. The scale bars = 50 μm.
Figure 2.6 also shows the PLGA on the surface of protruding microfeatures can be transferred onto PVA/slide while keeping their shapes and sizes. The microstructures on PVA were characterized using the optical profilometer. A cross-sectional profile of a typical microstructure produced from a 7 wt% PLGA/acetone solution is shown in Figure 2.7. The thicknesses at the centers of the microstructures on PVA were measured.

Figure 2.7: Optical profilometry characterization of PLGA microstructures on PVA produced using a stamp with round-cornered protruding microfeatures and 7 wt% PLGA/acetone solution. Left: Image of PLGA microstructures on PVA. The white dashed line delineates the cross-section where a profile was generated on the right diagram.
The statistical results on the thicknesses are listed in Table 2.1. Due to the significant lateral shrinkage of the PLGA solutions on the protruding microfeatures, the thicknesses of the microstructures produced from 1 and 3 wt% solutions were even higher than that of the microstructures produced from 5 wt% PLGA solution, which spread on the protruding microfeatures. Within each group (shrunk or spread), a higher concentration correlates to a greater thickness.

The lateral sizes of the microstructures were acquired from the optical micrographs. They are also shown in Table 2.1, indicating a positive correlation between the concentration of the PLGA solution and the lateral size of the microstructures. However, when the PLGA started to cover the entire top surface of the microfeatures, increasing concentration had a minimal effect of the lateral size of the microstructures.

<table>
<thead>
<tr>
<th>Conc. of PLGA solution</th>
<th>1 wt%</th>
<th>3 wt%</th>
<th>5 wt%</th>
<th>7 wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (μm, mean±STD, n=10)</td>
<td>0.99±0.09</td>
<td>1.75±0.08</td>
<td>0.76±0.05</td>
<td>1.16±0.08</td>
</tr>
<tr>
<td>Width (μm, mean±STD, n=10)</td>
<td>6.1±0.2</td>
<td>11.1±0.5</td>
<td>24.3±0.5</td>
<td>26.1±0.3</td>
</tr>
</tbody>
</table>

Table 2.1: The thickness and width of the PLGA microstructures on PVA produced using a stamp with round-cornered protruding microfeatures and PLGA/acetone solutions with concentrations of 1, 3, 5, and 7 wt%.
The microstructures produced from 5 wt% PLGA/acetone solution had almost the same size as the microfeatures of the stamp while 7 wt% solution produced microstructures slightly larger than the microfeatures on the stamp. However, PLGA whiskers were formed at the edges of the microstructures produced from 7 wt% PLGA/acetone solution as shown in Figure 2.6(D-2).

PLGA/acetone solutions of a series of concentrations were also applied on the stamp with cross-shaped protruding microfeatures to fabricate particulate microstructures. However, 1 wt% PLGA/acetone solution created fractal-like structures on the protruding microfeatures (Figure 2.8) that was not suitable for making particulate microstructures. PLGA solutions with higher concentrations produced uniform microstructures on the protruding microfeatures as shown in Figure 2.9. It is obvious that the PLGA coverage on the protruding microfeatures was proportional to the concentration of the PLGA/acetone solution and a full coverage was reached at 6 wt%. Particulate microstructures have been produced by printing the PLGA on the protruding microfeatures on PVA and releasing them in water.
The PLGA microstructures on PVA were characterized using the optical profilometer. A cross-sectional profile of a typical microstructure produced from 4 wt% PLGA/acetone solution is shown in Figure 2.10. The thicknesses at the centers of the microstructures on PVA are listed in Table 2.2. With the exception of 2 wt% solution, the thickness of the microstructures increased with the increase of the PLGA solution. The lateral sizes of the microstructures are also listed in Table 2.2. In general, the size increased with the increase of the concentration of the PLGA solution. At 6 wt%, the size of the microstructures was the same as the protruding microfeatures on the stamp.
Figure 2.9: Optical micrographs of PLGA on stamp (A-1, B-1, C-1, D-1, and E-1) and released PLGA microstructures in water (A-2, B-2, C-2, D-2, and E-2) produced from different PLGA concentrations: A: 2 wt%, B: 3 wt%, C: 4 wt%, D: 5 wt%, and E: 6 wt%. The scale bars = 100 μm.
Figure 2.10: Optical profilometry characterization of PLGA microstructures on PVA produced using a stamp with cross-shaped protruding microfeatures and 4 wt% PLGA/acetone solution. Left: Image of PLGA microstructures on PVA. The white dashed line delineates the cross-section where a profile was generated in the right diagram.

<table>
<thead>
<tr>
<th>Conc. of PLGA solution</th>
<th>2 wt%</th>
<th>3 wt%</th>
<th>4 wt%</th>
<th>5 wt%</th>
<th>6 wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (μm, mean±STD, n=10)</td>
<td>0.90±1.16</td>
<td>0.77±0.08</td>
<td>0.97±0.03</td>
<td>1.15±0.10</td>
<td>1.26±0.09</td>
</tr>
<tr>
<td>Width (μm, mean±STD, n=10)</td>
<td>156±4</td>
<td>176±2</td>
<td>177±3</td>
<td>192±1</td>
<td>198±2</td>
</tr>
</tbody>
</table>

Table 2.2: The thickness and width of the PLGA microstructures on PVA produced using a stamp with cross-shaped protruding microfeatures and PLGA/acetone solutions with concentrations of 2, 3, 4, 5, and 6 wt%.
2.3.2 PLGA Microstructures Produced Using a Stamp with Recessed Microfeatures

The microwell stamp used here consisted of 30 μm wide, 1.22 μm (STD = 0.01 μm, n = 10) deep square recessed microfeatures separated by 20 μm-wide ridges as shown in Figure 2.11. After being dipped in 1 wt% PLGA/acetone solution for 30 s and pulled out at a speed of ~1 cm/s, the stamp was covered by a network-like PLGA film on the ridges and hole-containing film in the microwells (Figure 2.12). Therefore, 1 wt% was too low to fabricate particulate microstructures with a well-defined structure. Dipping in 2 wt% PLGA/acetone solution produced more coverage of the PLGA on the stamp as shown in the Figure 13(A-1). Although the PLGA film was not continuous at the edge of the microwells, the bottom of the microwells were completely covered by PLGA. 3, 4, and 5 wt% solutions produced PLGA films apparently covering the entire stamp as exemplified in Figure 13(B-1). The results of the other critical steps in the fabrication using 2 and 3 wt% PLGA/acetone solutions are also shown in Figure 2.13. PLGA films on the ridges were transferred onto the glass slide while remaining their original shape and the microstructures printed on PVA from the microwells had the same shape and size as the microwells. Figure 2.14 shows the PLGA particulate microstructures produced from 4 and 5 wt% PLGA/acetone solutions.
Figure 2.11: SEM image of PDMS stamp with microwells. The scale bar = 50 μm.

Figure 2.12: Optical micrograph of PLGA on a microwell stamp dipped in a 1 wt% PLGA/acetone solution. The scale bar = 50 μm.
Figure 2.13: Optical micrographs of PLGA on stamp (A-1 and B-1), mesh-like PLGA film on PVA (A-2 and B-2), discrete PLGA microstructures on PVA (A-3 and B-3), and released PLGA microstructures in water (A-4 and B-4) produced from different PLGA concentrations: A: 2 wt%, B: 3 wt%. The scale bars = 50 μm.
Figure 2.14: Optical micrographs of released PLGA microstructures in water produced from PLGA solutions with concentrations of 4 wt% (A) and 5 wt% (B). The scale bars = 50 μm.

The microstructures in the microwells were characterized using the optical profilometer. A cross-sectional profile of a typical microstructure produced from a 7 wt% PLGA/acetone solution is shown in Figure 15. The thicknesses at the centers of the microstructures were measured. The statistical results on the thicknesses are listed in Table 2.3, indicating a positive correlation between the thickness and the concentration of the PLGA solution.
Figure 2.15: Optical profilometry characterization of a microwell on a bare stamp and with PLGA in the microwell but removed on the surrounding ridge area. The concentration of the PLGA solution was 3 wt%. Left: Optical profilometry image of a stamp with PLGA only in the microwells. The white dashed line delineates the cross-section where a profile was generated. Right: compounded cross-sectional profiles to show the thickness of the PLGA film in the microwell.

<table>
<thead>
<tr>
<th>Conc. of PLGA solution</th>
<th>2 wt%</th>
<th>3 wt%</th>
<th>4 wt%</th>
<th>5 wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (μm, mean±STD, n=10)</td>
<td>0.05±0.03</td>
<td>0.27±0.08</td>
<td>0.43±0.05</td>
<td>1.08±0.03</td>
</tr>
</tbody>
</table>

Table 2.3: The thickness the PLGA microstructures in the microwells of a stamp produced using PLGA/acetone solutions with concentrations of 2, 3, 4, and 5 wt%.
2.3.3 Chitosan Microstructures Produced from Aqueous Polymer Solution

Figure 16(A) shows the PDMS stamp used for preparing chitosan microstructures. It consisted of rectangular microwells with round corners which were 95 μm long, 20 μm wide, and 6.90 μm deep. The produced chitosan microstructures or microstrips had the same size and shape as the microwells as shown in Figure 16(B). Figure 17 shows the optical profilometry characterization of a microstrip in microwell. The thickness at the center of the chitosan microstrips is 0.15 ± 0.02 μm (n = 10).

![Figure 2.16](image)

Figure 2.16: (A) SEM image of the PDMS stamp with rectangular microwells. (B) Optical micrograph of released chitosan microstructures in water. The scale bars = 100 μm.
2.3.4 Poly(PEGMA-co-PEGDMA) Microstructures Produced from Liquid Reactive Resin

The PDMS stamp with an array of circular microwells with 5.0 μm-diameter opening, 3.5 μm-diameter bottom, 8.0 μm center-to-center distance, and 1.94 μm depth is shown in Figure 18(A). The produced poly(PEGMA-co-PEGDMA) microstructures are shown in Figure 18(B). Figure 19 shows the optical profilometry characterization of a
microstructure in microwell. The thickness at the center of the microstructures is $0.18 \pm 0.03 \, \mu m \ (n = 10)$.

Figure 2.18: (A) SEM image of the PDMS stamp with circular microwells. (B) Optical micrograph of released poly(PEGMA-co-PEGDMA) microstructures in water. The scale bars = 10 \, \mu m.
Figure 2.19: Optical profilometry characterization of a microwell of a bare PDMS stamp with circular microwells and the stamp after the Poly(PEGMA-co-PEGDMA) film was produced in the microwells. Left: Optical profilometry image of the stamp. The white dashed line delineates the cross-section where a profile was generated. Right: compounded cross-sectional profiles to show the thickness of the poly(PEGMA-co-PEGDMA) film in a microwell.

2.4 Discussion

Soft lithography is a collective name for a group of techniques. Among them, microContact Printing (μCP) [43,44], microTransfer Molding (μTM) [45], and microFluid Contact Printing (μFCP) [46] have been used to produce isolated microstructures, which can be released in the form of particulate microstructures, if printed on a sacrificial layer as demonstrated in this work.

μCP is usually used to generate an ultrathin (< 1 μm) functional film or molecular monolayer with micropattern [43,44,47]. A continuous film or monolayer covering the
face with topological microfeatures of a stamp is a prerequisite and the parts of the film on the raised areas are transferred onto a surface as a result of the conformal contact during printing. Therefore, the lateral size and shape of the microstructures produced by μCP replicate exactly that of the protrusions of the stamp. Elevated temperature has also been used to facilitate the polymer transfer as in this work [43]. However, to our knowledge, this technique has not been used with common hydrophobic polymers, probably because these polymers are not regarded as “functional” enough.

μFCP has been used to print amphiphilic, hydrophilic, and hydrophobic polymers [46]. In addition, the printing can be performed at room temperature because of the formation of isolated microstructures on the protrusions and the presence of residual solvent in the microstructures. However, this method is limited in producing microstructures that exactly replicate the top surface of the protrusions of a stamp because the shrinkage of the polymer solution is the basis for the formation of isolated microstructures.

μTM is able to produce microstructures that exactly replicate the 3-dimensional structures of microwells of a stamp. It is conducted by filling the microwells on a PDMS stamp with a liquid precursor, removing the excess precursor around the microwells, pressing the stamp against the substrate, solidifying the precursor, and removing the stamp. This technique typically results in a thin residual film connecting the produced microfeatures [41]. With regard to the fabrication of particulate microstructures, this residual film is particularly undesirable.

In this work, we used, modified, and extended the above methods for the production of particulate microstructures. μFCP was used directly by us to produce
isolated microstructures with the size control via adjusting the polymer concentration. Moreover, using the stamp with cross-shaped protrusions, we demonstrated for the first time that \( \mu \text{FCP} \) can produce microstructures with lateral shapes other than circular.

When the concentration of the polymer solution increased to a certain level, a partially continuous film was formed on the stamp with the discontinuities around the edges of the protrusions. As a result, transferring the polymer on the protrusions onto a substrate tended to pull off the film in the gaps as well. This problem was solved simply by printing at elevated temperatures. Heating is believed to play dual roles here: enhancing the polymer-substrate adhesion and lowering the cohesive force of the polymer by decreasing its viscosity. This process is slightly different from either \( \mu \text{CFP} \) or \( \mu \text{CP} \), seemingly filling the gap between these two methods nicely.

When the concentration of the polymer solution increased further to another level, a hole-free, thicker polymer film was formed on the stamp. Selective separation of the film thus required more reduction on the cohesive force of the polymer. This was achieved by further increasing the printing temperature. This process is actually \( \mu \text{CP} \) at elevated temperature. The thickness of the produced microstructures increased with the concentration of the polymer solution used. One of the merits of this work is to apply \( \mu \text{CP} \) for the production of thick (> 1 \( \mu \text{m} \)) microstructures made of common thermoplastics. However, as a thicker film was applied on the stamp, clean separation of the polymer on the protrusion from the rest of the film became more difficult. Defects such as whiskers at the edges of the microstructures as shown in Figure 2.6(D-2) were more likely to appear.
μTM was modified in two ways here for the fabrication of particulate microstructures. The first modification used μCP to remove the polymer on the ridges surrounding the microwells of a stamp. This technique has been used to create thermoplastic particulate microstructures with a tight control on their lateral size and shape. Again, the thickness was controllable by altering the polymer concentration.

The second modification used discontinuous dewetting [42] to selectively fill the microwells of a PDMS stamp with a liquid polymer precursor. At the cost of losing the control on the morphology at the thickness direction, the benefits obtained include the elimination of the step for removing excess liquid precursor between the microwells and the absence of the residual film between the produced microstructures. We used this modified μTM with two types of liquid polymer precursors, polymer solutions and reactive polymer resins to demonstrate the potentially wide applicability of this method to many polymers.

Each of the above methods has its own characteristics, strengths, and limitations in the fabrication of particulate microstructures. Much more versatility and capacity in terms of creating particulate microstructures with more than one components and/or with more complicated structures could be achieved if these techniques are used together and used with other polymer microfabrication techniques. For example, μFCP can be used to add a layer of small polymeric “dots” on a layer of larger microstructures produced by the modified μTM, creating particulate hybrid microstructures. Moreover, the modified μTM methods can be used to make particulate microstructures with multiple layers, which can be simply added into the microwells successively without the need for alignment.
Three polymers, PLGA, chitosan, poly(PEGMA-co-EGDMA) were used here to demonstrate the feasibility of the above approaches. PLGA/acetone represents systems consisting of a common thermoplastic polymer dissolved in an organic solvent that has comparable surface tension as PDMS and relatively high evaporation rate. This type of solution can form isolated microstructures on PDMS stamp when used at low concentrations and a pinhole-free film when the concentration increases to a certain level. Polystyrene/chloroform [46], poly(propyl methacrylate) (PPMA)/acetone [48,49] are the two other systems that have been used with μCFP and μCP, respectively.

Chitosan solution represents polymer solutions with a much higher surface tension than PDMS. This type of solutions could be filled only in microwells of a PDMS stamp due to discontinuous dewetting. Formation of the particulate microstructures can simply result from the evaporation of the solvent. Further treatment might be needed to render them stable in water as chitosan was crosslinked by glutaraldehyde.

The poly(PEGMA-co-EGDMA) was produced from a liquid reactive resin in this work. Although numerous polymers can be prepared in this way, some criteria must be met to use this modified μTM for producing particulate microstructures. First, the liquid resin must have high enough surface tension required by discontinuous dewetting. Second, the resin must have low enough evaporation rate to ensure no significant mass loss before it is fully polymerized. Third, the reactive agents in the resin should not diffuse into PDMS significantly. Otherwise they may react within the PDMS matrix, damaging the stamp and sticking the produced microstructures within the microwells. Nevertheless, we have produced particulate microstructures made of poly(hydroxyl
methacrylate) (PHEMA) and poly(methacrylic acid) (PMAA) [50]. Both of them are widely-used hydrogels in drug delivery.

Besides being used as model materials for the fabrication techniques, PLGA, chitosan, poly(PEGMA-co-EGDMA) are also important polymers in terms of drug delivery. PLGA is perhaps the most widely used class of synthetic polymers in drug delivery due to its excellent biocompatibility, controllable biodegradability, ease of processing, and commercial availability. Numerous formulations have been developed based on the PLGA microparticles as drug delivery vehicles. They are administered through a variety of routes to treat a large number of medical conditions. Chitosan has also been widely studied for drug delivery [51,52]. It is a polysaccharide carrying high-density of primary amine groups, rendering it soluble in aqueous acidic solutions. This property makes it possible to void the use of the hazardous organic solvents in chitosan processing. The primary amine groups are also readily available for crosslinking. Glutaraldehyde is a commonly used crosslinking agent for chitosan [53]. The poly(PEGMA-co-PEGDMA) synthesized in this work is a highly-crosslinked, PEG-based polymer. PEG has also been used extensively in drug delivery. Its water-soluble characteristic makes it a widely used material for hydrogels, whose volume-changing ability has been employed in various advanced drug delivery systems.

Polymeric particulate microstructures with a wide range of size (5 μm to 198 μm) have been fabricated in this work. This size range covers the majority of the microparticles currently studied and used for drug delivery. Moreover, since the size is determined by the microfeatures of the stamp used and the fabrication conditions, in
principle, particulate microstructures smaller than 5 μm and larger than 198 μm can be produced using the above methods.

A common feature of the particulate microstructures produced in this work is their plate-like geometry. This type of microstructures has more surface-to-volume ratio than microspheres, making them more likely to aggregate. On the other hand, the area available for bioadhesion of these plate-like microstructures is much larger than that of microspheres. Moreover, if attached to a biosurface, a plate-like microstructure would have smaller side area subject to the detaching force exerted by liquid flow and mechanical abrasion than a microsphere with the same volume. As a result, drug delivery microdevices constructed based on the plate-like microstructures may have stronger and longer adhesion on biosurfaces including the wall of the buccal cavity, esophagus, GI tract, genital tract, blood vessels, and the surface of the eyes.

This plate-like geometry should also render our microfabricated particulate microstructures with different aerodynamic and hydrodynamic properties from microspheres. Whether these properties can be utilized for the benefits of intravenous and pulmonary drug delivery remains an interesting question. In addition, controlling the lateral shape of particulate microstructures is a unique ability of the above techniques. This ability may be used to make integrated drug delivery devices with multiple components constructed in a sophisticated architecture.

Lift-off as a reliable and mild approach was used here to free the particulate microstructures. PVA was chosen as the sacrificial layer for its ease to form thin films, resistance to elevated temperatures, high solubility in water, and well established safety record for biomedical uses. Compared with the concentrated KOH etchant used to release
the silicon-based microdevices at elevated temperatures [11], PVA offers obvious advantages especially if labile biotechnology drugs are involved. Moreover, PVA becomes transiently tacky after absorbing water via a brief exposure to high humidity. This phenomenon has been used in this study to stick polymer microstructures out of microwells at room temperature. Other water-soluble materials such as glucose and sucrose have also been successfully used to make sacrificial layers.

A sacrificial layer may not be necessary to obtain free microstructures. For example, ultrasonic treatment can release microstructures printed on glass, but it may damage the microstructures with sophisticated and labile structures. In some cases, even the printing step can be spared. Some hydrogel microstructures can swell and spontaneously come out of the microwells of a stamp if immersed in water. However, printing microstructures onto a substrate facilitates further processing.

2.5 Conclusions

Soft lithography-based techniques have been employed successfully to fabricate polymeric particulate microstructures. These techniques are much more versatile than conventional microparticle manufacturing methods in controlling the size and shape of the microparticles while are less expensive, require milder processing conditions, and possess greater versatility in materials and processing approaches than silicon-based microfabrication techniques with respect to the fabrication of particulate microstructures for drug delivery. The particulate microstructures with highly uniform and controllable sizes, plate-like structure, and well-defined lateral shapes have been produced using widely-used polymers, offering them potential applications in drug delivery and as the components for the construction of multi-functional drug delivery microdevices.
3.1 Introduction

The previous chapter described the fabrication and characterization of single-layered particulate microstructures. To make a functional drug delivery device, however, more complex structures are usually required such as reservoirs created in the microfabricated drug delivery devices for drug loading [9-12]. In this chapter, we describe three processes for the fabrication of particulate microstructures with single or multiple reservoirs and capsule-like structure.

3.2 Experimental

3.2.1 Materials

The poly(dimethyl siloxane) (PDMS, T2) was purchased from Dow-Corning. The acetone, chitosan, poly(ethylene glycol methacrylate) (PEGMA) with an number average molecular weight (Mn) of 526, poly(ethylene glycol dimethacrylate) (PEGDMA) with Mn of 330, cold water soluble poly(vinyl alcohol) (PVA), 50 wt% glutaraldehyde
aqueous solution, acridine orange 8 (AO8), sucrose, and NaCl were purchased from Sigma-Aldrich. 2,2-dimethoxy-2-phenylacetophenone (Irgacure 651) was donated by Ciba Specialty Chemicals (Tarrytown, NY, USA). Poly(lactic-co-glycolic acid) (PLGA, lactic acid to glycolic acid ratio = 50:50, \( T_g = 48.5 \degree C \) according to the manufacturer) was purchased from Alkermes (Cincinnati, OH, USA). Fresh porcine intestine was obtained from the University Lab Animal Resources at the Ohio State University. It had been frozen at -20\(^\circ\)C and thawed at room temperature before use.

An optical profilometer (WYKO NT3300, Veeco Instruments, Woodbury, NY, USA) and a Scanning Electron Microscope (Hitachi S-3000H) were used to characterize the samples. Optical micrographs were captured using an Olympus BX 60 optical microscope, a Nikon Eclipse TE 2000-S inverted phase contrast optical microscope, and an Olympus BH-2 optical microscope.

### 3.2.2 PDMS Stamp Preparation

Silicon masters with the designed microfeatures were produced by standard photolithography using transparency masks. PDMS stamps were prepared by casting the PDMS resin and the curing agent at a 10:1 weight ratio against the masters for 48 hours at room temperature.

### 3.2.3 Fabrication and Characterization of Microstructures with Single Reservoirs

An UV-curable resin was used to make microstructures with single reservoirs. The resin consisted of PEGMA and PEGDMA with a weight ratio of 1:1 and 3 wt\% Irgacure 651 as photoinitiator. The fabrication process is shown in Figure 3.1. The PEGMA/PEGDMA resin was brushed with an applicator across the PDMS stamp with an array of ring-shaped microwells. The resin spontaneously trapped in the microwells
without wetting the surrounding areas except within the annuli. The stamp was then exposed to UV light at an intensity of 25 mW/cm$^2$ (EXFO Novacure 2000 Ultraviolet/Visible Spot Curing System) for 2 min under nitrogen environment. A glass slide covered with a thin PVA layer was prepared by casting a 2 wt% PVA/water solution on the slide. The stamp was placed on the PVA-covered slide immediately (< 5 s) after the slide was briefly (~5 s) exposed to water vapor generated from a 90 °C water bath. After maintaining the contact for about 1 minute, the stamp was peeled away, leaving the microstructures on the slide. Finally, by adding water, PVA was dissolved and the microstructures were released.

The thickness of the base layer of the microstructures containing single reservoirs was measured using the optical profilometer by measuring the heights of cured polymer above the micropillars in the microwells relative to the surrounding area. Depths of the reservoirs and the thickness of the base layer of the microstructures printed on the PVA-coated glass slide were also measured. Mean and standard deviation was calculated based on the randomly-chosen microstructures.

3.2.4 Rapid Filling of Single-Reservoir Microstructures with a Water Soluble Agent

A glass slide covered by PLGA rather than PVA was prepared by casting a thin layer of 2.5 wt% PLGA/acetone solution on the slide. A PDMS stamp with already cured polymer microstructures was placed on the slide on a hotplate at 120 °C, immediately followed by removing the stamp and slide away from the hotplate while maintaining the intimate stamp-slide contact. The stamp was peeled off from the slide after they cooled down to room temperature, leaving the reservoir-containing microstructures on the slide.
A 25 wt% NaCl aqueous solution was brushed onto the microstructures with an applicator. Crystals were formed in the reservoirs instantly.

Figure 3.1: Schematic illustration of the process for the fabrication of particulate microstructures with single reservoirs.
3.2.5 Fabrication and Characterization of Microstructures with Multiple Reservoirs

The fabrication process is schematically shown in Figure 3.2. First, the PDMS stamp was dip-coated in a 6 wt% PLGA/acetone solution. Second, the stamp was placed on a glass slide on a hotplate at 120 °C and peeled away immediately, transferring the PLGA originally at the area surrounding the microwells onto the slide. Third, the stamp was placed on a glass slide covered by a thin PVA film at 120 °C. A slight pressure was exerted to ensure complete conformal contact between the stamp and the slide. The slide and stamp were removed away from the hotplate while maintaining their close contact. After they cooled down in air, the stamp was peeled off from the slide, leaving PLGA originally in the microwells on the slide as discrete microstructures. The slide with the PLGA microstructures was then mounted on an Instron Microtester (Canton, MA 02021, USA) with a heating module. A flat PDMS block was used to flatten the PLGA microstructures at 100 °C with a pressure of $1.1 \times 10^5$ Pa for 1 minute. Then a PDMS stamp with micropillar array was used to emboss the microstructures at 100 °C with an approaching speed of 0.1 mm/s and a pressure of $2.2 \times 10^5$ Pa for 1 minute.

From the optical profilometry data on the microstructures with multiple reservoirs, ten microstructures were randomly chosen to measure lateral sizes of the microstructures. Four microreservoirs out of the ten microstructures were randomly picked up for measuring the depths of the wells and the thickness of the base layer. The thicknesses at the centers of the 40 microreservoirs were measured and to obtain the mean and standard deviation.
3.2.6 Fabrication and Characterization of Capsule-Like Microstructures

The fabrication procedure is shown in Figure 3.3. First, the PDMS stamp was dip-coated in a 6 wt% PLGA in acetone solution. An aqueous solution of 40 wt% sucrose and 1 wt% AO8 was brushed across the surface of the stamp with a cotton swab. The stamp was then placed in a desiccator under vacuum for 20 minutes to dry the sucrose solution. After that, the PDMS stamp was dipped again in the 6 wt% PLGA/acetone solution. The stamp was then brought in contact with a glass slide at 120 °C on a hot plate.
and peeled away immediately (contact time: ~1 s), transferring the PLGA originally on the ridges of the stamp onto the glass slide. Next, the stamp was brought in contact with a PVA-covered glass slide at 120 °C on a hotplate with a slight pressure to ensure complete conformal contact between the stamp and the slide. The slide and the stamp were removed away immediately (contact time: ~1 s) from the hotplate while maintaining their close contact. After they cooling, the stamp was peeled away from the slide, transferring the capsule-like microstructures, or microcapsules, originally in the microwells onto the slide. Water was added to release the microcapsules.

Figure 3.3: Schematic illustration of the process for the fabrication of capsule-like microstructures.
The thickness of each layer of the microcapsules was obtained by measuring the depth of microwells using the optical profilometer after each layer was applied on the stamp. To use the stamp surface as a reference, PLGA on the ridges of the stamp was removed by stamping onto a glass slide at 120 °C after each PLGA layer was applied. Multiple microwells were measured to acquire the averages and standard deviations. The thickness of each layer was calculated from the depths of the microwells.

3.3. Results

3.3.1 Microstructures with Single Reservoirs

Figure 3.4 shows SEM images of the PDMS stamp, cured polymer in the microwells, reservoir-containing microstructures on the PVA/slide, and an optical micrograph of the released microstructures in water. The stamp consisted of an array of ring-shaped microwells. As shown in Figure 3.5(A), the width of the microwells and the circular micropillars in the center of microwells are 80 and 40 μm, respectively. The center-to-center distance between two adjacent microwells is 160 μm. The depth of the microwells is 7.0 μm and the area surrounding the microwells has the same height as the micropillars. Figure 3.5(B) shows the optical profilometry measurement of cured polymer in a microwell of the stamp. The thickness of the base layer at the center point is 1.6 μm. Measurements on ten microstructures in microwells give a base-layer thickness of 1.6 ± 0.2 μm (n = 10). Figure 3.5(C) is the optical profilometry measurement of the depth of a reservoir and the thickness of its base layer of a microstructure on PVA. The depth and thickness at the center are 6.2 μm and 1.3 μm, respectively. Measurements on ten microstructures on PVA give a reservoir depth and base-layer thickness of 6.2 ± 0.1 and 1.2 ± 0.2 μm, respectively.
Figure 3.4: SEM images of (A) the PDMS stamp, (B) the PDMS stamp with cured poly(PEGMA-co-PEGDMA) in the microwells, and (C) the particulate microstructures on PVA/slide. (D) Optical micrographs of the particulate microstructures in water. The scale bar = 100 μm.

The lateral size and shape of the microstructures remained the same as that of the microfeatures of the stamp. However, the microstructures transferred onto the PVA/slide are 0.8 μm shallower than the depth of the microwells of the stamp. The pressure exerted during printing of the microstructures onto the PVA/glass might have caused this dimension reduction in the vertical direction. The thickness of the base layer is also reduced from 1.6 to 1.2 μm.
For a microstructure in Figure 3.5(C), the reservoir volume available for drug loading is approximately 7.8 pl (volume = \( \pi \times \text{diameter}^2 \times \text{depth} / 4 = 3.14 \times 40 \ \mu m \times 40 \ \mu m \times 6.2 \ \mu m \ / 4 \)). If erythropoietin (EPO) for treating anemia is used as a model drug with a typical dose of 0.03 mg per day for a 70 kg patient [4], the number of microdevices needed for one dose is approximately \( 2.3 \times 10^5 \) by assuming that an EPO solution of 50 mg/ml is filled in the reservoirs and a bioavailability of 33% can be achieved for oral delivery using these devices. Using the stamp in the study, which produces one microstructure per 160 × 160 \( \mu m^2 \) area, the total stamping area would be approximately 8 × 8 cm\(^2\). This area can be covered by a single silicon wafer that is commonly used to make the master for PDMS stamp fabrication.
Figure 3.5: Optical profilometry characterization of (A) a microwell on the stamp, (B) cured polymer in a microwell of the stamp (The PDMS stamp is compounded on the profile), and (C) a microstructure on PVA. The discrete sections of the profiles are a result of inability of the optical profilometer to acquire data from an inclined surface. The white dashed lines delineate the cross sections that profiles of the stamp were acquired.
Figure 3.6 shows the result of filling the microreservoirs with an aqueous NaCl solution. NaCl crystals were formed in all microreservoirs of the microstructures. The crystal sizes were relatively uniform across the whole filling area. Small crystals were also formed by the sides of the outer walls of the microstructures.

![Image of NaCl crystals in microreservoirs](image.png)

Figure 3.6. Optical micrograph of NaCl crystals in the microreservoirs of the microstructures printed on PLGA. The inset shows an enlarged microstructure containing NaCl crystals pointed by the arrow. The scale bar = 100 μm.
3.3.2 Microstructures with Multiple Reservoirs

Figure 3.7 shows SEM images of the PDMS stamps used. The square microwells are 30 μm wide, 10.7 μm deep, and 50 μm in center-to-center distance. The cylindrically shaped micropillars are 7.0 μm in diameter, 3.4 μm in height, and 8.3 μm in center-to-center distance.

![Figure 3.7: SEM images of the PDMS stamps with microwells (A) and micropillars (B).](image)

The results of the main fabrication steps are shown in Figure 3.8. The multi-reservoir microstructures transferred onto the PVA (Figure 3.8(C)) have also been used as a master to prepare a PDMS mold. Multi-reservoir microstructures were made through the photo polymerization method as described in the fabrication of single-reservoir microstructures.
Shown in Figure 3.9 is the optical profilometry characterization of a typical microstructure on PVA. It contains 14 enclosed reservoirs surrounded by a number of open reservoirs. Analysis of the optical profilometry data shows that the diameter of the microstructures with reservoirs is $45.5 \pm 0.5 \, \mu m$ ($n = 10$); the depth of the reservoirs is $3.05 \pm 0.05 \, \mu m$ ($n = 40$), and the thickness of the base layers is $0.35 \pm 0.03 \, \mu m$ ($n = 40$). The enclosed reservoirs can be used for drug loading. For the microstructure shown in Figure 3.9, the available volume for drug loading is approximately $1.7 \, pl$ (volume = $\pi \times \text{diameter}^2 \times \text{depth} \times \text{reservoir number} / 4 = 3.14 \times 7.1 \, \mu m \times 7.1 \, \mu m \times 3.05 \, \mu m \times 14 / 4$).

As a result, the number of microstructures needed for one-day dose EPO for a 70 kg patient is approximately $10^6$. Since one microstructure takes an average area of $50 \times 50 \, \mu m^2$, the total stamping area required for making such quantity of microdevices would only be $5 \, \text{cm} \times 5 \, \text{cm}$. 
Figure 3.8: Optical micrographs of PLGA microstructures on PVA (A) before and (B) after they were flattened, (C) after embossing, and (D) microstructures with multiple reservoirs released in water. The scale bars = 100 μm.
3.3.3 Capsule-Like Microstructures

The microwell stamp (Figure 3.7(A)) used to fabricate microstructures with multiple reservoirs was also used here. The produced microcapsules on PVA and in water are shown in Figure 3.10. The microcapsules appeared orange due to the encapsulated AO8, a dye used as a contrast enhancer. Sucrose in a 40 wt% aqueous solution was used as a model drug in this study because of its wide applications as a drug excipient and its high solubility in water.

The released microcapsules in water remained orange and swelled as time elapsed. The swelling of the microcapsules was likely caused by the osmotic pressure created by the encapsulated sucrose. This phenomenon indicates that 1) water can diffuse into the...
microcapsules; 2) the PLGA layers can be stretched significantly without breaking; and 3) the encapsulation of the microstructures is successful.

Figure 3.10: Optical micrographs of (A) microcapsules on PVA, microcapsules (B) just released and (C) 18 minutes later in water. Scale bars = 50 μm.
The microcapsules were also characterized using the optical profilometer after each step of fabrication. Figure 3.11 shows the compounded cross-sectional profiles of a microwell before and after each layer was applied. The layers were not flat, but they were relatively symmetrical to the center. Statistical results of the thickness of the layers are given in Figure 3.12. The average thicknesses of the first PLGA layer, the sucrose layer, and the second PLGA layer are 1.0, 3.3, and 1.3 μm, respectively. The mass fraction of the drug in these microcapsules is estimated to be 60% (= 3.3 / (1.0 + 3.3 + 2.3) × 100%), which is much higher than that of microspheres used as injectable drug depot system (11 wt%) [7].

Figure 3.11: Optical profilometry characterization of microcapsules. Left: optical profilometry image of the stamp with an array of microwells. The white dashed line delineates the cross section that profiles of the stamp were acquired after each layer was applied. Typical profiles are compounded in the right diagram.
Figure 3.12: Diagram showing the depths at the center of the microwells and the layer thickness after each layer was applied.

3.4. Discussion

3.4.1 Materials

The three polymers, PLGA, chitosan, and poly(PEGMA-co-EGDMA), used here to demonstrate the feasibility of above mentioned approaches are important polymers in drug delivery. PLGA is the most widely used synthetic polymer in drug delivery for its excellent biocompatibility, controllable biodegradability, ease of processing, and commercial availability. Numerous formulations have been developed based on PLGA microparticles that are administered through a variety of routes to treat many diseases. Chitosan has also been extensively studied for drug delivery [51,52]. It is a polysaccharide carrying high-density of primary amine groups, rendering its solubility in
aqueous acidic solutions. This property makes it possible to avoid the hazardous organic solvents in chitosan processing. The primary amine groups are also readily available for crosslinking. Glutaraldehyde is a commonly used crosslinking agent for chitosan [53].

The poly(PEGMA-co-PEGDMA) synthesized in this work is a highly-crosslinked, PEG-based polymer. PEG has also been used extensively in drug delivery. Its water-soluble characteristic makes it a widely used material for hydrogels. The volume-changing ability of hydrogels has been employed in various advanced drug delivery systems. Besides these three polymers, many other polymers can be used with the methods developed in this study.

3.4.2 Microstructures with Single Reservoirs

The geometries of the microstructures and their reservoirs are determined by the microfeatures on the stamp. In principle, a broad range of stamp microfeatures can be created in terms of the size, shape, depth of reservoirs, and number of reservoirs per microstructure, thus rendering this technique highly promising for specific drug delivery requirements. Compared to the photolithography-based techniques for fabricating reservoir-containing particulate microdevices [9-12], the method used in this study for is simpler, more environmentally friendly, and of lower cost. The cleanroom-based photolithography is not needed after the initial master mold-patterning. The filling of microwells with a liquid resin is selective, rapid, and parallel due to discontinuous dewetting [47]. Furthermore, micrometer-scaled alignment used for carving reservoirs in microplates is not required. Finally, water instead of concentrated (6M) KOH solution is used to release the microstructures.
Rapid filling of the microreservoirs with water-soluble agents was demonstrated with NaCl. PLGA, instead of PVA, was used to make the sacrificial layer to avoid unwanted release of the microstructures while applying the NaCl aqueous solution. Moreover, the hydrophobicity of PLGA is required for discontinuous dewetting [47]. Although not shown in this study, dimethyl sulfoxide (DMSO) can be used to release the microstructures by dissolving PLGA.

This method has some limitations. Selectively filling the microwells of a PDMS stamp with a liquid polymer precursor requires the liquid to have a higher surface tension than PDMS. Otherwise the liquid would spread on the PDMS stamp. The evaporation rate of the liquid should also be low enough so it would not evaporate much before being cured. Moreover, the toxic un-reacted monomers, crosslinkers, and initiators present in the cured polymers should be removed for drug delivery purposes. This might be achieved by rinsing the microstructures in water while they are on the PLGA sacrificial layer.

3.4.3 Microstructures with Multiple Reservoirs

The profile of the microstructures containing multiple reservoirs is mainly determined by the microfeatures of the two stamps. Since the microfeatures of a stamp can be produced with a broad range of shape, size, and depth, particulate microstructures with various geometries can be fabricated for specific drug delivery requirements. Compared to the single-reservoir microstructures, creating multiple reservoirs in one microstructure provides a possibility to incorporate multiple agents separately in a single device. In contrast to the method used above to produce single-reservoir microstructures, this approach does not involve the use of toxic reactive chemicals. The only non-aqueous
liquid involved in this method is acetone, which is a fairly benign organic solvent compared to \( \text{H}_2\text{SO}_4, \text{H}_2\text{O}_2, \) and concentrated KOH solution used by others [9-12].

A limitation of this method is the distortion of the microstructures at the regions away from the center of the embossing area, probably caused by the lateral deformation of the soft PDMS stamp during embossing. Use of a harder embossing mold made of metals or Teflon may solve this problem. Although uniform multiple reservoirs have been created on individual microstructures, a challenge is to load them with different agents.

3.4.4 Capsule-Like Microstructures

Compared to the conventional polymeric microspheres for drug delivery, the microfabricated capsules possess several advantages. First, they are uniform in size and shape, rendering them better control for precise drug release. Second, they have high drug loading capacity. Third, the microcapsules are absent of the burst effect typically associated with the conventional microspheres [14] because drug is totally encapsulated in the microcapsules. One of the potential applications of this type of particulate microstructures is as an injectable depot system for sustained drug release.

One potential drawback with this method is the organic solvent used for polymer film deposition, which may result in the diffusion of the organic solvent into the drug layer. This hazardous effect needs to be further studied. The elevated temperature required for stamping is also not desirable, but the exposure to the elevated temperature has been minimized to a few seconds. To achieve high drug loading, the drug solution used to fill in the microwells needs to have high concentration. This may be difficult for many drugs because of the limited aqueous solubility. One potential solution is to use concentrated suspensions of drug micro- or nano-particles.
3.5 Conclusions

Three types of particulate-like polymer microstructures have been produced for drug delivery applications by soft lithography. Two of them contain single and multiple reservoirs, respectively. The third type has a capsule-like structure. These microstructures have unique characteristics compared to conventional drug delivery microparticles, rendering them attractive for oral drug delivery and injectable sustained drug release.
4.1 Introduction

Mucus covers various internal surfaces of the body, including oral cavity, stomach, intestine, rectal tract, nasal cavity, respiratory tract, genital tract, and eyes. Medicines are administered into the body through all these surfaces under the collective name of transmucosal drug delivery.

Mucus plays important physiological functions, such as buffering the acid in the stomach, lubricating the movement of the luminal content in the gastrointestinal (GI) tract, trapping airborne particles in the respiratory tract, and preventing bacteria and viruses from contacting the cells in the GI tract. From a perspective of drug delivery, mucus can serve as a docking layer to which a mucoadhesive device can bind for 1) releasing drugs at a targeted location, 2) releasing drugs for an extended duration at a constant rate, and 3) creating locally high drug concentration gradient favoring drug absorption by forming intimate contact with the underlying tissue [54].
Among all the transmucosal drug delivery routes, oral administration is the most widely used one due to its convenience and non-invasiveness. Many drugs, however, are not suitable for this route with conventional formulations owing to 1) the rapid passage of the dosages through the desired absorption region such as the upper portion of the small intestine, 2) massive degradation of free drugs in the gastrointestinal lumen, and 3) low permeability of the drugs across the intestinal epithelium. To overcome these hurdles, various strategies have been taken. Some of them are based on the use of mucoadhesive materials that are able to stick to the mucus for a prolonged period of time.

A number of physiological conditions limit the application of mucoadhesive systems to oral drug delivery. Among them is the constant shed-off of the mucus that limits the retention of conventional mucoadhesive devices such as microparticles and patches which can only attach to the surface layer of mucus due to their relatively large sizes as schematically shown in Figure 4.1(A) [55]. It is also generally known that gastrointestinal mucus renews completely within a few hours [56], which apparently places an upper limit on the retention time of a mucoadhesive system. In addition, mucus layer can hinder the diffusion of drugs or drug carriers from the device to the absorption site [57,58].

However, what is usually not considered in the design of a mucoadhesive device is the bilayered structure of the GI mucus as displayed in rats [59,60]. One of the two layers of the mucus is on the lumen side and called loosely-adherent layer because it can be easily sucked away. The other is on the epithelium side and called firmly-adherent layer since it is tightly attached to the epithelial cells and resistant to suction. Experimental results indicate that the mucus that experiences full renewal in the
generally-regarded turnover time might solely be the loosely-adherent layer, and the firmly-adherent mucus probably has a longer turnover time. As a result, longer retention than a few hours may be achieved if a device can penetrate the loosely-adherent layer and adhere to the firmly-adherent mucus layer.

This chapter introduces a novel strategy in an attempt to solve these problems by using self-foldable microstructures. These microstructures were expected to be able to bind to and fold into the mucus layer as shown in Figure 4.1. As a result, these devices should be more resistant to the surface shed-off of the mucus layer than conventional mucoadhesive devices or even more desirable, to attach to the firmly-adherent layer and become independent of mucus turnover (Figure 4.1(B)). Another potential advantage of this type of microstructures is its ability to “grab” even more permanent biostructures --- the intestinal villi (Figure 4.1(C)). It is also possible that the epithelium layer under the mucus can be disrupted by the folded microstructures leading to increased drug permeation (Figure 4.1(D)). Finally, drug released from the parts that folded into the mucus would have a shorter diffusion distance to the absorption tissue.

4.2 Experimental

4.2.1 Materials

The PDMS (T2) was purchased from Dow-Corning. The chitosan, PEGMA with an average molecular weight (Mn) of 526, PEGDMA with Mn of 330, methacrylic acid (MAA), ethylene glycol methacrylate (EGDMA), cold water soluble PVA, 50 wt% glutaraldehyde aqueous solution, and ethanol were purchased from Sigma-Aldrich. 2,2-dimethoxy-2-phenylacetophenone (Irgacure 651) was donated by Ciba Specialty Chemicals. Fresh porcine intestine was obtained from the University Lab Animal
Resources at The Ohio State University. It had been stored frozen at -20°C and thawed at room temperature before use.

Figure 4.1: Schematic representations of (A) mucoadhesive microparticles and patch for drug delivery, (B) microstructures folded into the mucus layer in contact with the firmly-adherent layer, (C) foldable microstructures “grabbing” the intestinal villi, and (D) foldable microstructures mechanically disrupting the tight junctions between epithelial cells.
4.2.2 PDMS Stamp Preparation

Silicon masters with the designed microfeatures were produced by standard photolithography using transparency masks. PDMS stamps were prepared by casting the PDMS resin and the curing agent at a 10:1 weight ratio against the masters for 48 hours at room temperature.

4.2.3 Preparation of PMAA Microparticles

A PMAA film was prepared by the radical polymerization induced by UV light (4 mW/cm², 10 minutes). The resin consisted of MAA as monomer, TEGDMA as the crosslinking agent with a molar ratio of 100:1 (MAA to TEGDMA), 1 wt% Irgacure 651, 25 wt% water and 25 wt% ethanol as solvents. The crosslinked film was ground into fine powder and sieved to obtain particles around 100 μm diameter.

4.2.4 Fabrication of Single-Layered Chitosan Microstrips

The same stamp, chitosan solution, and protocol as preparing chitosan microstrips in Chapter 2 were used here.

4.2.5 Fabrication of Single-Layered Poly(PEGMA-co-PEGDMA) Microstrips

The same protocol as preparing the poly(PEGMA-co-PEGDMA) microstructures in Chapter 2 was used here. The stamp was the one used to prepare the chitosan microstrips. The resin consisted of PEGMA and PEGDMA with a weight ratio of 5:1 and 3 wt% Irgacure 651 was used as the photoinitiator.

4.2.6 Fabrication of Chitosan/Poly(PEGMA-co-PEGDMA) Bilayered Microstructures

Three resin mixtures with PEGMA to PEGDMA weight ratios of 1:1, 3:1, and 5:1 were prepared. In all three samples, Irgacure 651 was used as the photoinitiator with a concentration of 3 wt%. The fabrication process for the bilayered microstructures is
shown in Figure 4.2. Crosslinked chitosan film and poly(PEGMA-co-PEGDMA) were prepared in the microwells of a PDMS stamp consecutively using the same protocols as preparing single-layered microstrips. The stamp was placed on a PVA-covered glass slide immediately (< 5 s) after the slide was briefly (~5 s) exposed to water vapor generated from a hot water bath. The stamp and the slide were then maintained in contact for 10 min. After they cooled down to room temperature, the stamp was peeled off, leaving the microstructures on the slide. Water was added to dissolve PVA and the released microstructures folded spontaneously. In addition to the rectangular microstrips, microstructures with other shapes and sizes have been fabricated using the above protocol. One type of them was cross-shaped microstructures using the PEGMA/PEGDMA resin with PEGMA to PEGDMA weight ratio of 5:1. Another type was microstrips that were much longer than the above microstrips using the PEGMA/PEGDMA resin with PEGMA to PEGDMA weight ratio of 1:1.
Figure 4.2: Schematic illustration of the process for the fabrication of self-foldable bilayered microstructures.

4.2.7 Optical Microscopy

Micrographs of the microstructures were captured using an Olympus BX 60 optical microscope equipped with an Olympus DP 11-N CCD camera, a Nikon Eclipse TE 2000-S inverted phase contrast optical microscope equipped with a Photometrics CoolSNAP™ CCD camera, and an Olympus BH-2 optical microscope equipped with a Fujifilm Finepix 2600 digital camera.
4.2.8 Measurement of the Length of the Released Single-Layered Microstrips

The lengths of the single-layered microstrips in water were measured on the optical micrographs using an image analysis software: Image Tool (version 3.00, The University of Texas Health Science Center, San Antonio, TX, USA). Five microstrips were randomly chosen for each type of microstrips to obtain the averages and standard deviations.

4.2.9 Measurement of the Thickness of the Microstrips

An optical profilometer (WYKO NT3300, Veeco Instruments, Woodbury, NY, USA) was used to characterize the PDMS stamp and film thickness in the microwells. Depths of the microwells were measured before the chitosan solution was applied, after the chitosan layer was crosslinked, and after the poly(PEGMA-co-PEGDMA) layer was crosslinked. Cross-sectional profiles across the center of the microwells were acquired from optical profilometry data. For each microwell, the depths along the profile across the microwell were averaged as the depth of the microwells. Ten microwells at different areas of the stamp were randomly chosen to obtain the average depth and standard deviation. The depths were used to calculate the average and standard deviation of the thickness of chitosan and poly(PEGMA-co-PEGDMA) layers.

4.2.10 Measurement of the Deflection of Folded Bilayer Microstrips

The deflection of the folded bilayer microstrips was obtained through measurement on those microstrips that happened to reside on their sides vertically. Three microstrips were measured for each type to obtain the means and standard deviations.
4.2.11 Determination of Swelling Ratios

Poly(PEGMA-co-PEGDMA) samples were prepared as discs with 4 mm in diameter and 0.9 mm in thickness. Volume in the unswollen state was calculated from the buoyant force exerted on samples immersed in water measured using a dynamic contact angle analyzer (DCA 332, Chan Instruments, Cerritos, CA, USA). To obtain the volume of a sample in the swollen state, the sample was immersed in water for three hours. Silicone oil, which is immiscible to water, was used as the medium to generate the buoyant force. The volume swelling ratio was calculated by dividing the swollen volume by the unswollen volume. Three samples were used for each type of hydrogel to obtain means and standard deviations.

4.2.12 Measurement of Elastic Modulus

The elastic moduli of the dry crosslinked chitosan and poly(PEDGMA-co-PEGDMA) were measured using a Rheometrics System Analyzer (RSA III, TA Instruments-Waters LLC, New Castle, DE) at a displacement rate of 0.1 mm/s. Poly(PEGMA-co-PEGDMA) films with the same compositions and UV exposure conditions as those in the bilayer microstrips were prepared and cut into 5 mm by 30 mm rectangular strips with a thickness of 0.9 mm. The chitosan film was prepared by casting a solution of 1 wt% chitosan in 2 wt% acetic acid aqueous solution and cut into 5 mm by 30 mm rectangular strips with a thickness of 0.1 mm.

4.2.13 Flow Test on Mucoadhesion

The experimental setup for testing the mucoadhesion of the foldable microstructures under liquid flow is shown in Figure 4.3. A piece of ~1 × ~2 cm pig intestine was prepared and glued on a plastic film with its lumen side facing up. The
plastic film was then bound to a plastic trough with a width of ~2.5 cm, height of ~2 cm, and length of ~15 cm using a double-sided adhesive tape. The trough was mounted under an optical microscope for visual observation. The bilayered microstructures were first printed on a ~100 μm-thick PVA film prepared by casting a 10 wt% PVA aqueous solution. As shown in Figure 4.4, the sample was then placed on the intestine with the bilayered microstructures contacting the intestinal mucosa. A small amount of water was added to dissolve the surface layer of the PVA film, inducing the detachment of the bilayered microstructures from the PVA film, which was removed manually using a pair of tweezers. PMAA microparticles were placed on a different area on the same piece of intestine as control. Flow test was started by flushing distilled water from one end of the trough through the sample into a collector. The flow rate was controlled by a faucet and measured using a graduated measuring cylinder and a timer.
Figure 4.3: Left: schematic illustration of the experimental setup for the flow test on the mucoadhesivity of samples on the pig intestinal mucosa under water flow. Right: photograph of the experimental setup.
4.3 Results

4.3.1 Bilayered Microstrips

The PDMS stamp used in this study was the same as the stamp used to prepare single-layered chitosan microstrips in Chapter 2. It consists of an array of rectangular microwells, each 95 μm long, 20 μm wide, and 6.9 μm deep. Cross-sectional profiles of the microwells were acquired from optical profilometry data before the chitosan solution was applied, after the chitosan film was crosslinked, and after the PEGMA/PEGDMA) resin was cured. By compounding three typical profiles together, Figure 4.5 shows that
the thickness of the chitosan and the poly(PEGMA-co-PEGDMA) layer in the center are 0.10 μm and 4.58 μm respectively.

Figure 4.5: Optical profilometry characterization of rectangular self-foldable bilayered microstructures. Left: optical profilometer image of the stamp with an array of 20 × 95 μm rectangular microwells. The white dashed line delineates the cross section that profiles of the stamp were acquired before the chitosan solution was applied, after the chitosan was crosslinked, and after the PEGMA/PEGDMA resin was cured. These profiles are compounded in the right diagram.
Figure 4.6 shows single-layered chitosan and poly(PEGMA-co-PEGDMA) microstrips in water. The length of the chitosan and the poly(PEGMA-co-PEGDMA) microstrips in water are $96.2 \pm 1.2 \, \mu m$ ($n = 5$) and $117.2 \pm 1.1 \, \mu m$ ($n = 5$), respectively. Compared to the length of the microwells (95 \, \mu m), the chitosan microstrips showed little swelling while the poly(PEGMA-co-PEGDMA) microstrips swelled significantly. Figure 4.6(C) shows curled bilayered microstrips with PEGMA to PEGDMA weight ratio of 5:1. The averaged thicknesses of the chitosan and poly(PEGMA-co-PEGDMA) across the cross-section of the microwells are $0.27 \pm 0.03 \, \mu m$ ($n = 10$) and $4.54 \pm 0.24 \, \mu m$ ($n = 10$), respectively.

Figure 4.6: Optical micrographs single-layered chitosan (A), poly(PEGMA-co-PEGDMA) (PEGMA : PEGDMA = 5:1 by weight, B), and bilayered chitosan/poly(PEGMA-co-PEGDMA) microstrips (PEGMA : PEGDMA = 5:1 by weight, C) in water. The scale bars = 100 \, \mu m.
To control the degree of folding, we prepared samples with different PEGMA-to-PEGDMA ratios of the poly(PEGMA-co-PEGDMA) layer in bilayered microstrips. Besides a 5:1 ratio, microstrips with ratios of 3:1 and 1:1 were also fabricated. They showed different degrees of curling in Figure 4.7. A higher PEGMA to PEGDMA ratio led to a higher curvature of the self-folded microstrips.

Figure 4.7: Optical micrographs of the chitosan/poly(PEGMA-co-PEGDMA) bilayered microstrips in water with the weight ratio of the PEGMA to PEGDMA in the resins of 1:1 (A), 3:1 (B), and 5:1 (C). The scale bars = 100 μm.
4.3.2 Modeling

The folding behavior of bilayered microstrips in medium can be treated as bending of a composite beam with no slip at the interface [61]. Since the deflection of the structure is an order of magnitude larger than its thickness, the swelling in the thickness direction is negligible. Other assumptions include the neglected swelling for the chitosan layer and the linearly elastic deformation at small strains. The tip deflection, $y_{\text{max}}$, due to the swelling expansion can be expressed as equation (1):

$$y_{\text{max}} = 3 \cdot (\gamma_b - \gamma_a) \cdot (\nu - \nu_0) \cdot (t_a + t_b) \cdot \frac{L^2}{t^2} \cdot K = \frac{C}{2} L^2 \quad (1)$$

Here, subscripts a and b refer to the non-swelling and the swelling layers, respectively. $\gamma$ is the swelling coefficient of expansion and $t$ is the layer thickness. $\nu_0$ (equal to 1.0) is the volume-swelling ratio of bilayered structure at initial state. The volume-swelling ratio $\nu$ in equilibrium can be estimated by the volume-swelling ratios of layers a and b as shown in equation (2):

$$\nu = \frac{t_a}{t_a + t_b} \cdot \nu_a + \frac{t_b}{t_a + t_b} \cdot \nu_b \quad (2)$$

$K$ and $C$ are terms derived from the equivalent property of bilayered strips, and shown as follows:

$$K = 4 + 6 \cdot \frac{t_a}{t_b} + 4 \cdot \left(\frac{t_a}{t_b}\right)^2 + \frac{E_a}{E_b} \cdot \left(\frac{t_a}{t_b}\right)^3 + \frac{E_b}{E_a} \cdot \frac{t_b}{t_a} \quad (3)$$

$$C = 6 \cdot (\gamma_b - \gamma_a) \cdot (\nu - \nu_0) \cdot (t_a + t_b) / (t^2 \cdot K) \quad (4)$$

where $E$ is the elastic modulus. $L$ is the modified half-length derived from the initial
half-length of the microstrip $L_0$ as in equation (5):

$$L = \left( \frac{C \cdot L^2}{4} + \frac{1}{C} \right) \cdot \sin \left( \frac{2 \cdot L_0 \cdot C \cdot \nu^{1/3}}{C^2 \cdot L^2 + 4} \right) \quad (5)$$

By incorporating the swelling ratios, strip moduli, and strip thicknesses, the tip deflection for circularly folded microstrips can be given by equation (6):

$$y_{\text{max}} = \frac{3 \cdot (\nu_b^{1/3} - 1) \left[ \frac{t_a \cdot \nu_a}{t_a + t_b} + \frac{t_b \cdot \nu_b}{t_a + t_b} - 1 \right] \cdot (t_a + t_b) \cdot L^2}{(\nu_b - 1) \cdot t_b^2 \cdot [4 + 6 \cdot \frac{t_a}{t_b} + 4 \cdot \frac{t_a^2}{t_b} + \frac{E_a \cdot t_a^3}{E_b \cdot t_b^3} + \frac{E_b \cdot t_b}{E_a \cdot t_a}]} \quad (6)$$

The tensile tests for the dry gel samples were performed and the elastic modulus of a swollen cross-linked structure, $E_b$, was calculated based on the following equation: [62]

$$E_b = A \cdot e^{B \cdot X} \cdot \nu_b^{-1/3} \quad (7)$$

where $X$ is the PEGDMA fraction. The parameters $A$ and $B$ are determined by the measured swelling ratios and elastic moduli of poly(PEDGMA-co-PEGDMA) samples ($A = 42477$, $B = 3.745$). Since the chitosan layer is assumed non-swelling and its dry modulus ($E_a = 8.25$ MP) is an order of magnitude larger than that of the swelling layer, this value was used in the wet state. Substituting the measured and calculated parameters into the equation (6) ($t_a = 0.27$ μm, $t_b = 4.54$ μm, $\nu_a = 1.0$, $\nu_b = 1.15 \sim 1.45$, $L_0 = 47.5$ μm), the calculated self-folding of microstrips with 1:1 PEGMA-to-PEGDMA ratio exhibits almost the same profile as the experimental result (Figure 4.8(A)). As shown in Figure 4.8 (B), the calculated and experimental tip deflections agree well for all PEGMA to PEGDMA ratios studied in this work.
Figure 4.8: (A) Experimental and calculated results of curled bilayer microstrip with the higher-swelling layer made of a 1:1 PEGMA-co-PEGDMA weight ratio. (B) Experimental and calculated results of tip deflections of microstrips as a function of weight ratios of PEGMA to PEGDMA in the higher-welling layer. Error bar = SD, n=3.
4.3.3 Preliminary Mucoadhesion Test

4.3.3.1 Cross-Shaped Microstructures

Figure 4.9 shows a cross-shaped microwell on a PDMS stamp and a folded bilayered microstructures produced by the stamp. The stamp consists of an array of cross-shaped microwells. Each arm is 40 μm wide and 80 μm long.

Figure 4.9: Optical micrographs of (A) a cross-shaped microwell on PDMS stamp and (B) a folded microstructure in water produced using the stamp. The scale bars = 100 μm.

Figure 4.10 shows the compounded cross-sectional profiles of a single microwell before and after each layer was applied. Measurements on ten randomly chosen microwells show that the depth of the microwells at their centers are 6.82 ± 0.04 μm.
before the chitosan solution was applied, 6.55 ± 0.06 μm after the chitosan film was crosslinked, and 1.45 ± 0.15 μm after the PEGMA/PEGDMA resin was cured. The average thicknesses of the chitosan layer and the poly(PEGMA-co-PEGDMA) layer are thus 0.27 μm and 5.10 μm, respectively.

Figure 4.10: Optical profilometry characterization of cross-shaped self-foldable bilayered microstructures. Left: optical profilometry image of a cross-shaped microwell filled by crosslinked bilayer. The white dashed line delineates the cross section that profiles of the stamp were acquired before the chitosan solution was applied, after the chitosan was crosslinked and after the PEGMA/PEGDMA resin was cured. These profiles are compounded in the right diagram.
Figure 4.11 shows the cross-shaped microstructures folded on the pig intestinal mucosa, but whether the microstructures were folded into the mucus cannot be told from the image. The flow test result (Figure 4.12) shows about 40% folded microstructures were flushed away when the flow was started at 2 ml/s, indicating that about 60% microstructures were attached to the mucus. Assuming that a microstructure folded into the mucus would have certain degree of adhesion, those flushed away probably did not “grab” the mucus. About 50% PMAA microparticles were also flushed away. The number of both folded microstructures and PMAA microparticles remained unchanged as the flow rate was increased gradually from 2 ml/s in stepped increments. When the flow rate reached 35 ml/s, both types of the samples were flushed away.
Figure 4.11: Optical micrograph of folded cross-shaped microstructures on the pig intestinal mucosa. The scale bar = 100 μm.
Figure 4.12: Result of the flow test on the mucoadhesivity of the cross-shaped bilayered microstructures on pig intestinal mucosa. The arrow of a data line points to its corresponding vertical axis.

4.3.3.2 1 mm-Long Microstrips

1 mm-long bilayered strips were also fabricated and tested for their mucoadhesivity. As shown in Figure 4.13, the microwells of the PDMS stamp have a length of 1.0 mm and a depth of 6.93 μm. One edge of the microwells is saw-shaped with the width of 30 μm and 40 μm at the narrow and wide regions, respectively. Compounded typical profiles of microwells with the chitosan layer and the cured PEGMA/PEGDMA
layer are also shown in Figure 4.13. The thickness of the chitosan layer and poly(PEGMA-co-PEGDMA) are 0.28 μm and 3.00 μm at the center, respectively.

Folded long strips in water and on the pig intestine are shown in Figure 4.14. The strips on the intestine were barely seen because the intestine sample was nearly opaque and the bilayered strips were transparent. As a result, only a small number of long strips were tracked. Eight of them were shown in Figure 4.14(B, C, and D). The tracked strips stayed on the pig intestine throughout the 18-minute flow test with the flow pattern as shown in Figure 4.15.
Figure 4.13: Optical profilometry characterization of layer thickness of 1 mm-long self-foldable bilayered microstructures. Upper: optical profilometry image of the PDMS stamp. Bottom-left: optical profilometry image of a microwell of the stamp. The white dashed line delineates the cross section that profiles were obtained before the chitosan was applied, after the chitosan was crosslinked, and after the PEGMA/PEGDMA resin was cured. The profiles are compounded in the bottom-right diagram.
Figure 4.14: Optical micrographs of (A) folded long bilayered strips in water, (B, C, and D) folded long bilayered strips (pointed by arrows) on the pig intestinal mucosa. The scale bars = 100 μm.
Figure 4.15: Result of the flow test on the mucoadhesivity of the 1 mm-long bilayered strips on pig intestinal mucosa. The arrow of a data line points to its corresponding vertical axis.

4.4 Discussion

To produce bilayered microstrips, two different liquid precursors were crosslinked in microwells. The two layers had different swelling ratios, leading to the spontaneous folding of the microstructures in water. The degree of folding has been controlled by adjusting the PEGMA to PEGDMA ratio of the poly(PEGMA-co-PEGDMA) layer. Since PEGDMA is a crosslinking agent that inhibits hydrogel swelling and the difference of swelling ratios between chitosan and poly(PEGMA-co-PEGDMA) is the driving force of
self-folding, bilayered microstrips with a lower PEGDMA content curl more. These results have been fit into a simple mathematical model.

Excessive glutaraldehyde was used to crosslink chitosan. Since one aldehyde group reacted with one NH₂ group of chitosan and each glutaraldehyde molecule has two aldehyde groups, it is likely the crosslinked chitosan microstrips possessed unreacted aldehyde groups. Because aldehyde groups are toxic, they should be eliminated. Although not demonstrated in this study, it may be achieved by letting them react with methacrylic amide, which also has methacrylate groups that can react with PEGMA and PEGDMA to form covalent bonds between the two layers.

The cross-shaped foldable microstructures only showed insignificant enhancement of mucoadhesion in the experiment probably due to their small size. If the two opposite arms curled into a circle similar to the one in Figure 4.9 (B), the diameter of the circle would be around 80 μm (240 μm/π), which might be too small to achieve significant “grabbing” effect. We thus fabricated strips as long as 1 mm. The diameter of the curled strips was around 200 μm (Figure 4.13(A, C and D)) or larger (Figure 4.13 (B)). The long strips showed increased mucoadhesion than the small cross-shaped microstructures. Even though the highest and average flow rates of the flow test on the long strips were higher than that on the cross-shaped microstructures and the test duration was also longer, the long strips that were tracked were not flushed away in contrast to the small microstructures. To validate the results, however, both experiments should be optimized and repeated. For example, the temperature of the water should be maintained at the body temperature and a dye should be added into the bilayers for better imaging.
In this study, the foldable microstructures were placed on the pig intestine before the flow started. This is the case for applying the mucoadhesive drug delivery on the mucosal surfaces that can be easily accessed like oral and genital cavities. For oral administration, however, the device needs to attach to the mucosa before folding. A delayed folding mechanism is thus required. This might be achieved by binding a backing layer to the foldable microstructures. The backing layer should be able to hold the foldable microstructures at extended state and also slowly dissolve in water, allowing the released microstructures to fold. Another strategy is to cover the foldable microstructures with a front mucoadhesive gel layer that can bind the device to the mucosa first and become gel with time, allowing foldable microstructures to penetrate through it and fold into the mucus layer.

4.5 Conclusions

Bilyered microstructures were fabricated with the two layers having different swelling ratios, leading to the folding of the microstructures in water. This folding behavior may be utilized as a novel strategy for improving transmucosal drug delivery by enhancing mucoadhesion and promoting drug permeation. Preliminary tests on the mucoadhesion of the foldable microstructures were encouraging, but further experiments are required.
CHAPTER 5

TOWARDS FUNCTIONAL DRUG DELIVERY DEVICE BASED ON SELF-FOLDING

5.1 Introduction

The previous chapter introduced the use of self-folding mechanism in transmucosal drug delivery and describes the preliminary mucoadhesion test of the self-foldable microstructured on the pig intestine. To make a functional drug delivery device based on this concept, however, more components such as drug, foldable parts, and other functional parts must be integrated into a more complicated structure. This chapter describes a design and fabrication of a device with self-foldable microstructures and a model drug for transmucosal drug delivery.

5.2 Device Design

The designed device consists of three layers as shown in Figure 5.1(A). The top layer is an array of foldable strips, which consists of bilayered structure as demonstrated in Chapter 4. The middle layer contains drug. The bottom layer is a backing layer. It needs to be impermeable to the diffusion of the encapsulated drug. The fully assembled
device is shown in Figure 5.1(B). It is critical that the foldable strips must be placed partially on the drug layer and partially on the backing layer. The strips must remain bound to the backing layer while in water. In contrast, the drug containing layer must not bind to the strips while in water. Therefore, a water-soluble polymer can be used to make the drug-containing layer. When placed in aqueous environment, as the drug layer dissolves, the bilayered strips will fold as shown in Figure 5.1(C).
Figure 5.1: Schematic illustration of a design of a drug-containing device with foldable microstructures for transmucosal drug delivery. (A) Three layers of the device. (B) Fully assembled device. (C) Functioning device.
5.3 Device Fabrication

5.3.1 Materials

The PDMS (T2) was purchased from Dow-Corning. PEGMA with an average number molecular weight (Mn) of 526, PEGDMA with Mn of 330, cold water soluble PVA, AO8, polycaprolactone (PCL), and glutaraldehyde aqueous solution (50 wt%) were purchased from Sigma-Aldrich. Irgacure 651 was donated by Ciba Specialty Chemicals.

5.3.2 Fabrication Process

The fabrication process is illustrated in Figure 5.2. PCL was used to make the backing layer of the designed device. A PCL pellet was melt at 80 °C on a hotplate and pressed with a flat PDMS block to form a sheet 300 – 500 μm thick.

AO8 was used as the model drug in this study. A water solution of 10 wt% PVA and 1 wt% AO8 was cast and dried to form a film with a thickness of ~100 μm. It was cut manually into rectangular pieces with the size of ~1.5 × ~2.0 mm. One piece was placed on the molten PCL sheet at 80 °C and pressed with the flat PDMS block to push the PVA/AO8 film into the PCL.

The foldable bilayered strips were prepared using the same material and procedure as was used to produce 1 mm long strips for the mucoadhesion test in Chapter 4. The strips were printed onto the molten PCL sheet on the PVA/AO8 piece immediately after a brief (~5 s) exposure of the sheet to water vapor generated from a 90°C hot water bath.

The flat PDMS block was used again to press the strips on PCL into the molten PCL sheet. The sample was then cooled down to solidify the PCL and cut into a ~3 × ~4 mm-sized device using a razor blade.
Figure 5.2: Schematic illustration of the fabrication process of the drug-containing device with foldable microstructures for transmucosal drug delivery.
5.3.3 Characterization

Photographs of the fabricated devices were taken using a Fujifilm Finepix 2600 digital camera. Micrographs were captured using an Olympus BH-2 optical microscope and the Fujifilm Finepix 2600 digital camera.

5.4 Results

A photograph of a produced device is shown in Figure 5.3(A). The white frame is PCL and the red structure in the center is PVA/AO8. Figure 5.3(B and C) are micrographs of the device. PVA/AO8 is at the center of Figure 5.3(B). Figure 5.3(C) magnifies the left edge of the PVA/AO8 area in (B). It can be clearly seen that the strips are on both PCL area and PVA/AO8 areas.
5.5 Discussion

PCL was used to make the backing layer mainly for its relatively low melting temperature around 60°C. Molten PCL is highly viscous and sticky. It can bind to the PVA/AO8 layer and the foldable strips. PCL also meets the requirements for the backing layer to be water insoluble and not permeable to the carried drugs. Moreover, PCL has already been recognized as a safe material for drug delivery.
PVA was used to make the drug-containing layer for its water-solubility. However, dry PVA is not sticky as required for transfer of the bilayered strips from the PDMS stamp. Therefore it was exposed to water vapor before printing because PVA absorbed water and became tacky. PVA is also suitable for this application for its wide biomedical applications. In principle, many other water-soluble polymers can be used to make the drug-containing layer.

Use of a flat PDMS block to press the drug-containing layer into the PCL is to prepare a better substrate for printing the bilayered strips because an uneven substrate can induce incomplete printing and even fracture of the strips. Pressing the strips into PCL using the flat PDMS block is more important. Although the PCL was highly sticky in the molten state and could bind the strips tightly, a solidified PCL and the strips separated spontaneously in water. This is because PCL is a hydrophobic polymer and poly(PEGMA-co-PEGDMA) made up the strips is a hydrogel. They do not have strong attractions. In particular, the strips deformed in water while the PCL remained unchanged. The stress caused by this mismatch could detach the two structures. In order to hold the strips on the backing layer in water, the strips were thus pressed into the PCL to become embedded.

This device is similar to the mucoadhesive patch systems for drug delivery in applications and morphology, but it has a unique folding mechanism for enhancing mucoadhesion and drug permeation. This device needs to bind to the mucosal surface before it folds. For oral drug delivery, a mechanism to delay the folding until it attaches to the mucus is thus required. This may be solved by placing a thin layer of mucoadhesive gel on the foldable strips. The mucoadhesive gel can absorb water and become viscous. It
plays two functions by preventing premature folding of the foldable strips until enough
water is absorbed and binding the device to the mucus. However, for the mucosal
surfaces that are easily accessible such as oral cavity, the device can be placed on the
mucosa easily. Delayed folding is thus not required and this trilayered design may be
enough to make a functional device.

5.6 Conclusions

A microdevice incorporating self-foldable microstructures for transmucosal drug
delivery has been designed and fabricated. It is composed of a backing layer, a
drug-containing layer, and a self-foldable layer. The self-foldable parts are designed to
curl into the mucus to achieve enhanced mucoadhsion and drug permeation for the device.
Although much more study is needed to test this device, it represents an effort towards
the next-generation, highly engineered, multi-functional, and even “smart” drug delivery
microdevices.
6.1 Conclusions

Microfabrication technology has been used to produce particulate drug delivery microdevices with well-defined structures that cannot be created by microparticle manufacturing methods. For this application, the conventional silicon-based microfabrication suffers drawbacks such as the use of expensive cleanroom-based facilities, the relatively harsh processing conditions, and the use of materials that are not suitable for drug delivery. In contrast, many polymer microfabrication techniques are much easier and cheaper to perform. They generally also possess greater versatility in materials and processing approaches than silicon-based microfabrication techniques. In this dissertation, polymer microfabrication techniques are used to produce particulate microstructures for drug delivery applications. The microstructures can be made of a variety of widely-used biomedical polymers such as hydrogels and biodegradable polymers and have single-layered, reservoir-containing, capsule-like, and self-foldable structures. These microstructures have the potential to be used for various drug delivery routes such as ocular, oral, transmucosal, and injection with unique advantages, and the
polymer-based microfabrication technology holds promise for the creation of highly engineered, multi-functional, and “intelligent” next generation drug delivery microdevices.

6.2 Recommendations

Although the fabrication of various types of particulate microstructures for drug delivery applications have been successfully demonstrated in this dissertation, some of the fabrication protocols need to be optimized for higher yield and reproducibility. One example is the PLGA microstructures with multiple reservoirs. As suggested in Chapter 3, a hard embossing mold may be used to avoid the distortion of microstructures at the peripheral embossing area. Further characterization of some microstructures is needed. For example, the folding force of the self-foldable microstructures should be measured to see if it is large enough to disrupt cell layer for enhancing drug permeation. The mathematical model should also be used to determine the folding force.

Since drug delivery is the target application of these microstructures, incorporating drugs into the microstructures and characterizing drug release behaviors would certainly be the next phase of this research. Different strategies for drug loading should be taken for different microstructures. For single-layered microstructures, drugs can be simply mixed with the matrix polymer. For the microstructures with single or multiple reservoirs, drug may be filled by discontinuous dewetting and the reservoirs may be capped by polymer microprinting. Loading drug into microcapsules is straightforward. Efforts should be made in order to optimize the fabrication protocol to increase the yield and to control the parameters of the microcapsules such as the thickness of the coating layer in order to obtain desired drug release rate. Drugs may be either mixed into the two
layers that make up the bilayered self-foldable microstructures or incorporated into another layer to make the device proposed in Chapter 5.
REFERENCES


49. Appendix A.

50. Appendix B and H.


APPENDIX A

CONTROL OF THE LATERAL SHAPE OF THE PARTICULATE MICROSTRUCTURES

PDMS stamps with recessed “OSU” microfeatures were produced using a transparency film mask with an array of “OSU” letters. poly(propyl methacrylate) (PPMA) microstructures with lateral shapes of “O”, “S”, and “U” were produced as shown in Figure A.1. Using the method developed in Chapter 2, particulate microstructures with arbitrary lateral shapes can be fabricated.
Figure A.1: “O”-, ”S”-, and ”U”-shaped PPMA microstructures in water.
APPENDIX B

PH-SENSITIVE PARTICULATE MICROSTRUCTURES

PMAA pH-sensitive particulate microstructures were fabricated using the method for producing PEGMA-co-PEGDMA single-layered microstructures described in Chapter 2. The PDMS stamp had an array of 10 μm-wide square microwells. The resin consisted of MAA as the monomer, ethylene glycol methacrylate (EGDMA) as the crosslinker with a molar ratio of 100:1 (MAA to EDMA), 1 wt % Irgacure 651, and 40 wt% glycerin as solvent. The resin was exposed to UV light at 4 mW/cm² for 10 minutes with purging nitrogen. The cured PMAA microstructures were printed on a PVA-coated slide. Water was used to release the microstructures. HCl solution was added to lower the pH value, which was measured by a pH indicator as 0. The pH was increased by adding a large amount of buffer solution with pH of 10. The final pH was 10 measured by a pH indicator. The size of the PMAA microstructures was considerably larger at pH of 10 than at pH of 0 as shown in Figure B.1.
Figure B.1: PMAA microstructures at pH = 0 (A) and pH = 10 (B).
APPENDIX C

FABRICATION OF BILAYERED PARTICULATE MICROSTRUCTURES

Figure C.1 illustrates the process for preparing bilayered particulate microstructures. First, 1 wt% agarose/DMSO solution was filled in the 40 μm-wide, 1.4 μm-deep square microwells. A thin film of agarose formed in the microwells after DMSO evaporated. After that, the same procedure as preparing single-layered PPMA microstructures in Chapter 2 was performed. The final product was agarose/PPMA bilayered particulate microstructures as shown in Figure C.2.

This method can create particulate microstructures with two components as well as two surfaces with different properties. As demonstrated in this study, the bilayers had both hydrophobic (PPMA) and hydrophilic (agarose) surfaces. In principle, microstructures with more than two layers can be produced using this technique. From a perspective of drug delivery, micrometer-scaled multi-layered patch system may be prepared with each layer having different functions such as targeting, bioadhesion, and ensuring unidirectional drug release.
Figure C.1: Schematic illustration of the process for the fabrication of bilayered particulate microstructures
Figure C.2: Agarose/PPMA microstructures in water. The two arrows point two partially delaminated bilayered microstructures. The scale bar = 50 μm.
APPENDIX D

FABRICATION OF MICROPATTERNED PARTICULATE MICROSTRUCTURES BY DOUBLE PRINTING

The fabrication process is shown in Figure D.1. 30 μm-wide PPMA micropads were first printed on a PVA-coated glass slide using the same protocol as described in Chapter 2. μFCP was performed to print dot-like PLGA microstructures on the PPMA micropads. Figure D.2 shows dot-like PLGA microstructures on PPMA micropads and released micropatterned particulate microstructures in water.
Figure D.1: Schematic illustration of the process for the fabrication of micropatterned particulate microstructures by double printing.
Figure D.2: Optical micrographs of (A) dot-like PLGA microstructures on PPMA micropads on PVA and (B) released micropatterned particulate microstructures in water. Scale bars = 50 μm.
FABRICATION OF PILLOW-LIKE MICROCAPSULES

The same procedure as preparing microcapsules in Chapter 3 was performed using a stamp with 40 μm-wide, 1.4 μm-deep square microwells. The encapsulating layer was PPMA and the encapsulated material was fully-sulfornated polyaniline (FSPAN). Acetone and DMSO were used as solvents for PPMA and FSPAN respectively. The produced microcapsules swelled in water forming pillow-like microstructures as shown in Figure E.1.
Figure E.1: Optical micrograph of released pillow-like microcapsules in water. The upper left inset is the side-view of a swollen microcapsule. The scale bar = 50 μm.
APPENDIX F

3-D PARTICULATE HYDROGEL MICROSTRUCTURES

Bilayered hydrogel microstructures were fabricated by successively applying two layers with different swelling ratios in microwells. The resin for making the first layer consisted of 39 wt% glycerin as solvent, 1 wt% Irgacure 651 as photoinitiator, MAA as monomer, and EGDMA as crosslinker with a monomer-to-crosslinker molar ratio of 52 : 1. The formed PMAA was highly swellable. The resin was exposed to UV light (~ 2 mW/cm²) for 10 minutes with purging nitrogen. The resin for the second layer was EGDMA with 1 wt% Irgacure 651. It was also exposed to UV light (~ 2mW/cm²) for 10 minutes with purging nitrogen. This layer was non-swellable. The bilayered microstructures were printed on a PVA-coated glass slide and released in water. Various 3-D microstructures formed as shown in Figure F.1, F2, F.3, and F.4. A Nikon TMS inverted phase contrast optical microscope and a Fujifilm Finepix 2600 digital camera were used to capture the pictures.
Figure F.1: Optical micrographs of PMAA/polyEGDMA bilayered microstructures (A) on PVA and (B) in water. The scale bars = 100 μm.

Figure F.2: Optical micrographs of PMAA/polyEGDMA bilayered microstructures (A) on PVA and (B) in water. The scale bars = 100 μm.
Figure F.3: Optical micrographs of PMAA/polyEGDMA bilayered microstructures (A) on PVA and (B, C, and D) in water. The scale bars = 100 μm.
Figure F.4: Optical micrographs of PMAA/polyEGDMA bilayered microstructures (A) on PVA and (B) in water. The scale bars = 100 μm.
APPENDIX G

FABRICATION OF 3-D MICROSTRUCTURES BY INTERFACIAL TENSION DRIVEN SELF-FOLDING

This fabrication process is illustrated in Fig. G.1(A). 40 μm-wide square PPMA micropads were first printed on a chitosan-coated glass slide using the same protocol as described in Chapter 1. Soybean oil was then applied onto the slide, leaving oil droplets only on the PPMA micropads as shown in Fig. G.1(B) due to the polarity differences between oil, PPMA and chitosan. Finally, aqueous acetic acid solution was added to release the micropads by dissolving the chitosan sacrificial layer. The released micropads folded over the oil droplets spontaneously, forming dumpling-like microstructures as shown in Fig. G.1(C).

The exclusive wetting of micropads by oil is a result of minimization of interfacial energy of the system due to low surface tensions of PPMA and oil, and the high surface tension of chitosan. The folding of the micropads is also driven by free-energy minimization as water replaces air in the system. Since the oil-water interfacial tension is larger than that of oil-air and PPMA-water, the oil droplets that
initially spread on the micropads tend to shrink into a spherical shape to minimize their interfacial area in contact with water. Also due to the strong adhesion between oil and PPMA and the compliance of the micropads, the oil droplets force the released micropads to fold around them. The final structures are dumpling-like microcapsules with oil cores and PPMA coats.
Figure G.1: (A) Schematic illustration of the process for the fabrication of dumpling-like microstructures. (B) Oil droplets on PPMA micropads attached to a chitosan sacrificial layer. The upper-left inset shows PPMA micropads before oil was applied. (C) Dumpling-like PPMA microstructures in aqueous solution of acetic acid.
APPENDIX H

FABRICATION OF SELF-FOLDED HYDROGEL MICROSTRUCTURES

A PDMS stamp with 40 μm-wide, 1.4 μm-deep square microwells and a PDMS stamp with 30 μm-wide, 1.1 μm-deep square microwells were used to print the poly(hydroxyethyl methacrylate) (PHEMA) and PMAA micropads, respectively. The fabrication process is illustrated in Figure H.1(A).

The HEMA monomer (98.6 wt%) with Irgacure 651 (1.4 wt%) as photoinitiator in a closed bottle was exposed to UV (2.0 mW/cm\(^2\)) for 4 minutes until a viscous medium was obtained. The crosslinking agent triethylene glycol dimethacrylate (TEGDMA) with a molar ratio of 2.4 : 100 to the initial HEMA monomer was then added. The viscous prepolymer was brushed across the PDMS stamp using a cotton swab and trapped in the microwells due to discontinuous wetting. Since the liquid in the wells tended to bead up, it was immediately exposed to UV (2.0 mW/cm\(^2\)) under purging nitrogen for 10 minutes to complete its cure. The cured polymer in the microwells was printed out as micropads on a PVA-coated glass slide, which was prepared by brushing 1.5 wt % PVA solution in water on a glass slide with a cotton swab. The printing was carried out by placing the
stamp on the substrate and press manually at room temperature immediately after a brief exposure of the slide to water vapor. The water vapor was produced by 60°C water and it made the PVA sacrificial layer tacky to facilitate micropad transfer. Water was then dropped onto the slide to dissolve the PVA layer and the released micropads folded into roll-like microstructures spontaneously as shown in Figure H.1.(B).

The MAA resin was also partially crosslinked by exposing the resin, which is composed of MAA (53.2 wt%), DMSO as solvent (46.1 wt%), and Irgacure 651 as photoinitiator (0.7 wt%) to UV light (2.0 mW/cm²) for 8 minutes in a closed bottle. After that, the crosslinking agent TEGDMA with a molar ratio of 5.2 : 100 to the initial MAA monomer was added. The mixed viscous liquid was filled in the microwells by discontinuous wetting and exposed to UV (2.0 mW/cm²) for 20 minutes under purging nitrogen. The fully cured polymer in the microwells was printed out on a PVA-coated glass slide as micropads using the water vapor-assisted printing method described earlier. A buffer solution with a pH = 4.0 was dropped onto the slide to release the micropads. The released micropads folded into bowl-shaped microstructure spontaneously as shown in Figure H.1(C).
Figure H.1: (A) Schematic illustration of the process for the fabrication of hydrogel roll-like and bowl-shaped microstructures. (B) Roll-like PHEMA microstructures in water. (C) Bowl-shaped PMAA microstructures in buffer solution with a pH = 4.0. The upper-left insert shows a 30 μm PMAA square micropad on PVA with the same magnification as the micro-bowls. The upper-right insert shows a twice-magnified PMAA micro-bowl.
APPENDIX I

FABRICATION OF POLYMERIC MICROLENSES ARRAY

The process is shown in Figure I.1(A). PCL micropads were printed on a glass slide using the method developed in Chapter 2. The slide was immersed in hot water (~90 °C) for 1 minute and dried in air. PCL is a hydrophobic thermoplastic polymer with a relatively low melting temperature (~60 °C). In water, which has a high surface tension, the molten PCL beaded up to lower the polymer-water interface, forming the microlenses as shown in Figure I.1(B). The equilibrium curvature of the lenses is probably determined by the surface tensions between the melted polymer, the medium, and the substrate. Other thermoplastics may also be used with this method to create microlens array as long as 1) the polymer can be printed as isolated microstructures, 2) a medium can be found in which the polymer is not soluble and can be heated to melt, and 3) the molten polymer has a lower surface tension than the medium. As a benign medium, water may be used to prepare microlenses of a polymer with different curvatures via adjusting its surface tension by adding certain solute. The boiling point of water can also be increased by increasing pressure.
Figure I.1: (A) Schematic illustration of the process for the fabrication of microlens array. (B) SEM image of PCL microlens array.
As shown in Figure J.1(A), PCL micropads were printed on a glass slide using the method described in Chapter 2. ZnO powder was spread on the slide. The slide was placed on a hotplate at 100 °C to melt the PCL. A piece of flat PDMS block was placed on the slide and pressed for one minute. The slide was then removed from the hotplate, cooled down in air, and rinsed with water. The PCL micropads on the glass slide are shown in Figure J.1(B), and the micropatterned ZnO microparticles are shown in Figures J.1(C) and (D).

The molten PCL film served as a hot-melt glue to bind the ZnO microparticles to the glass surface with the stamped pattern. Since the thickness of the stamped polymer film was much thinner than the size of the ZnO microparticles, the microparticles are believed to be partially pressed into the molten PCL film and remained embedded as the film solidified. In principle, many other thermoplastic polymers can be used for the “sticky” layer. Microparticles of other materials that are stable at evaluated temperatures can also be easily patterned at micrometer scale using this method.
Figure J.1: (A) Schematic illustration of the processing procedure. (B) SEM image of PCL printed on glass slide. (C) SEM image of micropatterned ZnO. (D) A close-up SEM image of micropatterned ZnO.
A PDMS stamp with 7 μm-wide micropillar array was used in this study. The stamp was dipped in the aqueous solution of NaCl. Microcrystals formed on the micropillars. The size of the microcrystals was determined by the solution concentration. A higher concentration produced larger microcrystals (Figure K.1.). Figure K.2. shows a SEM image of the microcrystals on micropillars prepared from 25 wt% NaCl solution.

Microcrystals of NaCl can be easily obtained. With the concentration as low as 0.1 wt%, very small crystals (<1 μm) were produced. Although the exact size of the crystals has not been measured, it is possible to create crystals at the nanometer scale using this method. Aside from NaCl, sodium salt of PMAA and chitosan have been used to make microparticles on micropillars. It is likely that other substance such as various drugs can be used with this method to create either micro- and nano-crystals or micropatterned crystal array by transferring the crystals from micropillars to a flat substrate.
Figure K.1: Optical Micrographs of NaCl microcrystals on micropillars produced from NaCl solutions with the concentration of 25 wt% (A), 10 wt% (B), 1 wt% (C), and 0.1 wt% (D), respectively.
Figure K.2: SEM image of NaCl microcrystals on micropillars produced from the 25 wt% NaCl solution.
APPENDIX L

OVERSTRETCHING AND TRANSFERRING DNA

\(\lambda\)-DNA molecules labeled with YOYO-1 were stretched on a PDMS surface and were transferred onto the glass surface. A Nikon Eclipse TE 2000-S inverted phase contrast optical microscope equipped with a Photometrics CoolSNAP™ CCD camera was used to take the photographs. Since the contour length of \(\lambda\)-DNA is around 16 \(\mu\)m, a significant fraction of stretched DNAs was overstretched as shown in Figure L.1. The stretched DNAs have also been released from PDMS in water. Further experiments will use the stretched DNA as templates for adding multiple functional agents.
Figure L.1: Fluorescence micrograph of stretched λ-DNA molecules on glass surface.

The scale bar = 20 μm.
APPENDIX M

STRETCHING DNA-PEI COMPLEXES ON FLAT SURFACE

\( \lambda \)-DNA molecules labeled with YOYO-1 were mixed with poly(ethylene imine) (PEI) to make DNA-PEI complexes due to electrostatic attraction between negatively charged DNA and positively charged PEI. The complexes were stretched on the PDMS surface and transferred onto a glass surface as shown in Figure M.1. A Nikon Eclipse TE 2000-S inverted phase contrast optical microscope equipped with a Photometrics CoolSNAP™ CCD camera was used to take the photographs. The stretched DNA-PEI complexes had significant different morphology from the stretched DNA alone. Since PEI can efficiently condense DNA and facilitate DNA transfection in gene delivery, this method may open a new avenue for studying various DNA complexes and conjugates for gene delivery.
Figure M.1: Fluorescence micrograph of stretched λ-DNA-PEI complexes on glass surface. The scale bar = 20 μm.
APPENDIX N

MICROPATTERNING DNA AGGREGATES

The solution of λ-DNA labeled with YOYO-1 was applied on a PDMS stamp with 7 μm-diameter micropillar array. Bright particles were found on top of the micropillars in a highly regular pattern (Figure N.1.). Some stretched DNA molecules were originated from and bridged the bright particles, indicating the bright particles were likely to be aggregates of DNA. A Nikon Eclipse TE 2000-S inverted phase contrast optical microscope equipped with a Photometrics CoolSNAP™ CCD camera was used to take the photographs.
Figure N.1: Fluorescence micrograph of DNA aggregates on micropillars. The scale bar = 20 µm.
APPENDIX O

STRETCHING DNA ON MICROPILLAR ARRAY

The solution of $\lambda$-DNA labeled with YOYO-1 was applied on the PDMS stamp with 7 $\mu$m-diameter micropillar array. Stretched DNA molecules were found to bridge micropillars as shown in Figure O.1. A Nikon Eclipse TE 2000-S inverted phase contrast optical microscope equipped with a Photometrics CoolSNAP™ CCD camera was used to take the photographs.
Figure O.1: Fluorescence micrograph of stretched DNA on micropillars.
DYNAMIC STRETCHING AND RELAXATION OF TETHERED DNA BY ELECTRIC FIELD

YOYO-1-labeled λ-DNA molecules were tethered to an aminopropyl trimethoxysilane (APTES)-treated glass coverslip as schematically shown in Figure P.1. An electric field was applied to stretch the DNAs. Based on the lengths of the DNA molecules in the electric field (Figure P.2.), we calculated the charge density of the DNAs as 27 electrons per Kuhn step (Figure P.3.). This method offers a simple way to characterize DNA. It may be used to study the kinetics of complex formation of DNA and a number of agents of interest in gene delivery such as polyethylene imine (PEI) and chitosan.
Figure P.1: Schematic illustration of the stretching DNA by electric field.

Figure P.2: Tethered DNA Stretched by electric field.
Figure P.3: Experimental results and fixed curve of extension-electric field relation of DNA molecules stretched by electric field.