POLYMERIC MICRONEEDLES FOR
TRANSDERMAL DRUG DELIVERY

A Thesis
Presented to
The Academic Faculty

By
Jung-Hwan Park

In Partial Fulfillment
Of the Requirement for the Degree
Doctor of Philosophy in Biomedical Engineering

Georgia Institute of Technology
May 2004

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Date Approved by Chairman: March 5, 2014
To my wife, son and parents

I hope they are as proud of me as I am proud of them.
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<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda )</td>
<td>wavelength of the exposing radiation</td>
</tr>
<tr>
<td>A</td>
<td>area of circular section of a microneedle</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>b</td>
<td>Potowski constant</td>
</tr>
<tr>
<td>( b_{\text{crit}} )</td>
<td>half the grating period</td>
</tr>
<tr>
<td>BOE</td>
<td>buffered oxide etchant</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>c</td>
<td>distance from the neutral axis to the outermost edge of the microneedle</td>
</tr>
<tr>
<td>( C_c )</td>
<td>column constant</td>
</tr>
<tr>
<td>CD</td>
<td>circular dichroism</td>
</tr>
<tr>
<td>CHADD</td>
<td>controlled heat-aided drug delivery system</td>
</tr>
<tr>
<td>CMC</td>
<td>carboxymethylcellulose</td>
</tr>
<tr>
<td>d</td>
<td>diameter of a hole</td>
</tr>
<tr>
<td>D</td>
<td>diameter of circular section of a microneedle</td>
</tr>
<tr>
<td>( D_{\text{base}} )</td>
<td>base diameter of a microneedle</td>
</tr>
<tr>
<td>( D_{\text{equivalent}} )</td>
<td>equivalent diameter of a microneedle</td>
</tr>
<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
</tr>
<tr>
<td>( D_p )</td>
<td>diffusion coefficient of the drug in the medium</td>
</tr>
<tr>
<td>( D_{\text{tip}} )</td>
<td>tip diameter</td>
</tr>
<tr>
<td>E-beam</td>
<td>electron beam evaporator</td>
</tr>
<tr>
<td>E</td>
<td>Young’s modulus of material, and</td>
</tr>
<tr>
<td>EPT</td>
<td>electroporation therapy</td>
</tr>
<tr>
<td>f</td>
<td>void fraction</td>
</tr>
<tr>
<td>( F_{t_{\text{max}}} )</td>
<td>maximum transverse tip force</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>F_{\text{in}}</td>
<td>Insertion force</td>
</tr>
<tr>
<td>GBL</td>
<td>( \gamma )-butyrolactone</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>I</td>
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<td>L_path</td>
<td>path length</td>
</tr>
<tr>
<td>L_e</td>
<td>effective length</td>
</tr>
<tr>
<td>LiGA</td>
<td>lithographie, galvanoformung, und abformung (lithography, electroplating, and molding)</td>
</tr>
<tr>
<td>LM</td>
<td>light microscopy</td>
</tr>
<tr>
<td>LPLA</td>
<td>Poly (L-lactic-acid)</td>
</tr>
<tr>
<td>M</td>
<td>bending moment</td>
</tr>
<tr>
<td>M_0</td>
<td>amount of drug released at infinite time</td>
</tr>
<tr>
<td>MDT</td>
<td>mean dissolution time</td>
</tr>
<tr>
<td>MEAs</td>
<td>microenhancer arrays</td>
</tr>
<tr>
<td>MEMS</td>
<td>micro-electro-mechanical systems</td>
</tr>
<tr>
<td>M_t</td>
<td>amount of drug released from the particle at time t</td>
</tr>
<tr>
<td>MTS</td>
<td>microstructures transdermal system</td>
</tr>
<tr>
<td>N</td>
<td>flow rate of drug</td>
</tr>
<tr>
<td>n</td>
<td>diffusional exponent</td>
</tr>
<tr>
<td>n_m</td>
<td>number of microneedles</td>
</tr>
<tr>
<td>NL</td>
<td>nonlinearity</td>
</tr>
<tr>
<td>OVA</td>
<td>ovalbumin</td>
</tr>
<tr>
<td>PAGE</td>
<td>SDS-Poly-Acrylamide-Gel-Electrophoresis</td>
</tr>
<tr>
<td>PCL</td>
<td>polycapro lactone</td>
</tr>
<tr>
<td>P_cr</td>
<td>critical load by buckling</td>
</tr>
<tr>
<td>PDMS</td>
<td>poly-di-methyl-siloxane</td>
</tr>
<tr>
<td>PECVD</td>
<td>plasma enhanced chemical vapor deposition</td>
</tr>
<tr>
<td>PGA</td>
<td>poly (glycolic-acid)</td>
</tr>
<tr>
<td>PGMEA</td>
<td>propylene glycol methyl ether acetate</td>
</tr>
<tr>
<td>PI</td>
<td>photoinitiator</td>
</tr>
</tbody>
</table>
PLA  poly(lactic-acid)
PLGA poly(lactide-co-glycolide) (PLGA)
PR  Photoresist
PVA polyvinyl alcohol solution
R  theoretical resolution
R_{skin}  Resistance of skin
RF  radio frequency
r_{g}  radius of gyration
RIE reactive ion etching
s  gap between the mask and the photoresist surface,
SEM  scanning electron microscopy
SR  slenderness ratio
S_{y}  yield strength of material
TDD  Transdermal drug delivery
TDF transdermal fluid
UV  ultraviolet
V  volume
\Delta C  concentration gradient
\mu TAS  micro total analysis system
\tau  dimensionless time
SUMMARY

Although modern biotechnology has produced extremely sophisticated and potent drugs, many of these compounds cannot be effectively delivered using current drug delivery techniques. Transdermal drug delivery provides a potentially attractive alternative, but the permeability of skin to most drugs is very low. The primary barrier to transdermal transport is located in the outermost portion of skin and nerves are only found in deeper tissue. Previous studies have demonstrated that needles of microns dimensions can be painlessly inserted into skin and thereby increase skin permeability for transdermal drug delivery. However, the first generation of silicon needles can break and silicon-based MEMS techniques are time-consuming, expensive and low yield. The next generation of metal microneedles fabricated from nickel or nickel iron have strong mechanical properties for skin penetration but are not biodegradable.

Biodegradable polymers are attractive materials for microneedles because biodegradable polymers degrade to biocompatible monomers by hydrolysis. Even if they break off in the tissue or skin, biocompatible polymeric needles could be compatible with skin and degrade in the tissue safely. Chemically, biodegradable polymers allow additional functionality of the microneedles themselves. Polymeric microneedles that encapsulate drug can work as a delivery matrix itself and should not require medical expertise like injection.

However, polymers have weaker mechanical properties compared with previous materials used for microneedles, such as metals or silicon. To overcome this, polymeric microneedles were designed in this study to have enough mechanical strength to remain
intact while being inserted into skin by considering geometries and the mechanical properties of polymers. Beveled tip geometry, chisel tip geometry and cone shape tip, and tapered column geometry were fabricated. These needle tip geometries should provide a sharper needle to insert into skin with less pain and insertion force. The various geometries of tips were fabricated using MEMS techniques and an integrated lens technique for easy insertion. The tapered microneedles with high aspect ratio were fabricated using the lens technique to prevent failure of needles at their base by deformed skin and to increase the needle length for deeper insertion. The biodegradable polymer microneedles were mass replicated using a modified micromolding technique with ease of fabrication and expected cost effectiveness.

The mechanical properties of microneedles were characterized quantitatively. The insertion force of microneedles was characterized with respect to tip geometry. The relationship between the tip area of the microneedle and the insertion force was linear. The failure of polymeric microneedles by axial loading and transverse loading were characterized with respect to needle geometry and needle material. The failure force by axial loading was found to increase with the base diameter of a microneedle and the Young’s modulus of the material, and to decrease with the length of microneedle and encapsulating drug content in microneedles. The analytical model for the buckling failure predicted the tendency of failure force quantitatively. The transverse failure force by deformed skin was found to increase with the base diameter of a microneedle. The analytical model for lateral bending failure showed good agreement with the data. A comparison of the insertion force and the axial failure force of microneedles showed that biodegradable polymers including poly glycolic acid (PGA), poly L-lactic acid (PLLA)}
and high molecular weight of poly glycolic-co-lactic acid (PLGA) have satisfactory mechanical properties as needle materials and acceptable geometries for satisfactory mechanical behavior depends on the kind of polymer.

Solid polymer microneedles were used to increase skin permeability to calcein and bovine serum albumin (BSA). Arrays of 20 microneedles increased skin permeability to calcein and BSA by at least two orders of magnitude, while a 100-needle array increased permeability by almost three orders of magnitude. Transport rate was predicted using a porous membrane model and the prediction fit data well. Polymer microneedles that encapsulated calcein and BSA were used to investigate the release of model drugs from microneedles. PLGA microneedles encapsulating calcein showed rapid release from microneedles within hours. Carboxy-methyl-cellulose (CMC) was effective as a delaying agent for calcein release over days and LPLA microspheres encapsulating calcein within microneedles showed still slower drug release over weeks. The thermal stability of BSA was investigated when BSA was encapsulated in microneedles. Solid BSA particles were partially denatured by thermal exposure to a high temperature of 135 °C and the extent of irreversible and reversible denatured protein increased when the thermal exposure time was increased.

Polymer needles are potential transdermal delivery method to increase skin permeability with biological safety. Large size molecules can transfer through holes made by microneedles and the drug can be delivered without individual deviation. Polymeric microneedles encapsulating drug provide drug delivery matrix and drugs encapsulated in microneedles can be delivered out of microneedles after insertion into skin.
1. Introduction

Drugs are usually delivered to the human body through oral delivery or intravenous injection methods and they are still the most common methods for delivering drugs. But they have difficulties such as (1) high doses of drug cannot be injected into the body at one time, (2) intravenous delivery leads a high concentration of drug into the bloodstream and can create toxic side effects, (3) only a very small percentage of injected drug reaches the affected area in the body and hence multiple injections are often required for effective treatment (Chaubal 1997). Recently several technical advancements have been made in drug delivery. These techniques, including transdermal drug delivery and polymeric drug delivery, are capable of controlling the rate of drug delivery and targeting the delivery of drug to a tissue (Chien 1992).

Transdermal drug delivery (TDD) is the transport of drugs across skin (Chien 1987). Several TDD systems have been developed, aiming to achieve the objective of systemic medication through topical application to the intact skin surface. Transdermal products usually come in the form of a cream or a patch. The creams are used for short term, local treatment and are applied simply by rubbing them onto skin at the desired treatment site. The patches consisting of drug loaded polymer matrix and adhesive layers provide more controlled and lasting drug delivery through skin. But, transdermal drug delivery is limited by the extraordinary barrier properties of the stratum corneum, the outer 10 – 15 μm of skin (Schaefer 1996).

A new approach of transdermal drug delivery, microneedles, was recently introduced. Arrays of microneedles have been created to act as a bridge between conventional
injections and transdermal patches. Microfabrication technology has been adopted to allow the creation of arrays of needles capable of penetrating the stratum corneum without eliciting pain, due to their minute size. Solid silicon microneedles have been demonstrated to increase the permeability of in vitro human skin by three to four orders of magnitude for compounds ranging in size from 0.6 nm to 50 nm. Hollow, cylindrical microtubes have been demonstrated to increase the skin permeability of in vitro human skin (McAllister 2000; Davis 2003).

Most of the microneedles have been made of silicon or metal. Silicon has been attractive because it is common substrate in the microelectronics industry and therefore a great deal is known about its process. However, silicon is relatively expensive compared to metals and polymers, is based on sophisticated semiconductor process, is relatively fragile and is not a conventional biocompatible material. Metals are a more appropriate material for fabricating microneedles, but they are restricted from shear-induced breakage (Ratner 1996). Another potential problem is that metal can corrode to some degree when it is exposed to body fluid or blood for long time exposure (Ratner 1996). In comparison to silicon and metal counterparts, polymer has the mechanical advantage of improved resistance to shear-induced breakage due to the viscoelastic property. Previous microneedle fabrications required time consuming and expensive processes due to complicated procedure based on silicon process devices and they had low yield for producing microneedles.

We were motivated to make microneedles out of biocompatible and biodegradable polymer for biological safety. Even if they break off in the tissue or skin, biocompatible polymeric needles could be compatible with skin and degrade in the tissue safely.
Chemically, biodegradable polymers allow additional functionality of the microneedles themselves. Polymeric microneedles with drug internally encapsulated can work as a delivery matrix itself and should not require medical expertise like injection. Polymer microneedles can be produced by modifying conventional molding technique. Thus, biodegradable polymer microneedles are copied from various master structure using micromolding techniques for ease and economic performance.
2. Background

2.1 Transdermal drug delivery concept

The clinical effectiveness of delivered drug depends not only on its pharmacological properties but also on drug availability at the target sites. For the majority of drugs that are used to treat dermatological diseases, the target site lies within the viable layer of the skin. For systemic therapy, transdermal therapeutic systems have been designed to provide controlled continuous delivery of drugs via the skin to the systemic circulation. However, the stratum corneum, the thin outermost layer of the skin, obstructs the effective delivery of drugs to target sites (Loftsson 1998).

2.1.1 Anatomy of skin

Human skin is a complex tissue consisting of several distinct layers, each consisting of their own components and structure. The stratum corneum is the outer layer, generally between 10 and 50 cells, or between 10 and 20 μm thick, shown as Figure 2.1. Unlike other tissues in the body, the stratum corneum contains “cells” filled with bundles of cross-linked keratin and keratohyalin surrounded by an extracellular matrix of lipids. It is this structure that is believed to give skin its barrier properties. Below the stratum corneum is the viable epidermis, which is between 50 and 100 μm thick. The viable epidermis contains no blood vessels, and it exchanges metabolites by diffusion to and from the dermis. Beneath the viable epidermis is the dermis, which contains blood vessels, lymphatics, and nerves (Schaefer 1996).
Figure 2.1 Anatomy of skin (reproduced from http://www.pg.com/science/skincare/Skin_tws_9.htm)

Skin has been used as a route for drug delivery as shown Figure 2.2. Transdermal drug delivery has been preferred because of control of drug delivery rate, self-administration, non-invasive delivery, high patient compliance and easy removal of drug source. But the transport of drugs across skin depends on passive diffusion of drug and only small molecules can go through skin. The intact stratum corneum provides the main barrier; its “brick and mortar” structure is similar to a wall. The corneocytes of hydrated keratin comprise the ‘bricks’, embedded in a ‘mortar’, composed of multiple lipid bilayers of ceramides, fatty acids, cholesterol and cholesterol esters. These bilayers form regions of semicrystalline, gel and liquid crystal domains. Most molecules penetrate through skin via this intercellular microroute and therefore many enhancing techniques
aim to disrupt or bypass its elegant molecular architecture. Viable layers may metabolize a drug, or activate a prodrug (Barry 2001). Skin impermeability affects systemic delivery of drugs from transdermal delivery systems and can cause individual and unpredictable variations in transdermal delivery. (Loftsson 1998)

Figure 2.2 Simplified diagram of stratum corneum and two microroutes of drug penetration (Barry 2001).

Skin is composed of an outer epidermis and an inner dermis, cushioned on fatty subcutaneous tissue as shown in Figure 2.1 and each layer of skin has different mechanical behavior. To understand the drug delivery through the skin by passive and active transdermal system, the mechanical properties of each layer should be investigated. Skin behaves as a non-homogeneous, anisotropic, non-linear viscoelastic material subjected to a prestress. Experimentally obtained Young’s moduli of the layers vary considerably \( E_{\text{out, cor}} = 1000 \times E_{\text{dermis}} \) (Grebenyuk 1994). To gain better insight into the
overall skin behavior during insertion, the mechanical behavior of the different layers should be studied. The skin properties for young and aged people was studied and a computational model for studying the mechanical properties of skin with age was presented (Magnenat-Thalmann 2002). To understand skin folding, they approached a two-layer skin model consisting of epidermis and dermis and a three-layer model including stratum corneum, epidermis and dermis. They could predict the skin folding using three layers model. Skin properties for young and aged people are shown in Table 2.1.

Table 2.1 Skin properties for young and aged people (Magnenat-Thalmann 2002).

<table>
<thead>
<tr>
<th>Layer</th>
<th>Young</th>
<th>Aged</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First Layer – Stratum corneum</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Thickness (mm)</td>
<td>0.015</td>
<td>0.015</td>
</tr>
<tr>
<td>b) Elastic Modulus (N/mm²)</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td><strong>Second Layer – Epidermis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Thickness (mm)</td>
<td>0.050</td>
<td>0.2</td>
</tr>
<tr>
<td>b) Elastic Modulus (N/mm²)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Third layer – Dermis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Thickness (mm)</td>
<td>1.235</td>
<td>1.085</td>
</tr>
<tr>
<td>b) Elastic Modulus (N/mm²)</td>
<td>0.6</td>
<td>1</td>
</tr>
</tbody>
</table>
2.1.2 Transdermal drug therapy

Some methods have been developed for circumventing the stratum corneum barrier. Optimization of drug delivery through human skin is important in modern therapy. There are some methods for optimizing transdermal drug therapy and they can be categorized by skin barrier modification as shown Table 2.2.

Table 2.2 Methods for optimizing transdermal drug delivery, reproduced from ref. (Barry 2001).

<table>
<thead>
<tr>
<th>Drug/Vehicle interactions</th>
<th>Correct drug selection</th>
<th>Chemical potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesicles and particles</td>
<td>Liposomes</td>
<td>High velocity particles</td>
</tr>
<tr>
<td>Stratum corneum modified</td>
<td>Hydration</td>
<td>Chemical enhancer</td>
</tr>
<tr>
<td>Stratum corneum bypassed/removed</td>
<td>Microneedles array</td>
<td>Ablation</td>
</tr>
<tr>
<td>Energy assisted methods</td>
<td>Ultrasound</td>
<td>Electroporation / Iontophoresis</td>
</tr>
</tbody>
</table>

Drug and vehicle interactions are the simplest approach for transdermal drug delivery. Drug permeation rate through stratum corneum can be expressed as simple equation for steady flux.

\[
\frac{dQ}{dt} = \frac{ADC_s K}{h}
\]  
(2.1)
where $Q$ is the total amount of delivered drug through membrane, $t$ is time, $h$ is thickness of membrane $C_d$ is the concentration of drug in donor solution, $K$ is the partition coefficient of solute between membrane and bathing solutions, $D$ is the diffusion coefficient (Menczel 1995).

Drug is encapsulated in liposome bilayer vehicles to be delivered through skin. However, liposomes are not effective method to penetrate into viable skin, although occasional transport processes were reported (Marzda 1997). The PowderJet system fires solid particles (20 – 100 μm) through stratum corneum into lower skin layers, using a supersonic shock wave of helium gas (Burkoth 1999). This device does not use needles to penetrate into skin, so it can avoid skin damage and infection from needles. However, there have been problems with bruising and particles bouncing off skin surfaces (Barry 2001).

Hydration of stratum corneum increases the penetration rate of skin because water opens up the compact structure of horny layer (Menon 1994). Moisturizing additives, hydrophobic ointment and transdermal patches enhance the skin permeation and bioavailability of drug into skin (Barry 1995).

There are several types of chemical penetration enhancers. An enhancer, like ethanol, disrupts stratum corneum lipid organization (Menczel 1995). Ionic surfactants interact with keratin in cornocytes and open dense protein structure (Barry 1991). Many solvents enter stratum corneum, change its solution properties by altering the chemical environment, and thus increase partition coefficient in equation 2.1 (Barry 2001). Many chemical enhancements combine three mechanisms: lipid action, protein modification and partitioning promotion.
Stratum corneum can be bypassed by inserting of microneedles into skin (McAllister 2000). Drug can pass through holes which are created by microneedles. Several methods for stratum corneum abrasion have been developed. Microdermabrasion uses a stream of aluminum oxide crystals to remove the stratum corneum layer (Friedland 2000). Laser ablation is based on high powered pulses to vaporize a section of stratum corneum layer (Dover 2000).

Ultrasonic energy disturbs the lipid packing in stratum corneum by cavitation. Shock wave of collapsing vacuum cavities increases free volume space in bimolecular leaflets and thus enhance drug penetration into the tissue (Menon 1994). Iontophoresis, the electrical driving of charged molecules into tissue, passes a small direct current through a drug containing electrode in contact with skin. Charged species are driven primarily by electrical repulsion from the driving electrodes and the flow of electrical current may increase the permeability of skin (Naika 2000). Skin electroporation generates transient aqueous pores in the lipid bilayers by application of short and high voltage pulses of approximately 100-1000 V/cm (Prausnitz 1993). These pores provide pathways for drug penetration that travel through stratum corneum (Prausnitz 1995).

2.1.3 Transdermal drug products

As mentioned above, there are two types of transdermal delivery systems, passive and active. In passive transdermal systems, the drug diffuses through the stratum corneum and epidermis to the deep dermis where it can act locally or penetrate the capillaries for systemic effect. Active patches require a physical force to facilitate the movement of drug molecules across the skin. By using an applied force (such as an electrical potential or
ultrasound) active transdermal systems currently in development may be capable of delivering proteins and other large molecules.

Passive TDS devices can be categorized typically into three kinds as shown in Table 2.3. Drug-In-Adhesive type has adhesive polymer matrix containing drug. Reservoir type is characterized by the inclusion of a liquid compartment containing a drug solution or suspension separated from the release liner by a semi-permeable membrane and adhesive. The matrix system design is characterized by the inclusion of a semisolid matrix containing a drug solution or suspension which is in direct contact with the release liner. The component responsible for skin adhesion is incorporated in an overlay and forms a concentric configuration around the semisolid matrix (Nakano 2001; 3M 2003).

Table 2.3 Classification of passive TDS device, reproduced from ref. (Nakano 2001; 3M 2003).

Table 2.4 gives an overview of the passive systemic TDS currently available
worldwide. The majority are devices of the drug-in-adhesive type (Nakano 2001).

Table 2.4 Systemic passive TDS on the World market, reproduced from ref. (Nakano 2001; 3M 2003)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indications</th>
<th>Brand name (Drug-in-adhesive items in Bold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroglycerine</td>
<td>Angina pectoris</td>
<td>Herzer, Vasolator tape, Minitran, Nitroderm-TTS</td>
</tr>
<tr>
<td>Isosobide dinitrate</td>
<td>Angina pectoris</td>
<td>Frandol tape-S, Isopit</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Menopause symptom</td>
<td>Climara, Estrana, Estraderm-TTS</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Menopause symptom</td>
<td>Testoderm</td>
</tr>
<tr>
<td>Tulobuterol</td>
<td>Bronchial asthma</td>
<td>Hokunalin Tape</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Smoking cessation aid</td>
<td>Nicoderm, Nicodol, Habitrol</td>
</tr>
<tr>
<td>Clonidine</td>
<td>Hypertension</td>
<td>Catapres-TTS</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>Motion sickness</td>
<td>Transderm-Scop</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>Analgesic</td>
<td>Duragesic</td>
</tr>
</tbody>
</table>

Consequently, a variety of approaches for active transfer are being investigated by companies for enhancing transdermal drug delivery. They include the use of iontophoresis, ultrasound, elettroporation, heat and microneedles. Alza initiated in 1988.
the development of a self-contained, disposable and electrically-driven transdermal patch. However, to date, E-TRANS® of ALZA using electroporation has not been commercialized (ALZA 2003). Iomed’s iontophoretic device, Phoresor Dose Controller®, was FDA approved and commercialized (IOMED 2003). Elan has undertaken to develop a disposable iontophoretic patch, called PowerPatch®, for delivering calcitonin to treat osteoporosis. However, work on this device was discontinued (Berg 2002). The use of ultrasound for transdermal drug delivery is that acoustic energy vibrates molecules to generate flux and also caused cavitation which creates tiny holes in the skin surface through which drugs can pass. Sontra Medica, founded on research from the laboratory of Professor Robert Langer at Massachusetts Institute of Technology, is developing the SonoPrep® transdermal system for delivery of large molecules. However, The SonoPrep® device requires regulatory clearance and is not yet commercially available (SONTRA 2003).

The application of a short, carefully controlled, pulsed electric field to living cells causes a transient permeability in their outer membrane and the opening of pores that close after the electric field is removed. Genetronics Biomedical has developed the MedPulse® electroporation therapy system for use in delivering pharmaceuticals and genes. The development of the MedPulse® Electroporation Therapy (EPT) System was completed in 1998, which led to CE mark certification and ISO9001 certification in 1999. After the initial product release, feedback was solicited from clinical trials, focus groups and third party reviews (BIOMEDICAL 2003).

Zars is developing a controlled heat-aided drug delivery system (CHADD®) to enhance the transdermal passage of drugs. CHADD® system uses a thin heating device
which is attached to the top of a transdermal patch (ZARS 2003). Altea has developed a method for passing drugs through microscopic openings in the stratum corneum that are created using its MicroPor system. The minuscule pores are made using an array of elements that are pulsed at a high temperature for a few milliseconds. The thermal energy is emitted by an electrically heated filament and can be controlled. Altea is collaborating with Elan for the use of the MicroPor system to deliver DNA vaccines and for gene therapy. SpectRx has introduced an innovative way to measure glucose through the skin. They have developed an Altea MicroPor(TM) laser which creates microscopic pores in the stratum corneum, the outermost layer of skin (Figure 2.3). This painless procedure opens paths for interstitial fluid (ISF) to cross the outer skin barrier. Glucose in transdermal fluid (TDF) can be measured either with a dedicated sensor contained in the fluid collection device or by commercially available blood glucose measurement systems (Gebhart 2003).

![Image](image-url)

**Figure 2.3** (left) Minimally invasive glucose monitoring using laser poration and (right) Light microscope image of Micropore® cross section (Gebhart 2003)
NanoPass Technology is using MEMS (microelectromechanical systems) technology to develop hollow microneedle devices for painless transdermal drug delivery. NanoPump® includes a microneedle array, adhesive and tube system. It will be used in conjunction with insulin pumps for painless controlled release of transdermal insulin. NanoPump® is based on silicon needles, and will be regulated as a medical device (NanoPass 2003). Biovalve is developing products that are microfabricated using MEMS technology, based on the research of Professor Mark Prausnitz at Georgia Institute of Technology. Biovalve is also developing a transdermal patch, called e-Patch®, that employs an electrochemical reaction that can push a large dose of drug contained in a reservoir through a microneedle array. Alza is developing Macroflux®, a thin screen with precision microprojections (190 microprojections per cm² with each knife of 300 μm length). The screen is stamped from a sheet of titanium foil. The microprojections can be combined with Alza's active e-Trans device. Alza, in collaboration with Theratechnologies, is in a phase II trial using Macroflux device to deliver ThGRF peptide for treating endocrine and metabolic disorders (Theratechnologies 2003). Transdermal delivery of vaccines has also been reported by BD Technologies (Miksztta 2002). In this case, the goal is to deliver the antigen within the epidermis rather than to the systemic circulation. Microenhancer arrays (MEAs), silicon projections, disrupted mechanically stratum corneum and then vaccine was delivered epidermally. Microstructures transdermal system (MTS), utilizing plastic microstructures, was developed by 3M (Wick 2003). Coated MTS proprietary technology with protein release was introduced and immune response has been observed with extremely large antigens such as tetanus toxoid when co-administered with an adjuvant.
2.2 Microneedles

Intravenous injections exhibit a fast drug response. However, as previously described, single injection of drug into the body has disadvantages like the possibility of overdose, significant fluctuation in drug level and lack of targeted delivery. Transdermal drug delivery systems have greater patient compliance because there is no pain, no medical expertise is required and drug delivery can be sustained for the duration of therapeutic activity. However, transdermal drug delivery (TDD) can be applied to only a few kinds of drugs due to the barrier properties of stratum corneum. A combination of these two drug delivery techniques would yield a device that has high patient compliance and the ability to deliver a multitude of drugs. This hybrid device concept is an array of microneedles shown, for example, in Figure 2.4. The microneedles are sufficiently long and sturdy enough to penetrate the stratum corneum. Aqueous transport pathways across the stratum corneum can be created between the needle surface of a solid microneedle and the skin. These pathways may allow for diffusion of molecules across the skin barrier.

2.2.1 Solid microneedles

Microneedles were first created in 1997 based on the principle of the combination of needle injection and transdermal drug delivery (Henry 1998). These microneedles are the product of micro-electro-mechanical-systems (MEMS) and drug delivery research. Tiny silicon spikes are sharp enough to penetrate the skin easily and create pathways through skin. Figure 2.4 is a comparison of a conventional hypodermic needle and an array of microneedles at the same magnification. These silicon microneedles were created using a
modification of the Black Silicon Method (Jansen 1995). The required anisotropy in the etch rate can be accomplished by controlling the chemistry of the plasma in a dry etching process. By creating chromium disk masks on the silicon surface, a cone of silicon will be protected from the plasma. As the cone continues to grow in the vertical direction, it is slowly etched in the horizontal direction as well and eventually results in the disk being under-etched and falling off. McAllister et al. have demonstrated this concept by showing a marked increase in permeability for a variety of molecules and nanospheres (Henry 1998; McAllister 2000; McAllister In-press). The permeability of the skin was increased as much as four orders of magnitude when compared to an application without microneedles.

Figure 2.4 Microneedles compared to a conventional 26-gauge needle at the same magnification (Henry 1998; McAllister 2000).
Macroflux technology is a transdermal drug delivery method that ALZA corporation is developing for use with several transdermal delivery system (Lin 2001; Matriano 2002). This system incorporates a titanium microprojection array that creates superficial pathways through the skin barrier layer as shown in Figure 2.5. Macroflux microprojection array system has 330 μm length and 190 microprojections/cm². Ovalbumin (OVA) coated Macroflux penetrated into hairless guinea pig skin and higher antibody titers were observed compared to same subcutaneous or intramuscular dose (Matriano 2002).

Microenhancer arrays (MEAs), which were micron-scale silicon tapered structures, were introduced by BD technologies. MEAs breached the skin barrier, allowing direct access to the epidermis with minimal skin irritation. In mouse model, MEA-based delivery enabled gene transfer and genetic immunization through skin (Miksza 2002).

Figure 2.5 Scanning electron photomicrograph of the MacroFlux microprojection array (Daddona 2002)
2.2.2 Hollow microneedles

The inclusion of a hollow lumen in a microneedle structure expands its capabilities. The delivery of drug is not dependent on passive diffusion but convective transport. But the mechanical strength of hollow microneedles is weaker than solid ones and additional complexity is required to generate hollow structures. (Davis 2003).

Hollow silicon microtubes were fabricated by creating a tubular needle around the center lumen and the resulting microtubes increased the permeability of skin by orders of magnitude for a wide range of compounds (McAllister 1999). An extension of solid silicon pyramids of Hashmi was found to effectively withdraw blood through the lumen by capillary action, Figure 2.6. The fabrication process starts with several deep reactive ion etching (RIE) steps and then makes a beveled shape using silicon wet etching (Gardeniers 2002).

Figure 2.6 (Left) Hollow silicon microneedles used to withdraw blood by capillary force; (right) Blood sampling, using an array of needles. (white spots are droplets of blood) (Gardeniers 2002)
The hollow metal microneedles were fabricated using metal as shown Figure 2.7. Three dimensional arrays were first reported by McAllister (McAllister 1999). These structures increased the skin permeability by four orders of magnitude to calcein and bovine serum albumin, and nanospheres were also transported through the epidermis using these hollow structure (McAllister 2000). The hollow lumen of these metal structures made the transport possible while needles were still inserted in skin, but some hollow needles were blocked by skin tissue (Devis 2003).

Figure 2.7 Hollow metal microtubes. Microtubes were demonstrated to increase the permeability of human skin epidermis to calcein, and bovine serum albumin (McAllister 2000).

Tapered hollow metal microneedles based on laser micromachining and the LIGA process were developed as shown Figure 2.8 and these microneedles were used to successfully deliver insulin to living diabetic rats (Davis 2003).
2.2.3 Microneedles integrated device

In addition to the ability to make contact with or even puncture objects and membranes, microneedles offer the possibility of incorporating additional components to increase their functionality. The additive components include electrical and chemical sensors, and microchannels as well as pumps.

The simplest form of integrated microneedles is combined microchannels or microreservoirs with microneedles. A hollow silicon microneedle array integrated with fluidic microchannels for the extraction and analysis of biofluids was introduced (Mukerjee 2003). The needle fabrication is accomplished through a combination of dry and wet silicon etching, wafer sawing and anodic bonding to glass. The silicon microneedles are arranged in a two-dimensional array oriented normal to the silicon chip surface. This microdevice was reported to demonstrate the transdermal extraction of...
human interstitial fluid (ISF) using only capillary force. A microneedle array was combined with reservoirs and fluidic channels through wafer connection (Trautmann 2003). Microneedle fabrication depends on deep reactive ion etching of silicon with a suspended etch mask. The fabrication technology applies an etch mask which remains suspended above an etch cavity containing microneedle arrays and reservoirs. The MEMS syringe was fabricated based on an array of hollow-pointed silicon microneedles and a deformable polydimethylsiloxane (PDMS) reservoir for a suspension of lyophilized drug and it is illustrated in Figure 2.9 (Stoeber 2002).

Figure 2.9 (left) Concept of a disposable MEMS syringe (Right) A MEMS syringe with 8 microneedles and a PDMS container filled with a suspension of blue microbeads (Stoeber 2002).

Zimmermann et al. presented a disposable, minimally invasive, self-calibrating, continuous glucose monitor consisting of hollow out-of-plane microneedles to sample interstitial fluid from the epidermis, an integrated porous poly-Si dialysis membrane and an integrated enzyme-based flow through a glucose sensor (Zimmermann 2003).
Microneedles have been integrated with an on-chip MEMS positive displacement micropump for continuous drug delivery applications. The generation and collapse of thermally generated bubbles with flow rectified by directional check valves are used to achieve net pumping (Zahn 2001). However, the flow rates observed in this study were 1 nl/sec and this is about an order of magnitude lower than those previously observed in the pump without microneedles. This is likely due to an increased resistance to flow from the microneedle. The microneedle contributes more viscous losses to the pump. In addition, the small spacing between the needle and the seat will contribute to a very large drag on the fluid which limits flow rates.

A microdialysis microneedle is introduced that is capable of excluding large MW compounds based on size. These microneedles have been integrated into a planar microfluidic system capable of sampling and analyzing biological solutions. The integrated microfluidic system includes the assembly of microneedles with on-chip flow channels and electronics together with previously designed positive displacement micropumps, microvalves and a planar electrochemical sensor for biological detection. Multichannel fluidic control for biological sampling, sensor cleansing and recalibration is demonstrated with integrated sensor operation. Figure 2.10 shows an integrated microdevice (Zahn 2001).
Figure 2.10 Schematic of a three channel sensing chip with integrated components including micropumps, microvalves, glucose sensor and a microdialysis needle. White arrows show the direction of fluid flow in each channel (Zahn 2001).

2.3 Polymeric drug delivery

Design and synthesis of new biodegradable and biocompatible polymeric drug delivery systems based on both natural and synthetic polymers have been developed. Various synthetic as well as natural polymers have been examined in drug delivery applications. If the polymer matrix does not degrade inside the body, then it has to be surgically removed after it is depleted of the drug. Hence to avoid the costs as well as risks associated with multiple surgeries, the polymer used should be biologically degradable. Thus for a polymer to be used as a drug delivery matrix, it has to satisfy the following criteria: (1) It has to be biocompatible and degradable; (2) The degradation products should be nontoxic and should not create an inflammatory response; and (3)

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Degradation should occur within a reasonable period of time as required by the application (Chaubal 1997). Nature polymers have been studied for biodegradable polymeric drug delivery. Examples of these are collagen (Rubin 1973), cellulose and chitosan (Dyer 2002). One problem with natural polymers is that they often take longer to degrade in-vivo. Various synthetic polymers have been examined for their degradability and their applications in drug delivery. Extensive reviews on the use of synthetic polymers in drug delivery are available in the literature (Langer 1990a; Langer 1990b; Guy 1996; Ratner 1996). Over the past decade, dozens of hydrolytically unstable polymers have been suggested as degradable biomaterials. However, only a small fraction of those polymers are accepted as safe biomaterials through detailed toxicological studies in-vivo, investigations of degradation rate and mechanism, and careful evaluations of physicochemical properties (Ratner 1996). A small number of biodegradable polymer has been approved by the U.S. Food and Drug Administration (FDA). Poly (lactic-acid), poly (glycolic-acid) and their copolymers have been used routinely for a narrow range of applications in human medicine (Ratner 1996). Recent research has led to a number of well-established investigational polymers that may find practical applications as degradable implants within the next decade. Representative examples of these polymers are polyhydroxybutyrate (PHB), polycaprolactone (Allen 1998) and polyanhydrides (Hanes 1998). Their chemical structure is shown in Figure 2.11 and their mechanical properties are provided in Table 2.5.
Figure 2.11 Chemical structures of the degradable polymers
Table 2.5 Mechanical properties of biodegradable polymers, reproduced from ref. (Engelberg 1991; Ratner 1996; Middleton 1998)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Glass Trans. °C</th>
<th>Melting Temp. °C</th>
<th>Tensile strength (MPa)</th>
<th>Tensile modulus (MPa)</th>
<th>Elongation</th>
<th>Yield (%)</th>
<th>Break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly (glycolic acid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGA (M.W:50,000)</td>
<td>35</td>
<td>215</td>
<td>70</td>
<td>7000</td>
<td>~0</td>
<td>~0</td>
<td></td>
</tr>
<tr>
<td>PGA (M.W:100,000)</td>
<td>35</td>
<td>225</td>
<td>100</td>
<td>10000</td>
<td>~0</td>
<td>~0</td>
<td></td>
</tr>
<tr>
<td>Poly (lactic acid)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-PLA (M.W:50,000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-PLA (M.W:100,000)</td>
<td>54</td>
<td>170</td>
<td>28</td>
<td>1200</td>
<td>3.7</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>L-PLA (M.W:300,000)</td>
<td>58</td>
<td>155</td>
<td>50</td>
<td>2700</td>
<td>2.6</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>D,L-PLA (M.W:20,000)</td>
<td>59</td>
<td>178</td>
<td>48</td>
<td>3000</td>
<td>1.8</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>D,L-PLA (M.W:107,000)</td>
<td>50</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>D,L-PLA (M.W:550,000)</td>
<td>51</td>
<td>-</td>
<td>29</td>
<td>1900</td>
<td>4.0</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Poly caprolactone (M.W:44,000)</td>
<td>-62</td>
<td>57</td>
<td>16</td>
<td>400</td>
<td>7.0</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Poly (lactic-co-glycolic acid)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50/50 PLGA</td>
<td>45-50</td>
<td>n/a</td>
<td>30</td>
<td>3000</td>
<td>3-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly anhydrides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(SA-HAD anhydride)</td>
<td>n/a</td>
<td>49</td>
<td>4</td>
<td>45</td>
<td>14</td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

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There are three general mechanisms by which drugs are delivered from polymer: (1) diffusion of the drug species from or through the system; (2) a chemical or enzymatic reaction leading to degradation of the system, or cleavage of the drug from the system; and (3) solvent activation, either through osmosis or swelling of the system (Langer 1998). In one approach, the drug is physically entrapped inside a solid polymer that can then be injected or implanted in the body. Lactic/glycolic acid copolymers were used for drug delivery systems widely. In the case of PLGA, the combination of diffusion through pores as well as polymer matrix degradation allows control of release rates. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix, as shown schematically in Figure 2.11. For some degradable polymers, most notably the polyanhydrides and polyorthoesters, the degradation occurs primarily at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system (see Figure 2.12).

The most common formulation for these biodegradable materials is that of microparticles, which have been used in oral delivery systems and, even more often, in subcutaneously injected delivery systems. Given appropriate fabrication methods, microparticles of poly(lactide-co-glycolide) (PLGA) can be prepared in a fairly uniform manner to provide essentially nonporous microspheres, as shown in Figure 2.13(left). These particles will degrade through bulk hydrolysis in water or body fluids, yielding polymer fragments over time. The polymer microparticles in the human body are shown in Figure 2.13(right).
Figure 2.12 Mechanism of hydrolysis of biodegradable polymer, surface erosion and bulk erosion.

Figure 2.13 (Left) PLGA microparticles containing HBsAg and (right) 7 days after injection into ICR mouse muscle in-vivo (Lee 1997).
Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable, and progressively smaller, compounds. In some cases—as, for example, polylactides, polyglycolides, and their copolymers—the polymers will eventually break down to lactic acid and glycolic acid, enter the Kreb's cycle, and be further broken down into carbon dioxide and water and excreted through normal processes. Figure 2.14 shows the preparation of poly (glycolic acid) by ring opening polymerization and Figure 2.15 presents the chemical mechanism of hydrolysis of PLGA.

![Chemical structures](image)

**Preparation of Poly (glycolic acid) by Ring-opening polymerization**

Figure 2.14 Synthesis of poly (glycolic acid) by ring opening polymerization (Perrin 1997).
Figure 2.15 Hydrolysis of poly (lactic-co-glycolic acid) to lactic acid and glycolic acid (Ratner 1996).

2.4 PolymERIC Microneedles device concept

Previous studies have demonstrated that needles of microns dimensions can be painlessly inserted into skin (Kaushik 2001) and increase skin permeability for transdermal drug delivery (Henry 1998; McAllister 2000). These microneedles have been made of silicon or metal. We were motivated to make needles out of biocompatible and biodegradable polymer for ease of manufacture, safety and economic performance. Microneedles have not been made from biodegradable polymer before. We selected polyactic acid, polyglycolic acid and their copolymer because of their proven safety and effectiveness in drug delivery (Juni K. 1987). PolymERIC microneedles without drug can work by creating transport channels between the skin and needles, while polymERIC
microneedles with drug are expected to work as a drug delivery matrix after inserting into skin.

We were concerned that polymer needles might not be strong enough for skin penetration. We therefore wanted to make needles with sharp, beveled tips to reduce the force required to pierce into skin and tapered structures to increase the critical failure force. Microneedles with asymmetrically beveled tips and long tapered structures have not been made before, we needed to develop a novel fabrication technique to form master structures and a micromolding technique to copy polymeric microneedles from master structures economically.

2.5 Microfabrication background

To make polymeric microneedles, microfabrication techniques are needed: micromachining to make master structures and micromolding to replicate them. The micromachining techniques have been developed from existing microelectronics technologies. The basic techniques are pattern replication, material deposition and etching.

2.5.1 Silicon process

Silicon etching techniques have been used to make microneedles and can be adapted to make polymer microneedles.
2.5.1.1 Silicon dry etching process

Dry etching technology comprises three separate etching methods: reactive ion etching (RIE), sputter etching, and vapor phase etching. In RIE, the substrate is placed inside a reactor in which several gases are introduced. Plasma is struck in the gas mixture using a radio frequency (RF) power source, breaking the gas molecules into ions. The ions are accelerated towards and react at the surface of the material being etched, forming another gaseous material. In RIE, etch depths of hundreds of microns can be achieved with almost vertical sidewalls. Sputter etching is essentially RIE without reactive ions. In principle, this is similar to sputtering deposition systems, in which a substrate is subjected to ion bombardment instead of the material target used in sputter deposition. Vapor phase etching can be done with simpler equipment required by RIE. In this process the wafer to be etched is placed inside a chamber, in which one or more gases are introduced. The material to be etched is dissolved at the surface in a chemical reaction with the gas molecules (Maluf 2000). Dry etching is a good choice when good control over sidewalls for deep etchings in the substrate is desired. The major drawback of dry etching, however, is its high expense compared to wet etching or micromolding.

2.5.1.2 Silicon wet etching process

The etching of monocristalline silicon wafers along preferential directions is a typical process step in the fabrication of micromechanical devices. The capabilities of the anisotropic etch of silicon are well documented and reviewed (Bean 1979). Anisotropy can be obtained through different etch rates that selected chemicals exhibit against different crystalline planes. In silicon, the atoms laying on (111) planes appear more
densely packed than those on the (110) and (100) planes. As a consequence, certain etching formulations are favored in removing atoms from (110) and (100) planes. This enables achieving V- or U-shaped structures through an etch resistant mask layer. This is usually made of silicon dioxide or silicon nitride. A popular anisotropic wet etching bath is obtained with a solution of 40% by weight of potassium hydroxide (KOH) in isopropyl-alcohol (this acts as a "moderator"; i.e. slows down the etch). In the literature, etch speeds of 6000 Å/min at 80°C for (100) planes, 1000 Å/min for (110) planes and 60 Å/min for (111) planes are reported (Sze 1985). The etching rate for the above formulation is around 0.7 µm/min for (100) planes and 0.035 µm/min for (111) planes. For (110) planes the etching speed is roughly 4 times that of the (111) direction. The KOH solution has also a weak effect on the masking film, if this is SiO₂. A mask etch rate around 15 Å / min is reported in the literature, which is much slower than the etch rate of 1 µm/min for silicon. By assuming a safe 500:1 ratio between the etching speeds of silicon oxide, the maximum achievable silicon etch depth can be calculated for a given oxide thickness. For example, a 2000 Å thick oxide film can be used for obtaining a 100 micron deep structure on silicon. However, for larger depths a more resistant mask layer must be used, such as silicon nitride.

A variety of new biotechnologies have been developed based on the combination of dry etching process and wet etching process. For example, Lin et al. have fabricated single hollow silicon and polysilicon microneedles of 1 – 7 mm length and 30 – 200 µm diameter as hypodermic needles to reduce insertion pain and tissue damage (Lin 1993). Van der Berg et al. recently reported hollow silicon microneedles with triangular tip shape and 400 µm height (Van der Berg 2002).
2.5.2 Polymer process

Recently, a considerable effort has been focused on the use of polymers in microelectronics systems and MEMS. Polymers have been extensively used as both structural and functional materials for micro-devices. The particular features that make them attractive are: (1) Their flexibility and mouldability leading to ease of fabrication (maskless fabrication in the case of microstereolithography); (2) the interesting semiconducting (even metallic), magnetic and optical behavior in some functional polymers; (3) the broad ability to manipulate their molecular structure and to synthesize polymers with tailor-made properties; (4) biocompatibility; and (5) easy packaging and scalability (Gardner 2001).

Three dimensional structures require thick resist layers that are capable of high resolution and high aspect ratios. Polyimide, used in the manufacture of circuit boards, can be made thick and is a candidate for broad use in MEMS. Photosensitive polyimide is a negative tone resist and has been used as photosist. Polyamic acids are spun on the wafer and, upon exposure to UV light, cross-linking results (Madou 2002). But while these resists are useful in many applications, it is difficult to obtain high aspect ratio devices due to resolution limitations, high optical absorbency, and the difficulty in obtaining thicker layer than 50 μm in a single spin-coat (Shaw 1996). New resist systems specially designed for micromachining can be spin-coated as very thick films (up to 500 μm in a single coat) and have excellent sensitivity, high resolution, low optical absorption, high aspect ratio and good thermal, and chemical stability. A polymer in the class of new photoresists is an epoxy derivative of bis-phenol-A novolak, named as SU-8, Figure 2.16.
SU-8 is a negative, epoxy-type, near-UV photoresist based on EPON SU-8 epoxy resin (from Shell Chemical) that was originally developed and patented by IBM (Shaw 1989). SU-8 can be as thick as 2 mm and high aspect ratios of 20 have been demonstrated with standard contact lithography equipment. These astounding results are due to the low optical absorption in the UV range which only limits the thickness to 2 mm for the 365nm-wavelength where the photo-resist is the most sensitive (i.e., for this thickness 100% absorption occurs), Figure 2.17.

![Figure 2.16 Chemical structure of glycidyl ether of bisphenol A, SU-8](image)

SU-8 combines the desirable properties of high transparency at wavelengths above 340 nm, relatively fast photo-reaction speed, high lithographic contrast and resistance to plating bath chemistries, making it uniquely suited for the fabrication of high aspect ratio MEMS devices, Figure 2.17. Another important component of a resist is its solvent, which plays a critical role in determining viscosity (film thickness), coating quality
and cycle times for effective solvent removal. For standard SU-8, γ-butyrolactone (GBL) is the chosen solvent, primarily because of its high solvency, low cost and low toxicity. GBL, with a boiling point of 204°C has low volatility which is expected to result in longer drying times than might be the case with other more volatile resist solvents. The process consists of coating, soft bake under 95 °C, relaxation time, 365 nm UV exposure, post bake and development with propylene glycol methyl ether acetate (PGMEA).

Figure 2.17 Optical absorption of photoresist : Curve A, 25 μm thick SU-8; Curve B, 25 μm RISTON T-168 Resist; Curve C, 10 μm positive diazo resist (Shaw 1990).

Three-dimensional microstructures have been fabricated using SU-8. One example, which is based on the SU-8 process, is inclined SU-8 structure. The inclined SU-8 structures are introduced by using inclined UV lithography through transparent SU-8 (Han 2003; Yoon 2003). In this fabrication, microfilter and micromixer structures for microanalysis were fabricated from SU-8, Figure 2.18 (Yoon 2003).
Figure 2.18 Fabricated filter: (a) a single stage filter, (b) three integrated filters and channels for multiple diameter particle partitioning.

A biodegradable polymer was introduced for microstructure fabrication. By advantage of their ability to degrade in tissues, biodegradable polymers hold enormous potential as a new material for implantable biomedical microdevices. A process for fabricating biodegradable microstructure was previously reported (Arnani 2000). This study suggests three fabrication processes: (1) micro-molding process to form three dimensional microstructures in polycaprolactone (PCL) via a silicon micromachined structure; (2) a method of transferring metal patterns to surfaces of PCL substrates; and (3) techniques for sealing both dry and liquid filled PCL microcavities with a metal film. However, there is some difficulty applying this fabrication process to other biodegradable polymers including PLA, PGA and PLGA due to adhesion problem with the silicon substrate. Compared to PCL, PLA,PGA and their copolymers have different thermal expansion properties due to relative high young’s modulus and low elongation ratio and surface tension. In addition, this process can only make low aspect ratio microstructures.
2.5.3 Micromolding

After making master microneedles by wet and dry etching micromachining, these structures can be replicated by micromolding. In the micromolding process, microstructures are fabricated using molds to define the deposition of the structural layer, (i.e., the polymer). The structural material is deposited only in areas constituting the microdevice structure, in contrast to bulk and surface micromachining, which feature blanket deposition of the structural material followed by etching to realize the final device geometry. After structural layer deposition, the mold is dissolved in a chemical etchant that does not attack the structural material. One of the most prominent micromolding processes is the LIGA process. LIGA is a German acronym standing for lithographie, galvanof ormung, und abformung (lithography, electroplating, and molding). This process can be used for manufacturing high-aspect-ratio three dimension microstructures in a wide variety of materials, such as metals, polymers, ceramics, and glasses. Photosensitive polyimides are also used for electroplating molds. The photolithography process is similar to conventional photolithography, except that polyimide works as a negative resist (Mehregany 1999).

PDMS replication has been utilized mostly in microfluidics with patterned SU-8 as mold inserts (Madou 2002). A simple example procedure for making a PDMS stamp from a photolithographically patterned resist layer as master mold is outlined in Figure 2.19. PDMS structures also can be massively replicated using polymeric molds fabricated by the standard lithography-electroforming-micromolding (LIGA) process, because PDMS is durable, optically transparent, and inexpensive (Kim K.S. 2000). In soft lithography, a PDMS elastomeric stamp with patterned relief structures on its surface is

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used to generate patterns and structures with feature sizes ranging from 30 nm to 100 μm (Xia 1996). PDMS provides a convenient, effective, and low-cost material for formation and manufacture of microstructures.

Figure 2.19 Schematic diagram of PDMS micromolding process (Madou 2002).

2.5.4 Electroplating

Electroplating metal onto masters or molds allow the fabrication of metallic microstructures. Electroplating is the deposition of metal on a surface using a bath that consists of an electrolyte containing metal ions, an electrode that is usually the substrate to be metallized, and a counter electrode (Pandey 1996). A diagram of an electroplating bath with Ni chemistry and a constant power supply is shown in Figure 2.20. The anode
can serve as both a electrode for carrying the current as well as source to replenish the ionic species in the electrolyte bath. The Watts formulation is the dominant bath in use for nickel electroplating. The bath is a solution of nickel sulfate, nickel chloride, and boric acid. \( \text{Ni}^{2+} \) ions are reduced to \( \text{Ni} \) at the cathode, for sample. The anode is typically nickel foil which is dissolved from its ground state to replenish the ionic species consumed in the bath. Application of current causes anions to migrate towards the anode and metal ions to migrate towards the cathode and deposit on the sample. The bath contains nickel salts and boric acid. The electroplating process is performed at a constant current density between 3 and 10 A/cm\(^2\) (Schlesinger 2000).

Figure 2.20 Nickel plating bath. The position of the electrodes and the reduction of nickel from the ionized species in the bath onto the surface of the cathode is shown. An external power source supplies the electrons to reduce the nickel species.
3. Methods

3.1 Solid polymer microneedles

Solid silicon microneedles forms hybrid device proposed to have high patient compliance and the ability to deliver a broad variety of drugs. However, a few percent of silicon needles have been seen to be damaged near their tips (upper 5 – 10 μm) during insertion and removal (McAllister 2000a; McAllister 2000b). Silicon based micro-electro-mechanical systems (MEMS) techniques are expensive and therefore may not be suitable for bio-application. In addition, the yield of fabrication by silicon processes can be low, which drives the production cost higher. We propose that microneedles can be fabricated out of biocompatible and biodegradable polymer for safety, ease of manufacture and cost effectiveness. Micromolding processes are highly cost effective and their melting and welding process is antiseptic. Solid polymeric microneedles without drug can create pathways through the annular gaps or channels between the microneedles and skin. Microneedles with drug can serve as a drug delivery matrix. The tree of fabrication techniques is shown in Figure 3.1.
Figure 3: Tree diagram of the different fabrication techniques described in detail in this chapter and resulting type of microfabrication. The blue text in the dual columns lists the key techniques of each fabrication method.
3.1.1 SU-8 plasma etching fabrication

3.1.1.1 Beveled microneedles

Beveled microneedles are fabricated using an SU-8 photoresist process and reactive ion polymer etching process. A silicon wafer (300-350 µm thick, Nova Electronic Inc., Richardson, TX) is cleaned in 5 parts of deionized water (D.I. Water), 1 part of 30% hydrogen peroxide (J.T.Baker) and 1 part 27% ammonium hydroxide (NH₂OH, J.T.Baker) at 80 °C for 15 minutes to remove organic residue and then the wafers are dried in an oven (Blue M Electric, Watertown, WI) at 120 °C for 20 minutes. SU-8 epoxy photoresist with photoinitiator (SU-8100, MicroChem, Newton, MA) is coated onto a wafer at a thickness of 700 µm. The sample is then soft baked for 10 hours at 100 °C to remove all the solvent from the epoxy. Then UV light (365 nm, ~7000 mJ) is exposed through the circular transparent regions of an optical mask with 20 by 6 arrays of 100 µm diameter dots of a 400 µm in x-direction and 1400 µm in y-direction center to center spacing onto the SU-8 with photo initiator (PI, triarylium-sulfonium salts) using mask aligner (OAI: Optical Associates, Inc., San Jose, CA). The UV-exposed part becomes crosslinked.

Next, the sample is post-baked to cross-link the SU-8 on a hotplate for 30 minutes at 100 °C. After cooling, the non-crosslinked SU-8 is then developed with propyl glycol methyl ether acetate (PGMEA; Aldrich Chemical Co.). The spaces between the resulting cylindrical structures are filled with sacrificial polymer layer, which is thermoplastic poly(lactic-co-glycolic acid) (PLGA, 0.5 dL/g; Birmingham Polymers Inc. (BPI),
Birmingham, AL), up to the height of the cylindrical structures. This thermoplastic polymer is used to fill the space between cylinders to protect them from side etching of the cylindrical structure.

The sacrificial polymer layer with cylindrical structures of crosslinked SU-8 is coated with copper by electron beam metal deposition (Electron Beam Evaporator (E-beam); CVC Products). Next, the copper layer is patterned into 6 array-rectangles with 0.6 mm width and 10 mm length of 1.4 mm center to center spacing. This is done by first spin casting photoresist (1813; Shipley, Marlborough, MA) onto the copper layer at 3000 rpm for 30 seconds (CEE 100CB Spinner and Hotplate; Brewer Science Inc., Rolla, MO). The photoresist is then soft-baked in an oven at 100 °C for 10 minutes.

A standard photomask with the rectangular pattern is brought into contact with wafers using optical mask aligner (Hybralign Series 500; Optical Associates Inc., Milpitas, CA) and the photoresist is exposed to ultraviolet light (~100 mJ energy) through the photomask. The exposed photoresist is then removed in developer (Microposit 354 developer; Shipley) by soaking the wafers in it for 10 to 15 seconds. This unprotected copper is etched by acid solution (H₂O/H₂SO₄/H₂O₂ = 10/1/1; J.T.Baker) to make the desired pattern. The pattern only exposes one side of the cylindrical SU-8 structure. The exposed sacrificial polymer layer with cylindrical structures of crosslinked SU-8 is etched by reactive ion etching (O₂: 45 sccm, SF₆: 5 sccm, 150 W, 300 mTorr, RIE 700 series wafer/batch Plasma Processing System; Plasma Therm, St.Petersburg, FL) for 4 hours. Sacrificial polymer is removed by organic solvent, methylene chloride (Aldrich Chemical Co., St. Louis, MO), to yield the beveled etched SU-8 structure.
In some cases, Photoresist 1813 (Shipley, Marlborough, MA) is applied and spin coated at 700 rpm by spin-coater (CEE 100CB) to make curtained base of microneedles and then dried at a 100 °C oven for 20 minutes. This beveled structure is used to make a mold of PDMS that can be used to make a copy structure out of biodegradable material. The process is summarized in the Figure 3.2.
Figure 3.2. Process for beveled tip microneedles fabrication
3.1.1.2 Polymeric microneedles with notch

The microneedles are made following the beveled microneedle fabrication process, but using a different mask pattern with a notch in the circle. An optical mask has 20 by 6 arrays of 100 µm diameters dots with notches of 30 µm diameter, and it has 400 µm center to center spacing. UV light is exposed onto the SU-8 with photoinitiator using mask aligner as shown Figure 3.3. If the notch is left empty, drug can be delivered into the skin through the notch. These microneedles with notch are much easier to fabricate than hollow needles.

Figure 3.3 Process for microneedles with notches.

3.1.2 Silicon wet etching fabrication

Anisotropic etching of silicon in aqueous KOH solutions is an important technology in micromachining. The diaphragms of pressure sensors are fabricated by etching from the back side of the wafer. The back side of these diaphragms can be regarded as the {100} bottoms of anisotropically etched cavities. The formation of micropyramids on these etch bottoms is a well known and unwanted phenomenon (Seidel 1990). The
micropyramids are utilized to form tip part of microneedles.

3.1.2.1 Chisel tip solid microneedles

Silicon nitride is deposited onto a silicon wafer to a thickness of 4000 Å by chemical vapor deposition (5 sccm NH₃, 200 sccm SiH₄, 900 sccm N₂ at 30 W, 900 mTorr; PlasmaTherm PECVD, Plasma-Therm, St.Petersburg, FL) to make a hard mask for etching the silicon with KOH. Next, the silicon nitride layer is patterned into 15 by 15 arrays of 100 μm diameters square dots with a 600 μm center to center spacing. This is done by first spin casting photoresist (1813; Shipley, Marlborough, MA) onto the wafers at 3000 rpm for 30 seconds (CEE 100CB Spianer and Hotplate; Brewer Science Inc., Rolla, MO). The photoresist is then soft-baked in a oven at 100 °C for 10 minutes. A standard photomask with the 100 μm width of square opening is brought into contact with the wafers using an optical mask aligner (Hybralign Series 500; Optical Associates Inc., Milpitas, CA) and the photoresist is exposed to ultraviolet light (~100 mJ energy) through the photomask. The exposed photoresist is then removed in a developer (Microposit 354 developer; Shipley) by soaking the wafers in it for 10 to 15 seconds. The unprotected silicon nitride layer is etched using reactive ion etching with SF₆ + O₂ (22.5 sccm CHF₃, 2.5 sccm O₂ at 150 W, 45 mTorr; RIE, Plasma-Therm, St.Petersburg, FL). Subsequently the photoresist is removed using acetone. The structure is placed into KOH etchant (30 wt% KOH; Aldrich Chemical Co.), which is heated to 80 °C. Typical silicon etch rates for KOH are about 1.2 micron per minute, but this will vary with temperature and concentration. Silicon is etched to form pyramid structure holes terminating in a sharp point. SU-8 epoxy photoresist with photoinitiator (SU-8 100; MicroChem) is
coated afterwards onto the etched structure to form a 500 μm thick film. The sample is then soft baked for 10 hours at 100 °C to remove all the solvent from the epoxy. Subsequently, a second mask with is aligned with the wafer, so that a second square pattern with the 100 μm width of square opening can be transferred onto epoxy layer in vertical alignment with silicon nitride patterns. Then UV (365 nm, ~7000 mJ) is exposed through the square transparent regions of the optical mask onto the SU-8 with PI (photo initiator) using a mask aligner (OAI: Optical Associates, Inc., San Jose, CA). The UV-exposed part becomes crosslinked. Next, the sample is post-baked to cross-link the SU-8 on a hotplate for 30 minutes at 100 °C. After cooling, the non-crosslinked SU-8 is developed with PGMEA (Aldrich Chemical Co.). The non-crosslinked epoxy is developed with PGMEA, forming obelisk shaped structures. The spaces between the obelisk structures are filled with PDMS (Sylgard 184, Dow Corning). The crosslinked SU-8 is removed using RIE with O₂ (O₂, 45 sccm, 300 W, 300 mTorr; Plasma Therm, St.Petersburg, FL) for 12 hours, leaving only a PDMS mold. Subsequently, PDMS (Sylgard 186, Dow Corning) is poured into the PDMS mold and crosslinked to form polymeric microneedles having an obelisk shape to prepare the master structure for 12 hours in a 40 °C oven. This PDMS chisel structure is used to make a PDMS mold that can be used to make a copy structure from biodegradable polymer. The process is summarized in Figure 3.4.
Figure 3.4 Process for chisel tip microneedles fabrication I.
3.1.2.2 PDMS bonding

Instead of making PDMS mold layer on uneven silicon layer, PDMS layer is prepared separately. Prepared PDMS layer is aligned on silicon wafer layer and then bonded. The advantage of this process is eliminating RIE step to dry-etch SU-8 columns embedded in PDMS layer. The obelisk shaped structures are formed using PDMS bonding technique. A 300 Å chromium layer is deposited onto the glass substrate using a DC-Sputter (601 Sputtering System; CVC Products, Rochester, NY). Next, the chromium layer is patterned to have 20 by 20 arrays of 100 μm diameters square dots with a 300 μm center to center spacing using the same mask which is used to prepare micropyramids structures on silicon wafer. SU-8 epoxy photoresist with photoinitiator (SU-8 100; MicroChem) is coated afterwards onto the patterned glass substrate to form 506 μm thickness. The sample is then soft baked for 10 hours at 100 °C to remove all the solvent from the epoxy. Subsequently UV light (365 nm, ~7000 mJ) is exposed through the square transparent regions of the optical mask on the glass substrate from the bottom of the mask onto the SU-8 with Pi (photo initiator) using a mask aligner (OAI: Optical Associates, Inc.). The UV exposed part becomes crosslinked. Next, the sample is post-baked to cross-link the SU-8 on a hotplate for 30 minutes at 100 °C. After cooling, the non-crosslinked SU-8 is then developed with PGMEA (Aldrich Chemical Co.). The non-crosslinked epoxy is developed with PGMEA, forming hexagon shaped structures. The spaces between the hexagonal SU-8 structures are filled with the PDMS (Sylgard 184; Dow Corning) up to the height of hexagonal structures. The crosslinked SU-8 is removed by just peeling off PDMS layer. The PDMS mold and silicon substrate are cleaned by the Piranha process (3
parts of H₂SO₄ and 1 part of H₂O₂; Aldrich Chemical Co.) followed by rinsing in deionized water and drying at room temperature. Surface oxidation of PDMS is used to make a bond with the silicon nitride surface (Duffy 1998). Oxygen plasma treatment is performed using RIE (Plasma-Therm). The plasma parameters are power 100 W, vacuum pressure 100 mTorr, oxygen 40 sccm and operation time 30 seconds. As soon as PDMS mold is oxygen plasma treated in RIE, second square patterns of PDMS mold are aligned with square patterns on silicon wafer vertically and then leave bonding samples for a day. Subsequently, PDMS (Sylgard 186, Dow Corning) is poured into the PDMS-silicon mold and crosslinked to form polymeric microneedles having an obelisk shape to prepare the master structure for 12 hours in a 40 °C oven. The fabrication process is shown in Figure 3.5.
Figure 3.5 Process for chisel tip microneedles fabrication II.
3.1.3 Tapered cone shape solid microneedles

The use of an integrated lens substrate is introduced to produce unconventional exposure patterns in SU-8 (Park 2004). An illustrative application of this approach is the fabrication of tapered structures to increase the mechanical strength of polymer needles and deep-tissue microneedles, that require both sharply tapered tips and lengths exceeding 1 mm, from biodegradable polymers. In the case of a circular lens, development leaves a tapered-cone-shape pattern.

3.1.3.1 Integrated lens technique fabrication

The fabrication process is summarized in Figure 3.6. A layer of 5300 Å chromium is deposited on a glass substrate (4 x 4 x 0.06 inch, sodalime glass; Teic, Palm Beach Garden, FL) and positive photoresist, AZ1518 (Clariant Corp., Elgin, SC) is coated on the chrome layer. The chrome layer is patterned to have 20 by 10 arrays of 100 μm diameter dots with a center to center spacing of 400 μm in x-direction and 800 μm in y-direction on a glass substrate. A standard photomask with the circular pattern is brought into contact with photoresist on the glass substrate using an optical mask aligner (Hybralign Series 500; OAI) and the photoresist is exposed to ultraviolet light (~200 mJ energy) through the photomask. The exposed photoresist is then removed in a developer (AZ 400K developer; Clariant Corp.) by soaking the substrate in it for one minute. The unprotected chrome layer is etched using chrome etchant (CR-75; Cyantek, Fremont, CA).
Subsequently the back side of glass mask is coated with photoresist (1827, Shipley) to make a hard mask for etching the glass with HF-HCl solution. Isotropic wet chemical etching of the glass is then performed to create a concave pattern. The structure is placed in the HF-HCl solution (5 % BOE, 10 % HCl, 85% D.I.Water; Aldrich Chemical Co.) at room temperature for three hours. Long isotropic etch times can result in significant lateral underetch of the chromium, and subsequent chromium overhang. This overhang is either removed or left in place to control the light which passes through the original opening area in the chromium. Casting of SU-8 (SU-8 100, MicroChem) on this nonplanar surface results in a 700 μm thick film on the substrate with an underlying integrated microlens, due to the refractive index difference between glass and SU-8. After 12 hours soft-baking on the 100 °C hotplate, the film is exposed from the bottom (through the glass) using UV light (7,000 mJ; OAI). Due to the chromium layer on the glass, the substrate is opaque except in the lens regions. The light passes through the lens area to give a latent image in the SU-8. Next, the sample is post-baked to cross-link the SU-8 on a hotplate for 30 minutes at 100 °C. After cooling, the non-crosslinked SU-8 is then developed with PGMEA (Aldrich).
Figure 3.6 Process of integrated lens technique fabrication.
3.1.3.2 UV exposure from the backside of glass substrate

In this technique, the glass substrate between SU-8 and chrome mask layer works as lens. Spacing of glass layer between the chrome mask and SU-8 makes the diffraction of the transmitted light and it makes Gaussian distribution of light intensity in the SU-8 layer, and then forms tapered structure of SU-8 finally. A 5300 Å layer of chromium is deposited on a glass substrate (Telic) and positive photosist, AZ1518 (Clariant Corp., Elgin, SC) is coated on the chrome layer chrome layer is patterned on a glass substrate. A standard photomask with 15 by 15 arrays of 100 µm diameter square dots with a 600 µm center to center spacing is brought into contact with the photosist on the glass substrate using an optical mask aligner (Hybralign Series 500; OAI) and the photosist is exposed to ultraviolet light (~200 mJ energy) through the photomask. The exposed photosist is then removed in developer (AZ 400K developer; Clariant Corp.) by soaking the wafers in it for 30 to 45 seconds. The unprotected chrome layer is etched using chrome etchant (CR-75; Cyantek, Fremont, CA). Subsequently SU-8 (MicroChem) is cast on the opposite side of the chrome mask layer. After 12 hours soft-baking on the 100 °C hotplate, the film is exposed through the glass from the patterned chrome mask using UV light (OAI: Optical Associates, Inc., San Jose, CA). The non-crosslinked SU-8 is then developed with PGMEA (Aldrich Chemical Co.). The fabrication process is illustrated in Figure 3.7.
Figure 3.7 Process of backside of substrate UV exposure technique.
3.1.4 Micromold and replicate needle fabrication

Recently there have been many investigations on low-cost mass production-compatible microfabrication techniques for commercialization of MEMS devices. The MEMS research community has adapted conventional plastic replication techniques (such as hot embossing and injection molding) and modified those techniques to massively replicate micron-scale plastic MEMS devices (Kim 2000). PDMS has drawn the attention of the MEMS community and have been mainly used for microstructure replications in micro total analysis system (μTAS) (Jo 2000).

3.1.4.1 PDMS micromold fabrication

PDMS (Sylgard 184, Dow corning, Midland, MI) is poured over the master structure until it is about 5 to 10 mm thick so the mold can be easily handled. Vacuum (Laboport, KNF Neuberger Inc., Trenton, NJ) is applied for 30 minutes under 20 inHg vacuum pressure to remove air bubbles in the PDMS liquid. Once the trapped air is removed, the PDMS is cured by placing it a 40 °C incubator for 12 hours. The PDMS is then gently peeled off from the SU-8 master structure. The PDMS mold is placed in a 90 °C oven for 1 hour to make PDMS mold hardened.

3.1.4.2 Micromold filling

The PDMS mold is then filled with biocompatible polymer powder. Poly (glycolic acid) pellets (PGA, 1.4-1.8 dL/g; Birmingham Polymers Inc.) is put on the PDMS mold in the 230 °C oven (Vacuum Oven 1415M; VWR, West Chester, PA). The polymer pellets
are melted and the melt polymer is under 20 inHg vacuum pressure for 2 minutes to remove entrapped bubbles and fill up the melt into the PDMS mold. The sample is taken out of the oven and then cooled down to room temperature. The sample is put in the refrigerator at -10 °C for 30 minutes before releasing the polymer needles from the PDMS mold. The solidified polymer is removed from the PDMS mold and then the polymer needles are stored in the refrigerator. Alternatively, for needles made of PLGA 50/50, the oven is set at 140 °C and the same process of fabrication of PGA microneedles is used. The process is shown in Figure 3.8.
Figure 3.8 Process for replication using PDMS micromolding technique.
3.1.4.3 Electroplating methods

To provide an electrically conductive surface on polymer microneedles, Nickel is electroplated on polymeric microneedles to create a metal microneedles. The principles of electroplating are discussed in section 2.5.4. The electroplating is conducted using a Watts formulation bath (Technic, Cranson, RI). Arrays of polymer microneedles to be used masters are rinsed with alcohol solution (3 parts ethanol and 1 part D.I. Water) and dried. A seed layer is deposited onto the topside of microneedles by E-beam (CVC products). The seed layer is composed of stacked layers of titanium, copper, and titanium (Ti/Cu/Ti; 30 nm/200 nm/30 nm). A seed layer is necessary to provide a uniformly and highly conductive surface to electroplate metal onto. The copper layer provides this, and the upper titanium layer is to protect the copper from oxidizing and lower titanium layer is to provide good adhesion between the copper layer and the surface of polymer. Next, the top layer of titanium is removed in diluted HF solution (1% v/v, 3 – 5 seconds) to expose the copper layer. After the polymer needles with seed layers are prepared for plating, electric contact is made to the sample by an alligator clip. The counter electrode, nickel foil, is connected in a similar fashion to the power supply (E3611A DC Power Supply, Hewlett-Packard, Houston, TX). The paired electrodes are then submerged in the agitated (300 rpm) electroplating bath. The voltage is adjusted to maintain a constant current density of 10 mA/cm² and Ni is deposited on the sample for 30 minutes. This results in a 5 – 10 µm layer of Ni covering the surface of the polymer microneedles.
3.1.5 Insertion and failure test

3.1.5.1 Qualitative insertion test

Qualitative insertion testing is performed on each microneedle geometry. An array of solid microneedles is inserted into heat stripped human epidermis to test the ability of polymer needles to penetrate without breaking. As mentioned in Section 2.2.1, stratum corneum forms protective shield for covering the underlying viable epidermis and it has different mechanical behavior compared to dermis because Young's modulus of stratum corneum is 100 times or 1000 times higher than dermis (Van Duzee 1978). Thus, stratum corneum is mechanically main barrier for needle insertion. Heat stripped human epidermis is used to verify the creation of holes by polymer microneedles on epidermis without breaking of needles in-vitro.

Heat stripped human epidermis is prepared for insertion test and transdermal permeability test. Sections of human skin are obtained from autopsy (Emory University of Medicine Body Donor Program, Atlanta, GA) and plastic surgery procedures. The skin is stored at -80 °C (FZU-13; Thermotron Industries, Holland, MI) until use. Before separating the epidermis from the dermis using a heat stripping technique, the skin is removed from the freezer and allowed to thaw completely for 3 – 4 hours.

A heat stripping method is used to separate the epidermis from dermis (Hobson 1991). Once the skin is thawed, fat is removed from the dermis by scraping it with a scalpel handle (AliMed Inc., Dedham, MA). The skin is then submerged in a 60 °C water bath for 2 minutes. After removing the skin from the water bath, it is gently dried with paper wipes (Kimwipes EX-L; Kimberly-Clark Corp., Roswell, GA). The epidermis is then
slowly and gently peeled off the dermis using the curved edge of a spatula (VWR). The epidermis will spread out on the water surface with the viable epidermis side down due to the hydrophilic nature of the viable epidermis (Monteiro-Riviere 1991).

To view disruption of the skin barrier, the epidermis is placed on a quantity of layers of tissue paper (Kimwipes) or on top of dermis. The polymeric microneedles are put on to the epidermis, pushed in by 4 N force and removed. A blue hydrophobic dye (Trypan blue solution (0.4 %), Sigma Chemical Corp.) is applied on the epidermis. After ten minutes the blue dye is removed and the stratum corneum is patted dry with a wipe. The under side of epidermis is viewed by light microscopy (Olympus SZX12; Melville, NY) to determine if dye goes across the skin. The microneedles are also viewed by microscopy to determine if needles are damaged.

To view needles inserted into skin, solid PGA microneedles are inserted into heat stripped human epidermis to test the ability of biocompatible needles to penetrate without breaking. The epidermis is placed on multiple layers of tissue paper (Kimwipes). The polymeric microneedles are put on to the epidermis and pushed. The epidermis with needles is embedded in the 30% formaldehyde (Sigma) for 6 hours and then washed with D.I.Water to remove residual formaldehyde solution. Sample is dried out by ethanol process (Hobson 1991) for scanning electron microscopy (SEM) images. Water in skin sample is replaced with gradient ethanol solutions (25, 50, 75, 95, 100 % ethanol) and then ethanol remaining in skin is removed by vacuum drying (Laboport, KNF Neuberger Inc.). Dried sample is gold coated using EFF Sputter Coater (Ernest F. Fullam, Inc., Latham, New York) and then SEM images are taken using Hitachi 3500H SEM (Hitachi Corp. Tokyo, Japan).
Figure 3.9 Qualitative needle insertion to epidermis to test the ability of polymer needles to penetrate without breaking.

To perform an in-vivo skin penetration test, sterilized polyglycolide microneedles are inserted into human skin on a human subject. All procedures with human subject have been approved by the Georgia Tech Institutional Review Board. After insertion, microneedles are pushed at an angle to break the needles intentionally to check their insertion into the skin. The retention of tips in the skin is investigated using light microscopy (Olympus SZX12; Olympus USA, Melville, NY). Retained tips then removed using forceps.

To visualize holes in skin made by microneedles, skin puncture holes are copied using liquid Band-Aid® (skin-shield; Del Pharm. Inc., Farmingdale, NY) to investigate the change of hole-morphology over time by Hitachi 3500H SEM. Liquid Band-Aid® is applied on the needle-inserted skin area and then dried out to form film for 5 minutes. Then, the solidified film is removed from skin. The copy films are prepared at 0 min, 15
min and 30 minutes after insertion and removing microneedles from the human skin. Obtained copy films are gold coated for SEM investigation and SEM images are taken using Hitachi 3500H.

3.1.5.2 Quantitative characterization of the insertion force of microneedles into skin with respect to tip geometry

Understanding polymer needle insertion force and failure force, and their relationships to microneedle geometry is very important to design polymer microneedles. The material selection and geometry design for overcoming mechanical failure is considered based on the insertion force and failure force. Evaluating of millimeter-length metal microneedles insertion force into synthetic polymer was previously measured (Chandrasekaran 2002). Measuring and predicting the force necessary to insert hollow metal microneedles into human skin in-vivo was made by a force-displacement test station (Davis 2003). Davis tested the insertion of microneedles having a range of geometries into three Caucasian male subjects using a drop in electrical resistance of the skin to determine insertion into skin.

In this study, we follow the approach of Davis et al. and measure the force required to insert microneedles into the skin of human subjects using a displacement-force test station (Model 921A, Tricor Systems, Elgin, IL). This device measures the force applied to a needle, needle position and skin resistance during the needle’s translation to the skin, deflection of tissue around needle, and insertion into skin. The electrical resistance of the stratum corneum is much greater than the more aqueous deep tissues (Prausnitz 1996). The stratum corneum in contact with the needle provides the largest resistance in the
circuit. When the needle penetrates the stratum corneum, the measured resistance dropped suddenly.

As shown in Figure 3.10, a silver-silver chloride counter electrode (In Vivo Metric, Healdsburg, CA) is positioned on adjacent section of skin from needle injection spot, which is either mechanically stripped to remove stratum corneum using a scalpel blade or treated with electrode gel (Spectra 360, Parker, Fairfield, NJ) to ensure low-resistance electrical contact. To measure force and displacement associated with needle insertion, the test station forces down the microneedle against the subject’s hand at a rate of 1.1 mm/s. During this movement, the changes of electrical resistance and mechanical force acted on the microneedle are recorded. The microneedle stays moving into the skin until a predetermined maximum load (500 g) is reached. The recognition of insertion force is difficult because insertion force is too small to be measured by force-displacement station. The force, displacement, and electrical resistance data are then converted to an ASCII file to identify insertion force by skin resistance drop.
Figure 3.10 Procedure of measuring skin insertion force by Force-Resistance-Displacement test station. Skin resistance is calculated from parallel resistance equation. $R_{skin} = \frac{R_{measured} \cdot R_{resistor}}{R_{resistor} - R_{measured}}$.

3.1.5.3 Quantitative characterization of the failure force of polymeric microneedles with respect to needle geometry and needle material

Measuring and predicting the failure force of microneedles is a key factor to design polymer microneedles. The success of polymer microneedles depends on the ability to reliably insert microneedles into living subjects without microneedle failure. The understanding of failure force, its relationship to microneedle geometry and needle materials allows optimizing the needle design. The failure force must be more than insertion force.
3.1.5.3.1 Failure force measurement by axial force

The failure by axial force load is a primary failure mode of microneedles. The mechanical resistances of skin are due to the toughness of stratum corneum and supporting hard contact caused by bone. The failures by axial force can be explained by buckling failure by elastic instability and kneeling failure by inelastic instability. The failure force is measured by finding the ultimate stress using a displacement-force test station (Model 921A, Tricor Systems, Elgin, IL). The geometric parameters, including needle height (700 µm, 1 mm, and 1.5 mm) and column diameter (100 µm, and 200 µm), were considered in this study. The effect of polymer type including PLGA, PLA, and PGA (BPI), and molecular weight of PLGA (0.5 &dgr;g, and 1.2 dL/g), and the effect of calcin content in microneedles on failure force of microneedles were also measured. To measure stress and strain curves associated with needle failure, the test station presses the microneedles against a hard metal surface at a rate of 1.1 mm/s. During this movement, mechanical resistance experienced by the microneedle is recorded. The microneedle continues to move against the stainless steel surface until a preset maximum load (19.6 N) is reached. An array of microneedles is tested instead of using just one microneedle because (a) needle arrays can provide a planer surface to axial force (b) the measured force is averaged by the number of microneedles. After a test, the change of shape of microneedles is inspected by microscopy to confirm that all microneedles were pressed and deformed with the same magnitude across an array. The process is illustrated in Figure 3.11(a).
3.1.5.3.2 Failure force measurement by lateral force

The failure by transverse force caused by deformed skin due to misalignment of microneedles is also considered. The failure force by a transverse load is measured using a force-displacement station (Figure 3.11(b)). A row of 5 to 10 microneedles is mounted on a metal plate vertically using an epoxy adhesive. The tips of the polymer needles are pressed by the glass slide surface laterally while continuously measuring needle force and displacement. A PDMS layer (1 cm x 1 cm x 0.5 cm) is attached to the glass slide by a cyanoacrylate adhesive to control the interfacial area between the glass slide and the tips of the microneedles. The PDMS layer is aligned on the glass slide using a microscope and then bonded to the glass slide to make a stepped structure, as shown in Figure 3.11(b). The transverse failure force of PGA microneedles with 25 μm tip diameter, 1 mm length and 100 μm and 200 μm base diameter was measured. A row of microneedles was tested for bending in the vertical direction. After mounting a row of microneedles on the metal plate, the force was applied on the partial surface of the tips (from the end of the tip to 500 μm away from the tip) laterally until they are broken. During this movement, the force and displacement were recorded. After the test, the change of shape of microneedles was inspected by microscopy to confirm all microneedles were pressed and broken across a row.

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Figure 3.11 Procedure of measuring needle failure force by Force-Displacement test station

3.1.5.4 Quantitative drug delivery test with drug through Franz diffusion cell

The transdermal model chosen to determine skin permeability consists of heat stripped human cadaver epidermis mounted in Franz diffusion chambers. This is one of standard models to measure the delivery rate of drug through membrane in transdermal drug delivery research (Bronaugh 1982). This transdermal model was previously used to show that solid silicon microneedles increase skin permeability to various drug models (McAllister 2000). A Franz diffusion chamber (Vertical diffusion cell – Franz cell; Permegear, Hellertown, PA) is shown in Figure 3.12. The upper compartment is the donor compartment and it contains a solution of drug molecules to be delivered across the
epidermis (area = 1.766 cm²) into the receptor compartment below. The skin interfaces with two compartments are greased to prevent leakage when the chamber is assembled.

The epidermis is pierced by microneedles before hydrating skin because Young's modulus of stratum corneum depends on hydration (Van Duzee 1978). The epidermis is placed on ten layers of tissue paper (Kimwipes) or on top of dermis. The polymeric microneedles are put on to the epidermis, pushed in and removed. Pieces of epidermis and support mesh (300 µm polypropylene woven screen cloth; Small Parts, Inc) are loaded into the diffusion chambers and both compartments are then filled with phosphate buffer saline (PBA; Sigma Chemical Corp.). The donor compartments are sealed and then the loaded chambers are stored overnight at 4 °C to hydrate the skin. The following day the chambers are placed on the multi-water-immersible stirrer in a 37 °C water bath (Immersible multi-stirrer, Cole Parmer; Vernon Hills, Illinois). The donor compartment is in contact with the stratum corneum side of the skin. The lower compartment is the receptor compartment and it contains 5 ml of well-stirred phosphate buffered saline. It is in contact with the underside of the viable epidermis. Calcein is used for model drug. The PBS solution in the donor compartment is replaced with a calcein solution with 1 mM concentration. We assume that steady state of concentration in a receptor compartment achieves within an hour. After 1 hour, a portion of the receptor solution is sampled. Calcein intensity in receptor is analyzed using a spectrofluorometer (QM-1; Photon Technology International, South Brunswick, NJ) and calcein concentration is interpreted from a calibration curve. The epidermis is removed and placed on a glass slide. The size of holes that microneedles make is then inspected under a fluorescent microscope (IX-7, Olympus) to calculate flow flux of model drug through epidermis.

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Figure 3.12 (a) Design of an unassembled vertically oriented Franz diffusion cell. (b) The chamber is assembled and mounted in a heating/stirring unit to maintain both compartments and the epidermis at body temperature (37 °C) and to keep the receptor well mixed.

3.2 Polymeric microneedles that encapsulate drug

Microneedles are also made to insert into skin and slowly release drugs from the needle matrix. Biodegradable polymeric microneedles can be loaded to encapsulate drug or protein, which is released either by bulk diffusion of the drug or upon hydrolytic degradation of the needle.

3.2.1 Microneedles fabrication

Spray dried, and homogenized drug particles (i.e., calcein or BSA) with 1 to 100 μm diameter are prepared to be encapsulated in microneedles.

The BSA microparticles are prepared by spray drying method. A Buchi mini spray dryer (Model 191, Buchi Laboratoriums-Technik, Flawil, Switzerland) is employed for the spray drying process. 10 % (w/w) of BSA solution is sprayed by spray-dryer to create
1 to 10 μm diameter distribution of BSA particles. Parameters for operation are 110 °C inlet temperature, 60 °C outlet temperature, 25 °C collector temperature, and 10 ml/min spray flow.

The calcein powder (Sigma), BSA powder (Sigma), and BSA conjugated with Texas Red® (Molecular Probes, Eugene, OR) are suspended in acetonitrile with 10 % solid content (w/w) (Aldrich Chemical Corp.) and then homogenized for 5 minutes under 10,000 rpm speed by homogenizer(Fisher PowerGen 700; Fisher Sci. Corp.) to make microparticles with the distribution of 1 to 100 μm diameter.

The homogenized particles are filtered to get 1 to 60 μm size distribution. 60 μm size filter (Nylon Net Filter 60 μm; Millipore, Billerica, MA) is placed between the glass funnel and base (All glass filter holder; Millipore) and then clamp funnels is in place on filter holders. Suspension solution is poured into the funnels sufficient to cover the membranes; approximately 10 ml. Next, the vacuum pump is turned on to draw the sample solution through the membrane and into the filtrate collection flask. Then, the vacuum pump is turned off after all solution is filtered. The funnels are unclamped and removed, and filter kit is placed in sterilized hood for holding while membranes are removed. After removing filter and funnel, the filtered solution in the collection flask is filtered through 0.5 μm isopore membrane (Millipore) by repeating above filtering process. The particle cake on the filter is dispersed again in the acetonitrile solution to have high solid content (over 20 %). The resulting organic suspension solution with drug particles is poured into a PDMS microneedle mold. The organic solvent is dried under vacuum leaving drug particles in the mold.

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The drug particle filling in the mold without suspension in organic solvent is prepared by another method. After filtration, the dried out particles are spread out on the PDMS mold instead of re-dispersing in organic solution. Next, the master structure of PDMS mold is applied on the drug cake layer on the PDMS mold and then pushed in and removed to force drug particles into the mold. The particles remaining on the surface of the PDMS mold are removed by adhesive tape (3M). The process is illustrated in Figure 3.13. Biodegradable polymer is filled in the mold by the process mentioned in Section 3.1.4.2.
Figure 3.13 Process for encapsulating model drug in the microneedles.
3.2.2 Sustained release formulation

Controlled release technology is an important tool for improving performance and safety of bioactive chemicals. Active materials are encapsulated to form barriers so that the active substance can be delivered at an optimum time and rate. Usually specially designed polymers are used to make these barriers. Microencapsulation is an important sub-category of controlled release technology. Active materials are encapsulated in micron-sized capsules of barrier polymers. These materials are designed to control the rate of release by virtue of the permeability or solubility. Two formulations of encapsulated drug are made for medium term release and long term release. Gel formulation based on carboxymethylcellulose (CMC) is for medium term release and microparticle formulation based on the poly (lactic acid) is for long term release. Short term release can be achieved by encapsulating drug without further additions.

3.2.2.1 Gel formulation using carboxymethylcellulose

Carboxymethylcellulose (CMC) has been used to control the release rate of drug (Lee 1997). 0.5 gram of carboxymethylcellulose sodium salt (400-800 cP, 2 % aqueous solution; Sigma Chemical Corp.) is dissolved in 9.6 ml of D.I. Water for 12 hours on 50 °C hot plate by 300 rpm stirring and then 25 mg calcine (Sigma Chemical Corp.) is dissolved in CMC solution by stirring to form 1:10 ratio (w/w) of calcine and CMC solution. The final clear solution is poured on aluminum foil and dried out to remove water for 6 hours under 15 inHg of vacuum. The resulting film is pulverized by agate mortar and pestle (VWR Inc.) to form particles (few hundreds μm to few mm size distribution). These particles are dispersed in acetonitrile (Aldrich Chemical Corp.) and
then the suspension solution is homogenized for 5 minutes at 15,000 rpm (Fisher PowerGen Homogenizers 700; Fisher Sci. Corp.). The homogenized particles are filtered to get 1 to 60 μm size distribution by using filtering method mentioned in Section 3.2.1. These particles are then loaded into the mold as described in Section 3.2.1.

3.2.2.2 Microsphere formulation using LPLA microspheres

Biodegradable microparticles work as micrometric reservoir systems by releasing drug out of microparticles using hydrolysis of biodegradable polymer. An emulsion method has been applied to the entrapment of drugs within microparticles composed of biodegradable polymer (Ogawa 1988; Benoit 1996; Crotts 1997).

As shown in Figure 3.14, to fabricate microparticles, solvent evaporation method is used (Benoit 1996). This technique is based on the evaporation of the internal phase of an emulsion by agitating. Initially, the biodegradable polymer is dissolved in a volatile organic solvent. The active drug or protein is then dispersed in the organic solution to form a suspension, or an emulsion. In the following step, the organic phase is emulsified under agitating in a water phase, which is immiscible with the organic solvent. Once the emulsion is stabilized, agitation is maintained and the solvent evaporates after diffusing through the water phase. The result is the creation of solid microparticles. On the completion of solvent evaporation process, the microspheres in water phase are recovered by filtration or centrifugation and are washed and dried (Watts 1990).

The procedure for the encapsulation of calcine and BSA is based on the above technique. 50 mg of calcine is dissolve in 15 ml DI Water and then 0.2 gram of poly (L-lactic acid) (LPLA, 1.0 dL/g; BPR) is dissolved in 2 ml methylene chloride (Aldrich
Chemical Co.). 200 μl of calcein solution is emulsified in 2 ml of LPLA solution for 2 minutes under homogenizing with 15,000 rpm speed (Fisher PowerGen Homogenizers 700; Fisher Sci. Corp.). The 2 ml of water-in-oil emulsion (calcein in LPLA) is emulsified in 50 ml of external aqueous phase (0.1 % polyvinyl alcohol solution) leading to a water-in-oil-in-water emulsion under 2 minutes of 10,000 rpm homogenizing. The multiple emulsion solution is agitated for 3 hours by 300 rpm of magnetic bar stirring to remove organic solvent and solidify microemulsion. The organic phase acts as a barrier between the two aqueous compartments preventing the diffusion of calcein toward the external aqueous phase. The solidified microparticles are filtered to get 1 to 60 μm size distribution by filter method mentioned in Section 3.2.1. 60 μm pore size filter (Millipore) is used to get microparticles below 60 μm diameter size. Finally, 1 to 60 μm size distribution of microparticles are recovered by filtering suspension solution in collected flask through 0.5 μm isopore membrane (Millipore). These particles are then loaded into the mold as described in Section 3.2.1.
Figure 3.14 The principle of the preparation of microparticles following the solvent evaporation technique.

3.2.3 Transport experiment procedure

3.2.3.1 Release test with encapsulated drugs in saline

As shown in Figure 3.15, 37 °C of water bath should be prepared for release test. The water submersible magnetic stirrer plate (VarioMag Multiposition stirrer, ColeParmer) is placed into the water bath container (Plexiglas Tank, ColeParmer). D1. Water is filled into the water bath to the desired level, which covers the heating element of the water bath. The temperature of the water bath is set to 37 °C using water heating circulator (Immersion Circulator, VWR Inc.), so that the glass thermometer reads a steady state value of 37 °C. Before attaching the microneedles in the vial, the number of
microneedles is counted using a light microscope (Olympus SZX12). The microneedle array with 100 to 200 needles are attached to the bottom or side of vial containing filtered PBS, pH 7.4. Glass vials, PBS and magnetic stirrer bars are autoclaved before starting release test. The vial is filled with the desired volume of PBS (10 ml for calcine release test, and BSA release test; 5 ml for microsphere release test). The drug concentration in the vial during release test must be higher than the minimum detectable value of the drug measurement assay. The concentration of calcine and fluorescence in sampled 100 μl solution is above 2x10⁻⁵ M because the sample is diluted in a 1:20 ratio with PBS and the concentration of the diluted sample should be placed within linear calibration curve range, 1x10⁻⁴ to 1x10⁻⁷ M. The vials are incubated in 37 °C water bath with 300 rpm stirring. 100 μl of release medium is sampled from each vial periodically and analyzed to determine the amount of drug by spectrofluorometer (QM-I; Photon Technology International) and 100 μl of PBS is added to sample to keep sample volume. Each experiment is carried out in triplicate.

**Figure 3.15 Apparatus for release test of model drug from microneedles.**

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3.2.3.2 Release test with encapsulated drugs into skin in-vitro

To demonstrate the dissolution and release of drug or protein in actual skin, a confocal microscope study is performed (Figure 3.16). Polymer microneedles with calcein are applied to full thickness cadaver skin, and the skin/needle assembly is placed in a hydration chamber at a temperature of 4 °C for 8 hours. Skin is stored under 4 °C to avoid water dehydration in skin and retard the diffusion of calcein in skin to distinguish each volume occupied by calcein released from a needle. After 8 hours, the needles are removed and the residual calcein on the surface is removed away with wet tissue paper. The skin is viewed by fluorescent microscope (Olympus IX-70) to determine if calcein goes in the skin. Then, a series of confocal microscope images are taken by a confocal microscope (LSM 510, Zeiss, Thornwood, NY) and it allows depth profiling of the released calcein to be performed.

Figure 3.16 A diagram of skin in-vitro release test of microneedles with calcein.
3.2.3.3 Protein stability

During encapsulation drug within microneedles, unacceptable denaturation and aggregation can be induced by numerous stresses to which a protein in aqueous solution is sensitive; e.g., heating, agitation, freezing, pH changes and exposure to interfaces (Bunce 2000). Encapsulating drug in microneedles is exposed to polymer melting process, thus protein is opened to thermal shock. The protein stability must be analyzed to optimize encapsulation process. Dynamic light scattering, circular dichroism (CD) and SDS-Poly-Acrylamide-Gel-Electrophoresis (PAGE) are used to characterize BSA aggregation (Bulone 2001).

500 mg of homogenized BSA (Sigma Chemical Co.) is dispersed in PLGA 50/50 solution (2.5 gram PLGA in 25 gram methylene chloride) and then suspension solution is poured on the aluminum foil to form polymer film encapsulating BSA particles. Methylene chloride is dried out for 5 hours under 20 inHg vacuuming in the hood. PLGA film containing BSA on the aluminum foil is cut by 3x3 cm square size. 5 square samples are put in the 140 °C oven and then put out sample at 0 min, 10 min, 20 min, 30 min, and 2 hours. Samples are dissolved in methylene chloride again to dissolve PLGA and the resulting BSA particles in methylene chloride are recovered by filtration and are washed with methylene chloride and dried. Collected BSA particles is dissolved in PBS to have 80 μg/ml concentration. Protein concentration is determined by Lowry protein assay method (Lowry 1951).

To perform the Lowry assay, 1 g of sodium citrate (Na_3C_6H_5O_7·2H_2O; Sigma Chemical Co.) and 0.5 g of cupric sulfate (CuSO_4·5H_2O; Sigma Chemical Co.) are
dissolved in 100 ml D.I. Water to prepare solution A. 20 g of sodium carbonate (Na₂CO₃; Sigma Chemical Co.) and 4 g of sodium hydroxide are dissolved in 1000 ml D.I. Water to prepare solution B. Solution C is prepared by mixing 1 part of solution A and 50 parts of solution B. 0.2 N of Folin reagent is prepared by mixing 10 ml of 2 N Folin reagent with 10 ml water to make solution D. Add 500 µl of sample (sample + buffer = 500 µl) per tube. Next, 2.5 ml solution C is added to each tube. Then, each tube is vortexed and let stand at room temperature for 10 minutes. 0.25 ml of solution D is added to each tube and then incubated for 30 minutes at room temperature. The color intensity is read at 750 nm in a spectrophotometer (DMS 300 UV/Visible spectrophotometer, VARIAN, Palo Alto, CA). Finally, the concentration is calculated from a standard curve.

Circular dichroism (CD) measurements are performed on a J700 (Jasco instrument, Easton, MD). Different forms of regular secondary structure found in peptides and proteins exhibit distinct far-UV (190 – 240 nm) CD spectra (Kelly 1997). The structural properties of BSA intermediates are probed by CD measurements in the far-UV range. The observed change indicates a decrease of the ratio of α-helix/β-sheet components (Woody 1996). The CD spectra of each of solution with thermally exposed BSA are measured using the parameters below: (wavelength : 400 – 190 nm, step size : 2 nm, scan rate : 200 nm/min, accumulations : 2, bandwidth:10 nm, sensitivity: 20 mdeg, response time : 2 seconds). Each spectrum is displayed in the Standard Analysis software.

Dynamic light scattering (DLS), which is also known as "photon correlation spectroscopy" (PCS) or "quasi-elastic light scattering" (QELS), uses the scattered light to measure the rate of diffusion of the protein particles. This motion data is conventionally processed to derive a size distribution for the sample, where the size is given by the
"Stokes radius" or "hydrodynamic radius" of the protein particle. This hydrodynamic size depends on both mass and shape (conformation). Dynamic scattering is particularly good at sensing the presence of very small amounts of aggregated protein (Berne 2000). The intermediate of BSA is inspected by dynamic light scattering (DLS) measurements (Bulone 2001). Data relative to each thermal exposed BSA (0 min, 10 min, 20 min and 30 min) solutions at 25 °C and 800 µg/ml concentration are analyzed using CONTIN to obtain size distribution of each sample.

To concentrate BSA solution, Amicon filtration kit (Centricron YM-3, cut-off 3,000 DA; Amicon, Beverly, MA) is used. Sample reservoir is inserted into filtrate vial. 2 ml of 80 µg/ml BSA solutions is subjected to the sample reservoir. Covered device and attached filtrate vial is place into the centrifuge rotor centrifuge (Spinchron 15-R, Beckman) and then centrifuged under 6400 rpm for 40 minutes until 800 µg/ml BSA solutions is achieved. Centrifugal filter assembly is removed from centrifuge and then filtrate vial is separated from membrane support base. The about 200 µl of resulting solution in the sample reservoir is collected and then the concentrated BSA solution is filtered through a 0.8 µm hydrophilic filter (Millipore). 45 µl of filtered solution is put in a quartz cuvette (QS 45 mm, path length 3 mm; Proteion Corp., Piscataway, NJ) and then the cuvette is place in the holder to measure the size distribution using a dynamic light scattering device (DynaPro-MS/X; Proteion Corp., Piscataway, NJ). Size distribution is displayed on the software, Dynamics V5 (Proteion Corp.).

SDS-PAGE is performed under non-reducing conditions using Bio-Rad Mini-PROTEAN II electrophoresis system (Bio-Rad, Hercules, CA). Discontinuous gels are prepared with stacking and separating gels of 4 and 12 % polyacrylamide, respectively.
Protein solutions are diluted with a Tris-Buffer (Sigma Chemical Corp.) containing 2% SDS. Electrophoresis of samples is performed at constant voltage for an hour using a Bio-Rad power supply in a Tris/glycine/SDS buffer. The gels are stained with a 0.1 % Coomassie blue fixative solution, destained with an aqueous solution of 40% methanol and 10% acetic acid for an hour, and then dried by a gel drying equipment (Model 583 Gel dryer, Bio-Rad). A series of prestained protein markers (Bio-Rad) are used as low molecular weight standards. The protocol for the protein stability test is summarized in Figure 3.17.

Figure 3.17 Procedure for measuring stability of protein by thermal exposure.

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3.2.3.4 Transdermal drug delivery test with encapsulated drugs hairless rat skin *in-vivo*

Arrays of PGA microneedles are used to deliver model drug to hairless rats. Prior to experimentations, the rats are anesthetized as described (Davis 2003). The *in vivo* skin models are anesthetized normal Sprague-Dawley hairless rats (280 – 410 g) by intraperitoneal injection of pentobarbital sodium (50 mg/kg). Hairless rats are purchased from Charles River and received in filter cages. Rats are anesthetized and placed in the prone position. The microneedle arrays used in the *in-vivo* rat experiment are 750 μm tall, and they have a base diameter of 200 μm, and a tip diameter 12 μm. A blue hydrophobic dye (Tissue marking blue dye; Shandon, Pittsburg, PA) is applied on the epidermis, and then the polymeric microneedles are put on to the rat’s skin through blue dye, pushed in and removed. At the end of experiments, the animal is euthanized with Beuthanasia (390 mg pentobarbital sodium and 50 mg phenytoin sodium per 1 ml, 1 ml / 10 lbs at BW IP, Schering-Plough Animal Heath).

The needle-inserted skin specimen is removed from the rat and embedded in Tissue-Tek O.C.T. (Sakura® Fine-technical Co. Ltd, Tokyo, Japan). The freezing block is filled with some Optimal Cutting Temperature (OCT) solution and then place the skin inside the solution vertically. Add more OCT into the block to cover all the skin surface. Slowly lower the freezing block so that its bottom surface has contact with liquid nitrogen (for 5 s); then raise it in the air for 5 s; keep repeating this until almost all OCT is frozen. Then place the freezing block inside the -72 °C freezer before taking it for cryostat cutting. The frozen block is sectioned by cryostat microtome (HM 560, Microm) and the
section of the block is investigated to define blue dye injection profile in rat skin by microscope (IX-7, Olympus).
4. Results

4.1 Microfabrication Results

The microfabrication methods described in Chapter 3 allows the fabrication of many different types of microneedles composed of a variety of materials. Solid microneedles have been fabricated from biocompatible and biodegradable polymers. This approach is performed by the following steps: (1) fabricating microneedle masters by micro-electromechanical masking and etching, or modification of light path, (2) preparing a PDMS (poly-di-methyl-siloxane) mold or metallic mold from the fabricated microneedle masters and (3) injection molding copies of the microneedles made out of poly-glycolic acid, poly-lactic acid, and poly-glycolic-co-lactic acid. These needles can be loaded with drugs and used as a controlled release drug delivery matrix. The fabrication process to make beveled biodegradable structures, lens techniques to form tapered structures, and the process for drug encapsulation in microneedles are novel and improve upon prior fabrication processes in regards to ease of fabrication, mechanical properties, cost effectiveness, and ease of insertion.

4.1.1 Polymer microneedles

Solid microneedles are of interest because they have been used to disrupt the stratum corneum barrier, thus rendering skin significantly more permeable. The micro-fabrication methods described in 3.1 allow the creation of biodegradable microneedle structures with various geometries. We were concerned that the polymer materials might not be strong enough for microneedles to insert into skin without breaking. We therefore wanted to
make needles with sharp, beveled tips and chisel tips to reduce the force required to pierce into skin. A tapered column structure is designed and fabricated to increase the critical failure force by possible failure modes. A micromolding technique is used to multiply replicate microneedles from a single PDMS mold.

4.1.1.1 Master structure formation

The master structures are fabricated using a micro-electro-mechanical-system (MEMS) process. The master structures are used to form PDMS molds which can be utilized conventional plastic replication process. The results of the process for fabricating beveled tips, chisel tips, and tapered column structures are discussed.

4.1.1.1.1 Beveled microneedle

Figure 4.1 shows scanning electron micrographs of a section of a beveled poly (glycolic acid) microneedle array at different magnifications and angles. They are fabricated as described in Section 3.1.1.1. The microneedles are 600 μm in height, 160 μm at their bases, and 10 μm at their tips. The needles are positioned in a 20 by 6 array with a center to center spacing of 400 μm and 1400 μm. An entire array occupies an area of 9 mm by 9 mm. The properties of a microneedles array, such as needle-to-needle spacing, needle diameter, base diameter, base shape, can be controlled by adjusting the size, shape and spacing of the masks. The needle height is controlled by the thickness of SU-8 casting and RIE etching parameters. The tip sharpness is controlled by the etching parameters.
Figure 4.1 Scanning electron microscope (SEM) photomicrographs of an array of solid PGA microneedles with increasing magnification (a) to (b) and with different angle view: Left-Front (a) and (b), Left-Back (c) and Top-Left (d).
Figure 4.2 shows an example of over-etched, beveled microneedles because the sample is exposed to plasma etching for long period of time. The microneedles are 250 μm in height after about 6 hours of plasma etching. The needle geometry is changed by over-exposure in the RIE. The etching profile of SU-8 by RIE is not vertically straight because etching occurs vertically and laterally as well. By increasing vacuum pressure, the etching profile can be close to anisotropic etching by increasing free path of ions. Anisotropic etching proceeds in all directions so that part of the SU-8 under the mask is etched away, giving rise to hillocks, shown in Figure 4.3. Non-uniformity across the sample is most likely a result of macroloading (Mogab 1977). Macroloading is the reduction of etch rate due to the depletion of reactive species. This is seen when a greater amount of substrate is used. The issue we are concerned with is the variation in etch rate across a single wafer. The greatest etch rate exists near the wafer’s edge, and it decreases radially inward across the wafer. The reactant becomes depleted because the surrounding polymer reacts away the reactants faster than they are replaced. The solution to overcome non-uniformity is PDMS film with few hundreds micrometer thickness. PDMS is much less etched by oxygen plasma in RIE (Yatsui 1971). When the piece of PDMS film aligns and cover on the part of sample, which is etched more than other region on sample, the exposed part is etched further by plasma and the covered part is not etched. By adjusting opening area of sample by PDMS film on sample, the uniformity cross the sample can be controlled.
Figure 4.2 (a) Scanning electron microscope image of front view of the over-etched beveled microneedles and (b) image of side view. The microneedles are 250 µm tall and etched more laterally.

Figure 4.3 SEM photomicrographs of an array of solid SU-8 microneedles with increasing magnification from (a) and (b). The microneedles are 600 mm in height, 100 mm at their bases. The etching profile by RIE shows isotropic pattern under low vacuum pressure.
4.1.1.1.2 Beveled microneedle with notch

Figure 4.4 shows scanning electron micrographs of a section of a beveled poly(glycolic acid) microneedle array with notches at different magnifications. They are fabricated as described in Section 3.1.1.2. The microneedles are 600 μm in height, 100 μm at their bases, and 5 μm at their tips. The needles are positioned in a 20 by 6 array with a center to center spacing of 400 μm and 1400 μm. An entire array occupies an area of 9 mm by 9 mm.

A different mask pattern with a notch in the circle is used to leave an empty space in the cylindrical structure. The 30 μm diameter notch in the circle is located along the outside wall of the cylindrical structure, as shown in Figure 4.4. These microneedles with notches are much easier to fabricate than hollow needles. The dimension of the notch can be controlled by adjusting the size and geometry of the notch in the circle of the mask.

Figure 4.4 Scanning electron microscope (SEM) photomicrographs of an array of solid PGA microneedles with notch increasing magnification from (a) to (b).
4.1.1.3 Chisel tip microneedle

The chisel tip microneedles have been fabricated, as described in Section 3.1.2.1. The chisel tip parts with micropyramid shape are first fabricated and then column parts are fabricated on the micropyramid grooves. Figure 4.5(a) shows a scanning electron microscope picture of an array of plastic micropyramids made of PDMS from the micropyramids in etched silicon and Figure 4.5(b) shows a scanning electron microscope micrograph of an array of polyurethane microneedles. Each needle in Figure 4.5(b) is 570 μm in height and has a 100 μm in base width. The needles are arranged in a 15 by 15 array with 600 μm center to center spacing. The square openings, aligned with the (111) directions on the (100) wafer surface, result in a pit with well defined (111) sidewalls at angles 54.74 ° to the surface (height = width/√2). Figure 4.5(a) shows PDMS micropyramids copied from the silicon wafer and they have 100 μm width and 70 μm height.

Figure 4.5 Scanning electron micrograph images of: (a) an array of PDMS micropyramid structure from silicon mold; (b) a portion of an array of chisel tip microneedles.
4.1.1.4 Tapered column microneedles using integrated lens technique

As explained in Section 3.1.3.1, the tapered column structures are fabricated using integrated lens technique. The microlenses are fabricated on a glass substrate by wet etching and then tapered SU-8 structures are fabricated by exposing UV to SU-8 through lenses. Figure 4.6(a) shows an example of lenses etched by HCl-HF glass etchant for three hours. To ascertain the shape of the lens for subsequent ray-tracing analysis, a polydimethylsiloxane (PDMS) copy of the lens is made and is shown in Figure 4.6(b). The resulting round cavities have 200 µm diameter, 70 µm height and spacing of 400 µm between structures. The HF based etches usually result in a rough surface, but the recipe consisting of 10 % HCl, 5 % (Buffered Oxide Etchant) BOE and 85 % D.I.Water provides a smooth surface (Becker 1998).

![Figure 4.6 Scanning electron micrograph images of: (a) a portion of an array of convex glass lenses; (b) an array of PDMS concave lenses copied from the glass lenses.](image)

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Due to the chromium layer on the glass, the substrate is opaque except in the lens regions. The light passes through the lens area to give a latent image in the SU-8 on the uneven glass substrate as a ray trace of the convex lens. Development leaves a tapered, cone-shape pattern (in the case of a circular lens). The geometry of the resulting cones could be controlled by controlling the lens diameter and curvature, as well as the refractive indices of the substrate and photoresist. The pattern can be predicted by a straightforward analytic calculation or by using a ray-tracing software (IME software).

The geometry of the tapered structure is affected by the lens geometry. Long isotropic etch times can result in significant lateral underetch of the chromium, and subsequent chromium overhang. If this overhang is not removed, the light passes through only the original opening area in the chromium to form a shallow tapered structure according to the lens curvature. In contrast, removal of the chromium overhang results in structures with wider bottoms and increased angle. Figure 4.7 shows tapered microstructures created using the lens arrays of Figure 4.6(a). 1 mm thick SU-8 is applied to glass substrate with lenses and then fabricated as in Section 3.1.3.1. In the case of keeping the overhanging chromium, the microneedles are 1.197 mm height, 106 μm at their bases, and 3 μm at their tips. If the overhanging is removed, they have 1.197 mm height, 200 μm at their bases, and 30 μm at their tips. The needles are positioned in a 20 by 10 array with a center to center spacing of 400 μm and 800 μm. An entire array occupies an area of 9 mm by 9 mm. In order to show a theoretical ray trace, we simulate optical ray tracing using a ray trace simulator (IME software) with different lens openings in Figure 4.8. The refractive indices for the simulation of SU-8 and glass are 1.7 and 1.51, respectively. The light source used is 365 nm of wavelength (i-line). The radius of the curvature for the
microlens is 100 µm and the opening diameters are 100 µm, 150 µm, and 200 µm from the left to right in Figure 4.8, respectively.

Figure 4.9 shows different lenses fabricated with various etching times and the resultant tapered microstructure array in each case. Longer etching times yielded larger radii of curvature, which resulted in structures with longer focal lengths, as expected.

The resultant etching lengths of the microlens according to various etching times are summarized in Figure 4.10. Resulting change of length of the microlens is measured based on the original diameter of 100 µm according to various etching time. The lateral etching rate is 0.23 µm/min and vertical etching rate is around 0.37 µm/min from the Figure 4.10. The etching rate is not linear because contact area is increasing when etching time increases.

Also simulation results are shown in Figure 4.11 with two different radii of the curvatures. This simulates the size effect resulting from different etching time and the result of two different radii is matched with the geometry of structure shown in Figure 4.9.
Figure 4.7 Scanning electron micrograph images of a SU-8 tapered micro structure array.

Figure 4.8 Ray-tracing simulation with different lens opening.
Figure 4.9 Scanning electron micrograph images of glass lenses and the resulting SU-8 tapered structures.
Figure 4.10 Resulting diameter and depth of the microtuss with original diameter of 100 \( \mu m \) according to various etching time.

Figure 4.11 Ray-tracing simulation with different radii of the lens curvature.
To demonstrate the ability to create high aspect ratio of structures, the microneedles with 1.5 mm in length are fabricated using lens technique and then PGA needles are copied from PDMS mold. Microneedles for insertion into soft tissues (such as skin) for applications such as body fluid or blood extraction should have sufficient height to overcome the flexible deformation of the skin (Davis 2003). The master structures with long length are fabricated using the integrated lens technique. An array of 200 microneedles is prepared by the above-described process. Each needle had a bottom diameter of 200 μm, tip diameter of 20 μm and height of 1.5 mm (Figure 4.12).

Figure 4.12 Scanning electron micrograph images of a portion of an array of biodegradable polymeric microneedles.

The effect of stirring on etching rate is investigated. The glass substrate with 100 μm circular opening pattern is immersed in the described etchant for 2.5 hours without stirring and with 300 rpm stirring. Figure 4.13 shows that the diameter of microlens
opening without stirring is 170 \( \mu m \) and that of microlens openings with stirring is 190 \( \mu m \). The lateral etching rate is changed from 0.23 \( \mu m/\text{min} \) to 0.3 \( \mu m/\text{min} \) by stirring.

![Figure 4.13 Scanning electron micrograph images of an array of PDMS concave lenses copied from the glass lens (a) without stirring (b) 300 rpm stirring.](image)

Figure 4.14 shows an inclined column array exposed through microlenses with overhanging for 3 hours etching. The substrate is exposed at 45 degree of inclination from horizontal plane in order to form the inclined structure. The resulting inclined-tapered-columns with base diameter 150 \( \mu m \) and 70 \( \mu m \) tip diameter have been obtained. The inclined-tapered columns can be used to fabricate breakable needles after skin insertion.
4.1.1.5 Tapered column microneedles using backside exposure through

In addition to integrated lens technique, tapered columns have been fabricated using UV exposure through the glass mask, as previously described in Section 3.1.3.2.

In proximity printing, spacing of the mask away from the substrate makes the diffraction of the transmitted light that reduces the resolution (Madou 2002). The degree of reduction in resolution and image distortion depends on the gap between the mask and the photoresist surface.

\[ R = \frac{b_{\text{mask}}}{2} \sqrt{\frac{\lambda}{d}} \quad (4.1) \]

where \( R \) is theoretical resolution, \( b_{\text{mask}} \) is half the grating period and the minimum feature size transferable, \( s \) is gap between the mask and the photoresist surface, and \( \lambda \) is wavelength of the exposing radiation. The distorted UV is exposed on the 700 \( \mu \)m thick SU-8 using 365 nm exposing radiation through glass. The tapered structures with 200 \( \mu \)m
base width are formed through a glass mask with 100 μm width square openings and 1.52 mm thickness. The light distribution profiles on the photoresist surface after light passes through a glass mask as shown in Figure 4.15. Light profile on the SU-8 makes tapered structures.

![Diagram](image1)

**Figure 4.15 (a)** Light distribution profile on a photoresist surface after light passes through a mask containing an equal line and space grating (Willson 1994) (b) applying proximity printing technique to UV backside exposure on SU-8 through glass substrate.

Figure 4.16 shows a scanning electron microscope (SEM) micrograph of PGA microneedles copied from PDMS mold. The needle is 700 μm high and has a 200 μm
base diameter and 50 μm tip diameter. The needles are arranged as 15 by 15 arrays with 600 μm center to center spacing.

![Image](image.png)

Figure 4.16 Scanning electron micrograph images of an array of tapered columns with square cross section.

4.1.1.2 Copy structure formation

PDMS molds can be massively prepared from master structures and then copy structures can be replicated from PDMS molds using micromolding process. In this chapter, preparation of PDMS molds from master structures and replication of biodegradable copy structures from PDMS molds are discussed.

4.1.1.2.1 Mold formation

PDMS molds are made by casting molds from master structures (Sections 3.1.4). PDMS is able to create accurate molds of the silicon masters. For example, silicon

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microneedles fabricated using the black silicon method are shown in Figure 4.17 (a). A PDMS micromold prepared from silicon needle is shown in Figure 4.17 (b). This shows the PDMS mold can release silicon needles without breaking. In addition electroplated nickel structures with 300 mm in height and 15:1 aspect ratio are copied by PDMS (Kim 2002). This shows PDMS mold can be applied to copy other material structures too.

Figure 4.17 Scanning electron micrograph images of: (a) a portion of an array of silicon microneedles; (b) an array of PDMS mold from silicon microneedles.

Figure 4.18 shows an individual cavity of beveled microneedle. PDMS can copy SU-8 structures with 600 μm in height, 100 μm at their bases, and 10 μm at their tips, and PDMS mold can release high aspect ratio of SU-8 structures without breaking master structures. PDMS has good properties that make it suitable as mold material because it (a) has a low surface energy (21.6 dyne/cm), (b) is typically chemical inert, (c) is non-
hygroscopic, (d) has good thermal stability, (e) is optical transparent down to ~300 nm, (f) is durable and, (h) is an elastomer with $5 \times 10^6$ MPa Young’s modulus (Madou 2002).

Figure 4.18 Scanning electron micrograph image of cross section of microneedle cavity in PDMS mold from beveled microneedle.

4.1.1.2.2 Injection molding and demolding

As described in Section 3.1.4.1 and Section 3.1.4.2, a different modified injection molding method is introduced by applying vacuum instead of using high pressure. After air is removed from the micro cavities by vacuuming, the polymer melt occupies the empty cavities in which air used to be. The process is accomplished in the constant temperature oven, so the mold can be kept above melting temperature of polymer in the oven. It prevents the polymer from freezing before completely filling the mold. This is one of main problems of molding methods for microfabrication (McAllister 2000).

Figure 4.19(a) shows the PLGA enters the base of microneedle cavities, but they don’t fill the upper portions of needle mold without vacuuming and Figure 4.19(b) shows
entire structures that copied from whole portion of mold with 20 in Hg vacuuming. As shown in Figure 4.19(b), high aspect ratio of PLGA structures are copied from PDMS mold and release from mold without breaking.

Figure 4.19 Scanning electron micrograph images of: (a) a portion of an array of partially copied microneedles from mold without vacuuming; (b) a portion of an array of completely copied microneedles from mold under vacuum.

4.1.2 Polymer microneedles that encapsulate drug

Two model drugs, calcein and BSA, are encapsulated in microneedles for sustained release formulation. To further control the release pattern of calcein, a gel formulation and a microsphere formulation are introduced.

4.1.2.1 Drug encapsulation

To show the release of drug from microneedles, we first encapsulated drug (i.e., calcein and BSA) inside polymer microneedles made of PLGA. To retard the release of drug from microneedles, drug (i.e., calcein) is particularized with carboxymethylcellulose (CMC) and then encapsulated in polymer microneedles. As described in Section 3.2.1,
homogenized calcein particles with 1 – 30 μm size distribution were dispersed in acetonitrile and its suspension solution is poured in a microneedle PDMS mold and then dried under vacuum. After removing surface residual calcein, PLGA 50/50 powder is melted under vacuum to form PLGA microneedles with encapsulated calcein. Figure 4.20 shows optical microscopic images of beveled polymeric microneedles made of poly-lactic co-glycolic acid containing calcein. The microneedles encapsulating calcein were copied from PDMS mold of beveled tip microneedles. The needles are positioned in a 20 by 6 array with a center to center spacing of 400 μm in one direction and 1400 μm in the perpendicular direction. Most of the calcein is located near tip of needles and last of calcein is placed along the surface of cylindrical structures. This is because calcein is placed at the tip of microneedle cavities in a mold and along the wall of microneedle cavities when calcein particles is put into the mold cavities. 1 to 10 percent of contents of calcein are encapsulated in an array of microneedles and an array of 100 microneedle with 1 μg of calcein is fabricated for release test.

Figure 4.20 Photographic images of a portion of an array of PLGA 50/50 microneedles containing calcein.
Figure 4.21(a) shows microneedles with CMC-calcein (10:1 ratio) particles. The solution of CMC and calcein is poured on the aluminum foil and then dried out to get thin film of CMC and calcein. The film is pulverized and then homogenized as described in Section 3.2.2.1. Compared to other encapsulating solid particles like calcein and BSA, CMC-calcein particles are in form of micro-thin films, thus some of them are located close to the center of microneedles in addition to near surface because CMC-calcein film is less dense than calcein particle.

Figure 4.21(b) shows optical photomicrographs of a portion of an array of PLGA 50/50 microneedles containing BSA at their tips and SEM images of them (Figure 4.21(c), and (d)). The microneedles are prepared as described in Section 3.2.1. The needles are copied from a mold for beveled microneedles with notch. The optical microscope image shows that BSA particles are dispersed in microneedles (Figure 4.21(b)) but surface of microneedles are covered by biodegradable polymer as shown in Figure 4.21(d), so we can’t find BSA particles on the surface. Various contents of drug can be encapsulated in microneedles, and among them 1 μg of calcein with CMC and 1 μg of BSA, encapsulated in an array of 100 microneedles respectively are prepared for release test described in Section 3.2.1.
Figure 4.21  Photographic images of: (a) a portion of an array of PLGA 50/50 microneedles containing calcine-CMC; (b) a portion of an array of PLGA 50/50 microneedles containing BSA; (c) and (d) Scanning electron microscope (SEM) photomicrographs of an array of PLGA 50/50 microneedles with BSA.
4.1.2.2 Microsphere encapsulation

To further control release from microneedles, we first encapsulated drug (i.e., calcein) inside polymer microspheres made of PLA and then encapsulated the microspheres inside polymer microneedles made of PLGA. Poly (L-lactic acid) (LPLA) microspheres containing calcein are spherical in shape and polydisperse in size as shown in Figure 4.22(a). The diameter of these microspheres varies from 1 μm to 30 μm. Microspheres loaded about 5 to 10% calcein are used for making microneedles. Figure 4.22(b) shows typical population of these microspheres.

Figure 4.22(c) shows the optical photomicrographs of a portion of an array of PLGA 50/50 beveled microneedles containing microspheres with calcein, which are 600 μm in height and 100 μm diameter in base. Most microspheres are placed near the tip of the microneedles similar to previous microneedles with calcein only and CMC-calcein. To investigate the inside of microneedles the tip of a microneedle is sectioned as shown in Figure 4.22(d). The outside of needles is covered by thin polymer layer and the microspheres are encapsulated in the polymer microneedles without change of the shape of microspheres. An array of 100 microneedles with about 0.1 μg of calcein was prepared for release test as described in Section 3.2.2.2. If the polymer melt does not enough penetrated fully between microspheres, the cavity of microneedle mold is not fully filled by polymer melt and the microspheres are exposed outside microneedles, as shown in Figure 4.22(e). If polymer layer dose not cover the outside of microneedles, the shape of microneedles with microspheres is deformed while demolding microneedles out of PDMS mold.

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Figure 4.22 (a) Optical microscope image of microparticles made of LPLA containing calcein; (b) SEM image of microparticles with calcein; (c) Optical microscope image of an array of microneedles with calcein-encapsulating microparticles; (d) SEM image of a cross-section of the tip of a sectioned microneedle with microparticles; (e) SEM image of a partially copied microneedle with microparticles.
4.2 Insertion and failure force results

The force of insertion data and the force of failure data give insight into the mechanical behavior of microneedles and skin. To optimize the geometry of polymer microneedles and the type of polymer, two constraints governed by insertion force and failure force should be measured and predicted. Force of insertion of microneedle into human skin and failure force are discussed in this chapter.

4.2.1 Force of insertion

The relationship between insertion force and needle geometry is characterized to get information for design of microneedles. The methods to determine insertion force is described in Section 3.1.5.2. The force of insertion is determined by pressing microneedles against the skin of human subject and measuring the corresponding force upon insertion. The insertion is identified by a sudden decrease in resistance of an electrical circuit formed in the skin between the microneedle and a counter electrode. The decrease in resistance corresponds to the shorting of the resistance of the stratum corneum (Davis 2003).

4.2.1.1 Measuring force of insertion

To determine the force required to insert microneedles into skin, we measure forces of microneedle insertion into human skin as a function of contact area between the needle and skin. To determine force of insertion, a single metal coated polymer needle is pressed into the skin while continuously measuring needle force and displacement, as well as skin resistance. The minute size of microneedles and the deflection of skin around
microneedles during insertion make visual determination of insertion difficult. Instead, the electrical properties of the skin are monitored to determine penetration into the skin. Section 3.1.5.2 describes the method to determine the insertion force in detail. Polymer needles are not electroconductive. Because electrically conductive needles are needed to measure insertion, polymer needles were coated with nickel of 5 μm thickness for electro-conductivity as shown in Figure 4.23.

Figure 4.23 SEM image of metal coated microneedle.

Figure 4.24 shows typical data collected during the insertion of a microneedle into skin on the hand of a human subject. As a needle is pressed against the skin, the resistance of the needle-skin circuit decreased and then leveled off as the needle came into contact with the skin and deflected it. Upon insertion, skin resistance sharply dropped. A discontinuity in the force curve is difficult to find. The resistance measurement is preferred to identify when needle insertion occurs. Using this method, an insertion force of 0.036, 0.14 N and 0.27N were measured for needles with 341 μm², 1256 μm² and 2000 μm² interfacial area as shown in Figure 4.25
Figure 4.24 Insertion test of an array of three microneedles made of PLGA with 5 μm thick metal coating.

4.2.1.2 Modeling force of insertion

Insertion force was measured for microneedles with various tip diameter of microneedles (Davis 2003). The force of 0.8 N and the force of 1.29 N were measured for 55 μm and 115 μm diameter microneedle respectively and the microneedle insertion force was expressed by function of the tip area. The insertion force is measured based on the tip area and its relationship is shown in Figure 4.25. The quantitative relation between tip area and insertion force was calculated from Figure 4.25 and the result, equation 4.2 is compared with Davis’s data (Data is not shown) (Davis 2003) which were measured with identical force-displacement stage and same operating condition. The insertion force for

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small interfacial area between 200 to 5000 $\mu m^2$ based on experimental values and Davis's data can be expressed as equation 4.2.

$$F_i (N) = 0.00012 \times A (\mu m^2)$$  \hspace{1cm} (4.2)

where $F_i$ is an insertion force with a unit of $N$ and $A$ is the full cross-sectional area of the needle with a unit of $\mu m^2$. Equation 4.2 suggests that insertion force is linearly dependent on interfacial area for range of 200 to 5000 $\mu m^2$ interfacial area.

Figure 4.25 Insertion force has a linear dependence on interfacial area of microneedle.

4.2.2 Force of failure

In order to guarantee insertion of microneedles into skin, it is necessary to characterize the relationship between needle failure force and microneedle geometry and polymer type and thereby identify needle designs and minimum polymer strength that insert into skin at forces less than those which cause needle failure.
4.2.2.1 Measuring force of failure by axial force

Microneedle failure force is measured using compression failure tests. Section 3.1.5.3.1 describes the methods used to measure microneedle failure force. The typical mechanical behavior of microneedles is shown in Figure 4.26. Polymer needles are pressed at constant velocity into a hard metal surface while continuously measuring needle force and displacement. The applied force is divided by the number of microneedles and finally the force applied to a needle and its displacement is calculated. Microneedle failure is indicated by the drop in applied force and confirmed by visual observation during the test. After their first failure, the microneedles continue to move down against the hard surface initially recording a load lower than the ultimate load.

![Diagram](image)

Figure 4.26 Typical failure behavior of microneedles under axial load. The decreasing marks the failure of the microneedles.

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Figure 4.27 shows the variation of microneedle failure force with needle length. Three geometries of microneedles were prepared with different length, (0.7 mm, 1 mm and 1.5 mm) and the same tip diameter (25 μm) and base diameter (200 μm). Failure force decreases with increasing needle length as shown in Figure 4.27 (ANOVA, p<0.001). The failure forces for needles with three kinds of lengths are higher than the insertion force, 0.058 N, for 490 μm² of interfacial area. As shown in Table 4.1, safety factor defined by equation 4.2 between insertion force and failure force decreases by increasing the length of microneedle and there is small safety margin (n-1) between insertion force and failure force for a 1.5 mm tall microneedle.

Safety factor (n) = Actual failure force / Insertion force

Table 4.1 Safety factor of microneedle with 25 μm tip diameter and 200 μm base diameter regarding length of microneedle

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Insertion force (N)</th>
<th>Failure force (N)</th>
<th>Safety factor (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7</td>
<td>0.058</td>
<td>0.22</td>
<td>3.79</td>
</tr>
<tr>
<td>1.0</td>
<td>0.058</td>
<td>0.16</td>
<td>2.75</td>
</tr>
<tr>
<td>1.5</td>
<td>0.058</td>
<td>0.098</td>
<td>1.68</td>
</tr>
</tbody>
</table>
Figure 4.27 Effect of the length of microneedles on needle failure (200 μm base diameter, 25 μm tip diameter, PLGA 50/50).

Figure 4.28 provides the effect of base diameter on the failure force. Two geometries of microneedles were prepared with different base diameters, 100 μm and 200 μm, and same tip diameter of 25 μm and length of 700 μm. The failure force of the needle with 200 μm base diameter is 0.203 ± 0.014 and that of the 100 μm base diameter is 0.088 ± 0.024 N. This shows that the base diameter is a critical parameter to determine the failure force. When the microneedles are inserted into skin or on the deformed skin, the axial force or transverse force is applied to the middle of microneedle instead of tip. Thus, the increase of base diameter increases the bending failure of microneedles as needles insert into skin.
The effects of Young's modulus and yield stress on the failure force of polymer needles with 25 μm tip diameter, 200 μm base diameter and 1 mm in length are investigated using different polymer material and the result is shown in Figure 4.29. PGA have better mechanical strength with 10 GPa Young's modulus and 90 MPa yield strength compared to PLA with 5 GPa of Young's modulus and 70 MPa of yield strength, high molecular weight of PLGA with 3 GPa of Young's modulus and 50 MPa of yield strength, and low molecular weight of PLGA with 1 GPa of Young's modulus and 30 MPa of yield strength (Zhang 1995; BPI 2003). As expected, the polymer with better mechanical properties including Young's modulus and yield stress shows higher failure force. When a microneedle is 25 μm in tip diameter, 200 μm in base diameter and 1 mm
in length, three polymer types, PLGA 50/50 with high molecular weight, PLA and PGA, shows stable safety margin as shown in Table 4.2.

To further investigate the effect of intrinsic polymer mechanical properties, the failure force of two different molecular weight of PLGA (0.39 dL/g and 1.2 dL/g) was measured. Figure 4.29 provides the effect of molecular weight of PLGA 50/50 on failure force. There is relationship between molecular weight of polymer and failure force (ANOVA, p=0.001). At low molecular weight of PLGA 50/50, the failure force is close to the insertion force, thus it should not be used for fabrication of microneedles.

Table 4.2 Safety factor of microneedle with 25 μm tip diameter, 200 μm base diameter and 1 mm in length

<table>
<thead>
<tr>
<th>Polymer</th>
<th>E (GPa)</th>
<th>Sy (MPa)</th>
<th>Insertion Force (N)</th>
<th>Failure force (N)</th>
<th>Safety factor (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGA</td>
<td>10</td>
<td>90</td>
<td>0.058</td>
<td>0.314</td>
<td>5.4</td>
</tr>
<tr>
<td>L-PLA</td>
<td>5</td>
<td>60</td>
<td>0.058</td>
<td>0.206</td>
<td>3.5</td>
</tr>
<tr>
<td>PLGA 50/50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1.2 dL/g)</td>
<td>3</td>
<td>50</td>
<td>0.058</td>
<td>0.163</td>
<td>2.8</td>
</tr>
<tr>
<td>PLGA 50/50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.39 dL/g)</td>
<td>1</td>
<td>30</td>
<td>0.058</td>
<td>0.063</td>
<td>1.08</td>
</tr>
</tbody>
</table>

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Figure 4.29 Effect of Young's modulus on the failure force of a microneedle with 25 μm tip diameter, 200 μm base diameter and 1 mm in length.

To explore another aspect of needle material mechanical properties, the effect of calcein content on failure force was investigated. Failure forces of arrays of 100 microneedles with 1 μg calcein and 5 μg calcein are measured as shown in Figure 4.30. The failure force of PLGA 50/50 microneedles with 1 μg calcein is 0.0916 ± 0.0307 N and that of the needles with 5 μg is 0.4 ± 0.2 N which is lower than the insertion force. Calcein content has an effect on the failure force of microneedles (ANOVA, p=0.004). Calcein distribution is not uniform across the microneedle array and most of calcein is
placed in the tip of the needles. Failure probably happens at the interface between calcein and polymer mainly, thus adhesion between drug particles and polymer can be an important factor which determines failure force of microneedles with drug inside.

![Graph showing failure force vs. calcein content](image)

**Figure 4.30** Effect of calcein content in a microneedle of a microneedle with 25 μm tip diameter, 200 μm base diameter and 1 mm in length on the failure force.

4.2.2.2 Measuring force of failure by lateral force

The failure force by a transverse load is measured using a force-displacement station. Section 3.1.5.3.2 describes the methods used to measure transverse failure force. Typical failure behavior of a microneedle is shown in Figure 4.31. The tips of polymer needles
are pressed by the glass slide surface laterally while continuously measuring needle force and displacement. The applied force is divided by the number of microneedles and finally the force applying to a glass slide and displacement are calculated. Microneedle failure is indicated by the dramatically drop in applied force and confirmed by visual observation during the test.

The transverse failure force of PGA microneedles with 25 µm in tip diameter, 1 mm in length and 100 µm and 200 µm in base diameter is measured as shown in Figure 4.32 and the averaged maximum failure force is 0.058 ± 0.012 N and 0.245 ± 0.058 N respectively. The failure force by transverse load for 200 µm base diameter of PGA needle is smaller than or similar to 0.31 N of failure force of PGA microneedles with same geometries by axial load. This shows that the transverse bending is one of the critical failure modes. Even if needles are intend to be inserted vertically, and the following transverse load by deformed skin is smaller than the axial load, the needles can experience a significant transverse load due to misplacement of needles on the skin or skin deformation by microneedles.
Figure 4.31 Typical failure behaviors of microneedles under transverse load. The rapid decreasing marks the failure of the microneedles.

Figure 4.32 Effect of the base diameter of a microneedle with 25 μm tip diameter and 0.7 mm length on transverse failure force.
4.2.2.2 Modeling force of failure

Two possible failure modes of microneedles are considered including axial load and transverse load. We assume that the applied force is acting parallel to the microneedle axis. In the case of an axial load on the column structure, an analytical solution based on failure due to buckling caused by inelastic or elastic instability of the structure is developed. To predict the force required for needle failure due to buckling, the transition slenderness ratio, \( C_r \), defined by equation 4.4, should be determined.

\[
C_r = \sqrt{\frac{2\pi^2 E}{S_y}} \tag{4.4}
\]

where \( E \) is the Young’s modulus of the material, and \( S_y \) is yield strength of the material.

Then, the slenderness ratio of a column is computed from equation 4.5

\[
SR = \frac{L_e}{r_e} \tag{4.5}
\]

where the effective length, \( L_e \), and radius of gyration, \( r_e \), for a solid circular sectional column are defined as equation 4.6 and equation 4.7, respectively (Mott 1996).

\[
L_e = K \cdot L \tag{4.6}
\]

\[
r_e = \frac{D}{4} \tag{4.7}
\]

where \( L \) is the actual length of the column, \( K \) is the end-fixity factor, and \( D \) is a diameter of circular section. The end-fixity factor is a measure of the degree to which each end column is restrained against rotation. The value of \( K \) is 1.0, 0.7, and 0.5 for a pinned-pinned column, a fixed-pinned column and a fixed-fixed column respectively. The fixed column means that there is no vertical displacement or rotation and the pinned column means that vertical displacement is not allowed but node is free to rotation (Mott 1996).
For microneedle mechanical analysis, the end-fixity factors from $K=0.5$ to $K=1$ are scanned, because the needle bases are fixed to a support and the tips of needles are not allowed to rotate in any direction about any axis due to the friction with contacting surface.

If the actual slenderness ratio, $L_0/r_g$ is greater than the column constant, $C_C$, then the column is "long," and the Euler formula, defined in equation 4.8, should be used (Mott 1996) to predict the critical load, $P_{cr}$, at which the column would be expected to buckle.

$$P_{cr} = \frac{\pi^2 EI}{L_0^2} = \frac{\pi^2 EA}{(L_0/r_g)^2}$$  \hspace{1cm} (4.8)

$$I = \frac{\pi D^4}{64}$$  \hspace{1cm} (4.9)

where $I$ is moment of inertia for a circular section (equation 4.9), $D$ is diameter of the circular section and $A$ is area of the circular section.

As the column is short (i.e. $L_0/r_g \leq C_C$), it exhibit a tendency to fail at loads less than predicted value using the Euler formula. This has led to the development of a companion expression to properly account for failure in the intermediate region. This parabolic equation was first suggested by J.B. Johnson and the point at which the Euler formula is shifted to the Johnson formula is the column constant, $C_C$ (Mott 1996). If the slenderness ratio, $KL/r_g$ is less than column constant, $C_C$, and the column is short, the Johnson formula, equation 4.10 should be used (Pilkey 1989).

$$P_{cr} = AS \left[ 1 - \frac{S(L_0/r_g)^2}{4\pi^2 E} \right]$$  \hspace{1cm} (4.10)

The surface area of tapered column is varying with position along the column. The radius of gyration for a tapered column is determined by calculating the equivalent
diameter of the tapered column. The equivalent diameter of a linearly tapered column can be calculated from equation 4.11 to analyze a tapered member (OSC 2001). This equivalent value is used for computing the slenderness ratio, and the critical load from the Euler formula and the Johnson formula.

\[ D_{\text{equivalent}} = D_{tip} + \left( \frac{D_{tip} - D_{base}}{2} \right) \]  \hspace{1cm} (4.11)

For example, for a tapered column with a 25 \( \mu \text{m} \) tip diameter and a 200 \( \mu \text{m} \) base diameter, the equivalent diameter is 83.3 \( \mu \text{m} \). The slenderness ratio, \( \frac{L}{r_p} \), is 29.7 for fixed ends of a sway column (K=0.62) with 1 mm length and 83.3 \( \mu \text{m} \) equivalent diameter from equation 4.5. If a column is made of PLGA with a 3 GPa Young’s modulus and 50 MPa yield stress, the column constant is 34.4 from equation 4.4. Since the calculated slenderness ratio of 29.7 is less than the column constant of 34.4, the Johnson formula equation 4.10 should be used to predict a critical load, \( P_c \). If a column is a PLGA tapered column with 25 \( \mu \text{m} \) tip diameter, 200 \( \mu \text{m} \) base diameter, \( K \) of 0.62 and length of 1.5 mm, the slenderness ratio of 44.6 is greater than column constant of 34.4. Thus, the Euler formula, equation 4.8 should be used to calculate the critical load for failure.

There might be discrepancies between the theoretical critical load and measured data, because reading geometries from SEM figures for the calculation contains error, because we make assumptions that the column is tapered linearly, and because the needle restrains the movement of the needle at its ends.

The equivalent diameter is a critical factor to estimate the failure force from equation 4.8 and 4.10 and the equivalent diameter is a function of tip diameter and base diameter as shown in equation 4.11. Thus, an error in reading geometry from SEM figures causes
an error in the equivalent diameter and the estimated critical load. Microneedles have the same base diameter but different tip diameters. In order to reduce reading error of tip diameter, the averaged tip diameter from several samples is used to calculate the critical loads.

Another factor that complicates analysis is nonlinearity of tapered structures. As shown in Figure 4.33, the column is tapered nonlinearly, which may make an increase in experimental failure force compared with the calculated force by the Euler formula and the Johnson formula. The recognition of nonlinearity is very important because the geometry difference changes the failure force estimation. There is a difference in volume between the theoretical tapered column and an actual microneedle structure. Images of microneedles are imported from SEM figures (Figure 4.33(a)) and three dimensional images based on these images are drawn using AutoCAD (Figure 4.33(b)). Then, the volume of each structure is calculated (Figure 4.33(c)). This measurement from SEM figures is used to adjust the estimated values from the ideal assumption that structures are linearly tapered. The volume change term is added to the equivalent diameter, and it provides equation 4.12.

\[ D_{\text{equivalent, non-linear}} = D_{\text{equivalent}} \left(1 + \frac{\Delta V}{V_{\text{equivalent}}}\right) \]  \hspace{1cm} (4.12)

The volume correction term is expressed as the cubic root of the volume difference between a linearly tapered column and a non-linearly tapered column, because the volume correction factor is added to the equivalent length.

The end-fixity, the degree of restraint against rotation at the ends, determines the effective length and the critical loads. As mentioned above, the values of K=0.5 to K=1
are substituted in the Euler formula and the Johnson formula to find the value of K which
best fits data, by comparing the estimated loads and experimental data.

These considerations are applied to predict the effect of the length of a microneedle
on its failure, the effect of needle material on failure, and the effect of the base diameter
of a microneedle on failure, as shown in Figure 4.34, Figure 4.35, and Figure 4.36
respectively.

As shown in Figure 4.34, experimental data are placed between K=1 and K=0.5 and
they are well fitted by the line calculated at K=0.62. The effective length of a column for
use in the Euler and Johnson formula is 0.62 times the actual length. The distance
between the inflection points was reduced to a value less than actual length because of
the needle's tapered structure and friction with the surface. For example, the physical
meaning of an end-fixity factor of 0.7 is the combination of one fixed end and one pinned
end and the buckled shape of K=0.7 approaches the fixed end with a zero slope while the
pinned end rotates freely (Mott 1996). Fitting equation 4.8 and 4.10 using K=0.62 to
experimental data leads to good agreement between theory and experiment. The increase
of K value increases the effective length and the slenderness ratio according to the
equation 4.6 and moves the transition point, i.e., the intersection of the solid line from the
Euler formula and the dashed line from the Johnson formula as shown in Figure 4.33.
When the slenderness ration (SR) is smaller than Cc, the experimental data are fitted by
the Johnson formula, and the Euler formula predicts an excessively high critical load.
When SR is higher than Cc, the experimental data are fitted by the Euler formula and the
Johnson formula predicts an excessively low critical value.
The non-linear (NL) factor term expressed as equation 4.12 is considered as shown in Figure 4.35. The volume correction term for a needle with 25 μm tip diameter, 200 μm base diameter and 1 mm length, is 1.23 and its cubic root is 1.06. When a NL factor term of 1.05 is added to equivalent diameter, the equivalent diameter increases from 83.4 μm to 88 μm and the critical failure force also increases by increasing the equivalent diameter. An error associated with reading dimensions from SEM figures makes a deviation between the experimental data and calculated values. The deviation causes the error in the calculation of equivalent diameter and its effect has similar magnitude to the NL factor. The nonlinearity of the tapered needle structures provides better mechanical strength than linearly tapered needle structures because of the increase in equivalent diameter.

![Image](33x480 to 543x1174)

(a) 25 μm tip diameter
200 μm base diameter
1 mm length

(b) AUTOCAD images

(c) Dimensions from AUTOCAD images

Figure 4.33 Geometries of microneedles with 25 μm average tip diameter, 200 μm base diameter and 1 mm length. (a) SEM pictures of three geometries (b) three-dimensional image based on SEM pictures (c) Dimensions of each microneedles.

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Figure 4.34 Experimental and predicted force of failure for a microneedle with 25 \( \mu \text{m} \) tip diameter and 200 \( \mu \text{m} \) base diameter, as a function of length of the microneedle and the end-fixity.

Figure 4.35 Experimental and predicted failure force prediction by the Euler formula at a microneedle with 25 \( \mu \text{m} \) tip diameter and 200 \( \mu \text{m} \) base diameter, as a function of the length of the microneedle and the NL (non-linear term).
The effect of Young's modulus on the failure force of a needle with 25 μm tip diameter, 200 μm base diameter and 1 mm length, is predicted by the Euler formula and the Johnson formula using K=0.62 (Figure 4.36). The Euler region (SR > Cₐ) is changed to the Johnson region (SR < Cₐ) by the increase of Young's modulus and the calculated values from the Johnson formula and the Euler formula get close to each other and SR equals to Cₐ when E is near 3 GPa. The Johnson formula fits data better than the Euler formula in the region where the Young's modulus of the polymer is above 3 GPa and SR is smaller than Cₐ.

Figure 4.37 shows that the effect of the base diameter of a needle with tip diameter of 25 μm, and length of 1 mm, on the failure force is evaluated by the Euler formula and the Johnson formula using K=0.62. The Euler region (SR > Cₐ) is changed to the Johnson region (SR < Cₐ) by increasing base diameter and the transition point is near 100 μm base diameter. The Johnson formula fits the experimental data point of a microneedle with 200 μm base diameter.
Figure 4.36 Experimental and predicted force at microneedle failure as a function of the Young's modulus of polymer.

Figure 4.37 Experimental and predicted force at microneedle failure as a function of the base diameters of the microneedle.
In an ideal situation, microneedles insert with a completely axial force with negligible bending moment. The possibility of non-axial misalignment is minimized by using an array (e.g., with around 30 microneedles) instead of a single needle. However, in a real application, there is a chance that a needle can experience a bending moment generated by a transverse tip force due to misalignment and deformation of the skin as shown in Figure 4.38, while microneedles are inserted into the skin. We therefore consider needle failure due to transverse loading (i.e. perpendicular to the needle axis).

We assume that (1) Hook’s law is valid, (2) the transverse load is applied to the part of the tip on the flexible skin, (3) the part of the needle to which the transverse force is applied is a cylinder due to nonlinear tapered structure as shown in Figure 4.39. The maximum bending is expressed as equation 4.13 (Mott 1996).

$$\sigma = \frac{M \cdot c}{I}$$  

(4.13)

where c is the distance from the neutral axis to the outermost edge of the microneedle, M is the bending moment and I is the second moment of inertia. By replacing the bending moment with the tip force, \(F_t\), multiplied by the needle length, L, we can solve for the maximum transverse tip force which the needle can support

$$F_t = \frac{S_y \cdot I}{c \cdot L}$$  

(4.14)

where \(S_y\) is the yield bending strength of the material.

When 500 \(\mu\)m from the tip of a 1 mm tall PGA needle is engulfed into deformed skin, the maximum transverse force for a non-tapered cylindrical structure with 200 \(\mu\)m base diameter and 100 \(\mu\)m base diameter and 1000 \(\mu\)m length can be calculated from equation 4.14 and these values are compared with experimental values as shown in Figure 4.40.
Figure 4.38 Modeling of skin deformation by a microneedle having 25 μm tip diameter, 200 μm base diameter and 1 mm length under 0.04 N of insertion force.

Figure 4.39 Influence of bending moment on a microneedle applied into skin
Figure 4.40 Experimental and predicted force (eq. 4.14) at microneedle failure as a function of base diameter of a PGA needle with 25 μm tip diameter and 1 mm length.

Simulations using ANSYS (Canonsburg, PA) are employed to solve for the von Mises stress (sum of stress in all directions) with geometries matching the tested microneedles using three-dimensional models composed of tetrahedral elements. The lateral forces of 0.1 N, 0.2 N, 0.25 N, 0.3 N and 0.4 N are applied to the tip of a PGA microneedle with 25 μm tip diameter, 200 μm base diameter and 1 mm length to predict the bending stress by transverse force.

ANSYS simulation provides the stress distribution and the strain distribution in a microneedle. We assume that skin deformation causes a transverse force to be applied to
microneedles and the transverse load area depends on the amount of skin deformation by a microneedle. From the result of ANSYS simulation as shown in Figure 4.41, when an insertion force below 0.1 N is applied to a microneedle with 25 μm tip diameter, 200 μm base diameter and 1 mm length as a transverse force, PGA has enough strength to withstand it. The deformation and stress distribution depends on applied transverse force as shown in Figure 4.34. The bending strength of PGA is between 100 MPa and 214 MPa (Zhang 1995). The analysis for the same geometry is done with 0.3 N of tip load applied to the middle of a microneedle (500 μm from the end of the tip), which results in a maximum stress of 168 MPa. By comparison, the failure force due to the bending stress for same geometry is computed at 0.24 N analytically from equation 4.14.

![Graph showing stress distribution](image)

Figure 4.41 The analysis using ANSYS: 0.1 N, 0.2 N, 0.25 N, 0.3 N and 0.4 N of lateral force is applied on a PGA microneedle with 25 μm in tip diameter, 200 μm in base diameter and 1 mm in length.
As shown in Figure 4.46(a), when a shear force is applied to microneedles while needles are inserted into skin, the failure happens near the base of the microneedle. The microneedle tips can keep their shape without breaking, as shown in Figure 4.46(b). The contact area between a microneedle and its base substrate is one of the important parameters for determining failure of microneedles. The critical failure force by axial load and transverse load can be increased by increasing the base diameter of the microneedle. However, an increase of column diameter increases the resistance to insertion of needles into skin and may increase pain due to the increased cross sectional contact area. A tapered structure can create an increase of base diameter with narrowing tip.

4.3 Drug delivery results

This chapter will discuss an experimental study of transdermal drug delivery through holes pierced by microneedles, release test of drug out of microneedles and protein stability encapsulated in microneedles.

4.3.1 Microneedle-skin interaction

Transdermal patches are user friendly type of drug delivery as shown by their high patient compliance. One of main applications of microneedles is the transdermal delivery patch embedding a microneedle. Another application is drug encapsulated inside microneedles. To assess the possibilities of using polymer microneedles for these applications, qualitative insertion testing was performed. An array of solid microneedles
was inserted into heat stripped human epidermis to test the ability of polymer needles to penetrate without breaking. To perform an in-vivo skin penetration test, sterilized PGA microneedles are inserted into skin of human subject. Finally, PLGA polymer needles encapsulating calcein were inserted into full thickness human cadaver skin to test release of calcein into in-vitro human skin.

4.3.1.1 Solid Microneedles

We qualitatively assessed the ability of polymer microneedle to pierce into skin and increase skin permeability. The polymeric microneedles were put on to the epidermis, pushed in and then removed. Trypan blue was dropped on the treated area. After 10 min, Trypan blue dye was removed and the under side of the epidermis was examined by light microscopy. Trypan blue does not stain the stratum corneum, but will stain the viable epidermis (McAllister 2000). Figure 4.42 shows an optical photomicrograph of an array of blue dots on the underside of in-vitro epidermis showing trypan blue transport through the skin via pathways created when polymeric microneedles were inserted into and then removed from skin.

In additional experiments, the epidermis was placed on layers of tissue papers to simulate the epidermis mechanical properties. Beveled polymeric microneedles with and without notches were put on to the epidermis and pushed into the skin. The epidermis with needles was fixed in formaldehyde and dried out by a graded ethanol process. This experiment confirms that PGA polymeric needles are able to pierce epidermis, which is strongest layer in skin as described in Section 3.1.5.1, without breaking as shown Figure 4.43 and Figure 4.44.
Figure 4.42 Optical micrograph of the underside of human cadaver epidermis pierced by an array of polymer microneedles and subsequently exposed to Trypan Blue dye. The pattern of blue staining is the same as the array of microneedles, indicating the presence of transdermal transport pathways created in the epidermis by polymer microneedles.
Figure 4.43 SEM micrographs of beveled polymeric microneedles which have pierced across skin.

Figure 4.44 SEM micrographs of beveled polymeric microneedles with notches. Left panel shows the microneedles in the pierced skin. Right panel shows the holes left by the microneedles after insertion.
To carry on experiment in-vivo, skin puncture holes are copied using liquid BandAid after 0 min, 15 min and 30 min of insertion and removing microneedles on in-vivo human skin. Figure 4.45 contains SEM images of copies of a hole at different times after needle insertion and removal, and these images are inverse of real holes. The initial hole appears to have liquid including body fluid and blood, so the hole is partially copied. The opening of the hole is 100 μm in diameter and has a deep dent. After 15 min, the hole opening has a 90 μm diameter, but the size of dent decreases because skin hole is recovered. The size dent decreases at 30 minutes compared to those of the initial and 15 minutes. We can expect the piercing holes have initially liquid in dent and then they are recovered quickly.

Figure 4.45 Hole size change left by the microneedles after insertion according to time (a) 0 min; (b) 15 min; and (c) 30 min.

To further demonstrate insertion in-vivo, sterilized solid PGA microneedles with notches were inserted into human skin on the hand and pushed from the side, intentionally causing breakage. Most of the tips stayed in the skin while the base portion of the needles was separated and removed (Figure 4.46(a)). When inserted into human
skin, biocompatible microneedles pierced \textit{in-vivo} skin without failure. This shows that the beveled PGA microneedles have enough mechanical strength to remain intact during insertion. The inserted tips were removed and imaged by SEM to check for possible morphology changes after insertion. Most of the PGA beveled microneedles with notch were inserted without tip damage (Figure 4.46(b)).

Figure 4.46 Photomicrograph of a portion of intentionally broken polymeric microneedles inserted into human skin \textit{in-vivo} (a) and SEM micrographs of intact needle tips removed from the skin (b).
4.3.1.2 Microneedles containing drug

To demonstrate the dissolution and release of drug or protein in actual skin from microneedles that encapsulate molecules, a fluorescent microscope and a confocal microscope study were performed. Polymer microneedles with calcine were applied to full thickness cadaver skin, and the skin/needle assembly was placed in a hydration chamber at a temperature of 4°C for 8 hours. After 8 h, the needles were removed and fluorescent microscope images were taken to image the release of calcine out of needle into cadaver skin. The treated area has a strong calcine response as shown in Figure 4.47. This confirms that calcine diffuses out of microneedles and into skin. To investigate the distribution of calcine in the skin, a series of confocal microscope images were taken, which allowed depth profiling of the released calcine to be performed (Figure 4.48). Strong signals were detected over 200 μm deep into the skin and the intensity of fluorescents decreased gradually.
Figure 4.47 Fluorescent microscope image of calcine in human cadaver skin. An array of PLGA 50/50 microneedles with 1 μg of calcine in 100 microneedles was inserted into full thickness cadaver skin.

Figure 4.48 Confocal microscopic images of calcine at different depths in human cadaver skin.
4.3.2 In-vitro skin permeability

The enhancement of transdermal delivery rate by microneedles was evaluated. Transdermal permeability experiments were performed in vitro with calcine and BSA. A simple theoretical model was applied to predict transdermal delivery results.

4.3.2.1 Skin permeability measurements

To quantify changes in transdermal transport caused by polymer microneedles, the permeability of intact human cadaver epidermis to calcine (623 Da; 0.6 nm) and BSA (66 kDa; 3.5 nm) was compared to the permeability of epidermis pierced with arrays of 20 or 100 microneedles. As seen in Figure 4.49, insertion and removal of an array of 20 polymer microneedles increases skin permeability to calcine and BSA by at least two orders of magnitude, while a 100-needle array increased permeability by almost three orders of magnitude. There is a statistically significant difference between 20 and 100 microneedles for calcine (ANOVA, P = 0.006) and BSA (ANOVA, P=0.004) as well. This substantial increase in skin permeability is significant for transdermal drug delivery applications and similar to increases previously observed in experiments with solid silicon microneedles (McAllister 2003)
Figure 4.49 Calcein and BSA permeability of human cadaver epidermis left intact, pierced with an array of 20 microneedles, and pierced with an array of 100 polymer microneedles. The microneedles are 600 μm in height, 100 μm at their bases, and 10 μm at their tips. The needles are positioned in a 20 by 6 array with a center to center spacing of 400 μm and 1400 μm.
4.3.2.2 Skin permeability theoretical model

We assume that skin with hole created by microneedles is a porous solid membrane and drug moving through holes created by microneedles is much faster than through solid skin. Two parameters are used to describe the diffusion path through such a porous solid. The first is the void fraction, \( f \), defined as the ratio of pore area to total cross-sectional area. The other parameter is the tortuosity, but it is not important here because the path created by microneedle is straight and the diffusion length is short. Diffusion across porous skin plane has been presented as (Johnson 1999)

\[
N = \frac{f \cdot A \cdot D_p \cdot \Delta C}{L_{path}}
\]  

(4.15)

where \( N \) is the flow rate of drug, \( D_p \) is the diffusion coefficient of the drug in the medium, \( L_{path} \) is the path length, \( \Delta C \) is the concentration gradient and \( f \) is the void fraction.

With the pore dimensions and molecular diffusivities known, the above theory can be used to estimate the skin permeability to calcein and BSA pierced with solid microneedles. The permeability, \( k \), of skin that has been changed by microneedles is described by equation 4.25.

\[
N = k \cdot A \cdot \Delta C
\]  

(4.16)

\[
k = f \frac{D_p}{L_{path}}
\]  

(4.17)

where void fraction, \( f \) is the fractional area of skin containing holes from microneedles. The fractional area of holes for needles inserted and then removed, is calculated using following equation

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where \( n_m \) is the number of microneedles, \( d \) is diameter of a hole, and \( A \) is the area under the microneedle array. As shown in Figure 4.50, the diameter of a hole is determined by investigating fluorescent microscope images of holes on the skin created by microneedles. The skin permeabilities from theory and experiment data are shown in Table 4.3. The permeability decreases with increasing molecular radius. The number of holes increases the permeability as expected from equation 4.18. The experimental values have same order of magnitude of calculated values from equation 4.18.

<table>
<thead>
<tr>
<th>Microneedle Configuration</th>
<th>Permeability from theory (cm/hr)</th>
<th>Permeability from experiment (cm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 microneedles with Calcein</td>
<td>8.00 \times 10^{-3}</td>
<td>6.6 \times 10^{-4}</td>
</tr>
<tr>
<td>100 microneedles with Calcein</td>
<td>4.02 \times 10^{-3}</td>
<td>2.4 \times 10^{-4}</td>
</tr>
<tr>
<td>20 microneedles with BSA</td>
<td>1.47 \times 10^{-3}</td>
<td>2.2 \times 10^{-4}</td>
</tr>
<tr>
<td>100 microneedles with BSA</td>
<td>7.36 \times 10^{-4}</td>
<td>1.1 \times 10^{-3}</td>
</tr>
</tbody>
</table>
Figure 4.50 Fluorescent microscope image of holes left in the epidermis after transdermal delivery test using a Franz cell. From this image, the average diameter of hole is estimated to be 50 μm.

4.3.3 In-vitro release test result

The release rate of drug out of microneedles encapsulating drug is measured and compared with theoretical models to predict their behavior.

4.3.3.1 Polymer needle degradation

Figure 4.51 shows that 93 percent of the calcein was released from the microneedles within four hours in PBS, indicating this formulation of microneedles had fast release and its release pattern depended on calcein bulk diffusion rather than the slower polymer degradation because the degradation time of PLGA is 1 to 2 months. For BSA, 80 percent of BSA was released from the microneedles in approximately 5 days in PBS, suggesting that its release pattern depended on slower BSA diffusion. Microneedles with 1 μg of calcein and 1 μg of fluorescence conjugated BSA in 100 of microneedles respectively are used to measure release rate at 37 °C. Their morphological change is
investigated by light microscope over time as shown in Figure 4.52. The calcine in microneedles starts to diffuse out and most of the calcine located in the tip of microneedles is released out as shown in Figure 4.52(b). Microneedles are fully degraded in basic solution to determine the total amount of calcine in microneedles, and all calcine releases out and most of microneedles are degraded as shown in Figure 4.52(c). This morphological change is well matched with the in-vitro release test.

The morphological change of microneedles with BSA is investigated as well. An optical image of a microneedle with BSA after 12 hours in PBS at 37 °C shows BSA, located near the surface of the needle, releases out (Figure 4.53(a)) and SEM image confirms the release of BSA out of needle by showing holes on the surface (Figure 4.53(b)).
Figure 4.51 Cumulative release of calcein (●) and BSA-fluorescein (■) from microneedles in PBS.

Figure 4.52 The morphological properties of PLGA microneedles containing calcein during an in-vitro dissolution test. The samples are observed by optical microscope (a) initial preparation (b) after 9 hours in PBS and (c) after degradation in 0.1 N NaOH at 37 °C.
Figure 4.53 Optical image of a microneedle with BSA and SEM image of a microneedle with BSA after 12 hours in PBS at 37 °C.

For slower release, CMC is added to calcein to serve as a slow release formulation. CMC was used for retarding agent of drug out of microparticles (Lee 1997). Figure 4.54 suggests that CMC is effective in retarding calcein release. CMC is effective additive for few days release, however it is not for over one week. CMC-calcein particles are located also near the surface of the microneedles and it shows a relatively fast release rate as well. Figure 4.55 shows optical microscope images at different degradation stages: (a) immediately after preparation, and (b) after 2 days in PBS, pH 7.4 at 37 °C. After 2 days in PBS, the calcein placed in the tip of microneedles releases out and little calcein remains near center of needle.
Figure 4.54 Release profile of microneedles containing calcein, CMC-calcein and microparticles with calcein as function of time.

Figure 4.55 Optical microscope images at different degradation stages: (a) immediately after preparation, and (b) after 2 days in PBS, pH 7.4 at 37 °C.
To slow release even further, encapsulating calcine in LPLA microspheres that are encapsulated within PLGA microneedles, shows slow release over a month as shown in Figure 4.56. Although it has low drug loading concentration in microneedles (0.1 μg/100 microneedles) compared to 1 μg of calcine, BSA or CMC-calcine per 100 microneedles, this approach shows significant retardation of drug release from a few day release profile to a month-long release profile. The release pattern from microneedles depends on the release pattern of microspheres of PLA.

After 15 day of release test, the debris of microneedles is sampled and investigated by optical microscope and fluorescent microscope. The LPLA microspheres in microneedle have still strong fluorescent intensity of calcine (Figure 4.57). Figure 4.58 shows optical microscope images at different degradation stages: immediately after preparation, after 1 day and after 5 days in PBS, pH 7.4 at 37 °C. After 5 days in PBS, microneedles start to show a rough surface due to release of drug and surface erosion.

![Cumulative release profile of calcine from LPLA microspheres (●) and microneedles encapsulating microspheres (■) in PBS.](image)

**Figure 4.56** Cumulative release profile of calcine from LPLA microspheres (●) and microneedles encapsulating microspheres (■) in PBS.
Figure 4.57 (a) Optical microscope image and (b) fluorescent microscope image of a section of a microneedle containing microspheres with calcine after 15 days in PBS, pH 7.4 at 37 °C.

Figure 4.58 Optical microscope images at different degradation stages (a) immediately after preparation, (b) after 1 day and (c) 5 days in PBS, pH 7.4 at 37 °C.
4.3.3.2 Polymer needle degradation theory

To model the release from polymer microneedles, we used the simplest model of a drug-releasing particle; it assumes that the drug is initially uniformly distributed in a sphere made of material with a uniform diffusion coefficient. Drug placed in the tips of microneedles can be simplified to follow the release-time profile of spherical particles. The expected release-time profile for this model (Crank 1975) is

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp(-n^2 \pi^2 \tau)$$  \hspace{1cm} (4.19)

where $M_t$ is the amount of drug released in the particle at time $t$, $M_\infty$ is the amount of drug released at infinite time and the dimensionless time $\tau$, is given by

$$\tau = \frac{Dt}{r^2}$$  \hspace{1cm} (4.20)

$D$ is the diffusion coefficient of the drug in the polymer matrix and $r$ is the radius of the tip of the microneedle. This sum of exponential series provides a decay rate that initially is steeper than a single exponential, and at long times is slower than a single exponential. In order to simplify this model, simplifications of this equation have been used (Washington 1996). Expansion and truncation of this series provides

$$\frac{M_t}{M_\infty} = 6 \frac{\tau}{\pi}$$  \hspace{1cm} (4.21)

which suggests that the initial release rate is inversely dependent on the particle size and proportional to the square root of time. This latter relationship is often termed the Higuchi law. But square root law can’t cover data for longer interval. Addition of a further term in the series yields

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\[
\frac{M_t}{M_\infty} = 6 \frac{\sqrt{t}}{t} - 3t
\] (4.22)

which provides a fit for data at longer release times (Baker 1974).

The above equations can be written as the following general form

\[
\frac{M_t}{M_\infty} = kt^n
\] (4.23)

where the \( k \) is a kinetic constant that depends on the characteristics of the polymer matrix and drug, and \( n \) is the diffusional exponent, which defines the diffusion mechanism. Equation 4.23 is valid for the first 70\% of the fractional release (Shin 2003). For a slab, Fickian diffusion is defined by \( n=0.5 \), and non-Fickian diffusion is presented by \( n>0.5 \). Fickian diffusion from a cylinder and a sphere can be presented by the equation with \( n=0.45 \), and \( n=0.43 \), respectively (Ebrahim 2002).

Due to the differences in drug release kinetics and test conditions, the constant \( k \), though is one of the measures of release rate, should not be used for comparison (Reza 2003). Therefore, to characterize the drug release rate in different experimental conditions, mean dissolution time (MDT) was calculated from a kinetic constant, \( k \) and a diffusional exponent, \( n \), using the following equation (Lund 1994). Higher values of MDT indicates a higher drug retarding ability of the polymer matrix (Reza 2003).

\[
MDT = \left( \frac{n}{n+1} \right) \cdot k^{1/n}
\] (4.24)

As mentioned above, Fickian diffusion equations are valid for the 60\% to 70\% of the total release. Hence, Potowski has presented the following equation to cover whole release range.
\[
\frac{M(t)}{M_\infty} = 1 - \exp[-k(t + b)]
\]  
(4.25)

The presence of constant \(b\) in this equation makes it suitable for use in systems where the drug is partially located on the device surface \((b = 0)\), and also for systems with degradable films, whose removal is followed by drug release \((b < 0)\).

These different kinetic models for the release of calcein and BSA are compared with experimental data in Figure 4.59, 4.60, 4.61, and 4.62. The corresponding correlation coefficients for fitting experimental results with different models are summarized in Table 4.4.

Fitting Equation 4.22 (Baker model), 4.23 (Fickian model), and 4.25 (Potowski model) to the experimentally determined drug release rate from calcein loaded PLGA microneedles leads to good agreement between theory and experiment shown in Figure 4.59. The fitting procedure is based on the minimization of the resulting differences between experimental and theoretical values (least squares method).

The release curves are fitted with equation 4.23 and diffusional exponent \((n)\) and release rate \((k)\) are determined from fitting curve and they are summarized in Table 4.5. Results indicate calcein and BSA release can not be described as a simple Fickian diffusion process because the values of the diffusional exponents \(n\) are below 0.5, whatever the sample studied. Ritger and Peppas reported a value of \(n = 0.3\) in release systems composed of a mixture of microspheres with different sizes. They explained that the microspheres size distribution and the general shape of a distribution could influence the value of \(n\). The microneedles do not have uniform distribution of drug in the microneedles and most of drug is placed near surface of microneedle. The shape of microneedle is not spherical but close to a cone. Drug particles and Microspheres inside

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microneedles have a broad size distribution also. Even if the needles have same geometry but drug distribution in the microneedles is different cross the area, thus the actual volume occupied by drug in the microneedles is not uniform.

The release rates are found to be different according to formulation. The release rate depends on the matrix macromolecular network, and on characteristics of the drug (Grattard 2002). As shown in Table 4.5, a decrease in the release rate of calcein suggests the release kinetics of calcein is significantly affected by the water affinity of the matrix, solubility of drug and drug loading efficiency (Grattard 2002; Shin 2003). The microneedles with microspheres show much higher MDT value than CMC based system also shows higher MDT value. In the case of CMC based system, CMC works as the retarding agent for calcein due to its barrier to calcein diffusion. The higher drug-retaining ability of LPLA is due to its hydrophobicity, double coating by LPLA following PLGA 50/50 and lower loading of calcein (0.1 μg / 100 needles). The data are summarized in Table 4.5. There is a significant difference in median values among three groups. (ANOVA, P=0.001)

The apparent diffusion coefficient was calculated from fitting the equation 4.22 (Baker model) to experimental data (Faissant 2002). The following diffusion coefficients within system are obtained by regression of the theoretical curve line to experimental data and the determined diffusion coefficient of calcein in the PLGA 50/50 microneedles is $2.25 \times 10^{-6}$ cm$^2$/hr. This diffusion coefficient of calcein is about two fold less than diffusion coefficient in aqueous phase ($5 \times 10^{-6}$ cm$^2$/hr). This means that calcein is not dispersed uniformly in microneedle and located on surface, and calcein has high solubility in water. The diffusion of calcein decreases by adding CMC to calcein and

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encapsulating calcein in LPLA microspheres. When the calcein is mixed with CMC, the
calculated diffusion coefficient is $8.18 \times 10^{-8}$ which is almost two orders of magnitude
lower than without CMC. The diffusion coefficient of calcein encapsulated in LPLA
microspheres from microneedles is obtained from Figure 4.61, and its value is $3.3 \times 10^{-9}$
cm$^3$/hr which is almost three orders of magnitude over the calcein in water. The obtained
diffusion coefficient of BSA is $3.28 \times 10^{-8}$ and aqueous diffusion coefficient of BSA is
$9.2 \times 10^{-7}$.

Potowinski model (equation 4.25) agrees with experimental data well and it can be
used for modeling of this device. The resulting $b$ constants of this model are 0.054, 4.6,
7.33 and 173 for calcein only, CMC-calcein and LPLA microsphere respectively and all
of values are larger than 0. This indicates that drug is partially located on the surface of
microneedles and release by surface erosion is not dominant.
Figure 4.59 Comparison of semiempirical kinetic models with experimental results for calcine from PLGA 50/50 microneedles.

Figure 4.60 Comparison of semiempirical kinetic models with experimental results for CMC-calcine from PLGA 50/50 microneedles.
Figure 4.61 Comparison of semiempirical kinetic models with experimental results for calcanein from PLGA 50/50 microneedles encapsulating PLGA microspheres with calcanein.

Figure 4.62 Comparison of semiempirical kinetic models with experimental results for BSA from PLGA 50/50 microneedles.
Table 4.4 Comparison of semiempirical models with experimental data of drug release.

<table>
<thead>
<tr>
<th>Drug Formulation</th>
<th>Mode</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcein in PLGA 50/50 microneedles</td>
<td>Fickian Model</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Baker Model</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>Potowski Model</td>
<td>0.9721</td>
</tr>
<tr>
<td>Calcein+CMC in PLGA 50/50 microneedles</td>
<td>Fickian Model</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Baker Model</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Potowski Model</td>
<td>0.9652</td>
</tr>
<tr>
<td>Calcein-LPLA spheres in PLGA 50/50 microneedles</td>
<td>Fickian Model</td>
<td>0.971</td>
</tr>
<tr>
<td></td>
<td>Baker Model</td>
<td>0.9354</td>
</tr>
<tr>
<td></td>
<td>Potowski Model</td>
<td>0.9617</td>
</tr>
<tr>
<td>BSA in PLGA 50/50 microneedles</td>
<td>Fickian Model</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Baker Model</td>
<td>0.9946</td>
</tr>
<tr>
<td></td>
<td>Potowski Model</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Table 4.5 Apparent release rate (k), diffusional exponent (n) and mean dissolution time (MDT) for microneedle formulations.

<table>
<thead>
<tr>
<th>Drug Formulation</th>
<th>k (hr⁻¹)</th>
<th>n</th>
<th>MDT (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcein in PLGA 50/50 microneedles</td>
<td>0.66</td>
<td>0.19</td>
<td>1.422</td>
</tr>
<tr>
<td>Calcein+CMC in PLGA 50/50 microneedles</td>
<td>0.2019</td>
<td>0.3441</td>
<td>26.76</td>
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<tr>
<td>Calcein-LPLA spheres in PLGA 50/50 microneedles</td>
<td>0.0979</td>
<td>0.2874</td>
<td>1189</td>
</tr>
<tr>
<td>BSA in PLGA 50/50 microneedles</td>
<td>0.1151</td>
<td>0.4059</td>
<td>59.38</td>
</tr>
</tbody>
</table>
Table 4.6 Diffusion coefficients from equation 4.25 for needle formulations.

<table>
<thead>
<tr>
<th>Drug Formulation</th>
<th>Diffusion coefficient (cm²/hr) in water</th>
<th>Diffusion coefficient (cm²/hr) in polymer matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcein in PLGA 50/50 microneedles</td>
<td>5 × 10⁻⁶</td>
<td>2.2475 × 10⁻⁶</td>
</tr>
<tr>
<td>Calcein+CMC in PLGA 50/50 microneedles</td>
<td></td>
<td>8.18 × 10⁻⁸</td>
</tr>
<tr>
<td>Calcein-LPLA spheres in PLGA 50/50</td>
<td></td>
<td>3.3 × 10⁻⁶</td>
</tr>
<tr>
<td>microneedles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA in PLGA 50/50 microneedles</td>
<td>9.2 × 10⁻⁵</td>
<td>3.28 × 10⁻⁸</td>
</tr>
</tbody>
</table>

4.3.4 Protein stability test result

We wanted to know if proteins, such as BSA, remain stable when encapsulated in microneedles. In addition to detecting protein stability, we need to set up protein measuring protocol. Whenever we carry out an encapsulation process including high temperature injection molding, we need to measure encapsulated protein stability. Dynamic Light Scattering (DLS), Circular Dichroism (CD) and SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE) provides fast analysis. Even if we change the process, we can utilize these measurements to quantify the protein stability and identify the bioavailability of a process for encapsulating protein.

As described in Section 3.2.3.3, 10 mg samples of BSA after 0, 10, 20 and 30 min thermal exposure time at 135 °C were dissolved in 100 ml PBS respectively and then protein concentrations were measured to quantify irreversible aggregated protein. 100 μg/ml (0 min), 90 μg/ml (10 min), 82 μg/ml (20 min), and 68 μg/ml (30 min) of
protein concentration are measured. This indicates 10 %, 18 % and 32 % of solid BSA was denatured by heat exposure under 135 °C. After two hours, over 90% of BSA is denatured. In the case of 0 min exposed BSA, BSA is only exposed to acetonitrile and short term methylene chloride. The concentration of 0 min BSA is same as the untreated BSA standard. This means that acetonitrile is a good dispersing medium for protein such a BSA. The encapsulating process for BSA in microneedles by injection molding process is typically done within 20 minutes. To further test the stability of re-dissolved BSA in PBS was assayed using CD, DLS, and SDS-PAGE as described in Section 3.2.3.3.

CD measurement is used to characterize the presence of intermediate species (Bulone 2001). The structural properties of BSA intermediates are probed by CD measurements in the far UV range. CD spectra of each sample are shown in Figure 4.63. The observed spectra indicate no significant difference of the ratio of α-helix/β-sheet components. We can conclude that re-dissolved BSA keeps the same ratio of α-helix/β-sheet after thermal exposure. In contrast, if aqueous BSA is heated for 25 minutes under 67 °C and CD is measured, there is a significant change of the ratio of α-helix/β-sheet component (Figure 4.64). This shows that solidified BSA has much better thermal stability compared to aqueous BSA.
Figure 4.63 CD spectra of dissolved BSA in PBS after thermal exposure in the solid state. Consistent spectra indicate the same ratio of α-helix/β-sheet components.

Figure 4.64 An aqueous sample is heated to the denaturation temperature of the native protein (67 °C) for 25 minutes. The observed change indicates a decrease of the ratio of α-helix/β-sheet components. This shows that the solidified BSA has much better thermal stability compared to the aqueous BSA.
As a type of analysis, an inspection of the samples in solution is provided by DLS measurements. Results are shown in Figure 4.65 together with those obtained for native protein at the same temperature and concentration. A cumulative analysis of DLS data gives an average size of 4.14 nm ± 1.6 nm, 4.9 nm ± 1.39, 6.14 nm ± 3.3 nm, and 8.8 nm ± 5.1 nm after 0 min, 10 min, 20 min, and 30 min of thermal exposure time respectively. The bimodal distribution of each samples exposed for 20 - 30 min suggests that conformational change partially happens in BSA by these thermal exposures.

![Size distribution change of BSA by thermal exposure of solidified BSA under 135 °C](image)

As a final assessment of protein stability, SDS-PAGE results of thermally exposed BSA, run under non-reducing conditions, are shown in Figure 4.66. The non-reducing
conditions employed for this analysis would preserve all aggregates linked by disulfide bonds. A stained band of aggregated BSA is not found in these samples. This provides additional support for CD measurement showing intact protein, serious damage to covalent bonding, including disulfide linked aggregates, is not observed. But we can not say there is no additional covalent aggregation, because the DLS data shows a conformational change of BSA partially by thermal exposure. If the concentration of aggregated species is below the detection limit of the Coomassie blue staining method used for SDS-PAGE, whether the protein becomes covalently aggregated or not can’t be ascertained by SDS-PAGE analysis with Coomassie blue (Crotts 1997).

![Figure 4.66 SDS-PAGE results of BSA standard and thermal exposed samples in PBS](image)

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From the overall analysis, we conclude that BSA is partially denatured by thermal exposure to high temperature and the extent of irreversible denatured protein increases when exposure time is increased. The structure of dissolvable BSA is quite stable from CD, DLS and SDS-PAGE measurements. The stability of encapsulated model protein can be estimated by these measurements and these measurements provide the measurement of three dimension stability as well as two dimension stability. The thermal exposure process has to be minimized to avoid the change in protein structure. Solidified protein has much better stability as shown in CD measurements and we have minimized the property change of protein during fabrication process by using solidified protein.

4.3.5 In-vivo microneedle delivery

To demonstrate the application of polymer microneedles in-vivo, a blue dye solution was applied on the rat skin and then PGA polymer needles with 25 μm tip D.I, 200 μm base D.I and 1 mm length was pushed into the blue dye layer on hairless rat skin in-vivo and removed. This experimental model is for the patch containing solid polymeric needles. The treated surface was swiped with wet tissue paper to remove remaining dye on the surface. Figure 4.67(a) shows that blue dye went into the skin with microneedles and stayed inside the skin after removing needles. The penetration depth of blue dye was 200 μm below the skin surface and the spacing of blue spots was 1000 μm as shown in Figure 4.67(b). The spacing between the spots depends on the sectioning direction in x-y axis. All needles were inserted with blue dye and not one of the microneedles was broken after removing from skin (data not shown).
Figure 4.67 Transdermal drug delivery test using in-vivo rat. (a) blue dye in the rat skin after needle insertion (b) vertical skin section of injected site.
5. Discussion

Microneedles were fabricated out of biodegradable polymers for biological safety but polymer has mechanical weakness compared to previous microneedle material including silicon and metal. To solve these problems, the needle geometries and kind of polymer were considered. The effect of length of microneedles and column diameter on failure by axial force were measured and predicted by buckling failure mode. The polymer microneedle with wider base diameter showed they inserted into skin without breaking. Microneedles made of polymers with high Young’s modulus and yield strength showed better mechanical behaviors which are mechanically resistant. But the increase in base diameter might decrease insertion depth due to deformed skin resistance caused by the wider base. If the depth of deformed skin, by pressing needles until the interfacial tension between needles and skin reached the insertion force, is longer than the needle length, skin could not be punctured by needles. From the measurement of failure force, we suggested 150 – 400 μm base diameter and over 600 μm in length for high molecular weight PLGA, LPLA and PGA. PGA showed the best mechanical strength among the biodegradable polymers because of its high Young’s modulus of 10 GPa and yield strength of 90 MPa. Polymeric microneedles made of PGA could increase the applicable geometries of microneedles for insertion into skin without breaking. But the high melting temperature of PGA made encapsulating drug inside microneedles difficult.

The analytical solution by the Johnson formula and the Euler formula with K of 0.62 predicted the tendency as well as the quantitative axial failure force and this suggested the buckling failure caused by inelastic and elastic instability was the main failure mode.
The end-fixity of K=0.62 meant the effective length was 0.62 times the actual length of a column for use in analysis. The tip of the microneedle was not completely free to rotate as a result of friction, thus the effective length was reduced. A non-linear tapered column geometry reduced the distance between the inflection points to a value less than the actual length.

Also the lateral force caused by deformed skin made needle failure near its base by misalignment in-vivo. This was addressed by increasing the base diameter by fabricating a tapered column structure. The increased base diameter was clearly helpful to increase failure force, but the straight column increased deformed skin resistance, thus it made insertion depth short. The tapered column could increase base diameter without widening the tip interfacial area between tip part and deformed skin. The increase of drug content in microneedles decreased failure force of microneedles and 2 % drug content was the limit for insertion without serious failure. The failure happened at the interface between drug particles and the polymer matrix and the bad adhesion between them made the failure force less than predicted.

A solid porous membrane model was used to predict transport phenomena through skin pieced by microneedles. The good fitting of a kinetic model to measured data suggested that solid microneedles were effective to increase transdermal delivery rate and large molecule delivery.

The drug delivery pattern was controlled by the addition of retarding agents with the drug and it was predicted by a diffusion model. Calcein release from microneedles showed a fast release profile and the diffusion coefficient in the needle matrix was close to the value in water; this meant the drug was placed near the microneedle surface. CMC
and LPLA microspheres showed drug retaining ability due to creation of a diffusion barrier to the model drug, calcein. Addition of a retarding agent or encapsulation in microspheres controlled the release profile of the drug out of microneedles.

Thermal exposure damaged the properties of a model drug during the molding process. Increased thermal exposure increased the amount of irreversible and reversible denaturation of protein, but serious conformational changes like breaking of disulfide bonds was not found. The thermal exposure time should be minimized to prevent protein denaturation.
6. Conclusions

Currently, transdermal drug delivery is successful for a only a small number of drugs that possess certain properties. Many techniques have been developed to increase skin permeability to molecules more generally, but they have had limited success with macromolecules such as proteins. In this study, arrays of solid polymer microneedles and polymer microneedles encapsulating model drugs were created to form pathways for large molecules, to provide safer microneedles made out of biocompatible and biodegradable polymers, and to exploit the controlled release properties of biodegradable polymers.

Microfabrication techniques are utilized to fabricate microneedle arrays. Solid microneedles were fabricated from biocompatible and biodegradable polymers. This approach was achieved by micro-electromechanical masking and etching or modification of light paths, preparing a PDMS micromold and injection-molding copies of the microneedles made out of biodegradable polymers. These needles could be internally loaded with drugs and used as a controlled release drug delivery matrix. Especially, the fabrication process to make biodegradable microstructures, the lens techniques to form tapered structures, and the process for drug encapsulation in microneedles were novel and improved upon prior fabrication processes in regards to ease of fabrication, needle mechanical properties, cost effectiveness, and ease of insertion.

In this study, the force of insertion as a function of tip geometry was measured in human subjects and this analysis showed good agreement with previous work. Consistent
with previous findings, the microneedle tip diameter was the critical geometric parameter to determine the force of insertion.

The failure force by axial loading and transverse loading of microneedles as a function of their geometry and mechanical properties were also measured. The failure force by axial loading was found to increase linearly with the base diameter of a microneedle and the Young's modulus of the material, and to decrease with the length of the microneedle and encapsulating drug content in microneedles. The Johnson formula and the Euler formula were used to predict the axial failure forces and both models predicted the tendency as well as the quantitative axial failure force. The transverse failure force by deformed skin was found to increase linearly with the base diameter of a microneedle. The analytical solution and FEM solution were used to predict the transverse failure force and this analytical model demonstrated better agreement with the data. A comparison of the insertion force and the axial failure force of microneedles showed that biodegradable polymers including PGA, LPLA and high molecular weight of PLGA have satisfactory mechanical properties as needle materials.

Solid polymer microneedles were used to increase skin permeability to calcine and fluorescence-conjugated BSA. Array of 20 microneedles increased skin permeability to calcine and BSA by at least two orders of magnitude, while a 100-needle array increased permeability by almost three orders of magnitude. There was a statistically significant difference between using 20 and 100 microneedles for calcine and BSA delivery.

Polymer microneedles that encapsulated calcine and BSA were used to investigate the release of these model drugs from microneedles. PLGA microneedles encapsulating calcine demonstrated rapid release within hours. CMC works as a retarding agent for
calcein release over days by adding it to calcein during encapsulation. LPLA microspheres encapsulating calcein within microneedles showed slower drug release over weeks. The retarding ability of CMC and LPLA microspheres was caused by reduced diffusion of calcein in CMC and LPLA microspheres.

The thermal stability of BSA was investigated when BSA was encapsulated in microneedles. BSA was partially denatured by thermal exposure to high temperature of 135 °C and the extent of irreversible denatured protein increased when exposure time was increased. The structure of dissolvable BSA was quite stable as shown by CD, DLS and SDS-PAGE measurements. The thermal exposure process should be minimized to avoid changes in protein structure. Solidified protein had much better stability compared to aqueous protein. We minimized the property changes of the protein during the fabrication process by using solidified protein.
7. Recommendations

Building off the results presented in this thesis, I would recommend continuing research on the process development which reduces changes in protein structure during fabrication, because protein can be denatured by the thermal exposure while being encapsulated in microneedles. To overcome this problem, ultrasonic welding techniques should be useful to melt the polymer for short times which should cause less damage to the protein. Separate preparation of the mechanical polymer support part and the drug encapsulating polymer part of microneedles and then bonding two parts together may be a good solution.

Regarding the mechanical strength of polymer microneedles, the mechanical properties of polymer and the drug content in microneedles are of concern. It has been shown that polymer microneedles made of PGA, LPLA and high molecular weight PLGA are capable of penetrating the skin successfully, however there were geometric limitations for insertion without breaking. This limitation could be improved by increasing the Young's modulus and yield strength of the polymer. The mechanical strength of the polymer can be increased by using a polymer composite or self-reinforced polymer technique. For LPLA, a self-reinforced polymer has been developed and its Young’s modulus is almost three times greater than intact LPLA (Zhang 1995). It may be another good solution to use less brittle biodegradable polymers. The improved resistance to shear-induced breakage should be helpful to overcome the harsh transverse load due to the ductile viscoelastic property.
If the drug is concentrated at the tip of the needle, the needle may fail to insert into skin. The tip has to have less drug and more drug should be located further down the needle shaft. Also, an increase of adhesion between drug and polymer should be helpful to increase mechanical strength of microneedles with drug.
REFERENCES


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