HOLLOW MICRONEEDLES FOR
MOLECULAR TRANSPORT ACROSS SKIN

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HOLLOW MICRONEEDLES FOR
MOLECULAR TRANSPORT ACROSS SKIN

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To my parents,

for always believing in me and giving me the opportunity to explore and create
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LIST OF SYMBOLS AND ABBREVIATIONS

\( \alpha \)  
angle of micro needle wall

\( A \)  
area of micro needle and skin contact

\( A_A \)  
anular interfacial area of micro needle

\( A_F \)  
full interfacial area of micro needle

\( A_b \)  
cross sectional area of micro needle lumen

BGL  
blood glucose level

BSA  
bovine serum albumin

C  
Concentration

\( C_0 \)  
concentration in reservoir

\( C_b \)  
Concentration of insulin in blood

\( \delta A \)  
crack area

\( D \)  
diffusivity

\( d_B \)  
laser beam diameter

\( d_e \)  
entrance hole diameter

DI  
de-ionized

\( d_t \)  
tip diameter of micro needle

\( \delta W \)  
work input to create crack

\( d_x \)  
exit hole diameter

E  
Young’s modulus

F  
force

\( F_b \)  
buckling force
$F_i$  
insertion force

$F_f$  
fracture force

$G_s$  
fracture toughness

$G_p$  
puncture toughness

$h$  
height of microneedle

$L$  
diffusion length

$IR$  
Infrared

$k$  
edition rate constant

$K_i$  
permeability of fiber $i$

$K_{gs}$  
ground substance permeability

LIGA  
lithography, electroplating, and molding

PECVD  
plasma enhanced chemical vapor deposition

MEMS  
microelectromechanical systems

$M_{rat}$  
body weight of rat

$N_B$  
number of solute particles in blood

$n_i$  
number fraction of fiber $i$

PBS  
phosphate buffered saline

$R$  
radius of microneedle tip

$r_f$  
fiber radius

$r_s$  
solute radius

$\sigma_u$  
ultimate stress

SC  
stratum corneum

$S_{cf}$  
collagen fiber spacing
\( t \) \hspace{1cm} \text{microneedle wall thickness} \\
\( t_{\text{half}} \) \hspace{1cm} \text{half life} \\
\( T \) \hspace{1cm} \text{diameter of trepanned pattern} \\
\( UV \) \hspace{1cm} \text{ultraviolet} \\
\( \Phi_{\text{cf}} \) \hspace{1cm} \text{volume fraction of collagen fibers} \\
\( \Phi_t \) \hspace{1cm} \text{total volume fraction} \\
\( \Phi_i \) \hspace{1cm} \text{volume fraction of component } i \\
\( V_d \) \hspace{1cm} \text{volume of distribution} \\
\( x \) \hspace{1cm} \text{evaluation point}
SUMMARY

Transdermal drug delivery offers the opportunity to deliver therapeutic drugs without many of the drawbacks of conventional injections and orally administered drugs. However, the barrier property of the skin is extremely adept at preventing the penetration of therapeutic molecules as well as protecting the body from dangerous foreign material.

As a novel drug delivery technology, we have developed arrays of microscopic needles, which are capable of providing pathways for molecular transport across the skin without the pain associated with conventional injections. Pathways through the stratum corneum and viable epidermis created by hollow microneedles allow molecular transport regardless of molecular size and/or water solubility.

Previous generations of microneedles have been demonstrated to successfully create pathways to circumvent the barrier properties of the skin. The permeability of human epidermis was increased by up to four orders of magnitude, relative to intact skin, to a wide range of molecules. By creating these pathways in a minimally invasive fashion, due to their extremely small size, microneedles were shown to be painless in human volunteers.

Despite their success in previous work with in vitro tissue, microneedles have not been shown to reproducibly penetrate living skin and deliver molecules. The fabrication of the previous microneedles has also been limited in its ability to be tailored to desired microneedle geometries.

The fabrication of microneedles should be accomplished by a process that offers high degree of control over microneedle geometry without sacrificing reproducibility.
Additionally, a scalable process that can generate quantities of microneedles necessary for clinical testing is desirable. A fabrication scheme to create hollow tapered microneedles based on laser micromachining and the LIGA process has been created. Both metals and polymers were investigated as mold materials. The high quality, reproducible control over geometry, and ease of removal of polyethylene terephthalate molds make them the preferred choice.

To ensure the reproducible insertion of microneedles, the insertion force and fracture force of microneedles as a function of geometry was measured. Models to predict these values and the safety margin between the insertion and fracture of microneedles were also developed.

The insertion force of microneedles in the skin of human subjects was measured as a function of microneedle geometry. The relationship between the full interfacial area of the microneedle and the insertion force was found to be linear. This behavior agrees with a fracture mechanics-based model balancing the energy delivered to the skin and the energy necessary to puncture the skin. The puncture toughness of the skin was determined to be 30.1±0.6 kJ/m².

The fracture force of microneedles was measured as a function of microneedle geometry. Fracture force was found to increase with wall thickness, wall angle, and possibly tip diameter. This behavior agrees with analytical and finite element simulation models of fracture force. Both models predict the trends of the data with respect to geometry, but the analytical model was found to have better quantitative agreement with the data.
The purpose of microneedles is to deliver meaningful quantities of therapeutic compounds to living subjects. Well developed microfabrication processes and understanding the mechanical issues microneedles must overcome support this ultimate goal. Microneedles were used to successfully deliver insulin to living diabetic rats. Both pharmacokinetic and pharmacodynamic responses were measured. The blood glucose level of diabetic rats was reduced to 47% of its original value during four hours of insulin delivery through hollow microneedles. The concentration of insulin in the rat’s blood plasma was found to be 5.5±3 μU/ml after 30 minutes of insulin delivery and 12±4 μU/ml after 4 hours of delivery. The delivery of insulin through microneedles into the blood was then predicted by a simple Fickian diffusion model.
1 INTRODUCTION

Conventional drug delivery offers a range of techniques to move therapeutic molecules into the human body. By far the most common and convenient method is oral delivery [Barry, 2001]. While patient compliance is excellent for drugs taken orally, the molecule delivered must be able to withstand the harsh environment of the gastrointestinal tract and be of sufficient potency to remain effective after first pass metabolism. The primary alternative to orally administered drugs is hypodermic injection. Circumvention of the gastrointestinal tract often increases the number of molecules capable of being delivered and reduces the required dosage. Unfortunately the pain associated with injections and often the lack of medical expertise necessary for administering injections results in low patient compliance [Barry et al., 2001; Rosilio et al., 1998]. In addition, the bolus delivery of conventional hypodermics reduces the effectiveness of molecules that would benefit from delivery over long time periods.

Transdermal patches have recently emerged as a third option for drug delivery [1]. These patches deliver drugs through the skin without creating a pathway via puncture and they do not require medical expertise. The molecules are delivered via passive diffusion through the outermost layer of skin, stratum corneum. The stratum corneum acts as the primary barrier to the diffusion of molecules into the deeper tissues and excludes large or strongly charged molecules. Therefore, the number of molecules available for delivery via transdermal patches is severely limited.
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. Microneedles have been demonstrated to create pathways for a wide range of molecules through the stratum corneum and epidermis. 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 (calcein, bovine serum albumin, insulin, latex nanoparticles). Hollow, cylindrical microtubes have similarly been demonstrated to increase the permeability of in vitro human skin by an additional order of magnitude beyond that of solid microneedles for the same molecules (McAllister, 2000).

In this thesis hollow tapered metal microneedles were created to deliver molecules to living subjects. The prior successes of microneedles have occurred in environments that did not accurately represent living subjects (stripped, in vitro tissue). The mechanical issues that must be addressed for successful application of microneedles to living subjects: the force required to insert microneedles into living tissue, and the force needles can withstand before fracturing were measured and modeled. This understanding, and the flexible fabrication scheme developed, was then used to create arrays of microneedles for the delivery of therapeutic molecules to living animals. An example of these arrays is shown in Figure 1.7 with a conventional 27 gauge hypodermic needle for comparison.
Figure 1.2: A hollow metal microneedle array. The needles taper from a tip diameter of 75 µm to 300 µm at the base over their 500 µm length. A conventional 27 gauge hypodermic needle is shown for size comparison.
2 BACKGROUND

2.1 Transdermal Drug Delivery Background

Transdermal drug delivery is an attractive alternative to the most common routes of drug delivery: pills and injections. While both of these methods have demonstrated their capabilities and serve a large number of patients well, they also have a number of drawbacks. First pass metabolism and the harsh environment of the gastrointestinal tract reduces the number of compounds that are available for oral delivery and the effectiveness of those that are available. Conventional injections avoid these issues, but the pain associated with the injection and often the necessity for medical expertise lowers patient compliance.

Transdermal patches avoid many of the problems of injections and pills. Without the need for medical expertise and the simple application of the device patient compliance is not an issue. In addition, by delivering through molecules through the skin, they retain the benefit of injections by circumventing first pass metabolism [Kalia and Gay, 1998]. The large volume of blood flow to the skin (approximately 1/3 of all circulating blood) allows systemic delivery. Finally, without requirement of bolus delivery, transdermal patches can achieve delivery over long periods of time to maintain baseline levels of a drug in the body.
2.1.1 Drugs delivered transdermally

An increasing number of drugs are being delivered by the transdermal route. The most common include clonidine, estradiol, estrogen, fentanyl, isosorbide dinitrate, nethisterone, nicotine, nitroglycerin, scopolamine, testosterone [Finnin, 1999]. All of these molecules are delivered by passive diffusion through the skin. However, given the wide range of benefits offered by transdermal drug delivery, one might wonder why more molecules are not delivered in this fashion. The answer lies in the physical and chemical makeup of the drugs listed and the barrier properties of the skin. The drugs listed are relatively small (<400 Da), potent, lipophilic, and uncharged. These characteristics allow them to pass through the skin's natural defenses against foreign substances. If larger, charged molecules are to be delivered transdermally, the barrier presented by the skin must be diminished or bypassed.

2.1.2 Anatomy of Skin

Transdermal drug delivery of molecules other than those able to diffuse across unaltered skin is a challenging goal. Either the skin must be modified to allow the molecule in or a pathway past the skin's barrier properties must be created. Figure 2.1.1 shows the anatomy of skin. Skin may be roughly described by its three primary layers: hypodermis, dermis, and epidermis. The epidermis, in turn, is stratified into five separate regions (from deepest to superficial): stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. The outermost layer, stratum corneum, is made up of 15-20 layers of “dead” skin cells [Champion et al.]. It is the
stratum corneum (SC) that seals out foreign materials and prevents the diffusion of molecules.

![Diagram of skin anatomy](http://www.thebody.com)

**Figure 2.1.1: Anatomy of skin (reproduced from www.thebody.com)**

In order to allow molecular movement into the skin, a pathway must be created through the SC, such as through the barrel of a needle. As previously discussed, a conventional needle elicits pain during its insertion. This is due to primarily to the depth of the needle's penetration, but is also influenced by the diameter of the needle and other factors such as sharpness. The nerves located in the dermis and epidermis transmit the pain associated with an injection. Reducing the number of nerves contacted during insertion should reduce the pain. This can be accomplished by penetrating more shallowly and/or by passing through a smaller cross section of skin. For this reason, a device capable of creating a pathway in the SC large enough for molecules to pass through and shallow and narrow enough to minimize nerve excitation could allow transdermal delivery without the drawbacks of a conventional injection. This concept forms the basis of microneedle drug delivery systems.
2.2 Evolution of Microneedles

Over the past 15 years, the concept of microneedles for transdermal delivery and other uses has grown dramatically. A wide range of geometries have been created from a number of materials. A majority of the work remains rooted in the initial fabrication medium of silicon. However, as the fabrication tools for other materials has grown, so has the use of these materials, as constituent materials for microneedles. Also, as the number of materials has increased so has the complexity of both the fabrication sequences and the microneedles themselves. The field has grown from the initial work in solid silicon spikes to hollow microneedles with integrated pumps and sensors. When discussing such a broad range of work it is best to begin with the most simple and progress to the more complex.

Perhaps the simplest function of microneedles is to permeabilize objects by piercing. This is applicable to cells, tissues, or non-biological membranes. The goal is generally to create pathways in the target either for the transport of some material into or out of the target. The use of microneedles as opposed to a macroscale device is driven either by the size of the target or the benefit of creating the pathway in a minimally invasive procedure. Applications have included the transfection of cells, transdermal drug delivery, fluid extraction, electrical stimulation and acupuncture.

Inclusion of a hollow lumen in microneedles offers a simple extension of their ability to permeabilize objects. A hollow lumen allows greater transport area within the microneedle and a guided path for the deliverable. Drug delivery is a gain among the applications of this type of device as well as the sampling of biological fluids and tissues.
The specific organs targeted for drug delivery at this point have included the heart, brain, and skin.

The pathways created by microneedles are not limited to the delivery of liquids or biological applications. The conduction of electrical current has been explored with microneedles in their application as electrodes for the skin and brain. Furthermore, the delivery of gases has been investigated in the development of fluidic jets and microfluidic manifolds.

2.2.1 Solid Spikes

As stated above, in their simplest form microneedles are solid spikes. Besides being solid, their unifying characteristics include being very sharp and usually having fairly simple fabrication schemes. In regards to geometry, the side-wall profile of the needle can vary from pyramidal to strongly tapered and these spikes can be made from silicon, polymers, and metals.

2.2.1.1 Silicon

Hashmi et al. (1995) demonstrated one of the most basic designs in their formation of pyramidal silicon spikes, Figure 2.2.1. With the goal of delivering genetic material to cells, the small dimensions (tens of microns in height) and sharp tips (100 nm in diameter) were ideally suited. In addition, these microneedles were created in 3D arrays in order have large numbers of needle/cell interactions.
Figure 2.2.1: Solid silicon spikes used to deliver genetic material to plant and animal cells as well as nematodes [Hashmi et al., 1995].

These structures were used to successfully transfect cells belonging to plants (tobacco leaf cells) [Trimmer et al., 1995], nematodes, and mammals (smooth muscle cells from rat arteries) [Reed et al., 1998]. In addition, these pyramidal needles were also used to penetrate the internal elastic lamina of rabbit iliac artery when their length was increased to ~150 μm. This result supported the goal of the work, which was targeted delivery of anti-restenosis drugs to atherosclerotic vessels.

Henry et al. (1998) used a different approach to create similar silicon spikes for transdermal drug delivery, Figure 2.2.2. The application of microneedles to penetrate the upper layer of skin (stratum corneum) offered the ability to create pathways for molecules that would not normally be able to diffuse through the skin's barrier due to size or water solubility. Additionally, by creating microneedles that could not reach the deep nerve-rich layers of the skin, the possibility of painless needles became a possibility. Since the goal required penetrating a tissue rather than single cells, longer needles and

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higher aspect ratios were required, as demonstrated by the success of longer pyramids in the penetration of arterial tissue. The microneedles were ~80 μm at the base, 150μm tall, and have ~1 μm radius of curvature at the tip.

![Image](image.png)

**Figure 2.2.1: Solid silicon spikes used to increase the permeability of human skin by 3-4 orders of magnitude for calcine and bovine serum albumin (Henry et al., 1998).**

The arrays of microneedles created by Henry were demonstrated to increase the permeability of in vitro human epidermis by 3-4 orders of magnitude in comparison to intact skin for calcine and bovine serum albumin [Henry et al., 1998]. In addition, these arrays of microneedles were reported as painless when inserted into human volunteers [Kaushik et al., 2001].

### 2.2.1.2 Polymers
Polymers have also been used to form arrays of microneedles. In comparison to silicon counterparts, polymer microneedles offer the mechanical advantage of improved resistance to shear-induced breakage. Unfortunately, this comes at the cost of reduced sharpness at the tip of the microneedle due to the low modulus and yield strength of the polymers. Chemically, biodegradable polymers allow additional functionality of the microneedles themselves. Rather than simply piercing the skin to create pathways for therapeutic molecules, the microneedles themselves become drug depots implanted in the skin.

Park et al. (2003) demonstrated the fabrication of biodegradable polymer microneedles, Figure 2.2.3. The biodegradable microneedles were shown to possess the mechanical robustness and sharpness to penetrate in vitro human skin. Calcein and bovine serum albumin were loaded into the needles as model drugs and their release rate over five days was reported.

Figure 2.2.3: Biodegradable polymer microneedles. The needles were loaded and successfully released calcein and bovine serum albumin (Park et al., 2003).
2.2.1.3 Metals

Finally, spikes were fashioned from metals. Metals offer superior strength relative to polymers and reduced manufacturing cost relative to silicon. The wide range of metals available allows the mechanical and chemical properties as well as cost to be tailored to the desired application. A number of metal spike structures have been demonstrated, one of which is demonstrated in Figure 2.2.4 [McAllister, 2000].

![Figure 2.2.4: Solid metal spikes created from polymer molds. Solid metal structures have been used to deliver DNA and vaccines [McAlister, 2000].](image)

Solid metal spikes have been used to administer genomic therapies (antisense oligodeoxynucleotides) [Lin et al., 2001] as well as intact cutaneous antigens (ovalbumin) [Mariano et al., 2002] to animal models. The use of the spikes allowed the delivery of biologically active quantities of the nucleotide by either passive diffusion or in combination with iontophoresis. The antigen delivery, on the other hand, was accomplished with a coating of the antigen applied to the needles prior to insertion. The
immune response was found to be 50 times greater than that achieved with subcutaneous or intramuscular delivery.

2.2.2 Hollow Structures

The inclusion of a hollow lumen in a microneedle structure expands its capabilities dramatically. In regards to the delivery of fluidic in biological settings, the ability to deliver larger molecules and particles in larger quantities is a distinct advantage. In addition, the delivery of material is no longer dependent on passive diffusion, but may be driven by convective transport. The deliverable is also now protected from the surrounding environment and experiences only the storage device, the microneedle lumen, and the targeted site. This prevents the cross contamination of the deliverable and the surroundings, and insures highly localized delivery. Also, the movement of fluids is no longer confined to delivery, but may also be expanded to the withdrawal of material. Finally, the delivered material is no longer limited to liquids, but can be expanded to gases, or even energy (light). As one might expect, these benefits come at the cost of increased fabrication complexity.

2.2.2.1 Silicon

The additional complexity required to generate hollow structures lends itself to a material with a wide range of processing tools. As the base material for a large majority of microelectronic and microelectromechanical devices, silicon is just such a material. The most logical technique for the inclusion of a lumen in the silicon spikes presented is the addition of an etching step to form a fluidic channel.
Two of the simpler extensions of previously described work were found to be effective for both withdrawal and delivery of fluids. An extension of the solid silicon pyramids of Hashmi was found to effectively withdraw blood through the lumen by capillary action, Figure 2.2.5 [Gardeniers, 2002]. On the other hand, an extension to the solid silicon spikes of Henry was found to deliver both large particles (0.7 μm spheres) and dye (Lucifer Yellow) to chicken thighs under pressure driven flow, Figure 2.2.6 [Stoeber et al., 2000]

Figure 2.2.5: Hollow silicon microneedles used to withdraw blood by capillary action [Gardeniers, 2002].
Two other microneedle geometries which represent similar processing, but the opposite ends of the spectrum in regards to lumen placement were reported by McAllister et al. (1999), Figure 2.2.7 and Griss and Stemme (2002), Figure 2.2.8. As in the previous work, both of these shapes begin with a lumen etched through the wafer. McAllister then created a tubular needle around the center lumen to form hollow microtubes. Griss, on the other hand, created an intricate, cross-shaped needle design with a sharp solid tip and side openings for the lumen. The silicon microtubes have demonstrated the ability to increase the permeability of human epidermis by orders of magnitude greater than that possible with solid spikes. They have also been combined with microfluidic channels to create an air/fuel manifold for a miniature combustion system [Matta et al., 2001].
Figure 2.2.7: Hollow silicon microtubes used to increase the permeability of skin by orders of magnitude for a wide range of compounds [McAllister, 2000].

Figure 2.2.8: Silicon microneedles with side opening ports to prevent clogging at the needle tips [Griss, 2002].
The hollow silicon structures described up to this point have been created in three dimensional arrays, out of the substrate plane. An important shift in geometry is accomplished by creating the needles in the plane of the substrate. In this case the needle length is no longer limited to the thickness of the wafer, but instead by the width of the wafer (typically two orders of magnitude larger). In addition, working in the plane of the substrate simplifies the inclusion of additional electrical or mechanical components in the needle. These benefits are mitigated by the inability to form dense three dimensional arrays. The microneedles are limited to single devices, or two-dimensional arrays.

Both Chen and Wise (1997) and Lin and Pisano (1999) have detailed the fabrication of in-plane silicon needles for applications to neural measurement and drug delivery. The design of these microneedles incorporated a fluidic port on the side of the needle as well as area for the inclusion of pumping and/or sensing circuitry at the base of the needle, Figures 2.2.9 and 2.2.10. Chen and Wise demonstrates the ability to deliver both kainic acid and γ-aminobutyric acid to the nervous system of guinea pigs with this design.
Figure 2.2.9: Hollow silicon needle created in the plane of the substrate for neural drug delivery [Chen and Wise, 1997]

Figure 2.2.10: Hollow silicon microneedle for neural drug delivery with area available at its base for circuitry and micropumps [Lin and Pisano, 1999]
Similar structures were also created by Talbot and Pisano (1998), Figure 2.2.11. In this case the microneedles were created using an innovative polysilicon molding process. The resulting structure shared the common geometries of millimeter length scale and a hollow lumen.

![Microneedles](image)

Figure 2.2.11: Polysilicon microneedles created using the "polymolding" process [Talbot, 1998].

These in-plane silicon microneedles have all shared the common features of millimeter-scale lengths, sharp tips, and smooth tapers. Oka et al. (2001) varied this set of parameters by including a serrated taper in hopes in improving the insertion capabilities, Fig 2.2.12.
2.2.2.2 Polymers

As in the case of solid silicon spikes, the next materials for fabrication consideration are polymers. A very limited field of work has been investigated for hollow polymer structures. The primary limitation of this work is the basis of most polymer fabrication methods in molding processes. Molds that contain very high aspect ratio components, such as a solid rod to form the lumen in a needle, are quite difficult to release without damaging the parts due to the high degree of surface area between the mold and part.

An exception to the preceding paragraph is work by Stupar and Pisano (2001), Figure 2.2.13. In this case, a conformal coating of Parylene was deposited on the exterior, and in some cases interior of the silicon microneedles. The silicon microneedles were fabricated in similar fashion to the work previously described. This silicon/polymer hybrid can be used as fabricated to take advantage of the toughness of the polymer, or
have the silicon removed completely leaving behind a Parylene microneedle. The hybrid device offered the advantage of an encapsulating layer around the needle in case of failure. When the silicon microneedle was fractured, the exterior coating allowed fluid to continue pumping without leakage and permitted the withdrawal of the broken needle without leaving residual silicon in the skin. Pure Parylene needles, on the other hand, did not fracture even when bent to 180° angles. This feat was possible due the low modulus of the polymer, but also hindered insertion of pure Parylene needles due to buckling at low loads.

![Figure 2.2.13: Hollow polymer microneedles. The needles were created by coating silicon structures with Parylene and then dissolving the silicon (Shapar and Pisano, 2001).](image)

2.2.2.3 Metals

The final forms for hollow microneedles are those made of metal. As in the case of the hollow silicon microneedles, metal microneedles have been created both in three-dimensional arrays out of the plane and in two-dimensional in-plane arrangements.

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Three-dimensional arrays were first reported by McAllister et al. (1999) and later reproduced by Liu et al. (2001). Both works were focused on transdermal drug delivery and physiological fluid sampling, and generated similar structures, although the metal used by McAllister was nickel-iron while Liu used nickel-cobalt. Figure 2.2.14. These structures had a cylindrical cross-section as opposed to prior work with tapered structures.

Figure 2.2.14: Hollow metal microtubes. Microtubes were demonstrated to increase the permeability of human epidermis by four orders of magnitude to calcine, bovine serum albumin, and insulin [McAllister, 2000].

McAllister (2000) demonstrated that these structures could be used to increase the permeability of human epidermis by four orders of magnitude to calcine, bovine serum albumin, and insulin. Polystyrene nanospheres of 50 and 100 μm diameter were able transported through the epidermis using these hollow structures. Nanospheres were not capable of moving across the skin when used in conjunction with solid microneedles left in
the skin. However, the hollow lumen of these metal structures made the transport possible while needles were still inserted in skin.

Hollow metal microneedles for localized drug delivery were also created by Brazzle (1998). In this case the microneedles were in-plane and multiple needles were fluidically connected. These structures were laser modified to include multiple outlet ports along their length, Figure 2.2.15 [Brazzle, 1999].

![Image: Hollow metal microneedle with multiple outlet ports created in the plane of the substrate.](image)

**Figure 2.2.15:** Hollow metal microneedle with multiple outlet ports created in the plane of the substrate [Brazzle, 1998].

### 2.2.3 Integrated Components

Beyond 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 use of microelectronics fabrication techniques to create microneedles makes the inclusion of electronic components an obvious choice. In
addition, mechanical components from the MEMS community make ready companions for microneedles. The additive components that offer the greatest potential include electrical and chemical sensors as well as pumps.

2.2.3.1 Sensors

The simplest form of an electrical sensor can be created by adding electrical conductivity to a microneedle. This requires the addition of an electrically conductive material to one of the microneedle designs previously described. The metal microneedles make obvious choices for this work, however it was silicon microneedles that were first pressed into the duty as electrical sensors. Although not the first to create electrical sensors from microneedles, Griss and Stemme (2001) created the simplest form of the concept. They coated an array of solid silicon spikes with a layer of silver-silverchloride to create an electrode for the measurement of biopotentials, Figure 2.2.16. These sensors were demonstrated not only to have reduced skin impedance relative to conventional gel electrodes, but also to be capable of high quality electroencephalographic measurements.
Figure 2.2.16: Solid silicon microneedles coated with Ag/AgCl to create spiked electrodes [Grisw, 2001].

The manifold nature of microneedle arrays also suggests that each microneedle could serve as an electrode if it were electrically isolated from its neighboring needles. These devices would excel in applications that require the stimulation or recording of multiple sites within a small region. One such case is the stimulation of the visual cortex of subjects in order to restore sight, which was the goal of Campbell (1991) during their creation of such electrodes, Figure 2.2.17.
In addition to electrical sensors, chemical sensors have been incorporated into microneedle designs. There is a myriad of chemical species that can be sampled to allow, or improve upon, the diagnosis and treatment of disorders. One example of this work was the detection of potassium and hydrogen ions (pH) in cardiac tissue during cardiac surgery. Zine (2000) created solid silicon microneedles with pH, K⁺, and temperature sensors incorporated, Figure 2.2.17. The sensors were demonstrated to function over a wide range of concentrations in vitro.
2.2.3.2 Pumps

The development of hollow microneedles allows the use of pressure driven flow, as opposed to the diffusion-limited delivery of solid devices. However, in most cases the pressure driven flow through microneedles has been demonstrated by using macro-scale pumps. If the success of microneedles is tied to macro-scale pumps, some of the primary advantages (e.g., portability and ease of use) of the device are lost.

Soeber and Liepmann demonstrated the most basic form of an integrated pump in 2002. Their hollow silicon microneedles, whose fabrication has been previously discussed (Figure 2.2.6), were combined with a rudimentary membrane pump. Essentially, the backside of the microneedle array was capped with a poly-(di-methyl siloxane) reservoir. This reservoir was filled using a conventional hypodermic needle and when the top of the reservoir was deflected, it acted as a membrane pump by driving the filled fluid through the microneedle lumens.
A more complex form of pumping was demonstrated by Zahn (2001). A bubble-driven pump was incorporated with the hollow silicon needles fabricated by the "polymolding" process described earlier (Figure 2.2.11). This pump was fabricated in-plane with the microneedle and therefore allowed easy fluidic connection. The pump was driven by the expansion of a thermally generated bubble created by resistive heaters. As the bubble expanded, the fluid in the chamber was displaced. The fluid flow was rectified by a series of directional check valves to avoid withdrawal of fluid during the collapse of the bubble. After bubble collapse, a new bubble was generated and the pumping cycle continued. Initially, the pump was not able to generate enough pressure to overcome the surface tension of droplets at the tip of the needle during open-air operation. However, when the tension was diminished by wetting the needle tip, net flow rates of 1 μl/s were observed. The heaters driving the pump were demonstrated to operate for 18 hours (15,000 cycles) without failure. Although the flowrate is relatively low, the concept demonstrated here is an important first step to integrated pumping devices.
2.3 Microfabrication Background

Microelectromechanical systems (MEMS) are systems that have either mechanical or electrical (or both) devices, typically containing sub-millimeter feature sizes. MEMS first began to emerge from the microelectronics industry in the 1960’s when sensors were integrated with existing circuits. Throughout the 1970’s an evolution of fabrication techniques to create and integrate mechanical and electrical systems on the microscale occurred. In the 1980’s the field of MEMS became distinct division from microelectronics and commercial products became available. In 1982 the first mass produced MEMS products (automotive pressure sensors) were released [Kovacs, 1998]. It was at this point that pressure sensors, flowmeters, and accelerometers became common products, and industrial and government funding for research in the area became a reality [Senturia, 2001].

The MEMS products that create the bulk of the market include: pressure sensors, accelerometers, microgyros, ink jet nozzles, and hard disk drive heads. During the late 1990’s the sale of MEMS devices increased 15-20% per year, with approximately 3 billion dollars in 1999. This trend is expected to continue for the next several years. [System Planning Corp, 1999].

2.3.1 General fabrication

In their most basic form, most lithography-based MEMS fabrication processes are either additive or subtractive. One builds a block by either: (i) patterning and masking a region for deposition, depositing the material and removing the mask, or (ii) depositing a
material, masking a region for removal, and stripping away the deposited material. On an even more fundamental level, the processes include deposition of materials (additive), etching of materials (subtractive), and the enabling process of patterning. There are a myriad of techniques, as well as materials, available for these three elementary steps, however only the processes most relevant to the fabrication of hollow metal microneedles are discussed here.

2.3.2 LIGA

LIGA is a German acronym that stands for Lithographie, Galvanoformung, and Abformung. This translates to lithography, electroplating, and molding and was first introduced by E.W. Becker et al. in 1986, Figure 2.3.1. In general the process is to form a mold using lithography (b), electroplate into this mold to form a metallic structure (c), and then to remove the mold and reveal a completed structure (d). If the end result of the fabrication is a metal part the fabrication ends here. If the desired parts are polymeric, the metal structures in (d) serve as a mold for the polymeric parts. Polymers may be directly cast, embossed, or injection molded into the metal mold.
Figure 2.3.1: The LIGA process. A substrate is coated with a definable polymer (A) and is defined in a desired shape by lithography (B). Metal is then electroplated in the areas cleared by the lithography step (C) and the polymer is removed (D). The process may end here or may be filled with polymer to form a replica of the pattern defined in step (B).

In the past this was typically accomplished using polymethyl methacrylate and X-ray lithography, however, this technique was later broadened to include many other types of lithography and mold materials. In these LIGA-like processes, the general fabrication scheme remains the same, but the mold material may include any of a wide range of definable polymers (e.g. polyimides, epoxies, phenolic resins) [Ehrfeld, et al., 1994; Menz, et al. 1991]. The mold material then defines the lithography technique used to pattern the desired topology (electron beam, UV, extreme-UV) [Lawes, 1993; Qu et al., 1999]. After the formation of a mold, the metal structure is electroplated and released.

The ability to build high aspect ratios out of the plane of the substrate is a marked advantage for the LIGA process in the creation of MEMS devices. Where standard lithography offers patterning in the lateral dimension, the LIGA process offers projection in the vertical direction. The applications of the method reach into almost every area of MEMS [Baxter, 1995].

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2.3.3 Laser Micro-Machining

The light generated by a laser, or light amplification by stimulated emission of radiation, source is nearly monochromatic and highly coherent. The emission of light is caused by the excitation and relaxation of chemical species between metastable and stable energy levels [Young, 1992]. Two general classes of laser are of interest in this context. Gas-charged chambers with excitation electrodes along the length of the chamber generate a beam with a relatively uniform energy distribution across the beam itself. Their operational frequency is determined by the ability to charge and discharge capacitors across the electrodes. The halogen species used in the chamber are eventually depleted and can be refreshed when the emission intensity drops below a satisfactory level. Solid state lasers, on the hand, have a lamp (e.g. Kr or Xe lamps) exciting a rare earth crystal rod (e.g. Nd, Y, Li, F) to generate their emission. This can be a continuous emission, or be pulsed with the use of a switch device (e.g. crystal with variable index of refraction). These systems generate a Gaussian energy distribution and are capable of much higher operational frequency than the gaseous lasers.

One of the many applications of interest for lasers is their ability to ablate materials that absorb the wavelength of the laser wavelength [Miller, 1994]. Of particular interest were the first reports of micromachining using ultraviolet (UV) excimer lasers [Kawamura, 1982; Srinivasan, 1982]. For many devices laser ablation presents an attractive alternative to the typical photolithography, wet development, and etching process common in the microelectronics industry. The ability to create three dimensional structures of similar quality in a single, dry step is often reason enough to adopt laser fabrication techniques [Ricci, 1999]. The primary disadvantage of laser
patterning is the serial nature of the process. Where lithography offers batch-fabrication, laser ablation requires each feature to be patterned individually.

The first large scale incorporation of laser machining in microelectronics applications was in the drilling of vias for packaging applications. Siemens introduced the process in their multichip module in 1988 and it is now regarded as the most versatile, reliable, and high yield technology for creating the microvias [Gower, 1999]. Since then, the impact of laser machining on the MEMS community has been largely in the creation of ink jet printer heads. Most ink jet printer heads sold currently are excimer laser drilled and this accounts for $400 million in sales annually [Gower, 1999; System Planning Corp, 1999].

Beyond drilling holes, the versatile nature and high quality of the parts machined by lasers makes them natural tools for the MEMS community. Their application is just beginning to appear in the literature [Maeda et al., 1994; Yamagata, et al., 1996; Akedo, et al., 1997]

2.3.4 Electrodeposition

Electrodeposition is the deposition of a material initiated and propagated by an electrochemical reaction. Most commonly the term is used to identify the deposition of various metals on to a substrate via electroplating. However, the more general term can refer to the deposition of organics or inorganics by reactions driven by externally applied potentials (electroplating) or by in situ potential generated by chemical reactions (electrode-less, or electroless plating).
Hollow metal microneedles are formed by the electrodeposition of nickel into molds. This includes electroplating nickel to form the microneedle itself, and electroless plating of seed layers onto polymer molds.

2.3.4.1 Electroplating

The general principle guiding electroplating is the reduction of a metal species onto a desired part. The part is immersed in an electrolytic bath and an external power source supplies electrons for the reduction to occur. The anode can serve as both a electrode for carrying the current as well as a source to replenish the ionic species in the electrolyte bath.

Böttger created the first practical nickel electroplating bath in 1843 [Schlesinger and Paunovic, 2000]. However, the Watts formulation introduced in 1916 remains the dominant bath in use for nickel electroplating. The bath is a solution of nickel sulfate, nickel chloride, and boric acid and the reaction governing the deposition is:

\[ \text{Ni}^{2+} + 2e^- \rightarrow \text{Ni}^{0} \]

As previously described the \( \text{Ni}^{2+} \) species in the bath are reduced to nickel at the cathode, or substrate. The anode is typically nickel foil which is dissolved from its ground state to replenish the ionic species consumed in the bath. This process is illustrated in Figure 2.3.2 and typical operation conditions are shown in Table 2.3.1 (adopted from Schlesinger and Paunovic, 2000).
Figure 23.2: 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.
2.3.4.2 Electroless Plating

Electroless plating occurs in a manner similar to electroplating as described in section 2.1.3.1. However, rather than using an external power source to supply the electrons necessary for the reduction of ionic species, a chemical reducing agent is present in solution. The charge transfer that occurs between the reducing agent and the ionic species eliminates the need for an external source of electrons.

Electroless plating of nickel was invented by Brenner and Riddell in 1946 [Brenner, 1946]. The process used sodium hypophosphate as the reducing agent and this continues to be one of the primary reducing agents used today. At the expense of toxicity, formaldehyde has also been used extensively due to increased stability in solution.

An additional area of interest with regards to electroless plating is the ability to plate onto insulating surfaces since the necessity of the substrate serving as an electrode is obviated. In this case, the surface must be sensitized and catalyzed with some metal species prior to electroless plating. A typical sequence for plating onto plastics is the immersion of the sample in a sensitizing solution (e.g. acidic Sn), followed by catalyzing the surface with Pd (reduced by the Sn species). The catalyzed sample can then be plated. The reaction scheme of this process is:

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Table 2.5.1: The operating conditions of a semi-bright Watts nickel bath (Schlesinger and Faunovic, 2000).

<table>
<thead>
<tr>
<th>Operation condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel Sulfate Concentration</td>
<td>300 g/L</td>
</tr>
<tr>
<td>Nickel Chloride Concentration</td>
<td>25 g/L</td>
</tr>
<tr>
<td>Boric Acid Concentration</td>
<td>45 g/L</td>
</tr>
<tr>
<td>pH</td>
<td>3.5 to 4.5</td>
</tr>
<tr>
<td>Temperature</td>
<td>54°C</td>
</tr>
<tr>
<td>Current Density</td>
<td>3 to 10 A/dm²</td>
</tr>
</tbody>
</table>
Pd^{2+} + Sn^{2+} \rightarrow Sn^{4+} + Pd^{0}

Typical formulations for these baths are listed in tables 2.2 through 2.4 (adopted from Schlesinger and Paunovic, 2000). Tables 2.3.2 and 2.3.3 are the formulations for stock solutions of the sensitizing bath and catalyzing bath, respectively. The formulation for the operating baths are 0.5 mL of stock in 160 mL water for the Sn sensitizing bath, and 1.0 mL of stock in 200 mL of water for the Pd catalyzing bath.

Table 2.3.2: Composition of the stock solution for a tin sensitizing bath. 0.5 mL of this stock solution is combined with 160 mL of water to create the sensitizing bath (Schlesinger and Paunovic, 2000).

<table>
<thead>
<tr>
<th>Species</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stannous Chloride</td>
<td>10 g</td>
</tr>
<tr>
<td>Hydrochloric Acid</td>
<td>10 mL</td>
</tr>
</tbody>
</table>

Table 2.3.3: Composition of the stock solution for a palladium catalyzing bath. 1.0 mL of this stock solution is combined with 200 mL of water to create the catalyzing bath (Schlesinger and Paunovic, 2000).

<table>
<thead>
<tr>
<th>Species</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palladium Chloride</td>
<td>10 g</td>
</tr>
<tr>
<td>Hydrochloric Acid</td>
<td>10 mL</td>
</tr>
</tbody>
</table>

Table 2.3.4: The operating conditions of an electrolytic nickel bath (Schlesinger and Paunovic, 2000).

<table>
<thead>
<tr>
<th>Operation Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Hypophosphate</td>
<td>10 g/L</td>
</tr>
<tr>
<td>Nickel Chloride Concentration</td>
<td>30 g/L</td>
</tr>
<tr>
<td>Hydroxycetic Acid</td>
<td>35 g/L</td>
</tr>
<tr>
<td>pH</td>
<td>4 to 6</td>
</tr>
<tr>
<td>Max. Temperature</td>
<td>100° C</td>
</tr>
</tbody>
</table>
3 METHODS

3.1 Microfabrication Methods

The microfabrication background discussed earlier in chapter 2 gave an introduction to the techniques used to create solid and hollow metal microneedles. The details of the fabrication sequence for each type of microneedle will be described here. Hollow microneedles were created using a modified LIGA process incorporating laser micromachining to form the mold. Solid microneedles were made directly from metal shim stock using direct writing techniques.

3.1.1 Hollow Metal Microneedle Methods

The process to create tapered, hollow microneedles was quite similar to the LIGA process discussed in section 2.3.2. Initially a mold was created to mimic the desired shape of the microneedle. This required the formation of a tapered through-hole in a substrate. A through hole is required in order to generate a hollow structure with an open tip. A blind via mold would also generate a hollow structure, but the lumen would not be open. The substrates investigated here included both metals and polymers. After the formation of the mold, it must be masked to provide plating only on the inner region of the mold. In the case of conductive molds (metal), this means that the backside of the mold must be passivated, while an insulating mold (polymer) must have a conductive seed layer deposited on the topside and sidewalls of the through-hole. Both sputtered and electrolessly-plated seed layers were investigated. After masking, nickel was
electroplated on the topside and sidewalls to form the hollow metal microneedle. Finally, the mold was selectively etched to release the microneedle structure. This fabrication sequence is summarized in Figure 3.1.1.
Figure 3.1.3: Flowchart of hollow microneedle fabrication. The mold is laser machined with the desired geometry (a) and then masked with either a seed layer for a polymer mold, or a passivating layer for a metal mold (b). The masked mold is electroplated (c) and the needle is released by dissolving the mold (d).
3.1.1.1 Mold Formation Methods

The LIGA process discussed in section 2.3.2 is capable of generating high aspect ratio molds for the formation of a wide range of devices. UV exposure of SU-8 photoresist has been previously used to form microtubes. However, the binary nature of the photoresist, which was actively sought for microelectronic fabrication sequences, hinders the formation of tapered molds for microneedles.

Laser micromachining was used to form molds in materials other than conventional photoresists. The non-uniform distribution of energy in a laser beam results in the non-uniform removal of material from a drilled substrate. By tailoring the energy distribution the part experiences, the formation of tapered molds in both metal and polymer substrates has been accomplished.

3.1.1.1.1 Metal Mold Methods

Hollow metal microneedles were created from metal molds. A laser was used to drill tapered holes through both copper and titanium. Copper was chosen for its excellent electrical conductivity which results in high quality electroplated structures. Titanium was chosen for its ease of removal after the formation of nickel microneedles.

A substrate’s ability to absorb a particular wavelength defines the choice of a laser for the drilling process. In general, metals tend to absorb well in the infrared range. The absorption of energy in this case is a thermal event. The localized heating of the substrate vaporizes the metal. The vaporized species are either carried away by an external gas flow or are redeposited on the substrate. An infrared (IR) laser with a
wavelength of 1047 nm (Resonetics Maestro, Nashua, NH) was used to form metal molds for hollow microneedles. The laser was operated at 1000 Hz, and the energy density of the beam was 100 J/cm².

The shape of holes drilled by pulsing the laser is determined by the energy distribution in the beam. Regions of the beam that have high energy will ablate more material than regions with lower energy. The Gaussian energy distribution of the solid state IR laser used had a greater fluence at its center than at the edges of the beam and therefore a pulsed beam resulted in holes with significant taper, as shown in Figure 3.1.2. The figure shows that a Gaussian energy distribution across the beam (A) results in a similarly tapered hole through a material (B). If this is extended to drilling a through-hole, the geometry of the hole will have the same degree of taper (C). A typical geometry included an entrance diameter 120 μm, exit diameter of 30 μm, and length of 250 μm. The length of the hole was defined by the substrate thickness, and the entrance diameter and exit diameter were determined by the beam size (i.e. beam transmission).

Figure 3.1.2: Solid-state (IR) laser energy distribution's effect on drilled hole geometry. In A) the Gaussian energy distribution of the IR laser resulted in drilled geometries having the same shape B). When a through-hole was created, this shape is mimicked in the final hole geometry, C).
Material ejected by the plasma flume within the drilled region tended to redeposit on the top surface of the substrate. Due to the thermal nature of the ablation process in metals, the ejected material is at high temperature and readily bonds it to the surface of the substrate. Any redeposition of drilled material on the surface of the mold was removed with mechanical polishing (SIC 2000 grit, Leco, St. Joseph, MI).

3.1.1.2 Polymer Mold Methods

Hollow metal microneedles were created from polymer molds. An ultraviolet (UV) laser was used to drill tapered holes through polyethylene terephthalate (Mylar, Dupont, Wilmington, DE) and polyimide (Kapton HN, Dupont, Wilmington, DE). Mylar was chosen for its ease of removal after microneedle formation, and its relatively low cost. Kapton was chosen for its excellent thermal stability and its compatibility with other microfabrication processes.

Polymer molds were drilled with an excimer laser at 248 nm (Resonetics Micronmaster, Nashau, NH). The laser was operated at 200 Hz and an energy density of 2.0 J/cm². In contrast to the thermal overload method of material removal exhibited by an IR laser in metals, the wavelength of an UV laser is sufficiently energetic to break the chemical bonds of most materials. In this case small volumes of the substrate are ablated with each pulse and ejected by the plasma flume generated at the part.

As in the case of the IR laser, the geometry of drilled features was defined by the energy distribution in the beam. In the case of the KrF laser used in this application, the distribution was a "top hat" profile. Therefore, a lesser degree of taper was achieved in through holes created by pulsing the laser than when using the IR laser as shown in
Figure 3.1.3. The figure shows that a top hat energy distribution across the beam (A) results in a similarly tapered hole through a material (B). If this is extended to drilling a through-hole, the geometry of the hole will have the same, minimal, degree of taper (C).

\[ \text{Figure 3.1.3: Gaseous (UV) laser energy distribution’s effect on drilled hole geometry. In A) the “top hat” energy distribution of the UV laser resulted in drilled geometries having the same shape B). When a through-hole was created, this shape was mimicked in the final hole geometry, C).} \]

In order to achieve the desired taper of drilled holes, the process of simple pulsed laser operation was modified. To form a conical needle, it was desirable to have a circular beam with a region of high energy, or high fluence, at its center with a region of lower energy at its rim. Since changing the distribution of the energy in the beam was not readily achievable, the distribution of energy was modified at the substrate. This was achieved by altering the drilling program to allow trepanning, or tracing, of a circular path whose diameter was less than the diameter of the circular beam. The difference between the beam diameter and the trepanned diameter created a region of beam overlap in the center of the pattern (Figure 3.1.4). In this figure a 175 μm diameter beam was trepanned in a diameter of 125 μm. This overlap resulted in higher fluence at the center of the pattern than the edge, and a correspondingly tapered hole. In the case of Figure 3.3, this resulted in 300 μm diameter entrance hole and a 50 μm tip exit hole.
Figure 3.1.4: A 175 μm beam trepanned in a pattern whose diameter was only 125 μm. The difference diameters results in an overlap region 50 μm in diameter at the center of the pattern. The overlap region defines the exit hole of the tapered hole, while the entrance hole is defined by outside edges of the beam diameter during the trepanning.

The diameter of the beam and the trepanned pattern were calculated by simple geometry from the desired hole geometry as shown in Eq. 3.1.1 and Eq. 3.1.2:

\[ d_b = \frac{d_e + d_x}{2} \]  

(3.1.1)

\[ T = d_b - d_e \]  

(3.1.2)

where \( d_b \) is the beam diameter, \( d_e \) is the diameter of the entrance hole, \( d_x \) is the diameter of the exit hole, and \( T \) is the diameter of the trepanned pattern. These values, and the thickness of the substrate which determines the length, results in the final microneedle geometry. A typical geometry is a 250 μm diameter entrance hole and a 50 μm exit through a 500 μm sheet.

In instances in which the desired polymer thickness was not available from a vendor, multiple sheets of polymer were laminated to form the total desired thickness. Recent adhesive-less lamination techniques (Cirlex, Fralock, Canoga Park, CA) for
polyimide have made this unnecessary for Kapton. However, Mylar is limited to a thickness of 360 μm without special orders of mass quantities. The lamination of Mylar was accomplished with the use of a thin adhesive (Pyralux LF1500, Dupont, Wilmington, DE). This adhesive is available in 12 μm thickness and is heat- and pressure-sensitive. The thin sheet form and ability to bond at low temperatures made LF1500 ideal for Mylar applications due to its low melting temperature. To form the laminate, two sheets of Mylar were separated by an equally sized sheet of LF1500. The laminate was then bonded at 225°C and 300 psi for two hours (Vantage, Wabash MPI, Wabash, IN). The press was cooled to room temperature while pressure was maintained to ensure the planarity of the samples.

3.1.1.2 Mold Masking Methods

The electroplating of metal into the mold was controlled by masking regions of the mold for deposition. In the case of metal molds, a passivation layer was deposited on the backside of the mold to prevent electroplating. In the case of polymer molds, a conductive seed layer was deposited on the topside and sidewalls of the mold to allow electroplating. The seed layer was deposit either by direct current sputtering or electroless plating.

3.1.1.2.1 Passivation Layer

To prevent electroplating onto the backside of metal molds, a passivating layer of silicon nitride was deposited. The deposition occurred in a plasma enhanced chemical
vapor deposition system, PECVD (Multiplex CVC, STS, Redwood City, CA). The gas flow rates were 2000 sccm of 2% SiH₄ and 30 sccm NH₃. The system operated at a pressure of 650 mT and a temperature of 300°C. The power source generated 20 W at 13.56 MHz. Ninety minutes of deposition at these conditions resulted in a 1 μm thick layer of silicon nitride.

3.1.1.2.2 Direct Current Sputtering

To allow electroplating on the top side and sidewalls of the polymer mold, a conductive seed layer of titanium-copper-titanium was deposited. The deposition was made by a direct current sputtering system (CVC 601, Rochester, NY). The first titanium layer serves as an adhesion layer between the polymer and the copper. The copper layer is the metal that will actually be electroplated. The final titanium layer prevents the oxidation of the copper layer during processing or storage. The titanium was deposited from a 3” target at 300 W while the copper was deposited by a 8” target at 2000 W. The depositions occurred at a pressure of 6 mT. Deposition times of 350 s and 650 s resulted in 35 nm thick titanium layers and 650 nm thick copper layers.

3.1.1.2.3 Electroless Plating

To allow electroplating on the top side and sidewalls of the polymer mold, a conductive seed layer of nickel was deposited. The deposition was made by electroless
plating, the principles of which are described in section 2.3.4.2. The mold was first sensitized at room temperature for 2 minutes in the tin bath. The mold was rinsed for 30 seconds under DI water and then catalyzed at room temperature for 2 minutes in the palladium bath. The mold was then rinsed for 30 seconds under DI water and placed in the electroless nickel solution. Plating occurred at 65º C, with the application of ultrasound for the duration of the plating. The pH of the bath was 5.0. Plating for 3 minutes under these conditions resulted in a seed layer thickness of 450 nm.

### 3.1.1.3 Electroplating Methods

Nickel was electroplated into a masked mold to create a microneedle. The principles of electroplating are discussed in section 2.3.4.1. The electroplating was conducted using a Watts formulation bath (Technic, Cranston, RI).

When plating metal molds, any native oxide must be removed prior to electroplating to ensure uniform results. The rapid oxidation of titanium in atmospheric conditions makes electroplating directly onto the mold difficult. The oxide must be removed (or at least thinned) prior to deposition. Titanium molds were bathed in Buffered Oxide Etch (Brenntag, East Point, CA) for approximately 15 seconds and the mold was rinsed in DI water. The evolution of hydrogen bubbles when the etchant reached the base metal indicated the complete removal of the oxide. The removal of cupric oxide on copper molds was accomplished in a similar fashion with the use of 10% hydrochloric acid in place of the buffered oxide etch.

Polymer molds which have had seed layers sputter deposited were protected from oxidation by the upper layer of titanium. This layer was removed using dilute
hydrofluoric acid (2%) just prior to electroplating to expose the copper layer and then rinsed in DI water.

After the mold was prepared for plating, electrical contact was made to the mold by an alligator clip. The counter electrode, nickel foil, was connected in a similar fashion to the power supply (E3611A DC Power Supply, Hewlett-Packard, Houston, TX). The paired electrodes were then submerged in the heated (54°), agitated (300 rpm) electroplating bath. The voltage was adjusted to maintain a constant current density of 10 mA/cm².

Plating proceeded for 5 minutes under these conditions to form the initial “strike” layer on the mold. The mold was then removed from the bath and rinsed in DI water. A sheet of powder coating tape (Shercon, Santa Fe Springs, CA) was then placed across the backside of the mold to prevent any over-plating at the microneedle tips due to current crowding. The sample was placed back in the electroplating bath and plating was carried out to completion. The duration of plating defined the thickness of the microneedle walls and the array's base. The typical plating time was 60 minutes to generate a 10 μm layer of nickel.

In instances in which the sample size exceeded 5 cm², additional steps were taken to ensure uniform distribution of current, and therefore plated nickel, across the mold surface. After the remove of oxides, or protective layers, the sample was rinsed in DI and dried with nitrogen. A perimeter of copper tape was then applied to the mold to create a pathway for current during plating. This pathway was then insulated with powder-coating tape (Shercon, Santa Fe Springs, CA) to protect it electroplating in the bath. This
tape retains adhesion in high temperature and aqueous environments such as electroplating baths.

3.1.1.4 Mold Release Methods

The final step of hollow metal microneedle fabrication is the release of the completed needle from the sacrificial mold. The method of mold removal was completely dependant on the mold material and the ability to remove it selectively with regards to the microneedle (nickel).

Titanium molds were dissolved in 30% hydrofluoric acid while copper molds were dissolved in a saturated solution of cupric sulfate in ammonium hydroxide. The titanium molds were removed in approximately 3 minutes for 250 μm, while the copper molds generally took greater than 24 hours for the same thickness.

Mylar molds were removed in a concentrated caustic solution (1 N, NaOH, boiling) in approximately twenty minutes for a 350 μm thick mold. Kapton molds were destroyed using an oxygen-based plasma (700 series, PlasmaTherm, St. Petersburg, FL). The gas flow rates were 50 sccm of oxygen and 5 sccm of CHPs at 300 mT. With an operating power of 200 W, the etch rate was approximate 45 μm/hr, or a total etch time of nearly 8 hours for 350 μm.

3.1.2 Solid Metal 2D and 3D arrays

Solid microneedles were created using laser machining from stainless steel. The process was essentially direct-writing in that the beam traced the desired shape of the needle. The metal along the traced path was ablated and the resulting needles are created

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in the plane of the substrate. After cutting the needles were electro-polished to remove any slag from the drilling process and to sharpen the needle tip. The needles were used as free standing, 2D structures at this point, or they were subsequently bent out of the pane to form a 3D array.

3.1.2.1 Laser Micromachining

The shape of the microneedles and their orientation within an array was first drafted in a CAD file (AutoCAD, Autodesk, Cupertino, CA). This file was then converted to the file type required by the laser control software (Oregon Microsystems, Beaverton, OR). The stainless steel sample (SS 304, McMaster-Carr, Atlanta, GA) was drilled at 1000 Hz at an energy density of 20 J/cm². The pattern was traced at a rate of 1mm/s with a constant 30 psi airflow for cooling and debris removal.

3.1.2.2 Electropolishing

The drilled sample was electropolished to remove any debris or re-deposited slag. Initially the sample was cleaned, by polishing with detergent particles. Essentially, powdered detergent (Alconox, White Plains, NY) was placed on the drilled sample and ground into the sample manually. The abrasive nature of the powder and the cleaning agents in the detergent removed both organic and inorganic contaminants. The cleaned sample was rinsed thoroughly in DI water.

The electropolishing bath was a 6:3:1 mixture by volume of glycerin, phosphoric acid, and water (Fischer, Atlanta, GA). It was operated at 110°C and the sample was manually agitated during the polishing process. The sample was biased with a positive
charge relative to a copper electrode to generate a current density of 130 mA/cm². The sample was polished for 2.5 minutes and then rinsed for 15 seconds in DI water followed by 15 seconds in 15% nitric acid. This rinse cycle was repeated and then the entire cycle (polishing and rinsing) was repeated for a total polishing time of 5 minutes. After polishing the sample was rinsed with hot water for 1 minute followed by a thorough rinsing in DI water.
3.2 Insertion Force Methods

Measuring and predicting the force necessary to insert microneedles into skin is a key component to designing microneedles for drug delivery. The success of microneedles as a drug delivery device is dependent on the ability to reliably insert microneedles into living subjects. Understanding insertion force and its relationship to microneedle geometry allows intelligent design during fabrication.

Despite the importance of this testing, the mechanics of microneedle insertion into living humans has not been studied. Measurements of conventional needle insertion force have been limited to data necessary to simulate insertion for physician training and were not conducted on living subjects [Brett, 1997; Frick et al., 2001]. Chandrasekaran and Frazier (2002) and Stupar and Pisano (2001), on the other hand, made measurements of microneedle insertion force into synthetic polymers.

In brief, the measurement of insertion force was made by a force-displacement test station. Prior to testing, microneedle geometry was fully characterized. Once prepared, a single microneedle was advanced to, began deflecting, and finally punctured the skin on the hand of a human subject. During this movement, the force necessary to translate the needle was measured by a force transducer. The data was logged and then evaluated to determine the force of insertion.

3.2.1 Characterization of Needle Geometry

Prior to testing, all microneedles were imaged using scanning electron microscopy (Hitachi 3500, Pleasanton, CA) to determine their base diameter, tip
diameter, and wall thickness. Interfacial area (i.e. the effective area of contact between the needle and the skin) was then calculated in two ways: (i) the annular surface area at the needle tip

\[ A_f = \frac{\pi}{4} (2d_f t - t^2) \]  

(3.2.1)

and (ii) the full cross-sectional area at the needle tip.

\[ A_f = \frac{\pi \times d_f^2}{4} \]  

(3.2.2)

Needle wall angle was calculated as

\[ \alpha = \tan^{-1}\left( \frac{d_k - d_s}{2h} \right) \]  

(3.2.3)

where \(d_k\) is the outer diameter of the microneedle tip, \(d_s\) is the outer diameter at the needle base, \(t\) is the wall thickness and \(h\) is the height.

3.2.2 Microneedle Preparation

The preparation of microneedles prior to testing was done to ensure the safety of the subject, the electrical continuity of the microneedle assembly, and the mechanical robustness of the microneedle assembly. Microneedles were first cleaned by immersion in 70% ethanol for 15 minutes to sanitize the devices for human use. To electrically and mechanically connect the microneedle to the displacement-force test station, a 30-gauge copper wire was first soldered to the base of the microneedle. The base was then mechanically reinforced with adhesive tape (Blenderm, 3M, Saint Paul, MN). The microneedle was fixed to a 3-mm diameter brass post (Mcmaster-Carr, Atlanta, GA) using cyanoacrylate adhesive (Loctite, Rocky Hill, CT). The post/microneedle assembly
was inserted into the test station probe and the final electrical connection between the test station and microneedle was made to the soldered wire.

3.2.3 Insertion Protocol

In this study, we measured 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 measured the force applied to a needle, needle position and skin resistance during the needle's translation to the skin, deflection of tissue around the needle, and insertion into the skin.

Three Caucasian male subjects were used to test the insertion of microneedles. They ranged in age from 20 to 26 years old and gave informed consent. All testing was performed using a protocol approved by the Georgia Institute of Technology Institutional Review Board.

Each subject was seated at the test station and the subject's hand was fully hydrated by immersion in warm water for 5 min. A silver-silver chloride counter electrode (In Vivo Metric, Healdsburg, CA) was affixed to the back of the hand with adhesive tape (Shamrock, Bellwood, IL). A single drop of saline was placed at the insertion point, located at the proximal base of a knuckle, to improve electrical contact between the skin and the microneedle.

To measure force and displacement associated with needle insertion, the test station pressed the microneedle against the subject's hand at a rate of 1.1 mm/s. During this movement, the electrical and mechanical resistance experienced by the microneedle was recorded. The microneedle continued to move into the skin until a preset maximum

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load was reached (500 g). The force, displacement, and electrical resistance data were then logged to an ASCII file for evaluation.

3.2.4 Determination of Insertion

Because visual observation of needle insertion was extremely difficult, a drop in electrical resistance of the skin was used to identify needle penetration into skin. A similar technique was used by Young et al. (1987) to monitor needle insertion into deeper tissues during epidural block procedures.

An electrical circuit through the skin was created using the microneedle as one electrode and a Ag-AgCl counter electrode placed on a nearby section of skin that was either mechanically stripped of stratum corneum using a scalpel blade or treated with electrode gel (Spectra 360, Parker, Fairfield, NJ) to ensure low-resistance electrical contact. The electrical resistance of skin’s uppermost layer, the stratum corneum, is much greater than the more aqueous deep tissues, and skin resistance is a well-established measure of stratum corneum’s integrity [Yamamoto and Yamamoto, 1976; Kalía and Guy, 1995; Prausnitz, 1996]. In this way, the stratum corneum in contact with the needle provided the largest resistance in the circuit. When the needle penetrated the stratum corneum, the measured resistance dropped dramatically.
3.3 Fracture Force Methods

Measuring and predicting the force necessary to fracture microneedles is a key component to designing microneedles for drug delivery. The success of microneedles as a drug delivery device is dependant on the ability to reliably insert microneedles into living subjects without microneedle fracture. The understanding of fracture force and its relationship to microneedle geometry allows intelligent design during fabrication.

The mechanical strength of microneedles has rarely been tested quantitatively. For the most part, evaluation of mechanical strength has been limited to qualitative assessments of microneedle fracture after insertion. While these assessments are informative, generally the material chosen for penetration has not been living human skin (e.g. aluminum foil, potato, in vitro chicken thigh, and in vitro stripped human epidermis) which limits its applicability to clinical scenarios. The relatively few quantitative measurements conducted on the fracture of microneedles have been for structures millimeters in length in which buckling is the primary mode of failure [Oks, et al., 2001; Chandrasekaran and Frazier, 2002; Stupar and Pisano, 2001]. The alternative is fracture due applied stresses exceeding the material's strength, which is the mode of failure for the small microneedles in this work (see section 4.3.2.2.3).

In brief, the fracture force of microneedles was measured in this study by an axial load test cell. Prior to testing, microneedle geometry was fully characterized. A single microneedle was then fixed to the test station on a flat block. An opposing block was then advanced toward, made contact with, and eventually crushed the microneedle. During this movement, the force necessary to translate the needle was measured by a
force transducer. The data was logged and then evaluated to determine for the force of fracture.

3.3.1 Characterization of Needle Geometry

Prior to testing, all microneedles were imaged using scanning electron microscopy (Hitachi 3500, Pleasanton, CA) to determine their base diameter, tip diameter, and wall thickness. Needle wall angle was calculated as:

\[ \alpha = \tan^{-1}\left( \frac{d_b - d_t}{2h} \right) \]  (3.3.1)

where \(d_t\) is the outer diameter of the microneedle tip, \(d_b\) is the outer diameter at the needle base, \(t\) is the wall thickness and \(h\) is the height.

3.3.2 Fracture Force Measurement

The force required for mechanical fracture of microneedles was tested under an axial compression load using an axial load test station (ScopeTest1, EnduraTEC, Minnetonka, MN). The microneedles were attached to the testing surface using adhesive tape (Shercon, Santa Fe Springs, CA) around the base of the needle. The opposing test surface was a flat block of aluminum.

All microneedles used for mechanical fracture testing were 500 μm tall. The geometric parameters that were varied included tip diameter, wall thickness, and wall angle. Studies varying tip diameter had constant wall thickness of 12 μm and wall angle of 78.5°. Studies varying wall thickness had constant tip diameter of 85 μm and wall
angle of 78.5°. Studies varying wall angle had constant tip diameter of 60 μm and wall thickness of 10 μm, where desired wall angles were achieved by varying base diameter appropriately. Base diameter was not independently varied because it is not expected to affect the mechanical strength of microneedles, due to the concentration of stress and observed point of fracture at the tip of the needle. In a conical structure stress at a given point is inversely proportional to its radius [Roark and Young, 1975]. The stress is therefore maximized at the minimum radius, which in the case of microneedles occurs at the tip.

With the microneedle fixed to the testing surface, the axial load test station drove the microneedle against the testing surface at a rate of 0.01 mm/s. Microneedle fracture was observed through an attached microscope to evaluate the mode of failure and verify that the microneedle base remained fixed during the test. The test station measured the load applied to the microneedle during the test until a preset displacement was reached (500 μm). The force and displacement data were logged to an ASCII file and were used to determine the maximum force the needle withstood before failure.

3.3.3 Ultimate Stress Measurement

Additional tensile testing was conducted using the same axial load test station to determine the intrinsic mechanical properties of electroplated nickel. For this study “dog bone” shaped structures were electroplated into a polymer mold using the same plating conditions and nickel bath as used for the microneedle fabrication. The cross-section of the central test region for the dog bone measured 200 μm by 25 μm. These structures were tested under the same conditions as the microneedles, except that tensile loads were
applied rather than compressive loads, and cyanoacrylate adhesive was used to fix the samples rather than tape. To eliminate the possibility of buckling failure, ultimate stress is commonly determined using tensile testing and is expected to yield values applicable to compressive stresses too [Beer and Johnston, 1992].
3.4 Drug Delivery Methods

It has been shown previously that solid and hollow microneedles can increase the permeability of in vitro human epidermis by up to four orders of magnitude for molecules including calcine, bovine serum albumin, and insulin [Henry, 1997; M cAllister 2000]. However, these experiments were conducted on in vitro tissue that does not accurately represent the mechanical properties of living subjects. The methods described here were used to evaluate the delivery of insulin and 100 nm polystyrene spheres to rats in vivo and quantify the delivery capabilities of hollow microneedles.

The ease of handling and their previous use in drug delivery literature makes hairless rats good candidates for this work, despite the differences in anatomy between rats and humans [Bronaugh et al., 1982]. The goal of this work is not a comparison of drug delivery in humans and rats, but instead to quantify and characterize the drug delivery capabilities of hollow microneedles. The development of a model to predict the drug delivery capabilities into rats could possibly be expanded to humans in future work.

Drug delivery to hairless rats was initiated by first anesthetizing the animals and inducing diabetes in animals for insulin delivery. After a stabilization period, arrays of microneedles were inserted into the animal, and a chamber was sealed to the array. The chamber was then filled with the compound for delivery and passive diffusion ensued. Quantification of the delivery results was made either by analysis of blood glucose level or insulin concentration in plasma for insulin. Quantification of the delivery results for polystyrene nanospheres was made by spectrofluorometry. Qualitative analysis of the distribution of both insulin and nanospheres was attempted with confocal microscopy. Measurements of blood glucose levels were taken throughout the diffusion period, while
the other protocols required sacrificing the animals prior to sample collection. All procedures used in these animal studies were approved by the Georgia Tech Institutional Animal Care and Use Committee (IACUC).

The rats used in all experiments were Sprague Dawley Hairless Rats (Charles River Laboratories, Wilmington, M.A.). The animals were all male with body weights ranging from 250-350 g.

3.4.1 Animal Preparation

All animals were anesthetized prior to experimentation and remained sedated for the duration of the experiments. The level of anesthesia was judged regularly by evaluation of pedal and ocular reflexes. Animals to receive the insulin delivery protocol had diabetes induced prior to their anesthesia.

3.4.1.1 Anesthesia

The animal was first weighed to determine the correct dose of anesthesia. The animal was placed in a small plastic container and supported on a balance (BP2100, Sartorius, Goettingen, Germany). Anesthesia was induced by delivering 1300 mg per kg of animal body weight of urethane (200 mg/ml, Sigma, St. Louis, MO). The injection was made intraperitoneally, and the animal was then returned to its cage and moved from the animal care facility to the laboratory. The animal rested for one hour post injection to allow the anesthesia to take effect. The animal was then removed from its cage and placed on an absorbent pad. Its limbs were immobilized with medical grade tape.
(Shamrock, Bellwood, IL) to prevent movement during experimentation and its eyes were shielded from light sources by a folded portion of the pad.

3.4.1.2 Induction of Diabetes

Animals that were part of the insulin delivery protocol had diabetes induced one day prior to their anesthesia. The animal was first weighed to determine the correct dose of streptozotocin. The animal was placed in a small plastic container and supported on a balance (BP2100, Sartorius, Goettingen, Germany). Diabetes was induced by delivering 100 mg per kg of animal body weight of streptozotocin (Pfanstiehl Laboratories, Waukegan, IL). The injection was made intravenously in the animal’s tail as a slow bolus over 5-10 seconds and the animal was then returned to its cage. The animals rested for 24 hours post injection to allow the streptozotocin to take effect. The animal’s blood glucose level was then measured at the tail vein (Accu-Chek Compact; Roche Diagnostics, Indianapolis, IN) to confirm hyperglycemia (300-475 mg/dl) due to diabetes. If diabetes was successfully induced, the animal was then anesthetized as described in section 3.4.1.1. Animals in which diabetes was not induced were euthanized.

3.4.2 Diffusion Experiment

Arrays of hollow metal microneedles were used to delivery insulin or 100 nm polystyrene nanospheres to hairless rats. Prior to experimentation the rats were anesthetized and diabetes was induced in animals for the insulin delivery experiments as described in sections 3.4.1.1 and 3.4.1.2.
The microneedles arrays used in the diffusion experiments were 500 \( \mu \)m tall, had a base diameter of 300 \( \mu \)m, and a tip diameter of 75 \( \mu \)m. The needles had 10 \( \mu \)m thick walls and were spaced 600 \( \mu \)m center to center. The arrays contained 16 needles arranged in a 4 needle by 4 needle matrix. The total area covered by the needles was a 3 mm by 3 mm square, although additional area surrounding the needle array (10 mm by 10 mm total area) was left at the base for fluidic sealing purposes.

The experiment protocol for drug delivery is summarized in Figure 3.4.1. The microneedle array was first inserted into the animal’s skin with a pneumatic plunger (Bionic Technologies, Salt Lake City, UT). The array was placed on the animal’s skin and the plunger was placed on the array with enough pressure to hold the array in place. The plunger was then activated at 30 psi to generate a plunger head velocity of approximately 10 m/s [Rousche and Normann, 1992]. The inserted array remained fixed against the animal’s skin after insertion. A glass reservoir (6 mm diameter, 12 mm height, 25 mm diameter sealing surface) was prepared by coating its mating surface with adhesive (Loctite, Rocky Hill, CT). The prepared reservoir was then aligned to the array and pressed into place. The mating surface of the reservoir was sufficiently large to seal to both the array as well as the skin surrounding the array. The adhesive was allowed to dry for approximately 2 minutes and then a bead of adhesive was applied to the outer edge of the reservoir and the skin. The double layers of adhesive (mating surface and outer edge) ensured that no leaks occurred. An additional 2 minutes were allowed for curing of adhesive, and the reservoir was then filled with the target solution.
Figure 3.4.1: Drug delivery protocol. The microneedle array is inserted into the skin with pneumatic plunger. The mating surface of the reservoir is coated with adhesive and fixed to the array and skin (a). The outer edge of the reservoir is sealed with adhesive and the reservoir is filled (b). A cut-away view of the assembly is shown in (c).
The target solutions included human insulin and 100 nm polystyrene nanospheres. Insulin was chosen as a delivery target based on its clinical relevance. Nanospheres were chosen to represent very large compounds including vaccines and drug-loaded particles. The solution concentration and manufacturer varied depending on the analysis method used to quantify the delivery. Table 3.4.1 summarizes the solutions used in all experiments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Analysis Method</th>
<th>Concentration</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (Humalin-R)</td>
<td>Blood glucose</td>
<td>100 U/ml in PBS</td>
<td>Eli Lilly, Indianapolis, IN</td>
</tr>
<tr>
<td>Insulin (Humalin-R)</td>
<td>Plasma insulin</td>
<td>100 U/ml in PBS</td>
<td>Eli Lilly, Indianapolis, IN</td>
</tr>
<tr>
<td>Nanosphere (100 nm diameter)</td>
<td>Confocal microscopy</td>
<td>10^{-7} M in PBS</td>
<td>Molecular Probes, Eugene, OR</td>
</tr>
<tr>
<td>Nanosphere (100 nm diameter)</td>
<td>Spectrofluorometry</td>
<td>2.4x10^{12} beads/ml in PBS</td>
<td>Polysciences, Warrington, PA</td>
</tr>
<tr>
<td>Nanosphere (100 nm diameter)</td>
<td>Confocal microscopy</td>
<td>2.4x10^{14} beads/ml in PBS</td>
<td>Polysciences, Warrington, PA</td>
</tr>
</tbody>
</table>

3.4.3 Delivery Analysis

The delivery of insulin and polystyrene nanospheres through hollow metal microneedles was characterized using a number of methods. The two primary issues of interest include the quantity of molecules delivered as a function of time, and the distribution of the molecules in the skin. The former was determined using both direct and indirect measurement techniques. The reduction of blood glucose level due to insulin
delivery to diabetic animals was an indirect measurement of the delivery capability of microneedles. The insulin concentration in the blood and nanosphere concentration in the skin were directly measured. Finally, confocal microscopy was a direct measurement of the molecules distribution in the skin.

3.4.3.1 Blood Glucose Level

The ability of microneedles to deliver insulin to diabetic rats was evaluated using measurement of the animal’s blood glucose level. Although measurement of blood glucose is an indirect measurement of insulin delivery, it is an excellent demonstration of microneedles ability to deliver meaningful quantities of insulin and induce a systemic response.

The animals were prepared as described in section 3.4.1 and the diffusion experiment proceeded as described in section 3.4.2. After the reservoir was filled with 100 U/ml of insulin, measurements of the blood glucose level were taken every 30 minutes. Blood (~10 μl) was taken from tail vein lacerations and analyzed using a commercial test strip (Accu-Chek Compact; Roche Diagnostics, Indianapolis, IN). The same tail vein laceration was used for all measurements, and clotted blood was removed from the area by massaging the region and milking fresh blood. Measurements were taken for a total of 8 hours after the microneedle array was inserted. The reservoir remained filled with insulin for the first four hours and after this period the insulin was removed from the reservoir and filled with DI water. Measurements were then continued for the next four hours. After the completion of the experiment, the animal was euthanized by an intraperitoneal injection of pentobarbital (1cc).
3.4.3.2 Plasma Insulin Concentration

The ability of microneedles to deliver insulin to diabetic rats was evaluated using a measurement of the insulin concentration in the animal’s plasma. This technique is a direct measurement of insulin delivery and can be correlated to quantity delivered.

The animals were prepared as described in section 3.4.1 and the diffusion experiment proceeded as described in section 3.4.2. After the reservoir was filled with 100 U/ml of insulin, two blood samples were collected from the animal. The first blood sample was from an orbital collection 30 minutes after the microneedle insertion. The second blood sample was from an intracardiac puncture 4 hours after the microneedle insertion. Each blood sample was ~0.5 ml and was centrifuged at 5000 rpm for 5 minutes. The blood cells and serum were then stored at -70°C until the time of analysis. After completion of the experiment, the animal was euthanized by an intraperitoneal injection of pentobarbitol (1cc). Analysis of the insulin concentration in the animal’s plasma was conducted by radio immunoassay (Linco Research, St. Charles, MO).

3.4.3.3 Fluorescence Assay

The ability of microneedles to deliver 100 nm polystyrene nanospheres to rats was evaluated using a spectrofluorometric measurement of the concentration of nanospheres in the animal’s skin. The polystyrene nanospheres were fluorescently labeled with rhodamine. Nanosphere concentration in the skin was determined by comparing the intensity of fluorescent emission from nanospheres in the skin to known calibration.
curves relating nanosphere concentration and emission. This technique is a direct measurement of nanosphere delivery.

The animals were prepared as described in section 3.4.1 and the diffusion experiment proceeded as described in section 3.4.2. Multiple experiments were conducted simultaneously on a single rat. Because nanospheres are inert, multiple insertion sites on the same animal are unlikely to interact with each other. This reduces the number of animals required for testing. The methods described in section 3.4.2 were repeated for three separate delivery sites on the animals back with an additional site as an untreated control region. The timing of insertion for each site was adjusted to allow the four experiments to end at the same time. The delivery sites included: (i) control without any treatment, (ii) nanosphere suspension directly on the skin without needle insertion, (iii) microneedle insertion and nanosphere delivery for 30 minutes, and (iv) microneedle insertion and nanosphere delivery for 4 hours.

After insertion, the reservoir was filled with the nanosphere suspension (2.4x10^{13} beads/ml in PBS) and the requisite time period was allowed to elapse for delivery to occur. After completion of the experiment, the animal was euthanized by an intraperitoneal injection of pentobarbital (1cc). The reservoirs were removed from the animal and the delivery site was excised with a 6 mm diameter biopsy punch (Tru-punch, Sklar Instruments, West Chester, PA).

The excised tissue was dissolved in tissue digestion media (Solvable, Perkin Elmer, Loveland, OH) using 1.25 ml of digestion media for each tissue sample. The samples were then heated to 50° C, and stirred at 900 rpm for 12 hours. This process
resulted in liquid samples that were free of solids and suitable for spectrofluorometric analysis.

The volume of each sample was recorded and the samples were diluted with PBS for analysis. The diluted sample was excited at 535 nm and an emission spectrum from 550 to 595 nm was collected (QM-1, Photon Technology International, South Brunswick, NJ). The area under the spectrum was calculated and then compared to a calibration curve for determination of nanosphere concentration in the sample. To account for any fluorescence due to the digestion or dilution media, the spectrum of 1.25 ml diluted with PBS was also collected and subtracted from the experimental data prior to the determination of nanosphere concentration.

3.4.3.4 Confocal Assay

The distribution of insulin and polystyrene nanospheres in the skin of animals delivered by microneedles was evaluated with confocal microscopy. Both the insulin and polystyrene nanospheres were fluorescently labeled. The insulin was labeled with fluorescein isothiocyanate (FITC), while the nanospheres were labeled with rhodamine. The distribution within the skin of neither insulin nor nanospheres should be affected by other deliveries in remote locations. Therefore, multiple delivery sites were chosen on each animal. This reduced the number of animals required for testing.

The methods described in section 3.4.2 were repeated for three separate delivery sites on the animals' backs. The timing of insertion for each site was adjusted to allow the individual experiments to end at the same time. The delivery sites included: (i) control without any treatment, (ii) nanosphere suspension or insulin directly on the skin
without needle insertion, (iii) microneedle insertion and nanosphere or insulin delivery for 30 minutes, and (iv) microneedle insertion and nanosphere or insulin delivery for 4 hours.

After insertion, the reservoir was filled with the nanosphere suspension (2.4x10^{13} beads/ml in PBS) or FITC-tagged insulin (10^{-5} M in PBS) and the requisite time period was allowed to elapse for delivery to occur. After completion of the experiment, the animal was euthanized by an intraperitoneal injection of pentobarbital (1cc). The reservoirs were removed from the animal and the delivery site was excised by punch biopsy (Tru-punch, Sklar Instruments, West Chester, PA). The excised tissue was flash frozen in liquid nitrogen and stored at -70° C until the time of microscopic evaluation.

Tissue samples were viewed using a confocal microscope (LSM 510, Zeiss, Thornwood, NY) to determine the distribution of the delivered species in the skin. The excitation wavelength and collection wavelength spectrum was specific for each species. FITC-tagged insulin was excited at 488 nm and the detection band was from 505 to 555 nm. The fluorescent nanospheres were excited at 535 nm and the detection band was from 555 to 595 nm. The tissue was then scanned throughout its thickness to record areas of delivery.
4 RESULTS

4.1 Microfabrication Results

The microfabrication methods described in section 3.1 allow the creation of a wide range of microneedle geometries that can be tailored to specific applications, or varied systematically to further elucidate the performance of microneedles. Both hollow and solid microneedles have been fabricated using laser micromachining. The hollow structures were created by using a LIGA like process, with the initial formation of the mold accomplished through laser drilling. The solid structures were cut directly from stainless steel shim stock in the plane of the substrate. The use of laser micromachining to form solid and hollow microneedles is novel, and seeks to improve on prior generations of microneedles in regards to ease of fabrication and the ability to tailor microneedle geometry to the desired application.

4.1.1 Hollow Metal Microneedle Results

The creation of hollow metal microneedles required the initial formation of a mold with the desired geometry, the subsequent masking of the mold to allow controlled electrodeposition, the electrodeposition itself, and the final release of the structure by the dissolution of the mold. Each fabrication step required characterization and optimization to create an overall fabrication scheme that allowed relatively fast, reproducible, microneedle production while still allowing the variation of the microneedle geometry and arrangement in an array.
4.1.1.1 Mold Formation Results

The creation of metal and polymer molds for hollow microneedles is described in sections 3.1.1.1. The ability to form high quality conical molds is the first step to creating hollow tapered metal microneedles, and the primary differentiating factor between this work and previous work with microtubes.

4.1.1.1.1 Metal Mold Results

Copper and titanium molds were created using the methods described in section 3.1.1.1. Both materials were found to be amenable to 1047 nm laser drilling creating tapered profiles in the drilled region, Figure 4.1.1. Both materials were also found to have generated substantial amounts of slag on the entrance and exit sides of the mold as seen in the figure. However, it should be noted that the titanium mold offers a much more uniform taper whereas the copper mold is somewhat irregular.
The mold geometries created were bounded by the capabilities of the laser. During pulsed laser operation the entrance diameter was defined by the beam diameter. This was reduced from the maximum (180 μm) by reducing the energy of the beam with the use of filters. The taper of the hole is defined by the beams energy distribution and was fixed. The exit diameter was defined by the taper of the beam and the height of the mold. When the mold was thicker than the maximum penetration of the beam (~300 μm) then the hole did not exit the substrate and a closed mold was formed.

Although the metal molds created by IR drilling offered the tapered shaped desired for the final microneedle structure, the quality of the mold was poor in both cases. Additional polishing was required to remove the re-deposited slag on the mold prior to plating, thus increasing the complexity and production time required to create hollow metal microneedles. In addition, the sidewalls of the mold were heavily textured due to the combination of melting and ablation occurring in the laser plasma and the natural
irregularities in metal grain structure. These irregularities also resulted in non uniformities in mold geometry across large arrays.

4.1.1.2 Polymer Mold Results

Polyimide (Kapton) and polyethylene terephthalate (Mylar) molds were created using the methods described in section 3.1.1.1.2, Figure 4.1.2. Both materials were found to be amenable to 248 nm laser drilling. Both materials were also found to generate excellent quality molds with regards to re-deposition of material and sidewall roughness. The trepanning technique was demonstrated to be effective at creating tapered geometries whereas pulsed laser operation resulted in nearly vertical sidewalls.

![Image of UV drilled Mylar molds](image)

**Figure 4.1.2:** UV drilled Mylar molds (a. trepanning technique, b. pulsed operation) displaying excellent sidewall smoothness and almost no re-deposited slag. This sample is representative of both Mylar and Kapton molds.
The mold geometries had a much greater range than those created in metal due to the nature of the excimer laser. The flat energy distribution of the beam allows masking of the beam shape and size without reducing its energy density. During pulsed operation this allowed the variation of diameters from 50 µm to 400 µm. The long focal length of the beam also allowed very deep penetration into the substrate. The mold heights ranging from 50 µm to 1000 µm were created. Finally, the trepanning technique developed allowed control of the wall angle. Angles ranging from 45° to 97° were accomplished. The ability to vary beam size, the thickness of the substrate, and the diameter of the trepanned pattern allowed complete control over the microneedle mold geometry.

Polymer molds demonstrated excellent quality. Minimal re-deposition of slag material was observed. Any expelled material was cleaned from the surface with a methanol-soaked wipe. The sidewalls of the mold were uniform and smooth, and the uniformity of geometries across arrays of holes was excellent.

4.1.1.2 Mold Masking Results

Drilled molds must be masked appropriately to define the regions of electrodeposition of material. Metal should only be deposited on the topside of the mold and the sidewalls of the drilled features to form hollow microneedles. Therefore, the topside and sidewalls must be conductive, while the backside of the mold must be behave as an insulator. For metal molds that are conductive throughout, the backside was passivated, while insulating, polymer molds, had conductive seed layers deposited on the topside and sidewalls of the mold.
4.1.1.3 Passivation Layer

The plasma enhanced chemical vapor deposition of silicon nitride onto metal molds was described in section 3.1.1.2.1. Typical depositions of 1 μm were found to be sufficiently thick and pinhole free to prevent electroplating on the backside of molds. The deposition occurred on the backside of the mold which was essential a planar surface. This greatly eased the formation of high quality, uniform depositions.

4.1.1.4 Seed Layer

Seed layers of both titanium-copper-titanium and nickel were deposited as described in sections 3.1.1.2.2 and 3.1.1.2.3. While the deposition of material on the backside of molds is trivial, uniform deposition into high aspect ratio molds is far more difficult. While both techniques offer metal deposition into polymer molds, the mechanism and environment for each technique, plasma for sputtering and liquid for electroless plating, varies resulting in variable deposition characteristics.

To compare the ability of each method to conformally coat microneedle molds, depositions were made into molds with nearly vertical sidewalls (97°), a constant 500 μm depth, and varying diameter. The seed layers were then plated and released from the mold for examination under scanning electron microscopy to determine the depth of the deposition, Figure 4.1.3.

Since the mold depth was 500 μm the maximum metal deposition was also 500 μm. For larger diameters, and therefore small aspect ratios, the coverage of the mold was complete and uniform. As the diameter decreased the coverage for both methods decreased as well, however, the electroless layer maintained more uniform coverage and
deeper penetration at all diameters. At a diameter of 200 μm the sputtering system was still capable of depositing metal to the bottom of the mold, but the coverage became non-uniform. This non-uniformity remained for smaller diameters of the sputtered samples, while the electrolessly deposited layers were uniform regards of diameter.

![Comparison of Metal Deposition](image)

**Figure 4.1.3:** Comparison of the seed layer penetration dept during sputtering and electroless plating. The mold depth was 500 μm and the sidewalls of the mold were nearly vertical. The dashed line indicates the point at which the seed layer becomes non-uniform for the sputtered layer.
While the advantages of electroless deposition in regards to uniformity of coverage depth are clear, what is not obvious from this experiment is the effect of the actual deposition environment. Because sputtered depositions occur in a plasma environment and the sample resides on a flat substrate, deposition only occurred on the topside and sidewalls of the mold. Electroless plating, on the other hand, occurred in a liquid environment in which the any portion of the mold exposed to the bath experienced metal deposition. This helps explain why the uniformity of coverage and depth of coverage are much better for the electroless plating. However, it also resulted in the need to mask the mold during processing to prevent deposition of metal on the backside of the mold. If nothing was done to prevent backside plating the mold essentially reverted to a metal mold requiring additional masking for electroplating. Unfortunately the polymer mold, coated with metal, can not withstand the high operating temperatures of the passivation layer described in section 4.1.1.2.1. The inability to reproducibly mask polymer molds during electroless deposition prevented adoption of this technique for the final fabrication scheme. All microneedles formed from polymer molds were plated directly onto sputtered seed layers.

4.1.1.3 Electroplating Results

Prepared molds were electroplated as described in section 3.1.1.3. The deposition thickness was varied by adjusting the duration of the plating time. Plating thicknesses ranging from 2 μm to 20 μm were demonstrated.

As described in section 3.1.1.3 a technique of sealing the backside of the mold with adhesive tape after the strike layer formation was adopted. This was done in order
to reduce over-plating of the microneedle’s tip onto the backside of the mold. Although
the mold was an insulator, the current crowding that occurred at the interface of the seed
layer and the mold caused excessive growth of the plated material as illustrated in Figure
4.1.4.
Figure 4.1.4: Over-plating phenomena associated with current crowding at the tip of the mold. A) shows the crowding of the current lines near the tip, during plating this results in a mushrooming effect on the backside of the mold shown in B). C) is a scanning electron micrograph of the over-plating that occurs on the backside of an unprotected mold.
Sealing the backside of the mold after the strike layer was formed was found to alleviate this problem. Although this prevents backside plating, sealing the mold does introduce a problem of refreshing the electrolyte in the mold during long plating runs. For thicknesses up to 20 μm this was not observed to be an issue.

4.1.1.4 Mold Release Results

The mold release methods were discussed in section 3.1.1.4. Each mold material requires its own etch process in order to ensure selectivity relative to the microneedles material, nickel. Table 4.1.1 summarizes the techniques used for each mold material and the approximate etch rate. It should be noted that the removal of the Mylar is actually a release of the mold and not strictly a dissolution process. Therefore the removal rate is heavily dependant on microneedle packing density and bath agitation.

Table 4.1.1: Summary of the etchants used to remove mold materials and their respective etch rates.

<table>
<thead>
<tr>
<th>Mold Material</th>
<th>Etchant</th>
<th>Etch Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>CuSO4+H2O (saturated)</td>
<td>0.05 μm/min</td>
</tr>
<tr>
<td>Titanium</td>
<td>HF</td>
<td>85 μm/min</td>
</tr>
<tr>
<td>Kapton</td>
<td>O2:CHF3 Plasma</td>
<td>0.75 μm/min</td>
</tr>
<tr>
<td>Mylar</td>
<td>NaOH:H2O (1N), boiling</td>
<td>-20 μm/min (release rate)</td>
</tr>
</tbody>
</table>

4.1.1.5 Microneedle Results

Hollow metal tapered microneedles were created using the methods described in section 3.1. Figures 4.1.5 through 4.1.8 demonstrate microneedles from each mold material. After the formation of a mold, the subsequent fabrication steps were developed to allow creation of the microneedle, but had little impact on the overall quality or
geometry of the microneedles themselves. The mold material and its ability to be formed into high quality molds were the differentiating factors between the microneedles created.

Microneedles created from copper molds are shown in Figure 4.1.5. As in the case of the molds, the microneedles generated from the mold were generally of poor quality. The sidewalls were rough, very little taper was evidenced in the structure, and the structures were often non-uniform across multiple needles. The geometries investigated had a diameter of 50 μm and were 250 μm tall.

Figure 4.1.5: Hollow metal microneedle from copper molds. The poor quality of the mold is replicated in the microneedle structures. The structures are approximately 50 μm in diameter and 259 μm tall.

Microneedles created from titanium molds are shown in Figure 4.1.6. These structures showed the same characteristic rough sidewalls as microneedles generated from copper molds. However, the tapered design was accomplished and this was found to be reproducible over large arrays of microneedles. Typical geometries for
Microneedles created from titanium molds were 180 μm base diameter, 50 μm tip diameter, and 250 μm height.

![Microneedles from titanium mold](image)

Figure 4.1.6: Microneedles created from a titanium mold. The structures are 180 μm at the base, 50 μm at the tip, and 250 μm tall.

Microneedles created from polymer molds are shown in Figure 4.1.7. These structures did not exhibit rough sidewalls as seen from microneedles generated from metal molds. The smooth sidewalls and controlled tapered were reproduced in microneedles generated from polymer molds. The geometries and quality of the mold was consistent across large sample areas and quantity. Typical geometries for microneedles created from polymer molds were 250 μm base diameter, 50 μm tip diameter, and 500 μm height when the beam was trepanned. The geometries were all fully controllable as described in sections 4.1.1.2. Pulsed laser operation, on the other hand, yielded nearly straight sidewalls. The height and diameter of the microneedles was
also variable as described previously. Arrays of microneedles created from polymer molds were also created, Figure 4.1.8.

Figure 4.1.7: Microneedles created from polymer molds. On the left, a microneedle created by pulsed laser operation in Kapton. The microneedle has a diameter of 125 μm and is 180 μm tall. On the right, a microneedle created by trapped laser operation in Mylar. The microneedle has a base diameter of 250 μm, a tip diameter of 50 μm, and was 500 μm tall.
Figure 4.1.8: Arrays of hollow microneedles created from polymer molds. The needles taper from a tip diameter of 75 μm to 300 μm at the base over their 500 μm length. The wall thickness in this case is 10 μm. A conventional 27 gauge needle is shown for comparison.
4.1.2 Solid Metal Microneedles

Solid metal microneedles were created using the method described in section 3.1.2. An array of stainless steel microneedles is shown in Figure 4.1.9. The microneedles tapered from a base width of 250 μm to a point over their 1 mm length.

![Image of microneedles](image)

Figure 4.1.9: Solid microneedles cut from stainless steel stock. The needles are approximately 1 mm long and taper from a 250 μm base to a sharp point.

The fabrication sequence for solid microneedles was simple (2 steps) and yielded excellent quality microneedles. The independence of this method from electroplating allowed the expansion of constituent materials. Type 304 stainless steel was selected for its excellent mechanical properties and its pre-existing use as a biocompatible material.
As observed previously, the re-deposition of drilled material was an issue when producing high quality metal molds by IR laser micromachining. This problem was resolved by electropolishing the structures. The method for this process was described in section 3.1.2. and not only removed re-deposited material but sharpened the tips of the needles.

4.1.3 Discussion and Conclusions

The LIGA process has been modified to create hollow tapered microneedles. Metals and polymers have been drilled with infrared and ultraviolet lasers to generate tapered molds. The high quality and reproducible geometry of polymer molds is superior to the metal molds fabricated. Although the polymer mold quality was superior to metal molds, the desired tapered geometry was not accomplished with pulsed operation of the laser. The operation of the UV laser was modified to include a sputtering technique which was demonstrated to create a wide range of tapers in polymer molds. The high quality and geometry control of the polymers investigated in combination with the reduced expense and its ease of removal made polyethylene terephthalate the preferred mold material.

Seed layers of nickel and Ti-Cu-Ti were deposited by electroless plating and direct current sputtering, respectively. Electroless plating demonstrated superior coverage into deep molds in comparison to sputtering. However, difficulty in masking the backside of the mold to prevent plating by the solution hindered the full utilization of the technique. Despite the promise of electroless plating, direct current sputtering was preferred for simple control of deposition area.
Nickel electroplating was demonstrated to yield uniform deposits within the molds. To prevent backside plating due to current crowding, a technique of “blinding” the vias was adopted. After the initial strike layer of nickel was deposited, the backside of the mold was sealed with adhesive tape. The technique was successful in preventing backside plating while still allowing uniform plating at the needle tip.

The removal of the mold was specific to the mold material. As previously stated, the removal of Mylar in a hot, caustic bath is simple and relatively fast. This etch bath is selective with regards to the nickel microneedles.

Arrays of hollow, tapered, metal microneedles were created. The quality of the mold was found to have the most significant impact on the quality of the microneedle array. As previously stated, the quality of polymer molds was superior to metal molds and this holds true for the microneedles generated from each kind of mold as well. For these reasons the preferred microneedle fabrication scheme is the UV drilling of Mylar, direct current sputtering of a Ti-Cu-Ti seed layer, nickel electroplating, and mold removal in a hot, caustic bath.
4.2 Insertion Force Results

This chapter discusses the results of measuring and modeling of microneedle insertion force in human subjects. The relationship between insertion force and microneedle geometry was characterized in order guide needle design for reproducible insertion. This information can facilitate the intelligent design of microneedles for transdermal drug delivery.

The methods used to conduct the experiment are detailed in section 3.2. The force of insertion was determined by pressing microneedles against the skin of human subjects and measuring the corresponding force upon insertion. The insertion event was 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 corresponded to the shorting of the resistance corresponding to the stratum corneum. To facilitate interpretation of experimental results, a fracture mechanics-based model was developed to predict insertion force as a function of microneedle geometry and compared to experimental data.

4.2.1 Force of Insertion

To determine the force required to insert microneedles into skin, we measured forces of microneedle insertion into the skin of human subjects as a function of microneedle geometry. To determine force of insertion, a single microneedle was pressed against, and ultimately, into the skin while continuously measuring needle force and displacement, as well as skin resistance, which served to identify the point of needle
penetration into the skin. Section 3.2.3 describes the methods employed to determine the force of insertion in this manner.

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 were monitored to determine the penetration of the skin. Section 3.2.4 describes the methods employed to determine the insertion event in this manner.

Figure 4.2.1 demonstrates typical data collected during the insertion of a microneedle into skin on the hand of a human subject. The force required to press the needle against the skin increased with needle displacement, then showed a discontinuity upon insertion into the skin, and finally increased further when pressed deeper into the skin. After full insertion of the microneedle, the base metal and mounting post contacted the skin and continued to deflect the tissue. The slope of the curve increased sharply probably due to deflection limited by supporting bone structure beneath the test site.

As a needle was pressed against the skin, resistance of the needle-skin circuit decreased and then leveled off as the needle came into contact with the skin and deflected it (Figure 4.2.1.) Upon insertion, skin resistance rapidly dropped and then slowly decreased further when pressed deeper. Because the discontinuity in the force curve was not always as dramatic as shown in Figure 4.2.1, we used the more reliable drop in resistance to identify when needle insertion occurred.

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Insertion force was measured for microneedles with geometries varying from tip diameters of 60 to 160 μm, and wall thickness ranges from 1.5 μm to solid tips (equivalent to 55 μm). As shown in Figure 4.2.2, force of insertion was measured for different needle geometries in three human subjects. Forces of insertion ranged from 0.08 to 3.04 N, all of which could be easily applied by hand. When plotted versus...
microneedle interfacial area, insertion force increased linearly, albeit with considerable scatter. A linear fit data ($r^2=0.81$) was described by:

$$y = 0.002x - 0.657$$  \hspace{1cm} (4.2.1)

![Graph showing the relationship between insertion force and microneedle interfacial area. The solid line represents the linear regression of the data ($r^2 = 0.81$) and the upper and lower bounds of the 95% confidence level are plotted as dashed lines.]

Figure 4.2.2: Force to insert a microneedle into skin of a human subject as a function of microneedle interfacial area. Insertion force had a linear dependence on interfacial area. The solid line represents a linear regression of the data ($r^2 = 0.81$) and the upper and lower bounds of the 95% confidence level are plotted as dashed lines.

Theoretical considerations suggested that insertion force should depend on interfacial area (see section 4.2.2). However, it was not clear whether the area should be calculated as the annular ring of metal at the needle tip or the full cross-sectional area, independent of the lumen size, at the needle tip. The area of skin-needle contact depends
on the behavior of the skin during its interaction with the microneedle. If the skin deforms conformally around the inner and outer walls of the microneedle, the area of contact would be annular in shape. If the skin is insufficiently flexible to dimple into the needle bore and deforms only around the outer walls, the area of contact would be circular.

To determine the appropriate calculation of interfacial area, the dependence of insertion force on microneedle wall thickness and tip diameter was analyzed. When the outer diameter of a needle is constant, the size of the lumen defines the wall thickness. If the insertion force has a correlation to wall thickness the skin must be able to deform around the walls and the area should be calculated as an annular region. Likewise, if insertion force is independent of wall thickness, the skin must not be able to deform around the needle walls and must experience the microneedle tip as if it were a solid indenter. Therefore, the area would be calculated as a circular region corresponding to the full area at the tip.

The data collected did not show a clear dependence on wall thickness ($r^2 = 0.5$). To further elucidate the relationship between insertion force and wall thickness, microneedles with solid tips were tested. Hollow microneedles with a tip diameter of 115 µm and wall thickness ranging from 11-17 µm inserted with an average force of 1.79±0.3 N. Solid microneedles with 115 µm tip diameter inserted with an average force of 1.29±0.2 N. The dependence of insertion force on wall thickness should be positive, whereas a possibly insignificant (t-test, p=0.09) or even negative dependence is demonstrated in the data. This observation confirms the independence of wall thickness and insertion force.
The independence of insertion force with respect to wall thickness and the linear
dependence on microneedle tip diameter is consistent with the microneedle acting as a
solid indenter during insertion (i.e. the skin was not flexible enough to deform around
both the inner and outer regions of the microneedle lumen), and the interfacial area was
therefore calculated as a circular region, \( A_p \) in Equation 3.2.2, as opposed to an annular
region, \( A_a \) in Equation 3.2.1 as described in section 3.2.1.

4.2.2 Modeling Force of Insertion

As a companion to experimental results, we also modeled the force of insertion as
a function of microneedle properties. To predict the force required for insertion into skin
based on microneedle geometry, we postulated that when the energy delivered to the skin
by the needle exceeds the energy necessary to create a tear in the skin, the needle will
insert into the skin. Pereira et al. (1997) used a similar approach to model tears in the
skin initiated and propagated with scissors in vitro. In this case the stored potential
energy released during cracking was set equal to the surface energy of new surfaces
created. Equation (4.2.2) relates the work necessary to initiate a crack per unit area,
referring to as crack fracture toughness, \( G_c \), to the total work input to propagate the crack,
\( \delta W \), and the surface area of the new fracture, \( \delta A \).

\[
G_c = \left( \frac{\delta W}{\delta A} \right)_{\delta A}
\]  

(4.2.2)

For microneedles, we were interested in the energy to puncture the skin rather
than the energy to propagate a fracture. Therefore, the work input, which was the change
in potential energy between unaltered skin and the skin just prior to needle penetration,
was calculated as the area under the load-versus-displacement curve before fracture as opposed to the area after fracture in the case of crack propagation (Gurson and Hunt, 1966).

In this case, $\delta W = \int F dx$, where $F$ is the force applied by the needle, $x$ is the axial position of the needle, and the boundaries of integration are from the point of needle contact with the skin to the point of needle insertion. Correspondingly, the change in fracture area is the difference between the puncture area prior to insertion (i.e., zero) and the area after puncture (i.e., the area of contact between the needle and the skin, which we have called the interfacial area of the needle, $A$). Equation 4.2.2 can therefore be rewritten as:

$$\int_{x_0}^{x_f} F dx = G_p A$$  \hspace{1cm} (4.2.3)

where $G_p$ is the puncture fracture toughness. The left side of Equation 4.2.2 is the summation of energy applied to the skin during its deflection. The right side of the equation is the energy required to initiate a tear in the skin.

To calculate the summation of energy applied to skin during its deflection, the integration of the force-displacement data was simplified by fitting to an exponential equation (i.e., $F = 6e^{nx}$) and integrating. The correlation between the exponential fit and the data was excellent (average $r^2=0.99$).

$$\int_{x_0}^{x_f} \Theta e^{nx} dx = G_p A$$  \hspace{1cm} (4.2.4)

$$\frac{\Theta e^{nx}}{r} = G_p A$$  \hspace{1cm} (4.2.5)
\[ F_p = \tau G_p A_p + \Theta \]  
(4.2.6)

where \( \Theta \) is the pre-exponential term, \( \tau \) is the exponential term, \( F_p \) is force evaluated at the displacement at which penetration occurs (i.e., insertion force), and \( A_p \) is the full cross-sectional area as described in Equation 3.2.2. The substitution of \( A_p \) for \( A \) was made based on the justification offered in the previous analysis of the relationship between insertion force and wall thickness. As shown in Equations 4.2.4-4.2.6, the slope of the insertion force and interfacial area data corresponds to the product of the penetration toughness of the skin and the exponential factor, \( \tau \), in the equation fit to the force-displacement data. Therefore, with the integration of the force and displacement data and the linear fit of Figure 4.2.2 the puncture toughness, \( G_p \), could be evaluated.

### 4.2.2.1 Integration of Force and Displacement

To calculate the penetration toughness of skin, the physical properties of the skin and its behavior during deflection and penetration were evaluated. The most common method for evaluating a material's physical properties under load is a measurement of stress as a function of strain. Stress is the force per unit area upon which the force is acting. Strain is the ratio of the change in length induced by the stress to the original length. Because this technique is a direct measurement of the material's physical properties, the relationship between stress and strain is constant regardless of the sample geometry.

In the specific case of microneedle insertion testing, the applied force is actually acting on a larger area than the microneedle tip due to the viscoelastic nature of the skin.
In addition, it is impossible to know the original length of the sample due to the variations in skin thickness and basement tissue supporting the skin. However, in the ideal case of a single subject, with testing occurring at the same location under the same conditions, these two parameters (i.e., area and original length) would be constants. Therefore, the plot of stress and strain would be reduced to a plot of force and displacement.

Figure 4.2.3 demonstrates multiple (n=15) insertions of various geometry microneedles on the same subject in the same location. Force is applied to the microneedle and the skin as described in section 3.2.3. In order to capture the behavior of skin at low loads, the data collection begins before the microneedle contacts the skin. Therefore, the displacement data is relative to an arbitrary point, and not the top of the skin. Therefore the absolute value of displacement is not known. This results in curves of similar shape, but dissimilar location on the displacement axis.

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Figure 4.2.3: Force as a function of displacement for a microneedle pressed against the skin of a single subject. Over the 15 data sets shown, the exponential factor of the curves is approximately constant which facilitates analysis of Equation 4.2.5.

For the purposes of the fracture mechanics model, and specifically the evaluation of puncture toughness, the approximately constant nature of the exponential factor describing force as a function of displacement simplifies the calculation. A single value of the exponential factor was used to calculate puncture toughness regardless of microneedle geometry. The exponential factor of the force deflection relationship was taken as the average value across all insertions, 6.25±0.6 mm⁻¹.
4.2.2.2 Calculation of Puncture Toughness

Using the experimental data in Figure 4.2.3 and the value for the exponential factor, Equation 4.2.6 can be used to determine the puncture fracture toughness of skin. The puncture toughness in the range tested was determined to be $G_p=30.1\pm0.6$ kJ/m$^2$.

4.2.3 Discussion and Conclusions

The insertion force of microneedles in human subjects was found to vary linearly with microneedle interfacial area. The interfacial area was calculated as the full cross-sectional area at the microneedle tip, independent of lumen geometry. A fracture mechanics-based model presented and compared to the data. The model agreed with the linear relationship in the data and combined with constant nature of the physical properties of the skin was used to calculate the puncture toughness of human skin. The puncture toughness of skin was determined to be $G_p=30.1\pm0.6$ kJ/m$^2$.

As previously stated this value is not directly comparable to measurements of fracture toughness due to the manner in which it was calculated, the experimental protocol, and the viscoelastic nature of the skin. Despite these differences, it was interesting to note the close agreement with Purslow's (1983) measurement of rat skin's fracture toughness, $26.9\pm2.73$ kJ/m$^2$. Purslow's experiment measured fracture toughness during crack propagation which also includes the crack blunting effect of a viscoelastic material (as in our puncture test). However, our measurements are an order of magnitude greater than those taken from scissors cutting test ($1.7\pm0.6$ kJ/m$^2$) in which the cutting edge of the blade reduces the effect of crack blunting due to the viscoelastic behavior [Pereira, 1997].
The value of puncture toughness determined in this testing not only yields mechanistic information regarding the penetration of biological membranes, but is the necessary link between the insertion force of microneedles and their geometry. When compared to values of fracture toughness collected in more traditional manners, it was determined that the soft underlying tissue and the viscoelastic nature of skin enhances the skin's toughness by blunting the formation and propagation of cracks. This was demonstrated by the agreement between the puncture toughness determined from microneedles and the mechanically similar crack propagation tests of Purslow. Of even greater interest, specific to microneedle design, is the ability to predict the insertion force of microneedles based solely on their geometry. This understanding can now be used to design microneedles that insert with the minimum force necessary. In combination with similar information regarding the fracture of microneedles, one could determine a range of geometries that not only inserted with minimal force, but retained the mechanical strength to do so reproducibly.
4.3 Fracture Force Results

This chapter discusses the results of the measurement of microneedle fracture force. In order to guarantee reproducible insertion of microneedles in skin, it was necessary to characterize the relationship between fracture force and microneedle geometry and thereby identify needle designs that insert into skin (see section 4.2) at forces significantly less than those which cause fracture. This can facilitate intelligent design of microneedle for transdermal drug delivery.

The methods used to conduct the experiment are detailed in section 3.3. The fracture force of microneedles was determined using an axial load compression cell. An opposing block was applied against the microneedle tip while the force applied was recorded. The point of microneedle fracture was characterized by the sudden decrease in supported load. Both an analytical as well as a finite element simulation model were developed to predict fracture forces as a function of microneedle geometry and compared to experimental data.

4.3.1 Fracture Force

Microneedle fracture force was measured using compression failure tests. Section 3.3 describes the methods used to measure microneedle fracture force. The typical fracture behavior of a single microneedle is shown in Figure 4.3.1. As the test block pressed against the microneedle tip the axial load increased with the block’s displacement. This continued until the ultimate load of the microneedle was reached, at which point the microneedle fractured. Microneedle fracture was indicated by the
discontinuity in the applied force and confirmed by visual observation during the test. After fracture, the block continued to press against the crushed microneedle registering a load substantially lower than the ultimate load.

![Force vs. Displacement Graph](image)

**Figure 4.3.1**: Typical fracture behavior of a microneedle under axial load. The discontinuity marks the failure of the microneedle.

Fig 4.3.2 shows the variation of microneedle fracture force with needle geometry. Fracture force does not appear to increase with increasing tip diameter (ANOVA, $p=0.82$), Fig. 4.3.2a. Increasing tip diameter effectively increased the area over which the applied force was distributed which should result in lower stresses. Fracture force increased with increasing wall thickness (ANOVA, $p<0.001$), Fig. 4.3.2b. Increasing the wall thickness also effectively increased the area over which the applied force was distributed resulting in lower stresses. Fracture force increased with microneedle wall angle as well, although the trend in the data was not as clear as in the cases of wall
thickness (ANOVA, p=0.03), Fig. 6c. As the wall angle approaches vertical, and the shape of the needle approaches a cylinder, and a larger portion of the total stress becomes oriented along the plane of the micronneedle wall, as opposed to normal to the wall, and should result in increased force of fracture.
Figure 4.3.2: Force at failure as a function of microneedle geometry. Mechanical strength increased with tip diameter when wall thickness and angle were constant (A), wall thickness when tip diameter and wall angle were constant (B), and possibly wall angle when tip diameter and wall thickness were constant (C).
4.3.2 Modeling Fracture Force

As a companion to experimental measurements, both analytical and finite element simulation models were developed to predict force of microneedle fracture. To develop an analytical model, we made a thin shell assumption to determine the stress in an axially loaded cone. This approach requires wall thickness to be at least 10 times thinner than tip radius and provides solutions only for stresses in the plane of the microneedle wall. Finite element solutions, on the other hand, do not have such geometry constraints and they are capable of calculating stresses in all directions. However, the necessity of individual simulations for each geometry under consideration increases the computation time required to form continuum predictions.

4.3.2.1 Ultimate Stress

Before either of these approaches to modeling the fracture of microneedles was undertaken, the mechanical properties of the electroplated material constituting the microneedles were determined. While the Young’s modulus of electroplated nickel is quite similar to bulk nickel (~200 GPa), the yield and ultimate stress of electroplated materials have been found to be significantly greater in plated materials [Mazza et al., 1996; Sharpe et al., 1997].

Previous work in the field has focused on sulfamate baths for electroplating nickel. In this work, a Watts formulation was employed. Therefore, a direct measure of the ultimate stress of our electroplated nickel was made as described in section 3.3.3. Using this approach, the ultimate stress of our electroplated nickel was found to be 1.2 GPa. As expected, this value far exceeds bulk nickel’s ultimate stress of 0.32 GPa.
(Metals Handbook, 1990) and is closer to literature values for electroplated nickel from sulfamate baths of 0.50-0.80 GPa (Mazza et al., 1996; Sharpe et al., 1997).

4.3.2.2 Analytical Solution

We first modeled fracture of microneedles using analytical solutions simplified by basic assumptions. In the case of high aspect ratio structures, two types of failure must be considered. First, a solution based on fracture due to stresses in the structure exceeding the ultimate stress of the constituent material was developed. Then, a solution based on fracture due to buckling caused by elastic instability of the structure was developed.

4.3.2.2.1 Stress Failure

To predict the force required for fracture due to failure of the constituent material, we modeled microneedles as thin shells. Following the approach of Roark and Young (1975):

\[ F_f = 2\pi Rt\sigma_u \sin(\alpha) \]  \hspace{1cm} (4.3.1)

where \( F_f \) is the axial force required for needle fracture, \( R \) is the radius at the needle tip, \( t \) is the wall thickness, \( \sigma_u \) is the ultimate stress of the needle material (1.2 GPa), and \( \alpha \) is the needle wall angle. This approach assumes needles fail when the membrane stress generated in the plane of the needle walls exceeds the ultimate stress of the needle material. Predictions made using this approach are shown in Figure 4.3.3. as the lower plane.
The thin-shell assumption used in Equation 4.3.1 requires the ratio of tip radius to wall thickness to be greater than ten, which assures a uniform stress distribution across the wall thickness [Roark, 1975]. Because the microneedle geometries used in this study had radius-to-thickness ratio of 2.5 to 10.5, the thin-shell assumption is generally invalid. This means that predictions generated by this model overestimate fracture forces. This is because the thin-shell assumption of uniform stress distribution does not account for heterogeneous peak stresses and stresses in directions other than in the plane of the needle walls. Due to the complexity required to account for these factors, computational methods are commonly employed. However, predictions generated by this model are useful for creating an upper bound representing the maximum fracture force expected.

4.3.2.2.2 Buckling Failure

To predict the force required for needle failure due to buckling, Haurath and Dittoe (1962) have developed a widely used empirical modification to an analytical solution:

\[ F_s = 0.277 \left( \frac{2\pi E r^2 \sin^2 \alpha}{\pi} \right) \]  

(4.3.2)

where \( F_s \) is the axial load to cause buckling and \( E \) is Young’s modulus of the structure’s material (200 GPa). Equation 4.3.2 has been shown to predict 90% of tested literature values examined by Haurath and Dittoe with a 95% confidence level. Predictions made using this approach are shown as the upper plane in Figure 4.3.3.
4.3.2.3 Comparison of Stress and Buckling Failure

Comparison of fracture force predictions by constituent material failure and buckling modes indicates that buckling should only occur when wall thickness is less than one to two microns (Figure 4.3.3). In the figure, the upper plane represents the force necessary to cause failure due to buckling, while the lower plane represents the compressive force necessary to create a stress in excess of the ultimate stress of nickel. Because microneedle wall thickness varied in this study from 4 to 15 μm, the buckling mode of failure was excluded from our analysis. This conclusion is consistent with visual observations during experimental studies of needle fracture, where the observed failure mode was consistently material failure (i.e., collapse at the tip) and not buckling (i.e., bending at some location along the needle shaft) (data not shown).
4.3.2.3 Finite Element Simulation

As a companion to analytical solutions, we also predicted the force of microneedle failure using finite element simulations. These simulations accounted for all stresses within the needle wall regardless of their orientation or distribution, but required longer computation times, and therefore, additional effort to generate continuum predictions.

Simulations using ANSYS (Canonsburg, PA) were employed to solve for the von Mises stress (sum of stress in all directions) in cones with geometries matching the tested
microneedles using three-dimensional models composed of tetrahedral elements. The force applied to a microneedle was varied iteratively until the simulation predicted a stress equal to ultimate stress of the microneedles constituent material (i.e. 1.2 GPa) in a single element. Theoretically, exceeding the ultimate stress at any point in the microneedle structure should result in failure. Practically, some finite area within the needle must exceed the ultimate stress for failure to occur. The choice of a single element's stress as the failure criterion results in the most conservative estimate of fracture force and will therefore form a lower bound.

4.3.2.4 Comparison to Data

Analytical and finite element models were developed to predict the force of fracture. The analytical model was expected to over-predict fracture force since it did not account for stresses out of the plane, nor did it account for peak stresses in the structures. The finite element model took into account all stresses and stress non-uniformities. It was expected to under-predict the force of fracture due to the conservative choice of a single element's stress as the failure criterion.

Figure 4.3.4 shows a comparison between each of the two modeling approaches and the fracture force experimental data. In the case of tip diameter, both modeling approaches predict a moderate increase in fracture force as the tip diameter increases. This trend is not evident in the data. The finite element model under-predicted the fracture force by an average of 21% while the analytical solution over-predicted the fracture force by 23%. In the case of wall thickness, both modeling approaches predict a sharp increase in fracture force as the tip diameter increases. The data were not
completely bounded by the finite element simulations and the analytical solution. As the wall thickness continued to increase, the measured fracture force increased to an even greater extent than either model predicted. The average percent difference between the finite element simulation and fracture force was 50%, while the analytical solution was typically within 28%. Finally, in the case of wall angle, both the finite element model and the analytical model agreed that fracture force should modestly increase with increasing wall angle. This trend was evident in the data collected. The finite element model under-predicted the fracture force by an average of 130% while the analytical solution over-predicted the fracture force by 20%.

Microneedle fracture force increased with increasing wall thickness, wall angle, and possibly wall tip diameter. The finite element simulations and analytical solutions both predicted the trends in the data. In addition, the quantitative agreement between the analytical solution and the data was typically 20-30%, while the finite element predictions varied in quality of agreement from 23% to 130%. Considering the improved quality of agreement with data, the simplicity of the calculation, and the ability to generate continuum predictions, the thin-shell analytical model should be used if only one prediction is to be made. However, the ability to form a set of upper and lower boundaries in the predictions lends additional value to combining the finite element method and the analytical calculation.
Figure 4.3.4: Experimental and predicted force at microneedle failure as a function of microneedle geometry. Model predictions indicate that mechanical strength increases with tip diameter (A), wall thickness (B), and wall angle (C). Experimental data are consistent with these predictions. Thin shell analytical solution is presented as a dashed line and the ANSYS finite element simulation prediction is shown as solid line. Experimental data are presented as points with standard deviation error bars.
4.3.5 Safety Ratio

Individually, the force of insertion data (section 4.2) and the force of fracture data (section 4.3) give insight into individual mechanical events involving microneedles. However, the comparison of these values was the ultimate goal of this study and permits determining the range of geometries over which an appropriate safety margin exists between insertion force and fracture force (Figure 4.3.5). The upper plane in Figure 4.3.5A represents the force necessary to cause microneedle fracture as predicted by the analytical solution (which represents the more accurate, estimate). The lower plane in the plot represents the upper 95% confidence level of the insertion force as predicted by the fitted puncture resistance value (the conservative upper bound estimate). Figure 4.3.5B illustrates the margin of safety (i.e., ratio) between the failure and insertion forces of microneedles. This analysis shows that microneedles that have small diameters and large wall thickness offered a significantly greater margin of safety between insertion and fracture force where insertion forces were many-fold smaller than fracture forces. Microneedles' behavior as solid indenters can explain this, because strength increased as a function of wall thickness while insertion force was unaffected.
Figure 43.5: Comparison of insertion force to fracture force (A) and the safety margin (i.e., ratio) between the insertion and fracture forces of the microneedles (B). In (A) the upper surface is the analytical solution for the force at microneedle fracture (Equation 4.3.1) while the lower plane (B) is the force necessary to insert microneedles at the 95% confidence level (Equation 4.2.1).
4.3.4 Discussion and Conclusions

The fracture force of microneedles was found to increase with wall thickness, wall angle, and possibly tip diameter. Analytical models of fracture due to excessive stress and fracture due to elastic instability (buckling) were developed. Buckling failure was predicted only for microneedles with wall thickness on the order of 1-2 μm for the range of geometries tested. Therefore, fracture due to buckling was ignored as a possible mode of failure for the microneedle geometries under consideration. Finite element simulation modeling of fracture due to excessive stress was developed as a companion to the analytical model.

Both analytical and finite element simulation models were found to predict the trend of the fracture data as well as predict quantitative fracture force for the range of geometries tested. The analytical model was found to bound the fracture force data with predictions of the maximum fracture force while the finite element simulations generated a lower bound. Although, the analytical model demonstrated better agreement with the fracture force data (~20%), the ability to form a set of upper and lower boundaries in the predictions lends additional value to combining the finite element method and the analytical calculation.

The ratio between the force of fracture and the force of insertion, or margin of safety, was found to increase with both wall thickness and tip diameter. Increases in either geometry parameter increased the force the needles can withstand before fracturing. However, increases in wall thickness did not increase the force of insertion and therefore created a greater margin of safety in comparison to increases of tip diameter. This data suggests that in order for microneedles to insert with the minimum
amount of force and retain an acceptable safety margin, they should be created with the minimum tip diameter possible and the maximum wall thickness acceptable.
4.4 Drug Delivery Results

This chapter discusses an experimental study of drug delivery through hollow microneedles to rats \textit{in vivo}. Although previous work has demonstrated the ability of microneedles to permeabilize \textit{in vitro} human epidermis (McAllister, 2000), the mechanical issues facing needle insertion and delivery of molecules to living subjects are significantly different. All experiments in this study were conducted on hairless rats \textit{in vivo} in order to better simulate the mechanical environment faced by microneedles in future medical applications.

The methods for delivery of insulin and polystyrene nanospheres to rats through microneedles as described in section 3.4. Both the amount delivered and the distribution of molecules in the skin were evaluated. A simple theoretical model was developed to predict the transepidermal delivery results. Also described are observations of the interaction between the skin and microneedles and possible mechanical issues to overcome in the future.

4.4.1 Insulin Delivery

There are currently 12 million Americans diagnosed with diabetes and it ranks as the fifth leading cause of death in the United States. The total expenditures attributable to diabetes in 2002 totaled $91.8 billion [American Diabetes Association, 2003]. Treatment of diabetes involves administration of insulin. Unfortunately, the size of insulin (5808 Da [Brown et al., 1955]) and its tendency to polymerize into larger macromolecules (hexameric at pH=7.0 [Jeffry and Coates, 1966; Carpenter, 1966]) make it a challenging
molecule to delivery transdermally. The combination of this difficulty and the large segment of the population affected by diabetes strongly motivates the need for effective, patient-compliant delivery methods for insulin. For these reasons, insulin delivery to diabetic rats was chosen as the first test of the ability of hollow metal microneedles to deliver drugs into skin. The effects of delivered insulin were analyzed to provide to both pharmacodynamic (reduction in blood glucose level) and pharmacokinetic (insulin concentration in plasma) data. In addition, the distribution of insulin within the skin during transdermal delivery was investigated.

4.4.1 Blood Glucose Level

Insulin was delivered to diabetic rats through hollow metal microneedles. Their blood glucose level was monitored over time and used as an indication of the pharmacodynamics of insulin delivery. The methods for the animal preparation, diffusion experiment, and sample collection are described in sections 3.4.1, 3.4.2, and 3.4.2.1 respectively.

Figure 4.4.1 shows the variation in reduction of the animals' blood glucose level (BGL) with respect to time after microneedle insertion and insulin application. Blood glucose values in the figure are normalized relative to the rat's average BGL during the 1.5 hour period prior to treatment, BGLo. Animals were treated either with microneedles and insulin (●) or insulin without the application of microneedle (○).
Figure 4.4.1: BGL reduction due to Insulin delivery to diabetic rats. The animals BGL drops rapidly when microneedles are inserted (●), while animals with just insulin and no microneedle insertion show no change (○). After removal of the insulin, the BGL of rats treated with microneedles and the untreated rats remains constant. The average standard deviation for the animals treated with insulin without microneedle insertion is 0.30.
After the introduction of insulin, and an approximately 30 minute lag time, BGL was reduced at a constant rate for the four hours of insulin application to 47% of its original value (ANOVA, p<0.001). Animals that did not have microneedles inserted into their skin, but did have insulin placed on their skin did not demonstrate a statistically significant variation (ANOVA, p=0.99). After the initial four hour delivery period, the insulin was removed from the reservoir for the microneedle treated animals. For the next four hours without insulin, BGL was constant in both treated and untreated animals (ANOVA, p=0.999).

The effect of anesthesia must also be considered when evaluating the success of insulin delivery through microneedles. Urethane has previously been shown to have a hyperglycemic effect on the blood glucose level of rats and rabbits [Reinf, 1964; Sanchez-Pozo, 1988]. Recent work has demonstrated that although urethane has a hyperglycemic effect in normal rats, it does not increase the hyperglycemic BGL of diabetic rats [Wang, et al., 2000]. This conclusion is supported by the constant BGL demonstrated in the control animals in Figure 4.4.1. Although urethane was not found to impact the BGL level in diabetic rats, it was found to reduce the hypoglycemic effect of injected insulin. The reduction in BGL of rats treated with insulin without urethane was nearly twice as large as the reduction of BGL in rats anesthetized with urethane in Wang's work. This suggests that the BGL reduction reported in this work is likely muted by the use of urethane as an anesthesia. It is possible that without the use of urethane the reduction in BGL accomplished by insulin delivery through microneedles would be even more significant.
4.4.1.2 Plasma Insulin Concentration

In addition to measurements of the pharmacodynamic effects of insulin delivered through microneedles, the pharmacokinetics of the process were also measured. Insulin was delivered to diabetic rats through hollow metal microneedles. The insulin concentration in the animal's blood stream was analyzed for three time points and used as an indication of the pharmacokinetics of the insulin delivery. The methods for the animal preparation, diffusion experiment, and sample collection are described in sections 3.4.1, 3.4.2, and 3.4.3.2 respectively.

Figure 4.4.2 shows the concentration of insulin in rat plasma at the time points evaluated. The analysis of insulin concentration was specific to the human insulin delivered by microneedles, so that endogenous rat insulin was not detected by the assay. As expected, no delivery of human insulin was detected in untreated rats (data not shown). After thirty minutes of delivery, the plasma concentration of human insulin was found to be 5.5±3 μU/ml. Four hours of delivery resulted in an insulin concentration of 12±4 μU/ml. The final data set collected at eight hours was not for continuous delivery during the entire time period as in the previous data sets. Instead the data was collected after four hours of insulin delivery and an additional four hours of microneedles inserted without insulin in the reservoir. The plasma insulin concentration after this time was 12.5±6 μU/ml. These data confirm that insulin was delivered by microneedles and is most likely the cause of the observed reduction in BGL.
Figure 4.4.2: Concentration of insulin in plasma of diabetic hairless rats after 30 min, 4 h, and 8 h of delivery through microneedles. The 30 min and 4 h data was collected immediately after delivery while the 8 h delivery included 4 h of insulin delivery and 4 h without insulin.
4.4.1.3 Transdermal Diffusion Model

The change in insulin concentration is the bloodstream as a function of time can be determined from the summation of the rate of insulin transport across the skin to the capillary bed and the elimination of insulin from the bloodstream. Rates of insulin transport across the skin to the capillary bed, or flux, can be predicted using Fick's laws of diffusion. Rates of insulin elimination from the bloodstream can be predicted using a first order rate constant. This constant is determined from the half-life of insulin in the bloodstream of humans as report by Eli Lilly (2002).

In this approach we have assumed that diffusion is occurring in an isotropic media (i.e., diffusivity is independent of the direction of transport). The use of hollow microneedles bypasses the diffusion barrier presented by the stratum corneum and viable epidermis and creates a source of molecules within the dermis (Figure 4.4.3). The microneedle penetrate through the upper layers of skin (stratum corneum and epidermis) to create a conduit for insulin into the dermis. Molecules must first move from the reservoir to the needle tip (length H). They may then diffuse from their source at the microneedle tip through the dermis to the capillary bed. (length L). We assume that diffusion from the needle tip to the capillary bed is the rate limiting step in the transport through the needle to the capillaries. This assumption is based on the similar diffusion lengths (H and L in Figure 4.4.3) for each step of the process, and the reduced diffusivity of solutes is the structural environment of the dermis relative to the aqueous environment within the needle. This assumption allows the needle tip to act as a region of constant

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concentration, $C_0$ and the capillary bed to behave as a region of zero concentration at all times.

![Diagram of dermal layers](image)

**Figure 4.4.3:** Microneedle enhanced transdermal diffusion. A microneedle creates a pathway to deeper tissues and bypasses the diffusion barrier of the stratum corneum and viable epidermis. A diffusing solute at initial concentration $C_0$ moves through the needle across length $H$ to the needle tip and then across length $L$ to the capillary bed where it is absorbed into the bloodstream.

As a first order approach, diffusion is assumed to occur in a single dimension at an unsteady state. While two dimensional and three dimension models would likely increase the accuracy of the prediction, the modeling of the capillary bed as an infinite slab at a particular depth captures the key elements of the transport process. Thus, the additional complexity of the calculations relative to the minor improvement in accuracy does not justify their use in the initial model. With these assumptions, and the relative
simplicity of the calculation, the flux, and therefore the number of molecules entering the bloodstream, can be calculated.

Given the geometry of the microneedle, the key system parameters that must be known to make a prediction are the diffusivity of the solute in dermis, and the length scale over which the diffusion occurs. A prediction of diffusivity is determined from a fiber matrix model, while the length scale is determined by direct measurement.

4.4.1.3.1 Diffusivity

The path of a solute diffusing in the dermis is tortuous. The dermis is a stratified network of collagen fibers surrounded by an extracellular matrix. Both materials restrict the movement of molecules, but when the spacing of the collagen fibers is much greater than the diameter of the solute, the extracellular ground substance is the rate limiting material. To our knowledge, no direct measurement of the diffusivity of insulin in dermis has been made.

As a frame of reference, the diffusivity of hydrocortisone was measured in dermis as $4.40 \times 10^{-6}$ cm$^2$/s [Tojo, et al, 1987]. Thus, we should expect the diffusivity of insulin to be of the same order of magnitude. An estimate for insulin's diffusivity in free solution using Polson's semi-empirical equation yielded $1.3 \times 10^{-6}$ cm$^2$/s. Obviously the diffusivity of the molecule in the structural environment of dermis should be less than this estimate. A direct measurement of insulin's diffusivity in agar gel (isotropic gelatin) has been used as an estimate of diffusivity in subcutaneous tissue, $9.65 \times 10^{-7}$ cm$^2$/s [Hildebrandt, 1985]. In this case, the molecule is moving through an isotropic media, but the uniform nature of the gelatin does not capture the environmental interaction that a
solute experiences during diffusion through the stratified network of collagen and extracellular matrix within dermis. As an improvement on these previous values, the diffusivity of insulin in dermis was predicted based on a fiber matrix model.

Edwards and Prausnitz (1998) have proposed a fiber matrix model for molecular diffusion in the sclera and corneal stroma of the eye. The sclera and cornea are similar to dermis in that they are made up of a fibrous network of collagen (and elastin) fibers surrounded by ground substance. The ground substance is composed of glycosaminoglycans (GAGs) and proteins that may be free or associated with the GAGs. In this case, the primary factor determining the diffusivity of the solute is its size relative to the geometric spacing of the fibrous network, which is strongly influenced by the degree of hydration in the tissue. The water content of the dermis is 65.3% [Duck, 1990] and is 70.0% for the sclera so the hydration factors of the model are similar. The level of hydration varies the volume fraction of the remaining components of the skin and thus varies the geometric spacing of the fibrous network.

The general process for calculating the effective diffusivity of the solute in dermis (adopted from Edwards and Prausnitz, 1998) is an initial calculation of the hydrodynamic permeability which can then be used to determine the diffusivity in the ground substance. This value in turn can be used to calculate the diffusivity in the matrix of the collagen fibers and ground substance.

The permeability of the ground substance, $K_p$, is calculated as:

$$K_p = \left( \frac{n_1}{K_1} + \frac{n_2}{K_2} \right)^{-1} \quad (4.4.1)$$

where $n_i$ is the number fraction of the fiber (1=GAGs, 2=associated proteins) and is calculated as:

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\[ n_i = \frac{\phi_i / r_i^2}{\phi_i / r_i^2 + \phi_j / r_j^2} \]  (4.4.2)

where \( \phi_i \) is the volume fraction of the fiber (excluding collagen) and \( r_i \) is the radius of the fiber. \( K_i \) is the individual permeability of the solute in the fibers and is calculated as:

\[ K_i = \frac{3r_i^2}{20\phi_i} \left[ -\ln \phi_j - 0.931 \right] \]  (4.4.3)

where \( \phi_f \) is the sum of \( \phi_1 \) and \( \phi_2 \).

The effective diffusivity within the ground substance, \( D_\mu \), can then be calculated as:

\[ D_\mu = D_\infty \frac{e^{-f} e^{-2\phi_f r_i^2}}{1 + \frac{r_i^2}{K_\mu} + \frac{r_j^2}{3K_\mu}} \quad \text{where} \quad f = \phi_i \left( 1 + \frac{r_i^2}{r_j^2} \right)^2 \]  (4.4.4)

where \( r_i \) is the radius of the solute, \( r_j \) is the radius of the GAG fiber, and \( D_\infty \) is the infinite dilution diffusivity in water as determined from the Poisson (1950) relationship. Finally, the effective diffusivity in dermis, \( D \), was calculated as:

\[ D = \frac{D_\mu \left( 1 - 2\phi_d \right)}{1 + \phi_d \frac{C_1 \phi_d^2}{1 - C_2 \phi_d^2} - C_3 \phi_d^2} \quad \text{where} \quad \phi_d = 2\pi \left( \frac{r_d + r_i}{S_d} \right)^2 \]  (4.4.5)

where \( r_d \) is the radius of the collagen fibers, \( S_d \) is the center-to-center spacing of the collagen fibers, and \( C_1=0.075422, C_2=1.060283, \) and \( C_3=0.000076 \) are given constants.

Values of the physical parameters used in the calculation are tabulated in Table 4.4.1. With the exception of collagen fiber radius and diffusivity at infinite dilution the values were adopted from Edwards and Praunitz (1998). The volume fractions were modified to account for the hydration content of the dermis and the physical parameters.

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of the ground substance components were assumed the same as in sclera. The radius and spacing of collagen fibers in the dermis was taken from Saidl, et al (1995). The diffusivity at infinite dilution was calculated from the semi-empirical relationship of Polson (Polson, 1956).

Table 4.4.1: Summary of physical parameters used to calculate diffusivity of solutes in the dermis.

<table>
<thead>
<tr>
<th>Physical Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume fraction of GAGs, (\Phi_1)</td>
<td>0.37%</td>
</tr>
<tr>
<td>Volume fraction of proteins, (\Phi_2)</td>
<td>1.11%</td>
</tr>
<tr>
<td>Radius of GAGs, (r_1)</td>
<td>0.5 nm</td>
</tr>
<tr>
<td>Radius of Proteins, (r_2)</td>
<td>0.35 nm</td>
</tr>
<tr>
<td>Average ground substance fiber radius, (r_f)</td>
<td>0.37 nm</td>
</tr>
<tr>
<td>Radius of collagen, (r_{cf})</td>
<td>1400 nm</td>
</tr>
<tr>
<td>Spacing of collagen, (S_f)</td>
<td>4300 nm</td>
</tr>
<tr>
<td>Diffusivity at infinite dilution, (D_e)</td>
<td>1.22 x 10^{-9} cm²/s</td>
</tr>
</tbody>
</table>

Figure 4.4.4 shows the effective diffusivity of solutes in the dermis as a function of solute radius from the fiber matrix model (assuming the solute mass is similar to insulin). Insulin's radius of 2.5 nm (as a hexamer) [Blundell et al., 1972] results in a prediction of 3.95 x 10^{-8} cm²/s for its diffusivity in dermis.
4.4.1.3.2 Diffusion Length

The distance over which the rate-limiting diffusion occurs is the separation between the tip of the microneedle and the capillary bed. This value requires estimates of needle insertion depth and capillary bed depth. The diffusion length of solute particles strongly affects the time necessary for a steady state to be reached in the process. Thus, over short time periods diffusion length has little impact on the calculated flux. However, as the diffusion time approaches the time necessary to accomplish steady state, the impact of diffusion length becomes more important.

The insertion depth of microneedles was found to be ~100 μm based on histology photos of microneedle insertion into human cadaver skin. Figure 4.4.5 shows a single needle, which is part of a 16 needle array, inserted into human cadaver skin. Although the skin deflects around the microneedle to some degree the tip penetrates through the stratum corneum and epidermis and into the dermis.
Figure 4.4.5: A microneedle after insertion into human cadaver skin. The microneedle is part of a 16 needle array. The skin was fixed after insertion and diced to reveal the cross-section of the needle.

The depth of capillaries was determined from histology data for rat skin (courtesy of Nancy Monteiro, N.C. State). Figure 4.4.6 shows the location of capillaries in rat skin. These are the upper capillary loops that nourish the epidermis. They are ~200 μm from the surface of the skin. The capillary plexus feeding these loops was observed near the dermal subcutaneous junction (~1.8-2.0 mm from the surface of the skin). In comparison, human skin has similar loops near the epidermal-dermal junction fed by an upper plexus 1.0-1.5 mm from the surface of the skin and a lower plexus at the dermal-subcutaneous junction [Braverman, 1997].
The diffusion length of solute particles in the dermis, L, was calculated as the difference between the needle insertion depth and the capillary bed depth as shown in Figure 4.4.3. This value was calculated to be 100 μm based on the histology data collected.

4.4.1.3.3 Flux into the blood

Having calculated the diffusivity of insulin in dermis and determined the length over which diffusion is occurring, the flux of insulin molecules can be calculated. The concentration at any point in a non-steady state, one-dimensional diffusion process with a fixed concentration source and zero concentration sink was calculated as [Crank, 1975]
\[ C = C_0 - C_{\infty} \frac{x}{L} + \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{C_0}{n} e^{-\left(\frac{\pi n}{L}\right)^2 t} \left( \sin\left(\frac{n \pi x}{L}\right) \right) \left( f(x) \sin\left(\frac{n \pi x}{L}\right) \right) \]  
(4.4.6)

where \( C_0 \) is the concentration of solute in the microneedle (1.38 x 10^{-3} \text{ M}), \( L \) is the diffusion length (100 \text{ \mu m}), \( t \) is time, \( D \) is the effective diffusivity in dermis (3.95 x 10^{-8} \text{ cm}^2/\text{s}), \( f(x) \) is the initial distribution of the solute in the skin and \( x \) is the point at which the concentration is evaluated. During the first time period, in which the insulin remains in the reservoir during the delivery and no initial distribution is present in the skin, Equation 4.4.6 simplifies to:

\[ C = C_0 - C_{\infty} \frac{x}{L} + \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{C_0}{n} e^{-\left(\frac{\pi n}{L}\right)^2 t} \left( \sin\left(\frac{n \pi x}{L}\right) \right) \]  
(4.4.7)

After the initial delivery period, the insulin was removed from the reservoir and the boundary conditions of the equation are revised to reflect the zero concentration at the top surface and an initial distribution in the skin, \( f(x) \), as predicted by Equation 4.4.7 evaluated at \( t=4 \text{ hr} \) (14400 \text{ s}). This simplifies Equation 4.4.6 to:

\[ C = \frac{2}{L} \sum_{n=1}^{\infty} \frac{C_0}{n} e^{-\left(\frac{\pi n}{L}\right)^2 t} \left( f(x) \sin\left(\frac{n \pi x}{L}\right) \right) \]  
(4.4.8)

\[ f(x) = C_0 - C_{\infty} \frac{x}{L} + \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{C_0}{n} e^{-\left(\frac{\pi n}{L}\right)^2 t} \left( \sin\left(\frac{n \pi x}{L}\right) \right) \]  
(4.4.9)

With the concentration of the solute in the skin as a function of time defined for each set of boundary conditions, Fick’s Law may then be used to calculate the number of molecules entering the skin [Crank, 1975]:

\[ \text{Flux} = A_L D \frac{\partial C}{\partial x} \]  
(4.4.10)

where \( A_L \) is cross-sectional area of the needle’s lumen (0.062 mm^2 for 16 needles).
4.4.1.3.4 Concentration in the blood

As previously stated, the change in insulin concentration in the bloodstream as a function of time can be determined from the summation of the rate of insulin transport across the skin to the capillary bed and the elimination of insulin from the bloodstream. This results in the linear, ordinary differential equation:

\[
\frac{\partial N_s}{\partial t} = A_t D \frac{\partial C}{\partial x} + kN_s
\]

(4.4.9)

where \( k \) is the elimination rate constant defined by the half life of insulin (e.g. when \( C_{\text{half}} = 0.5C_{\text{initial}} \)) in the bloodstream, \( t_{\text{half}} = 1.5 \) hours:

\[
k = \frac{\ln(0.5)}{t_{\text{half}}}
\]

(4.4.10)

The solution of this linear ODE was found using an integrating factor \( (e^t) \) and evaluated by MathCAD at each time point to determine the number of insulin molecules in the bloodstream.

Finally, the number of molecules in the bloodstream was related to the concentration in the bloodstream by the volume of distribution for insulin. This is a measure of the degree of partitioning for insulin between the various bodily fluids. Insulin’s fraction volume of distribution in humans is 0.26-0.36 L/kg [Eli Lilly, 2002]. The median of this range (0.31 L/kg) was scaled to a value of 0.40 L/kg for rats using the tabulated relationship between human and rat volumes of distribution of Baehmann (1996). The concentration in the blood stream, \( C_b \), was calculated as:

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\[ C_t = \frac{N_o}{V_o \cdot M_{tot}} \] (4.4.11)

where \( V_o \) is the fractional volume of distribution, and \( M_{tot} \) is the bodyweight of the animal.

Figure 4.4.7 shows the theoretical prediction compared to the experimental insulin concentrations. The model predicted 2.4 \( \mu \text{U/mL} \) after 30 minutes of delivery, 12.4 \( \mu \text{U/mL} \) after 4 hours of delivery, and 2.1 \( \mu \text{U/mL} \) at the 8 hour time period. The prediction at 30 minutes is just outside the standard deviation of the data, while the 4 hour prediction is within 1% of the mean. The 8 hour prediction is substantially lower than the data collected. The model predicts that minimal flux of insulin in the bloodstream occurs after the removal of insulin from the reservoir while elimination continues to reduce the concentration in the plasma. Given the simplicity of the model, the level of agreement is quite good. Improvements in the estimation of diffusivity (preferably a measurement) and the volume of distribution would likely improve overall prediction. Beyond improving the estimation of physiological parameters, the model could be improved by the inclusion of other rate influencing steps such as protein binding and degradation.

Previous work (Shkumatov, 1989; Miyazaki, et al., 2001) has demonstrated that the inclusion of a second, peripheral compartment in addition to the primary plasma compartment can be used to accurately model the pharmacokinetics of insulin in rat plasmas. The additional compartment of this model adds a depot effect which would like reduce the rate of elimination at the latest time period. Finally, the migration from a one dimension model to a three dimensional model would more accurately represent the diffusion process occurring.
4.4.1.4 Confocal Imaging

In addition to quantitative data regarding the diffusion of insulin through microneedles, the distribution of insulin within the skin was evaluated as described in section 3.4.3.4.

Although the quantitative data described in sections 4.4.1.1 and 4.4.1.2 clearly demonstrated the flux of insulin into the skin, no evidence of FITC-tagged insulin was found during confocal analysis of skin. Fluorescence was present in microscopy images, but it was impossible to distinguish any regions of delivery from the background fluorescence of the skin.
Upon further investigation, it was determined that the donor concentration of FITC-tagged insulin was below the detection limit of the confocal microscope. The donor concentration determination was based on the manufacturer's claims of 1 FITC molecule per insulin molecule. Based on this claim, and the confocal microscope's normal detection limit of $10^{-9}$ M, $10^{-7}$ M FITC-insulin was expected to be three orders of magnitude greater than the detection limit. It was later determined, through spectrofluorometry, that the emission intensity of the tagged insulin was four orders of magnitude less than claimed by the manufacturer. This resulted in the emission intensity of the delivered insulin to be an order of magnitude below the detection limit of the microscope. For this reason, confocal microscopy studies did not yield useful information.

### 4.4.2 Nanosphere Delivery

In contrast to insulin, polystyrene nanospheres are not direct therapeutic agents with waiting markets. However, nanospheres represent two important classes of transdermal deliverables. They are of similar size to deactivated viruses used for vaccines. They also represent a growing number of delivery vehicles that include drug loaded nano- and microspheres [Kreuter, 1996; Tolbo et al., 1998]. In these cases the sphere acts as a depot and releases its drug payload over time. For these reasons, nanosphere delivery to rats was chosen as the second test of the ability of microneedles to deliver drugs into skin. The quantity of delivered nanospheres was measured as a function of time using spectrofluorometric techniques. In addition, the distribution of nanospheres within the skin during transdermal delivery was investigated.
4.4.2.1 Spectrofluorometry

The delivery of 100 nm nanospheres to diabetic rats through hollow metal micro needles was attempted. The delivery sites were excised from the animal postmortem and dissolved to form liquid suspensions. The liquid suspensions were then analyzed for nanospheres by spectrofluorometry. The methods for the animal preparation, diffusion experiment, and sample collection are described in sections 3.4.1, 3.4.2, and 3.4.3.3 respectively.

Figure 4.4.5 shows the concentration of nanospheres as determined by spectrofluorometry for 30 minute and 4 hour deliveries, as well as nanospheres applied to the skin without microneedles. The samples without microneedles applied had an average nanosphere concentration of $1.39 \pm 0.28 \times 10^{10}$ spheres/ml. The nanospheres in these samples are most likely residual spheres left on the skin surface after the experiment. The total number of spheres in the samples corresponds to approximately two monolayers of uniform coverage across the area of the skin sample. The nanosphere concentration for the microneedle applications for 30 minutes and 4 hours were $2.77 \pm 0.33 \times 10^{10}$ spheres/ml and $3.07 \pm 0.33 \times 10^{10}$ spheres/ml respectively. Although the samples without microneedles are distinct from the samples with microneedles ($p<0.001$), the microneedle samples at 30 minutes and 4 hours are indistinguishable from each other ($p=0.907$). Not only are these values indistinguishable from each other, but they are both less than the delivery without microneedles.
Figure 4.4.8: The concentration of nanospheres in the skin as a function of time. The spheres in the samples without microneedles were entrained on the surface of the skin. The application of microneedles eliminated surface exposure to spheres, and without diffusion to carry sphere into the skin almost no spheres were delivered.

When microneedles are inserted, the only path for the nanospheres to enter the skin is through the lumen. If microneedles are not able to diffuse into the tissue, no delivery will occur. The constant concentration with time indicates that no diffusion is occurring or that diffusion is so slow that it does not exceed the detection limit. In addition, because the spheres can only reach the tissue through the lumen, no spheres will be in contact with the upper surface of the skin and therefore the concentration found in the samples without microneedles is greater than those with microneedles.

The lack of diffusion of nanospheres is supported by the previously proposed diffusion model in section 4.4.1.3. The diffusivity of the species is based on the ability of
spherical solute to diffuse through a fiber matrix. Clearly, the size of the solute relative to the area available for diffusion between fibers has a strong effect on this process. As the molecular radius is increased, the diffusivity of the species is reduced drastically. Solute with a radius of 10 nm are predicted to have a diffusivity of $10^{-23}$ cm$^2$/s. The nanospheres’ diameter of 100 nm reduces the diffusivity prediction to practically zero ($10^{-250}$ cm$^2$/s) in agreement with the experimental evidence.

4.4.2.2 Confocal Imaging

In addition to quantitative data regarding the diffusion of nanospheres through microneedles, the distribution of spheres within the skin was evaluated as described in section 3.4.3.4.

The quantitative data suggesting that no net flux of nanospheres into the skin at either time period was confirmed during the confocal analysis. No spheres were found within the skin at any depth.

4.4.3 Observations

In addition to the characterization of drug delivery through hollow microneedles, some important observations were made during the experiments. The ability of microneedles to penetrate human and rat skin has been demonstrated in both sections 4.2 and 4.3 and was supported by the drug delivery results presented in this chapter. However, what has not been fully appreciated up to this point is the interaction between the skin directly in the path of the microneedle and the hollow lumen of the microneedles. During insertion there are two possible routes for tissue in regards to an intruding
microneedle. The tissue can either be displaced to either side of the needle, or the tissue can be cored by the microneedle and fill the lumen. In either case, a passage through the stratum corneum has been produced and diffusion may take place. However, the latter case would greatly increase the difficulty of pressure driven flow through hollow microneedles and possibly the diffusion of nanospheres.

Following the animal experiments, the microneedles were evaluated for possible clogging and fracture of microneedles. While the failure of microneedles during insertion was rare, filling of the hollow lumens with tissue was very common. In nearly all cases of insertion (~90%), the microneedle tip was found to contain some tissue. Fig 4.4.7 shows an array of 16 microneedles after their insertion into rat tissue. All 16 microneedles have tissue filling their tips. Upon further evaluation, these tissue plugs found to be approximately 70 µm in diameter and 60 µm in length. Figure 4.4.8 shows a plug of tissue after it has been removed from the microneedle tip.
Figure 4.4.9: An array of microneedles after insertion into a rat. All 16 needles have tissue trapped at the tip of the microneedle.
4.4.4 Discussion and Conclusions

Insulin was delivered to hairless, diabetic rats through hollow metal microneedles. Both pharmacodynamic as well as pharmacokinetic responses were measured. The blood glucose level of the rats was reduced to 47% of its original value after 4 hours of insulin delivery. After the removal of insulin from the delivery reservoir, the blood glucose level of the rats remained stable for 4 hours. The use of urethane as an anesthesia possibly hindered the ability of the delivered insulin to reduce blood glucose level; suggesting that the overall reduction in blood glucose level may have been even more significant if another anesthesia was used.
The concentration of human insulin in the plasma of diabetic rats was found to be 5.5±3 μU/ml after 30 minutes of insulin delivery and 12±4 μU/ml after 4 hours of delivery. After the removal of insulin from the delivery reservoir and the passage of an additional 4 hours, the concentration was found to be 13±6 μU/ml.

The delivery of 100 nm polystyrene nanospheres through hollow metal microneedles was not successful. The concentration of nanospheres found after 30 minutes and 4 hours of delivery were statistically indistinguishable. Samples in which microneedles were not inserted into the skin were found to contain approximately four times as many nanospheres (equivalent to two monolayers) as microneedle treated samples. These results are consistent with the deposition of nanospheres on the surface of skin as opposed to delivery of nanospheres into skin.

An analytical model based on Fickian diffusion was developed to predict the diffusion of solutes through the dermis to the capillary bed. The diffusivity of solutes in the dermis was predicted using a fiber matrix model to more accurately represent the physical environment of the dermis. The diffusivity of insulin in the dermis was predicted to be $3.95 \times 10^{-8}$ cm$^2$/s and the diffusivity of 100 nm spheres was predicted to be on the order of $10^{-20}$ cm$^2$/s. The prediction of insulin delivery to the bloodstream was of the correct order of magnitude although it over-predicts delivery at long time scales. This over-prediction is most likely due to the lack of inclusion of an elimination term in the model to account for the removal of insulin from the bloodstream. The diffusivity prediction for 100 nm nanospheres is consistent with the lack of delivery found experimentally.
5 CONCLUSIONS

Transdermal drug delivery has a number of advantages over the most common drug delivery methods today, pills and injections. Primarily, transdermal delivery avoids first-past metabolism and retains excellent patient compliance. Despite these advantages, the barrier properties of the skin limit the number of molecules that can be successfully delivered by this route. In this study, arrays of hollow, tapered, metal micro needles were created to form pathways for molecules that are either too large or are too strongly charged to move through the stratum corneum on their own.

Microfabrication techniques were adopted to manufacture microneedle arrays. Essentially, the fabrication scheme is a modified LIGA process in which laser micromachining has been used to form the initial mold. This process allows faster production than prior microneedle fabrication schemes and is less expensive in regards to manufacturing equipment and materials. This process also allows greater control over the geometry of microneedles so that they can be tailored to specific applications.

Both metals and polymers were investigated as microneedle mold materials. While the titanium molds had the tapered profile desired for microneedles, the quality of the mold was poor. Excessive material was deposited on the mold surface during the drilling process and the sidewalls of the mold were non-uniform. Polymer molds on the other hand exhibited excellent mold quality in regards to slag deposition and sidewall quality. However, the flat energy distribution across the beam resulted in the sidewalls having very little taper. This was corrected by adopting a taperspacing technique which allowed simple calculation of the drilling parameters based on the desired microneedle geometry.

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In addition, this process allowed control over the geometry over a wide range. The ability to create high quality molds with controllable geometry made polymers, specifically polyethylene terephthalate, the preferred mold material.

Successful drug delivery using microneedles is dependent on the ability of microneedles to penetrate living skin and withstand the force of insertion. Although prior work demonstrated microneedles' ability to penetrate stripped human epidermis, this did not accurately represent the in vivo mechanical environment for which microneedles were intended.

In this study, the force of insertion as a function of microneedle geometry was measured in living subjects. A linear relationship was found between the force of insertion and the interfacial area of the microneedle. This relationship was independent of the geometry of the hollow lumen. Therefore, the microneedle tip diameter was the sole geometric parameter needed to determine the force of insertion.

A model to predict the force of insertion in living subjects as a function of microneedle geometry was developed. This fracture mechanics-based model balanced the energy delivered to the skin during its deflection by a microneedle and the energy necessary to initiate a tear the size of the microneedle tip in the skin. The model predicted a linear relationship between insertion force and microneedle interfacial area, which was supported by the data. The model also allowed determination of the puncture toughness of the skin which was then used to predict insertion force as a function of microneedle geometry.
The fracture force of microneedles as a function of their geometry was also measured. The fracture force was found to increase linearly with wall thickness, wall angle, and possibly tip diameter over the range of geometries tested.

Both analytical solutions and finite element simulations were developed to model the fracture of microneedles. Both models predicted the trends as well as the quantitative forces of failure. However, the analytical model demonstrated better agreement with the data and was found to create continuum predictions much more readily.

A comparison of the insertion force and fracture force of microneedles demonstrated that needles with small diameters and large wall thicknesses yielded the largest margin of safety. This was due to the independence of insertion force on wall thickness. A decrease in the diameter of the microneedle tip reduced insertion force and fracture force, but the mechanical strength of the needle was reinforced by the larger wall thickness.

As a demonstration of microneedles ability to deliver drugs to a living subject, insulin and nanospheres were delivered to rats. Insulin delivery was confirmed by both a reductions in the animals' blood glucose levels as well as increases in plasma insulin concentrations. The animals' blood glucose level was reduced to 47% of its original value over 4 hours of insulin delivery. The animals' plasma concentration of human insulin was found to be 5.5±3 μU/ml after 30 minutes of delivery, 12±4 μU/ml after four hours of delivery, and 12.5±6 μU/ml after four hours of rest following the delivery period. The low diffusivity of nanospheres within the dermis prevented the delivery of significant quantities of the nanospheres.
A Fickian diffusion model was developed to predict the quantity of molecules delivered to the blood stream of an animal as a function of time. The one dimensional, unsteady-state, model was found to agree with the plasma insulin concentration data.

The promise of microneedles as a transdermal drug delivery device is evident from the work presented here. The device is capable of delivering molecules to living animals that would not enter the skin by passive diffusion alone. In addition, the delivery was accomplished without pressure driven flow and demonstrated systemic responses in the animals.
6 RECOMMENDATIONS

Building on the results presented in this thesis, I recommend that continued effort be devoted to refining the geometry of microneedles and the fabrication techniques required to support this refinement. The long term goals for hollow microneedles include the pressure driven flow of molecules to allow higher delivery rates into the skin. In addition, an ideal drug delivery system would include an integrated sensing and delivery component to form closed loop control scheme.

The fabrication scheme presented in this work is capable of producing microneedles with lengths up to 500 µm easily, and with some additional effort microneedles up to 1 mm in length. Above 500 µm, the availability of thick polymer stock that is capable of withstanding all the processing steps and is not cost prohibitive is limited. The lamination scheme described in this work is capable of creating thick polymer laminates, but the additional time and effort and the possibility of introducing a defect region in the needle wall is problematic. Investigation into other polymers may present options not considered in this work.

Surpassing 500 µm microneedle heights also presents difficulty in preparing uniform seed layers in polymer molds. Electroless plating was demonstrated to be capable of depositing seed layers into deep molds, but the masking of parts to prevent plating on the backside of the mold has not been satisfactorily resolved. Further investigation to resolve this issue would practically remove the upper bound on length of microneedles created in this fashion.

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It has been shown that 500 μm microneedles are capable of penetrating the skin and successfully delivering molecules in meaningful quantities. Therefore the previous recommendations are necessary only for applications in which longer needles are required for some other purpose than overcoming the deflection of the skin.

Of far greater concern, in regards to microneedle geometry, is the coring of tissue at the tips of microneedles. This issue must be resolved if delivery is to be accomplished by means other than passive diffusion. There are at least two alternatives to resolve this issue. Either the lumen must exit the microneedle somewhere other than the tip (side wall, or tip with a leading edge to penetrate first) or a leading edge concentric to the lumen, that is withdrawn post insertion to leave a clear pathway, must be included. Without the benefit of a clear tip, pressure driven flow will not be possible.

Finally, investigations should begin in the development and integration of sensing and pumping components for microneedles. Although substantial literature exists on each of these subjects, limited work actually demonstrating the successful integration of these devices with microneedles has been published. For a truly complete drug delivery system, these items and control circuitry must be included to close the delivery loop.
REFERENCES


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VITA

Shawn Paul Davis was born in Spokane, Washington on February 20, 1976. He graduated from Eau Gallie High School in Melbourne Florida in 1994. He then completed a Bachelor of Science in Chemical Engineering at the Florida Institute of Technology in Melbourne, FL. He graduated with highest honors and was named a “Florida Tech Distinguished Graduate” (valedictorian equivalent). In August of 1998 he attended Georgia Institute of Technology, Atlanta, GA. His dissertation title was “Hollow Microneedles for the Molecular Transport Across Skin.” He defended his thesis on May 13, 2003 and obtained his Ph.D. in Chemical Engineering with a Minor in Management on August 1, 2003.