FABRICATION OF OUT-OF-PLANE MICRONEEDLES FOR DRUG DELIVERY AND BIOSENSING

by

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ABSTRACT

Hollow microneedles can be used to painlessly inject drugs or extract dermal interstitial fluid for biosensing. However, their fabrication so far has been associated with costly and time-consuming steps restricting their batch production as a viable option. This thesis presents novel methods for fabricating inexpensive hollow microneedles, and investigates new methods of characterizing the drug delivery and interstitial fluid sampling using microneedles.

First, a method is presented for fabrication of hollow polymer microneedle arrays. Microneedles are formed during a solvent casting process, which leaves a polymer layer around pillars in a pre-fabricated mold. Arrays of microneedles with lengths up to 250 µm have been fabricated. The strength of the microneedles was evaluated to ensure reliable skin penetration and their suitability for drug delivery was demonstrated by injection of fluorescent beads into a skin sample.

A second fabrication method is presented for making metallic microneedles with high aspect ratios. Solvent casting was used to coat a mold with a conductive polymer composite layer, which was then used as a seed layer in a metal electrodeposition process to form 500 µm tall microneedles. Some fabrication process steps were characterized and the strength of the microneedles was evaluated. Their usefulness for drug delivery was also demonstrated by injection of fluorescent microspheres into animal skin.
Designing effective microneedles requires understanding the drug diffusion process in skin. Here, a novel method is used to characterize diffusion of a chemotherapeutic drug injected with microneedles into skin. Using confocal microscopy, the concentration distribution of the drug was measured over time and then compared to an analytical diffusion model to obtain the drug’s diffusion coefficient. Using this method, different skin storage conditions were evaluated. It was concluded that using previously frozen skin should be avoided for transdermal drug delivery studies.

Finally, using the proposed processes, hollow and solid microneedles were fabricated for sampling interstitial fluid for biosensing applications. Minimal removal of the interstitial fluid was achieved with a solid microneedle design as well as a hollow metallic microneedle array attached to a vacuum probe, while no trace of the fluid was observed when using hollow polymer microneedles.
PREFACE

The research presented in this dissertation was carried out at the University of British Columbia (UBC), in the Stoeber laboratory at the department of Electrical and Computer Engineering, under supervision of Dr. Boris Stoeber. This research was performed in close collaboration with Dr. Urs O. Häfeli from UBC’s Faculty of Pharmaceutical Sciences. A portion of the research was also conducted at the BC Child and Family Research Institute in collaboration with Dr. Jan Dutz and Dr. Jacqueline Lai from UBC’s Department of Dermatology & Skin Science.

Chapter 1 of this thesis starts with a brief overview of the transdermal drug delivery and the anatomy of the mammalian skin. Next, after discussing the basic concepts of microelectromechanical system (MEMS) fabrication technologies, it is discussed how MEMS can be used to fabricate microneedle systems which can significantly enhance transdermal drug delivery by overcoming the barriers and limitations of traditional drug delivery systems. This discussion is then followed by a detailed literature review on the previous fabrication methods used to make microneedle devices with different shapes and from various structural materials.

Chapter 2 is based on the work published as the following journal article:

This work was also presented in the following workshops and conferences:


These papers present novel fabrication methods for hollow out-of-plane polymer microneedle arrays used for painless administration of compounds into the skin. Being cost effective and applicable for batch processing, the presented process uses an inexpensive polymer solvent casting technique to form disposable microneedle arrays that can be used for a variety of transdermal drug delivery applications. The proposed procedure was used to make microneedles with heights of up to 250 µm. The devices were characterized through drug delivery trials and their strength was evaluated to ensure reliable penetration into skin.

Chapter 3 of this thesis is based on the work published as the following journal article:


This work was also presented in the following workshops and conferences:


In addition, a provisional patent application has been filed based on the work in this chapter:


In this chapter, a process is presented for fabricating inexpensive hollow out-of-plane microneedle materials from metals. The proposed process is a combination of solvent
casting and metal electroplating. It allows fabricating microneedles with large aspect ratios. The microneedles can be much longer than the polymer needles in the previous chapter and their length is only limited by the height of the solvent casting mold structure. Some process parameters were characterized and the needles fabricated were tested for drug delivery and mechanical strength. My contributions in the corresponding journal publication as a first author are: designing the fabrication procedure, fabricating microneedles, characterizing the plasma etching process, carrying out some of the conductivity and resistivity measurements, carrying out mechanical tests, performing skin penetration and injection trials, performing a detailed literature review, and writing the manuscript.

Chapter 4 of this thesis is based on a manuscript that is ready for submission to a journal:


This chapter focuses on the drug delivery application of microneedles. More specifically, metallic microneedles are used to inject a chemotherapeutic compound into the skin. Using confocal microscopy, the drug diffusion in skin is investigated and compared to an analytical diffusion model to measure the diffusion coefficient of the drug in the skin. My contributions in this journal publication as a first author would be: performing the calibration tests, preparing the injection setup, carrying out all of the injection tests,
carrying out the imaging process, carrying out the diffusion measurements and analysis, performing a detailed literature review, and writing the manuscript.

Chapter 5 of this dissertation is based on the research performed towards using the microneedles for biosensing applications. More specifically, the needles fabricated through the processes similar to those presented in Chapters 2 and 3 were used to extract interstitial fluid from skin. A version of one of the techniques presented in this chapter was presented in the following conference:


The last chapter gives a summary of the previous chapters as well as a discussion of possible future works than can be pursued to improve the presented microneedle technology.

The scientific contributions of the work in this thesis can be summarized as following:

1. Demonstrating a novel fabrication method, based on solvent casting technology, to make hollow polymer microneedle arrays for drug delivery and biosensing. The proposed process is among the most inexpensive proposed fabrication techniques for polymer microneedles and is suitable for commercial adoption of the microneedle technology. It allows fabricating microneedles with any polymer material that can be cast, and allows a wide range of dimensions.
II. Demonstrating a novel fabrication process, based on metal electrodeposition onto cast conductive polymer film, to make hollow metallic microneedle arrays for drug delivery and biosensing. The proposed process is among the most inexpensive proposed fabrication techniques for metallic microneedles and is suitable for commercial adoption of the microneedle technology. It allows fabricating microneedles with any electrodepositable metal and allows a wide range of dimensions and spacing.

III. Demonstrating a novel technique to characterize transdermal drug delivery with hollow microneedles. The proposed technique uses fluorescent imaging to investigate diffusion of a microneedle-injected drug in skin. This process can be used to analyze the usefulness of microneedles for delivery of any stable fluorescent compound. This technique also allows measuring the diffusion coefficient of the compound in artificial and biological skin models.

IV. Evaluating three potential interstitial fluid extraction techniques using solid and hollow microneedles, to demonstrate which technique is more promising for developing microneedle-based biosensing systems.
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LIST OF VARIABLES

δ  Membrane thickness
ρ  Resistivity
∇ Laplace operator
A  Area of cast cylindrical structures
B  Characteristic skin membrane constant
C  Concentration
D₀  Diffusivity of a hypothetical drug molecule with zero volume
D  Diffusion coefficient of drug
F₆  Microneedle failure load
I  Electrical current
J  Steady-state flux of solute
Kₘ  Solvent-membrane distribution coefficient
l  Thickness of cast cylindrical structures
N₀  Initial mass
r  Radial distance from diffusion source
R  Resistance
τ₁  Duration of time between the end of the injection process and taking the first confocal image
τ  Time
τᵢ  Duration of drug injection
V  Molecular volume of drug
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DEDICATION

To my parents

&

my wife
CHAPTER 1

INTRODUCTION

Finding novel and effective methods of delivering medicinal compounds into the human body and monitoring and controlling these compounds has been an ongoing field of research since the emergence of the medicine. When it comes to delivering an agent, the most effective administration method depends on the type of the drug itself as well as the location and properties of the target medium. In another view, the pharmacokinetics and the pharmacodynamics of the drugs determine how they should be administered. Common methods of drug administration use parenteral, enteral, or topical routes or a combination. Parenteral administration is direct injection of drugs into specific tissues using traditional hypodermic needles attached to drug-filled syringes. This method is usually invasive and painful as often no local or general anaesthesia is used. The main advantage of this method, however, is that it allows delivery of large and controlled quantities of drugs into specific target areas. Enteral administration is based on delivering drugs to the digestive system orally, rectally, or through feeding tubes. Oral and rectal administrations are often easy to
practice and can be carried out by the patients themselves. Most of the over the counter medication uses this method of administration. The main disadvantage of this method is that there is less control over how efficiently the drug can target a specific region of the body. In addition, many drugs might cause damage to the digestive system before they are absorbed or disposed. Moreover, a lot of patients (especially small children) may find this method of administration uncomfortable. Topical administration routes are among the least invasive methods and therefore more comfortable for patients. Some examples of topical administration methods include: transdermal, rectal, inhalational or through eye drops, eardrops, or mucus membranes.

In general, a crucial aspect of drug delivery is determining the type of drug that the patients need in the first place, which is often done by physicians; next, is deciding on the amount to be administered. In some cases, deciding on the amount requires knowing how much a certain compound (ex. the same drug) already exists in the blood prior to administration. This is especially important for therapeutic drug monitoring (TDM) whose main focus is keeping drug concentrations within a therapeutic window in order to avoid under- or over-dosing. While there have been numerous methods of determining the concentrations of compounds in the body, the non-invasive methods (such as optical methods) are often limited to a very small number of compounds. The most common method of analyzing compounds in blood relies on taking blood samples from patients using hypodermic needles, which can be painful and uncomfortable for many patients or even unethical for small children.
1.1 Transdermal drug delivery

Among the topical drug administration methods, transdermal drug delivery is becoming increasingly popular because it is the easiest to practice and not associated with the potential risks and pain of traditional hypodermic needles [1]. This method relies on the transport of drugs across the skin into the blood stream. The structure and properties of the skin are important factors for this method of administration.

1.1.1 Structure of skin

Skin is a protective membrane that makes up the outer covering layer of animals’ body and guards the underlying muscles, bones, and internal organs. The general structure of mammalian skin is shown in Figure 1.1.

Figure 1.1: Structure of mammalian skin [2].
The skin mainly consists of two layers, the epidermis and the dermis, which are attached to the hypodermis that connects the skin to the underlying bone and muscle. The epidermis, (shown in more detail in Figure 1.2) constitutes the superficial layer of the skin. It is mainly made of keratinocytes, protective cells that serve as building blocks of a barrier against physical environmental damage, pathogens, heat, radiation, and water loss. The keratinocytes in the epidermis are arranged in multiple layers, with the stratum basale being the innermost and the stratum corneum (SC) being the outermost layer. Through a process called cornification, the fresh live keratinocytes in the stratum basale gradually lose their internal nuclei and organelles, produce keratin (a structural fibrous protein), and become flat. Different stages of this process correspond to the different epidermal layers, with the SC being the fully keratinized, flat, and rough cells that are gradually pushed outwards. The average thickness of the human SC layer is 14.8 ± 4.8 µm while the average thickness of the viable epidermis is 68.9 ± 17.0 µm [3].

The dermis is the layer underneath the epidermis, and is made of connective tissue that connects the epidermis to the hypodermis (or subcutaneous, fatty) tissue. The dermis is a flexible membrane that protects the body from stress and strain. The collagen and elastic fibers in the dermis provide tensile strength and give it elasticity. The dermis also contains blood capillaries, lymphatic vessels, sweat glands, hair follicle, and nerve fibers. The dermis layer is generally much thicker than the epidermis, and its thickness varies widely throughout the human body and can range anywhere from 0.6 mm up to 3 mm in adults. In addition to the structural membranes and fibers, both the viable epidermis and the dermal
layers contain interstitial fluid (ISF). The ISF is a water-based medium that surrounds all the cells and is responsible for transferring nutrients and ions to and from the cells.

![Skin Structure Diagram](image)

**Figure 1.2: Structure of epidermis [4].**

The elasticity and thickness of both the dermis and the epidermis are also a function of gender, age, and ethnicity [5-7]. The mechanical properties of the skin depend on the nature and organization of the dermal components such as the collagen and elastic fibers as well as the water, proteins, and the macromolecules embedded in the extracellular matrix [8]. Skin exhibits viscoelastic behaviour when undergoing deformation. There have been many proposed techniques to measure the mechanical properties of skin. According to a recent study that used indentation test methods, the average value of the skin’s Young’s modulus is between 4.5 kPa to 8 kPa [9].
1.1.2 Adhesive skin patches

One method for transdermal drug delivery uses adhesive skin patches. In this method, a pharmaceutical compound is absorbed through the skin after attaching a patch (filled with the formulation) to the skin surface by means of an adhesive layer. It is estimated that currently more than one billion transdermal adhesive patches are being manufactured each year [10]. Various types of pressure sensitive adhesive patches (PSA) made from polyisobutylene, acrylic, and silicone are commercially available for use on skin [11] for applications such as smoking cessation (nicotine delivery) [12], pain relief (morphine or fentanyl delivery) [13, 14], and high blood pressure treatment (glyceryl trinitrate delivery) [15].

Adhesive patches are associated with limitations that restrict their usage for drug delivery. Among the skin layers, the epidermis and more specifically the SC plays the most important role in governing the diffusion of compounds from these patches into the body, due to their lower permeability compared to the dermis [16-18]. The SC barrier contains mostly tightly arranged flat cells filled with keratin, and it is essentially lipophilic. It is impermeable to most hydrophilic compounds but lipophilic molecules are generally better accepted by the SC [19]. Ideally, the drug molecules have to be small (less than 500 kDa) and have to exhibit both lipoidal and aqueous behaviour in order to be able to liberate from the formulation, go through the lipid cellular matrix of the SC, and then move to the more aqueous epidermis underneath [19]. In other words, if the drug is too hydrophilic, it will not diffuse into the SC, and if it is too lipophilic, it will stay in the SC and not diffuse to the viable epidermis and
dermis. When it comes to mathematically modeling the diffusion, using the familiar Stokes-Einstein equation is invalid since this model describes the movement of spherical particles in uniform fluid medium whereas for the skin, the biological cell membrane is a gel-like viscous lipid matrix that does not move around the particles and contain a staggered arrangement of cells. A model for diffusion across skin membrane

$$D = D_0 \times \exp(-\beta \cdot V) \quad (1)$$

is presented by Potts et al. [17], where $D$ is the diffusivity of the drug within the membrane, $D_0$ is the membrane diffusivity of a molecule with zero volume, $\beta$ is a characteristic membrane constant, and $V$ is the molecular volume of the drug. The permeability of the skin membrane can then be described in the terms of the steady-state flux of solute

$$J = \frac{K_mD \Delta C}{\delta} \quad (2)$$

obtained from Fick’s law [18], where $\Delta C$ is the drug concentration difference across the membrane, $\delta$ is the membrane thickness, and $K_m$ is the solvent-membrane distribution coefficient obtained from the ratio of the drug concentration sorbed on the SC over the drug concentration in the patch. In general $D$ and $J$ are small for most therapeutic compounds for diffusion through the SC [16-19], which limits the usefulness of skin patches.

Another limitation is that to deliver compounds using patches at useful dosages, these devices often have to rest on the skin for prolonged durations, from a few hours to days and even weeks. Long exposure times can potentially induce skin sensitivity reactions [20].
One type of these skin reactions is the non-immunological contact sensitivity such as irritant dermatitis [20]. Long-term applications of transdermal patches cause hydration of the epidermal layer, which leads to accumulation of sweat on the surface; such a moist environment promotes yeast and bacteria growth on the surface. Both the bacterial overgrowth and the sweat accumulation contribute to the irritation reactions. Another type of skin reaction is allergic contact sensitivity, which is caused by the response of the immune system to the transdermal patch components such as the adhesive, the membrane, the solvent, the enhancer, and the active drug [20].

Different methods have been previously proposed for evaluating skin permeability for different drugs exposed to the skin surface. One method involves measuring the change in electric properties of skin due to compound absorption [21-23]. Kasting et al. [21] used this method to evaluate the change in electrical resistance of skin with respect to a change in sodium ion content. A similar method was used by Dick et al. [22] who measured pig ear skin conductivity to evaluate its usefulness as an in vitro model for human skin permeability studies. Karande et al. [23] also used skin electrical impedance measurements to evaluate the effect of chemical enhancers on skin permeability. A more common method involves using diffusion cell instruments where the skin is mounted between a donator bath and a receptor bath and the change in concentration of the baths is monitored over time. Bath concentrations are measured by different methods including fluorescence imaging, radioactivity measurements, or high-performance liquid chromatography [24-35]. Using this method, Khalil et al. [31] measured glucose diffusivity in the viable epidermis of human skin. Williams et al. [32] used a diffusion cell to measure the diffusion coefficient of
fluorouracil (an anti-cancer drug) in the human epidermis when administered with different penetration enhancers. Kreilgaard et al. [33] used a diffusion cell to evaluate the usefulness of microemulsions for delivery of a lipophilic compound (lidocaine) and a hydrophilic compound (prilocaine hydrochloride) across rat skin. Similarly, Hansen et al. [34] studied the permeability of different human skin layers to flufenamic acid and caffeine compounds. A diffusion cell was also used by Mitragotri [35] to evaluate the influence of various enhancers including ultrasound and chemicals on the delivery of the five drugs estradiol, naphthol, aldosterone, lidocaine, and testosterone across the human epidermis.

Confocal microscopy is also a useful approach to image diffusion of fluorescent drugs into skin [36-40]. This method has been previously used by Schätzlein et al. [37] to characterize transfersome-based delivery of fluorescently labeled compounds into mouse skin. Scarmato De Rosa et al. [38] used confocal microscopy to visualize the penetration of 5-aminolevulinic acid (a compound used in photodynamic therapy (PDT) of skin cancers) into mouse skin. Verma et al. [39] used confocal microscopy to evaluate penetration of carboxyfluorescein into abdominal human skin incubated on a diffusion cell. A. P. Raphael et al. [40] used confocal microscopy to characterize diffusivity of macromolecules delivered using a microprojection array through epidermal and dermal skin layers.
1.2 MEMS for biomedical applications, drug delivery, and biosensing

The field of microelectromechanical system (MEMS) research deals with the development of very small (often less than 1 mm) electromechanical structures [41-43]. This technology uses the conventional semiconductor processing tools used for electronic chip manufacturing to create systems that are used for a variety of applications such as power generation, sensing, and actuation. The small size of these devices provides many advantages over large-scale systems in terms of power usage, sensitivity, cost, and space occupation [41-43].

Common materials used in MEMS technology are silicon, polymers, metals, and ceramics, and the basic techniques used in MEMS fabrication are material deposition, patterning, and etching. Material deposition leads to the formation of layers of materials with controlled thicknesses ranging from a few nanometers up to hundreds of micrometers on a substrate [41-43]. Physical vapor deposition (PVD) is a physical deposition technique that evaporates material and deposits the vapor onto a surface. Examples are deposition of metals by sputtering or evaporation. Another physical deposition method is solvent casting in which a polymer solution is applied to a surface and after evaporation of the solvent a polymer layer coats the surface. In chemical deposition methods (such as chemical vapor deposition, CVD) a stream of gas or plasma reacts on a substrate surface to grow the desired material, or alternatively the gas species first react and then deposit on the substrate.
The patterning process involves transferring a pattern onto a substrate [41-43]. The most common type is photolithography in which the pattern is transferred to a photosensitive material (Figure 1.3). Through this process, an extruded form of the pattern is created with the photosensitive material (i.e. photoresist). The photoresist can be positive (light weakens the adhesion between polymer chains) or negative (light cross-links the polymer chains). The photolithography process can be used for making etch masks (i.e. temporary layers) or permanent mold structures, and is a common fabrication step in almost all MEMS processes.

The remaining MEMS fabrication step involves etching or removing material by dry etching or wet etching methods [41-43]. This is often performed to pattern functional layers or to remove a sacrificial material for facilitating the lift-off of a structural layer. The wet etching process uses chemicals to dissolve the materials. Some examples are isotropic etching of silicon dioxide using hydrofluoric acid (HF) or anisotropic etching of pure silicon using potassium hydroxide (KOH). In the dry etching technique, chemically reactive gases are used to remove material from substrate surface. Reactive ion etching (RIE) of polymers is an example of dry etching process that uses high energy plasma. Deep reactive ion etching (DRIE) is a highly anisotropic etching process, based on multiple RIE steps, used to form deep channels in silicon. Xenon difluoride is an example of a highly reactive gas used for isotropic dry etching of silicon.
The area of MEMS with applications to the life sciences and biomedical engineering is referred to as BioMEMS [44]. Cheaper and smaller devices can be fabricated using this technology for diagnosis and treatment of various conditions. Miniature medical implants, drug delivery systems, on-chip assays, cell sorting devices, and DNA sequencers are some examples of BioMEMS devices.

1.3 Microneedles as a minimally invasive interface with the body

A transdermal approach to drug delivery using microneedles can potentially circumvent the limitations of adhesive skin patches. Microneedles are sharp mechanical structures made through MEMS technology. Microneedles have been used for transdermal drug delivery [45-47] and extraction of biofluids for sensing applications [48], or as neural probes and
microelectrode arrays [49]. They can have solid or hollow structures, and can be arranged in out-of-plane or in-plane arrays (Figure 1.4). Out-of-plane microneedles are often less than 1 mm tall while in-plane devices are usually longer than 1 mm.

![Diagram of in-plane and out-of-plane microneedles](image)

**Figure 1.4:** Comparison of orientation of in-plane and out-of-plane microneedles, illustrated on a wafer substrate in stereoscopic view. In-plane needles are arranged along the plane of the substrate while out-of-plane needles are arranged perpendicular to the plane of the substrate.

For drug delivery applications, unlike traditional hypodermic needles, microneedles can be used in a way that does not require a high level of training, and they bear a low risk of device contamination through blood. Furthermore, microneedles can facilitate fluid injection and sampling painlessly or with minimal pain sensation (mostly from the applied pressure) if they do not penetrate deep enough to reach the nerve endings in the skin’s dermal layer. Previous studies have demonstrated painless injection of drugs into the skin using microneedles [50]. Microneedles can potentially replace hypodermic needles for delivery of many compounds including vaccines and drugs that target the blood circulation system. Microneedles can also be used to substantially increase the rate of delivery of drugs currently administered via adhesive patches. In some case where the drug properties (such as molecular weight) make it difficult to administer via adhesive patches, microneedles can overcome the SC barrier and substantially improve the delivery rate of these compounds.
Microneedles are also useful for targeted drug delivery into the skin for treatment of various skin conditions such as skin cancers.

Since the early 1990s, different concepts for the fabrication of microneedles with a variety of dimensions and geometries have been developed. These devices have been fabricated out of single or polycrystal silicon [51-65], glass or silica [66-68], metals [69-76], and polymers [77-82].

1.3.1 In-plane solid and hollow microneedles

In-plane microneedles are planar structures formed along the surface of a base substrate and are developed for drug delivery, biosensing, and neural stimulation [51-54]. These devices are often longer than 1 mm and some are designed for targeted delivery at the cellular level. They are usually integrated with additional microfluidic and electronic circuitry to make more complete lab-on-a-chip systems.

Chen et al. [51] has presented a 4 mm silicon in-plane hollow probe for neural stimulation and targeted drug delivery. The fabrication process uses multiple boron doping and silicon dioxide patterning steps as well as anisotropic etching of silicon along wafer plane using ethylene-diamine pyrocatechol (EDP), to create the hollow needle structure. A similar fabrication process is presented by Lin et al. [52] in which needles, made from silicon nitride, are formed through silicon dioxide patterning, boron doping, silicon nitride deposition, and EDP etching of silicon.
An alternative fabrication method for in-plane microneedles is presented by Zahn et al. [33]. This fabrication method is based on forming a polysilicon microneedle structures onto a silicon micromold, with silicon dioxide being used as sacrificial release layer. The silicon mold is a planar channel formed by aligning and attaching two patterned silicon wafers together. The main advantage of the process is that the silicon micromolds are reusable, but each fabrication run requires precise alignment of the wafers. Paik et al. [54] presented a similar fabrication technique to make polysilicon needles but without alignment, however, the process required multiple deep reactive ion etching steps (DRIE) to form deep silicon channels.

Fabrication of polymer in-plane microneedle has been demonstrated by Takeuchi et al. [77]. A flexible single needle has been made by deposition of polyimide on an AZ photoresist sacrificial layer that corresponds to the inner channel. The proposed design reduces the fabrication costs compared to the previous designs, but the needle by itself was too flexible and weak and not capable of penetrating skin. To solve this, the authors proposed filling the needle channel with a temporary polyethylene glycol (PEG) layer to increase the needle rigidity for skin penetration.

Finally, to make metallic in-plane needles, Brazzle et al. [69] used electroplating to form needles on sacrificial photoresist layers. This process is among the cheapest fabrication methods for in-plane needles, but still requires multiple masking and metal sputtering steps.
Although the usefulness of in-plane needles have been demonstrated in these works, they have not been commercially adopted since the manufacturing processes are too expensive for single-use systems. In addition, these processes are useful for making single needle devices or one-dimensional arrays, which may not be sufficient for delivering larger quantities of drugs. Developing cheaper two-dimensional arrays, containing larger numbers of out-of-plane microneedles, is thus crucial for overcoming these limitations.

1.3.2 Solid out-of-plane microneedles

Solid out-of-plane microneedles are designed to increase the skin permeability by piercing the SC and exposing the underlying skin layers to the drugs that are later applied to the skin surface, or alternatively to the drugs that already coat the surface of the needles or are embedded in a biodegradable polymer that is the structural material of the needles [10]. Solid microneedles provide an improvement to the existing skin patches by overcoming the SC barrier. It is shown that solid microneedles can increase skin permeability by almost four orders of magnitude [20, 75]. However, the disadvantage of these devices is that they provide a passive drug delivery method that is slow which may not be useful for delivery of many compounds.

Griss et al. [83], Hashmi et al. [84], Shikida et al. [64], and Ji et al. [85] all present fabrication processes for making large arrays of solid out-of-plane microneedles from silicon. The needles are cone-shaped and sharp, and formed through isotropic or anisotropic etching of silicon that created undercuts beneath silicon dioxide mask layers (Figure 1.5).
Figure 1.5: Fabrication of sharp cone-shaped structures using silicon etching.

Park et al. [79] present solid biodegradable microneedle arrays designed to dissolve inside the skin and release the drugs that are integrated within the structural polymer matrix. For the fabrication, a master mold is first made, often through photolithography, which contains an array of pillars. Next, the inverse of the master mold is made by a flexible molding material such as polydimethylsiloxane (PDMS). A solution of the biodegradable polymer (polyglycolic acid, PGA or polylactice-co-glycolic acid, PLGA) was then filled in this mold leading to the replicated biodegradable pillar arrays. Similar technique was used by Han et al. [80] but instead of casting the polymer solution, polycarbonate (PC) needles were made through hot embossing.

Solid metallic microneedle arrays have also been made and already used for commercial applications [86]. One fabrication method uses a laser to create needle-shaped patterns in metallic sheets; the needles are then bent perpendicular to the needle plane to form the needle array.

1.3.3 Hollow out-of-plane microneedles

Hollow out-of-plane microneedles can pierce through the SC and provide a passage for the injection of lipophilic and hydrophilic compounds of small and large molecular weights into
the skin (Figure 1.6). In contrast to adhesive patches, hollow microneedles are not limited by skin permeability, and unlike solid microneedles, they can be potentially used to deliver larger amounts of drugs into the skin.

Figure 1.6: Concept of drug delivery using hollow microneedles.

Transdermal drug delivery through hollow out-of-plane microneedles has been demonstrated in clinical trials, where methyl nicotinate was injected using hollow microneedles [50]. During this study, the volunteers confirmed that the method was painless and they only felt a slight pressure during the injection. In a different study, hollow metal microneedles were used to deliver insulin to diabetic hairless rats [73]; a 4-hr delivery of insulin injection resulted in 47% reduction in the blood glucose.

Hollow silicon microneedle arrays were the first hollow designs that were demonstrated and tested in clinical applications. Stoeber et al. [55, 56] present a process for making arrays of 200-250 μm tall microneedles with sharp tips and wide bases (Figure 1.7). The fabrication
process is based on first creating a deep channel (corresponding to the needle lumen) from one side of a silicon wafer using DRIE (Figure 1.7a-1–3) and then using isotropic etching of silicon on the other side of the wafer to create the cone-shape needle structure (Figure 1.7a-4–7). The proposed process also allows producing sharper needles by dislocating the centerline of the patterned silicon dioxide layer in Figure 1.7a-5 with respect to the centerline of the channel. This results in one side of the needle to be etched more than the other side resulting in pointed tip shapes shown in Figure 1.7c.

Figure 1.7: a) silicon microneedle fabrication process, b) SEM image of microneedles with symmetrical tips, c) SEM image of microneedles with pointed tips [56].

Similar concepts were used by other groups such as Griss et al. [63], Gardeniers et al. [60], and Mukerjee et al. [48] for producing silicon microneedles. In these works, the deep needle lumens are created through masking steps and DRIE of silicon, and the needle shapes are formed through isotropic and anisotropic wet or dry etching steps. DRIE of
silicon has also been combined with multiple deposition and etching steps to form silicon dioxide microneedles [66].

Hollow polymer microneedles are not generally as strong as silicon designs but they are less expensive and thus more suitable to be used as single-use disposable devices. Moon et al. [78] have used two successive inclined photoresist exposure steps and a hot embossing step using a PDMS mold to make 750–1000 µm tall microneedles from poly(methyl methacrylate) (PMMA). This process is time-intensive and requires sensitive alignment steps to make the needles. An even longer fabrication process is proposed by Huang et al. [81] that involved making a patterned PDMS base layer on another patterned glass assembly, and then forming needles on the PDMS layer from SU-8 (an epoxy type negative photoresist) through photolithography. The resulting needles (~200 µm tall) are not sharp and their mechanical strength for reliable skin penetration is not sufficiently demonstrated.

To make hollow microneedles from metal, Kim et al. [70] first make a mold (containing an array of pillars) from SU-8 on a glass substrate and then after deposition of a metallic seed layer, a thick nickel layer is electroplated on the mold. The electroplating step also covers the pillar tips resulting in non-hollow structures. To remove the nickel covering the tips, an additional mechanical polishing step is used with an extra layer of unexposed SU-8 used as planarizing layer (to assist with uniform polishing of the tips). After polishing, the planarizing SU-8 layer is removed in developer. The microneedle array (400 µm tall) is separated by first dry etching the SU-8 mold and then wet etching of the electroplating seed layer. The proposed process is time consuming and expensive since there are multiple
wet and dry etching steps and also the SU-8 molds are not reusable. In addition, the process yield is low since removing SU-8 in deep metallic channels using dry etching is not highly efficient, which results in a lot of the SU-8 pillars getting stuck in the nickel needles.

A different process proposed by Davis et al. [73] creates metallic microneedles by electroplating nickel on cone shaped through-holes (drilled by laser) in polymer substrates. The microneedle array is separated from the polymer substrate by dissolving the polymer in solvent. The resulting needles are 500 µm tall. The main disadvantage of this process is that it requires drilling individual holes on single-use polymer substrate, which is a time consuming process especially for large needle arrays.

Fabrication of ultra-high-aspect-ratio metallic microneedles (taller than 1 mm) was presented by Li et al. [76] and Lee et al. [87]. To create microneedle arrays, first drawing photolithography technique was used to make a base layer containing tall pillar arrays from SU-8. Drawing lithography takes advantage of the higher viscosity of the unexposed SU-8 material to form elongated pillar structures between two parallel plates. After making the base layer, the pillars were used as mold for metal seed layer deposition and subsequent nickel electroplating. The hollow needle tips in [87] were achieved by deposition of a nonconductive material onto the pillar tips prior to nickel deposition, while in [76] it is achieved by cutting the individual needle tips with a laser. In both cases, the needles were removed from the mold by removing the base SU-8 layer. These processes are also long (due to processing individual tips with laser) and expensive (due to the single-use of the SU-8 molds).
In addition to drug delivery applications, hollow microneedles have been shown to be promising devices for biosensing applications. For this purpose, the hollow needles are used to sample ISF or blood from the skin in order to analyze the sample for a particular compound. Biofluid extraction using hollow microneedles is either facilitated through capillary forces or by applying a vacuum through the needle lumen. Mukerjee et al. [48] used 200 µm tall silicon microneedles to collect ISF using capillary forces from human earlobe. The presence of glucose was demonstrated in the sampled fluid using commercial glucose strips.

Extraction of blood requires longer needles in order to reach the blood vessels in the dermis or in the hypodermal layer. Due to penetration into deeper skin layers, this process is often associated with pain. The amount of pain partly depends on the number of nerves stimulated during the insertion and extraction process, which depends on the needle shaft’s outer diameter. Therefore, thin microneedles may still have an advantage over the traditional hypodermis needles in this application.

Blood extraction is shown by Li et al. [76] in which the authors use a single 1800 µm tall metallic microneedle to extract 20 µL of blood from a mouse tail. The extraction force is provided by a negative pressure of 13.45 kPa through the needle lumen.

Another proposed approach for using microneedles to extract blood or ISF is to puncture holes on the surface of the skin using microneedles and then applying vacuum to the damaged surface after removal of the needles. Wang et al. [88] use a similar technique by creating holes in skin using 700-1500 µm long microneedles, and then extracting 1-10 µL of
ISF using a 200-500 mm-Hg vacuum source after 2-10 min. This method does not directly use microneedles to sample the fluids and therefore it is not possible to integrate a detection system with the microneedles.

To conclude this section, the hollow microneedle offer a better alternative to other microneedle designs for injection of drugs in terms of the amount of liquid delivered as well as the injection rate. They also can be used to sample biological fluids for sensing applications. However, until now, the commercial use of hollow microneedles for biomedical applications has been encumbered by the expensive fabrication techniques mentioned above, such as deep reactive ion etching of silicon needles [48, 56, 60, 63], sequential formation of disposable polymer molds for electroplating [70, 73, 76, 87], and multiple UV exposure or mold transfer and assembly steps to form polymer needles [78-82]. An inexpensive alternative to the costly fabrication techniques proposed in the literature would facilitate faster adoption of these systems in the commercial market.

In addition to the fabrication processes, previous literature on hollow microneedles lacks an in-depth analysis of what happens to the drug once it is injected into the skin tissue and how long it takes the drug to reach target skin layers. This is mainly because there has not yet been a method proposed to quantify the drug diffusion process in skin when the drug is delivered using hollow microneedles. Only after utilizing such a method, effective hollow microneedle systems can be designed for clinical drug delivery applications, and the drugs that are suitable for transdermal injection with hollow microneedles can be chosen.
Furthermore, although the potential for painless biosensing has been revealed in a limited number of publications, there is still a need for further analysis of the potential ISF extraction methods and investigate which ones are the most promising.

This thesis aims at addressing some of the gaps and limitations in the previous literature on hollow microneedles. More specifically, the objectives pursued in this work can be summarized as follows:

I. Develop cost effective fabrication processes applicable for large scale manufacturing of robust microneedles.

II. Use the microneedles for biomedical applications:
   
   a. Demonstrate transdermal drug delivery using the fabricated microneedles.

   b. Assess the possibility of using microneedles for ISF sampling and biosensing applications.

III. Develop a method useful for studying drug transport in skin and comparing different skin models.
CHAPTER 2

FABRICATION OF POLYMER MICRONEEDLES BASED ON SOLVENT CASTING

Here, we present a simple solvent casting method for the fabrication of hollow out-of-plane polymer microneedles. Solvent casting has been a common method for more than a century to form polymer films [89]. In this method, a polymer solution is deposited onto a mold, and after evaporation of the solvent, a polymer layer remains on the mold which is then separated by physical or chemical means. In MEMS, solvent casting has been used to coat microchannels with polymers such as poly(vinyl alcohol), poly(ethylene oxide), polyacrylamide, and poly(N-hydroxyethylacrylamide), mainly for modifying surface properties or for protein and DNA separation [90]. Solvent casting has also been used to make polylactic acid microstructures for tissue engineering [91] as well as polystyrene microcantilever beams for sensing applications [92]. Additionally, solvent casting has been used to fabricate microneedles with solid structures [93]. We have previously shown that
the polymer profile formed in a mold during solvent casting can be adjusted by controlling
the solvent casting process parameters such as temperature [94].

Solvent casting is a fast and repeatable fabrication process for microneedles, and it requires
only one step of photolithography, eliminating the need for mask alignment. In this chapter
we present the selection of the clay concentration for maximum strength of the polymer-clay composite that serves as the structural material for the needles, followed by the
detailed fabrication process of the microneedles made using solvent casting. This is then
followed by a study of the mechanical strength of the fabricated microneedles, and by the
demonstration of fluid injection into rabbit ear skin.

2.1 Fabrication of hollow out-of-plane polymer microneedles

2.1.1 Microneedle material selection

Polyimide was chosen as the structural material for the microneedles due to its high
Young’s modulus (8.5 GPa) relative to that of other polymer materials [95]. The polyimide
type PI-2611 (HD Microsystems, Parlin, NJ) used in this work was diluted with its solvent N-
methyl-2-pyrrolidone (NMP) for better deposition and handling, at a weight ratio of 4:3 (PI-
2611:NMP). In order to further increase the strength and rigidity of the polyimide,
montmorillonite nanoclay powder (Nanocor, Hoffman Estates, IL) was added to the polymer
as reinforcement. A series of tests was performed to determine the optimum clay
percentage in the polyimide that would result in the highest strength under compressive
loading. For preparing the composite mixtures, first, the nanoclay powder was mixed with
the polyimide solvent NMP for 5 seconds using a Model 100 Sonic Dismembrator (Fisher Scientific) at 5 W output power. Then, this suspension was added to the PI-2611 polyimide solution and mixed using a stir bar for several minutes. The mechanical tests were performed on cone shaped test structures made through solvent casting the polyimide-nanoclay composite from a PDMS mold. For each investigated clay percentage in the composite, three test structures were created using the same mold. The structures were 280 µm tall, and had tip and base diameters of 65 µm and 115 µm, respectively. In order to apply compressive loads, a Physica MCR rheometer (Anton Paar, Ashland, VA) was used, which recorded force vs. displacement for each compression test (Figure 2.1). The rheometer’s test geometry was set to move vertically downwards onto the test structures at a constant velocity of 2 µm/s. The force was recorded at a resolution of 1 mN.

An example of a force vs. displacement graph is shown in Figure 2.2. In this plot, the sharp peak corresponds to the failure load that leads to bending of the structure under compressive stress. The test results showed that the drop in force after the peak is steeper for higher clay content, indicating a more brittle behaviour. The average of the maximum failure loads for the different clay content are shown in Table 1, which indicates that the 2 wt% clay reinforced polyimide has the highest failure load and was therefore chosen as the composite for the fabrication of microneedles.
Figure 2.1: Setup used for compression tests.

Figure 2.2: Typical compression test curve for a test structure with 2 wt% clay reinforcement.
Table 2.1: Mean failure loads for different clay content in polyimide, obtained from three tests per clay content.

<table>
<thead>
<tr>
<th>Clay content [weight %]</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>10</th>
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<td>Average failure load [N]</td>
<td>0.144</td>
<td>0.109</td>
<td>0.169</td>
<td>0.130</td>
<td>0.127</td>
<td>0.107</td>
<td>0.153</td>
</tr>
<tr>
<td>Standard deviation [N]</td>
<td>0.009</td>
<td>0.014</td>
<td>0.016</td>
<td>0.006</td>
<td>0.012</td>
<td>0.011</td>
<td>0.010</td>
</tr>
</tbody>
</table>

2.1.2 Fabrication process

SU-8 photoresist (Microchem, Newton, MA) was used for the mold. For fabrication of the mold, a 450 µm layer of SU-8 2150 was first spin-coated onto a 300 µm thick Pyrex® glass substrate, and then soft-baked on a hotplate at 65°C for 10 min and at 95°C for 2 h. The SU-8 was then exposed to 5300 mJ cm⁻² UV light (performed in several 2 minute intervals with 30 s cooling breaks in between) through a dark field mask that contained arrays of circular transparent regions with diameter of 40 µm. The exposure was performed through the Pyrex® substrate, as shown in Figure 2.3a, in order to take advantage of light diffraction caused by the gap between the mask and the photoresist [96]. This method of exposure resulted in tapered pillar structures with the wider bases attached to the Pyrex® base plate. After exposure, the sample was baked on a hotplate at 65°C for 5 min and at 95°C for 25 min. Next, the sample was immersed in SU-8 developer for approximately 45 min and then washed for 5 min with fresh developer and isopropanol. The resulting structure was an array of slightly tapered cylindrical pillars (Figure 2.3b and Figure 2.4) with base
diameters of 60 µm, tip diameters of 40 µm, and a center-to-center spacing of 500 µm. These pillars constituted the mold and they would eventually form the lumens of the needles. The entire mold structure was then coated with a 4 µm layer of Parylene C (Specialty Coating Systems, Indianapolis, IN). This layer improved adhesion of the pillars to the Pyrex® substrate and provided a protective surface on the mold for consecutive fabrication steps. Consecutively, a thin layer of PDMS (Dow Corning, Midland, MI) was spin-coated onto the mold structure at a base layer thickness of 30 µm and cured at 65°C for 2 h (Figure 2.3c). This layer improved the strength and rigidity of the mold structure and also had a poor adhesion with the structural material of the microneedles, which therefore allowed easy removal of the microneedle array from the mold. The complete mold structure contained an array of 14 pillars and was surrounded by a square (8 mm × 8 mm) of vertical walls (Figure 2.5). The mold structure at this stage could be used for consecutive solvent casting of microneedles. For more detail on mold fabrication and the photolithography process see Appendices A.1 and A.2.

For fabrication of the microneedle arrays, first, the mold was treated for 30 s with O₂ plasma in a RIE/PECVD tool (Trion Technology, Clearwater, FL), with a power setting of 30 W, an oxygen flow rate of 50 sccm, and a chamber pressure of 500 mTorr. The plasma treatment temporarily improved the surface wetting of PDMS by the polymer solution used for solvent casting and therefore resulted in sharper and taller needles. Without plasma treatment, the polymer solution does not cover the pillars entirely and leads to shorter and blunter structures. Consecutively, 35 µL of the 2% clay polyimide/NMP mixture was deposited into the mold structure (Figure 2.3e). During a soft baking process in an oven at
65°C for 2 h, NMP evaporated and the microneedles were formed around the pillars in the mold with wide bases and sharp tips (Figure 2.3f, Figure 2.6). The obtained structures would be suitable for use as solid out-of-plane microneedles, in which case the molds would not be reusable for consecutive fabrication.

To achieve hollow structures, the array was separated from the mold by mechanical force (Figure 2.3g). It was observed that the separation of the polyimide/clay layer became easier if sufficient time had passed after the soft baking process (more than 48 h), which may have been due to a slight swelling of the polymer film due to moisture uptake [97-99] or deformation due to further solvent evaporation at room temperature. After separating the needle structure from the mold, a thin layer of the polyimide/clay composite resided on top of the needles from the polymer solvent casting step obstructing the needle lumen. This layer was removed (Figure 2.1h) by either an O₂/CF₄ plasma etching step (200 W power, 700 mTorr pressure, 80 sccm O₂, 20 sccm CF₄, and duration of 300-500 s at 25°C) or by polishing the tips using fine 3 µm aluminum oxide polishing film (3M, St. Paul, MN).
Figure 2.3: Fabrication process using solvent casting for hollow out-of-plane polymer microneedles, a & b) fabrication of pillars from SU-8, c) PDMS deposition, d) O2 plasma treatment of the mold, e) deposition of a clay/polyimide suspension in NMP, f) Evaporation of NMP, g) removing of the microneedle array from the mold, h) opening of the microneedle tips.

Figure 2.4: SEM image of an array of pillars in a mold used for microneedle fabrication.
Figure 2.5: A complete mold used for microneedle fabrication.

Figure 2.6: SEM image of an array of solid microneedles formed in a mold after solvent evaporation.

Figure 2.7 shows SEM images of the microneedles fabricated through this process. Figure 2.7a shows an array of 250 µm long microneedles with tip diameters of 50.4 ± 4.2 µm (mean ± standard deviation) and the same pitch of 500 µm as the pillars. The slight variation in the microneedle tip diameter is mainly due to the variation in thickness of the spin coated PDMS layer on the SU-8 pillars, which may lead to small differences in the force
required for the penetration of the microneedles into skin. The wider bases of the pillars, due to the backside exposure of the SU-8, result in larger channel openings on the backside of the backing plate as shown in Figure 2.7b. In Figure 2.7c, a single microneedle is shown with its tip opened through plasma etching, while Figure 2.7d shows a single microneedle with the tip opened by sanding.

![SEM images of fabricated microneedles](image)

**Figure 2.7:** SEM images of fabricated microneedles: a) Array of 250 µm long needles, b) microneedle channel openings, c) a needle tip opened by plasma etching, and d) a needle tip opened by sanding. For more SEM images see Appendix A.3.

The dimensions of the microneedles can be adjusted by changing the dimensions of the mold structure during the photolithography steps. For instance, longer pillars with smaller diameter result in longer needles with smaller channel diameters.
2.1.3 Contact angle measurement of NMP on a PDMS surface

In order to characterize the polyimide-PDMS interaction after plasma treatment of the mold and during solvent casting, a series of tests was performed to observe the change in contact angle of polyimide solvent on a PDMS surface over time after the PDMS is plasma treated and covered with a layer of polyimide, as during the casting step in the fabrication process as shown in Figure 2.3e, f. For these tests, first a layer of PDMS was spin coated on a microscope slide and then baked to cure. The PDMS surface was then treated with oxygen plasma as explained above. A thin layer of polyimide was then spin coated on the PDMS surface and left in an oven to soft bake at 65°C for 2 h. The polyimide layer was peeled off from different samples after different time intervals (6 h, 8 h, 1 day, 2 days, 3 days, and 6 days) following solvent casting. The advancing contact angle was measured immediately after peeling off the polyimide layer from the PDMS surface (Figure 2.8a) using a Theta tensiometer (Attension / Biolin Scientific, Espoo, Finland). The measurements were performed on 4 µL NMP droplets and were repeated several times over the duration of 30 min, each time in a different location on the PDMS sample.

Figure 2.8b shows the measurement results. It was observed that regardless of when the polyimide layer was removed, the immediate contact angle of NMP on PDMS was constant and low (about 10-15°). The angle only increased when the surface was in contact with air, and this increase occurred at a similar rate regardless of how long after solvent casting the polyimide layer was removed.
Figure 2.8: NMP contact angle on a PDMS surface; a) contact angle measured 3 days after the PDMS surface was coated with a polyimide layer, 13 minutes after removing the polyimide layer; b) contact angle measurements for NMP on a PDMS at different times after removing a polyimide layer that was deposited between 6 hours and 6 days prior to removal.

The wetting behaviour of NMP on PDMS remains constant as long as the PDMS surface is in contact with the polyimide composite layer. The wetting only changes when the PDMS is in contact with air, regardless of when the polyimide composite is removed from the PDMS surface. This showed that the surface wetting behaviour of NMP on PDMS remains identical throughout the evaporation process, which would result in needles with the desired geometry.
2.2 Experimental procedures for needle characterization

2.2.1 Microneedle robustness tests

In order to test the robustness of the fabricate microneedles, a series of compression tests were performed on individual 2 wt% clay-reinforced polyimide needles, with tips opened by plasma etching. The test procedures were identical to the robustness tests performed on test structures described in section 2.1.1. Individual needles were made using the process shown in Figure 2.3. A total of ten 250 µm-long needles where tested under compressive loading and the corresponding force vs. displacement plots were obtained for each test.

2.2.2 Microneedle injection tests

The capability of the fabricated devices for drug delivery and skin penetration was demonstrated through injection trials on rabbit ear skin. Rabbit ear skin was used for this study because it is a reasonable human skin model for in vitro transdermal permeation studies [100]. Traditionally, pig skin has been used as a skin model for permeation studies due to its biochemical similarity with human skin [101, 102]. However, pig skin and other traditional skin models are associated with a high permeability compared to human skin, especially with respect to hydrophilic agents. In contrast, rabbit ear skin has a considerably lower permeability to hydrophilic compounds compared with traditional skin models, and is therefore considered to be a suitable human skin model for in vitro trials [100].
Arrays of 250 μm-long microneedles, with tips opened by plasma etching, were bonded to modified plastic female luer-to-barb fittings (McMaster-Carr, Cleveland, OH), using Loctite Super Glue (Henkel, Avon, OH), and then attached to conventional 1 mL syringes (Becton Dickinson, Mississauga, ON) as shown in Figure 2.9a. A 0.025 wt% suspension of 0.21 μm polystyrene fluorescent beads (Bangs Laboratories, Fishers, IN) in water was prepared. For each trial, the syringes were filled with the suspension containing fluorescent beads, and were then pressed against the inner rabbit ear skin for about 10 seconds while a moderate pressure was applied to the plunger. Figure 2.9b shows the skin after application of the microneedles. A total of six injection trials were carried out. After each test, the injection site was imaged using a D-Eclipse C1 confocal microscope (Nikon). Using the confocal microscope, the skin was scanned down to a depth of 200 μm to investigate the penetration of the fluorescent beads into the skin.
2.3 Results and Discussion

2.3.1 Microneedle robustness

Figure 2.10a shows an example of a force vs. displacement plot obtain for a compression test on a single needle. The plot shows a sudden increase in force upon contact of the
compression test tool with the microneedle tip followed by a sudden drop in force. The peak force corresponds to the failure load of the microneedle similar to the changing force seen for the solid structures in Figure 2.1. A needle tip after failure is shown in Figure 2.10b. In this Figure, the tip of the microneedle has collapsed and bent almost at the midpoint of the needle shaft, while the needle base is still rigid, which explains the increase in force again as the needle is compressed further. From these tests, it was observed that on average, the microneedles can sustain compressive loads of up to 0.32 ± 0.06 N (mean ± standard deviation).

![Figure 2.10](image)

**Figure 2.10:** a) A needle displacement under axial loading, b) a failed needle after loading.

Davis et al. investigated the relationship between the cross sectional area of a microneedle tip (interfacial area) and the force required for penetration of the microneedle into skin [103]. The experimental results presented by Davis et al. seem to suggest that the needle strength obtained experimentally for polymer/clay composite needles with a 50 µm tip diameter in this work, $F_N = 0.32$ N, is sufficient to penetrate human skin.
2.3.2 Results of injection tests

Figure 2.11 shows the fluorescent beads at different depths under the skin surface after an injection test using microneedles. For each confocal scan, an intensity histogram distribution was obtained by measuring the total bead fluorescent intensity at each depth using MATLAB for image processing. Figure 2.12 shows the intensity distribution for the confocal scan shown in Figure 2.11.

![Confocal scan of skin after injection of fluorescent beads. The skin surface is at 0 µm. The confocal slice thickness is 13.4 µm.](image)
Figure 2.12: Histogram of the intensity distribution of the fluorescent beads obtained from the confocal scan in Figure 2.11.

For each injection test, an average injection depth and an injection range were calculated from these intensity measurements corresponding to the mean and the 95% confidence interval, respectively. The results from six injection trials are shown in Table 2.1. The average delivery depth for all the trials was $104.8 \pm 15.6 \mu m$ with an average range of $119 \pm 25.8 \mu m$, indicating successful delivery into the epidermis, past the stratum corneum.

Table 2.2: Mean injection depth and 95% confidence interval for the fluorescent intensity distribution of injected beads as in Figure 2.12.

<table>
<thead>
<tr>
<th>Trial</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean depth [µm]</td>
<td>110.3</td>
<td>113.9</td>
<td>83.7</td>
<td>108.7</td>
<td>123.9</td>
<td>88.1</td>
<td>104.8</td>
</tr>
<tr>
<td>Upper range [µm]</td>
<td>169.4</td>
<td>176.7</td>
<td>121.8</td>
<td>171.0</td>
<td>180.3</td>
<td>166.3</td>
<td>164.3</td>
</tr>
<tr>
<td>Lower range [µm]</td>
<td>51.3</td>
<td>51.1</td>
<td>45.5</td>
<td>46.4</td>
<td>67.4</td>
<td>9.9</td>
<td>45.3</td>
</tr>
</tbody>
</table>
Since the microneedles used for these tests are 250 μm long, the average delivery depth of 104.8 μm indicates that the majority of the microneedle height could not penetrate due to the flexibility of the skin. This penetration behaviour might be different for human skin and additional experiments have to be done in vivo to observe the penetration depth in human skin. Additionally, in all of the confocal images, the distribution of the fluorescent beads was localized to distinct regions corresponding to each individual needle in the array. The distance between the needles therefore seems to be sufficient to have them act independently as individual needles, and injection of more material, with controlled volume, could thus be accomplished by proportionally adding more needles.

2.4 Conclusions

A new fabrication technique, based on solvent casting, has been presented for fabrication of hollow out-of-plane microneedles on a reusable mold. This fabrication procedure is inexpensive and applicable for mass production of polymer microneedles. The proposed process is flexible in terms of the possible dimensions of the microneedle array as well as the needle material. By adjusting the photolithography parameters and therefore controlling the pillar dimensions in the mold, microneedles with various heights and lumen diameters can be made. Also, other polymers can be used in this process for hollow microneedle fabrication by adjusting the process parameters.

Clay-reinforced polyimide was used in the proposed process to fabricate 250 μm long microneedles. A series of compression tests were carried out on solid structures to find the optimum clay percentage in the composite, leading to the maximum compressive strength.
The robustness of the fabricated microneedles has been measured, and the needles were found to be strong enough for penetration into human skin. The delivery of a suspension of polystyrene beads into the epidermis of rabbit skin was also successfully demonstrated.
CHAPTER 3

FABRICATION OF METALLIC MICRONEEDLES USING

ELECTRODEPOSITION OF METAL ONTO CONDUCTIVE

POLYMER FILMS

The fabrication process demonstrated in the previous chapter allowed fabrication of the microneedles with a variety of polymeric materials with heights of up to 250 µm. However, the proposed process did not allow fabrication of taller devices with sufficient strength for skin penetration. Taller microneedles (i.e. up to 1 mm) may be ideal for some medical applications in which the target delivery site is far below the skin surface or the skin is too flexible or too thick. A good structural material for tall hollow microneedles is metal due to its high strength and low cost. However, previous fabrication processes for metallic microneedles often require sequential formation of disposable polymer molds for electroplating. In addition, in most cases, metallic microneedles are opened through laser
cutting of individual needle tips, which can be time costly for making arrays containing a large number of microneedles.

In this chapter, we have used a solvent casting process to form a thin conductive polymeric layer on reusable molds that contain arrays of vertical pillar; the conductive polymer layer is then used as a seed layer in an electroplating process to fabricate metallic microneedles. Several polymers have been previously shown to be capable of conducting electricity [104, 105]. Although their conductivity is not as high as the conductivity of metals, they are used in many small-scale industrial applications because they are easy to process. In addition, non-conductive polymers can also be modified to conduct electricity by adding conducting particles to their matrix. Nickel-coated particles, copper powder, carbon black, and graphite are some examples of fillers used to increase polymer conductivity [106–111].

In order to further characterize the fabrication process, some experiments were carried out to investigate the conductivity of the polymer layer, its plasma etching rate, and the metal electroplating process. The fabricated metallic microneedles were also tested for robustness through a series of mechanical compression tests, and their failure loads were compared with forces required for skin penetration. The microneedles’ adequacy for skin penetration and drug delivery was then proven by using them to inject fluorescent beads into animal skin.
3.1 Fabrication of hollow metallic microneedles

3.1.1 Fabrication of the mold

The fabrication process of microneedles is summarized in Figure 3.1. It starts with making a mold through photolithography for which the epoxy-type negative photoresist SU-8 (MicroChem, Newton, MA) was used. A 700 µm thick layer of SU-8 2150 was first spin coated onto a 300 µm thick Pyrex® wafer. The wafer was then soft-baked for 10 min at 65°C and 2.5 h at 95°C. The SU-8 was then exposed to 9200 mJ cm² UV light (performed in multiple 3 min intervals with 20 s cooling breaks in between). The exposure was performed through a dark field mask containing arrays of transparent circles with 40 µm diameter. These circular regions lead to SU-8 pillars on the mold which are used as the basic structures for microneedle formation, and therefore, their spacing and size defines the final spacing and the approximate channel diameters of the microneedles in the array. In addition, the exposure was carried out from the back side of the Pyrex® substrate. This method of exposure takes advantage of the gap between the mask and the photoresist and leads to cone shaped pillars, which eventually translates into microneedles with wide channel openings and sharp tips [96]. The sharpness of the pillars can also be further increased by performing additional dry etching steps on the pillars to isotropically reduce the pillars’ width to achieve a desired sharpness. Characterization of such dry etching steps was not pursued for the purpose of this work. After exposure, the sample was baked for 5 min at 65°C and 35 min at 95°C. Finally, the sample was placed in a developer bath for 50 min to remove the unexposed photoresist.
Figure 3.1: Fabrication process for making hollow metallic microneedles, a) fabrication of the mold containing cone shaped pillars using backside exposure of SU-8 photoresist, b) deposition of PMMA/CB + NMP solution, c) evaporation of NMP at 80°C, d) O₂-CF₄ plasma etching of dried PMMA/CB, e) electrodeposition of the metal layer, f) removing the microneedle array by dissolving the PMMA/CB in NMP.

A final hard-baking step was carried out in an oven set to 175°C for 1 h. For convenience, the Pyrex® wafer was cut into smaller 1×1 cm² pieces, with each piece containing an array of pillars corresponding to a single microneedle array. For more detail on the photolithography process and the mask design see Appendices A.1 and A.2.
In order to further increase the strength of the pillars and their bonding with the Pyrex®
substrate an additional SU-8 layer was cast on the mold pieces. For this step, SU-8 3025 was
first diluted with cyclopentanone to make a 6.7 wt% solution, and then 45 µL of the solution
was cast on the mold at 95°C for 20 min, leading to a 30 µm thick coating on the base
substrate. This layer was then cured with 900 mJ/cm² of UV light and then baked at 95°C for
5 min followed by 190°C for 1 h. At this stage, the molds were complete and used multiple
times for the fabrication of microneedles (Figures 3.1a and 3.2a).

![Figure 3.2: Image of a mold used for microneedle fabrication, a) before coating with
PMMA/CB, b) coated with PMMA/CB.](image)

### 3.1.2 Deposition of the polymer-based conductive seed layer

Poly(methyl methacrylate) (PMMA, Polysciences, Warrington, PA), with molecular weight of
25 kDa, seeded with carbon black (CB, VULCAN XC72R, Cabot, Boston, MA), with a primary
particle size of about 150 nm, was used as the conductive polymer composite. Although,
other particles such as copper particles or graphite can be used as conducting fillers, CB was
used in this work mainly due to availability of the material and its low cost. Using any other
filler would suffice as long as the conductivity of the deposited film is high enough to
facilitate the electrodeposition of the metal layer. However, particles of higher density than CB have been observed to settle quickly during the evaporation process leading to a depleted region near the top of the pillars.

To prepare the PMMA/CB polymer solution / particle suspension, first 0.3 g of PMMA was dissolved in 5 g of N-methyl-2-pyrrolidone (NMP). Next, 0.135 g of CB was mixed with the solution. Subsequently, 0.015 g of sodium dodecyl sulphate (SDS, Sigma-Aldrich, Oakville, ON) was added as surfactant. The solution was then placed in an ultrasonic bath for 30 min. The SDS surfactant prevents the formation of CB particle clusters and therefore leads to a uniform suspension of the particles in the solution, which consequently results in a uniform distribution of the particles within the polymer matrix once the material is cast on the mold; this is necessary for getting a uniform conductivity and therefore metal coating on its surface. The resulting fluid had a solid concentration of 9 wt% with the CB accounting for 30% of the total solid content.

After fabrication of the mold, 20 µl of hexamethyldisilazane (HMDS, Sigma-Aldrich, Oakville, ON) was applied to the mold at room temperature to improve its surface adhesion. Next, 40 µl of the 9 wt% PMMA/CB mixture was deposited into the mold and then heated to 80°C for 3 h to evaporate the NMP and fully dry the PMMA/CB composite (Figures 3.1b, 3.1c, and 3.2b). The thickness of this layer was 100 µm on the base plate which gradually decreased towards the tip of the pillars. The PMMA/CB composite layer was then used as a seed layer in a metal electroplating process. This layer also served a sacrificial layer, which was later removed to separate the metallic microneedle structures from the mold. It has previously
been shown that the thickness of a polymer layer on the pillars that was deposited using solvent casting can be adjusted by controlling the solvent casting process parameters such as the temperature and the dew point [94]. The thickness of the PMMA/CB seed layer on the pillars can therefore be adjusted in order to tune the microneedle shapes. After the PMMA/CB casting step, a thin PMMA/CB layer covered the top surfaces of the pillars; this layer was removed using an O$_2$/CF$_4$ plasma etching step (O$_2$: 80 sccm, CF$_4$: 20 sccm, pressure: 500 mTorr, temperature: 25° C, power: 200 W, and duration: 200 s) in order to expose the SU-8 pillar tips (Figure 3.1d). This etching step ensures that the non-conducting needle tips would be open once the needles are formed through the electroplating process.

### 3.1.3 Metal deposition

The next fabrication step involved deposition of metallic layers that constitute the microneedle array structure. This process was done in two steps: first a thick nickel layer was electroplated onto the conductive polymer layer (Figure 3.1e), which made up the main structural material; then, the nickel layer was coated with a thin layer of gold to cover the outer surface of the microneedles. Nickel is inexpensive and has a high compressive strength and Young’s modulus compared with polymers, silicon, and many other metals [112, 113]. Electrodeposition of nickel has been used in numerous applications and is a well-established process. Since nickel has been found to cause allergic reaction upon skin contact in some people [114], it is coated with a gold layer to improve its biocompatibility [115].
For nickel deposition, after the microneedle mold piece was coated with the conductive polymer, it was positioned parallel to a pure nickel anode at a distance of 2.5 cm inside an electroplating solution consisting of nickel chloride \((25 \text{ g l}^{-1})\), nickel sulfate \((170 \text{ g l}^{-1})\), and boric acid \((15 \text{ g l}^{-1})\). Figure 3.3 shows the schematic of the electroplating setup. Since the conductive layer had a lower conductivity than the contact wire connected to the power supply, the top portion of the coated mold piece was kept outside of the electroplating solution in order to prevent a point of high field strength resulting in an accumulation of nickel on the contact wire. The power source was set to provide a constant current of 2 mA and the process was set to run for 150 min, which resulted in a 70 µm thick backing layer. After deposition of nickel, a 20 nm layer of gold was sputtered on the top surface of the microneedle array.

Figure 3.3: Schematic of the setup used for electroplating of nickel. The wire contact point to the PMMA/CB layer is kept out of solution to prevent uneven nickel deposition.
3.1.4  Microneedle array lift-off

Removing the microneedle array was finally facilitated by dissolving the PMMA/CB layer in NMP and in an ultra sonic bath for 60 min (Figure 3.1f). Once the microneedle array was separated, the mold could be further cleaned with acetone or NMP to remove any leftover CB particles and used again for fabrication. In addition, the dissolved PMMA/CB layer can be potentially reused for fabrication after evaporating part of the NMP to reduce its concentration to an amount applicable for solvent casting. Figure 3.4 shows images of microneedles fabricated using this process.
3.2 Experimental procedures

3.2.1 Conductivity measurements of PMMA/CB composites

A series of experiments were carried out to investigate the effect of CB content on conductivity of the PMMA/CB composite. For these experiments, PMMA/CB suspensions in
NMP were prepared with a CB concentration in the final solid varying between 0 to 50 wt%. All suspensions had a total solid concentration (CB, SDS, and PMMA) of 9 wt%. After the solutions were prepared, they were deposited into cylindrical cavities with diameters of 4 mm to create disk-shaped composite films for conductivity measurements. The film thicknesses were then obtained based on deposition volume and the solids volume fraction in the solution, and were also verified using a digital micrometer. A copper layer was evaporated on the top and bottom surfaces of the films. The electrical resistance values $R$ of the films were then measured using a conventional multimeter, by placing the meter probes on the top and bottom copper surfaces. The resistivity values

$$\rho = R \frac{A}{l} \quad (3)$$

were calculated from the thickness $l$ of the cast film, the measured resistance $R$, and the area $A$ of the circular cross section of the cylinders. The conductivity values were obtained by inversing the resistivity values. See appendix A.4 for more detail.

### 3.2.2 Dry etching of the PMMA/CB layer

The dry etching step in the fabrication process impacts the height of the microneedles as well as their tip diameter; it is therefore necessary to precisely control this process to ensure there will be enough polymer removed from the pillar tips so that the microneedle tips will be open, while maintaining the sharpness and aspect ratio of the microneedles.
Through a series of experiments, the plasma etch rate of the PMMA/CB composite films were measured for different gas flow settings. It had previously been shown that O₂ and CF₄ plasma etching is an effective dry etching technique for removal of many organic polymers including PMMA [116, 117]. Here, a Trion RIE/PECVD (Trion Technology, Clearwater, FL) was used to investigate the etch rate of the PMMA/CB composite in plasma. First, 100 µm thick layers of PMMA/CB composite, with 30 wt% CB in solid, were cast on 1×1 cm² glass chips. The surfaces were then partially covered with an adhesive polyimide tape to partially protect them from the plasma. The glass chips were then placed in the plasma etcher and were etched with different combinations of O₂ and CF₄ volumetric flow rates. The total flow rate was adjusted to 100 sccm. The power was set to 200 W, and the chamber pressure and temperature to 500 mTorr and 25°C, respectively. The etching process was set to run for 300 s for all the trials.

For determining the etch rates, first, the amount of polymer that was removed by plasma was measured. For this purpose, once the process was complete, the polyimide tape was removed from the polymer surface, and then a 40 nm layer of gold was uniformly evaporated onto the surface. A Wyko NT1100 optical profilometer (Veeco, Plainview, NY) was then used to measure the height difference between the areas exposed to the plasma and the areas covered with the tape, which corresponded to the etch depth. The etch rate was then calculated by dividing this height difference by the duration of the etching process. See appendix A.5 for more detail.
3.2.3 Characterization of the nickel electroplating process

The electroplated nickel constitutes the main structural material of the microneedles and its deposition thickness controls the rigidity and wall thickness of the microneedles, as well as the back plate of the array.

Through a series of tests, the effect of the process duration and the electric current density on the nickel thickness was investigated, in order to determine the deposition rate of the nickel with respect to these parameters. For this purpose, 100 µm layers of PMMA/CB, with 30% CB in solid, were cast on glass chips, which were then immersed in the electroplating solution and used as seed layers similar to the microneedle electroplating fabrication steps. Once the electroplating process was complete for each trial, an Alpha Step 200 profilometer (1% accuracy, KLA-Tencor, Milpitas, CA) was used to measure the deposited metal thickness. See appendix A.6 for more detail.

3.2.4 Mechanical compression tests on the fabricated microneedles

The strength of the microneedles was measured through a series of mechanical compression tests and compared with literature data. The schematic of the test setup is shown in Figure 3.5. A Physica MCR 301 Rheometer (Anton Paar, Ashland, VA) was used to apply vertical compressive loads to 500 µm tall microneedles. The rheometer tool was set to move at a constant velocity of $5 \, \text{µm/s}$ throughout the tests, and force vs. displacement data was obtained for analysis of the failure loads. After each experiment, the needles were
visually observed to make sure they did not buckle. These tests were carried out on 5 individual microneedle samples made through the process shown in Figure 3.1.

![Diagram of microneedle setup](image)

Figure 3.5: Schematic of the setup used for mechanical compression tests on microneedles.

### 3.2.5 Fluid delivery into sample skin using the fabricated microneedles

The capability of the metallic microneedles for skin penetration was realised by applying a 500 μm tall microneedle to pig skin and then taking histology sections to investigate the penetration depth and skin surface damage. The capability of the microneedles for transdermal drug delivery was assessed through an injection of fluorescent beads into pig skin. For this purpose, a 500 μm tall microneedle was bonded to the tip of a conventional 1 ml syringe. After filling the syringe with a 0.01 wt% suspension of 2.28 μm fluorescent beads in water, the microneedle was pressed against the skin, and then a force of approximately 2 N was applied to the syringe plunger for 5 min. The skin surface near the injection site was then washed with water and dried with a wipe. A Nikon Eclipse C1
confocal microscope (Melville, NY) was then used to scan the distribution of the fluorescent beads inside the skin. Pig skin was used for transdermal analysis due to its similarities to human skin in terms of skin layer thickness and elasticity, which makes it a suitable model for in vitro studies [118].

3.3 Results and discussion

3.3.1 Conductivity measurements

The resistivity of the polymer is shown in Figure 3.6 as a function of CB content. This plot shows a significant decrease of the resistivity as the CB% increases from 0 to 50%; this is consistent with previous works investigating resistivity for lower CB contents [106, 107, and 119]. Although using the PMMA/CB composite with the highest conductivity could facilitate faster electroplating, using CB beyond 30% leads to cracks in the polymer film during the casting step. As the higher CB content makes the polymer more brittle, the cast films become more sensitive to internal stresses that develop during the evaporation process. Using CB above 30 wt% creates cracks mostly around the pillar bases and around the edges of the mold in areas of high thickness variations. The 30 wt% CB concentration, therefore, was chosen in this process to prevent crack formation while providing a sufficiently high conductivity for electroplating.
Figure 3.6: The resistivity of the PMMA/CB polymer film as a function of CB content. Each data point represents the average value from three experiments and the error bars indicate ± standard deviations.

3.3.2 Dry etching of the PMMA/CB layer

The plasma etching rates of the PMMA/CB composite is shown in Figure 3.7 for different gas flow settings. It has been previously shown that the addition of fluorine-containing gases can lead to an increase in the oxygen atom density in the plasma and therefore enhance the polymer etching rates up to a certain fluorine concentration [116]. In Figure 3.7, the etch rate peaks for CF$_4$ flow rate approaching 20%, which is consistent with the peak locations for some other polymers [116]. The 20% CF$_4$ flow rate has then been used for the microneedle fabrication.
Figure 3.7: Etch rates for PMMA/CB composite (with 30 wt% CB) for different ratios of CF$_4$ to total gas flow rate. The total gas flow rate was kept constant at 100 sccm. Each data point represents the average value from three experiments and the error bars indicate ± standard deviations.

The plasma etching step could potentially be eliminated from the fabrication process if the PMMA/CB solution concentration was kept below a certain value for specific pillar heights. It was seen during the experiments that for low solution concentrations (less than 5 wt% total solid concentration) the polymer would not cover the pillar tips and the solution does not wet the pillars entirely. This could be due to a moving contact line of the polymer solution during the evaporation process, which occurs for lower polymer concentrations or taller pillars. The resulting electroplated layer would be expected to be uniform on the base plate and metal would not cover the pillars tips.
3.3.3 Nickel electroplating process

The thickness of the nickel layer over time is shown in Figure 3.8, for a constant current source of 2 mA, and a cathode area of 1×1 cm² positioned 2.5 cm from the nickel anode. In Figure 3.9, the nickel thickness is shown for different current source settings and a deposition time of 90 min with the same electrode size and orientation as the previous experiment. The change in nickel thickness is linear in both cases within the range of the tested parameters. The nickel deposition rate of 0.49 $\frac{\mu m}{min}$ at 2 mA was found from the slope of the linear fit in Figure 3.8, and the thickness as a function of current of 21.9 $\frac{\mu m}{mA}$ over 90 min was found from the linear fit in Figure 3.9, yielding a current-dependent deposition rate of 0.24 $\frac{\mu m}{mA \ min}$.

![Graph](image.png)

**Figure 3.8:** The thickness of electroplated nickel on a PMMA/CB layer over time, with 2 mA supply current. The slope of the linear trend line is 0.49 $\mu m/min$. 

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Figure 3.9: Thickness of electroplated nickel as a function of power source current, for process duration of 90 min. The slope of the linear trend line is 21.9 μm/mA.

The recorded thickness values correspond to the thickness of the microneedle array backing plates and not the microneedle structures themselves; as seen in Figure 3.4a, the thickness of the nickel at the microneedle tip is much less than that of the 70 μm backing plate, which shows that the nickel coating is not isotropic. This could be due to the orientation of the mold pillars as well as the lower conductivity of the polymer at the pillar tips due to decreased thickness. Since the pillars’ orientation in the electrolyte is perpendicular to the anode electrode plane, there could be less Ni\textsuperscript{2+} accumulation on their tips due to the perpendicular movement of the charged particles to the microneedle plane. In addition, the lower conductivity at the pillar tips could lead to lower current density in those regions which results in a slower metal coating at the beginning of the electroplating process.
3.3.4 Mechanical compression tests

Figure 3.10 shows an example of a force vs. displacement measurement used to determine the microneedle failure load. In this figure, the first peak in the force graph corresponds to the tip failure under compressive loading. From the five compression tests, the average failure load was 4.2 ± 0.61 N. A plot with all measurements is provided in Appendix A.7. In a previous study by Davis et al. [103], the authors have studied the fracture force of hollow metallic microneedles with respect to the needle tip radius, wall thickness, and wall angle. For this purpose, the authors have compared the experimentally obtained fracture force data with an analytical solution estimating the force, as well as a finite element model.

![Failure load](image)

**Figure 3.10: A needle displacement under axial loading.**

The analytical model assumes thin-shell needles, which requires the ratio of the tip radius to the wall thickness to be larger than ten. This model is therefore not applicable to the needles presented in this work, since this ratio is much smaller.
The finite element analysis was also found to underestimate the fracture force due to a conservative failure criterion of the needle material. However, the experimental data in [103] for needles with a similar material, deposition method, and height as in the present work can be compared with the experimental results obtained here.

It was found in [103] that the fracture force is insensitive to tip radius for radii ranging from 40 μm to 65 μm, the fracture force increases significantly with wall thickness from 4 μm to 15 μm and slightly with wall angle from 60° to 70°. Extrapolating the data in [103] following these trends would lead to a fracture force above 5 N for the needle geometry in the present work (tip radius: 28 μm, wall thickness: 15 μm, wall angle: 80°), which is slightly higher than the measured 4.2 ± 0.61 N. This slight difference in strength can be a result of a different nickel nanocrystalline structure formed during the electrodeposition [113].

In addition to the fracture force measurements in [103], the forces required for the penetration of microneedles into human skin have been investigated experimentally for microneedles with different tip diameters. According to this study, the forces required for tip diameters of less than 50 μm is below 1 N; the failure load here is well above this force indicating that the needle will most likely not break upon insertion into skin.

3.3.5 Transdermal fluid delivery into sample skin

The histology image in Figure 3.11 shows the skin surface damage caused by the microneedle penetration. The confocal scan of the injection site, shown in Figure 3.12, indicates delivery of the fluorescent beads to a depth of 250 μm into the pig skin, which
shows the usefulness the microneedles for transdermal delivery of drugs including suspensions.

![Histology image](image1)

**Figure 3.11:** Histology image of a microneedle insertion site on pig skin showing the skin damage caused by a 500 µm tall microneedle.

![Confocal scan](image2)

**Figure 3.12:** Confocal scan (514 µm × 514 µm) of injection site on pig skin showing the distribution of fluorescent beads under the skin surface. The test was carried out using a 500 µm tall microneedle.

To verify that the skin is not permeable to the fluorescent beads without the aid of the microneedles, a series of control tests were performed by applying the fluorescent beads
solution to skin surface for 10 min and then scanning the skin after washing the surface. The control tests did not show any fluorescent microspheres penetration below the skin surface.

3.4 Conclusions

A new fabrication process was demonstrated for making metallic microneedles. The process uses a conductive polymer as the seed layer for the electrodeposition of metal. Open microneedle tips are achieved through a single plasma etching step prior to deposition of the metal layer. This process can potentially allow fabrication of microneedles with a wide range of dimensions and spacing. This process can also be used for batch fabrication of microneedles, since large arrays of hollow needles can be formed without the need of opening the individual needles’ tips, a process which is often time and labour intensive and requires methods such as laser micromachining. In addition, the molds can be reused for multiple fabrication runs which would substantially reduce the materials and labor costs for mass production. The molds in this work, for instance, were used up to three times without seeing any degradation of the needle shape and structure.

PMMA filled with CB has been used as the conductive polymer layer during the microneedle preparation. The conductivity of this polymer composite was characterized to find the optimum CB content which was 30 wt%. Also, from a series of O₂-CF₄ plasma tests, a 20% CF₄ flow rate composition was found to result in the fastest etch rate for the PMMA/CB composite with 30% CB. The thickness of electroplated nickel was found to be linear with
respect to both process time as well as supply current. The current-dependant deposition rate was measured to be $0.24 \frac{\mu m}{mA \text{ min}}$.

Through mechanical compression tests, the average strength of the fabricated microneedles was found to be above 4 N, showing that these needles can pierce through human skin without breakage. In addition, the usefulness of the microneedle devices was demonstrated by successful delivery of 2.28 μm fluorescent microspheres into pig skin to a depth of about 250 μm, using 500 μm long microneedles.
CHAPTER 4

STUDYING DIFFUSION OF A MICRONEEDLE-INJECTED DRUG

INSIDE SKIN: INJECTION OF DOXORUBICIN INTO PIG SKIN

Drug transport across the epidermis is often the time limiting step for drug transport across the skin due to the dry and dense cellular structure of the epidermis, which makes drug diffusion difficult. The dermis, in contrast, is much less dense and has more ISF, which makes drug diffusion much easier compared to the epidermis. The dermis also contains the blood capillaries and is thus often considered the target medium in the transdermal drug delivery process. Previous methods investigated drug transport from the skin surface. Microneedles, however, release the drug below the SC layer, and thus require different methods of analysis to follow the drug movement. Most of the previous works on hollow microneedles focus on the fabrication technologies but not much has been invested on studying the drug delivery process. Although these devices overcome the SC barrier, drug
delivery using microneedles still relies on the diffusion of drugs through the epidermal and the dermal layers.

Developing microneedle systems capable of replacing traditional injection methods require additional research on the drug absorption process in the skin and the factors that affect this process, such as the injection rate, the diffusion medium, and the drug chemistry. In the case of the diffusion medium (i.e., the skin) many have proposed using the skin of animals as models for studying transdermal drug delivery. Ideally for in vitro experiments, freshly excised skin would be the most representative of in vivo skin. However, obtaining fresh skin may not be feasible or ethical when a study involves a large number of experiments. Researchers, therefore, often have to use skin specimens previously stored in refrigerators or freezers and it is not clear how the storage procedure will affect the absorption capability of the skin. Besides the diffusion medium, understanding the effects of the drug injection rate and its chemical and physical influences on the diffusion process is essential in order to maximize the process efficacy.

Analysing all these factors, however, first requires developing a method that can facilitate studying injection with microneedles. So far, in the previous works on microneedles, researchers injected compounds into the skin with microneedles and then determined the compound concentrations in blood [10, 50 and 120] or simply injected fluorescent dye (or particles) into an animal tissue and measured the penetration depth of the dye [121]. However, it is instructive to investigate drug transport inside the skin right after injection to understand how fast the drug diffuses through the skin to reach blood vessels.
This chapter uses confocal microscopy to study drug delivery with hollow microneedles for the first time, by characterizing the diffusion of a fluorescent drug injected into pig skin using microneedles. Confocal microscopy is used to obtain the fluorescence intensity images at different skin depths after the drug is injected. The drug concentration distribution is calculated using an intensity-concentration calibration. The change in the spatial concentration distribution is monitored over time and then used to calculate the diffusion coefficient of the drug in skin and to validate an analytical diffusion model. In addition, this method was used to compare drug diffusion in skin specimens from three different storage conditions (fresh skin, refrigerated skin, and frozen skin) in order to evaluate their usefulness for in vitro drug delivery studies. Many previous studies have used skin after any of these three storage methods; however, as it was observed through some experiments that they may not be equal in terms of skin permeation properties, a comparison study is pursued here to compare the refrigerated and frozen skin with the most physiologically correct case (i.e. fresh skin). This is achieved by comparing the diffusion coefficients obtained for refrigerated and frozen skin to that of fresh skin.

### 4.1 Experimental procedures

#### 4.1.1 Materials

For the injection tests, domestic pig skin was chosen, since it is a useful model for human skin in terms of the mechanical properties as well as cellular structure and drug permeability [118]. The skin from the belly of one animal was freshly excised immediately
after animal sacrifice. Injections were carried out into skin specimens after storing them in three different ways; first as fresh skin (within eight hours after sacrifice), secondly as refrigerated skin (after three days of storage), and thirdly as frozen skin (after twelve days of storage). The skin pieces were placed in sealed vials before being placed in the refrigerator or the -20 °C freezer in order to maintain their moisture. Before injection, the skin samples were removed from the refrigerator or the freezer, and they were kept sealed until they reached room temperature.

Doxorubicin (or 14-hydroxydaunorubicin) was chosen as the drug for studying the diffusion process. Doxorubicin is a chemotherapeutic drug that works by intercalating DNA molecules (binding between DNA strands) to inhibit cellular replication [122-124]. It is a water-soluble compound and has the chemical formula of \( \text{C}_{27}\text{H}_{29}\text{NO}_{11} \) and a molar mass of 579.98 g/mol. Doxorubicin is commonly used to treat a large variety of cancer conditions including leukemias and lymphomas, as well as cancers of breast, lung, prostate, bladder, stomach, ovaries, thyroid, soft tissue sarcoma, and others [125, 126]. Doxorubicin is among the most cytotoxic compounds and is associated with many adverse side effects such as skin reactions, digestive system disorders, and even fatal heart damage [125, 126]. Precise delivery of the drug is thus critical to avoid over- or under-dosing. In some cases, it is advantageous to deliver the drug via dermal routes especially in situations that involve targeting tissues close to the skin. In addition, reaching effective doses of the drug sometimes requires multiple hypodermic injections into the blood system within a short time period, which can be very uncomfortable for patients, especially small children. Microneedles can be useful in this case since they can provide a targeted delivery
mechanism to the skin while being painless. However, in order to determine the amount of doxorubicin that reaches the target area after injection with microneedles, it is important to measure the drug’s diffusion rate within the skin. Doxorubicin is a fluorescent compound with excitation and emission peaks of 480 nm and 580 nm, respectively [127]. It is therefore possible to image the drug and investigate its diffusion within tissue using fluorescence microscopy.

A series of experiments was performed to measure the fluorescence intensity of the drug at different concentrations and to construct a calibration curve. For this purpose, eight calibration solutions were prepared using distilled water and doxorubicin powder (Polymed Therapeutics, Houston, TX; assayed purity >98.0%; MW 579.98 g/mol). The solutions were deposited on a hemocytometer gridded region (to maintain a consistent volume) and then imaged using the confocal microscope to measure fluorescence intensity. The concentrations were 0, 25, 50, 150, 300, 900, 1500, and 3000 µM. To prepare the solutions, first, 5.2 mg of doxorubicin powder was weighed and dissolved in 3.00 mL of distilled water to make a 3000 µM solution. The remaining solutions were then prepared via serial dilution with the appropriate volumes of distilled water. A vortex mixer was used after each dilution to ensure adequate mixing.

After measuring the intensities, it was found that the drug’s fluorescence intensity changed linearly up to concentrations of 150 µM (Figure 4.1). Injected concentrations were therefore kept within this linear range to obtain quantitative distribution data from the intensity measurements.
4.1.2 Injection setup

Different systems have been used in the past to inject compounds with microneedles. Stoeber et al. [121] proposed a microneedle syringe concept in which the needles are attached to a drug container, and the drug is ejected by pushing on a flexible membrane (Figure 4.2). This setup was used by Häfeli et al. [120] to inject compounds into live mice with silicon microneedle arrays.
Figure 4.2: Microneedle syringe concept presented by Stoeber et al. [121].

Another alternative method is to simply glue a microneedle array to the tip of a conventional syringe, and then injecting the drug by pushing the syringe plunger, similar to the injection tests described in Chapters 2 and 3. The disadvantage of these methods is that there is less control over the rate of drug injection. For investigating drug delivery with microneedles it is better to use a more controlled injection mechanism in order to keep track of flow rates. For the injection tests here, the setup shown in Figure 4.3 was used to inject doxorubicin into pig skin.

Figure 4.3: a) schematics and b) actual image of injection setup used for delivering drugs at defined flow rates. The syringe is placed in a commercial syringe pump were the plunger speed is controlled.
Single 500 µm long microneedles were bonded to the luer ends of 23G steel needles, with the steel needle connected to a 1 ml syringe via flexible capillary tubes. After filling the syringe with drug solution, it was placed on a syringe pump (PicoPlus, Harvard Apparatus, Holliston, MA, USA).

Generally, when microneedles penetrate the skin and the injection process begins, the skin tends to resist fluid flow. As a result, if the injection rate is too high, the drug may leak along the side of the needle shafts to the skin surface (Figure 4.4).

![Microneedle array](image)

**Figure 4.4: Concept of drug backflow along outside of the needles to the skin surface.**

To prevent the fluid flowing along the microneedle shafts to the surface, the injection pressure has to stay below a threshold that is controlled by skin elasticity around the microneedle tips. The injection rate of 200 nL/min was chosen for our experiments to ensure there was no backflow. This rate was also found to be the effective rate for delivery of compounds into animal skin *in vivo*, using microneedles [120].

A custom skin holder, made from the cap of a polypropylene microcentrifuge vial, keeps the skin in tension during the injection and the imaging process in order to maximize microneedle penetration depth into the skin (Figure 4.5).
Figure 4.5: Schematic of setup used to stretch skin and keep it moist during injection and imaging processes.

After mounting the skin on the holder and injecting the drug, the holder was flipped and placed on the confocal microscope’s inverted stage. Water was added to the backside in order to keep the skin as moist as possible during imaging (Figure 4.5).

4.1.3 Injection procedure

After preparing the solution and setting up the syringe pump and the skin, the injection process was carried out three times for each skin storage condition. For each trial, the microneedle was pressed against the skin with a force of approximately 3 N. The syringe pump was then set to run for 5 min (~2 min lag time before liquid came out of the microneedle, due to compliance of flexible tubing). The needles were then removed from the skin surface and the skin was transferred to a confocal microscope for imaging. Transferring the skin to the microscope, finding the injection spot, and adjusting the confocal range took between 5 - 12 min.
4.1.4 Confocal imaging

The confocal images were obtained using a TCS SP5 system (Leica Microsystems, Wetzlar, Germany). An argon laser source was used for excitation. The excitation wavelength was set at 488 nm at 50% illumination intensity. A HyD1 detector was used to collect fluorescence in the wavelength range of 535-625 nm. The system’s smart gain was adjusted to 280% to enhance the fluorescence brightness, and the pinhole size was set to 60 µm. The confocal thickness was set at 9.5 µm and the X-Y image size was 512×512 pixels (corresponding to 775 µm × 775 µm physical image size).

4.2 Data analysis

4.2.1 Diffusion model

The steady-state diffusion flux

\[ J = -D \nabla C \quad (4) \]

can be described by Fick’s first law [128], where \( \nabla C \) is the concentration gradient in three-dimensional space. The diffusion coefficient \( D \) depends on the drug properties (i.e., MW, ...) as well as the properties of the skin (i.e., viscosity, porosity, ...). The Fick’s second law of diffusion

\[ \frac{\partial C}{\partial t} = D \nabla^2 C \quad (5) \]
describes how the diffusion causes the concentration to change with time [128]. Solving the differential equation (5) gives the spatial concentration distribution over time. The initial boundary condition describing the source can be continuous or limited, each resulting in a different solution. In addition, sometimes it is simpler to solve this equation in spherical coordinates rather than Cartesian. This is especially useful when the initial boundary condition is a point source or spherical source that spreads symmetrically in all directions. The solution to equations (4) and (5) in spherical coordinates for a limited point-source deposited at time zero at the origin is given by the Gaussian distribution [129]

\[ C(r,t) = \frac{N_0}{(4\pi Dt)^{3/2}} \exp\left(-\frac{r^2}{4Dt}\right), \]  

where \( N_0 \) is the initial mass. Equation (6) assumes isotropic diffusion from the source with the same \( D \) in all directions. This equation describes how concentration is distributed in space, at different distances \( r \) from the source. In addition, it describes how the spatial concentration distribution changes over time. The negative sign of the exponential term indicates how the concentration drops with distance. As the value of \( Dt \) increases over time, the term before the exponential decreases indicating a depleting source. For a continuous source, this term is a constant \( N_0 \) which does not change over time and the exponential function is replaced by the complementary error function.
The diffusion process for injection with microneedles can be described in two steps:

1. A constant-source diffusion step, while the microneedle is inserted into the skin and the drug is ejected.

2. A limited-source diffusion step, which starts upon removal of the needle from the skin.

It is difficult to image the skin during the first step since the microneedle would block the optical path. The confocal imaging is, therefore, done only during the second step, where the diffusion process can be modeled as a spherical source, positioned below the microneedle insertion spot, spreading in all directions (Figure 4.6). Equation (6) can then be used to describe the diffusion. Here the injection step is much shorter than the duration of the diffusion experiments. We therefore assume the injection step is associated with only a small amount of diffusion, so that the measured diffusion can be modeled with equation (6).

![Diagram showing diffusion process](image)

**Figure 4.6: Concept of diffusion of drugs, injected with microneedles, through the epidermis.**

Equation (6) also assumes a constant D independent of direction of diffusion. In reality, since the density of skin layers changes with depth, the value of D also changes with depth. In addition, upon application of pressure from the injection process, the skin tissue gets
squeezed which might result in a variation in $D$ near the needle surface. However, $D$ is expected to stay constant when spreading in planar direction at each depth. Confocal microscopy gives planar images; therefore, since the analysis is always done in the same depth, assumption of constant $D$ is justified.

### 4.2.2 Data processing

In order to use equation (6) to describe the diffusion, the concentration distribution of the drug must be first obtained from the intensity data for the depth corresponding to the spherical source illustrated in Figure 4.6. For spherical diffusion, the concentration or intensity distribution should normally be the same in all directions originating from the source at each depth plane. However, in this case, the distribution may not be perfectly symmetrical due to the natural non-uniformity of the skin`s cellular matrix which might lead to non-uniform spreading of the drug. The data should thus be averaged. To do this, the total intensity is first obtained for narrow hypothetical rings spreading from the source, and then divided by the number of pixels in the rings to get the average intensity for each individual ring (Figure 4.7). In order to measure the intensities with the process shown in Figure 4.7, the MATLAB image processing toolbox was used. The code used to calculate the average values in rings spreading from the source is shown in Appendix A.9.
Figure 4.7: Calculating concentration distribution from confocal images, by measuring the average intensity within hypothetical thin rings (with thickness of 10 pixels) at different distances originating from drug source.

4.3 Results and discussions

4.3.1 Confocal data

Figure 4.8 shows an example of a series of confocal images obtained for a fresh skin sample injected with the doxorubicin solution. In this image, the trace of the drug can be observed down to a depth of 236 µm under the skin surface. The change in intensity of the drug at each depth indicates drug diffusion over time. The hole created by the microneedle insertion can be observed in the image series corresponding to the surface and depths of 47 µm and 95 µm. The high brightness of the skin surface is due to the exposure of the skin to some doxorubicin that was dried on the microneedle array’s outside surface before insertion. In addition, through a control test, it was verified that there is no drug diffusion through the SC when the drug was applied to the skin surface.
Figure 4.8: Confocal images obtained for a fresh skin sample injected with 87 µM doxorubicin solution. The images are 775 µm × 775 µm. The rows correspond to different scan depths while the columns correspond to the scans over time. The time difference between two columns is 5 minutes.

The first column on the left, which correspond to the start of the imaging procedure (i.e. t1), was taken almost 12 min after the injection procedure stopped, which is the amount of time it took to take the sample to the microscope and prepare it for scanning. The row corresponding to the depth of 142 µm is the depth corresponding to the spherical source of drug below the injection spot; this depth corresponds to the deeper epidermal layers or the
upper dermis layers which is a suitable depth for releasing the drug since it is close to the target blood vessels. Figure 4.9 shows the cross-section view that has been reconstructed from the confocal image in Figure 4.8, and the 142 µm depth images correspond to the yellow dashed line in the figure.

![Image of cross-section view](image)

**Figure 4.9: Cross-section of the injection location, reconstructed from confocal image shown in Figure 4.8, corresponding to t1.**

### 4.3.2 Diffusion measurements

The resulting intensity distribution for all time steps, obtained through the procedure detailed in section 4.2.2, for the experiment corresponding to Figure 4.8 is shown in Figure 4.10. In this plot, the origin of the x-axis corresponds to the origin of the spherical source. As expected from equation (6), for each time step, the intensity drops exponentially moving away from the source. As time passes, the intensity levels drop. The confocal images include a low level of noise with a standard deviation of the measured intensity around 5.8 in regions of zero fluorescence. Figure 4.11 shows the corresponding concentration distribution obtained from Figure 4.10. The concentration distributions for limited source diffusion in 3D spherical coordinates look different from the more familiar concentration distribution for linear diffusion. In 3D diffusion, the curves don’t intersect while in the case of linear diffusion, the curves intersect.
The calibration curve in Figure 4.1 is found using a confocal setting different from what was used to detect the drug distribution in the skin in Figure 4.8. The detected intensity level in the skin attenuates due to the light absorption and scattering effects, and is thus weaker than the intensity levels for the pure solution on the hemocytometer slide for the same confocal setting. The concentrations values in Figure 4.11 were estimated from the highest intensity region for the image with the smallest \( t_1 \); the corresponding concentration was assumed to be equal to the initial injected concentration (see Appendix A.8 for details).

![Graph showing intensity distribution](image)

**Figure 4.10:** Intensity distribution for the confocal images corresponding to the depth 142 \( \mu \text{m} \) in Figures 4.8 and 4.9, and obtained through the method illustrated in Figure 4.7.

Equation (6) can be used as the theoretical model to describe the change in concentrations in Figure 4.11. Using the MATLAB curve fitting toolbox, a Gaussian function describing diffusion is fitted to the data in Figure 4.11.
The fit parameters then yield the diffusion coefficient and initial mass. The value for D in equation (6) can be obtained by comparing the exponential terms. Here, comparing the t1 and t1 + 5 min curves gives a value of $5.86 \times 10^{-9}$ cm$^2$/s for D while t1 and t1 + 10 min curves gives $8.23 \times 10^{-9}$ cm$^2$/s. However, for comparing the different skin types here, only the values of D for t1 and t1 + 5 min are used. This is because as time passes the skin properties such as moisture content might change due to the exposure to the heat of the confocal laser. Therefore using the initial time steps gives the most realistic representation of the diffusion value. Using this technique, the value for D was obtained for each trial and for each skin storage condition (see Appendix A.10 for the confocal images for all trials).

![Figure 4.11: Doxorubicin concentration distribution in skin obtained from Figure 4.10. The theoretical fitted curves (i.e. Gaussian diffusion, eq. (6)) are included as red lines.](image)

Table 4.1 summarizes the diffusion coefficients obtained for the different skin types.
Table 4.1: Doxorubicin diffusion coefficient in epidermal tissue with units of \([\text{cm}^2/\text{s}]\)

<table>
<thead>
<tr>
<th>Skin type</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>5.82×10^{-9}</td>
<td>5.86×10^{-9}</td>
<td>2.16×10^{-9}</td>
<td>4.61×10^{-9}</td>
<td>2.12×10^{-9}</td>
<td>0.461</td>
</tr>
<tr>
<td>Refrigerated (3 Days)</td>
<td>6.02×10^{-9}</td>
<td>1.07×10^{-8}</td>
<td>2.25×10^{-8}</td>
<td>1.31×10^{-8}</td>
<td>8.47×10^{-9}</td>
<td>0.648</td>
</tr>
<tr>
<td>Frozen (12 Days)</td>
<td>1.44×10^{-8}</td>
<td>9.10×10^{-8}</td>
<td>2.09×10^{-8}</td>
<td>4.21×10^{-8}</td>
<td>4.24×10^{-8}</td>
<td>1.008</td>
</tr>
</tbody>
</table>

The diffusion coefficient values in Table 4.1 are plotted in Figure 4.12.

![Figure 4.12: Doxorubicin diffusion coefficient for different skin types (fresh, refrigerated for 3 days, frozen for 12 days). Two of the data points for fresh skin overlap.](image)

Here, an increasing trend in D can be observed from the fresh skin to the frozen skin, indicating that the freezing and thawing process can have a significant impact on D. This observation is consistent with another study that showed how permeability of skin to sodium ion was slightly higher for previously frozen skin compared to fresh skin [130]. A similar observation was reported by another investigator who compared the permeability
of chromone acid in fresh cadaver skin to that of frozen skin [131]. A possible reason for the change in D could be a change in the skin’s water content as a result of freezing, which could influence drug diffusion through the cellular matrix. Another reason could be the formation of ice crystals during freezing in the cells, which damages the skin such that after thawing gaps and holes remain within the skin. To prevent that, a much more controlled freezing could be used. The difference between the refrigerated skin and the fresh skin is not large; however, the slight difference could be due to the skin’s partial water loss when stored in the refrigerator. The value for D for doxorubicin has been previously obtained in different media both analytically and experimentally. Table 4.2 summarizes the values reported in the literature.

**Table 4.2: Comparison of doxorubicin diffusion coefficient in different media**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Diffusion Coefficient [cm²/s]</th>
<th>Method</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>2.96×10⁻⁵</td>
<td>Analytical</td>
<td>[132]</td>
</tr>
<tr>
<td>Mouse liver tumor</td>
<td>5.01×10⁻⁷</td>
<td>Experimental</td>
<td>[133]</td>
</tr>
<tr>
<td>Normal mouse liver</td>
<td>6.70×10⁻⁷</td>
<td>Experimental</td>
<td>[134]</td>
</tr>
<tr>
<td>macroscopic diffusion coefficient in interstitial network</td>
<td>1.20×10⁻⁸</td>
<td>Analytical</td>
<td>[135]</td>
</tr>
<tr>
<td><strong>Pig belly skin (fresh)</strong></td>
<td><strong>4.61×10⁻⁹</strong></td>
<td>Experimental</td>
<td>this work</td>
</tr>
<tr>
<td>macroscopic diffusion coefficient in cellular network</td>
<td><strong>2.22×10⁻⁹</strong></td>
<td>Analytical</td>
<td>[135]</td>
</tr>
<tr>
<td>MDA-468 cell nuclei</td>
<td><strong>2.70×10⁻¹⁰</strong></td>
<td>Experimental</td>
<td>[135]</td>
</tr>
</tbody>
</table>
According to Table 4.2, the value measured for doxorubicin diffusion in pig skin is between the macroscopic diffusion coefficients obtained for an interstitial network and the cellular network. As the skin’s epidermis is a relatively dry medium with a limited amount of interstitial fluid, it is expected that the measured value of D for skin would be closer to that of cellular network.

The value of D for some other compounds in the skin has been obtained from diffusion cell experiments. One work measured $7.5 \times 10^{-8} \text{ cm}^2/\text{s}$ for glucose diffusion in epidermis [31], which is larger than the value obtained here for doxorubicin. Although glucose has a smaller diffusion coefficient in PBS ($6.8 \times 10^{-6} \text{ cm}^2/\text{s}$, obtained from the Einstein-Stokes equation [110]) its faster diffusion (i.e. larger D) in the epidermis can be associated with its smaller molecular weight giving the molecules more mobility within the cellular matrix. The value obtained analytically by the Einstein-Stokes equation is not applicable here since it only considers the molecular size and describes diffusion in a purely liquid medium, while in this case the drug interacts with a solid cellular array. The diffusion coefficient in the SC has also been measured for fluorouracil, caffeine, and flufenamic acid to be $7.2 \times 10^{-12} \text{ cm}^2/\text{s}$, $1.1 \times 10^{-11} \text{ cm}^2/\text{s}$, and $2.2 \times 10^{-11} \text{ cm}^2/\text{s}$, respectively [32, 34]. All these compounds also have smaller molecular weights, but their smaller values of D can be explained by the lower permeability of the SC compared to the viable epidermis.

By using the calculated diffusion coefficient D in equation (6), and using the curve fit terms for the concentration distribution curve in Figure 4.11, the value for $N_0$ is calculated to be $6.32 \times 10^{-15} \text{ kg}$. The analytical concentration distribution model from equation (6) can then
be plotted (Figure 4.13). The drug distribution obtained experimentally (shown as scattered data in Figure 4.13) has close resemblance to the analytical distribution, which shows the validity of model for prediction the drug concentration.

![Graph showing concentration distribution over distance](image)

**Figure 4.13: Doxorubicin concentration distribution obtained from analytical model (shown as lines) and the experimental data for all the time steps (shown as scatter plots).**

A characteristic diffusion length (obtained from $\sqrt{D_t}$ where $t_i \sim 3$ min is the duration of the injection process) for the first constant-source diffusion step is also calculated to be 9.11 µm. This length describes how much the drug progresses in the skin before the second limited-source diffusion step starts. Given the much longer distances of drug progression in the second step (i.e. more than 150 µm) the assumption of instantaneous source for the second diffusion step can be justified.

The method presented here is used to study diffusion microscopically, while understanding the effect of drug chemical properties on the diffusion process requires investigating the
interaction of drug at the molecular level. The skin samples used here were excised and contained dead cells. Drug uptake might be different for live cells compared to dead cells, which would have an impact on the diffusion rate of the drug. A useful study would be investigating the drug diffusion behaviour in presence of live epidermal and dermal cells at the microscopic level, which should be considered for future studies.

4.4 Conclusions

This chapter presents an optical method to investigate the diffusion of a drug injected with microneedles inside the skin. Single 500 µm tall microneedles were used to inject doxorubicin into pig skin. The drug intensity distribution was then measured using a confocal microscope. By obtaining the concentration distribution, using intensity-concentration calibration curves, and then fitting the analytical diffusion model to the data, the diffusion coefficients were obtained. A theoretical model was then compared with the experimental data, indicating that the model is valid for predicting the drug concentration distribution in skin. Using this technique, the doxorubicin diffusion coefficient was obtained for three skin conditions: fresh skin, refrigerated skin (for three days), and frozen skin (for twelve days). Overall, there was no significant difference between the values for fresh skin versus the refrigerated skin, while the diffusion coefficient for frozen skin was considerably larger, suggesting that the use of frozen skin should be avoided when performing similar diffusion studies.
CHAPTER 5

EXTRACTION OF INTERSTITIAL FLUID USING MICRONEEDLES

Microneedles can be used for painless or less painful (compared to hypodermic needle) sampling of biological liquids from the body for therapeutic measurements. One target liquid is ISF, which is present all over the body and is a water-based medium. Its composition is similar to blood plasma (the liquid component of blood that holds the blood cells in suspension), and only lacks the large proteins that cannot pass through the capillary membrane.

![Diagram of interstitial fluid](image)

Figure 5.1: Conceptual sketch of interstitial fluid (ISF) in body.
ISF is responsible for transferring nutrients and ions from the blood vessels to tissue cells and vice versa (Figure 5.1), and its composition is regulated by diffusion of these nutrients through the semipermeable vessel wall. Due to its similar composition compared to blood plasma and the constant exchange of compounds between the ISF and the blood, ISF can be used to indirectly measure the concentration of many blood components. Previous work has demonstrated the ISF/blood correlation for various compounds [136]. In many cases, the change in concentration in ISF lags that of blood by several minutes and should be taken into account when estimating the blood concentrations [136].

Hollow microneedles can be used to sample ISF or blood from the skin using capillary forces or by applying a negative pressure through the needle lumens. Apart from the SC, all the skin layers contain live cells that depend on nutrients; therefore, the presence of ISF in skin is crucial to maintain the cells’ life cycle. The epidermis is however, a relatively dry medium compared to other body tissues with the ISF making up about 25% of its composition [137]. In contrast, ISF makes up a larger portion of the dermal tissue, 40% [137], which is mainly due to the presence of blood capillaries in this layer. Extraction of ISF from the dermis is therefore easier than from the epidermis, but the presence of nerves may cause a sensation of pain upon needle penetration. ISF extraction from the skin using microneedles was first demonstrated by Mukerjee et al. [48], where 200 µm tall silicon microneedles were used to collect the liquid using capillary forces from human earlobe. To prove successful extraction of ISF, the authors used commercial glucose strips and demonstrated a color change in the presence of the collected sample.
This chapter presents some ISF extraction procedures carried out with the needles produced in the previous chapters as well as a new solid microneedle design. Hollow polymer and metallic microneedles were used on animal skin to evaluate their usefulness for ISF extraction. In addition, the solvent casting process in Chapter 2 was modified to make solid microneedles with a water absorbent layer embedded in its structure, and needles were then applied to animal skin for ISF sampling.

5.1 Experimental procedures

5.1.1 ISF extraction using solid polymer microneedles

A new method is presented for the extraction of dermal ISF that uses solid microneedles with an absorbent polymer layer embedded in its structure. Through a solvent casting process solid microneedles were formed, composed of mainly three layers and a base support structure to facilitate insertion of the needles into the skin. The solvent casting is performed on a support structure made from SU-8 photoresist that consists of an array of cone shaped pillars (Figure 5.2a, b, and Figure 5.3a).
Figure 5.2: Fabrication process using solvent casting for solid out-of-plane polymer microneedles for ISF extraction; a & b) fabrication of pillars from SU-8; c) clay/polyimide + NMP solution deposition; d) evaporation of NMP; e) solvent casting of hydroxyethyl cellulose layer; f) solvent casting of poly(ethylene-co-vinyl acetate) layer.

The cone shaped pillars are created by backside exposure of the SU-8 layer through a Pyrex® base plate, similar to the mold fabrication steps in Chapters 2 and 3. Then a polymer composite layer is deposited, made of 2% clay-reinforced polyimide (Figures 5.2c and 5.2d) with high rigidity and mechanical strength. Next, a water soluble/absorbent hydroxyethyl cellulose (HEC) layer is cast on top of the polyimide layer (Figure 5.2e). Finally, a thin poly(ethylene-co-vinyl acetate) (PEVA) layer is cast on top of everything (Figure 5.2f). Since PEVA is hydrophobic, when cast on top of the hydrophilic HEC layer, it does not cover the needles during the drying process and only covers the backing plate. This layer improves the
biocompatibility of the microneedle array [138] and also provides a protective layer for the HEC layer from the dead cells, fat, and hair on the skin surface; and it can be later removed either by its solvent or by peeling it off from the HEC layer. Figure 5.3b shows the final microneedle array made through this process.

Once the microneedle array is inserted into the skin, the dermal ISF is absorbed by the HEC layer, which is highly water absorbent, causing it to swell and expand. The agent of interest, which includes biomarkers and drugs, can then be analyzed either directly in the HEC layer, or after dissolving the HEC in water.

Figure 5.3: a) A base structure used for solvent casting of solid microneedles, consisting of an array of tall pillars made from SU-8; the distance between the pillars in the array is 500 µm, b) An array of 250 µm-long microneedles made through the process shown in Figure 5.2.

Mechanical tests, similar to the tests demonstrated in Chapters 2 and 3, were performed on the microneedles to evaluate their strength. The average failure load of single needles from three compressions tests was measured to be 0.28 N, which should be sufficient to pierce human skin given the needle dimensions [103]. In addition to strength tests, extraction of dermal ISF was demonstrated using the fabricated microneedles. For this purpose, the
microneedle arrays were applied to the inner rabbit ear skin and they were held in place for 5 min. Figure 5.4 shows a microneedle insertion site for an extraction experiment, as well as a swollen 14-microneedle array after absorption of the dermal ISF. Three ISF extraction experiments were carried out on the rabbit ear skin; and the microneedle arrays were weighed before and after application to the skin (with measurement resolution of 0.01 mg) to measure the amount of the extracted fluid. Control tests were also performed with polymer surfaces having no needles, to estimate the amount of fat, dead cells, and hair that accumulates on the surface.

![Figure 5.4: Extraction of dermal ISF using the fabricated microneedles; a) insertion site on the inner rabbit ear skin; b) swelling of the hydroxyethyl cellulose layer upon application to the skin as a result of ISF absorption](image)

**5.1.2 ISF extraction using hollow polymer microneedles**

Removing ISF using capillary forces requires a hydrophilic surface in the microneedle lumens. In this work, we have modified the casting process to make microneedles with hydrophilic channels to extract ISF from the skin through capillary action. Hydrophilic
polymers are often water-soluble or swell in the presence of water (or ISF), which is a problem since microneedles can soften during the extraction procedure, thus lose their mechanical rigidity. To solve this, they can be further cross-linked by chemical treatment to improve their mechanical strength and substantially reduces swelling caused by the ISF.

![Fabrication process for making hollow PVA microneedles](image)

**Figure 5.5:** Fabrication process for making hollow PVA microneedles; a) fabrication of a mold consisting of an array of SU-8 pillars coated with a PDMS layer, b) deposition of PVA + BA solution, c) solvent evaporation, d) casting of a PMMA layer, e) separation from the mold, f) opening the tips using O₂/CF₄ plasma etching.

Here, the microneedles are fabricated from cross-linked polyvinyl alcohol (PVA) (average MW: 124,000 to 186,000, 99% hydrolyzed) with boric acid (BA) used as the cross-linking agent (Figure 5.5). A thin poly(methyl methacrylate) (PMMA) layer is cast on top of the PVA in order to protect the PVA during the plasma etching step that opens the tips. Figure 5.6 shows the PVA microneedles made using solvent casting.
A series of mechanical tests were performed to evaluate the strength of the fabricated microneedles under compressive loading. It was found that the needles can sustain compressive loads of up to $0.21 \pm 0.04$ N which is sufficient to pierce human skin given the needle dimensions [103]. A series of contact angle measurements were also carried out to evaluate the surface hydrophilicity of the cross-linked PVA. For this purpose, the material was spin coated on a microscope slide. After baking the polymer, the contact angle measurements were carried out (Figure 5.7). From five measurements, the average contact angle was observed to be $48.6^\circ$ showing sufficient hydrophilicity. This is confirmed in Figure 5.8 where an array of microneedles is exposed to a dyed water solution at its tips and capillary action has transported the liquid from the microneedle tips along their lumens to the backside of the chip.
Figure 5.7: Contact angle measurement of a 4 µL water droplet on a cross-linked PVA surface shows that the surface is hydrophilic.

Figure 5.8: Dyed water transfer through the microneedle lumens when the tips are exposed to the liquid, a) schematic of experiment, b) microneedle backside showing capillary driven flow reaching the channel openings.

Finally, extraction of ISF from skin using the microneedles was attempted by applying an array of 14 microneedles attached to a glucose test strip (BETACHEK VISUAL from National Diagnostic Products Pty Ltd, Sydney, Australia) against rabbit ear skin for 30 min (Figure 5.9). The strip was tested several times to ensure it only changes color in response to blood
or interstitial fluid and not sweat or other fluids. To evaluate sampling, the glucose strips were observed under a microscope for signs of color change.

![Figure 5.9: Setup used for ISF extraction with hollow polymer microneedles.](image)

### 5.1.3 ISF extraction using hollow metallic microneedles

The 500 µm tall microneedles fabricated in Chapter 3 were used for extracting ISF from pig skin using vacuum. For this purpose, a vacuum-operating extraction device was made which contained a hollow solid tube attached to a microneedle array on one end and to a vacuum source in the other end (Figure 5.10). The microneedle array contained six 500 µm tall needles. The microneedle array needle opening side rested on a small cone-shaped cavity that was connected to a capillary tube on the narrow end. The capillary tube was directed at a commercial glucose strip (BETACHEK VISUAL from National Diagnostic Products Pty Ltd, Sydney, Australia). The vacuum was set to 10 psi and was chosen based on a previous literature that used vacuum to sample ISF from the skin surface [88]. The device was then pressed against pig skin for 10 min. After this period, the glucose strip was removed from the tube and observed under microscope. To ensure no blood was extracted instead of ISF, the capillary tube was carefully observed under a microscope to ensure there was no sign of blood. This process was repeated five times.
Figure 5.10: a) Schematic of custom vacuum device made for ISF extraction using the 500 µm metallic microneedles, b) image of the actual vacuum device.

5.2 Results and discussion

5.2.1 Solid microneedles

The results of the three experiments (Table 5.1) show an average extracted ISF volume of 0.77 µL. Extraction of larger amounts of ISF can be achieved by proportionally increasing the number of microneedles in the array, which can be realized by simply increasing the number of pillars in the base structure. Although this technique may be useful for removing the liquid from the skin, analyzing the ISF absorbed in the cellulose layer is more difficult
compared to analyzing the pure ISF. One approach can be dissolving the layer in a solvent and then investigating the concentrations. Another approach can be developing systems that directly measure the compound in the cellulose layer.

**Table 5.1: Summary of the ISF extraction tests using 14 solid microneedles with a cellulose functionalization; the net extracted volume is calculated by subtracting 0.18 mg (average mass transferred during the control tests) from the mass differences, and using density of water at 25°C (0.997 g/ml) instead of that of ISF**

<table>
<thead>
<tr>
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<th></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>167.70</td>
<td>168.56</td>
<td>0.86</td>
<td>0.68</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>116.01</td>
<td>117.07</td>
<td>1.06</td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>3</td>
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<td>131.67</td>
<td>0.94</td>
<td>0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>Control test 1</td>
<td>112.87</td>
<td>113.06</td>
<td>0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control test 2</td>
<td>114.23</td>
<td>114.40</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**5.2.2 Hollow polymer microneedles**

No signs of color change were observed after inspecting the glucose strips. Successful extraction, therefore, was not demonstrated with these needles. Possible reasons could be the uptake of the liquid by the polymer upon application or simply the duration required for sampling the liquid is much longer than what was tried. A possible approach to solve this is to replace the material with a polymer that has no liquid uptake upon application to the skin.
5.2.3 **Hollow metallic microneedles**

After observing the glucose strips under a microscope, two trials showed traces of color change due to presence of ISF on the glucose strip. However, the area on the strip that changed color was small and not enough for estimating the exact concentrations. These experiments demonstrate the potential and the usefulness of the vacuum and metallic microneedles for ISF sampling. However, only very small amounts of ISF were extracted which may not be sufficient for many applications. To improve this, it is possible to increase the number of needles in the array to target a larger skin area. Other possibilities are increasing the duration of application as well as the vacuum pressure in the tube.

5.3 **Conclusions**

In this chapter, ISF sampling was attempted with three microneedle designs: solid polymer array, hollow polymer array, and hollow metallic array. Solid microneedles showed successful removal of the ISF with an average volume of 55 nL per needle. However, it is difficult to measure the compounds in the ISF removed with this technique since it is embedded in the cellulose layer. Hollow polymer microneedles did not show any liquid removal while the hollow metallic microneedles showed minimal removal of the ISF using vacuum.

Overall the proposed methods suggest the potential of using microneedles for this application; however, additional investigation should be carried out to study the limitation
of this technique before implementing experimental procedures. Also, other methods that enhance ISF sampling should be investigated and combined with the proposed techniques.
CHAPTER 6

SUMMARY AND FUTURE WORK

6.1 Summary

This dissertation presents research performed towards developing manufacturing processes for hollow microneedle devices used for drug delivery and biosensing applications. A major focus of the research objectives was developing cost effective processes that are applicable for batch manufacturing, thus providing the possibility of widespread adoption of microneedles and replacing traditional painful hypodermic needles. In addition, this thesis presents a novel method of studying drug delivery using microneedles, which can help future researchers to evaluate the usefulness of microneedle for administration of various clinical compounds.

Chapter 1 of this thesis, first, provides an overview of the skin anatomy as well as the transdermal drug delivery. It is shown how adhesive skin patches facilitate drug transport
across skin and what are the important parameters and formulas controlling this process. Next, this chapter discusses the emerging microneedle technology and how they can be more effective over the traditional adhesive patches for transdermal drug delivery. It is also shown how they can be useful over traditional needles due to the minimal pain associated with them.

In Chapter 2, we present a process based on solvent casting process to make hollow out-of-plane polymer microneedles. Through photolithography of SU-8 photoresist, molds are created containing arrays of cone-shaped pillars 450 µm tall. A thin layer of PDMS is then deposited and cured followed by a plasma treatment step. Next, a solution of polyimide and nanoclay suspension is cast on the mold. The optimum clay percentage of 2 wt% in solid was found from a series of compression tests on pillar (made of polyimide with varying clay content) of similar dimension scale as microneedles. The polyimide/nanoclay layer constitutes the microneedle structure and is separated from the mold by mechanical force. Through an additional plasma etching step, their tips are opened. An alternative technique is demonstrated for opening the tips using mechanical polishing of the tips with fine lapping paper. The microneedles prepared through this process are 250 µm tall. The needle strength of 0.32 N, found from compression tests, is shown to be sufficient for skin insertion without failure. To test the needles for drug delivery, fluorescent beads are injected six times into rabbit ear skin and their distribution is observed in deep skin tissue using confocal microscopy. The average injection depth of 104.8 µm is found from the mean value of the intensity distribution. It is shown that the microneedles clearly facilitate the delivery of the compounds past the stratum corneum. The process in Chapter 2 allows
making needles up to a height of 250 µm which may not be sufficient for some applications; the natural result of the polymer casting process is a curved-shaped profile that gets thinner closer to the pillar tips. For taller than 250 µm structures, this thickness is so small that the microneedles would not be mechanically rigid and thus their tips would bend or buckle upon attempting skin insertion.

Chapter 3 of this thesis presents a fabrication procedure for making metallic microneedle with taller structures compared to the polymer ones in Chapter 2. The solvent casting step is used to create an electrically conductive seed layer for subsequent metal deposition, to create metallic microneedles strong enough for skin penetration even at very tall dimensions. The fabrication process uses a similar array of molds presented in Chapter 2, but with an additional structural layer added to the molds to improve their strength. PMMA filled with carbon black is used as the cast seed layer. The optimum carbon black percentage of 30 wt% in solid is found from a series of conductivity measurements. Through a plasma etching process, the conductive layer covering the pillar tips are physically etched exposing the pillar tips which later resulted in hollow needles. Next, nickel is electroplated on the conductive layer. Through some tests, the electroplating process is characterized for optimum deposition rate. After the electroplating, the microneedles are lifted off from the molds by chemically etching the conductive layer. Using this process, 500 µm tall needles were made. The mechanical strength of the needles was evaluated to be 4.2 N and drug delivery was demonstrated by injection of fluorescent beads into pig skin.
Chapter 4 of this thesis presents a novel method used to characterize drug delivery with hollow microneedles. For this purpose, a fluorescent drug (doxorubicin) is first injected into pig skin using metallic microneedles, and then using confocal microscopy the intensity distribution of the drug is measured over time in the skin. The concentration distribution of the drug was then calculated from intensity data, and then compared to an analytical model based on Fick’s laws of diffusion. Through this comparison, the diffusion coefficient of the drug in the skin’s epidermal layer is obtained ($4.61 \times 10^{-9}$ cm$^2$/s for fresh skin) and then compared with the values reported in the literature for other tissues. The diffusion coefficient of doxorubicin is also compared in skin specimens treated with three storage conditions (fresh, refrigerated, and frozen). It is found that freezing the skin may considerably alter the rate of drug diffusion within the skin, and thus should be avoided when performing similar studies.

In the last chapter, we evaluate some potential ways of removing ISF from the skin using microneedles, through experimental trials. First, the solvent casting process used in Chapters 2 and 3 was modified to make solid microneedles from a highly water absorbent polymer material (HEC). Extraction of ISF was then demonstrated by applying the water absorbing microneedles to skin samples in vitro. Using arrays of microneedles (with 14 needles), an average volume of 0.77 µL is removed from skin. The solvent casting process was also used to make hollow microneedle made of a hydrophilic polymer (PVA) that can withstand swelling upon exposure to water or ISF. The purpose of developing these systems was using capillary forces to attract ISF from skin. However, successful sampling was not demonstrated through several trials. And finally, the metallic microneedles (array of 6
needles) made in Chapter 3 are used to remove small amount of ISF from skin by applying vacuum pressure using a custom made vacuum probe. Traces of ISF are observed on glucose strips positioned in the probe. However, the probe was not able to attract large enough amounts required for glucose concentration measurements.

6.2 Future work

This section discusses some future work that can be pursued to help improve the microneedle fabrication repeatability and yield, as well as its efficiency for drug delivery sampling of biological liquids.

6.2.1 Microneedle process improvement

6.2.1.1 Mold

The mold is the backbone of the microneedle formation process and the shape of mold pillars affects the final shape and sharpness of the microneedles. At this stage, the mold structure is the most important factor that influences the process repeatability. The inconsistency in the photolithography process is reflected in the variation observed in the height and width of the pillars even on the same wafer. This is mainly due to the poor performance of the photolithography equipment used in the cleanroom facility (i.e. the spinner, hotplates, and mask aligner). Therefore, for better molds, better photolithography equipment must be used. Alternatively, the mold can be made from other materials and through other fabrication techniques, such as the ones used for making solid microneedle systems.
In addition, as the mold pillars are subject to mechanical stress upon needle lift-off, using a stronger mold alternative is recommended. Right now, the molds may be usable up to four or five runs, but increasing the usability can substantially reduce the costs in an industrial setting. In addition, having less number of pillars on the mold would increase the chances of more successful microneedle lift-off leaving intact mold for subsequent fabrication.

6.2.1.2 Structural polymers and polymer solutions

Although the current material used for polymer microneedle fabrication is among the strongest polymers, investigating other options for the structural material would be useful especially for developing polymer microneedle taller than 250 µm. Possible options are using carbon nanotube-based composites. In addition, for the conductive polymer in Chapter 3, using other solvents (such as PM Acetate) instead of NMP eliminates the need for surfactant and also results in a more uniformly coated layer on the pillars.

6.2.1.3 The electroplating process

Nickel, which is the current metal used for microneedle fabrication, is not biocompatible as discussed in Chapter 3, and the needles are coated with an extra layer of gold for biocompatibility. While most electrodepositable pure metals that are inexpensive are not biocompatible, it is possible to form biocompatible alloys through the electroplating. This would eliminate a final gold coating step and reduce the fabrication duration. Some possible options for the metals are nickel-chromium or cobalt-chromium alloys.

In addition to the material, it is recommended to use an electroplating station designed for MEMS application, for better control of the distances, electrode sizes, supply currents, and
electrolyte solution (i.e. its pH and purity). In addition, it was observed that adding a brightener component to the nickel bath would not facilitate coating of the thinner conductive polymer parts, which should be taken into account.

6.2.2 Investigating the optimum needle height, diameter, spacing, and array size

Until now, the hollow microneedle technology has mostly focused on the fabrication techniques. Little has been done to study the optimum height, diameter (outer and inner), and spacing of the microneedle arrays that could facilitate effective drug delivery or fluid extraction. This also requires studying the skin mechanics in detail. Using taller needles to reach the dermis might be beneficial for both injection and extraction due to the low density of the dermis and its large amount of ISF, but at the same time it may cause pain. Using needles with larger diameters may be useful for injecting/extracting at faster rates but it may cause more nerve damage and be painful. In addition, using more densely arranged needles might be beneficial in terms of space occupation but at the same time it could lead to a bed-of-nails effect causing no skin penetration by the needles. One main advantage of the proposed processes here is that they are flexible and allow making microneedles with a wide range of dimensions, which would be useful when investigating the optimum dimensions.
6.2.3 Drug delivery using microneedles

As discussed in Chapter 4, the microneedle technology requires extensive research into the drugs and pharmaceutical agents to be delivered. As this technology relies on drug diffusion through the skin, understanding the chemical structure of drugs and their physical properties is important to design useful systems. In addition, additional research has to be carried out to find optimum injection pressures, rates, and volumes for specific skin layers.

6.2.4 Biofluid sampling using microneedles

The studies of fluid sampling with microneedles are at a preliminary stage. To develop consistent systems, more research has to be done to find the optimum target layer in the skin as well as the right needle material and dimensions. In addition, further research has to be carried out to investigate possible ways of treating skin to facilitate faster and more reliable fluid sampling. In addition, since we are dealing with very small amounts of liquid, very sensitive assays have to be developed in order to extract useful information from the sampled liquids.
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APPENDICES

A.1 Detailed photolithography parameters and mold fabrication

Fabrication of the SU-8 molds for polymer microneedles in Chapter 2:

Equipment used (located in UBC Nanofabrication Facility):

- Spinner: LAURELL WS-400-6NPP-LITE
- Mask aligner: CANON DOUBLE SIDE 100MM ALIGNER
- Dicing saw: MA1006 Disco Saw
- Plasma etching: Trion RIE/PECVD

Parameters

- Substrate: 300 μm thick Pyrex®
- SU-8 type: 2150
- Spinner: 500 RPM for 10 s (110 RPM/s), 1100 RPM for 35 s (330 RPM/s), 0 RPM for 10 s (110 RPM/s)
- Soft bake: 65°C for 10 min, 95°C for 2 h
- Rest: 5 min
- Exposure: 5300 mJ cm⁻² UV light; backside exposure as described in Figure A.1.
  (Performed in multiple 3 min intervals with 20 s cooling breaks in between)

![Figure A.1: Setup used to perform backside photoresist exposure.](image-url)
Post exposure bake: 65°C for 5 min, 95°C for 25 min
Rest: 5 min
Developer: ~50 min
Rinse: Fresh Developer + IPA
Hard bake in oven: ramp up temperature to 175°C, maintain for 30 min, ramp down to RT
Resulting thickness: 450 µm ± 10 µm

**Wafer dicing**

The wafer was first glued to a silicon wafer (as base support) using AZ photoresist. It was then cut along the + patterns on the wafer to give square mold chips. The mold chips were then separated from the silicon wafer using acetone to remove AZ photoresist.

**Pillar diameter reduction**

To achieve better aspect ratio for the pillars which leads to sharper microneedles, a plasma etching tool can be used after or before cutting the wafer into square pieces to etch the pillar structures. The etching process is not fully anisotropic or fully isotropic. But it leads to the decrease in the height of the pillars as well but not substantial with respect to the reduction in diameter. One should take into account that having thinner pillar structure also means less mechanical strength for the pillars, which would increase the chance of pillars getting damaged during microneedle lift-off.

Images below (Figure A.2 and A.3) compare arrays of pillars before and after the plasma etching process:
Figure A.2: A microneedle mold array before plasma etching.

Figure A.3: A microneedle mold array after plasma etching.

For the needles produced in chapter 2 and 3, the following parameters were used with the Trion RIE/PECVD to achieve pillars with 35 µm tip diameter (Figure A.3):

\[
\begin{align*}
O_2: & \quad 90 \text{ sccm; } CF_4: 10 \text{ sccm; temperature: } 25^\circ\text{C; power: } 200\text{W; pressure: } 500 \text{ mTorr;} \\
duration &= 1200 \text{ s}
\end{align*}
\]

Fabrication of the SU-8 molds for metallic microneedles in Chapter 3:

Similar process as the molds described for polymer microneedles but with the following parameters:

- Substrate: 300 µm thick Pyrex®
- SU-8 type: 2150
Spinner: 800 RPM for 45 s (110 RPM/s), 0 RPM for 10 s (110 RPM/s)
Rest: 3 min
Soft bake: 65°C for 10 min, 95°C for 2.5 h
Rest: 5 min
Exposure: 9200 mJ cm\(^{-2}\) UV light; backside exposure as described in Figure A.1.
(Performed in multiple 3 min intervals with 20 s cooling breaks in between)
Post exposure bake: 65°C for 5 min, 95°C for 30 min
Rest: 5 min
Developer: ~50 min
Rinse: Fresh Developer + IPA
Hard bake in oven: ramp up temperature to 175°C, maintain for 30 min, ramp down to RT
Resulting thickness: 680 µm ± 30 µm
A.2 Photolithography masks used for mold fabrication

For mold fabrication 100 mm dark field masks were used (Figure A.4). The masks were divided into square sections by “+” marker, each square corresponding to a single mold chip. Each square contains an array of 40 µm circular regions. Masked were designed in Clewin 4 software. Once the mold wafer was made, it was cut into the square sections along the + marks, using a dicing saw.

![Figure A.4: A 100 mm mask used for making microneedle molds.](image-url)
A.3 SEM images of polymer microneedles

This section includes some additional SEM images of molds and polymer microneedles made through the process in Chapter 2.

Figure A.5: An array of polyimide/clay microneedles made through the process in Chapter 2.

Figure A.6: An array of polyvinyl alcohol microneedles made through the process in Chapter 2.
Figure A.7: An array of polyvinyl alcohol microneedles made through the process in Chapter 2.

Figure A.8: Top view image of a hollow polymer microneedle showing the needle lumen.
A.4 Conductivity measurements

The effect of CB content on conductivity of the PMMA/CB composite was investigated. For these experiments, PMMA/CB suspensions in NMP were prepared with a CB concentration in the final solid varying between 0 to 50 wt%. After the solutions were prepared, they were cast into cylindrical cavities with diameters of 4 mm to create disk-shaped composite films for conductivity measurements (as shown in Figure A.9).

![Figure A.9: PDMS mold used to make cylindrical pieces for PMMA/CB conductivity measurements.](image)

Using an e-beam evaporator a thin copper layer was deposited on the top and bottom surfaces. The resistance of the pieces was then measured using a multimeter and then used to calculate conductivity and resistivity values (Table A.1).

Table A.1: Conductivity and resistivity data for different CB content (three samples each).

<table>
<thead>
<tr>
<th>Carbon black content</th>
<th>0%</th>
<th>5%</th>
<th>10%</th>
<th>15%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resistivity [ohm x cm]</strong></td>
<td>∞</td>
<td>1.55E+06</td>
<td>2.81E+03</td>
<td>1.63E+03</td>
<td>9.36E+01</td>
<td>3.54E+01</td>
<td>1.53E+01</td>
<td>1.45E+00</td>
</tr>
<tr>
<td><strong>Conductivity [S/cm]</strong></td>
<td>0</td>
<td>6.50E-07</td>
<td>3.99E-04</td>
<td>8.44E-04</td>
<td>1.10E-02</td>
<td>3.02E-02</td>
<td>8.61E-02</td>
<td>7.07E-01</td>
</tr>
</tbody>
</table>
A.5  Plasma etch rate measurements

The etch rate for PMMA/CB composite, with 30 wt% CB in solid, was measured for different gas flow settings (Table A.2). The O₂ and CF₄ flow rates were varied while the other parameters were kept constant:

Equipment: TRION RIE/PECVD at UBC Nanofabrication Facility

Power: 200 W

Pressure: 500 mTorr

Temperature: 20°C

Table A.2: Etch rates for different CF₄ proportion in O₂/CF₄ gas combinations.

<table>
<thead>
<tr>
<th>CF4 content %</th>
<th>etch rate [µm/min]</th>
<th>Standard deviation [µm/min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.290</td>
<td>0.069</td>
</tr>
<tr>
<td>10</td>
<td>0.415</td>
<td>0.032</td>
</tr>
<tr>
<td>20</td>
<td>0.423</td>
<td>0.036</td>
</tr>
<tr>
<td>30</td>
<td>0.337</td>
<td>0.051</td>
</tr>
<tr>
<td>40</td>
<td>0.102</td>
<td>0.023</td>
</tr>
<tr>
<td>60</td>
<td>0.032</td>
<td>0.026</td>
</tr>
<tr>
<td>80</td>
<td>0.010</td>
<td>0.001</td>
</tr>
<tr>
<td>100</td>
<td>0.008</td>
<td>0.005</td>
</tr>
</tbody>
</table>
A.6 Nickel electroplating tests

The change in thickness of nickel was measured for different source currents (Table A.3).

The setting used for the plating process was as follows:

Time: 90 min

Area: 1 cm × 1 cm

Electrode-electrode distance: 2.5 cm

Solution: nickel chloride \( \frac{25 g}{L} \), nickel sulfate \( \frac{170 g}{L} \), and boric acid \( \frac{15 g}{L} \)

<table>
<thead>
<tr>
<th>Electric Current [mA]</th>
<th>Nickel thickness [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>22.94</td>
</tr>
<tr>
<td>2</td>
<td>46.88</td>
</tr>
<tr>
<td>3</td>
<td>70.8</td>
</tr>
<tr>
<td>4</td>
<td>92.35</td>
</tr>
<tr>
<td>5</td>
<td>108.1</td>
</tr>
<tr>
<td>6</td>
<td>131.5</td>
</tr>
<tr>
<td>7</td>
<td>156.1</td>
</tr>
</tbody>
</table>

Table A.3: Nickel thickness from electroplating for different supply currents.

The thickness of nickel was measured for different process durations (Table A.4). The setting used for the plating process was as follows:

Current: 2 mA

Area: 1 cm × 1 cm

Electrode-electrode distance: 2.5 cm
Solution: nickel chloride (25 $g_l$), nickel sulfate (170 $g_l$), and boric acid (15 $g_l$)

Table A.4: Nickel thickness from electroplating for different process durations.

<table>
<thead>
<tr>
<th>Process duration [min]</th>
<th>Nickel thickness [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>6.19</td>
</tr>
<tr>
<td>30</td>
<td>11.45</td>
</tr>
<tr>
<td>60</td>
<td>31.04</td>
</tr>
<tr>
<td>90</td>
<td>46.88</td>
</tr>
<tr>
<td>120</td>
<td>58.69</td>
</tr>
<tr>
<td>150</td>
<td>72.18</td>
</tr>
<tr>
<td>180</td>
<td>85.84</td>
</tr>
</tbody>
</table>
A.7 Additional data for the mechanical tests

Figure A.10 shows all the curves obtained for the compression tests performed on the 500 µm metallic needles presented in Chapter 3.

![Compression test plots for 500 µm tall microneedles (made through the process in Chapter 3) showing the compressive forces as a function of displacement; the first load peaks indicate tip failure.](image)

Figure A.10: Compression test plots for 500 µm tall microneedles (made through the process in Chapter 3) showing the compressive forces as a function of displacement; the first load peaks indicate tip failure.
A.8 Doxorubicin intensity-concentration calibration

Finding the linear region in the intensity-concentration plots

Calibration experiments performed to measure the intensity of various doxorubicin concentrations on a hemocytometer slide. For each test, a droplet is placed on the gridded region and then covered with a coverslip. The Hemocytometer is then flipped and placed on the confocal microscope stage. Figure A.11 below shows the setup and the slide orientation with respect to the objective.

![Figure A.11: Schematic of the setup to measure doxorubicin intensity. Confocal setting was: objective 10x, frame size: 512x512 pixels, 1.55 mm x 1.55 mm, speed: 600 Hz bidirectional, laser: 488 nm @ 10% intensity, detector: HyD1, step size: 2.06 µm.](image)

For each concentration, up to five measurements are carried out, and for each measurement, two scans are obtained and averaged. The Hemocytometer is washed carefully with soap and DI water between each test. Figure A.12 shows examples of intensity plots obtained for some of the concentrations.
Figure A.12: Examples of intensity measurements (in arbitrary units) from hemocytometer for different concentrations.
Moving from left to right on the x-axis correspond to moving from top (grids) to bottom (away from the hemocytometer) as described in Figure A.13.

**Figure A.13: Locations of scanning on an examples intensity plot.**

**Results:**

Based on the curves in Figure A.12, three calibration curves were plotted: one based on intensity readings from the grid region, one from near the coverslip, and one in the middle of the two (Figure A.14). The linear regions (0-150 μM) are the same for all the curves and beyond the linear region, the curves diverge.
Finding the intensity corresponding to the initial concentration for the tests in Chapter 4

The calibration curve in Figure A.14 is found using a confocal setting different from what was used to detect the drug distribution in skin. The detected intensity level in skin is influenced by the light absorption and scattering effects in skin, and is thus weaker than the intensity levels in hemocytometer slide for the same setting. Fluorescence detection in skin, thus, requires a higher laser power setting for excitation. The calibration curve here is, therefore, only useful for finding the linear range of intensity change with respect to concentration but not useful for calculating the concentration values in confocal images.

To estimate the concentration values in confocal images taken from skin, the t1 image taken for the experiment with the smallest t1 (5 min) was used to estimate the intensity of
the injected drug concentration at the start of the diffusion process (Figure A.15). The concentration of the very bright center of the diffusion source was assumed to correspond to roughly the initial concentration injected into the skin (i.e. 87 µM).

![Image showing bright region in the center corresponding to the initial concentration](image)

**Figure A.15:** Initial concentration calculated from the average intensity at the bright center of the diffusion source. Measured average intensity was 120.4 for 87 µM concentration.
A.9 MATLAB code used to calculate intensity distribution in the confocal images

```matlab
aveintensity(1,numImages) = 0;
%aveintensity = resulting average intensity distribution of rings
%numImages = number of images for timesteps
i = 1;
p = 2;
while i<6

I = imread(strcat('T= ',int2str(i),'.tif'));
%Igray = image in grayscale
Igray = rgb2gray(I);
%Image size
SZ = size(Igray);
%circular boundary under investigation:
boundary=min(min(c),min(SZ)-max(c));
%c = matrix coordinates for the center of source (found independantly)
ix=SZ(1);iy=SZ(2);

%center coordinates of the mask from c (being the center of interest)
cx=c(1,1);cy=c(1,2);
%ringthickness:
rt = 10;

%circular mask generating function
%http://www.mathworks.com/matlabcentral/newsreader/view_thread/146031
numcircles = int64(floor((boundary/rt)));

%previous masked images;
previousmasked = Igray.*0;

%ring average intensity
ringav = 0;
k=1;
for n=1:numcircles

   [x,y]=meshgrid(-(cx-1):(ix-cx),-(cy-1):(iy-cy));
c_mask=((x.^2+y.^2)<=(n*rt)^2);
maskedring = im2uint8(c_mask)/255 - previousmasked;
maskedimage = Igray.*maskedring;
ingerav = sum(sum(maskedimage))/nnz(maskedring);
previousmasked = im2uint8(c_mask)/255;
aveintensity(k,p) = ringav;
aveintensity(n,1) = n;
k = k+1;
end

i = i + 1;
p = p + 1;
end```

A.10 Confocal data for all of the injection trials

Figures below show the confocal images corresponding to the depth where the spherical source below the injection spot was located. The average injection depth for all the trials was $121.9 \pm 22.5 \, \mu m$.

Figure A.16: a) and b) confocal images for fresh skin samples injected with 87 \, \mu M solution of doxorubicin using 500 \, \mu m tall microneedles, time difference between images: 5 min; a) first image taken 9 min and 30 s after injection; b) first image taken 10 min and 45 s after injection. Size of images: 775 \, \mu m \times 775 \, \mu m.
Figure A.17: a), b) and c) confocal images for refrigerated skin samples injected with 87 µM solution of doxorubicin using 500 µm tall microneedles, time difference between images: 5 min; a) first image taken 12 min and 20 s after injection; b) first image taken 9 min and 40 s after injection; c) first image taken 8 min after injection. Size of images: 775 µm × 775 µm.
Figure A.18: a), b) and c) confocal images for frozen skin samples injected with 87 µM solution of doxorubicin using 500 µm tall microneedles, time difference between images: 5 min; a) first image taken 10 min and 29 s after injection; b) first image taken 5 min and 47 s after injection; c) first image taken 5 min and 4 s after injection; Size of images: 775 µm × 775 µm.