Fabrication of micro-needles for transdermal drug delivery

Ng, Hui Beng

2009

Ng, H. B. (2009). Fabrication of micro-needles for transdermal drug delivery. Master's thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/47084>

<https://doi.org/10.32657/10356/47084>

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FABRICATION OF MICRO-NEEDLES FOR TRANSDERMAL DRUG DELIVERY

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A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Master of Engineering

2009

Abstract

Alternative means of delivering drugs such as insulin to patients sometimes include the usage of high voltage pulsing or electrical current. However, such systems are limited by their ability to push drugs across the stratum corneum at therapeutic rates. One promising solution could be the usage of a micron scale needle, to create micro-pores in the skin which can allow faster diffusion of drugs with greater molecular weight or polarity into the human body. In combination with a transdermal patch, an array of micro-needles can be created to enhance the intake of drugs as well. With rapid advancement in MEMS (Micro Electro Mechanical Systems), silicon micromachining had enabled the development of such devices and result in various micro-needle designs to date. In addition, micro-needles had been fabricated with the inclusion of a fluid channel along the structure to allow controlled diffusion rates. However, the steps in fabricating such structures are often complex which lowers the process reliability and repeatability. By further proposing certain fabrication protocols and improvising on the design of micro-needles, the process can be made simpler and result in more efficient manufacturing routes.

An extensive study by researchers have revealed certain requirements of the micro-needle during drug injection, various fabrication techniques for different kind of materials as well as the possibilities of mass manufacturing processes. These areas are widely investigated and a particular area of study uses the soft lithography techniques in fabricating polymeric micro-needles. This requires the fabrication of a master mold either from silicon or commonly nickel. The research focus in this report is devoted to fabricating an array of silicon micro-needles which can double as usages for direct drug applications or as master molds for soft molding. A novel, multiple-sacrificial approach has been demonstrated as suited to the fabrication of at least 150 μ m long micro-needle bodies with positive profiles. By changing the mask design for base diameters from 90 μ m to 240 μ m, the needle profile can be engineered to give a suitable tip size for penetration, as well as a broad needle base to facilitate the creation of either single or multiple-through holes. This approach allows the mechanical properties of the otherwise brittle needles to be optimized up to buckling loads of 28mN. In

comparison, silicon micro-needles had also been tested to be painless which is consistent with literature review and capable of skin penetration.

Based on the experimental investigations and finite element simulations presented in this report, the objectives of creating and utilizing silicon microneedles had been met and various future work proposed. These include soft lithography on silicon microneedles, in vitro testing on humans, rats and porcine skin samples for feasibility of silicon micro-needles and finite element modeling of micro-needles.

Acknowledgements

The author would like to express his gratitude and appreciation towards these following people for their great help during the project and the willingness to share their knowledge and experiences with regards to their fields of study. The author had an enriching experience being part of a team to research on the fabrication of micro-needles for future Bio-Mems applications. First of all, he would like to thank his supervisor, Professor Christopher Shearwood for his valuable advice, patience and assistance in guiding the author during his project.

The author would also like to thank:

- The research students and lab technicians from Micromachines Centre for their help during the fabrication process.
- Co-worker (Melissa Teo Ai Ling) from DSO National Laboratories for her help and patience during the animal testing.
- The research students (Tan Tong Tat, Alicia Toh Guek Geok and Leo Cheng Seng) and technicians in Material Lab A for rendering their assistance in preparation of samples and measurements for the various equipments used.
- Research students from Metrology laboratory for their moral support and help throughout the research process.
- FYP students (Seet Pei wen and Tan Hong Siang Sharon) for their assistance throughout the whole project.

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List of Papers

The presented thesis is based on the following papers:

1. H.B. Ng, C. Shearwood, *A Systematic Approach to fabricate High Aspect Ratio Silicon Micro-needles for Transdermal Drug Delivery*, Proc SPIE Int Soc Opt Eng, 6799, 2007.
2. H. B. Ng, C. Shearwood, T. J. White, L. G. Yu, K. A. Khor, *Spark Plasma sintering of Silver nanopowder*, Proc SPIE Int Soc Opt Eng, 6799, 2007.

Chapter 1 Introduction

This chapter presents a brief introduction on the research project undertaken and the outline of the report. The background of the research area is accompanied by the project motivation, research objectives and the originality of the research. A final layout of the report is also included.

1.1 Background

The concept of transdermal drug delivery (TDD) refers to the ability to penetrate drugs across the main skin barrier and deliver drugs to the bloodstream for therapeutic effects [1]. As transdermal drug delivery could control the release of the drug into the user, a steady profile of the blood level can be obtained with the improved efficacy over other forms of drug dosage [2]. Moreover, the transdermal patch is often painless and handy as well as offering the ease of usage for a better patient compliance [3].

Although the development of transdermal drug delivery systems had undergone the next phase, the approval of these medical techniques is still keeping the pharmaceutical industry confused. Coupled with a short regulatory history compared to other dosage alternatives, the world wide transdermal patch market is based on fewer than 20 drugs [4]. However, due to rapid developments in the Micro Electro Mechanical Systems (MEMS), a new field referred to as BioMEMS had emerged to specialize in applying such technologies to biomedical applications [5]. A market study by the WTC Wicht Technologies Consulting firm for an article [6] had revealed that there is still an upcoming trend and demand in BioMEMS applications recently which had resulted in significant growth in MEMS. Although projected revenues for the U.S. market is set as 54 million by year 2012 (see Figure 1-1), mass market for such transdermal drug delivery systems has not yet materialized due to barriers from healthcare authorities as well as the cost of manufacturing.

Figure 1-1 Total Market for MEMS products adapted from WTC [6].

Thus one crucial component in realizing the targeted growth for TDD systems is the availability of suitable manufacturing processes and materials for producing the required parts and products economically and reliably. Continuous effort is needed to optimize the existing techniques and development of a systematic approach to cater to the future manufacturing needs in TDD systems.

Although the earliest approval usage of transdermal patch was in 1981 to prevent nausea and vomiting due to motion sickness [7], its true commercialization was brought about by the recent launch of the 7-day contraceptive TDD product (OrthoEvra™) in the US. The main difficulty in using TDD systems lies with the skin barrier to limit drugs that can be delivered through passive diffusion from an adhesive patch [8]. Thus various system innovations often include the use of energy sources to increase drug diffusion.

These systems are non-invasive and focused on either changing the skin barrier or increasing energy of molecules by utilizing mechanical energy to increase drug flux. These transdermal technologies include the iontophoresis [9] – using a low voltage electric current to create ionization of drugs to penetrate the tissue, electroporation [10] – usage of high voltage but short pulses of electric current to create aqueous pores in

the skin, sonophoresis [11] – using low frequency ultrasonic waves to disrupt the skin tissue for enhanced diffusion. However, among the mentioned technologies, only the iontophoresis technique had been successfully launched in the market where several similar systems are still in clinical development. These systems often rely on the structural disruption or alterations of the stratum corneum layer to allow greater diffusion of drugs. But the outermost layer is the most dominant rate-limiting factor which is detrimental for most TDD systems. To date, drugs being transdermally delivered shared the following constraints [12]: (1) less than 500 Da molecular weight (2) immense lipophilicity (3) small dosage up to few milligrams. Often, drugs with very high molecular weight and polarity are unsuitable for passive administration. Another limitation is due to the hydrophobic nature of the skin which could prevent drug penetration. As a result, drugs with a lipophilic character are more suited for TDD systems. On the other hand, recent developments in the choice of compounds targeted the more hydrophilic drugs that were almost undeliverable [13]. A summary of the limitations can be seen in Table 1-1.

Table 1-1 Problematic areas observed in TDD systems

No.	Limitations of TDD systems
1	Low permeability of the stratum corneum
2	Unsuitable drugs with high molecular weight and polarity
3	Hydrophobic nature of the skin
4	Most useful drugs are hydrophilic nature to date
5	Local irritation may appear at the site of application
6	Disposal of transdermal patch as 95% of drug still remains after usage
7	Damaged Patch may result in toxic rate release of drugs

A very promising area of transdermal research is in the development of a miniaturised combination of a hypodermic needle and transdermal patch to produce an array of micro-needles. By addressing certain limitations of the former systems, this biomedical device can be used to create micropores in the stratum corneum without causing any bleeding and pain sensation. As reported by Henry *et al* [14], such micropores tend to provide less resistance to intake of drugs compared to normal skin. They are also capable of better drug diffusion rates typically macromolecules up to 100,000 fold [15].

Various materials had been widely applied in the fabrication of micro-needles ranging from metallic (electroplated nickel alloys [16-19], stainless steel sheets [16], palladium

[17], copper [18] and titanium [19]) to polymeric materials (polycarbonate PC [20], Poly-Methyl-MethAcrylate PMMA [21-23] and SU-8 negative photoresist [21, 22]. Due to the great potential of using silicon for biomedical devices, a significant amount of successful research on the fabrication of silicon micro-needles [21-27] had been done and a few systems are available in the market.

1.2 Motivation

The determination to replace present hypodermic needles with micro-needles for drug delivery is overwhelming. Though coupled with safety issues as well as limited approval of drugs for transdermal drug delivery, the ultimate goal of utilising such bio-devices at a mass-scale level can only be attained by an efficient manufacturing process.

1.3 Objectives

The research work is conducted with the following underlying objectives:

1. To demonstrate the reproducible microfabrication of silicon micro-needles.
2. To develop finite element modelling for the structural aspects of the silicon micro-needle.
3. To show the transdermal penetration of the fabricated silicon micro-needle for feasibility studies.
4. To explore the various ways in which silicon micro-needles could be used as mold inserts or mold masters.

1.4 Originality of research

1. Systematic approach to fabrication of micro-needles. The existing works in the fabrication of micro-needles require complex steps in attaining required tip sharpness and geometry. Various micro-needle designs are created by changing the machine parameters in order to achieve a more robust shape. This report demonstrates a simplistic way of changing the mask design during photolithography to alter the shape of the micro-needle. The outcome could improve the mechanical properties as well as the safety aspects of using micro-needles for drug delivery.
2. Structural aspects of the micro-needle. Although a vast amount of theoretical work had been done on micro-needles, the design of micro-needles is often tailored to a simplified model in order for better comparison. In view of this, it is necessary to use FEM software on more complex designs to obtain fracturing forces for fabricated results without tailoring to a simpler model.
3. In vitro testing on humans and animals. Works on animal testing had shown disparity among researchers as certain groups had claim drug delivery on such small scale needles while others do not. This could be due to design issues or machine limitations. However, promising results from preliminary investigations had shown penetration across stratum corneum.

1.5 Outline of report

Following the introduction in Chapter 1, a critical literature review on the major aspects of the micro-needle and fabrication techniques to date are presented in Chapter 2. Chapter 3 will cover the experimental methodology and fabrication techniques used to describe the machined features. Experimental results are presented in Chapter 4 together with a discussion on the experimental observations. Finally, Chapter 5 will present the conclusion and the proposed future work.

Chapter 2 Introduction

Chapter 2 is divided into three main sections, comprising the working principle of the micro-needle, technological advancements in fabrication of micro-needles and outcome of the literature review. The working principle of the micro-needle would consist of the history of TDD systems, the need for micro-needles as well as the various limitations faced to date. The section on technological advancements would be reviewing the various types of materials used for micro-needles, various types of fabrication techniques, the types of successful delivery systems fabricated to date and soft lithography. An outcome of the literature review respect to the whole of this chapter is presented. Figure 2-1 shows the outline of Chapter 2.

Figure 2-1 Outline of Literature Review

2.1 Working Principle of Micro-needle

This part of the chapter covers a few sections which may seem trivial but yet crucial to the design of an ideal micro-needle. It encompasses the history of the transdermal drug delivery systems and how it leads the author in choosing micro-needles as the best route to transport drugs. With a better understanding of the anatomy of the skin structure and health issues, it helps to decipher the fundamental issues pertaining to micro-needles.

2.1.1 History of Drug Delivery systems

The history of transdermal drug delivery dates back to the use of plasters and ointments applied directly on the local skin sites or the underlying tissue. During the 20th century, several plasters were recognized under the United States Pharmacopeia and National Formulary [21]. The application often involves a slow diffusion process due to the concentration change between the delivery system and the human skin. Thus it must often be in continuous contact with the skin layer for a longer period of time.

Due to possible circumstances such as the setting up of public immunizations caused by disease outbreaks [22], campaigns for immunization in the developing countries and probable bioterrorism events [23], the possibility of using the transdermal route for delivery drugs systematically has been recognized. Although FDA (Food and Drug Administration) approval of the transdermal patch stretches back to 1981, its recent popularity was mainly due to the successful launch of certain products such as the 7-day contraceptive TDD product and nicotine patch to curb smoking habits.

Until then, the launch of various drug delivery systems had been observed such as the jet injectors which are able to deliver liquid drugs or vaccines using a high speed and pressure stream to pass through the skin layer [24]. However, mixed results had been reported for this device as some had reported slight pain during vaccination while others reported pain intensity similar to that of hypodermic needle injection [25].

Second form of drug intake is the common mucosal routes through oral, nasal and lung intake of drugs. Although this form of medication is still widely adopted, their effectiveness is highly reduced due to drug degradation by enzymes and low drug absorption [26]

Although various TDD systems such as the iontophoresis [9], electroporation [10], sonophoresis [11] aim to deliver drugs across the skin, their ability to push the drugs across the stratum corneum at therapeutic rates are severely limited. As such, studies had shown on the possibility of combining a transdermal patch and hypodermic needle to produce an array of micro-needles. When inserted into the skin surface, they provide pathways of micron size holes across the stratum corneum to transport macromolecular drugs which can be undertaken by the capillaries in the deeper tissues to the vascular system [27]. Despite their rather large feature size compared to clinical drug dimensions, it is proposed that the perforated holes are safe in the skin due to the fact that they are smaller than holes created by conventional needle or minor abrasion of the skin caused during daily routines .

The concept of using micro-needles was first stated in 1970s [28]. The possible usage of micro-needles was brought about by a research group at Georgia Institute of Technology in 1998 where they fabricated an array of 20 by 20 silicon micro-needles as shown in Figure 2-2.

Figure 2-2 A scanning electron micrograph of the fabricated micro-needles by Georgia Tech [29].

An enormous amount of fabrication techniques had evolved and will be covered in the later sections. The current research focus is on the feasibility of using micro-needles in the mass market typically in clinics, hospitals and among human community. Issues such as biocompatibility, safety compliance and the mass manufacturing process had been pertinent in order to reach commercial viability of such bio-devices. As such, it is necessary to have a common understanding of how the human skin structure interact with such a medical device, its basic requirements and then further focus on developing reliable manufacturing strategies to attain the objectives.

2.1.2 The need for micro-needles

This section touches on how micro-needles are capable of delivering drugs by studying the skin structure (section 2.1.2.1) and attempts to compare it with the conventional hypodermic needle (section 2.1.2.2). Problems include the compliance and discomfort of the patient, hazards posed by needle-stick injuries and the amount of cost and training involved for using current delivery system.

2.1.2.1 Anatomy of the skin structure

Figure 2-3 Anatomy of skin structure.

As stated in previous sections of the report, the stratum corneum (part of the epidermis layer) seen in Figure 2-3 serves as the main physical barrier to transdermal drug delivery. The epidermis layer is approximately 50-150 μm thick and contains a large amount of cells called keratinocytes while renewing and constantly moving up to the external environment. Through this process, the outermost layer (approximately 10-20 μm) of the epidermis contains a region of packed, dead, cornified cells called the stratum corneum. These cells are called corneocytes and are flat, hexagon-shaped and overlap each other with an approximately 30 μm diameter. By mechanical coupling to

each other and stacking with the layers of lipid, they form a very resistant scaffold which also contributes to preventing water from diffusing into the skin [30].

The dermis layer contains mostly collagen fibers and little elastin to serve as connective tissue and is very much thicker than the epidermis layer. This layer of tissue provides the tensile strength and pliability of the skin as well as supplying the epidermis with nutrients through a vascular network. In providing support for the nerve network, it contains Pacinian corpuscles (free nerve endings), blood vessels, nerve fibers, sweat glands and hair follicles.

The hypodermis or (sometimes called the subcutaneous layer) lies below the dermis. It is composed of loose connective tissue with fat. By serving as a protective cushion for the skin, its thickness varies over different parts of the body and between humans.

It is widely known that any foreign micro-objects are prevented from entering the human body by the skin's resistant and flexible structure. By understanding the anatomy of the human skin, it is possible to design micro-needles which could enable a safe yet painless drug delivery by just slight penetration across the stratum corneum. As the outer skin does not contain any nerve, skin anatomy provides the opportunity to pierce needles across the skin without stimulating nerves. Although it is often evident that micro-needles pierce through the stratum corneum as well as the viable epidermis where nerves are present and in some cases into the superficial dermis. Nevertheless, they are still reported as painless, most likely to be due to their small size which result in a reduced probability in encountering a nerve or causing it to create a painful sensation [26].

2.1.2.2 Use of the conventional needle compared to the micro-needles

To date, the general practice of delivering drugs into patients still employs the standard method of parenteral administration by using a hypodermic needle. As of the current medical records, particularly in regards to insulin delivery for diabetic patients, the smallest needles that are commercially available for injection are the 30 gauge conventional syringes and 31 gauge for the pen injectors with corresponding outer diameters of 305 and 254 μm respectively. Despite its effectiveness in delivering drugs or vaccines, typically completing an injection within a minute, this method of administration has its own disadvantages. Three such major issues are briefly discussed in Figure 2-4.

Figure 2-4 Disadvantages of using conventional injection

(a) Compliance and Discomfort

In using the hypodermic needle, there is typically a poor compliance with the schedule which is of a parental concern due to the number of injections administered in children and to “needle phobia”. Several studies had estimated 20% of the children often suffered serious distress during vaccinations [31] and “fear of injections” had been reported by approximately 8.2% of young adults [32]. When the needle is penetrated through the skin barrier, free nerve endings would detect the pain inflicted by the protrusion during drug delivery, often resulting in trauma or discomfort to the patients. Using micro-needles is painless due to the short length but yet sufficiently long enough to deliver drugs.

In comparison, a bolus injection of drug from conventional administration into the body can cause rapid increase in the blood plasma concentration followed by a drastic exponential decay due to metabolism and excretion. However, micro-needles allow the

possibility of controlled release over time at therapeutic rates which could lower the risk of side effects as well as insufficient medication shown in Figure 2-5.

Figure 2-5 A schematic diagram of drug concentration in blood plasma as a function of time for different administration schemes [33] .

(b) Safety

The risk of transmitting infectious diseases through the use of hypodermic needles had been a primary concern for the patients and healthcare providers. This is especially important in developing countries where the administration of drugs or vaccines often pose as an occupational risk due to needle-stick injuries [34]. It was reported that approximately 3 million workers under the healthcare sector are injured with a sharp device that is often contaminated with the hepatitis C virus, hepatitis B virus as well as the human immunodeficiency virus (HIV) annually [35]. This is mainly due to the fact that improper injection practices are carried out and cause a higher risk of blood-borne pathogen transmission. This includes the re-use of contaminated needles and syringes lacking sterilization in between patients, change of needles but not the syringes as well as the incorrect disposal of used needles and syringes which could put the public at risk of needle-stick injuries [36].

Even in developed countries where occupational risk due to needle-stick injuries is relatively low under normal circumstances, the risk may drastically increase in cases where mass vaccinations or medication is needed during bioterrorism or natural

pandemics [22]. Recurring incidents can only be resolved by using auto-disable technology which could help prevent the needle re-use and is common among the medical community in much of the world. Alternative solutions to drug delivery such as micro-needles could also help alleviate such issues as they have a distinct advantage of reducing transmission of blood-borne pathogen.

(c) Cost and Training

The current expensive medical service should not be burdened with additional costs due to newer drug delivery systems. Although the basic material costs can be as low as \$0.06 per injection for vaccination using conventional needles and syringes [36], it is also paramount to take into account the differences in cost from medical attention needed for complications by blood-borne pathogen transmission. For example, it was reported that the cost of medical attention due to pathogen transmission and productivity losses when included could increase the societal cost per injection to approximately \$26.77 per injection [36].

Beside the increasing cost of medical administration, there is a need to train healthcare workers in using syringes and needles to give drug relief. In using micro-needles, the problem could be resolved as patients could perform such injections by themselves without any formal training. This ease of drug delivery is especially useful when mass medication or vaccination is required in natural pandemics or bioterrorism emergencies as well as in developing countries where shortage of healthcare workers is often present [37].

2.1.3 Limitations of micro-needles to date

This part of the chapter comprises of certain limitations that are pertinent to the fabrication of the micro-needles. Although a significant number of micro-needles have been fabricated to date, not all results are safe or usable for drug delivery. The author will attempt to identify and review certain aspects of the micro-needles produced by various research groups. It is by no means exhaustive but gives a critical assessment of how a better micro-needle should be produced in the future. This assessment includes two major aspects – mechanical and human compatibility as shown in Figure 2-6.

Figure 2-6 Overview of the possible types of limitations of micro-needles

2.1.3.1 Mechanical Aspect of micro-needles

(a) Tip sharpness

In order to reduce skin damage due to the piercing reaction to the skin, micro-needles with very sharp tips are required [38]. In comparison with other technologies, such tips are often easily obtainable through the usage of silicon bulk micro-machining as they are able to achieve higher aspect ratios and sub-micron resolution [26]. As the exact mechanical properties of human skin is not sufficiently quantified, probably due to differences in age and races, the exact sharpness required for penetration through the stratum corneum of the skin structure is yet to be formulated [39]. In addition, it had been reported [40, 41] that the tip sharpness of micro-needles could be a limiting factor during skin insertion on diabetic animals. Although positive penetration results have often been reported, micro-needles are often carried out on cadaver skin [14], chicken breast [39], polyimide film [40] and silicone rubber [41], which are not representative of live human skin. As such, it is necessary to give an evaluation on the cross sectional area of the micro-needles' tips that had successfully penetrated the human stratum corneum which could give rise to possible drug delivery. A range of tip sizes shown in Table 2-1 and Table 2-2 is being listed down in order to give a better comparison.

The tables give a range of tip sharpness for out-of-plane micro-needles excluding in-plane designs as they usually result in single rows of needles. In-plane needles are developed with their shafts parallel to the substrate surface [19, 41]. Generally, most authors consider out-of-plane needles whereby their shafts are perpendicular to the substrate because they allow a more efficient drug distribution over a large surface area of the human skin. Although in-plane micro-needles can be aligned together in the same plane to form an array, it is also more economically and technologically convenient to use out-of-plane designs to attain an array of micro-needles.

As observed in Table 2-1 and Table 2-2, with respect to silicon bulk micro-machining, tip sharpness can be kept very low [29, 42-45]. Often enough, the tip diameter can also be controlled to the required sharpness.

As for polymeric materials used for micro-needles, the focus of using such polymers are on the biocompatibility with human, mass scale production and reduced possibility of shear induced breakage due to the viscoelastic property [33]. The possibility of obtaining very sharp polymeric micro-needles is shown in Table 2-1. It mainly utilises a synchrotron radiation source of X-ray for lithography which is capable of achieving nano resolution due to its short wavelength [46]. However, to incorporate mass scale production techniques, the use of a LIGA mold is necessary and often results in a more blunt tip and shorter height of micro-needle [47] as shown in Table 2-2. It was reported by Yoshimura *et al* [47] this defect could be due to the increase in resin viscosity during the filling process and also possible air trapped in the cavity.

In fabricating metallic hollow micro-needles, nickel electroplating is often adopted. The features were often fabricated following a master mold made from polymeric material such as SU-8 or Mylar. From Table 2-1, it was noted that such micro-needles were much less sharp compared to using other techniques, mainly due to the opening of the lumen diameter not offset from the middle of the needle. Thus, the delivery of drugs would need to be assisted with a pneumatic plunger to help penetrate the skin due to its viscoelasticity [48].

In conclusion, although tip diameters greater than 75 μm are usable for drug delivery, it is generally preferred to have a tip that is as sharp as possible to lower the penetration force for using hand insertion.

Table 2-1 A comparison of hollow micro-needles with possibility of piercing skin.

Hollow Needle Reference	Davis <i>et al</i> [48]	Strober <i>et al</i> [42]	Griss <i>et al</i> [43]	Gardenier <i>et al</i> [44]	Moon <i>et al</i> [46]	Kim <i>et al</i> [49]	Khumpuang <i>et al</i> [40]
Needle Dimensions							
Tip Diameter (µm)	75	12	10	0	0	58-121	0
Tip Area (µm ²)	4418	113	100	0	0	2640-11500	0
Base Diameter (µm)	300	324	160	250	190-400	92.6-170	300
Height (µm)	500	200	200	350	750-1000	200-400	350
Lumen Diameter (µm)	55	40	60	70	70-100	34-101	40
Lumen Opening from tip (µm)	0	20	100-160	60-190	70-90	0	0-58
Array Size	16	8	100	100	25-60	100	100
Type of Material	Ni	single crystal silicon	single crystal silicon	single crystal silicon	PMMA	Ni	PMMA
Remarks	In vivo Insulin Delivery into diabetic rats with pneumatic plunger	Penetrate skinless chicken breast	Penetrate inner forearm of human without breaking after subsequent rounds of testing .	In vivo Insulin Delivery into Diabetic rats	Penetrate back of hand with extraction of blood sample	No testing on any animal models	No breakage of needle tip while inserting into polyimide film

Table 2-2 A comparison of solid micro-needles with possibility of piercing skin.

Solid Needle Reference	Henry <i>et al</i> [29]	Yoshimura <i>et al</i> [47]	Wilke <i>et al</i> [45]	Park <i>et al</i> [50]
Needle Dimensions				
Tip Diameter (µm)	0	15-17	0	10-20
Tip Area (µm ²)	0	178-227	0	80-315
Base Diameter (µm)	50	100-200	300-800	100-200
Height (µm)	150	300	280-300	570-1500
Array Size	400	900-4400	145-150/cm ²	120-225
Type of Material	single crystal silicon	PLA (Polylactic Acid)	single crystal silicon	PLA (Polylactic Acid), PGA (Polyglycolic acid), PLGA (Polylactic-co-glycolic acid)
Remarks	Skin permeability to calcein of 1000 fold.	Penetration rate of 60-80% on pig skin	electrodes for cancer therapy (electroporation) and for delivery of vaccines into skin	Increased skin permeability of human cadaver epidermis

(b) Skin penetration force

Figure 2-7 shows that the smaller the tip area, the smaller the penetration force. Although this relationship is true to a certain extent, the insertion forces are also subjected to environmental conditions such as the skin roughness and viscoelasticity. Thus, these result in scatter of the experimental data in Figure 2-7. In particular, Davis *et al* [51] modeled the insertion force by equating the amount of work input due to the change in the potential energy to the change in fracture area between intact skin and skin just after penetration. This relationship is shown in equation (1),

$$\int_{x=0}^{x=x_i} F dx = G_p A \quad (1)$$

where G_p is the puncture fracture toughness and x_i is the displacement where insertion occurs. An exponential equation for the force versus displacement graph was fitted and derives the insertion force in equation (2):

$$F_i = \tau G_p A + \theta \quad (2)$$

Equation (2) predicts that the insertion force varies linearly with needle tip area. This was confirmed by experimental method where they obtain a value of 0.1N – 3.0N for a single needle penetration. It is noted the author reported that the insertion force depended on the outer radius of the micro-needle tip. Thus it is suggested that the insertion force do not vary with wall thickness instead of the full cross sectional area.

Figure 2-7 Insertion forces plotted against interfacial area. Solid line shows the linear regression of experimental data while dashed lines showed the lower and upper 95% confidence limits [51].

Table 2-3 Penetration forces for micro-needles fabricated out-of-plane.

Hollow Needle Reference	Davis <i>et al</i> [51]	Griss <i>et al</i> [52]	Kim <i>et al</i> [53]
Needle Dimensions			
Tip Diameter (μm)	30-80	0	20-60
Full cross sectional area (μm2)	710-5030	0	310-2830
Height	720	400	90-150
Lumen Diameter (μm)	25-75	60	0
Lumen Opening from tip (μm)	0	100-160	0
Average Insertion Force per needle (N)	0.1-3	0.01	2.08e-6-0.00324
Array Size	1	25	5X5-25X25
Type of Material	Ni	single crystal silicon	Silicon Dioxide
Remarks	micro-needle inserted on back of hand mechanically stripped of stratum corneum or treated with electrode gel with drop of saline	micro-needle inserted into upper hand of human that is kept dry	Micro-needle inserted onto cadaver skin

Generally, as silicon bulk micromachining techniques enable the possibility of obtaining sub-micron tips [52, 53], the usage of silicon micro-needles often require only a slight penetration force (approximately tens of milli-newtons) compared to micro-needles made from other materials. However, to enable a safe drug delivery, besides measuring the insertion forces, fracture forces of the micro-needle is also needed to be taken into account so that needle tips do not break inside the human skin.

(c) Required length

With reference to the section 2.1.2.1 anatomy of the skin, there had been different views on the required height of the micro-needle. It was observed by Teo *et al* [54] that in vivo characterization of transdermal transport in a diabetic rat was unsuccessful which could be due to the insufficient height of the micro-needle. Though very short needles had been successful in penetrating the stratum corneum [53], this could be mainly due to the tip sharpness which was addressed earlier and the type of experimental setup being adopted mainly (1) hydrating the skin which could alter the mechanical properties of the skin before conducting drug delivery which allows lower penetration force [55] (2) using a pneumatic plunger [48] to aid in protrusion of the skin barrier.

In recent years, there had been a trend for the fabrication of long micro-needles. Although this was often only possible for in plane micro-needles fabricated from silicon wafers [41, 56, 57], other techniques such as micromolding [50] and using deep X-ray lithography [46] for polymer micro-needles has resulted in out-of-plane micro-needles as long as 1000 μm . As such, a few studies have differentiated the micro-needles into two categories- transdermal drug delivery and transdermal blood extraction. In the former case, drugs are diffused by penetrating the stratum corneum but not long enough to reach the dermis layer. As such, it is painless as there are no nerves before the dermis layer. In the latter case, micro-needles penetrate to the end of the epidermis layer which will come into contact with the blood vessels thus enabling the blood to be extracted by capillary forces. However, this will result in a slightly painful delivery as the needle will also be in contact with nerves. Moon *et al* [46]

reported that the sensation will be minimized due to a smaller invasive surface compared to a conventional needle.

In view of this, different authors had different viewpoints on the required length mainly due to the viscoelastic nature of the skin for each application. Thus it is paramount that the author should identify micro-needles that had successfully deliver drugs and based the needle length according to the relevant literature review. As this report is based on the transdermal drug delivery of drugs, it is suggestive that the author takes on the required length of a micro-needle to lie between 150-200 μm which will ensure a painless delivery of drugs from Table 2-4.

Table 2-4 Length of micro-needle for transdermal drug delivery and blood extraction by various researchers.

	Moon <i>et al</i> [46]	Ma <i>et al</i> [58]	Stoeber <i>et al</i> [39]	Shikida <i>et al</i> [59]	P'erenne's <i>et al</i> [60]	Gardeniers <i>et al</i> [44]	Henry <i>et al</i> [29]
Author's view of Required length for transdermal drug delivery (µm)	300-400	150-250	>100	>50 <200	150-200	>130	<150
Author's view of Required length for blood extraction (µm)	700-900	N.A.	N.A.	>200	N.A.	>1000	N.A.
Fabricated length (µm)	750-1000	200	200	120-250	400-700	150-350	150
Pain Sensation	yes	N.A.	N.A.	N.A.	N.A.	no	no
Remarks	Successful blood extraction	No review on drug delivery or skin penetration	Penetrate skinless chicken breast up to 100micron	No review on drug delivery or skin penetration	No review on drug delivery or skin penetration	Successful delivery of insulin through 150micron needle	Skin permeability to calcein of 1000 fold.

(d) Mechanical Model

By taking into considerations the various types of forces or the modes of loading (compressive force, buckling force and free bending forces) that are acting on the micro-needle, it will be easier to verify the design and safety usage of the fabricated structures. The constraints include the minimal micro-needle dimensions, the various withstanding forces as well as overcoming the skin insertion force in section 2.1.3.1. In this section, the author will review some of the theoretical concepts in the first section and experimental work done by various mechanical studies in the second section.

Firstly, the theoretical establishments between the 4 modes of loading are being discussed.

(i) Compressive force

When loaded with a coaxial force, the maximum compressive force that a micro-needle can withstand without breaking is given by equation (3):

$$F_{compressive} = \sigma_y A \quad (3)$$

where σ_y = fracture strength of the material and A = cross-sectional area of the micro-needle.

Davis *et al* [51] had also proposed an alternative solution to the compressive force for their fabricated results.

$$F_f = 2\pi r_t t \sigma_u \sin \alpha \quad (4)$$

where F_f = axial force for needle fracture, r_t = radius at needle tip, t = wall thickness, σ_y = ultimate stress of the material, α = needle wall angle.

They have modeled their micro-needles as thin shells [61] but they had reported that Equation (4) may overestimate the fracture forces as it does not take into account for peak stresses and stresses that are normal to the needle walls [51].

(ii) Buckling force

During micro-needles insertion into human skin, one of the main mechanical failures is due to buckling. This happens when the moment of inertia is insufficient relative to the length of the micro-needle. From practical experience the needles are seen to fail well before the ultimate stress is achieved by a buckling failure mode during insertion [14, 62]. In order to consider the critical buckling force experienced by the micro-needle, a simple cylindrical geometry can be considered and from the Euler formula in equation (5):

$$EI \frac{d^2 v}{dv^2} = -P(\delta + v) \quad (5)$$

Assuming linear elastic behavior, in this case; one end fixed and one end free shown in Figure 2-8, solving equation (1) will yield equation (6):

Figure 2-8 Free body diagram of the simple beam or cylinder with constant cross sectional area during buckling.

$$P_{cr} = \frac{\pi^2 EI}{4L^2} \quad (6)$$

where E is Young's modulus, I is moment of inertia of the cylinder cross section, v is the lateral deflection, δ is the lateral deflection at the tip and L is the length of micro-needle.

A few modified buckling models are capable of giving more accurate solutions and are discussed below:

Davis *et al* [51] used the buckling model developed by Hausrath *et al* [63] which is a empirical approach to the analytical solution for tapered cone nickel plated micro-needle as shown in equation (7).

$$F_b = 0.277(2\pi Et^2 \sin^2 \alpha) \quad (7)$$

where F_b = axial load to cause buckling and E = Young modulus of the structure material.

Kim *et al* [49] modeled their nickel plated hollow micro-needles with reference to a tapered truncated hollow cone column in equation (8). A variation of the critical buckling load against the length of the micro-needle was simplified to equation (9). Using the formula developed by Smith [64] for a fixed-free tapered column:

$$P_{cr} = \frac{\pi^2 E}{2L^3} \int_0^L \sum_{i=0}^n k_i z^i \cos^2\left(\frac{\pi z}{2L}\right) dz \quad (8)$$

where area moment of inertia $I(z) = \sum k_i z^i$ can be expressed as a polynomial of z along the columnar structure.

$$P_{cr} = \frac{E}{80\pi L^2} * [\frac{5\pi^4}{16} (d_o^4 - d_i^4) + (5\pi^2 + \frac{5}{4}\pi^4)(d_o^3 - d_i^3)L \tan \alpha + (15\pi^2 + \frac{5}{2}\pi^4)(d_o^2 - d_i^2)L^2 \tan \alpha^2 + (120 + 30\pi^2 + \frac{5}{2}\pi^4)(d_o - d_i)L^3 \tan \alpha^3] \quad (9)$$

where d_o = outer diameter of the cone, d_i = inner diameter of the cone, α = tapered angle to the vertical.

Ji *et al* [65] had modeled their fabricated results using the same equation (8) to obtain the critical buckling load as shown in equation (10).

$$P_{cr} = \frac{E}{240\pi^2 L^2} [\pi^4 (b^4 + b^3 a + b^2 a^2 + b a^3 + a^4) + 20\pi^2 (b - a)(a^3 + b^3) + 120(a + b)(b - a)^3] \quad (10)$$

where a = tip length and b = base length.

(iii) Free bending force

In an ideal application, a micro-needle would be inserted into the skin with an axial force without any bending. However, in the real life situation, a needle could experience a lateral force between the skin tissue and the micro-needle during insertion [62]. The micro-needle is subjected to a transverse force where the maximum bending moment is given by equation (11):

$$F_{Maxfreebend} = \frac{\sigma_y I}{cL} \quad (11)$$

where c is the distance of the neutral axis to the outermost edge of the micro-needle.

Ji *et al* [65] had modeled the micro-needle with an ideal pyramidal structure yielding the maximum normal stress through the use of the flexure formula stated in equation (12):

$$\sigma(z) = \frac{M(z)}{S(z)} \quad (12); \quad S(z) = \frac{I(z)}{c} \quad (13); \quad c = \frac{H(z)}{2} \quad (14)$$

where $S(z)$ is the section modulus, $H(z)$ is the base length of the structure at height z .

By applying the flexure formula equation (11) and differentiating the equation, the maximum normal stress can be obtained in equation (15):

$$\sigma_{\max} = \frac{2P_t L}{(ba)a^2} = \frac{P_t}{a^2 \tan \alpha} \quad (15)$$

where P_t is the bending force.

(iv) Skin Resistance

It was proposed that the theoretical pressure to pierce the human skin is approximately 3.183×10^6 Pa [14]. In order to puncture the skin, the applied force on the micro-needle must be greater than the opposing skin resistance force given in Equation (16):

$$F_{\text{resistance}} = P_{\text{piercing}} A \quad (16)$$

where A is the cross-sectional area of the micro-needle.

Once the skin is penetrated, the skin resistance force decreases drastically to a value equivalent to the frictional force due to clamping of the skin tissue. The reduction in the skin resistance force also decreases the piercing pressure from 3.18 MPa to approximately 1.6 MPa [66, 67].

Secondly, experimental testing on different micro-needles designs had been carried out. Though most of the reports are in-plane needles, a mechanical study by Davis *et al* [51] serves to provide a very detailed relationship between the needle geometry as well as the needle fracture force.

Figure 2-9 (a) Successful penetration of cadaver skin (b) Unsuccessful penetration of cadaver skin using nickel plated micro-needles by Davis *et al* [51].

It was shown in Figure 2-9 that a micro-needle could fracture during insertion into cadaver skin. Thus if the insertion forces are greater than the critical fracture forces, such a catastrophic failure could happen. Davis *et al* [51] reported that the fracture forces is unlikely to be dependable on the range of tip radius considered although an increasing tip radius should increase the amount of area that the force is acting on.

However, for the case of wall thickness, both experimental data and modeling showed a drastic increase in the fracture force as the increase in area helps to distribute the forces resulting in lower stresses. This is shown in Figure 2-10.

Figure 2-10 Fracture forces of hollow nickel micro-needles with varying wall thickness. Bold line shows the ANSYS finite element simulation. Dotted line shows the thin shell analytical solution. Experimental data are shown as average forces with standard deviations [51].

Kim *et al* [53] had reported a fracture force of approximately 23.3 mN with a square micro-needle made of SiO₂ with dimensions of length 125 µm and width 40 µm. Although observed fracture force readings for varying length or width dimensions were not in inverse relationship according to the Euler formula, the author had mentioned it could be attributed to small fractures such as microscopic crack defects which could lead to the observed values. These stress concentrators could have induced in the material to fracture before it reaches the theoretical strength.

2.1.3.2 Human Aspect associated with micro-needles

(a) Pros and Cons of current materials

As mentioned in the introductory sections, various types of materials had been reported for the usage in micro-needles. However, the biocompatibility of such micro-needles is always an important issue and is under the jurisdiction of the FDA. (Food and Drug Administration). In particular, this section will review some of the materials mainly silicon, polymeric and metallic materials that have been developed successfully.

One of the earliest material proposed as suitable for micro-needles even up to this present day is silicon [29, 41-43, 68, 69]. This material is especially suited to creation of very sharp tips compared to other material systems. Although silicon is an attractive material with extensive processing techniques in the microelectronics industry, it is brittle and yet to be proven as a biocompatible material [70]. More than often, fabrication of silicon micro-needles is also time consuming and expensive due to multiple cleanroom processes [71]. It is necessary to attempt on a systematic approach towards fabricating micro-needles that are sharp yet robust. In minimizing the steps taken, the cost of fabrication as well as the time taken can be drastically reduced without compromising the safety issues for instance the breakage of silicon tips during application. One of the major concerns regarding bulk silicon micromachining is in the optimization of the process parameters. This is partly due to various parameters used in the etching process which often results in machine issues or contamination. By increasing the process reliability and repeatability, it can help improve the manufacturability of the silicon micro-needles as well as provide alternative routes to attain commercial viability.

Polymeric materials such as PMMA (Poly(methyl methacrylate) in Table 2-1) and PLA (Polylactic Acid in Table 2-2) had been gaining wide popularity in recent years due to the possibility of mass production. In comparison to silicon bulk machining, it is relatively easy to attain greater lengths which can be used to extract blood. It is also proposed that micro-needles made from such biocompatible materials could also

fracture without any health hazards and needle tips could dissolve and break down in the blood stream [50]. However, the low tensile strength of polymeric materials may render them useless in drug delivery as well as the difficulty in attaining very sharp tips during mass molding techniques [47]. In addition, the equipment used for the master molds such as DEEP X-ray lithography is often expensive and requires special alignment techniques [46]. Certain research groups have proposed that polymers could serve as one of the successful candidates in the future as they can ensure a very robust production route with wide availability most importantly, the ease of integration with other devices such as micro-pumps for lab-on-chip devices.

The property of metallic materials having high tensile strength and high ductility is one of the determining factors for use in micro-needles. To date, the usual techniques adopted for obtaining metallic micro-needles are through the use of laser etching techniques [16] as well as the relatively straight forward method of using electroplating technology [18, 48, 49, 59, 72]. By modifying the LIGA (acronym for **L**ithographie (lithography), **G**alvanoformung (electroforming) and **A**bformung (moulding)) process, metal material is being electrodeposited on the silicon substrate with the developed resist. Although a very smooth finish can be obtained through variation of the process parameters such as the pH and temperature of the bath and the current density, the range of metals (typically copper, gold, nickel and nickel alloys) is limited due to the conditions of the plating systems [73]. An important issue will be in the biocompatibility of such metallic materials. More than often, nickel micro-needles are often electroplated for medical applications and nickel is not biocompatible. It is reported that nickel metal is a potent allergen and results in hypersensitivity reactions for humans [74]. Although nickel molds can be fabricated for micro injection molding of micro-needles, such molds are often subjected to high tool wear rate especially at elevated molding temperatures and pressure [75].

(b) Micro-needle-Skin Interaction

Although a significant number of studies on the mechanical characterization of micro-needles have been reviewed, characteristics of the micro-needle design and the interaction between the skin and needle are still lacking. The complexity of the problem lies in the fact that the skin is a multi-phasic material which requires a substantial amount of experimental data for skin properties. In addition, the problem is compounded with the effect of age, race as well as sexuality.

A study by Baron *et al* [76] had modeled the skin structure and investigated the effect of micro-needle design on skin penetration. They used numerical simulator ANSYS to model the skin behaviour using a non-linear approach. The skin structure consists of three layers whereby the stratum corneum is considered as linear elastic isotropic material with Young modulus (6 MPa) and Poisson ratio (0.33) [77]. The epidermis and dermis layer are assimilated by the Ogden model (hyperelastic material model) which are often being used for soft tissues. The Ogden function contains a strain energy potential term equivalent to a combination of left-Cauchy strain tensor, λ_i given in equation (17):

$$\phi = \frac{2\mu}{\alpha^2} (\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) \quad (17)$$

where α and μ are skin properties from experimental data [78].

(i) Effect of needle length on skin

The human skin can withstand a lot of strain due to its viscoelasticity. With a very short needle length, it was observed that the maximum Von Mises Stresses in the skin structure occur at around the diameter edge of the micro-needle as well as the stratum corneum which are in contact with the micro-needle. However, numerical simulations show that a longer length often results in Von mises stresses higher than any of those former stresses mentioned.

It was also shown recently by Wu *et al* [79] that 150 micron height micro-needles should be assisted by an external applicator to attain reproducibility in creating conduits on human skin for drug delivery. A retainer ring was installed on the applicator to reduce the effect of skin deformation during insertion and further details can be found in the report. Micro-conduits with depth between 50 μ m to 80 μ m were shown in Figure 2-11 and drug delivery was shown to be dependent on the area and time of insertion. Although certain reports often show conduits created on skin samples, these results are often limited to micro-needles with length typically more than 400 μ m [16, 52, 80] without any external aid.

Figure 2-11 Florescence image of human skin after application of 150 μ m height micro-needles with an external applicator. Micro-conduits correspond to sites of insertion.

(ii) Effect of inter-needle distance on skin

Simulation of hollow needles was varied between 400 μm and 600 μm with a fracture skin limit value of 3.1 MPa. It was shown by Baron *et al* [76] that the inter-needle distance between two needles maybe of importance as it was shown that the array of needles did not manage to pierce the skin at certain distances. The choice of a good inter-needle distance can prevent the “bed of nails” effect where short and closely spaced micro-needles can cause a secluded deformation with reaching the skin rupture limit.

(iii) Effect of micro-needle diameter on skin

It was stated by Davis *et al* [51] that the average insertion force for a hollow micro-needle is similar to a solid needle of the same tip diameter. This was further verified by Baron *et al* [76] who observed the variation of the inner lumen diameter do not change the Von Mises stresses created in the skin. Thus it was proposed by both groups that the skin does not deform into the lumen during drug delivery.

(iv) Effect of hydrostatic pressure

Roxhed *et al* [52] had observed the stress field distribution of the micro-needle-skin interaction assuming skin to be single phasic material to investigate on the drug delivery properties. The flow pattern or diffusion of drugs is greatly related to the hydrostatic pressure. During micro-needle application, compression forces generated causes a negative hydrostatic pressure in the tissue. They claim that if this pressure persists for a certain time, fluid phases are squeezed out of the tissue and results in dehydration coupled with a decrease in the tissue’s permeability. Experimental tests had also show similar results in other reports [81, 82]. By carrying out finite element simulation on three micro-needle designs, they shown that all needles are bounded by a negative hydrostatic pressure at the tip which could prevent sufficient diffusion of drugs into the skin. However, as their hollow needles incorporate a side opening

(instead of a lumen through the centre of the needle), it was proposed that an effective drug diffusion could still be obtained. This is because a positive hydrostatic pressure is still present at the opening site of the micro-needle when inserted into the skin as seen in Figure 2-12.

Figure 2-12 Simulated distribution of the hydrostatic pressure for micro-needle by (a)Stoeber *et al* [39] (b)Davis *et al* [48] (c) Roxhed *et al* [52] Black color –negative p, white color –positive p.

At the points where the needle interacts with the skin, negative hydrostatic pressure is generated and thus a possible sealing could have been created and prevents the leakage of the liquid out from the skin tissue. With the lumen in the middle of a hollow needle, it is necessary to use a higher pressure in order to overcome the deformed regions ahead of the needle tip to transport drugs to the underlying tissues.

(c) Fluid flow

A few studies have reported on the fluid flow in the lumen of the micro-needle which gives an insight into the amount of drug that a micro-needle can deliver. The current review is limited to the fluid mechanics of the liquid flow in a micro-needle as well as the theoretical pressure needed.

By deriving from the Navier Stokes equation and assumptions made as follows:

1. Distance between micro-needles is large enough so that fluid mechanics of one needle is not affected by the neighbouring micro-needle.
2. Laminar flow is obtained with consideration of Reynolds number.
3. Fluid used is incompressible.

The Hagen-Poiseuille law is applied to derive equation (18) relating the amount of pressure required to the flow rate in the micro-needle:

$$\Delta P = \frac{128\eta L_t Q}{\pi d^4} \quad (18)$$

where Q is flow rate (m^3/s), viscosity η ($\text{N}\cdot\text{s}/\text{m}^2$), diameter of lumen d (m), length of the lumen L_t (m), ΔP is pressure drop (N/m^2).

Minor losses during the flow should also be taken into account by the equation (19):

$$C_D = \frac{F_D}{\frac{1}{2}\rho v^2 A} \quad (19); \quad Q = Av \quad (19)$$

$$\Delta p = \frac{1}{2} C_D \rho \left(\frac{Q}{A}\right)^2 \quad (20)$$

where density of the fluid ρ (kg/m^3), mean velocity v (m^3/s), C_D is the drag coefficient, A is the cross-sectional area (m^2).

Both groups Stoeber *et al* [39] and Griss *et al* [43] had applied equations (18) and (20) in deriving the pressure drop for their corresponding micro-needles. For vaccine delivery, typically 100 μ l of drugs would only need a pressure difference of 5kPa for 18s to be delivered with eight needles (fabricated by Stoeber *et al* [39]) and less than 2kPa for 2s to be delivered with 21 needles (by Griss *et al* [43].) The flow rate per needle or time required could also be reduced by increasing the needle density in both cases.

Although a more complete picture of how the liquid flow through micro-needles should include aspects such as drug diffusion by the epidermis and motion of the fluidic drug due to cell structures, it would still require an extensive research into this field. Although greater delivery rates can be improved by injection and further retraction to relieve skin compaction [83], the amount of drug delivered is highly limited by skin absorptivity. It was observed by Roxhed *et al* [84] that too high a flow rate could result in the backflow of the drug administered thus a micro-needle device will most likely require an extended delivery time for a particular dose.

2.1.4 Concluding Remarks

The major findings and requirements of a good micro-needle are summarized as below:

1. needle tip kept as sharp as possible to enable a low insertion force.
2. depending on the type of usage - short micro-needles can used for painless transdermal drug delivery and length lies from 130 μm -400 μm .
3. mechanical models for buckling and failure due to the constituent material are highly considered during skin insertion due to geometry of micro-needle.
4. fluid flow rate is dependent on the change in pressure due to Hagen-Poiseuille law and friction factor, number of micro-needles and diameter of perforated micro-channels.

2.2 Technological Advancements in fabrication of Micro-needles

Silicon micro-needles can be very brittle in nature and expensive to fabricate using conventional cleanroom processes [71]. Generally, metallic components have good conductivity, thermal and mechanical capabilities and perform better than silicon in the MEMS technology [85]. Typical examples include micro gears, RF components and fuel injection nozzles using high performance metallic materials. Thus it is necessary to adopt alternative manufacturing methods to increase higher economic efficiency and cost reduction. This chapter includes a brief review of the various machines and techniques used by current researchers to fabricate different types of micro-needles, from silicon, polymer and metallic materials. In each section, each fabrication technique is elaborated as well as the corresponding needles produced in a tabulated form for easy viewing. A summary of the various delivery systems is also presented at the end for the overall packaging of the micro-needle device. A case study of soft lithography by Moon *et al* [86] for possible mass production of micro-needles is also included.

2.2.1 Silicon Micro-needles

The first micro-needle to appear in the research field was made from silicon [29]. Silicon still serves as a primary material as it is greatly used in the cleanroom processes. There are two forms of silicon etching mainly wet and dry etching techniques. Wet etching of micro-needles usually involves the reaction of the KOH (potassium hydroxide) with (100) silicon at elevated temperatures. As the formation of micro-needles are highly dependable on the anisotropic etching characteristics in KOH governed by the crystal orientation, it also requires an intricate design of the mask in order to optimize the needle density and height [87]. Although this form of etching produces micro-needles with very robust profiles and high reproducibility, the formation of hollow micro-needles using such a technique is yet to be reported.

Thus the adoption of using dry etching is often the preferred route as it enables a greater control over needle design, as well as the formation of high aspect holes which act as fluidic channels for hollow micro-needles. Many researchers used DRIE (Deep reactive ion etching) to fabricate micro-needles. Most often, the Bosch process uses a cyclic etch/passivation method invented by Lärmer and Schilip [88]. In this process, high etching rates as well as anisotropic profiles with high selectivity to conventional photoresist masks are found. Three primary steps with two different types of gas are used. Firstly, sulfur hexafluoride (SF_6) is used to etch the silicon anisotropically. Secondly, after a certain period of etching, the process switches to deposition where C_4F_8 is used to deposit a passivation layer over the whole surface. This polymer layer protects the substrate from chemical attack and further etching. Lastly, the process switches back to etching with SF_6 where the energetic plasma creates a vertical stream of ions that removes the passivation layer from the bottom of the previously etched trench, not from the sides. This can be seen in Figure 2-13.

Figure 2-13 Etch and Passivation cycle during the DRIE process

A table for the various types of micro-needles produced by both wet and dry etching is being shown in Table 2-5:

Table 2-5 A summary of the various silicon micro-needles fabricated to date.

references for out of plane silicon micro-needles	technique used	Picture of micro-needle	Remarks
Wilke <i>et al</i> [87]	Wet etching of KOH with (100) silicon		Hollow needles are yet to be found.
Henry <i>et al</i> [29]	Black silicon method using DRIE		First solid micro-needles fabricated.
Stoeber <i>et al</i> [39]	DRIE using isotropic etch		The hollow micro-needle is tapered till it reaches the silicon nitride layer.
Griss <i>et al</i> [43]	DRIE using various isotropic and anistropic DRIE etch		Tip is sharpen by consecutive oxidation and stripping.
Gardeniers <i>et al</i> [44]	Combination of Wet and Dry etching by DRIE		Tip shapes can be determined by different mask design
Shikida <i>et al</i> [69]	Combination of wet etching and mechanical dicing		No lithography is involved but the surface is very rough with several different tip sizes in a single array.
Ji <i>et al</i> [65]	Combintion of dry etching by DRIE and electrochemical etching		A porous tip is produced to enable biocompatibility of micro-needle tip if broken in the skin

2.2.2 Polymer Micro-needles

As polymeric materials are easily available and biocompatible, various techniques such as conventional cleanroom processes, micro-molding and injection molding had been used for micro-needles fabrication. In particular, PMMA is the most common material being used for direct fabrication by deep X-ray lithography. A synchrotron radiation source is often being used with double or triple exposures adopting the PCT (plane pattern to cross section transfer) technique [40, 47] or inclined X-ray exposure technique [20, 86]. Although the fabricated micro-needles are very sharp and of controllable height, the alignment issues and expensive machine usage had lead to production of PMMA master molds instead for further mass production.

Micro-molding is often used to produce replicated pattern of the master molds which can be made from SU8 [50] or PMMA micro-needle master molds. A soft silicone material is then vacuum cast on the master mold for replication. This is often done for solid polymeric micro-needles [50] due to the lack of stiffness [60] during peeling off of high aspect micro-channels. As the soft elastomer is stable above the transition temperatures of certain biocompatible polymers, hot embossing or vacuum casting can be used to obtain the replicated solid polymeric needles. The type of materials being used include PC sheets or grains [20], PLA pellets [50] and PMMA solution [60].

A table for the various types of micro-needles produced by X-ray lithography and hot embossing is being shown in Table 2-6.

Table 2-6 A summary of the various polymer micro-needles fabricated to date

references for out of plane polymer micro-needles	technique used	Picture of micro-needle	Remarks
Moon <i>et al</i> [46, 86]	Inclined X-ray lithography		Various tip shapes, needle length and hole diameters are fabricated
Khumpuang <i>et al</i> [40]	PCT X-ray lithography		Various position of hole to the micro-needle tip was fabricated to investigate fracture limits
Han <i>et al</i> [20]	Inclined X-ray lithography and hot embossing		Out of plane micro-needles were embossed by using few rows of in plane micro-needles
Park <i>et al</i> [50]	Vacuum molding and casting		Various tip shapes were fabricated (Beveled-tip, Chisel-tip, Cone-tip)
Hsu <i>et al</i> [89]	Wet etching and casting		SU8 micro-needles from casting of silicon molds

2.2.3 Metallic Micro-needles

Electroplating is the most widely adopted and straight forward technique to obtain metallic micro-needles. This usually involves the fabrication of a master mold made from SU8 [49] or silicon molds [48, 59]. Nickel is often used as it has a good fracture strength and likely to prevent the breakage of tips in the skin. However, a disadvantage of using such a technique is in the lost of the molds after electroplating. They are dissolved in chemicals such as KOH to release the final electroplated part.

As reported by Gil *et al* [16], stainless steel micro-needles can be fabricated by using laser cutting. Various geometries and design can be implemented but laser cutting often results in very rough edges deposited by slag. Thus an additional electropolishing step is needed to obtain sharp, clean and smooth micro-needles.

The various types of micro-needles produced by nickel electroplating and laser cutting are shown in Table 2-7.

Table 2-7 A summary of the various metallic micro-needles fabricated to date

references for out of plane metallic micro-needles	technique used	Picture of micro-needle	Remarks
Kim <i>et al</i> [49]	Nickel electroplating using SU8 micro-needle mold		Various dimensions of micro-needle were obtained to analyse the critical buckling loads.
Tan <i>et al</i> [18]	Copper electroplating using silicon mold		Tip dimension is as large as 100µm which may not penetrate the skin barrier.
Davis <i>et al</i> [48, 51]	Nickel electroplating using silicon mold		Various tip dimension and needle geometry to study its effect on the skin insertion force.
Shikida <i>et al</i> [59]	Nickel electroplating using silicon mold		Techniques involve the usage of dicing and wet etching to create silicon molds.
Gil <i>et al</i> [16]	Laser cutting of stainless steel sheets		Out of plane micro-needles were obtained by deforming in plane needles out of the sheet

2.2.4 Successful micro-needle devices to date

Few studies had implemented micro-needle device as a glucose monitor or biochemical analyzer successfully. The device often contains few components which include the fabrication of the micro-needle, micro-pumps and valves in combination. One typical example is the device created by Zahn *et al* [90]. Their overall delivery system consisted of a delivery micro-needle for input of insulin as well as a sampling micro-needle for monitoring of the glucose level. Their design had allowed a tight control over the injection flow depending on the drug concentration. In addition, the planar micropump was created with check valves using silicon microfabrication combined with resistance heaters to create vapour bubbles to drive fluid out of the micro-needles.

Mukerjee *et al* [91] reported the extraction of interstitial fluids with an in situ measurement of glucose level. The design consists of a bilayer silicon and glass chip whereby collecting reservoirs and channels are created on the latter while micro-needle tips on the former. They showed that fluids can be drawn by capillary action while the micro-needle resides in the skin sample. The design concept is similar to Zimmermann *et al* [92] whereby hollow micro-needles sample interstitial fluids from the epidermis and through an integrated porous polysilicon membrane; the glucose level is monitored by an enzyme based sensor.

Taking into account the current working technology for portable insulin pumps, Ma *et al* [58] had miniaturized the common lancets used by diabetic patients to micro-needles for insulin delivery. The research had also integrated a PZT (piezo-electric transducer) to regulate the dosage and delivery rate from a drug reservoir which was vacuumized. Additional work was also done on the device by a remote control device using RF signals.

One of the interesting micro-needle devices to date had been undertaken by Roxhed *et al* [84]. Their compact design encompasses the cost of materials, the accuracy of the dosage rate and an in vivo testing of diabetic rats for continuous active infusion. The

usage of a silicone composite filled with micro-spheres is heated by a PCB (printed circuit board) to push insulin out from the drug reservoir. Thus the dosage rate can be controlled and varied in precise rates by the power applied. As the expansion of the silicone composite is irreversible, the micro-needle device is a one-time usage to prevent sharing of micro-needles and transmission of diseases due to needle-stick injuries. In their study, particular attention had also been given to the fabrication of silicon micro-needles which were mentioned in section 2.2.1. The work done by this group had been extensive and future direction focuses on the packaging of such a device.

A summary of the various micro-needle devices is shown in Table 2-8:

Table 2-8 Summary of the micro-needle devices to date

references for micro-needle device	Technique used	Picture of micro-needle device
Zahn <i>et al</i> [90]	Silicon microfabrication with micropumps, microvalves and resistance heater
Mukerjee <i>et al</i> [91]	Interstitial fluid extractor by silicon microfabrication with silicon to glass bonding
Zimmermann <i>et al</i> [92]	Glucose monitor by silicon microfabrication with porous polysilicon membrane and enzyme based glucose sensor
Ma <i>et al</i> [58]	Portable insulin delivery by silicon microfabrication for micro-needles and commercial PZT transducer to regulate dosage rate.
Roxhed <i>et al</i> [84]	One time usage transdermal patch by silicon microfabrication incorporating expandable composite to push fluid out from the reservoir upon heating.
Kim <i>et al</i> [93]	Nickel Electroplating with SU8 and conventionally machined PMMA interconnector
Khumpuang <i>et al</i> [94]	Portable device with usage of Deep X-ray lithography and vacuum tank to create a pressure difference for blood extraction

2.2.5 Soft Lithography

Although advanced techniques such as extreme UV (EUV) lithography [40, 46, 86], focused ion beam (FIB) lithography are used for the fabrication of micro-needles and are capable of generating very small tips (as small as nanometers), their development into the mass-manufacturing sector still requires substantial effort. Thus by duplicating mold inserts for micro-needles, it is possible that production cost can be greatly reduced. By using a non-lithographic set of micro-fabrication methods call soft lithography, three dimensional structures with rather complex geometries can be generated with a wide selection of materials [95].

Figure 2-14 Molding PMMA micro-needles using PDMS to produce PLLA micro-needle by Moon *et al* [46].

The key element to soft lithography lies in the usage of an elastomeric block which often contains patterned relief structures. This elastomer is called poly(dimethylsiloxane) (PDMS) and is usually prepared by cast molding. A master structure is often fabricated using various techniques such as photolithography, e-beam writing, micro-machining such as EDM and from already available relief structures. The master is silanized prior to molding and can be used to replicate stamps repeatedly (more than 50 times by Xia *et al* [95]). A typical procedure for fabricating PDMS

micro-needle molds and for molding PLLA micro-needles can be observed as in Figure 2-14.

After molding master molds with relief structures, PDMS is subjected to elevated temperatures to allow immediate curing for few hours by hydrosilylation reaction between hydrosilane (SiH) and vinyl (SiCH=CH₂) [96]. Besides its elasticity, properties of PDMS that makes it very useful also include:

1. Low interfacial free energy (~21.6 dyn/cm) and good chemical stability. Most patterned mixture do not adhere or react with the surface of PDMS.
2. Non-hydroscopic (no swelling with humidity).
3. PDMS allows gas to pass easily.
4. Good thermal stability up to 186°C in air allowing prepolymers being molded to be cured.
5. Optically transparent to approximately thickness of ~300nm where curing of prepolymers can be done by UV cross linking.
6. Isotropic and homogenous which can be deformed easily to alter patterns and relief structures in the surfaces [97].
7. Durable and multiple usages without noticeable degradation in performance.
8. Surface properties of PDMS can be changed by modifying the prepolymers or treating it with plasma to create different interfacial interactions.

2.3 Outcome of the literature review

The literature review highlighted the importance of micro-needles as part of drug delivery systems and their potential in transporting drugs painlessly as well as extracting blood. Certain aspects of the micro-needle such as the needle sharpness and length requirement are studied in the literature review and are highlighted to enhance the injection process. To enable a greater market outreach for micro-needles in different usages, be it mass vaccination or self administration, it is necessary that cheaper forms of fabrication techniques be explored without compromising the mechanical aspects. One such process enhancement could be using PDMS to pattern relief structures of the silicon master mold. Various types of polymeric microneedles are successfully produced using PDMS mold by hot pressing, vacuum casting or hot embossing.

The outcome of the literature review helps to identify the various aspects that are lacking in the usage of micro-needles and are summarized as follows:

The review on current technological advancements made in mechanical aspects of micro-needles revealed the following gaps:

- Fabrication of a suitable silicon master mold (solid and hollow micro-needles) that can be used for molding purposes.
- Blunt needles that penetrate into the body are often assisted by high speed injectors. There are limited works on the tip sharpness or length of micro-needle to penetrate skin barrier with normal application by hand.
- As slim tips are often encountered for different micro-needle designs, buckling models are used with modified cross sectional area of inertia or approximated.

The outcomes of research works into the above-mentioned areas would provide the following contributions, respectively:

- Adopting the usage of DRIE machine to enable both solid and hollow needles to be fabricated that can be used for drug delivery purposes.
- Evidence from in vitro animal or human testing for penetration of skin barrier with application by hand,
- It is necessary that a more accurate model (in this case non linear buckling load) be used for fabricated results by author. An ideal isotropic etching model could be put forward for the fabricated shape of the micro-needle.

Chapter 3 Experimental Methodology and Characterization Techniques

Extensive research revealed that in order for safe usage of micro-needles with optimal skin penetration, the design of the micro-needle must encompass certain aspects (i) sharp tips, (ii) biocompatibility, (iii) sufficient length, and (iv) side opening. Few silicon micro-machining techniques had been used to form micro-needles but only a few research groups had place emphasis on these requirements. Thus a relatively straight forward and systematic fabrication technique was adopted and takes into account of the above mentioned criteria.

This chapter covers the experimental methodology for the fabrication of a solid micro-needle followed by preliminary investigations into hollow micro-needles. This includes the standard cleanroom processes followed by the use of DRIE (deep reactive ion etching) which is capable of producing micro-needles with different profile variation. In addition, characterization of fabricated structures is done using the scanning electron microscopy. Results of animal testing and micromolding of silicon master mold had been successful

The chapter is divided into three main sections comprising of details on the type of cleanroom processes undertaken with special emphasis on the etching process, investigations on animal testing and chapter summary as shown in Figure 3-1.

Figure 3-1 Outline of Literature Review

3.1 Standard Cleanroom Processes

During the fabrication process, 4 inch (100 crystal orientation) P type double polished silicon wafers with thickness of about 700 μ m are used. All wafers are cleaned in standard Piranha bath to remove any contaminants. The description includes the design of the mask layout for lithography processes, the usage of thicker photoresists for etching and lastly, the optimum exposure time for photolithography.

3.1.1 Mask Fabrication

L-edit is a software used by the semi conductor industry to design mask patterns. This project required the fabrication of solid pillars with double annular rings that could be used to form solid micro-needles. An initial investigation uses a low cost transparency mask with minimal feature size of $20\mu\text{m} \pm 2\%$ due to budget constraints. The base diameters were varied from $90\mu\text{m}$ to $240\mu\text{m}$. Positive photoresist AZ 9260 is preferred thus dark features with a clear field mask is used for micro-needle fabrication. Negative photoresist such as SU8 is not considered as it often had adhesion problems after spin coating and is unable to dissolve in any suitable solvent. A transparency mask is as shown with arrays of different diameters shown in Figure 3-2.

Figure 3-2 Mask Pattern with different base diameters.

3.1.2 Photoresist coating

The purpose of wafer cleaning is to remove any source of impurities or contamination and to ensure reproducibility during subsequent photolithography process.

The wafers were immersed into a concentrated solution of sulphuric acid (H_2SO_4) at 120°C for 20 min. Hydrogen peroxide (H_2O_2) was added into the solution. The hydrogen peroxide reacts with sulphuric acid to produce effervescence which acts as a mechanical abrasive to clean the wafers. The wafers were subsequently rinsed in de-ionized water. Finally, the wafers were then spun at 2300 rpm for 60 sec. The wafers were then left on a hotplate at 110°C to evaporate any source of moisture. Next, the wafers were primed with Hexamethyldisilazane (HMDS) vapors to improve adhesion of the resist during spin coating. The machine should only be used when the system reaches 100°C and the liquid level of the HMDS exceed a certain prescribed level to ensure consistency in priming.

Photoresist was dispensed from a viscous polymer solution to the centre of the wafer. The wafer is spun at 1100 rpm to achieve a uniform film thickness of approximately $18\mu\text{m}$. The resist used was a positive resist AZ-9260.

After spin coating, the wafers were placed onto a hot plate at 110°C and soft baked for 4 min to promote adhesion and improve uniformity.

The wafers were then exposed using the Karl Suss MA6 mask aligner; a double-side contact and proximity mask aligner system for pattern down to $1\mu\text{m}$. The mode of exposure was hard contact printing and the exposure time used was 70 seconds. The UV source was a broadband, emitting between 350nm and 450nm (i- and h-line).

After UV exposure, the wafer was placed in a tray with developer solution of diluted AK400 ratio 1:2.5 to ensure uniformity in developing. The glass tray was agitated for about 4minutes or when wafer surface becomes shiny and formation of the relief image could be seen.

The wafer was then rinsed gently along the edge to remove the excess developing solution. The development stage transform latent resist image into relief image, dissolving soluble area of the positive photoresist. The wafers were then spun dry.

3.1.3 Deep reactive ion etching

Deep Reactive Ion Etching (DRIE) was carried out to create micro pillars of about 150µm-200µm height. This is accomplished by using a switched gas scheme that alternates between a passivation and etch step, otherwise known as the Bosch Process [98]. A typical DRIE system has an inductively coupled power (ICP) source to provide high-density plasma, and an independent substrate power bias to provide directional ion bombardment during the etching step.

Figure 3-3: Schematic Diagram of an STS Inductively Coupled Etch System used for ASE.

As the DRIE uses various parameters to attain high aspect ratio features, the influence of each parameter plays an important role during etching and can be summarized in Table 3-1:

Table 3-1 Effect of various parameters for the DRIE machine.

S/No.	Parameters		Effect on Etched Profile or process
1.	Pressure	High	high etch rates due to the increased number of fluorine radicals. But as the pressure is increased further, the ion energy and flux is reduced and the etching rate drops., promote isotropic profiles [99]
		Low	Reducing the pressures will result in an increase in the maximum ion energy and hence more vertical profile, though compromising in the selectivity [100, 101]
2.	Platen Power	High	high power promotes anisotropy and higher etch rate resulting in an increase in the mask erosion rate due to the increase in ion energy [100-102].
		Low	the lack of electrode power results in the closing up of features.
3.	Coil Power	High	The silicon etching rate increases with coil power because of the increase in the ion flux density [101]. Vertical etching increases as well with respect to lateral etching for higher coil power settings,
4.	SF6 Flow Rate	High	etch rate increases due to the increase in amount of fluorine radicals or increase in etching active cycle.
		Low	etch rate decreases due to concentration of fluorine etchant is limited and the concentrations of SiF ₄ products that redeposit increases.
5.	C4F8 Flow Rate	High	etching rate decreases when the C ₄ F ₈ flow and/or passivation active cycle duration increases. Increase in deposition of C4F8 will also result in the bowing and closing up of features towards the bottom.

Although it can be concluded that the profile of a feature is dependent on the parameters mentioned earlier, it is an iterative task to obtain a particular profile. More often than not, it is required to vary various parameters in order to achieve the desired profile.

Before any etching is carried out the STS-ICP machine has to be oxygen cleaned for 60mins to remove organic compound. The recipe is given in Table 3-2:

Table 3-2 Recipe for oxygen cleaning DRIE chamber

Oxygen Cleaning Recipe	
SF6 Flow rate	0 sccm 5%
O2 Flow rate	40sccm 95%
Pressure	Manual APC 0.1%
Coil Power	500W Tolerance 10%
Platen Power	40W Tolerance 10%

In order to attain at least 150µm height micro-pillars, it is necessary to use the deep recipe, a proprietary process which involves switching between SF₆ etching gas and C₄F₈ passivation gas. The recipe is given in Table 3-3 and is carried out for 2 hours. The DRIE etch rate under these conditions was approximately 2.5µm/min.

Table 3-3 Recipe for anistropic etching in DRIE machine

Anistropy Etching Deep Recipe	
SF6 Flow rate	130sccm 33%
C4F8 Flow rate	100sccm 33%
O2 Flow rate	13sccm 33%
Pressure	Manual APC 71%
Coil Power	Etch 800W Passivate 800W
Platen Power	Etch 14W Passivate 0W
Etching Time	14s
Passivating Time	8.0s

After resist stripping and wafer cleaning, the wafer was subjected to another isotropic etch to polish the micro-pillars into micro-needles with sharp tips. The process is subjective to etching coverage and is time dependent. Each etching time interval is monitored with the use of the scanning electron microscopy and the process step is halt once the required sharpness is reached. The recipe for isotropic etching is given in Table 3-4:

Table 3-4 Recipe for isotropic etching in DRIE machine.

Isotropic Etching	
SF6 Flow rate	130sccm 95%
O2 Flow rate	0sccm 5%
Pressure	Manual APC 0.1%
Coil Power	800W
Platen Power	0W

3.1.4 Scanning Electron Microscopy (SEM)

The Scanning Electron Microscope (SEM) that creates magnified images using electrons in a highly vacuum chamber. The principle of SEM works by focusing a relatively high energy electron beam on a specimen feature. Low energy secondary electrons are emitted from the location where electron beam is directed. A detector locates and counts these electrons and then sends the signal to an amplifier. The final image is built up from the number of electrons emitted from each location on the specimen. The Hitachi S-3500H SEM is used for the imaging and study of the micro-needle height and geometry. Certain parameters such as the magnification and emission voltage were kept constant at 250X and 25kV respectively.

1. The angle of tilt of the wafer samples in the chamber was kept at 90° anti-clockwise while using the scanning electron microscope to document the cross sectional profile.
2. The angle of tilt of the wafer samples in the chamber was kept at 60° anti-clockwise while using the scanning electron microscope to document the isometric cross sectional profile.

3.2 Animal/ Human In vitro Testing

Animal and human testing was carried out to evaluate the feasibility of micro-needles with such short length. It is necessary to show that needles do penetrate the stratum corneum in order for the transportation of drugs into the bloodstream.

A male Sprague Dawley rat (300-350g) was used for the in vitro study as shown in Figure 3-4. It was first anaesthetized and shaved clean at the dorsal region. The rat was then placed on an acrylic platform and secured by applying tape to its legs. The dorsal region is then sectioned into ten square parts. A fluorescent marker chemical was applied onto the micro-needle tips. The needle array was being pressed into the skin using finger pressure and then removed. After ten trials with the micro-needle, the dorsal region was harvested for cross-sectioning and viewing under the optical microscope for evidence of skin penetration.

Figure 3-4 Harvest of rat skin for cross-sectioning

In using skin prick tests (SPT) on human for penetration capability, histamine solution (Sigma Aldrich, St. Louis, Mo.) was applied on the targeted site followed by pressing the silicon arrays onto the skin. For additional comparison, a skin site was treated with histamine formulation in the absence of micro-needle injection. The application of histamine often leads to a local wheal with redness and warm sensation. It results in the irritation of nerve endings often causing itchiness. The result of the skin prick test

is evaluated after 10mins for the maximum reaction by visual inspection. Intermittent inspections were carried out if the reactions at the treated site were positive.

3.3 Microcasting of PDMS

Variable micro-needle diameters were used for the casting of PDMS, to investigate the likelihood of silicon tips or needle breakage in the soft mold rendering the master mold unrecyclable. Master molds were supported on wafers spun with AZ9260 photoresist and heated at 110°C to serve as an adhesion layer. The wafers are then sent into the DRIE machine to coat a layer of C₄F₈ passivation layer. The fluorine containing plasma is used to decrease the surface energy and increase the hydrophobicity of the needle surface. Silicone rubber is commercially available as Sylgard 184/182 which consists of a curing agent and an epoxy from Dow Corning. The mixture was prepared by mixing the elastomer base to curing agent in a 10:1 weight ratio and thoroughly mixed. The bubbles were then removed by a vacuum chamber. The mixture is then poured gently over the silicon micro-needle mold. The setup is degas again to ensure bubble formation during pouring is eliminated. It is then placed on a hot plate at 60°C for two hours prior to demolding.

3.4 Summary

The experimental techniques used to fabricate the solid micro-needles are presented. In addition, techniques used to characterize the micro-needles were described with additional results on the in vitro studies and mold casting carried out.

Chapter 4 Results and Discussion

This chapter reports on the results of the fabricated techniques used to create arrays of micro-needles with the possibility of changing profile geometries. In vitro studies are presented with fracture analysis of the micro-needle using finite element simulation software, ANSYS. An overview of the chapter is shown in Figure 4-1.

Figure 4-1 Outline of Chapter 4

4.1 Fabrication of micro-needles

A systematic and straight forward approach is needed to fabricate needles that are sharp with side openings to enable drug delivery. The properties had been discussed previously in section 2.1.3. During the fabrication process, certain protocols were noted and fabrication techniques were improvised to ensure the reproducibility of the needles.

4.1.1 Fabrication procedures

A schematic diagram of the fabrication process to produce hollow micro-needles is shown in Figure 4-2. A mask design with 90µm base diameter was used to explore the various problems that might arise and resolved in this primary investigation.

Figure 4-2 Schematic of the micro-needle fabrication process (a) photoresist patterned for fluidic channels (b) DRIE to produce eventual blind holes (c) rear side alignment and photolithography (d) DRIE to define micro-needle body and sacrificial annuluses (e) photoresist stripping (f) isotropic polishing etch in SF6.

An initial investigation was carried out on shorter and solid micro-needles of length less than 100µm with slim diameters of approximately 90µm. A cross sectional view of a typical micro pillar is illustrated in Figure 4-3. In this image, the wafer has been cleaved to confirm the suitability of the solid body profile for further isotropic etching given in Table 3-4.

Figure 4-3 Wafer cleaved to reveal the X-section of the micro pillar after anisotropic etching.

The profile of high aspect ratio structures depends sensitively on their separation from neighboring structures. If variable pitch arrays of needles are fabricated on the same wafer then satisfactory profiles can be produced only when the needle pitch is small. By contrast, for large needle pitches or even isolated needles, the profile is often negative [103]. After the anisotropic Deep Reactive Ion Etch (DRIE), the micro-needle body must have either a vertical or positive profile. A positive profile is easily achieved by surrounding the micro-needle by a sacrificial annulus. In using this novel approach, two annuluses were used to provide redundancy and enable the geometry of longer needle bodies to be controlled. After the anisotropic etch, the photoresist is stripped and an isotropic etch is applied to the micro-needles. Since the profile is positive this process will sharpen the needles rather than thinning them at their bases.

4.1.2 Effect of Platen power, O₂ flow rate and mask design during isotropic etching

Figure 4-4 Outline of the parameters used during the fabrication process.

(a) Variation of platen power

As it was initially believed that the profile of the needle with the mask still attached would be completely isotropic, the effect of the platen power variation was studied. Thus photoresist was not removed in this set of experiments to study its effect on the etching profile. Platen power was chosen to be 10, 50, 100W for a 90µm base diameter micro-needle.

Wafers were placed in the DRIE machine for a short period of time and then taken out to document the degree of etching done and height of inner core cylinder using the scanning electrode microscope.

Figure 4-5 Diameter of core cylinder against Etching time

From the graph above, it can be observed that the etching rate of the sample at 100W is the highest as expected. This is due to the greater ion bombardment energy possessed by the ion S_xF_y during the dissociation of the etchant [104]. However, the effect of the platen power is more significant on the etching of the photoresist as reported by Ayon *et al* [99]. This is also observed in the SEM images of the various samples etched at different platen power. It is reported that the increase of the platen power increases the photoresist etching rate. This is due to the increase of the ion bombardment energy which gives rise to etching anisotropy but a lowering of the selectivity.

Figure 4-6 SEM Image of Needle profile at 50W platen power

From the SEM images, it is observed that photoresist is left behind after etching. Although the etching process was stopped when most of the resist had been removed, it was noticed the needle profile often thinned fastest near the middle of the feature. As such, it often results in a point of breakage when the silicon can no longer support the resist (See Figure 4-6). It leads to different length of needles and sharpness formation. One point to take note is silicon underneath the photoresist is not completely etched due to the shadowing of fluorine neutrals by the photoresist. As such, it is not possible to obtain consistent height and sharpness of the micro-needles throughout the whole wafer.

(b) Variation of O₂ flow rate

Parameters in the isotropic recipe are kept constant except increasing the flow rate of the oxygen to 13sccm and platen power at 50W. This parameter was used as it was shown by Tao *et al* [105] to control the sidewall verticality of the silicon tips and plays a crucial role in the isotropic etching of silicon.

Figure 4-7 SEM Image of Needle profile at 13 sccm flow rate of O₂

In this study, it was observed that increasing the flow rates of O₂ do not assist in the isotropic profile of the needle. This is most probably due to the different dimensions of silicon needles produced by the author and the silicon tips produced by Tao *et al.* Increasing the oxygen content in the chamber decreases the etching rate of the silicon as the amount and probability of atomic fluorine reaching the silicon surface is being decreased. Moreover, oxygen causes a dramatic increase in the etching of photoresist as it is often used to clear away any residue or to strip off photoresist. When the platen power was reduced to 0W, it was also observed increasing the O₂ increases the surface roughness of the micro-needle. Although oxygen was often reported in the fabrication of micro-needles during the isotropic etch step [39, 106], the usage of oxygen causes the wall roughness to increase drastically which could no longer be polish into micro-needles as observed by the author.

(c) Effect of 0W platen power

To improve reproducibility, it was decided to remove the PR and set platen power to 0W. In this way, the problems (i) photoresist causing fracture of silicon when it is being thinned down near the middle (ii) inconsistency in height of the fractured needles will be reduced. This is because platen power would provide greater bombardment energy to the ions and cause the height of the features to shorten with the removal of the photoresist. By comparing the height of the features before and after final etching, the height of the micro-needles is not compromised at 0W platen

power. This can be observed in Figure 4-8. The removal of photoresist after DRIE is difficult due to the cross-linking induced by the ion bombardment. Although acetone can be used to remove AZ9260 photoresist, stubborn residues of the phtotoresist is still being left behind after the DRIE process which can cause undesirable features on the wafer. This problem is resolved using oxygen plasma etching (March PX-500 Plasma Cleaning System) using parameters in Table 4-1.

Table 4-1 Recipe for O₂ plasma

Input Power	Oxygen flow rate	Input Time
500W	50sccm	300s

Figure 4-8 The following micrographs show the various stages of the isotropic etch, after the definition of the needle-body and sacrificial annulus by DRIE. Etch times are a) 0, b) 2.5, c) 6 and c) 14 minutes.

Figure 4-8 (a)-(d) shows a sequence of micrographs taken at various stages in the SF₆ polishing process. No *through*-holes were fabricated for these test needles. The 90µm

diameter needle-body was polished for around 14 minutes. During the isotropic sharpening process both the outer and the inner annuluses will develop negative profiles and will be removed from the substrate subsequently. Afterwards, only the needle body structure will remain (see Figure 4-8 (c)). The tip-radius can be made sub-micron in diameter if necessary (see Figure 4-8 (d)) making then suitable for intracellular applications [103]. Micro-needles which are too sharp are unsuited for transdermal drug delivery since they are mechanically too weak. In addition, it is not possible to create through-holes of a suitably large diameter for fluidic applications. Logically, a balance between the requirements of a sharp tip and wide-base is required and this is where profile control becomes important. A sharp tip is required only to penetrate the skin. The remainder of the needle can be larger in diameter and will easily pass through the perforated skin. This significantly improves the fracture toughness of the structure and permits the fabrication of single or multiple fluidic holes of reasonable diameters. Moreover, these holes are now located away from the debris field created by the initial penetration.

(d) Variation of core cylinder diameter

Upon the successful fabrication process of the $<100\mu\text{m}$ long micro-needle, a longer DRIE anisotropic etch time approximately 2 hrs is used to produce micro-pillars with greater height. To enable sufficient height, a thicker layer of about 18 microns photoresist is needed for DRIE. Due to non-uniformity of the DRIE machine [107], it is difficult to produce a uniform range of sizes for the micro-needles. However, the minimum criterion of at least 150 microns is met throughout the wafer. This is observed from the cross section images shown in the later section. An approximate feature model without any annular features can be established due to the isotropic etch recipe. This is shown in Figure 4-9.

Figure 4-9 Feature model of micro-pillar etching into a micro-needle (i) $W < 2H$ (ii) $W = 2H$ (iii) $W > 2H$

The diagram shows the micro-needle formed without the sacrificial annuluses after performing the isotropic etch. As the minimum height of micro-needle for transdermal drug delivery is $150\mu\text{m}$,

$H = 150\mu\text{m}$ with three cases considered.

(i) if $W < 2H$,

Resultant needle profile is shown in the above Figure 4-9 (i). It is noticed that the length of the needle tip decreases as the base diameter (W) increases.

(ii) if $W=2H$,

The resultant needle profile has a sharp tip with minimal length observed when the radius of curvature is exactly 2 times greater than the required micro-needle height.

(iii) if $W>2H$,

The resultant needle profile has a very broad base with unnecessary isotropic etching needed to attain the required sharpness.

As indicated in the Figure 4-9, during the isotropic maskless etch, the height of the micro-needle is preserved but results in thinning of the bulk wafer. A new mask set was thus produced to study the effect of the base diameter on the shape profile of the micro-needle. This is because the robustness of the needle would also be dependent on the shape or profile of the micro-feature from the ANSYS results. The mask consists of four different base diameters of $90\mu\text{m}$, $140\mu\text{m}$, $190\mu\text{m}$ and $240\mu\text{m}$ with each needle density of 14 by 14. Alternative approaches to the fabrication of micro-needles have been described, [106, 108] but the approach presented here is simpler, only requiring a change in the diameter of the needle body mask rather than adjustments to process parameters from previous sections (a), (b) and (c). An ideal isotropic etch helps to reduce the surface roughness of the micro-pillar after DRIE as reported by Larsen *et al* [109]. In addition, it aids in tapering the profile to enhance the fracture toughness of the micro-needle by controlling the mask/base diameter.

(e) Geometrical Study of micro-needle profile due to annular rings by isotropic etching

Figure 4-10 X-Y coordinates of final etching profile

Equation of line 1: $y-H = g(x-a) \Rightarrow y = gx+H-ga$

Equation of curve: $(x-b)^2+(y-R)^2=R^2$

To solve the point of intersection (x_1, y_1) between the line and the curve, equation of line was substituted in the equation of curve.

$$\underbrace{x^2(1+g^2)}_c + \underbrace{x(2gH-2g^2a-2gR-2b)}_d + \underbrace{H^2-2gaH-2RH+g^2a^2+2gaR+b^2}_e = 0$$

As we have equal real roots for the equation, $d^2-4ce=0$, we can find the radius of the circle R.

$$(2gH-2g^2a-2gR-2b)^2-4(1+g^2)(H^2-2gaH-2RH+g^2a^2+2gaR+b^2) = 0$$

$$g^2R^2+R(2gb+2H-2ga)+(-2gHb+2g^2ab-H^2+2gaH-g^2a^2-g^2b^2) = 0$$

Equation of line 2: $y-H = g(x-a) \Rightarrow y = (-1/g)x+(b/g)+R$

Solving Equation line 1 and 2 gives coordinates of the point of intersection (x_1, y_1).

Table 4-2 Coordinates of the tangent point for different base diameters

Diameter of mask pattern (μm)	x-coordinate (μm)	y-coordinate (μm)
90	9.8	32.9
140	8.1	57.8
190	6.4	82.8
240	4.8	107.7

The author observed that the coordinates of the tangent point (x_1, y_1) increases along the y-axis which also decreases the length of the slim tip observed for the micro-needles fabricated shown in Figure 4-11 (a)-(d). These values can be inserted into the FEM software ANSYS for further modeling of buckling forces as well as fracture forces due to the constituent material.

Figure 4-11 Scanning electron micrographs of “inverted funnel shaped” micro-needles. Base diameters are (a) 90µm (b) 140µm (c) 190µm (d) 240µm.

Figure 4-11 (a)-(d) show micro-needles fabricated with variable diameter bases. The process parameters were kept constant (see Table 3-4) and only the original diameter of the micro-needle body, and consequently the geometry of the two annuluses, were changed. As the base diameter increases, the length of the needle tip decreases. As expected, the micro-needle with the biggest base diameter (see Figure 4-11 (d)) has the shortest tip. Of the four results, the 140, 190 and 240 μm base diameters are wide enough to allow the fabrication of either single or multiple micro-channels (through-holes). The force required to insert a needle into the skin is a function of the tip sharpness and depends only weakly on the base diameter of the needle [51]. Therefore, even though a larger needle diameter may be required for the perforation of micro-channels, it should not hinder their insertion as long as their tips are sharp.

The greater the length of the silicon micro-needle tip, the higher the chance of fracture during or after insertion. A compromise is therefore required between the base diameter and tip length.

4.1.3 FEM results on fabricated micro-needles

With reference to 2.1.3.1, it is often reported from practical experience that silicon micro-needles can fracture in two ways : either *buckling* [14, 110] or when the stress reaches the ultimate tensile strength (UTS) of silicon [29].

(a) Critical Buckling load

Buckling is due to elastic instability of the structure and become more likely for micro-needle with longer tips. For example, consider a tip of length 150 μm and average diameter 3.77 μm (i.e. without a broad base), from the Euler formula, assuming elastic behaviour,

$$P_{cr} = \frac{\pi^2 EI}{4L^2} \quad (6)$$

yields a critical buckling force of about $P_{cr} = 0.18\text{mN}$ when the cross sectional diameter is 3.77 μm , Young Modulus $E = 169\text{GPa}$ and area moment of inertia $= \pi r^4/4 = 9.92\text{e-}24\text{m}^4$.

This is then verified by using FEM simulation software (ANSYS) for computational analysis. Eigenvalue Buckling Analysis is used to obtain the theoretical buckling strength of the different micro-needles by using the ‘Block Lanczos’ method. Assumptions made during the simulation are as follows:

- Valid only for structural degrees of freedom.
- Only linear behavior is valid.
- Nonlinear elements, if any, are treated as linear.
- Material properties are linear, isotropic and constant
- Nonlinear properties, if any, are ignored.

The simulation was performed in the structural mode and uses the finite element

SOLID 186. The silicon material was considered to be isotropic with fracture strength of 7 GPa, Young modulus of 169 GPa and Poisson ratio of 0.22 [56]. The results are summarized as below.

Table 4-3 shows the theoretical buckling strength of each micro-needle. As expected, the buckling load increases with an increase in the base diameter. This can be observed from the equation (5) as well. When the area of moment of inertia increases, the expected buckling load increases. Although a 240 μ m single micro-needle can only deliver a buckling strength of about 28mN, an array of 15 by 15 micro-needles will be sufficient to deliver drugs without the micro-needles fracturing in the skin [51].

Besides considering Eigen buckling, a non linear approach will be needed as it gives a better approximation towards the buckling load of the micro-needle taking into account large deflections during static analysis. It employs the Newton-Raphson solver by increasing the applied load to a level where it becomes unstable.

Table 4-3 Eigen buckling and Non-linear buckling for micro-needles fabricated. Force required to fracture micro-needles due to structure stresses exceeding the ultimate stress of silicon

Structure	Isolated tip	Micro-needles with variable base diameters (µm)			
		90	140	190	240
Analytical approach (mN)	4				
	0.183	-	-	-	-
Eigen Buckling load (mN)	0.184	9.99	10.6	11.6	31.5
Non-Linear loading (mN)	0.144	9.46	10.1	10.1	28.0

Force to UTS (mN)	-	80.9	80.9	81.1	87.0

(b) Structure stresses exceeding the UTS of silicon

For the second mode of failure, for stresses exceeding the ultimate tensile strength of the micro-needle, simulations using ANSYS were used to determine the Von Mises stress of the different shapes of micro-needles (see Figure 4-12). The force experienced by the micro-needle tip was varied iteratively until the fracture strength of the silicon material was exceeded.

The fracture force increases gradually with an increase in the micro-needle base diameters (see Figure 4-12). In all cases, the buckling force is significantly lower than these values. Thus the micro-needles developed in this work are mainly limited by the needle tip rather than by the intrinsic strength of silicon. The matter is further complicated by the reduction in fracture toughness created by the DRIE etching process. The surface roughness is quite sensitive to the etching parameters. Future work will need to include an experimental setup suitable for obtaining the fracture force to verify the above results.

Figure 4-12 Plot of critical loads experienced by micro-needles against base diameter. The buckling force and force corresponding to the UTS are indicated by the symbols ● and □ respectively.

4.1.4 Investigations for inclusion of hollow channels along fabricated micro-needles.

Hollow micro-needles are inevitably very useful compared to their solid counterparts. This is because they allow controlled diffusion rates as well as the possibilities of storing drug reservoirs before medication is needed in the human body. The field of hollow micro-needles had been thoroughly studied in certain aspects mainly the inclusion of side openings for holes [39], the therapeutic rates of certain drugs for example insulin [52], the fluidic mechanism and pressure flow of the perforated holes [43] as well as the integration of pump mechanisms for drug delivery [58, 84].

This section comprises of three parts (i) fabrication result of a hollow micro-needle (ii) in vitro testing for silicon solid micro-needles and hollow micro-needles (iii) PDMS casting. It serves to establish some primary stages for future work as well as feasibility of the micro-needles during injection.

4.1.4.1 Fabrication result of hollow micro-needle

In choosing 240 μm base diameter micro-needle for its better mechanical properties, step (a) and (b) from Figure 4-14 were carried out to etch through-holes into the micro-needle by rear-side alignment. Initial investigations on the centre to centre spacing were kept at 10 μm and 60 μm for 20 μm blind-holes, as shown. It was shown that a hollow micro-needle with a sharp tip can be obtained with needle length of 150 μm maintained. However, it was observed that the micro-needle tips were not sharp in Figure 4-13 due to the overflow of photoresist AZ9260. Silicon wafers for isotropic etching are being bonded using photoresist onto a secondary wafer for additional support during isotropic etching. This helps to prevent the etched wafer from collapsing into the machine due to stresses or cracks created during the process. As the holes appear during isotropic etching process, photoresist would flow out of the 20 μm holes and subsequently protect the micro-pillar from further etching. SEM pictures taken show only few successful micro-needles being created using this technique as shown in Figure 4-13.

Figure 4-13 Scanning electron micrographs of micro-needles taken at 250X with holes perforated through the micro-needle (a) spacing of 10 μm (b) spacing of 60 μm .

The author had thus proposed an additional oxidation step to ensure reproducibility of hollow microneedles by keeping the micro-pillars capable of the subsequent isotropic etching step. The schematic diagram is as shown in the Figure 4-14. The oxidation

layer ($\sim 1.8 \mu\text{m}$) would prevent the overflow of the photoresist onto the front side of the wafer.

Thus additional masks were created for a 15 by 15 microneedle array keeping the centre to centre spacing at $30\mu\text{m}$ for $40\mu\text{m}$ blind-holes. Two $20\mu\text{m}$ blind-holes at centre to centre spacing of 20 micron were also created in order to create multiple channels. It was noted that a greater hole diameter or by increasing the number of channels along the micro-needle were created so that more fluids could be injected for transdermal drug delivery. The holes were all kept side perforated in order to prevent the clogging of the lumen by compaction of skin tissue along the outlet of the hole [83] and thus results in a positive hydrostatic pressure to create sufficient drug flow into the vascular system [52].

Figure 4-14. Schematic of the micro-needle fabrication process. (a) Photoresist patterned for fluidic channels, (b) DRIE to produce eventual *blind-holes*, (c) thermal growth of silicon oxide, (d) Rear-side alignment and photolithography, (e) DRIE to define micro-needle body and *sacrificial annuluses*, (f) Photoresist stripping, (g) isotropic polishing etch in SF_6 , (h) silicon oxide etch.

The depth of the blind hole would first need to be investigated so that it would be long enough to appear during the isotropic etch step. By making use of the recipe as shown in Table 4-4 for 3hours, cross sectioning was carried out on the wafers in order to obtain the depth of the hole.

Table 4-4 Anistropy Etching Deep Recipe for hole formation

Anistropy Etching Deep Recipe	
SF6 Flow rate	130sccm 33%
C4F8 Flow rate	100sccm 33%
O2 Flow rate	13sccm 33%
Pressure	Manual APC 71%
Coil Power	Etch 800W Passivate 800W
Platen Power	Etch 14W Passivate 0W
Etching Time	12s
Passivating Time	8.0s

Figure 4-15 Cross-Sectional View of (a) 20 micron hole (b) 40 micron hole after 3 hours of anisotropic etching

The depth achieved for both 20 μm and 40 μm holes were 380 μm and 620 μm respectively. Although a longer etching time can be used, the depth of the hole would not change due to insufficient energy needed to propel the incident ions into the bottom of the hole. In addition, as the aspect ratio of the perforated holes are very high, there is a decreased transport of the reactive species and thus a very low etch rate at the bottom of the hole is only attained [99, 111].

Figure 4-16 Array of hollow microneedles during isotropic etching (a) after 2hrs of DRIE and isotropic etching for 10mins (b) after 60mins of isotropic etching with appearance of oxidative layer (c) after 76mins of isotropic etching with full appearance of oxidative layer (d) after removal of oxidative layer by buffered oxide etch for 20mins.

Figure 4-16 (a)-(d) shows an array of hollow micro-needles being etched during the isotropic process with the additional oxidation step. A single, 40 μm blind hole was fabricated by rear side alignment to each micro-needle for this set of array. As

observed earlier, both the outer and then the inner annuluses will develop negative profiles and will then be removed from the substrate during the isotropic process. Upon more etching time, the $240\mu\text{m}$ pillar would taper into a micro-needle with a SiO_2 pillar with an inter-spacing of $30\mu\text{m}$ to the tip of the micro-needle. The SiO_2 pillar is then being removed in a buffered oxide etch for 20 minutes in order to reveal the perforated holes as shown in Figure 4-17. However, for the two $20\mu\text{m}$ blind-holes rear aligned to the micro-needle front side, it was observed that the SiO_2 pillar did not appear even after the micro-needle tip attained its sharpness. This is mainly due to the insufficient hole depth created during the deep reactive ion etch step. The depth of the $20\mu\text{m}$ hole is generally shorter than the $40\mu\text{m}$ hole due to the effects of the aspect ratio dependent etching which is shown in Figure 4-15.

Figure 4-17 Holes aligned at $30\mu\text{m}$ inter spacing to the centre of the microneedle tips.

ANSYS results carried out on this set of micro-needle array had also shown similar buckling results to solid micro-needles as the sharp tip are the same and the point of maximum stress occur at the same location as observed in Figure 4-18. In spite of a single $240\mu\text{m}$ micro-needle only capable of withstanding a load of about 28mN , an array of 15 by 15 microneedles would have enough strength (6.3N) to overcome the

maximum 3N insertion force reported by Davis *et al* [48] to assist in the delivery of drugs without fracturing. Thus, new sets of microneedles were being fabricated to investigate the feasibility of penetration into the skin tissue for the experiments carried out in 4.1.4.2.

Figure 4-18 Von mises stresses of microneedle upon non-linear buckling simulation by ANSYS.

4.1.4.2 In vitro testing of silicon solid and hollow micro-needles

The penetration of the stratum corneum layer is necessary in order to transport drugs from an external environment to the internal vascular system. As such, it is vital to check upon whether needles from previous fabrication techniques could pierce the skin by carrying out in vitro experiments.

In conducting the rat testing illustrated in Figure 3-4, 9 sets of solid silicon micro-needles with various heights (150-160 μ m), base diameters (90,190 and 240 μ m) and array size (1-9 micro-needles) were used with sharpness kept as small as 4 μ m (tip diameter). A new mask was utilized and the array was kept as small as possible to ease the problems due to cross-sectioning of the rat skin.

Figure 4-19 Histology samples of the rat (a) by 190 micron micro-needle (b) by 90 micron micro-needle (c) 90 micron needle (d) a 3 by 3 micro-needle array sample used during histology.

After removing samples from the neutral buffered formalin which is used for preservation, they are cross-sectioned as shown in Figure 4-19. Out of the 9 samples, only one histology trial manages to yield positive result whereby the micro-needle had penetrated the skin to reach the dermis layer. This is shown as the marker chemical stain in Figure 4-19 (c). Although micro-needle penetration should show the disruption of stratum corneum, this was not obvious which could be due to the dicing of the skin sample away from the small penetrated hole. It was interesting to take note that all the tested micro-needles did not fracture due to either of the two causes mentioned in section 4.1.3. This could be due to the fact that rat skin is one-third thinner than human skin which needed a smaller insertion force [48]. In addition, the subcutaneous fat and muscle layers below the stratum corneum could provide a cushioning effect during the needle insertion [54]. These two factors could have resulted in the anomaly during the injection.

Porcine skin samples had been used in few transdermal studies [16, 80]. Due to budget constraints, porcine skin tissues instead of live rats and human cadaver skin samples were freshly obtained to ascertain the postulated results from Figure 4-19 (c). The fresh porcine cadaver skin samples were subjected to in vitro testing immediately after harvesting. Porcine skin samples were pierced for 1 minute with food dye and were frozen in OCT compound. The skin samples were cut into 5µm thick slices using a cryostat. During the piecing of the porcine skin by hollow micro-needles shown in Figure 4-16 (d), it was also observed that excess food dye leaks to the surface of the skin during the insertion. This was also observed by Roxhed *et al* [84] as the amount of liquid that can be absorbed by the skin tissue is limited. A slow delivery rate of 2µl/hr is necessary in order to prevent the leakage of insulin for diabetic rats. In addition, it should be noted that the spacing of the perforated hole to the tip centre is paramount as it determines whether the entire hole is being sealed between the skin surface and the micro-needle to prevent leakage. The possibility of creating a seal between the micro-needle and skin surface is also being investigated by Roxhed *et al* [52].

Upon the penetration test of the porcine skin, some micro-needle tips had fracture into the skin tissue as shown in Figure 4-20(a) by a light microscope. Although the biocompatibility of silicon had not been studied for micro-needles, it had been used in implantations for days to weeks [112-114]. It is also worth taking note that fractured silicon tips may not serve as potential hazards as the skin renews constantly between 30-60 days [115].

Figure 4-20 (a) Fractured microneedle tips in porcine skin observed under a light microscope (b) a 15 by 15 hollow micro-needle array with fluid channel for attachment to syringe

Figure 4-21 Cross Section of Porcine skin sliced at 5μm under stereo microscope.

Most of the histology trials show pictures as depicted in Figure 4-21 (a) due to the difficulty of cryo-sectioning at the micro-needle tip. However, Figure 4-21 (b) shows an exact profile of the micro-needle with the annular features observed at the surface of the skin tissue. The penetration ability of the micro-needles fabricated in accordance to the techniques in this report had been established and thus it is capable of delivering drugs for transdermal drug delivery. The result shows one perforated hole and is not representative of the whole array. It was reported that insertion results for 150μm height micro-needles had to be assisted by a pneumatic applicator to create an impact force during the process [79]. The enhancement of the transdermal permeability of drugs had increased by 10^4 to 10^5 times compared to intact skin and is influenced by the area and time of insertion. It could only be postulated in this stage that fabricated micro-needles are capable of penetration by hand application but the number of insertions had been irreproducible. Further work can be carried out to find out the number of conduits created during an insertion by using fluorescence dyes and confocal laser scanning microscopy but is not covered in this report.

In order to reaffirm the result of penetration, histamine testing was also carried out on part of a living hand to prove that the stratum corneum had been disrupted to allow diffusion of drugs.

Histamine solution results in vigorous inflammation of the skin when stratum corneum is disrupted or abraded. However, the limitation of such a test is the reproducibility of the result. It was stated by Trautmann *et al* [116] that the same reactions were not observed during histamine application using a commercial lancet. Twelve sets of new micro-needles were fabricated and used on each of the square sections as observed on both human hands in Figure 4-22.

Figure 4-22 Square section corresponds to a new set of micro-needle penetration in (a) and (b) and (c) shows a 15 by 15 solid micro-needle array

It was first observed that the sites treated without micro-needle injection do not display any reaction and skin is still intact with complete absence of swelling. This set of needles had various length (90-200 μ m), tip sizes (1-24 μ m) and array size 15 by 15. Site A had shown a square wheal after the application of micro-needles which is highly probable that the needles had penetrated the skin barrier. However, site B only show a partial square shaped wheal. All sets of micro-needles applied had shown partial or complete swelling due to histamine solution diffusing into the skin by visual inspection. The disparity between the results had shown possible penetration to deliver drugs but the number of micro-needle insertions had been irreproducible and random.

Thus it is highly probable that micro-needles of such short height need to be assisted by an impact force in order to ensure whole array is inserted.

The test person did not report any pain sensation and no blood stain was observed. Tested micro-needles were observed under the SEM and none of the sets had shown any fracture due to buckling or the ultimate tensile strength of the silicon material. This goes to show that the array size of 15 by 15 had sufficient strength to prevent any breakage during application.

4.1.4.3 PDMS casting of silicon micro-needles

As it was initially thought that high aspect ratio micro-needle or their slim and sharp tips could fracture during demolding of the soft elastomer, PDMS was cast onto 90 μ m diameter base micro-needle. It was observed such phenomenon did occur during demolding as expected. Micro-needles were also observed under SEM and were found to be unsuited for further replications.

By applying a hydrophobic layer of C_4F_8 onto the micro-needle master mold, PDMS was successfully demold without fracturing the needles. Moreover, repeated replications were done on the same mold set and were successful. The scanning electron micrographs of the 140 μ m master micro-needle insert shown no defects as shown in the Figure 4-23. The various base diameters from 90-240 μ m micro-needles had been successfully replicated. As such, these PDMS molds could serve to fabricate polymeric micro-needles by soft lithography depicted in section 2.2.5.

Figure 4-23 Master micro-needle mold and replicated mold inserts for soft molding. Micrographs are taken after etching (middle), after first replication (left) and second replication (right)

4.2 Summary

Through the fabrication of micro-needles by the DRIE machine, it was observed:

- A positive profile of the micro-needle was maintained by surrounding the micro-pillar with sacrificial annuluses.
- It is necessary that photoresist be stripped off before isotropic etching is being carried out in order to prevent notching effect of the micro-pillar.
- Platen power is kept low to prevent shortening the height of the micro-needle.
- A variation of the base diameter from the mask design result in micro-needles with better mechanical properties. The final profile geometry was governed by an ideal isotropic etching mechanism of the micro-pillar.
- Finite element simulation by ANSYS showed that the greater the base diameter the higher the critical buckling force of the micro-needle. The needle with base diameter $240\mu\text{m}$ yield a value of 28mN fracture force.
- An additional oxidation step helps to keep the size of perforated fluidic channel consistent as well as the micro-needle tip sharp to prevent backflow of photoresist from supporting wafer.
- In vitro testing of the micro-needle had shown promising result in penetrating the stratum corneum to deliver drugs by both animal and human skin testing. However, the number of perforations created is irreproducible and random due to viscoelasticity of the skin.
- Successful microcasting of PDMS to ensure that micro-needle tips do not fracture in soft mold inserts and is capable of replication.

A systematic and straight forward approach was used to fabricate solid micro-needles with different geometries. As the various micro-needles would fail by buckling instead, an array size of 15 by 15 was used for drug delivery.

Chapter 5 Conclusion and Future work

This chapter covers a conclusion to the report and proposed future works. The coverage of these works is selected based on the information gathered from available literature review as well as results obtained to date.

5.1 Conclusion

In chapter 2, a thorough literature review was performed on topics that included the requirements of the micro-needle during drug injection, various fabrication techniques for different kind of materials and included the possibilities of mass manufacturing processes. Thus the fabrication of the silicon micro-needle was first carried out to double as usages for direct drug applications or as master molds for soft molding. An initial investigation was carried out on shorter and solid micro-needles of length less than 100 μm with slim diameters approximately 90 μm where annular structures were incorporated to sustain a positive profile throughout the isotropic etching. Although certain process parameters were varied which was thought to give a pure isotropic etch, fabrication results were unsuccessful.

By maintaining the platen power of the isotropic etches to 0W and stripping off the photoresist, the length of the micro-needle is maintained whilst the tip is being polish to give a sharp tip. By changing the mask design instead of varying process parameters, a broad needle base micro-needle was created to facilitate the creation of either single or multiple-through holes. Through the usage of FEM software ANSYS, it was found that all micro-needles would fail by buckling and the non linear buckling load for the broadest micro-needle is 28mN. Thus an array of 15 by 15 micro-needles will be strong enough to penetrate the skin without any fracturing.

Fabrication of hollow micro-needles was performed by an additional oxidation step to ensure the reproducibility of the fabrication process. The holes were all kept side perforated in order to prevent the clogging of the lumen by compaction of skin tissue

along the outlet of the hole. In addition, silicon micro-needles had been tested to be painless in consistent with literature review. In vitro testing carried out on animal and human skin samples had shown possible penetration of stratum corneum but results had been irreproducible. This is due to the elasticity of the skin and the height of the micro-needles as reported. Experimental investigations had been carried out to verify silicon micro-needle tips do not fracture in soft mold insert and is capable of replication for soft lithography.

5.2 Future Work

Based on the experimental investigations and literature review which indicates a potential for the mass manufacturing of polymeric biocompatible micro-needles, various future works are proposed. These include:

- (1) To replicate polymeric micro-needles from soft molds by using hot embossing/press or vacuum casting. A parametric study would also need to include the effects on the aspect ratio and the sharpness of the polymeric micro-needle.
- (2) To carry out soft molding on hollow silicon micro-needles whereby fluid diameters are sufficiently big enough for ease of demolding. If fluid diameters are kept small, the casting of a soft elastomeric mold would prove futile. Thus it is necessary to investigate on a possible fluid diameter size that allows easy demolding without the micro-pillars formed from soft molding to collapse. Replication techniques can then be applied to attain hollow polymeric micro-needles.
- (3) To setup on site monitoring of micro-needle failure during skin penetration. This helps to verify ANSYS results generated in this report although they are consistent with values reported in literature review. Additional structural studies can be conducted to compare silicon and polymer micro-needles. In view of mass production and biocompatibility, the author felt that polymeric micro-needles will serve as a promising candidate for future commercialization.

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Appendix 1

Problems encountered during micro-needle fabrication

(a) Silicon grass and Contamination

Plasma chamber was oxygen cleaned before etching to remove organic contaminant but Figure A shows that chamber needs more cleaning. The impurities affect the rate of etching and also the outcome of profile as results vary with the same parameter used. In this case, the structure is without resist and being etched with 0W platen power. There was no visible sharpening of tip, instead the height of the cylinder decreased.

Figure A Contaminant on wafer

Silicon grass was formed on some wafers (see Figure B) and it was believed that the growth was due to the chamber condition. Some research has attributed the growth with the SF_6 flow rate. There is a minimum value of SF_6 flow rate before formation of silicon grass starts. The density and height of silicon grass will also increase with SF_6 flow rate. The exact reason behind grass formation is not clear yet. However, it is thought that formation of silicon grass is due to the localised concentration of incoming fluoride ions. A short SF_6 etch in the RIE will get rid of the silicon grass but etches other features more than oxidization.

Figure B Growth of Silicon grass

Figure C Contaminated wafer

Figure C shows a layer of contaminant left on the wafer after being etched in the STS machine. From previous experiments conducted, it was noted that this layer of contaminant will greatly affect the result obtained as needle profile will not result.

(b) Thermal Stress

Figure D Cracks on wafer and results in shattering

Cracks started to appear after the initial isotropic etching process as shown in Figure D. It was believed that the cracks are results of thermal stresses introduced during the wafer re-bonding process. Wafer was separated after the anisotropic etching when it was soaked in acetone to remove the photoresist. Then resist was coated on a new wafer and placed together with the etched wafer before hot baking for 2mins. Stresses during this process probably caused the cracks to appear as when the re-bonding process was not done, crack was not seen too.

Figure E Shifting of annular rings

A point noted during the experiment was that after baking, the wafers must be placed on insulated surface instead of cool surfaces such as metal table top so as to reduce the thermal cooling gradient. Initially some wafers were placed in direct contact with the metal table surface. With the continuation of experiments, problem such as poor adhesion of resist was observed when resist rings starts falling out of place after the

development process. Hence, rapid cooling of wafers caused the resist to fall off during processing shown in Fig. E.

Appendix 2

Printed Papers

A Systematic Approach to fabricate High Aspect Ratio Silicon Micro-needles for Transdermal Drug Delivery

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ABSTRACT

The successful development of micro-needles can help transport drugs and vaccines both effectively and painlessly across the skin. However, not all micro-needles are strong enough to withstand the insertion forces and viscoelasticity of the skin. The work here focuses on the micro-fabrication of high aspect ratio needles with careful control of needle-profile using dry etching technologies. Silicon micro-needles, 150 μ m in length with base-diameters ranging from 90 to 240 μ m have been investigated in this study. A *novel*, multiple-sacrificial approach has been demonstrated as suited to the fabrication of long micro-needle bodies with positive profiles. The parameters that control the isotropic etching are adjusted to control the ratio of the needle-base diameter to needle length. By careful control of geometry, the needle profile can be engineered to give a suitable tip size for penetration, as well as a broad needle base to facilitate the creation of either single or multiple-through holes. This approach allows the mechanical properties of the otherwise brittle needles to be optimized. Finite element analysis indicates that the micro-needles will fracture prematurely due to buckling, with forces ranging from 10 to 30mN.

Keywords: transdermal drug delivery, silicon, micro-needles, MEMS, DRIE

1. INTRODUCTION

Medicinal properties of drugs administered orally are often degraded in the gastrointestinal tract or simply eliminated from the body by the liver. Hence intravenous injection is often a preferred alternative to oral drug delivery. However, such a practice had its limitations mainly due to the pain caused by the hypodermic needle and lack of expertise in performing such an injection. This is particularly important in diabetic patients as insulin delivery is mainly self-administered. Often, insulin is not taken at prescribed times due to the inconvenience of such injections. Thus an alternative approach is required such as transdermal drug delivery across the skin.

Although various techniques using electroporation [1], penetration enhancers [2], and low-frequency sonophoresis [3] have been used to improve transdermal drug delivery (TDD), the effective transport of drugs particularly, with high molecular weight and polarity, are still severely limited by the stratum corneum of the skin structure. With the use of micro-fabrication technologies, a combination of the hypodermic needle and transdermal patch had been used to develop an array of micro-needles. When inserted into the skin surface, they provide pathways of micron size holes across the stratum corneum to transport macromolecular drugs which can be undertaken by the capillaries in the deeper tissues to the vascular system [4].

Both solid and hollow micro-needles had been fabricated in various materials including metals, silicon, silicon dioxide, polymers. In many cases, drugs are delivered into the body by coating the solid micro-needles before insertion into the skin. Studies by Henry [5] had shown that the skin permeability increases up to more than four orders of magnitude. Inclusion of a hollow lumen in a micro-needle structure extensively expands its usage, especially in delivering drugs effectively by pressure driven flow [6].

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Although various authors had reported on the insertion or fracture forces of their fabricated micro-needles [7] or simulated results [8, 9], few have actually shown the effect on the profile of the micro-needles after insertion. Typically,

Davis [10] had shown that a micro-needle could fracture during insertion into the cadaver skin tested. This is possible as not all geometries for micro-needles reported are suitable for insertion without fracturing. Reported insertion forces vary between 0.1 to 3.0 N with a linear dependency on tip area. By contrast, fracture forces range between 0.5 to 6N increasing strongly with needle wall thickness but remaining independent of tip radius.

There are few examples of the application of hollow micro-needles. One such example involves the delivery of insulin into rats by using a 75 μ m tip diameter hollow micro-needle [6]. Although metallic micro-needles with tip diameter as large as 100 μ m have been fabricated by Kim [11], Polla reported that the lack of tip sharpness implied that the micro-needles may have not penetrated the epidermis layer. Hence the tip diameter of the micro-needles need be kept as small as possible to allow easy piercing of the skin and the provision of fluidic channels to enable controlled delivery of drugs.

Our work focuses on the micro-fabrication of silicon micro-needles with the needle geometry controlled by dry etching. Dry etching is preferred as it offers a higher level of anisotropy and aspect ratio control compared to wet etching. This is especially important in the creation of fluidic *through-holes* in otherwise solid needles. By changing the mask design, as well as controlling the parameters used during the isotropic etching of the silicon micro-needles, we developed a range of micro-needles with sharp tips for good insertion and a range of different base diameters which can help accommodate multiple fluidic channels. To achieve a minimum length of at least 150 microns, deep reactive ion etching is used, combined with a second isotropic etch step to sharpen the tips as well as giving it a slightly tapered profile for smooth insertion.

The profile of high aspect ratio structures depends sensitively on their separation from neighboring structures. If variable pitch arrays of needles are fabricated on the same wafer then satisfactory profiles can be produced only when the needle pitch is small. By contrast, for large needle pitches or even isolated needles, the profile is often negative [13]. After the anisotropic Deep Reactive Ion Etch (DRIE), the micro-needle body must have either a vertical or positive profile. A positive profile is easily achieved by surrounding the micro-needle by a sacrificial annulus. In this work we use two annuluses to provide redundancy and enable the geometry of longer needle bodies to be controlled. After the anisotropic etch, the photoresist is stripped and an isotropic etch is applied to the micro-needles. Since the profile is positive this process will sharpen the needles rather than thinning them at their base.

2. FABRICATION

The micro-needles were fabricated from double-sided polished, 700 μ m thick, 4-inch, p-type Si (100) wafers. These are a little thicker than usual but allow longer needles to be created. A schematic of the process is illustrated in Fig. 1. A thickness of 20 μ m of AZ9260 Photoresist was applied throughout the process, according to the manufacturers' instructions.

Initially, an anisotropic etch to create the *through-holes* was made by a proprietary process involving switching between SF₆ (flow-rate 130 sccm) and C₄F₈ (flow-rate 100 sccm) gases, using a STS Multiplex ICP system. More details can be found elsewhere [12]. These holes (of diameter 20 μ m) were positioned off-axis to facilitate drug delivery with a reduced likelihood of blockage. The coil power and platen power, for both etching and passivation cycles, were set to 800 and 14W respectively. Etch and passivation times were optimized at 14 and 8 seconds. The DRIE rate under these conditions was 2.5 μ m/min.

After resist stripping and wafer cleaning, the wafer was then inverted to enable definition of the micro-needle body and *sacrificial* annuluses. Rear-side alignment was made to the through-holes and the photolithography and anisotropic DRIE step was repeated. The resist was then stripped and the *near-cylindrical* needle body polished using SF₆ (flow-rate 130 sccm and platen power set to 0W).

In this initial study, the base diameters were varied from 90 to 240 μ m with constant length of 150 μ m to facilitate transdermal drug delivery. The length of the micro-needles is only dependent on the micro-

needle body, defined by the anisotropic DRIE step. Platen power is not applied during the isotropic etching step as it decreases the aspect ratio of the micro-needles.

Fig. 1. Schematic of the micro-needle fabrication process. (a) Photoresist patterned for fluidic channels, (b) DRIE to produce eventual *through-holes*, (c) Rear-side alignment and photolithography, (d) DRIE to define micro-needle body and *sacrificial annuluses*, (e) Photoresist stripping, and (f) isotropic polishing etch in SF_6 .

3. RESULTS

Fig. 2 (a)-(d) shows a sequence of micrographs taken at various stages in the SF_6 polishing process. No *through-holes* were fabricated for these test needles. The $90\mu\text{m}$ diameter needle-body was polished in around 14 minutes. During the isotropic sharpening process both the outer and then the inner annuluses will develop negative profiles and will then be removed from the substrate. Afterwards, only the needle body structure will remain (see Fig. 2 (c)). The tip-radius can be made sub-micron in diameter if necessary (see Fig. 2 (d)) making then suitable for intracellular applications [13]. Micro-needles which are too sharp are unsuited for transdermal drug delivery since they are mechanically too weak. In addition, it is not possible to create through-holes of a suitably large diameter for fluidic applications. Logically, a balance between the requirements of a sharp tip and wide-base is required and this is where profile control becomes important. A sharp tip is required only to penetrate the skin. The remainder of the needle can be larger in diameter and will easily pass through the perforated skin. This significantly improves the fracture toughness of the structure and permits the fabrication of single or multiple fluidic holes of reasonable diameters. Moreover, these holes are now located away from the debris field created by the initial penetration. This study represents the first systematic approach to this topic.

Alternative approaches to the fabrication of micro-needles have been described [14], [15] but the approach presented here is simpler, only requiring a change in the diameter of the needle body mask rather than adjustments to process parameters.

Fig. 2 The following micrographs show the various stages of isotropic SF_6 etch, after the definition of the needle-body and *sacrificial* annuluses by DRIE. Etch times are a) 0, b) 2.5, c) 6, and c) 14 minutes. The magnification is $\times 250$.

Fig. 3 (a)-(c) show micro-needles fabricated with variable diameter bases. The process parameters were kept constant and only the original diameter of the micro-needle body, and consequently the geometry of the two annuluses, were changed. As the base diameter increases, the length of the needle tip decreases. As expected, the micro-needle with the biggest base diameter (see Fig. 3 (c)) has the shortest tip. Of the three results, all base diameters are wide enough to allow the fabrication of either single or multiple micro-channels (through-holes). The force required to insert a needle into the skin is a function of the tip sharpness and depends only weakly on the base diameter of the needle. Therefore, even though a larger needle diameter may be required for the perforation of micro-channels, it should not hinder their insertion as long as their tips are sharp.

The greater the length of the silicon micro-needle tip, the higher the chance of fracture during or after insertion. A compromise is therefore required between the base diameter and tip length.

4. MECHANICAL STRENGTH ANALYSIS

Silicon micro-needle can fracture in two ways: either *buckling* or when the stress reaches the ultimate tensile strength (UTS) of silicon. Buckling is due to elastic instability of the structure and become more likely for micro-needle with long tips. For example, consider a tip of length $100\mu\text{m}$ and average diameter $4\mu\text{m}$ (i.e. without a broad base). An analytical approach, based on the Euler formula and assuming linear elastic behavior [16], suggest that a force of only 0.18mN will lead to buckling failure (see Table 1).

Fig. 3 Scanning electron micrographs of “inverted funnel shaped” micro-needles. Base diameters are (a) 140μm, (b) 190μm, and (c) 240μm.

Structure	Isolated tip(μm)	Micro-needles with variable base diameters (μm)			
		90	140	190	240
Analytical approach (mN)	0.183	-	-	-	-
Eigen Buckling load (mN)	0.184	9.99	10.6	11.6	31.5
Non-Linear loading (mN)	0.144	9.46	10.1	10.1	28.0
Force to UTS (mN)	-	80.9	80.9	81.1	87.0

Table 1 Eigen buckling and Non-linear buckling for micro-needles fabricated. Force required to fracture micro-needles due to structure stresses exceeding the ultimate stress of silicon

Eigenvalue buckling analysis was used to obtain the theoretical buckling strength of the different micro-needles by using the ‘Block Lanczos’ method. The simulation was performed in the structural mode and uses the finite element SOLID 186. The silicon material was considered to be isotropic with fracture strength of 7GPa, Young modulus of 169GPa and Poisson ratio of 0.22. The results are summarized in Table 1.

Fig. 4 Plot of critical loads experienced by micro-needles against base diameter. The buckling force and force corresponding to the UTS are indicated by the symbols ● and □ respectively.

For the “*inverted funnel*” micro-needles, the buckling load increases with increasing base diameter (see Fig. 4). The 240μm micro-needle has a buckling strength of about 28mN. However, in TDD, an array of 20×20 micro-needles will be used and this will have sufficient to deliver drugs without the micro-needles fracturing in the skin [10]. In addition to eigen buckling, a non-linear approach was also applied to the analysis since it gives a better approximation towards the buckling load of the micro-needle and takes into account large deflections during static analysis. It employs the Newton-Raphson solver by increasing the applied load to a level where it becomes unstable. A non-linear approach gives a better estimation of the buckling strength (see Table 1).

For the second mode of failure, for stresses exceeding the ultimate tensile strength of the micro-needle, simulations using Ansys were used to determine the Von Mises stress of the different shapes of micro-needles (see Fig. 5). The force experienced by the micro-needle tip was varied iteratively until the fracture strength of the silicon material was exceeded.

Fig. 5 ANSYS measurements of the Von mises Stress. Base diameters are (a) 90μm and (b) 240μm.

The fracture force increases gradually with an increase in the micro-needle base diameters (see Fig. 4). In all cases, the buckling force is significantly lower than these values. Thus the micro-needles developed in this work are mainly limited by the needle tip rather than by the intrinsic strength of silicon. The matter is further complicated by the reduction in fracture toughness created by the DRIE etching process. The surface roughness is quite sensitive to the etching parameters. Future work will need to include an experimental setup suitable for obtaining the fracture force to verify the above results.

5. PRELIMINARY RESULT OF HOLLOW MICRO-NEEDLE

An example of a micro-needle with off-axis fluidic channel is shown in Fig 6. The base diameter is $240\mu\text{m}$ and needle length $150\mu\text{m}$. Optimization of the number of *through*-holes, as well as *through*-hole diameter, and displacement of *through*-hole from the micro-needle axis is presently in progress and will be reported elsewhere, along with FEA of this more complex geometry.

Fig. 6 Scanning electron micrograph of micro-needles with single through-hole, displaced off-axis by $10\mu\text{m}$.

6. SUMMARY

Silicon micro-needles, $150\mu\text{m}$ in length with base-diameters ranging from 90 to $240\mu\text{m}$ have been fabricated and analyzed in this work. A *novel*, multiple-sacrificial approach has been demonstrated as suited to the fabrication of long micro-needle bodies with positive profiles. The parameters that control the isotropic etching are adjusted to control the ratio of the needle-base diameter to needle length. By carefully controlling the geometry, the needle profile can be engineered to give a suitable tip size for penetration, as well as a broad needle base to facilitate the creation of either single or multiple-through holes. This approach allows the mechanical properties of the otherwise brittle needles to be optimized. Finite element analysis indicates that the micro-needles will fracture prematurely due to buckling, with forces ranging from 10 to 30mN . Future work will focus on the optimization of the diameter, placement and number of *through*-holes, as well as experimental qualification of the FEA results.

ACKNOWLEDGMENTS

The authors kindly acknowledge the financial support of ACRF grant number RG 23/05. The assistance of Ms Tan Hong Siang Sharon and Seet Pei Wen are also gratefully acknowledged.

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Spark plasma sintering of silver nanopowder

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ABSTRACT

The spark plasma sintering behaviour of silver nanopowder prepared by the electro-explosion method was investigated. Consolidation was carried out from 50°C to 800°C for 5 mins at 34 MPa with differential scanning calorimetry indicating a sintering onset temperature as low as 160°C and an activation energy of 86 ± 1 kJ/mol. Near full density resulted from treatment at 300°C, and at higher temperatures a normal Hall-Petch relation is obeyed. The enhancement of Vicker's hardness to 1000MPa for materials sintered at 300°C is three times greater than for silver annealed in a conventional way. While polysynthetic twinning contributes to superior hardness, the primary cause is the sub-micron grain size.

Keywords: nanocrystalline materials, sintering, silver, nanopowder, grain growth, hardness, twinning

1. INTRODUCTION

Micro-needles for transdermal drug delivery [1] are under development for a range of military and civilian uses. Although small in dimensions, they are sufficiently strong and long to deliver drugs across the stratum corneum (10-15 μm thick) of human skin. Due to the potential application of MEMS in developing miniature medical devices, most micro-needles are fabricated from silicon but this material is brittle. Metallic microneedles are prepared using electroplating technology which is limited to materials such as copper and nickel based alloys [2]. Silver is a suitable alternative due to its antimicrobial properties and biocompatibility [3]. However, it is relatively malleable and a significant limitation of the needles is poor strength and rigidity. Thus, strengthening mechanisms including grain refinement, solid solution alloying, introduction of a second reinforcing phase, or plastic straining can be applied. In addition, the introduction of crystallographically coherent twins that limit deformation [4, 5] can enhance mechanical properties.

Silver nanopowder is being extensively investigated owing to its superior electrical and thermal conductivity, stability against oxidation, and microbial properties. Certain research groups have been experimenting with silver nanoparticles rather than using flake powders or fibres as filler materials for polymer matrix composites (PMC) [6]. They serve to enhance the electrical conductivity of the polymer interface which could find application in battery electrodes, biosensors and microelectronic devices. In addition, metal substitutes for solder are being considered despite lead free alloys being widely used in the electronics packaging industry for interconnections [7]. Compared to silver, such solders have poorer conductivities and lower strength. Hence silver nanoparticles that sinter at $<300^\circ\text{C}$ are promising die attach materials for high temperature applications in wide bandgap semiconductors ($>350^\circ\text{C}$).

Aside from semiconductor applications, silver has in recent years been applied in the medical industry. Its present widespread use is boosted by the fact that new strains of bacteria resistant to current antibiotics are being discovered [8]. Antibacterial agents found in consumer products such as household cleaners, often contain triclosan to combat pathogens, but this ingredient contributes to the creation resistant bacteria strains that pose a public health risk. Thus by combining the well known anti-bacterial properties of silver with the technology for production of nanopowders, materials are created that catalytically interact inhibit the ability of bacteria to replicate. It is worth noting that silver has been incorporated with silica gel microspheres to produce plastics with lasting antibacterial properties [9]. In

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addition, nanocrystalline silver is an ingredient of a burn dressing developed by NUCRYST Pharmaceuticals to eliminate fungi and bacteria and reduce inflammation for up to seven days [10].

Although a significant number of reports have described the fabrication and use of microneedles, the commercial viability of such a medical device is dependent on a mass-scale manufacturing process not yet demonstrated. Therefore, alternative fabrication routes for silver powder metallurgical processing to replace classical cold pressing and sintering [11] and metal injection molding [12] are of significant interest. In spark plasma sintering (SPS), a high energy, low voltage pulsed current momentarily generates localized inter-particle temperatures, up to tens of thousands of degrees, that causes surface vaporization and melting. In this way, the compacted powder can sinter to high density at relatively low bulk temperatures in a shorter time (typically a few minutes) compared to conventional methods. At lower temperatures, solid state diffusion of atoms is quite slow and therefore lattice defects created in the wire exploded precursor are expected to be preserved in the dense product leading to hardening.

Nanopowders often show desirable properties compared to materials with larger grain sizes. A larger surface area leads to higher reactivity [13], and such particles can exhibit lower sintering temperatures with the possibility of attaining micro-miniaturization for medical devices. Nanopowder produced by the electro-explosion of wire (EEW) process generally has a metastable and crystalline microstructure. This complex crystallographic structure often retains high thermal energy enhanced by a high density of crystal defects, contrary to powder produced by evaporation and condensation methods.

This work focuses on the consolidation of silver powder derived from EEW by SPS. In combination, these techniques allow some measure of control over the nanostructure of the silver that enhances mechanical properties and makes the product ideal for medical applications.

2. EXPERIMENTAL PROCEDURES

Silver powders (100nm diameter) were prepared by the electro-explosion of Ag wire in an Ar atmosphere (99.999% purity) delivered by Argonide, Nanomaterials, USA and stored under N₂. Specimens were compacted in a glove box prior to thermal analysis and SPS.

Calorimetric measurements were carried out in argon to avoid oxidation, using a Perkin Elmer differential scanning calorimeter (DSC-7), operated at variable heating rates (1, 2, 5, 10, 15, 20, and 50 K/min). Temperature and heat flux were calibrated by melting a high purity indium standard (mp=156.6°C).

The nano-powder was processed in a Dr. Sinter[®] Spark Plasma Sintering System SPS 1050 shown in Fig. 1. Four grams of silver were loosely packed into a 13 mm diameter graphite die and punch unit lined with carbon paper and placed between the upper and lower electrodes. Sintering was carried out in a vacuum of ~4 Pa with the temperature measured using a thermocouple. During consolidation, a pulsed voltage (860 ms on-time, 140 ms off-time, 0-1750 amp, 0-5 volts) applied across the powder compacts with a uniaxial pressure of 34 MPa, yielded discs ~12.8 mm in diameter and 3 mm thick. Two sets of specimens were produced. The first were treated isochronally (5 minute hold time) at temperatures from 50°C - 800°C, while the second were isothermal experiments (SPS temperature of 300°C) for 1 - 20 mins. Five additional specimens were sintered at 700°C for 5 mins to establish specimen-to-specimen reproducibility.

Fig. 1 Schematic diagram of the spark plasma sintering system.

Specimen surfaces were polished back by 0.5 mm to remove carbon contamination before Vickers diamond indentation (Matsuzawa Seiki Co Ltd) was measured at loads of 50 g for 15 s (according to BS ISO 4498), with 20 repetitions. Density was obtained by the Archimedes method with 5 repetitions. The microstructures of the powder and sintered specimens were studied by scanning electron microscopy (SEM, Hitachi S5300N) and transmission electron microscopy (TEM, JEOL JEM-2010FX). SEM data were collected in the secondary electron imaging (SEI) mode at a voltage of 20 keV. Selected area electron diffraction (SAD) patterns, bright field images and high resolution imaging (HRTEM) were conducted at 200 keV. X-ray photoelectron spectra (XPS) were collected with a Kratos-Axis instrument using monochromatic Al K α (1486.71 eV) X-ray excitation. Survey spectra in the range 0-1100 eV were recorded in 1 eV steps, followed by high-resolution data of the Ag 3d^{5/2} and C 1s spectral lines collected in 0.1 eV steps, from which the surface composition was calculated.

3. RESULTS AND DISCUSSION

3.1 Morphology of as-received Ag nanopowder

The silver nanopowder is composed of ~100 nm euhrdal (clearly-faceted) and anhedral crystals (Fig. 2). Banded contrast of some crystals arises from polysynthetic twinning. Inter-crystal welding creates a continuous network, as distinct from other metals and alloys prepared by wire-explosion, where the particles are invariably spherical without intergrain sintering [14, 15]. Secondary electron imaging of the precursor powder revealed large silver aggregates ranging from a few nanometers to tens of microns. Turker [16] report that the presence of oxygen during powder production results in the formation of separated spherical shape particles. Since argon is the carrier gas during EEW, it is postulated that contamination by surface oxides is minimised which promotes necking between the irregularly shaped particles.

Fig. 2 A bright field TEM image of Ag nanopowder with anhedral and euhrdal crystals welded in a continuous network.

Fig. 3 (a) Plot of heat against temperature at variable heating rates (b) Application of Kissinger's equation. The symbols ● and ○ correspond to T_p and T_o respectively.

3.2 Calorimetric Measurements of Ag Nanopowder

DSC revealed an exothermic peak in the range 120-175°C (Fig. 3) similar to earlier reports [8,9]. This feature is believed to be associated with surface sintering of the nanoparticles and promotes abnormal grain growth, as it is not present if the powder was pre-annealed at 150°C for 30 mins. Accelerated heating displaces the exothermic transition towards higher temperatures in a process that according to Kissinger [10] can be described by the function:

$$\ln\left(\frac{T^2}{b}\right) = \frac{E_c}{RT} + C$$

where b is the heating rate (K/min), R is the gas constant (8.3143 JK⁻¹mol⁻¹), E_c the activation energy, and C is a constant. An estimate of E_c can be made by plotting $\ln(T^2/b)$ against $1/RT$ (Fig. 4). Temperatures T_o and T_p were measured corresponding to the *onset* and *peak* temperatures. Activation energies for T_o and T_p of 86±1 kJ/mol and 85±1 kJ/mol respectively are comparable to the values of 85-90 kJ/mol reported for grain boundary self-diffusion [8]. Moon [17] had reported an exothermic peak at 100°C due to moisture adsorbed on the particle surface and a weight loss of 1.75% was observed from thermogravimetric analysis. For the EEW silver nanopowder, the weight loss due to moisture was measured to be 0.07% and would make an insignificant contribution to the exothermic peaks at 100°C.

3.3 Microstructure of sintered Ag compacts

Stacking faults, dislocations, and twins were observed in both the precursor powder and SPS specimens. In particular, [101] twinning between larger 2C silver crystals was common, and displayed a characteristic pairing of $\{111\}_c \equiv \{002\}_{\text{hex}}$ reflections. This is not unexpected as the twin $\{111\}_c \equiv \{002\}_{\text{hex}}$ composition plane coincides to the polytypic intergrowth plane in Fig 4 (a). As shown in Fig. 4 (b), selected area electron diffraction (SAD) patterns were found to contain additional spots and diffuse intensity indicative of unit-cell scale disorder. While common in the precursor powder, this became increasingly rare at higher sintering temperatures.

To observe crystal size in the sintered particles, images were collected by two methods. Method 1 involved TEM on ion-milled samples and method 2 using SEI of surfaces prepared by fracturing under liquid nitrogen. In either case, a statistical analysis was made from at least twenty images as shown in Fig. 5. When measurements were made by both SEM and TEM approaches on the same specimen, grain size measurements were found to be in agreement.

Fig. 4 (a) A high resolution image of polysynthetically twinned silver. The twin planes, evident through the appearance of mirror planes in the parent cubic structure are indicated by arrows. A Fourier transform of this region yields a power transform (inset) whose intensity distribution reflects the twin relation observed in macroporous twins. This is expected as the composition plane $\{111\}_{\text{cu}} \equiv \{001\}_{\text{hex}}$. (b) The selected area diffraction (SAD) pattern show macroscopic twinning of cubic silver leading to pairs of $\{020\}$ and $\{111\}$ reflections that can be separated as arising from twin individuals.

Fig. 5 (a) Representative SEI of a fracture specimen. (b) Typical grain size distribution of a specimen (sintered at 800°C).

Fig. 6 Grain size as a function of variable (a) SPS temperature and (b) hold time. Symbols $\square$ and $\bullet$ correspond to TEM and SEM observations. Error bars correspond to the standard deviation of each grain size distribution.

Fig. 7 Relative density as a function of variable (a) SPS temperature and (b) hold time. The error bars are calculated using multiple measurements made at different points on the same specimen.

Crystal growth is favoured for samples sintered at higher SPS temperatures as illustrated in Fig. 6 (a). Above 300°C, grain growth approaches a mean value of 1.2 μm at 800°C. As anticipated, Fig. 6 (b) also showed that isothermal measurements made at 300°C indicate a small increase in grain size with time. Although rapid grain growth has been reported in other studies of silver nano-powder [17, 18], though not as obviously observed as in this work, the effect could be due to the aggregation of the nano-powder before sintering [19, 20].

3.4 Mechanical Properties of sintered Ag compacts

Fig. 7 (a) gives the variation in relative density (RD) against the SPS temperature. Isochronal measurements show that the relative density increases to a maximum at 300°C, and above this temperature, remains constant. Below 200°C, RD is dominated by the porosity of the nano-silver powder compact. Above 200°C, there is rapid closure of porosity and crystal growth [11, 17]. Fig. 7 (b) indicates isochronal measurements made at 300°C. As expected, the RD remains constant at 0.98 regardless of the holding time; a phenomena commonly observed in agglomerated nano-powders for which the application of modest compressive loads is insufficient to remove large pores [21].

Fig. 8 (a) shows the Vickers hardness (HV) is small at SPS temperatures <200°C since there is little compaction, minimal reduction in porosity, and the particles are not bonded together effectively. Above 200°C, the hardness rises rapidly to 1,000MPa (an enhancement of approximately three times compared to the hardness of silver according to ASTM Standard B413-89), peaking at 300°C, and then slowly decreases with increasing temperature. The hardness of the specimen SPS at 800°C is seen to be a little lower than expected due to partial melting. Fig 8 (b) also shows isothermal measurements at 300°C indicating a small decrease in hardness with time due to the continued grain growth.

Fig. 8 Vickers Hardness as a function of variable (a) SPS temperature and (b) hold time. The error bars are calculated using multiple measurements made at different points on the same specimen.

Fig. 9 Hall-Petch relation applied to SPS temperatures above 300°C for which porosity changes are not a factor.

Depth profiling using XPS showed that solid solution strengthening, due to the incorporation of carbon to be absent in the bulk for SPS temperatures below 800 °C. Therefore the possibility of chemical impurities promoting twinning can be discounted. XPS analysis of the anomalous specimen produced at 800 °C indicated the presence of 2.5 at% C. This is believed to arise due to partial melting of the specimen. In fact, the reduced hardness and density of this specimen can be accounted for by presence of amorphous carbon at the grain boundaries [22].

Hardness, being a microstructure sensitive property, depends on many factors including impurities, porosity, and defects. A model linking hardness to grain size G and fractional sintered density ρ_s can be described as follows [23]:

$$HV = \rho_s \left(H_0 + \frac{\Theta}{\sqrt{G}} \right)$$

where H_0 and Θ are constants and G is given in units of μm . For temperatures greater than 300°C, the change in porosity is negligible as depicted in Fig.7a; thus a normal Hall-Petch relation can be inferred.

Fig. 9 gives a plot of hardness against $\sqrt{G}$ for SPS temperatures >300°C. A best fit gives a Hall-Petch constant of 21.3 GPa.(nm)^{1/2}, as compared to a value of 4.2 reported by Qin [11]. Their specimens were synthesized by the cold compaction of silver nano-particles prepared by inert gas condensation. In fact, an interpretation of the hardness solely in terms of grain growth might be insufficient due to the dislocation pinning provided by twins. A modified form of the Hall-Petch expression that takes into account twin interfaces has been suggested in recent studies on silver [24].

The addition of small alloying agents to silver combined with SPS may provide a very precise way to control polysynthetic twin density and therefore hardness. In this way, stability towards higher temperatures could be achieved. For instance, the mineral allargentum ($\text{Ag}_{0.88}\text{Sb}_{0.14}$) is a hexagonal structure with a Vickers hardness of 1,890MPa [25]. In other words, an order of magnitude difference in hardness is achievable by the addition of tiny amounts of Sb.

CONCLUSION

Partial sintering of Ag nanopowder can be concluded to have already occurred at room temperature due to signs of necking from the microscopy images. Thermal analysis of the powder by DSC at variable heating-rates gave an activation energy of 86 ± 1 kJ/mol, consistent with grain boundary self-diffusion. Silver nano-powder prepared by the wire explosion method has a high density of twins that provides a potential strengthening mechanism.

Silver nanopowder produced by the electro-explosion of wire process was used to obtain compacts by spark plasma sintering from 50°C to 800°C. For SPS conducted at >300°C, 98% full density is reached and the relationship between sample hardness and grain size can be described by a normal Hall-Petch Relation. Indentation results show a maximum peak hardness of 1,000 MPa for specimens SPS at a

temperature of 300°C which is an enhancement of approximately three times compared to annealed silver. The observed enhancement to the hardness is believed to be mainly due to grain refinement.

ACKNOWLEDGEMENTS

The authors would like to acknowledge contributions from Mr Leau Ming Tong Norman and Mr Aw Song Sen as well as ACRF funding (grant number RG 23/05).

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