Pain, sensation and biological responses following human skin puncture with microneedles

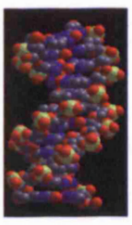
Mohammed Inaam-ul Haq

A thesis submitted to Cardiff University in accordance with the requirements for the degree of

DOCTOR OF PHILOSOPHY

Gene Delivery Research Group
Department of Drug Delivery
Welsh School of Pharmacy
Cardiff University

March 2011



ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

NOTICE OF SUBMISSION OF THESIS FORM: POSTGRADUATE RESEARCH

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

DECLARATION

This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

Signed ..... (candidate) Date....23/08/2011

STATEMENT 1

This thesis is being submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (PhD).

Signed ..... (candidate) Date ..23/08/2011.

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated.
Other sources are acknowledged by explicit references.

Signed ..... (candidate) Date ..23/08/2011.

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed ..... (candidate) Date ...23/08/2011.

ACKNOWLEDGEMENTS

In the name of God the most Beneficient the most Merciful.

It gives me great honour to have been supervised by Dr. James Birchall and Dr. Dai John, both of whom have been nothing but pillars of support and advice during my PhD. James has, especially, has been aware of my needs whilst entrusting me with laboratory and department responsibilities.

I must also thank our collaborators, Dr. Alex Anstey, Mr. Christopher Gateley and Dr. Manju Kalavalaat the Royal Gwent Hospital; Dr. Anthony Morrissey, Tyndall National Institute, for the supplying microneedles; Dr. Rebecca Porter for her assistance during wound healing analysis, and Miss. Emma Smith who dedicated hours assisting with the trial. I would also like to thank the staff at the School of Optometry for their assistance with the cryostat and Dr. Antoine Hann for his assistance with the scanning electron microscope.

I would like to thank the British Skin Foundation for their financial support of the clinical trial, and Cardiff University for my PhD studentship.

I must also thank numerous people in the department, not least Keng, Marc, Bal, Barbara and Chris for their friendship, as well as Prof Baxter's group for saving my desk space.

Finally, lifelong gratitude will be insufficient for the inspiration from my Father, may he rest in peace, and support from my Mother, twin sister and her family for supporting me always!

ABBREVIATIONS

25G	25 gauge hypodermic needle
180MN	180μm length microneedles
280MN	280μm length microneedles
AIDS	Acquired immune deficiency syndrome
Botox	Botulinum toxin type A
CRF	Case report forms
CTA	Clinical trial authorisation
CD68	Cluster of differentiation 68
DAB	DAB chromogen
DMEM	Dulbecco's modified eagle medium
EGF	Epidermal growth factor
EOPI	Evaluative overall pain index
GCP	Good clinical practice
H & E	Haematoxylin and eosin
HIV	Human immunodeficiency virus
IHC	Immunohistochemistry
IL-1β	Interleukin 1 beta
K14	Keratin 14
K16	Keratin 16
K6	Keratin 6
MMPs	Matrix metalloproteases
MPQ	McGill pain questionnaire
MHRA	Medicines and Healthcare products Regulatory Agency
mRNA	Messenger ribonucleic acid
MEMS	Microelectromechanical systems
MN	Microneedles
OCT	Optical coherence tomography
OCT	Optimal cutting tissue compound embedding media
PBS	Phosphate buffered saline
pDNA	Plasmid deoxyribonucleic acid
PDGF	Platelet derived growth factor
PCL	Polycaprolactone

PDMS	Polydimethylsiloxane
PCR	Polymerase chain reaction
KOH	Potassium hydroxide
SEM	Scanning electron microscopy
MPQ-SF	Short-form McGill pain questionnaire
SEWREC	South East Wales Research Ethics Committee
SD	Standard deviation
SC	Stratum corneum
SG	Stratum germinativum
SGR	Stratum granulosum
SL	Stratum lucidum
CDC	The Centers for Disease Control
TEWL	Transepidermal water loss
TGF	Transforming growth factor
TNF-α	Tumour necrosis factor alpha
TNF	Tumour necrosis factors
UV	Ultraviolet
VAS	Visual analogue scale
WSP	Welsh School of Pharmacy
WHO	World Health Organisation

SUMMARY

Injections using hypodermic needles cause pain, discomfort, localised trauma and apprehension. As an alternative, microneedles facilitate drug delivery without significantly impacting on pain receptors or blood vessels that reside within the dermis. In this study we aimed to investigate, for the first time, whether two silicon microneedle arrays (36 equally spaced 180μm or 280μm length microneedles) elicit pain and sensory response when applied to human volunteers. In addition to *in-vivo* clinical testing of silicon microneedles, alternative polymer microneedle designs were characterised and tested *ex-vivo*.

Prior to applying silicon microneedles clinically, ethically approved testing of applicator devices determined that inverted-syringe plungers caused minimum discomfort when applied to human volunteers. Microneedle arrays mounted onto inverted-syringe plungers reproducibly created microconduits through the stratum corneum of *ex-vivo* skin. Following ethical approval, 12 subjects received single-blinded insertions of a 25G hypodermic needle and both microneedle arrays. A visual analogue scale (VAS), perception questionnaire and audio-recording collected descriptions of the pain intensity and sensory perception following each application. The creation and temporal retention of skin microchannels was assessed over 24 hours by external dye staining and measurement of transepidermal water loss (TEWL). Characterisation of wound healing markers, including keratin K16, was carried out by immunohistochemistry.

Mean VAS scores, verbal descriptions and questionnaire responses showed that the 180μm and 280μm silicon microneedles caused significantly less pain and discomfort than the hypodermic needle. Dye staining and TEWL analysis confirmed that microchannels were formed in the skin following microneedle application with repair and resealing apparent at 8-24 hours post-application. A spring-loaded applicator device was developed to reproducibly accelerate polymer microneedles into the skin along a trajectory perpendicular to the skin surface.

Microneedles provide a less discomforting method of skin penetration than hypodermic needles. Future work should optimise the design of microneedle devices for clinical delivery of active molecules.

CONTENTS

LIST OF FIGURES

LISTS OF TABLES

Chapter 1

General Introduction

1.1 Introduction

Hypodermic needles have been used for the therapeutic delivery of medicaments and drugs since Francis Rynd invented the hollow needle in 1845 (Schorr 1966). The delivery method was further developed over the 19th century and became commonly used in clinical practice. A child in the UK will receive at least 15 injections from birth to adulthood in an effort to vaccinate against infectious disease (NHS 2008). However, hypodermic needle use is not exclusive to vaccinations and their use is set to increase further with the rising prevalence of diabetes (Katz et al. 2009; Knip 2008; Turner et al. 2009), the emergence of biotechnology derived medications that are not orally active (Jin et al. 2008; Nandedkar 2009) and for use in the delivery of cosmetic agents, such as botulinum toxin type A (Botox) (Carruthers and Carruthers 2007; Frankel and Markarian 2007; Sepehr et al. 2010). Whilst the use of hypodermic needles is clearly commonplace in healthcare, their use poses a variety of issues such as difficult storage and disposal, possibility of cross-contamination, intentional or accidental reuse and patient phobia to needle injection (Hogan 2006; Kleinknecht 1994; Nir et al. 2003; Pandit and Choudhary 2008).

The main focus of this study was to determine, in a pilot clinical study, the pain and sensations caused by the use of a hypodermic needle and compare those responses against a less invasive alternative to conventional injection (microneedles). The study will explore the wound healing responses to skin injury caused by these devices and learn more about appropriate application techniques for microneedles in human skin. This introductory chapter considers the structure of human skin, the major methods of facilitating transdermal drug

delivery (including the use of microneedles), the measurement of pain and changes to skin barrier function and processes of wound healing.

1.1.1 The anatomy of the skin

The skin is considered the largest organ of the body and forms a multifaceted barrier between the internal and external environments of the body. The skin varies in thickness, sensitivity and metabolic activity depending on its location, age, and exposure to its environment (Fenske and Lober 1986; Levi et al. 2009; Mancini 2004). Skin provides physical protection from harmful ultraviolet (UV) rays and regulates body temperature by altering blood flow and sweat excretion (Stocks et al. 2004; Tadokoro et al. 2005). There are two main areas of skin. The non-hairy smooth (or glabrous) skin, such as found on the soles of the feet and palms of the hands, is characterised by thicker epidermis and a dermatoglyphic configuration of troughs and ridges (El Gammal et al. 1999; Moore and Munger 1989). The other skin areas consist of both follicular ducts and sebaceous glands. The surface hairs provide insulation whilst intra-epidermal, intradermal and subcutaneous immunological defences react to pathogens (Proksch et al. 2008). In all types of skin, the presence of nerves enables individuals to feel sensations of touch, heat and cold, and pain. This enables the body to respond to the external environment through regulating the internal environment, for example, vasodilatation of blood vessels under skin in times of heat or vasoconstriction when cold, or the regulation of hair follicles and sweat glands (Stocks et al. 2004).

All healthy skin can be divided into two main layers, the epidermis and dermis, each with a distinct role in the function of the skin (Goldsmith 1991; Montagna and Parakkal 1974). The dermis is further attached to the underlying hypodermis or panniculus adiposus, which comprises mostly of adipose tissue

for fat storage (Figure 1.1) and is separated from the striated muscle by the panniculus carnosus (Goldsmith 1991; Montagna and Parakkal 1974).

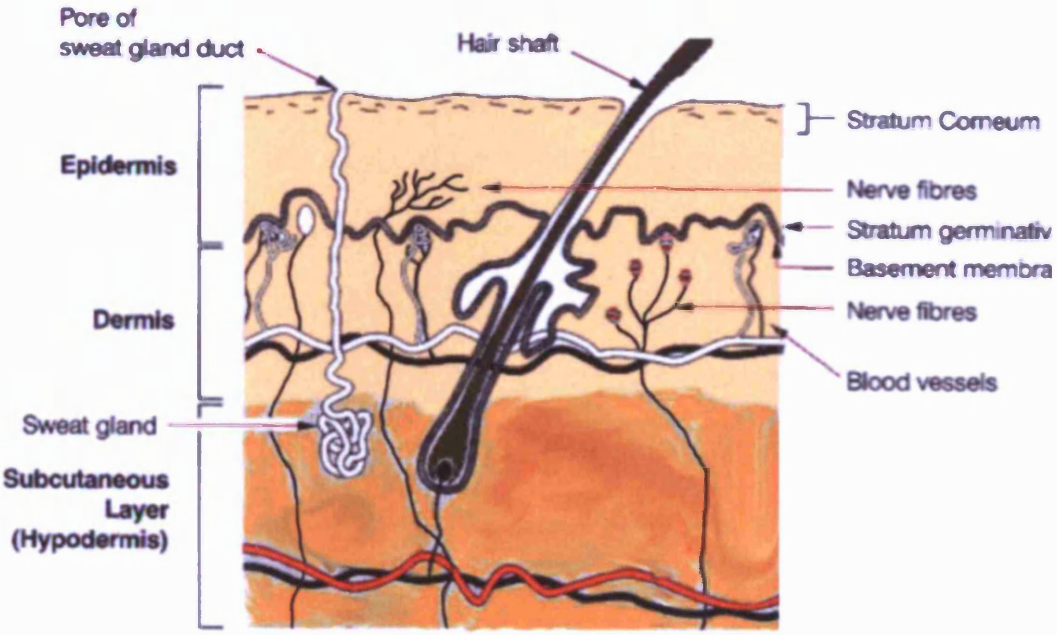


Figure 1.1. Schematic showing the various layers of the skin.

1.1.1.1 The viable epidermis

The viable epidermis is approximately 50 to 100μm thick and forms the primary barrier of the skin. This layer is histologically sub-divided into 5 strata (layers): starting from the outermost surface with the stratum corneum (SC), stratum lucidum (SL), stratum granulosum (SGR), stratum spinosum, and stratum germinativum (SG), which is separated from the dermis by a thin basement membrane (Ko and Marinkovich 2010; Montagna et al. 1992; Rook and Burns 2004; Schellander and Headington 1974). The cells in the SG undergo mitotic division to provide cells which constantly regenerate the epidermis (Montagna et al. 1992; Rook and Burns 2004). Subsequently cells from the SG progressively mature and keratinise as they migrate through the strata (Rook and Burns 2004). Keratinocytes slowly migrate through the strata whilst

undergoing histological changes. For example, in the SGR, keratinocytes accumulate dense basophilic keratohyalin granules which contain lipids (Gray and Williams 1974) .

The SG also hosts the majority of melanocytes, which are the pigment-producing cells in the skin (Blair 1968; Sulaimon and Kitchell 2003). Melanocytes contain melanin pigment throughout their dendritic structure (Busam et al. 2001; Lin and Fisher 2007; Sulaimon and Kitchell 2003). These reside in the SG in an approximate ratio of 1 melanocyte to 10 keratinocytes (Baxter et al. 2009; Lin and Fisher 2007). Another important cell population of the epidermis are the Langerhans cells, which as part of the immune system, act as a primary defence against pathogens, regulating reactions to foreign substances (Elias 2005; Harding 2004; Rook and Burns 2004).

Epidermal thickness varies depending on age, the regional function, exposure and frictional forces on the surface (Batisse et al. 2002; Giangreco et al. 2008; Holbrook and Odland 1974; Montagna and Carlisle 1979). For example, the eyelids have the thinnest epidermis, on average about 0.05mm, whilst the palms of the hands and soles of the feet have the thickest on average 1.5mm (Dutton et al. 2007). As keratinocytes migrate towards to the SC, they are filled with keratin filaments aligned into disulphide cross-linked fibres (Fuchs 2007; Jensen and Proksch 2009; Proksch et al. 2008). These keratinocytes lose their nuclei and cytoplasmic organelles to form corneocytes. At the SC the desmosomal junctions degrade and corneocytes are bound together by intracellular lipids.

The SC is an average of 10-20μm thick across the main torso and limbs of an adult, consisting of 10 to 20 layers of flattened, closely packed corneocytes (Jensen and Proksch 2009; Nouveau-Richard et al. 2004; Proksch et al. 2008; Welzel et al. 2004). In healthy skin, keratinocytes proliferate and migrate through the layers of the epidermis in homeostatic balance to the constantly shedding SC (Brysk and Rajaraman 1992). Corneocytes break away from the SC by desquamination when the intracellular lipids are degraded by mechanical or biochemical insult from the environment (Brysk and Rajaraman 1992; Madison 2003; Pierard et al. 2000). The usual life cycle of skin cells is approximately 28 days, thereby enabling efficient healing and repair of the skin (Blanpain and Fuchs 2006; Madison 2003).

1.1.1.2 The dermis

The dermis is separated from the epidermis by the continuous basement membrane which is usually one cell thick (Gruson and Cohen 2004; Ko and Marinkovich 2010; Uitto and Pulkkinen 1996). The dermis is approximately 2-3mm thick and maintains thermoregulation of the body and vascular supply for nutrient exchange to the skin tissue (Briggaman 1982; Briggaman and Wheeler 1968). The dermis can be subdivided into two areas, the papillary dermis and the reticular layer. The dermis forms from mesenchymal cells that differentiate into vasculature and connective tissue cells (Fuchs and Horsley 2008; Sellheyer and Krahl 2009; Smith and Holbrook 1982), whilst, fibroblasts in the dermis secrete elastins and collagens to assist in providing elasticity of the skin (Fuchs 2007; Fuchs 2008; Rook and Burns 2004).

The dermis also contains populations of immune cells. The dermal dentritic cells (DDCs), act as antigen-presenting cells and represent a population of

immunoresponsive cells that recognise and process foreign antigens (Brand et al. 1992; Cerio et al. 1989; Meunier et al. 1995; Zaba et al. 2007). DDCs can associate to T lymphocytes and migrate through the dermis to the lymph vessels where they are transported to the lymph nodes to generate a whole-body response to an antigen. Other immune cells, such as macrophages, act by engulfing foreign cells by phagocytosis. Therefore macrophages prevent foreign cells invading through the dermis (Akimoto et al. 1998; Cameron et al. 2002; Groneberg et al. 2005; Gruschwitz et al. 1991; Gupta et al. 1989; McLellan et al. 1998).

The papillary dermis contains blood vessels and provides the avascular epidermis with nutrients, whilst also functioning as a major thermoregulatory system of the body (Abdel-Naser et al. 2007; Branzan et al. 2007; Braverman 1983). The papillary dermis also contains free nerve endings and Meissner's corpuscles, which are highly sensitive areas for sensory recognition (Holbrook et al. 1982; Holbrook and Odland 1975). The reticular layer consists of dense connective tissues to increase skin strength and elasticity whilst protecting the epithelial derived structures such as hair follicles, sweat and sebaceous glands (Caspers et al. 2003; Hristakieva et al. 2000; Hwang and Baik 1997; Lazarova et al. 2000; Lindberger et al. 1989).

1.1.2 Wound healing in the skin

The various strata of skin provide humans with a protective barrier that can systematically respond to trauma. Generally wound healing proceeds, after trauma or microbial insult, via imbrications of inflammation, epithelialisation, granulation, and matrix and tissue remodelling (Epstein et al. 1999; Midwood et al. 2004; Werner and Grose 2003). These processes are mediated on a molecular level with interactions from interstitial and intracellular cytokines

being released through keratinocyte damage (Barrientos et al. 2008; Li et al. 2007). These cytokines initiate and orchestrate the inflammation and healing responses in the wounded skin (Gillitzer and Goebeler 2001; Hackam and Ford 2002; Pradhan et al. 2009).

Response to cutaneous injury has been modelled in the skin of patients suffering from chronic disease related wounds (Usui et al. 2008) or wounds caused by injury (Evers et al. 2010; Merz et al. 2010; Miller and Nanchahal 2005). Such wounds tend to cause damage to multiple blood capillaries from which the coagulation factors and platelets are released as a rapid-fire response to prevent blood loss (Evers et al. 2010; Meijer-Jorna et al. 2002; Schiech 2002). When intradermal lesions damage blood vessels, the aggregation and degranulation of platelets within fibrin clots induces the plethora of cytokines and growth factors to be released from surrounding skin cells (Epstein et al. 1999; Falanga 2005; Servold 1991). It is these factors, for example cytokines from the transforming growth factor (TGF) and tumour necrosis factors (TNF) super-families, and platelet derived growth factor (PDGF) that initiate and influence inflammation (Schmid et al. 1993; Yonei et al. 2007). However, for minor insults to the skin, the response is expected to be less as fewer or no blood vessels are damaged (Diegelmann and Evans 2004; Li et al. 2007). Therefore, when compared to larger wounds, minor wounds initiate smaller quantities of cytokines to be released from damaged cells causing reduced inflammation, proliferation and healing (Diegelmann and Evans 2004; Li et al. 2007).

1.1.2.1 Inflammatory response during wound healing

Acute cutaneous injury incites a combination of different cytokine signalling pathways which progresses skin healing causing inflammatory responses;

therefore, several cytokine pathways have been researched to understand their roles in wound healing (Barrientos et al. 2008; Gillitzer and Goebeler 2001). Fibroblasts are pivotal in wound healing as they undergo various phenotypic transitions to become myofibroblastic, thus contracting skin tissue to close and form scars (Sorrell et al. 2007, 2008; Werner et al. 2007).

Fibroblasts express TGF-β1, which is a 112 amino acid polypeptide member of the TGF family of cytokines (Lawrence 1996). TGF-β1 is released by degranulating platelets and is activated by proteolytic and non-proteolytic mechanisms and influences the formation of clots, deposition of matrix and remodelling of the tissue (Gabrielli et al. 1993; Martinez-Ferrer et al. 2009; Myers et al. 2007). TGF-β1 induces the release of additional cytokines including tumour necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β). These cytokines drive inflammation, whilst also mediating the maturation and activation of dendritic monocytes and macrophages (Abe et al. 2000; Amento and Beck 1991; Amjad et al. 2007). TGF-β1 has been investigated thoroughly and appears to instrumental in all types of wound healing. However other cytokines such as TNF-α are released from multiple cellular sources (Arnett et al. 2001; Grimstad et al. 2011). TNF-α is a 157 amino acid length protein of 17kDa which drives transcription of inflammatory proteins (Barrientos et al. 2008; LaDuca and Gaspari 2001).

1.1.2.2 Secondary responses during wound healing

Monocyte populations increase as neutrophil recruitment peaks at around 24 to 48 hours post-wounding (Kim et al. 2008; Weber-Matthiesen and Sterry 1990). Wounding causes activation of monocytes to release chemokines, which recruit more monocytes leading to amplified release of cytokines (Barrientos et al. 2008; Gillitzer and Goebeler 2001; Werner and Grose 2003). Antigen

stimulation drives lymphocytic recruitment and activation. In conjunction with neutrophil, monocytes, macrophage and lymphocytes, mast cells also propagate at the site of injury (Bulat et al. 2009; Groneberg et al. 2005; Jarvikallio et al. 2003). Macrophage assist in clearing cell debris and foreign agents, whilst re-epithelialisation restores functional integrity of the skin (Raja et al. 2007; Rodero and Khosrotehrani 2010; Spiekstra et al. 2007).

1.1.2.3 Re-epithelialisation of the skin during wound healing

Re-epithelialisation is an important stage for optimal wound healing to ensure wound contraction and reformation of the cutaneous barrier (Steffensen et al. 2001). Chemokines released by keratinocytes instigate the dissociation of matrix metalloproteases (MMPs) from the basement membrane and the migration of keratinocytes across the wound bed (Strodtbeck 2001).

As keratinocytes proliferate from the basal, stratum germinativum cell layer, they release various growth factors including TGF-β1, EGF, IL-1β and TNF-α (Barrientos et al. 2008; Choi and Fuchs 1990; Suter et al. 2009). Upregulation of TNF-α and IL-1β are also mediated by macrophages in a paracrine fashion and influences fibroblast development (Epstein et al. 1999; Rodero and Khosrotehrani 2010; Strodtbeck 2001).

Re-epithelisation begins 8 to 12 hours after the injury (Li et al. 2005; Raja et al. 2007; Spiekstra et al. 2007). Keratinocytes detach from the underlying basal lamina and progenitor cells proliferate and migrate into the clot matrix (Garlick and Taichman 1994b; Martin and Leibovich 2005). Keratinocytes at the wound edges react to chemical signals, such as, keratinocyte growth factor, TGF-α, TGF-β1 and EGF, which are produced by the clot, inflammatory cells, and neighbouring keratinocytes and fibroblasts (Garlick and Taichman 1994b;

Martin 1997; Werner and Grose 2003). As a result, keratinocytes begin to produce new integrin and keratin subtypes, which reorganise the actin cytoskeleton and produce MMPs, which dissolve the matrix to make space for the migrating cells (Larjava et al. 1993; Martinez-Ferrer et al. 2009). Subsequently, as keratinocytes cover the wound surface and stop migrating, the basal lamina and epidermis structures are re-established (Ahn et al. 2009; Bae et al. 2002; Chen et al. 1993; Martin 1997). Nedlec and Ghahary et al. (2000) discussed how at 3 or 4 days post-wounding, the dermal fibroblasts adjacent to the lesion begin to proliferate causing underlying connective tissues to contract, closing the wound during re-epithelisation.

1.1.3 Delivering medicaments through the skin barrier

Transcutaneous drug delivery (TDD) can be defined as the delivery of drugs through the skin into the systemic circulation. TDD differs from topical delivery as molecules permeate through the skin for systemic affect (Naik et al. 2000). TDD may provide several advantages over the oral delivery route for certain molecules in both safety and efficacy. For example TDD avoids the risk of first pass elimination by the liver (Chien 1984). Furthermore, TDD offers increased control, whereby removal of the TDD device allows rapid termination of treatment to prevent overdosing (Karande et al. 2005; Panchagnula et al. 2005; Varvel et al. 1989; Yang and Zahn 2004). Disadvantages of this approach when compared with intravenous administration may arise due to slower therapeutic effect or reduced bioavailability as the molecule has to diffuse through hydrophilic and lipophilic domains for systemic effect (Barry 1999; Knepp et al. 1987). TDD patch-based methods are used for various drugs including nicotine for smoking cessation (McNeil et al. 2010; Schnoll et al. 2009; Shiffman et al. 2008), oestradiol for hormone replacement, testosterone for hypogonadism

(Busse and Maibach 2011; Mazer et al. 2005; Rutledge et al. 2006) and nitrates for angina and ischemic chest pain (Abrams 1984; Berner and John 1994; Todd et al. 1990).

The rate of transcutaneous transport follows Fick's law, such that molecules passively diffuse through the skin across a concentration gradient (Hadgraft 2001b; Hadgraft and Valenta 2000; Knepp et al. 1987). Therefore, transport of molecules through the skin depends on drug concentrations, the thickness of the SC and the diffusion constants of the molecule in the skin. If the molecule is applied to a greater surface area of skin, the amount of molecule permeating the skin will increase, whilst thicker skin will reduce the permeation of the molecule through the skin (Ansari et al. 2006; Henning et al. 2008; Sloan et al. 2006). Furthermore, owing to the free fatty acids composing the SC, the skin has a pH of 4.5-6 (Hadgraft and Valenta 2000). This weak acidic environment could change the degree of ionisation of a molecule, as inonised molecules do not permeate the SC as rapidly as nonionised molecules. In particular weak bases such as fentanyl which ionise more as the pH decreases (Grond et al. 2000; Guy and Hadgraft 2003; Hadgraft and Valenta 2000)

However TDDs can employ topical preparations containing permeation enhancers to improve systemic drug delivery through the skin (Hadgraft and Lane 2005). For example, gels or solutions can contain alcohol, which can increase permeation of hydrophilic molecules by reducing the hydration of the SC (Sugibayashi et al. 1992); whilst ointments are occlusive and hydrate the skin (Gabbanini et al. 2009; Sloan et al. 2006). Water hydrates the skin and disrupts the lipid structure of the SC, thereby improving the solubility of hydrophilic molecules (Alonso et al. 1996; Bjorklund et al. 2010; Michaels et al.

1975). Once a molecule diffuses through the SC and permeates the dermis, it is absorbed by the capillary plexus and transferred into the system circulation, though some molecules may pass through the dermis into underlying subcutaneous tissue (Bos and Meinardi 2000; Busse and Maibach 2011; Knepp et al. 1987; Varvel et al. 1989).

The skin surface presents a formidable barrier consisting on average of 10-15 layers of corneocytes (Anderson and Cassidy 1973; Holbrook and Odland 1974). The skin is often described as a 'brick and mortar' structure, whereby the keratinocytes are connected by desmosomes and comprise the 'bricks' which are stacked in a 'mortar' of intercellular lipid matrix composed of free fatty acids, long chain ceramides and cholesterols (Guy et al. 1987; Hadgraft 2001a; Heisig et al. 1996). The corneocytes form polygonal flat structures from 0.2-1.5μm thick, 34-46μm in diameter (Anderson and Cassidy 1973; Mathur et al. 2010; Scheuplein 1967). The intercellular matrix is formed by lamellar content released from keratinocytes as they migrate from the middle to upper SG (Brody 1989; Elias et al. 1977). The lamellae associate into lipid bilayers, thus forming a lipid rich out skin surface (Madison 2003; Pierard et al. 2000; Van Hal et al. 1996).

Transport through skin can potentially occur via three main routes: transappendageal, transcellular and paracellular. The percutaneous diffusion of therapeutic moieties through the skin is considerably limited by the lipid rich SC as well other factors, such as the release of sebum and sweat, skin pH, fluidic pressures and the small cross-section of sweat ducts (Barbero and Frasch 2005; Degim 2006; Lademann et al. 2005; Moser et al. 2001). Early transdermal studies investigated transport through the skin's appendages

(sweat ducts and hair follicles). However, skin appendages comprise less than 0.1% of the skin surface area, thus the transappendageal pathway has been determined to have minimal contribution to the steady state flux of most molecules (Bronaugh and Maibach 1999, 2002; Johnson et al. 1997; Schaefer and Lademann 2001).

Alternatively, molecules can undergo transcellular transport, whereby molecules enter the cytoplasm thought the cellular membrane, travel though the cytoplasm and cellular membrane of neighbouring cells (Barbero and Frasch 2006). Therefore, molecules would have to diffuse through lipophilic and hydrophilic phases as they transition the cell membrane and cytoplasm of keratinocytes. Furthermore, molecules would need to transfer to adjacent keratinocytes through intercellular spaces consisting of lipid lamellae (Bonte et al. 1997; Corcuff et al. 2001; Elias et al. 1977; Potts and Francoeur 1991).. Additionally, transcellular transport through keratinocytes in the epidermis requires molecules to be stable to avoid degradation by intracellular enzymes such as the cytochrome P-450 family and reductases, or be metabolised as they pass through multiple cells (Devine et al. 2009; Gibaldi et al. 2007; Khan et al. 1989; Vyas et al. 2006).

Paracellular transport, whereby small molecules administered topically may passively diffuse through the tight junctions in between skin cells for systemic effect (Hadgraft 2001b). Paracellular transport requires molecules to pass through virtually impermeable intercellular apical tight junctions. Apical regions contain claudins and occludins, which are proteins that associate with other proteins in the intracellular side of the cellular membranes of neighbouring cells (Kirschner et al. 2010; Morita and Miyachi 2003). Tight junctions contain several

strands of associated proteins that maintain cellular polarity and prevent ion transport through the intracellular fluid.

Given the aforementioned challenges, it is unsurprising that only a few molecules can traverse the skin barrier. These molecules, such as nicotine, should have low molecular weight (<500Da), low melting points for better solubility and high lipophilicity (Bos and Meinardi 2000; Donnelly et al. 2010; Kligman 1984). Extending the range of candidates that can be delivered across the skin requires reduction of skin barrier properties to enable more efficient permeation or penetration (Sugino et al. 2009; Wille 2006; Xing et al. 2009). Reduction of the skin barrier can be physically and chemically aided by various techniques: skin ablation and abrasion to remove the SC (Birchall et al. 2006; Moser et al. 2001; Sasaki et al. 2009); biolistics using high velocity propulsion to penetrate skin (Lee et al. 2008b; Liu 2006; Yang et al. 2001); sonication to vibrate and disrupt the plasma membrane (Escobar-Chavez et al. 2009b; Lee at al. 2009); electrical modulation to alter the isoelectric point or charge of the skin (Escobar-Chavez et al. 2009a; Wong et al. 2005); and perforation to make artificial conduits through the skin (Badran et al. 2009; Kim and Colton 2005). These methods however, have yet to make significant clinical impact and require complex and expensive equipment making their use cumbersome and often impractical for clinical use (Asbill et al. 2000; Prausnitz and Langer 2008). Furthermore they can cause skin irritations and discomfort due to prolonged exposure (Brown et al. 2006; Huang et al. 2004; Karande et al. 2005).

1.1.3.1 Drug delivery by injection

Hypodermic injections represent the standard established method for delivering many different types of therapeutics and vaccines. There are currently over 15 billion injections administered globally each year, of which at least 40 million are curative or therapeutic injections being administered worldwide daily. Only 5% of injections are used for vaccinations, whilst the vast majority including opioids, atropine and adrenaline, are prescribed for other medical requirements. Injections using hypodermic needles are essential for many forms of treatment as they provide reliable and effective interventions (WHO 1999, 2004).

There are many incidences of injection abuse or improper use, especially in the developing world (Reeler 1990; WHO 2004). Patients can be misdiagnosed or wrongly prescribed an injected therapeutic, whilst others may have the injection administered by someone with poor injection technique (Flaskerud and Nyamathi 1996; Foege and Eddins 1973; Hutin et al. 2003; Janjua et al. 2005; Kermode 2004; Marilyn 2002; Meit et al. 2004; Simonsen et al. 1999). Poor injection practices have been a major factor in the spread of acquired immune deficiency syndrome (AIDS) across Africa, where over a million cases of human immunodeficiency virus (HIV) and viral Hepatitis B and C have been associated to the lack of proper sterilisation of injection equipment and the reuse of needles (Apetrei et al. 2006; Kane et al. 1999; Newman et al. 2004; Reid 2009; Ross et al. 2008; Simonsen et al. 1999; Whitworth et al. 2007). Reuse of needles has been shown to increase transmission of blood borne pathogens from patient to patient, or to health care workers (Kermode 2004; Ndinya-achola et al. 1986; Priddy et al. 2005). The World Health Organisation (WHO) bulletin (October 1999) shows there to be only 30% regulated recycling of the needles and syringes used throughout the world, whereby plastics and metals

are melted down and recycled for other use. Thus, inappropriate disposal of injections also causes an unnecessary waste of resources and an increase in dangerous sharps. In addition to these administration and disposal issues, vaccine safety, storage, transport and handling, all form key challenges of immunisation programmes in developing countries.

Regulations published by the WHO and national health bodies aim to control the safe production, storage, administration and disposal of hypodermic needles and provide trained clinicians to administer injections (Dodoo et al. 2007; Gisselquist 2009; WHO 2004). The chances of needle-stick injuries increases with the number of personnel handling hypodermic needles. For example, healthcare workers may place used needles on a surface prior to disposal and either forgets or a second person removes these, they may prick themselves if not taking suitable precaution, such as being double-gloved (Bolyard et al. 1998; Clever and Omenn 1988; Jagger et al. 1988). Thus needle-stick can be attributed to the accidental spread of diseases from patient to patient or clinician, particularly in developing countries, such as in African nations, where needle disposal is improperly managed (Delobelle et al. 2009; Pandit and Choudhary 2008). 'The Centers for Disease Control and Prevention' (CDC) (USA) estimate that in the US alone during 1994 nearly one thousand healthcare workers contracted work-related Hepatitis B infection, and each year between 100-200 healthcare workers die from Hepatitis B (Bolyard et al. 1998; Clever and Omenn 1988; Jagger et al 1988).

There are also many psychological issues to consider with regard to injections. Whilst the use of various needles in a variety of devices has led to a culture of acceptability in their application, equally many patients experience needle-

phobia or 'belonephobia' that can inhibit patients from seeking medical and dental help due to fear of pain and injury (Deacon and Abramowitz 2006; Fredrikson et al. 1996; Hart and Yanny 1998; Kettwich et al. 2007; Kleinknecht 1994; Marks 1988; Milgrom et al. 1997; Nir et al. 2003; Ost 1992). In relation to needle-phobia, the psychological affects of needle insertion can cause physiological responses such as vasovagal shock or transient cardiac asystole (Catanzaro et al. 2006; Deacon and Abramowitz 2006; Hart and Yanny 1998; Sprung et al. 1998). Physiological problems with injections also exist in relation to the actual injury by the needle. These include local hypersensitivity with regular users such as insulin users or illegal substance abusers; lipohypertrophy in children, where fats and fibrous tissues accumulate at injections sites; pain and discomfort caused by the force and pressure of administering several millilitres of therapeutic agent or vaccine into muscle or veins; and bruising and bleeding after intramuscular injections (Ipp et al. 2007; Montgomery et al. 2005; Okawa et al. 2005; Yomtoob et al. 2009).

Injections of therapeutics and many immunisations given daily have saved numerous lives, however, the cost of producing, administering and disposing of these injections is prohibitively high in the developing world (Bridges et al. 2000; Cutts et al. 1990; Taddio et al. 2009). Therefore, it is important to develop a safer, cheaper and easily administrable needle device, to potential increase the use of drugs and vaccines (Hutin et al. 2003).

1.1.3.2 Drug delivery using microneedles

Recently, microneedles have been explored as a simple alternative method to deliver molecules and macromolecules into the skin epidermis (Ding et al. 2009; Henry et al. 1998; McAllister et al. 2003). In the last 20 years silicon-based microchip technology has rapidly developed through great advances in

microelectromechanical systems (MEMS) and microfabrication techniques (McAllister et al. 2000; Park et al. 2005; Teo et al. 2005; Wang et al. 2009; Wilke et al. 2005a). These advances have helped progress the development of microfluidic and microneedle device technologies for healthcare applications. Microneedles are needles with a length of less than one millimetre that can pierce the skin in a minimally invasive manner to increase the repertoire of drugs deliverable transcutaneously in a less painful manner than hypodermic needles (Kaushik et al. 2001; Sivamani et al. 2009; So et al. 2009). Upon applying microneedle arrays to the skin, each microneedle on the array pierces the skin to create micro-conduits across the stratum corneum and into the epidermis. Therefore, larger arrays with more microneedles have the capacity to produce multiple micro-conduits to enhance skin permeation. Microneedles provide a direct route for transport of drugs and vaccines into the skin.

Microneedles can benefit patients and clinicians alike by providing a method of controlled and targeted drug delivery, causing negligible pain, no bleeding and potentially at reduced cost of production, storage and transport (Prausnitz et al. 2009; Wermeling et al. 2008). Currently, a search of the U.S. National Institutes of Health clinical trial database highlights there are 9 microneedle-based clinical trials, 2 of which are completed, 2 are active, 3 are recruiting and 2 are not recruiting (Health 2011). These studies are varied, looking at the delivery of different molecules for different purposes. For example, a study in Hong Kong evaluated the safety of a low-dose H1N1 influenza vaccine (Trial Identifier NCT01049490) (ClinicalTrials.gov 2010a), whilst a Phase I study by 3M is aiming to investigate the tolerability of the application of microneedle structures (Trial Identifier NCT01257763) (ClinicalTrials.gov 2010b).

1.1.3.2.1 Types of microneedles

Microneedles can be manufactured from silicon, glass, metal, and biodegradable polymers (Donnelly et al. 2010). The needles are either solid or hollow with bevelled or tapered tips of various sizes and shapes (Prausnitz 2004). In the case of silicon, chemical and electronic engineers use MEMS techniques to fabricate microneedles. Dry-etch and wet-etch microfabrication techniques have both been used to manufacture microneedles. Dry-etching refers to a process, such as deep reactive ion etching, whereby a material is bombarded through a masked pattern with a plasma of gas contain various ions of fluorocarbon, fluorine, chlorine and oxygen (Bessel et al. 2003; Cakmak 2002; Chekurov et al. 2009; Wilkinson and Rahman 2004). Deep reactive ion etching has been used to create microneedles on silicon wafers (Ashraf et al. 2010; Coulman et al. 2010; Hafeli et al. 2009; Mikolajewska et al. 2010; Yan et al. 2010; Yeshurun 2010). Dry-etching is limited to only certain materials and process scale-up is difficult due to expensive equipment costs, which require specialist training due to many operating parameters and slow etch rate (Madou 2001).

Wet-etch microfabrication methods utilise the anisotropic characteristics of silicon in potassium hydroxide (KOH) solution (Pearton et al. 2007; Wilke et al. 2005b). Wet-etch techniques are not as expensive as dry-etching and are therefore more amenable to mass production of microneedles (McAllister et al. 2000). Wet-etching of silicon relies on aligning the crystal planes of the silicon structure and the lithographically patterned mask prior to exposing the silicon wafer to KOH solution. The design of the square mask causes the silicon at the edges to etch very slowly, whilst the corners are under-etched and thus form the octagonal shape of the microneedle. When the corners meet, the point of

the microneedle is formed and the etching is stopped. This technique is limited by the planes within the silicon structure and thus alterations in geometry or density are significantly harder to make when compared to dry-etching techniques (Wilke et al. 2005a). Once the silicon wafers are etched to form microneedles, these are then coated by platinum to provide rigidity and though this is an expensive treatment (Park et al. 2005). Platinum-coated silicon microneedles have been shown to be of appropriate dimensions to create microconduits, approximately 50μm in diameter, extending through the SC and viable epidermis (Birchall et al. 2005).

Stainless steel microneedles are easily and cheaply manufactured using laser-etching techniques to cut thin stainless steel sheets, after which the etched needle is folded out of the plane of the sheet (Davis et al. 2004; Gill et al. 2008; Kim et al. 2009b; Martanto et al. 2004; McAllister et al. 2000; Song et al. 2010). Initially steel microneedles were used to perforate the skin to enable topically applied solutions, for example insulin, to diffuse through the microconduits (Martanto et al. 2004). However, increasingly the focus is on coating steel microneedles to deliver a dosage upon application to the skin (Cormier et al. 2004; Gill and Prausnitz 2007; Prausnitz et al. 2009; Quan et al. 2009). Glass microneedles have also been produced using conventional drawn-glass micropipette techniques. Such microneedles may offer advantages, as these techniques can be adapted for large-scale manufacture (Lee et al. 2003; Martanto et al. 2006; McAllister et al. 2003; Wang et al. 2005b). However, glass microneedles may be limited in their distribution and use due to fragility and physical stability of the glass structure (Martanto et al. 2006). Polymers, such as polyvinylpyrrolidone (PVP) (Sullivan et al. 2010) and sugars including maltose and galactose (Donnelly et al. 2009; Kolli and Banga 2007) have been

used to produce dissolving microneedles. Dissolving microneedles offer the advantages of reducing bio-hazardous waste as they dissolve and are completely absorbed into the skin (Ito et al. 2006b; Lee et al. 2011; Sullivan et al. 2010). However, dissolving microneedles remain unstable at changing temperature and humidity, thus causing global logistic and distribution related issues (Donnelly et al. 2009; Ito et al. 2006a; Lee et al. 2008a). Meanwhile other polymers, such as the photoreactive acrylate-based polymer eShell 200 (Gittard et al. 2009) and the mucoadhesive copolymer Gantrez® AN-139 (Donnelly et al. 2011) are commonly used in the pharmaceutical industry, and when combined with two-photon polymerization (2PP) microfabrication or laser engineering method, produce structurally stable and functional microneedles.

1.1.3.2.2 Transcutaneous drug/vaccine delivery by microneedles

Solid microneedles can be used for drug and vaccine delivery by coating the therapeutic onto the microneedle surface and inserting into the skin whereby the coated drug then dissolves upon contact with interstitial and cellular fluids (Cormier et al. 2004; Gill and Prausnitz 2007; Kim et al. 2009b; Pearton et al. 2010; Shirkhanzadeh 2005; Teo et al. 2005; Widera et al. 2006a; Zhu et al. 2009). Microneedles have been shown to be robust enough to penetrate skin and dramatically increase skin permeability to macromolecules and nanoparticles (Coulman et al. 2009; Donnelly et al. 2010; McAllister et al. 2000; McAllister et al. 2003). Microneedle devices have been used to successfully administer a variety of model compounds, including lower-weight compounds such as methylene blue (Li et al. 2010), cascade blue (Verbaan et al. 2007), calcein (Oh et al. 2008), as well as, macromolecular model compounds such as fluorescein isothiocyanate (FITC)-dextrans (Verbaan et al. 2007; Wonglertnirant et al. 2010), bovine serum albumin (BSA) (Henry et al. 1998; Park et al. 2006)

and fluorescent polystyrene nanoparticles (Chabri et al. 2004; Coulman et al. 2009; McAllister et al. 2003),

Low-molecular weight drugs including diclofenac (Banks et al. 2011), naltrexone hydrochloride (Banks et al. 2008) and methyl nicotinate (Sivamani et al. 2005) have been successfully administered through microneedle-punctured skin. Furthermore, macromolecules such as insulin (Martanto et al. 2004; McAllister et al. 2003) human immunoglobulin G (IgG) (Li et al. 2010) and desmopressin (Cormier et al. 2004). Coulman et al (2006) investigated the ability of microneedles to create microconduits through the stratum corneum of *ex-vivo* skin which could facilitate the delivery of charged macromolecules and plasmid DNA. Macromolecules such as β-galactosidase and fluorescent nanoparticles were delivered to the viable epidermis. It was concluded that further research into applicator morphology, application technique and therapeutic formulation are essential to optimise delivery through microchannels.

Microneedles have been widely investigated as a means for facilitating minimally invasive immunisation. Microneedle vaccines may confer stability, storage, transport and administration advantages to improve vaccine distribution for mass-vaccinations (Koutsonanos et al. 2009; Li et al. 2009; Quan et al. 2009; Van Damme et al. 2009; Zhu et al. 2009). Zhu et al. (2009) investigated the different coating capabilities of solid microneedles, showing that up to 10μg of viral protein could be coated onto their array of 5 microneedles. Quan et al. (2009) demonstrated that microneedles can be coated with stabilized vaccine to produce superior protective immune response to conventional intramuscular vaccination. Van Damme et al. (2009) conducted

a randomised trial in 180 healthy adults to evaluate the safety and immunogenicity of low-dose influenza vaccines delivered intradermally by microneedle devices. The resultant data showed that the intradermal microneedle vaccination caused significant local reactions, though these remained transient and mild in their nature, whilst the immunogenic responses were similar to those of a full-dose intramuscular vaccination.

As microneedle technology continues to develop, microneedles of different dimensions, varying array designs and material composition are being produced for clinical use. Therefore, the way microneedles interface with patients is becoming an essential consideration. It is hoped that in the future microneedles will be valuable alternative to current vaccine and drug delivery methods.

1.1.4 Pain

1.1.4.1 Defining pain

The Oxford English Dictionary (2009) defines pain to be "a strongly unpleasant bodily sensation such as is caused by illness or injury" and as "mental suffering or distress". Pain has also been defined by the International Association for the Study of Pain (2009) as "An unpleasant sensory and emotional experience usually associated with actual or potential tissue damage, or described in terms of such damage". Whilst sensation is defined in the Oxford English Dictionary (2009) as a "a feeling that one gets when something affects your body" or "the ability to feel through your sense of touch" or "a general feeling or impression that is difficult to explain".

Pain can be comprised of various sensations though not all of a negative nature (Chambers et al. 2009; Grob and Mannion 2009; Jeba et al. 2009). Pain can be

constant, sporadic, regular or random and can be felt as either sharp or dull sensations (Chen et al. 2009b; Hanley et al. 2009; Leppert 2009). Pain can exist in several or single anatomical locations which may represent the area of physical injury or damage (Ivanusic et al. 2009; Kobayashi et al. 2009; Lane et al. 2005; Quevedo and Coghill 2009). Pain provides a vital clue to the location of any injury or external forces that might be acting on the body. This enables treatment of the primary cause of pain or at least alleviation of the pain by means of medical treatment. Therefore the physiological and psychological triggers and receptors of pain form the patients' perception of interventions such as injections (Bergh et al. 2008; Cox et al. 1996; Dunwoody et al. 2008; Harvima et al. 1993; Jamison 1996; Uman et al. 2006; Uthaikhup et al. 2009). However, extensive literature searches highlights that the severity and intensity of the overall sensations and pain specifically felt during an injection have not been measured or quantified.

1.1.4.2 Measuring pain

There are many different pain intensity and pain measuring devices discussed in the literature (Barakatt et al. 2009; Grob and Mannion 2009; Ho et al. 1996; Holtan and Kongsgaard 2009; Katz and Melzack 1999; Snow et al. 2009). It is important to take certain key attributes into account before choosing a measuring instrument: The instrument must have been used in the clinical setting previously and it must be valid and reliable in measuring that particular type or aspect of pain for which it is intended. In this context validity is the extent to which a measuring instrument actually measures what it is designed to measure and reliability is the extent to which a measuring instrument remains consistent regardless of minor changes in conditions. The instrument of pain measurement must also be efficient and able to collect maximum information for the type of pain it is measuring. Most pain assessment instruments can be

combined to give maximum data, though to remain efficient it is advisable to use an instrument from each dimension (pain description, responses to pain and impact of pain) (Carnes 2006; Grotle et al. 2004; Guyatt et al. 1987; Vierck et al. 2008).

1.1.4.3 Reliability and validity of pain measuring instruments

A reliable device will be able to measure pain consistently without being effected by minor changes in environment, administration or circumstances, yet would register a change if the pain was to change. Guyatt et al (1987) discussed the importance of responsiveness to change and how a reliable pain measuring instrument will provide similar information in between readings unless the pain changes. This is known as 'intrarater reliability'. The instrument will also measure very similarly, if not identically, for a given situation even if the administrator changes. This is known as 'interater reliability'.

However, the data collected are often context specific, such as, specific to a certain pain condition, population, ethnicity or demographic, and this limits the instrument's range of uses in general populations or all pain conditions. Thus it is important for the administrator to determine which measurement instrument is appropriate for the specifics of the given situation in which it will be used (Al Jarad et al. 1999; Bimstein et al. 2009; Blomkalns et al. 2005; Greenwald 1991; McCrory and Fitzgerald 2004; Melzack 2005; Vierck et al. 2008).

Certain instruments may be more reliable but time consuming to administer thereby making them impractical in a clinical setting. Thus in many clinical settings it is important to account for the complexity and duration of the pain/perception-measuring instrument. A valid instrument is one that is designed for that situation and will measure that type of pain and not another.

However, deciding which pain measurement instrument is valid for a given situation is a contentious issue (Caraceni et al. 2009; Carnes 2006; Clark et al. 2003; Margolis et al. 1986; Voorhies et al. 2007).

1.1.4.4 Different types of pain measuring instruments

There are three main types of pain measurements: self-reporting, physiological and observational. Self-reporting requires the individual pain sufferer to complete various key items of information and/or maintain a diary to monitor the pain experienced. Such instruments often consist of a variety of rating scales to rate, for example, from 'the least pain felt' to 'worst pain imaginable' (Alves et al. 2009; Balbierz et al. 2006; Bartell et al. 2008; Choiniere and Amsel 1996). Dairies and long term self-report forms can assist in classifying the pain as sporadic or persistent, and acute or chronic. Dairies also provide insight into how the sufferer copes with the pain and its impact on the sufferer's life (Jamison et al. 2009; Lewandowski et al. 2009).

Physiological measures are often used to assess the biological responses in relation to pain. Pain can cause muscular tension, increased blood pressure, and alterations in heart rate, sweating, breathing and other stress responses. Such changes in physiology can be used to indirectly measure acute pain, but are prone to stabilising as the body becomes accustomed to the pain stimuli. Therefore, even if the pain remains, the biological characteristics may have returned to normal (Jamison 1996; Jensen et al. 1989; Sim and Waterfield 1997; Tunks et al. 2008).

Observational measurements usually rely on a person close to the sufferer completing a measurement, for example their perception of the sufferer's mood, expression, physical mobility or activity (Apolone et al. 2009; Nijrolder et al.

2009; Veresciagina et al. 2009). These can often be used with the self-reporting and physiological measures to determine any missing features of the pain or its cause.

It is clear that no single method or measuring instrument is totally accurate or encompassing. Each provides perception and description that can be used in varying degrees to determine the cause, site and impact of pain on the sufferer's life.

1.1.4.5 Different techniques for measuring pain in a clinical setting

Most measuring instruments rely on self-reporting as that is regarded as the best standard for understanding and assessing pain. This requires the sufferer to describe their pain through a variety of measurement instruments, which can either be questionnaires, rating scales, visual analogue scale (VAS), drawings or a combination of some or all. Pain can be described by its intensity, descriptive words and location on the body (Dias et al. 2008; Huskisson et al. 1976; Ipp et al. 2009; Katz and Melzack 1999; Melzack R 1994; Okawa et al. 2005; Rokyta et al. 2009). Self-reporting is a very commonly used instrument as it provides subjective descriptions consistent with the objective nature of pain and measures the sufferer's personal perception to pain. However, due to the variations in individual perception, self-reported inter-individual data cannot be easily compared, but can provide details for intra-individual comparisons (Bringuier et al. 2009; Cohen et al. 2009b; Jensen et al. 1989; Serpell 2002; Spielberg et al. 2003). Research by Jamison (1996) shows that self-reporting of pain intensity is a very valid and reliable method.

The VAS is a simple quantifying tool consisting of a 10cm line marked at one end with "no pain" and the other with "worst pain imaginable". The sufferer is

asked to mark the intensity of pain felt on the 10cm scale. The VAS has been shown to be reproducible, sensitive and universally simple to understand and administer (de Boer et al. 2004; Harding et al. 2009; Vieira et al. 2005). Numeric rating scales are commonly used alongside a VAS (Choiniere and Amsel 1996; Fauconnier et al. 2009; Hirsh et al. 2009; Jennings et al. 2009; Katz and Melzack 1999). Jensen et al (1989) suggests that acute pain is best measured by the 11-point box, whilst linear numerical rating is better for chronic pain.

Pain drawings are a useful method of gauging pain and sensation by marking on a picture of the anatomy (Parker et al. 1995). Sometimes these can be scored or used to indicate the type of pain felt in different regions of the body. The scoring and marking of these pictures can differ considerably depending on use, though they essentially consist of outlines drawings of the human body, front and back, onto which the patient either marks using defined symbols or shading, the type or location of pains (Margolis et al. 1986). Attempts to rate such drawings as physical manifestations of psychological distress have been unreliable, though pain drawings are still a clinically useful method of understanding the location and distribution of pain (Carnes 2006; Giske et al. 2009; Parker et al. 1995; Voorhies et al. 2007; Wenngren and Stalnacke 2009).

The McGill pain questionnaire (MPQ), developed by Melzack et al. (1975), measures dimensions of pain quality by including a VAS, a set of descriptor adjectives, an intensity scale and pain drawing. Patients are asked to rate 20 adjectives that describe various pain sensations. These descriptors are divided into 4 categories: sensory (categories 1-10), affective (categories 11-15) and evaluative (category 16) and miscellaneous (categories 17- 20). Thus the MPQ

provides multidimensional evaluation of pain (Melzack 2001). It can be quantitatively evaluated using the pain intensity (VAS), individual rating indexes for each descriptor category and an evaluative overall pain index (EOPI). This makes it one of the most widely used of all pain measurement tools. In 1987, Melzack developed the short-form version of the MPQ (MPQ-SF), which uses key adjectives from the longer MPQ (Melzack 1987). As shown by Melzack (1994), the MPQ and MPQ-SF provide a reliable and valid method of assessing the qualitative nature of an individual's pain experience, thereby differentiating between subtle and dramatic clinical changes, as well as recording any unexpected features.

Audio recordings are used in the social sciences to collect data from focus groups and interviews. Everything said by the participants is potentially important to improve understanding and further develop the topic being discussed (Arber 2007; de Salis et al. 2008; Maikler 1991). In the case of a clinical study, literature searches provide no evidence of audio-recordings being used as a data source in the clinical assessment of drugs or medical technologies, though Van Tilburg et al. (2009) used audio and video recording to assess a home-based, guided imagery treatment protocol. However, when assessing pain, especially transient pain, audio recording may add another dimension to the details of the pain and sensation felt.

1.1.4.6 Measuring pain caused by microneedle applications

To better understand the complex nature of pain and sensation during needle-based injections it is important to appreciate the various layers of skin through which a needle may penetrate. For example, the epidermal layer of the skin contains multitudes of nerves that enable us to feel sensations of touch, heat and cold and regulate pain sensing. These physiological details are covered in

Section 1.1.1 but their implications will always present as intra-and inter-individual differences in the intensity of each sensation felt.

Microneedles have been tested to determine their potential benefits in the delivery of vaccines and other medicaments. Whilst it has been purported that microneedles can facilitate delivery of insulin, nicotine and therapeutic antibodies into humans without stimulating underlying pain receptors or blood vessels, only a few studies have measured qualitatively or quantitatively the pain related to microneedle insertion into human volunteers (Gupta et al. 2009; Kaushik et al. 2001; Li et al. 2009; Prausnitz et al. 2009; Sivamani et al. 2009). Bal et al. (2008) investigated the penetration and pain of solid microneedle arrays of 400μm and 200μm lengths on human volunteers. Microneedle penetration of skin was confirmed by significant increase in transepidermal water loss (TEWL) upon application. The microneedle devices caused minimal pain when measured on a 10-point measuring index, though little information is provided on how pain was measured.

Other assessments of pain caused by microneedle insertion use the VAS, which is an efficient, reliable and validated measure of 'pain intensity', but provides limited information on sensation or an overall assessment of the human perception of microneedle application. Gill et al. (2008) tested single microneedles 480μm to 1450μm length with varying base widths, as well as, microneedle arrays containing 5 to 50 microneedles. The microneedles were inserted into the volar forearms. Gill et al. (2008) demonstrated that microneedle length had the greatest effect on pain, whilst the number of microneedles applied on an array had less influence on pain. Microneedle morphology, for example the thickness, base width and tip angle, did not

significantly influence pain. However, all microneedles tested caused significantly less pain than 26-gauge hypodermic needles.

Kaushik et al (2001) measured pain response using VAS to the application of an array of 400 microneedles, each approximately 150μm in length. The pain responses were statistically insignificant when compared to the application of a smooth surface, and statistically insignificant to the pain responses following the insertion of a 26G hypodermic needle. However, Kaushik et al. (2001) did not attempt to simultaneously demonstrate both microneedle functionality and pain response, as there was no *in-vivo* assessment during the study to verify skin puncture due to microneedles in relation to level of sensations.

Sivamani et al. (2005) compared *in-vivo* human injections of 1μl methyl nicotinate, using 200μm length hollow needle arrays, to topical application. The data revealed increased blood flux post-application of microneedles whilst comments from the volunteers describe the application of microneedles as a feeling of "*pressure but no pain*". Miyano et al. (2005) described that 500μm length detachable and biodegradable microneedles manufactured from maltose do not cause any pain on skin insertion. Shirkhanzadeh (2005) reported that volunteers tolerated microneedles coated with porous calcium phosphate, although pain scoring was not performed. Further reports suggest that microneedles do not cause any significant pain when used for extraction of interstitial fluid or blood for glucose monitoring (Smart and Subramanian 2000; Wang et al. 2005).

These aforementioned studies did not use instruments such as the MPQ or either real-time or reflective audio commentary of the participants to clearly define the extent and type of pain felt during microneedle insertion.

1.1.5 Transepidermal Water Loss (TEWL) as a measure of skin penetration

The non-aqueous SC restricts water loss from the deep, water-rich layers of the skin. This creates a water gradient that increases gradually from the dermis to higher strata of the epidermis. Water vapour diffuses continuously through the skin through pores and hair follicles because vapour pressure is higher in the dermis than in the external environment (Morgan et al. 2003; Richards et al. 2003; Rodrigues et al. 2004; Schwindt et al. 1998; Singh et al. 2001). The amount of water diffusion through skin is not only regulated by environmental humidity but also by regulatory responses from the skin.

TEWL measurement is a standardised method of determining water loss in the skin and is frequently used in the cosmetics and dermatology industry to test safety and irritancy of topical creams (Berardesca and Distante 1994; Loden 2003; Robinson and Perkins 2002). As TEWL is a process of passive diffusion, the rate of water vapour diffusion across the SC depends not only on the ambient relative humidity and temperature but also on the thickness and integrity of the SC (Pinnagoda et al. 1990; Roskos and Guy 1989; Shah et al. 2005). Therefore, the measurement of TEWL provides an assessment of skin barrier function. The skin surface is surrounded by a microenvironment formed by a vapour boundary. This forms a physical barrier against the environment and enables transition of moisture and heat from the body to the ambient surrounding. This transition can be expressed in terms of the vapour pressure gradient (Grove et al. 1999; Pinnagoda et al. 1990). High TEWL values

correspond to dry or damaged skin whilst low TEWL correlate to healthy undamaged skin (Bashir et al. 2001; Bornkessel et al. 2005). The measurement of TEWL in this study will provide information concerning the compromised integrity of the epidermis following application of microneedles.

There are, however, limitations with TEWL measurement. TEWL apparatus are very sensitive and subject to disturbances by ambient airflow due to physical moment, windows, breathing or opening doors near the probe whilst taking measurements (De Paepe et al. 2005; Miteva et al. 2006; Pinnagoda et al. 1990; Rosado et al. 2005a). It is also acknowledged that TEWL varies considerably with anatomical locations and differences in skin physiology (Aramaki et al. 2002; Atrux-Tallau et al. 2009; Fluhr et al. 2006; Garcia Bartels et al. 2009; Rosado et al. 2005b; Schwindt et al. 1998; Singh et al. 2001).

1.1.6 Aims and objectives

This thesis aims to determine pain response, sensory perception and skin barrier damage, following hypodermic and microneedle applications in human volunteers and inform the development of a reliable application method for microneedle administration.

Objectives:

- To assess the ability of microneedles to puncture *ex-vivo* and *in-vivo* human skin.
- To use appropriate pain measurement instruments to characterise pain post-application of microneedle devices and hypodermic needles in human volunteers.

- To determine the degree and breadth of sensations felt during microneedle and hypodermic needle applications.
- To investigate the wound healing responses within the first 24 hours following clinical applications of hypodermic and microneedle devices.
- To develop a suitable application method that allows for efficient and reproducible application of polycarbonate microneedles to human skin.

Chapter 2

Pre-clinical testing of potential microneedle applicators and characterisation of silicon microneedles

2.1 Introduction

This chapter will aim to characterise the microfabricated silicon microneedle arrays, as well as test simple applicators for administering the microneedles to human skin *ex-vivo* and *in-vivo*. The results of this chapter will thereafter assist in defining the appropriate methods for applying microneedles, and assessing microneedle penetration, in Chapter 3.

2.1.1 Defining Pain

Pain has been defined in Section 1.1.4. as "a strongly unpleasant bodily sensation such as is caused by illness or injury" and as "mental suffering or distress" (Waters and Bull 2006). It has been reported that physiological and psychological aspects of pain are apparent in patients when they are subjected to injections (Arendt-Nielsen et al. 2006; Chambers et al. 2009; Ipp et al. 2009; Lane et al. 2005; Lawes et al. 2008; Price et al. 2009; Shah et al. 2009; Taddio et al. 2009; Uman et al. 2006). However, as outlined in Section 1.1.4, few research groups have conducted detailed studies into the intensity and nature of pain and other sensations, specifically experienced during microneedle injections (Gill et al. 2008; Kaushik et al. 2001; Sivamani et al. 2005).

2.1.2 Measuring Pain

The various techniques of assessing and quantifying pain and sensory perception are described in Section 1.1.4. Each technique aims to collect multidimensional data on the perception of pain. Thus the measurement and manifestations of pain and associated perceptions, and the complexities in describing pain (Benoliel et al. 2009; Fabbri et al. 2009; Lundqvist et al. 2009; Melzack 1987; Snow et al. 2009; Strand et al. 2008; van Tilburg et al. 2009)

were carefully considered when developing the instruments for data collection during the clinical study.

2.1.3 Imaging to characterise microneedles to be used in the clinical study

As discussed in Chapter 1, microneedles come in many different shapes and sizes as a result of different material composition and manufacturing process (Ayittey et al. 2009; Badran et al. 2009; Coulman et al. 2009; Kolli and Banga 2008; Ramasubramanian et al. 2008; Wang et al. 2009). To assess microneedle morphology, magnified imaging techniques such as scanning electron microscopy (SEM) are used. SEM provides greater surface detail than simple light stereomicroscopy by using electrons instead of light to form an image (Moore 2009; Xiong et al. 2009). The advantage of SEM compared with light microscopy is that the former has a larger depth of field which improves focus over a greater surface, also the ability to use higher resolutions allows for greater magnification. Using the magnetic field to control electrons, the user is able to minutely alter magnification and resolution. SEM requires vacuum conditions, thus sample preparation is important. For example, all samples must be dehydrated and as the electron beams will only give a clear image off an electron dense surface, non-metal surfaces usually have to be sputter-coated with gold (Goodhew et al. 1997; Lopes et al. 2009; Risnes 2003; Xiong et al. 2009).

2.1.4 Measuring transepidermal water loss (TEWL) to establish skin penetration

The measurement of TEWL provides an assessment of the integrity of the epidermis under normal and various disease situations, for example dermatitis and eczema (Shimada et al. 2009; Wynne et al. 2002). TEWL is a measure of the degree of evaporation of water immediately above the stratum corneum

(Faurschou et al. 2007; Rosado et al. 2005b; Schmid et al. 2005). Evaporation from the skin surface is measured using a TEWL probe and meter, which relies on two hygrosensors and thermistors placed in a chamber (De Paepe et al. 2005; Miteva et al. 2006; Rosado et al. 2005a). When placed on the skin the open chamber forms a seal on one end to the skin whilst remaining open at the other end. The resulting vapour flow from the skin surface to the ambient air is measured by specific electro-sensors in the chamber. The degree of evaporation is determined by measuring the vapour pressure at one point of the chamber. Another sensor measures the humidity levels in the chamber close to the skin surface. As water vapour builds in proximity to the skin surface, the humidity in the chamber increases in comparison to the humidity in the ambient air. This creates a humidity gradient in the chamber that is measured by the two measuring sensors inside the open chamber. The vapour flow evaporating from the surface of the skin can be calculated by diffusion equations from the measured humidity gradient (Cohen et al. 2009a; Miteva et al. 2006). The humidity and vapour pressure gradient is approximately constant in the absence of external airflow. Thus under steady-state condition it is proportional to the amount of water vapour passing through the transition layer per unit of time multiplied by the area over which evaporation from the skin surface occurs. The proportionality constant is a measure of the permeability of the skin to water. TEWL meters measure at two different fixed heights perpendicularly above the skin surface and within the transition zone and calculate TEWL on the basis that the difference between the vapour pressures at the two points is approximately proportional to the vapour pressure immediately above the skin (Cohen et al. 2009a; Fluhr et al. 2006; Miteva et al. 2006; Nuutinen et al. 2003; Shah et al. 2005; Tagami et al. 2002).

2.1.5 Aims and objectives

The method of application of microneedles is important to achieve successful penetration through the skin. The use of an applicator will help to ensure that the microneedles will penetrate efficiently. Thus, prior to the clinical study detailed in Chapter 3, it was necessary to perform a preliminary study to assess the puncturing efficacy of microneedles when mounted onto different applicator designs and ensure that the applicator itself did not cause pain upon application.

Objectives:

- To characterise the morphology of silicon microneedle arrays.
- To determine whether silicon microneedles penetrate *ex-vivo* human skin and create microconduits.
- To design and test a simple, clinically acceptable, method of applying the microneedles to human volunteers.
- To assess pain and sensation induced by different applicator devices when applied to human volunteers.

2.2 Materials and Methods

2.2.1 Materials

Araldite epoxy resin (Huntsman International LLC, Basel, Switzerland); TEWL probe and DERMALAB data unit (Cortex Technology, Handsund, Denmark); Sanyo TRC-525M dictaphone (Sanyo, Japan); methylene blue dye powder (Sigma-Aldrich Ltd, Dorset, UK); Olympus DP-10 Camera (Olympus Optical, London, UK); Olympus TH3 Power unit (Olympus Optical, London, UK); Philips XL-20 Scanning Electron Microscope (Philips, Eindhoven, Netherlands); Schott KL1500 fibre optic light source (Schott UK Ltd, Stafford, UK)

The 180μm and 280μm wet-etched silicon microneedle arrays used in this study were provided by Anthony Morrissey at The Tyndall National Institute, Cork, Ireland. These microneedles were manufactured from silicon wafers that were coated with silicon nitride on a silicon oxide layer and wet-etched using potassium hydroxide (KOH). Square shape patterns were transferred into the masking double layer by standard photolithography, after which, the patterned silicon wafer was etched using a 29% KOH solution at a temperature of 79°C. The needle formation was based on convex-corner under cut. Each microneedle array was comprised of 36 equally spaced pyramidal shaped microneedles of either 280μm or 180μm length with a base diameter of approximately 180μm and microneedle tip of 1μm wide. The silicon arrays were then coated in 0.3μm platinum. These microneedle arrays have been used *ex-vivo* in a number of previous studies (Birchall et al. 2005; Coulman et al. 2006; Pearton et al. 2008; Wilke et al. 2005a).

Outline designs for the aluminium applicator rods, onto which the microneedle arrays were mounted, were provided to Professor David Barrow at the Cardiff School of Engineering who kindly agreed to machine the applicator rods. The addition of foam and rubber was undertaken in the laboratory.

2.2.2 Selecting a suitable applicator and application method for the clinical study

Before applying microneedles to humans, it was important to establish a suitable method of application to the skin. Initially 9 potential applicator devices were designed and tested.

2.2.2.1 Designing various applicator rods for the application of microneedle arrays

Applicators for the clinical application of silicon microneedles were designed with the aim of increasing skin penetration of microneedles whilst reducing applicator induced pain and sensation. Square-end ($6mm^2$) [Y] and cylindrical [Z] aluminium rods where engineered so that the microneedle array ($6mm^2$) was not overhanging the end of the rods when mounted (Figure 2.1).

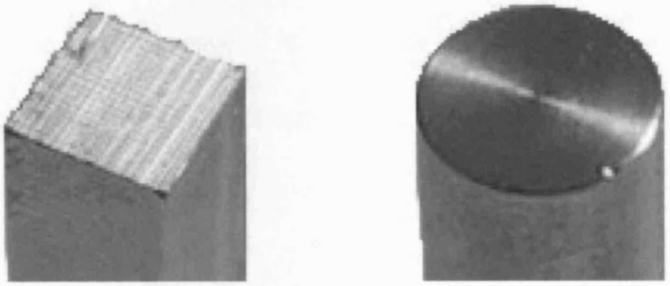


Figure 2.1. Square-end applicator [Y] and cylindrical applicator [Z].

In addition, 7 potential new applicators were designed using plastic syringes or cylindrical applicator rods as their base structure (Figure 2.2). They were as follows:

[A] An inverted 2ml plastic syringe with the plunger surface heated and smoothed to remove any protrusions.

[B] Cylindrical aluminium rod applicator with rubber (3mm x 2mm) wrapped around at the application end.

[C] Cylindrical aluminium rod applicator with elastic (4mm x 1mm) wrapped around at the application end.

[D] Cylindrical aluminium rod applicator with rubber removed from a syringe bung (2mm x 1mm) wrapped around the application end.

[E] Cylindrical aluminium rod applicator with foam measuring (5mm x 4mm) wrapped around the application end.

[F] Cylindrical aluminium rod applicator with rubber (6mm x 2mm) wrapped around the application end with 6mm by 9mm diameter foam on the application face.

[G] Cylindrical aluminium rod applicator with 6mm diameter foam attached on the application face.

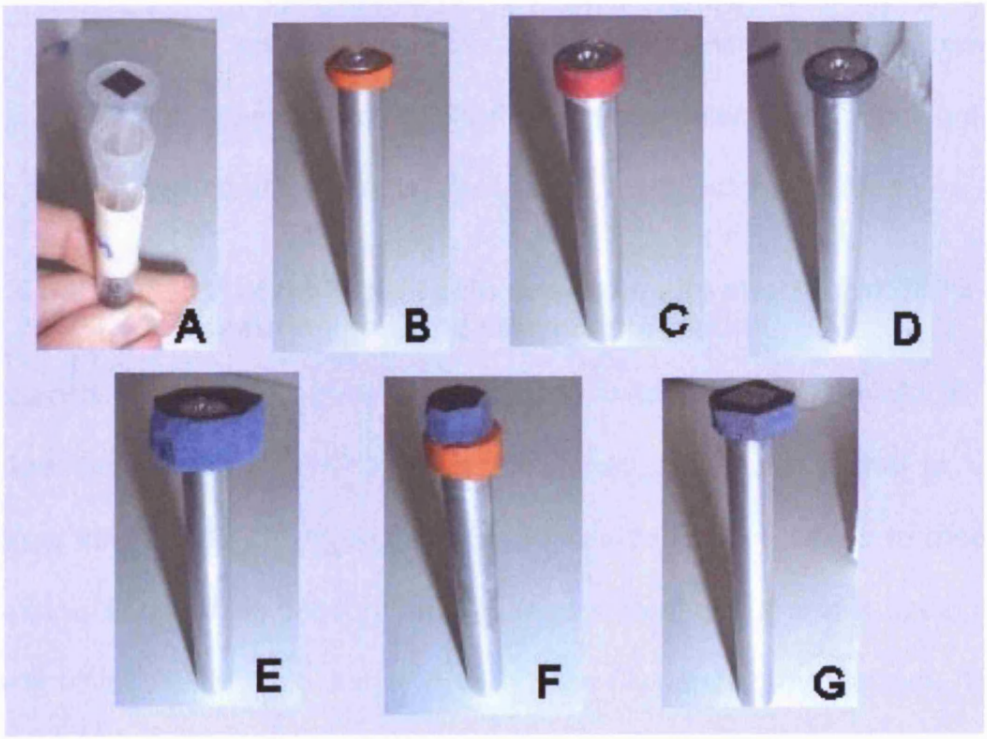


Figure 2.2. Photographs of the microneedle applicator designs.

2.2.2.2 Ethical approval and participant consent approval

Prior to beginning testing of the applicators, ethical approval was obtained from the Welsh School of Pharmacy (WSP) Research Ethics Committee for the administration of applicators to volunteers and for recording their oral commentary. Informed consent (Appendix I) was obtained from each participant in adherence with the Declaration of Helsinki and a written explanation of the study provided at least one week before their first visit. At the first visit each participant read and signed consent forms after the nature of the study had been explained. Each participant was informed that they could withdraw from the study at any stage without any explanation from them. Subjects were also given the opportunity to ask questions before deciding to participate.

2.2.2.3 Audio recording of participants

Throughout each session with each participant, two dictaphones were placed in close proximity to the participant to record the entire dialogue between participant and coordinator. At the start of each session, the participant was asked to *"describe clearly what they feel as each applicator is applied".*

2.2.2.4 Design of the McGill pain questionnaire short-form (MPQ-SF) for measuring pain and sensory perception

The pain and sensation questionnaire used was the standard McGill pain questionnaire short-form (MPQ-SF) as described in Chapter 1, that is, visual analogue scale (VAS) to measure pain, 15 key descriptive words to measure sensation (representing sensory and affective words) and a present pain index or evaluative overall pain index (EOPI) (see Appendix II) (Melzack 1987). Immediately post-application of each applicator, the VAS was registered first by participants marking on a 10cm horizontal line marked at opposing ends with 'No pain' and 'Worst pain imaginable'. Subsequently the participant was asked to rate each descriptive word of the MPQ-SF: *"As each word is read out to you*

please state if it describes a sensation you felt during or after the application. Rate it as either none, mild, moderate and severe."

2.2.2.5 Scoring the MPQ-SF

As the MPQ-SF used was a reliable and validated questionnaire, it was scored using the criteria as laid out by Melzack (1987) (Wright et al. 2001). Each VAS was measured in millimetres starting at the 'No pain' end along the length of the 100mm to where the participant had marked the horizontal line. Each descriptive word was scored by giving a score of 0 for words the participants had responded to as 'none'; 1 for the words marked as 'mild'; 2 for those marked as 'moderate'; and 3 for those marked as 'severe'. All the scores were totalled for the sensory descriptive words, affective words and subsequently combined for an overall total score for all 15 descriptor words. The EOPI was scored from 1 to 5 corresponding to the numerical rank of the terms 'No pain = 0' 'Mild = 1', 'Discomforting = 2', 'Distressing = 3', 'Horrible = 4' and 'Excruciating = 5'. Thereafter the VAS, scores from key descriptive words, and EOPI scores were totalled to enable comparison of each of the 4 applicators. Thus the applicators could be ranked in order of lowest to highest score as a measure of least painful and sensation inducing, to highest pain and sensation inducing.

2.2.2.6 Assessment of pain and sensation caused by different applicator designs

To test the applicator designs for the pain and sensations perceived by human volunteers, the volar forearm was deemed a suitable anatomical region as it was easily accessible on participants and represented an area where microneedle devices may, in future, be clinically administered. However, during the clinical study (Chapter 3), ethics approved the buttock as the most suitable region for needle administration, due to the subsequent biopsies of the

administration sites (Chapter 4). Initially all seven, [A] to [G] applicator devices were applied to one volunteer (V1). V1's oral comments were audio-recorded and the two least discomforting of the seven applicators were chosen for comparison with the square-ended [Y] and cylindrical [Z] aluminium rods (Figure 2.1). Subsequently 12 additional participants were recruited making the total 13 participants (6 male and 7 female, though recruitment was not gender specific).

Testing the applicators on the 13 volunteers took place over two sessions: the first session informed and consented the participants before both square-ended [Y] and cylindrical [Z] applicators (Figure 2.1) were applied in a rolling fashion (as explained in Section 2.2.3) to either their left or right volar forearm in a single blind and randomised manner. The participants were asked to look away during each application to ensure single-blind application. After one application, the MPQ-SF in its standard form was administered (Appendix II). Subsequently the remaining two applicators were applied to the other forearm and the MPQ-SF repeated.

The second session was conducted at least 1 hour after the first to allow the participants to have time between applications so that their perceptions between applications were not overlapping. Only the 2 specific applicators, [A] and [E], from the 7 applicator designs [A] to [G] in Figure 2.2 were assessed. Each applicator was applied to either the right or left forearm and the MPQ-SF repeated. Recovery time of at least 1 hour between applications to the same forearm ensured previous applications had not increased the sensitivity of the forearm. This whole process of application and recovery was repeated for each

application. Each session was audio-recorded and participants were advised to vocalise their comments throughout the applications.

2.2.3 Mounting microneedle arrays to applicators in preparation for application to skin

A thin coat of Araldite epoxy was applied to the top of the applicators and, using tweezers, a single array of microneedles was carefully placed in the centre of the top of each applicator. Subsequently the applicators were left standing upright for 18 hours with the array facing upwards. Light microscopy inspection of the array surface was used to determine whether the epoxy had bonded the base of the array to the applicator and confirm that the microneedles had not been adversely affected.

2.2.4 *Ex-vivo* testing of microneedle arrays mounted to the two least painful applicators

Full-thickness human breast skin was obtained from mastectomy or breast reduction with Gwent Healthcare NHS Trust ethical committee approval and informed patient consent. Skin was excised from a variety of donors ranging from 45 to 65 years of age at the Royal Gwent Hospital, Newport. Excised skin was transported from the operating suite direct to WSP in Dulbecco's Modified Eagle Medium (DMEM) culture media (44.5ml DMEM, 5ml 10% foetal bovine serum and 0.5ml 1% penicillin and streptomycin) (Coulman et al. 2006; Pearton et al. 2008). Skin was stored at -20°C for a maximum of 6 months, before being left to thaw to room temperature for 1 hour prior to use. All experiments using *ex-vivo* skin samples were performed in a Containment Two designated laboratory. In all cases, the skin was pinned down to a corkboard support before the application of microneedles mounted to inverted-syringe plunger and the foam-tipped applicator.

The 2 least painful applications, that is the 2 with the lowest MPQ-SF scores were the foam-tipped and inverted-syringe (Figure 2.3). Therefore these were then tested to determine if they could effectively apply a microneedle array to facilitate effective skin puncture in human skin samples *ex-vivo*.

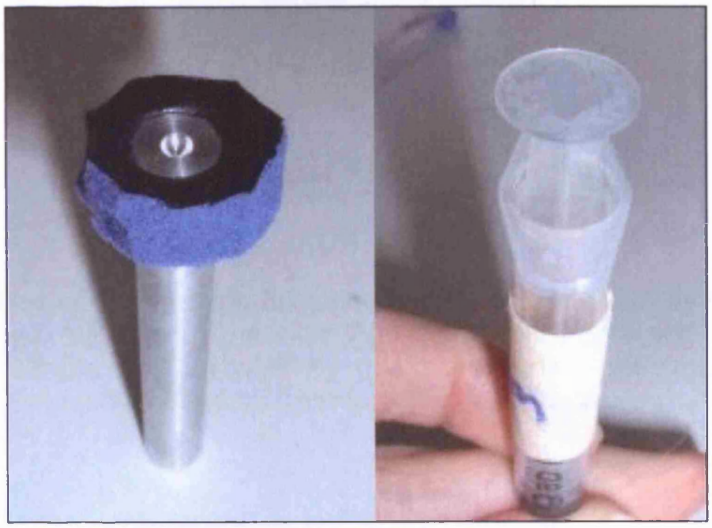


Figure 2.3. Foam-tipped applicator [E] and inverted-syringe applicator [A].

Three different application techniques were selected and tested to determine which was the most effective method:

1] Rolling the array firmly onto the skin from a 45° angle to the skin surface, massaging the applicator for 10 seconds whilst in the vertical position and then rolling the array off the skin 45° from the skin (Figure 2.4)

2] Pushing the applicator straight down perpendicular onto the skin, massaging the applicator for 10 seconds then lifting it off the skin.

3] Stabbing the applicator down perpendicular on to the skin with force, holding down for 10 seconds and then lifting off the skin.

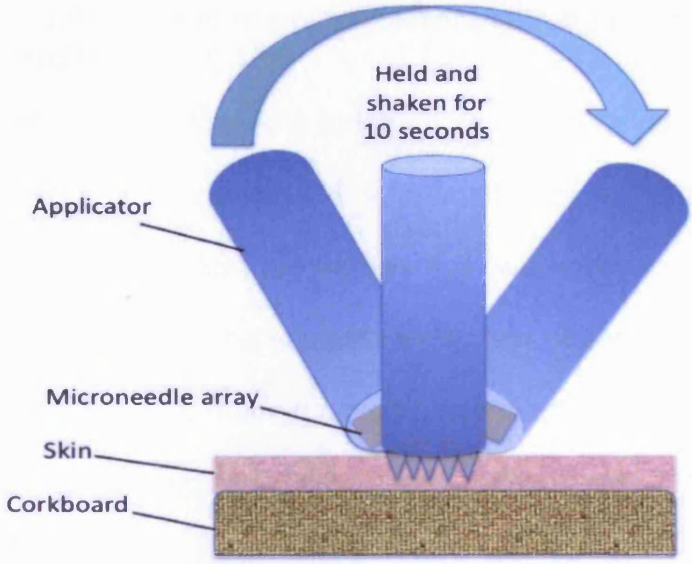


Figure 2.4. Method of applying the microneedle arrays to the skin by mounting on an applicator rod and rolling onto the skin. Applicator rolled onto skin at a 45° angle through to the upright position whereby the microneedles penetrate the skin and massaged for 10 seconds, before being rolled off at a 45° angle.

2.2.4.1 Pre-clinical assessment of microneedle puncture efficiency using TEWL

Transepidermal water loss (TEWL) is used clinically as a measure of skin permeation. Thus TEWL was used to assess skin permeation post-application of 280μm microneedles to *ex-vivo* skin samples. Immediately after each application, testing the techniques in Section 2.2.3, TEWL measurements were taken using a DERMALAB TEWL probe and unit from Cortex Technology (Cohen et al. 2009a). The TEWL probe was rested firmly on the skin surface and held perpendicular to the skin with care taken not to blow or disrupt the airflow over the probe. Humidity and temperature readings were taken at regular intervals to ensure the temperature or humidity in the containment room did not increase significantly. If the temperature altered from a range of 18-20°C the reading was discarded and repeated once the temperature returned within range.

2.2.4.2 Topical assessment of microneedle punctures post-application to skin

Immediately after the microneedle-mounted applicator device had been applied, the skin was topically treated with 10% methylene blue dye solution for a minimum of 5 minutes before the excess dye was wiped from the skin surface using 70% ethanol wipes. The samples were then observed *en-face* under light microscopy. The puncture made by each microneedle array for each application was visualised by the blue staining, counted and recorded.

2.2.5 Microscopic characterisation of the microneedle arrays to be used for clinical trial

Each microneedle array can vary in morphology whilst some may be damaged during production, thus prior to use each microneedle on every array was thoroughly inspected by SEM and light microscopy and each array catalogued into a database.

2.2.5.1 Characterisation by light microscopy

Due to the cost of SEM, each array was initially assessed under light microscopy to examine for any larger defects to the surface or morphology of the microneedle arrays. The mounted microneedle array was carefully positioned on the stage of the Olympus BX-50 microscope, illuminated with a fibre optic light source and photographed. The angle and orientation of the each array was altered systematically to visualise every side of each microneedle on each row.

2.2.5.2 Characterisation by scanning electron microscopy

All microneedle arrays used in this study were characterised by scanning electron microscopy (SEM). SEM is a high magnification, high resolution, imaging technique containing a heated tungsten filament with an electron gun above the sample stage sealed in a vacuum chamber. The sample microneedle

array was mounted onto an aluminium stub and placed on the stage of the Philips XL-20 Scanning Electron Microscope. Sputter coating with gold was not required as the platinum-coated microneedles were sufficiently electron dense. The sample was irradiated by a thin beam of electrons resulting in a secondary emission of electrons from the sample surface that were collected by the detector and produced a signal which was translated into a two-dimensional image.

2.3 Results

Thirteen volunteers were used to test the levels of pain and sensation caused by 4 application devices. Initially, two applicator designs were selected from the 7 designs that were administered to Volunteer 1 (V1) based on their level of comfort during application. These applicators, the foam-tipped rod and inverted-syringe plunger, were compared to the square-ended [Y] and cylindrical [Z] metal rods. Subsequently, *ex-vivo* testing of 280μm length microneedles mounted to the syringe plunger determined a suitable application technique for consistent skin puncture.

2.3.1 Pain and sensation assessment of different applicator devices on human volunteers

A preliminary study aimed to establish the simplest method of applying microneedles with the resources available. As microneedles were already being applied in the laboratory by means of mounting an array onto a rod, variations on this setup were used to establish the simplest applicator which caused the least sensation and pain. Initially 7 applicator devices (Figure 2.2) were applied only to V1, who then commented what they felt during the application of each device. Based on this rudimentary assessment, the foam-tipped and inverted-syringe applicators (Figure 2.3) were compared to the original square-end [Y]

and cylindrical [Z] applicators (Figure 2.1). These 4 applicators were tested on 13 human volunteers, including V1.

2.3.1.1 Audio-recorded comments from applying all seven applicator devices to a single volunteer

Recorded comments made by the subjects during the application of the applicator devices were verbatim transcribed (Table 2.1). These comments determined which 2 applicator devices would be compared against the aluminium square-ended [Y] and cylindrical rods [Z].

Table 2.1 verbatim-transcribed comments vocalised by Volunteer 1 (V1) when each applicator [A]-[G] was tested on the forearm.

Device	Verbatim-transcribed comment
[A]	*"Does not feel sharp on the skin. Pressure can be felt but no pain."*
[B]	*"Causes less sharpness than the square applicator but feels very similar to the round applicator."*
[C]	*"Similar to applicator B, causes less sharpness than the square applicator but feels very similar to the round applicator."*
[D]	*"Feels the same as the round applicator."*
[E]	*"Feels soft on the skin. Causes no pain or does not feel sharp."*
[F]	*"Cannot really feel the applicator on the skin. Causes no pain."*
[G]	*"Similar to applicator F, causes no pain."*

2.3.1.2 Assessing pain and sensation using the MPQ-SF post-application of 4 applicator devices

The pain and sensation of 4 applicators, the 2 original applicators of square-end [Y] and cylindrical [Z] morphology and the 2 new applicators (inverted syringe plunger [A] and foam-end cylindrical rod [E]) were compared against one another to determine which of the 2 were the least painful upon administration to the volar forearm of the 13 (including V1) study participants.

Table 2.2 summarises the overall scores and percentage from the MPQ-SF the participants completed immediately post-application. The MPQ-SF was scored in accordance with Melzack's scoring (1987) whereby the VAS measurement from 0-100mm was summed with the scores, none = 0, mild = 1, moderate = 2, severe = 3, for each descriptor word and the present pain index of 0-5.

Table 2.2. Overall totals calculated from the MPQ-SF for simple comparison of pain and sensation between the types of applicators.

	Applicator type			
	Square-ended [Y]	**Cylindrical [Z]**	**Foam-tipped [E]**	**Inverted-syringe [A]**
Total Score	182	117	44	79

The standard square-ended [Y] and cylindrical [Z] rods both caused greater sensation and pain when compared to either the foam-ended [E] or inverted-syringe [A].

2.3.1.3 Audio-recorded comments when 4 applicator devices were applied to 13 human volunteers (including V1)

All volunteers were asked to vocalise any sensation felt during the application of each device, though participants 12 and 13 required further prompting by the researcher asking *"anything?"* or *"did you feel anything then?"* at the time of application to encourage oral commentary. Table 2.3 contains all the verbatim-transcribed comments that each participant vocalised during the application of the 4 different applicator devices.

Table 2.3. Verbatim-transcribed comments vocalised when 4 applicator devices were applied to 13 human volunteers

Participant No.	Applicator device			
	Cylindrical metal rod [Z]	**Square-end metal rod [Y]**	**Foam-tipped metal rod [E]**	**Inverted-syringe plunger [A]**
1	"Cold feeling. Doesn't feel too bad but I felt a bit of a sharp edge and some pressure."	"That feels quite sharp. When the device went on it felt sharp uh then when you applied further pressure it felt sharper but I can't feel anything now."	"Something being applied to my skin but no real sharp sensation. Doesn't feel too bad at all. It's just like someone pressing their finger against your arm"	"Not feeling anything at the moment. Can feel the device going onto my arm. Doesn't hurt just felt it going on really. Didn't feel any pain"
2	"Just slight pressure, no pain."	"That felt colder. No pain but it felt a bit sharp"	"I can just feel a slight pressure but there's no pain."	"No pain, colder than the first one [foam]. Slight pressure again but felt different to the first one [foam]. The pressure felt more concentrated."
3	"A bit of pressure but I think that's your finger. Oh now something a little bit sharp."	"Much sharper. The applicator is cold. Definitely felt that."	"Can't really feel much. Can feel something cold. A bit of a touch and a shake. No pain."	"Can feel a bit of a pressing sensation. Little bit of sharpness but nothing painful as such. Now I can't feel anything."
4	"Feels a bit cold. I can feel something there but it's not as pressing as the last one [square]"	"I can feel something. It's not painful but something pressing. Now I can't feel anything."	"Can't feel anything. That's cold. Pressing a bit but not as much as the others."	"Something is in contact with my skin but there's no pain or discomfort."
5	"Um same as before really [square]"	"Cold, kind of a pressure sensation but nothing major"	"Same as the last one [syringe]. Nothing at all. I'd say the first two [square and round] were a slight sensation but not really pain and nothing for the last two [syringe and foam]."	"No pain at all. Probably less than the first 2 [square and round]"
6	"I can feel something touching my skin. I know something's there."	"Something really sharp. That was quite painful."	"Just a gentle touch."	"There is something touching my skin. There is no pain."

Table 2.3 continued...

Participant No.	Applicator device			
	Cylindrical metal rod [Z]	**Square-end metal rod [Y]**	**Foam-tipped metal rod [E]**	**Inverted-syringe plunger [A]**
7	"I can feel you hand, I think now something pressing. Something wiggling around a bit. I can feel a kind of pressure."	"Something with a sharp edge, wiggling around. Pressure and sharpness."	"I can feel your hands then a cold sensation."	"Pressure, a little bit sharp."
8	"Can't really feel much at all. No, nothing. Just pressure."	Can't really feel much at all. Little bit of a pin prick feeling but not painful."	"No nothing, well a little bit of pressure but no sharpness or pain or anything."	"Can't really feel much again, like the second one [round]."
9	"No pain."	"Oh, that was quite sharp."	"Nothing that's fine.	"Could feel it but no pain at all."
10	"Nothing, just a bit of pressure."	"That feels a bit sharp."	"I can feel something there. Just a little bit of pressure."	"Can't really feel anything."
11	"Nothing, bit of pressure."	"Felt stabbing."	"Nothing at all."	"A prick."
12	[prompted with "Did you feel anything then?"] "No, nothing at all."	[prompted with "Anything with this one?"] "Um yes, it didn't actually hurt but I could feel something touching my arm."	[prompted with "Anything?"] "Nothing at all."	[prompted with "Did you feel anything?"] "No, not really. Little bit of pressure I guess."
13	"[Prompted with "Anything then?"] "Not really, little bit more than the other one but nothing really"	"Ooh a bit sharper than the others but it didn't hurt."	[Prompted with "Did you feel anything?"] "Not really, little bit of pressure, but no pain."	[Prompted with "Anything?] "Nothing."

Whilst 7 participants stated the square-end [Y] applicator felt *"sharp"* or *"sharper"*, the foam-tipped applicator [E] elicited comments of *"no pain"* from 4 participants. Overall the verbal comments imply the least painful and discomforting applicators are the foam-tipped [E] and the inverted-syringe [A].

Based on these results, the foam-tipped applicator [E] and the inverted-syringe applicator [A] were selected for potential use as applicators for the clinical trial. However, due to the ease and convenience of supply, the inverted-syringe applicator was the only one used for *ex-vivo* testing. The syringes were simple inverted, surface ridges on the plunger were smoothed down with heat and an array was adhered using epoxy to the surface. Finally each applicator was visually inspected under light microscopy to be of the same design.

2.3.2 *Ex-vivo* skin testing of applicators

A 280μm silicon microneedle array was mounted to the inverted 2ml syringe plunger. This was then applied to excised human skin using 3 different application techniques explained in Section 2.2.3. An example *en-face* photograph of methylene blue stained skin post puncturing when microneedles were applied using the rolling technique is shown in Figure 2.5. Whilst the microneedles appeared to puncture the skin efficiently, the intensity and spread of blue stain at each channel was not completely consistent within a single array pattern. The percentage increase in TEWL post-application of microneedle arrays using the 3 differing application techniques, as well as the visualised number of methylene blue stained punctures caused by the microneedle applications are graphically represented in Figure 2.6. Figure 2.6

evidences that punching the applicator onto the skin surface elicited greater TEWL from the skin surface, though all the microneedles did not penetrate the skin surface.

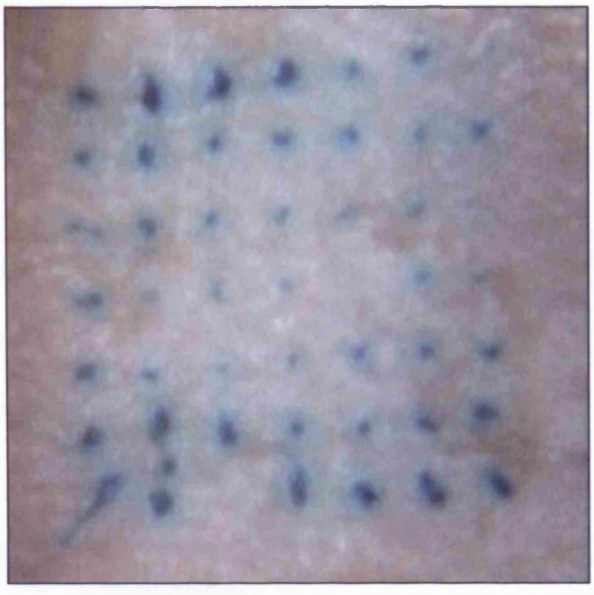


Figure 2.5. Example photograph of methylene blue stained channels visualised under the light microscope post-application using the rolling technique of a 7 by 7, 280μm microneedle array to *ex-vivo* skin.

As TEWL is a difficult technique to use and often prone to variance in the external environment (Cohen et al. 2009a) TEWL measurements were repeated with 25 separate applications: 5 different samples, each from a different female donor (as described in Section 2.2.4) and each had 5 separate applications. 'Rolling' the microneedles by rotating an array through 45° onto the skin provided a simple and consistent method of application with mean TEWL increase post-application of device of 85% (standard deviation (SD) = 5%) and mean of 35 (SD= 1) (out of 36 possible) punctures stained by methylene blue. Pushing and punching the microneedle device onto the skin resulted in a mean TEWL increase of 34% (SD= 15%) and 81% (SD= 6%)

respectively, whilst the number of stained microneedle punctures were 16 (SD= 7) and 23 (SD= 5), respectively for the pushing and punching application techniques. The only method that resulted in all 36 of the 280μm length microneedles penetrating the skin was the rolling technique.

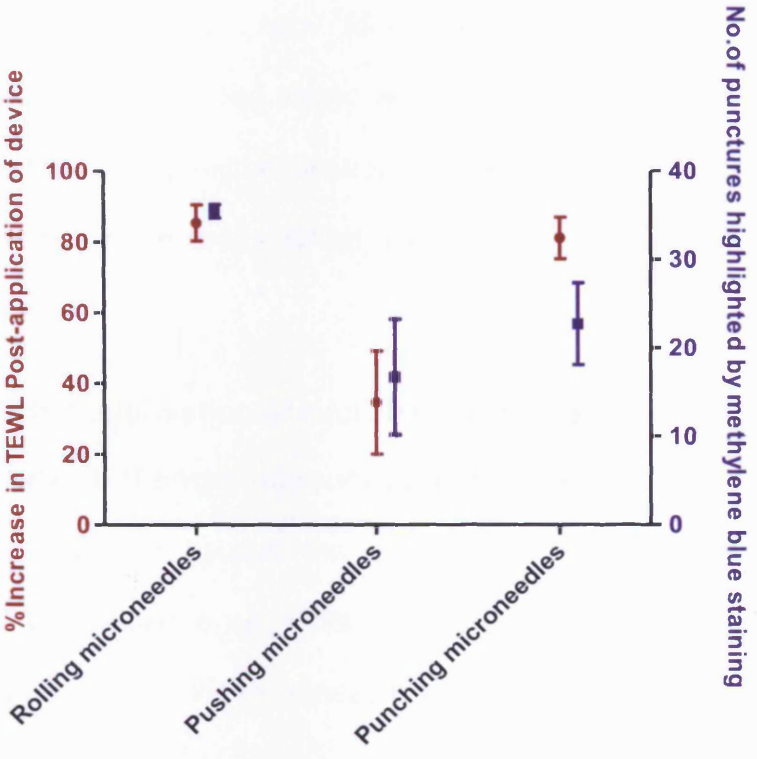


Figure 2.6. Testing the puncture efficiency of 3 application methods. Each of the 3 application methods was tested *ex-vivo* on human skin to see which produced consistent punctures from a total of 36 microneedles. TEWL measurements (Red) were taken prior to methylene blue staining (blue) and the percentage increase post-application of the microneedle device was recorded. The rolling, pushing and punching techniques had a mean increase in TEWL of 85% (SD= 5%), 34% (SD= 15%) and 81% (SD= 6%) respectively. Whilst the number of identifiable punctures following the rolling, pushing, punching techniques was 35 (SD= 1) 16 (SD= 7) and 23 (SD=5) respectively.

2.3.3 Microscopic analysis of the microneedle arrays pre-application

All the 280μm and 180μm microneedle arrays used in the clinical trial where checked for damage by SEM and stereomicroscopy prior to use. Example images, in Figure 2.7, of 180μm and 280μm microneedle arrays demonstrate the robust and precise production etching process, which produces arrays consisting of 36 intact and identically shaped octagonal-pyramid microneedles. Three damaged arrays were removed from the stock that was to be used in the clinical trial because sharper and stable microneedles would penetrate the skin more easily, whilst also reducing the risk of microneedles breaking in the skin.

2.3.4 Characterisation of microneedle arrays post skin application

Single applications of silicon microneedle arrays onto the skin without using an attached applicator caused the base of the fragile array to fracture. However when applied once whilst attached to the applicator rods and inverted syringe plunger, the microneedle arrays did not fracture or fragment.

2.4 Discussion

All novel technologies and drugs for human use are required to be extensively tested in the laboratory prior to administering to human volunteers. Therefore, trials are conducted in phases for laboratory, animal and human testing. For successful ethical approval, supporting data of all the techniques and the relevance of all treatments to participants must be documented. Therefore, pre-clinical testing is conducted in the laboratory to establish safe working parameters. In this chapter, the application of microneedles to human

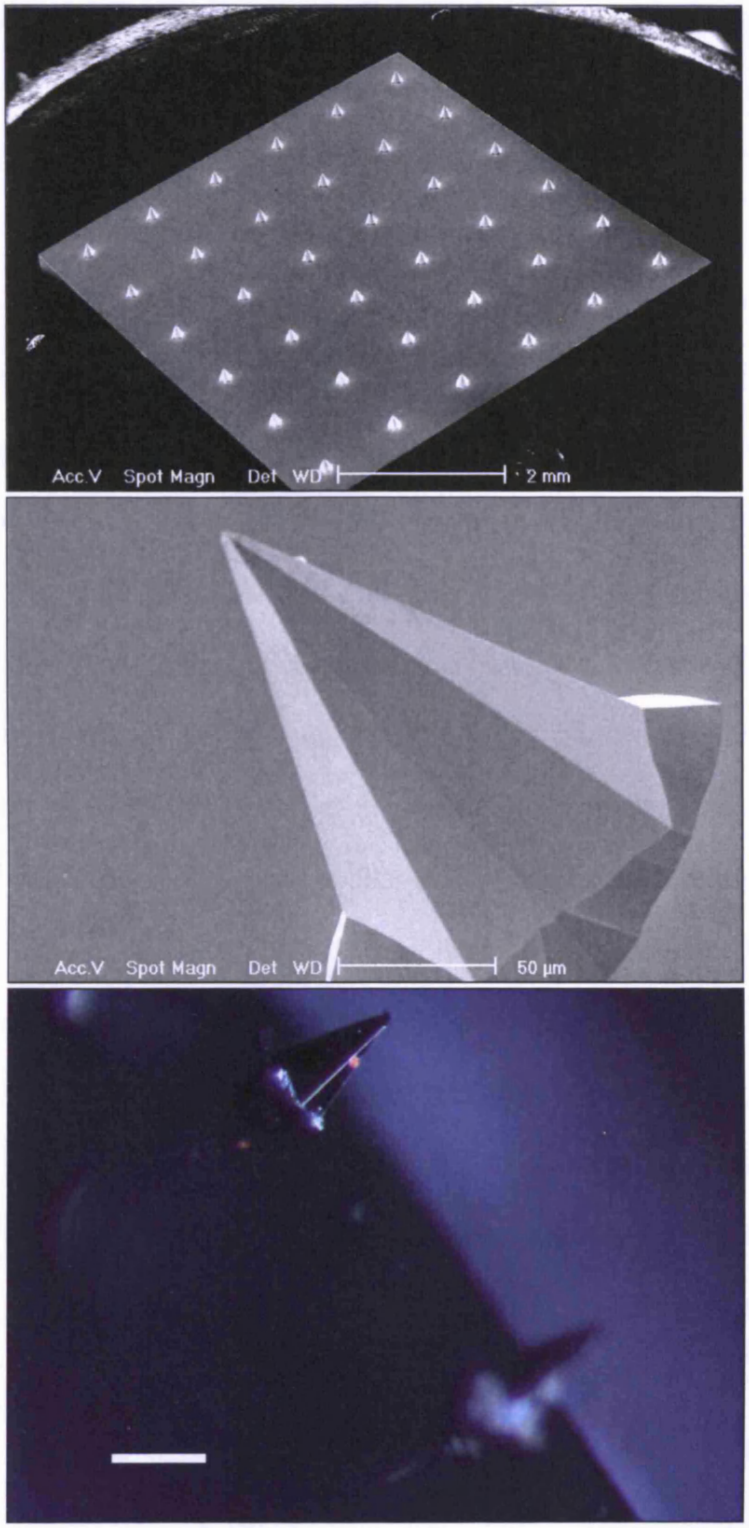


Figure 2.7. SEM micrographs and stereomicroscopy of the microneedle arrays pre-application. [A] SEM of array consisting of 36-microneedles each of 280µm length.[B] 280µm length single microneedle. [C] Stereomicroscopy image of intact 280µm length microneedle (bar = 280µm).

volunteers was considered. Thus a rudimentary applicator was developed to apply microneedles to the skin and tested, *ex-vivo*, to ensure the applicator improved skin penetration.

Whilst single hollow microneedles penetrate the skin successfully (Alarcon et al. 2007), the morphology and design of microneedle arrays is being optimised (Al-Qallaf and Das 2009). However, to-date, *in-vitro* animal studies (Koutsonanos et al. 2009; Li et al. 2009), and *ex-vivo* (Coulman et al. 2006) and *in-vivo* (Kaushik et al. 2001; Wermeling et al. 2008) studies on human skin have administered microneedle arrays by pushing the array into the skin surface without any specialised applicator design. Though metal or wooden rods (Coulman et al. 2006; Henry et al. 1998) and syringe barrels (Sivamani et al. 2005) have been used as applicators. Therefore, to understand and standardise the application process for applying microneedles to human skin, 9 applicator designs ([A]-[G], and [Y] and [Z]) were tested without microneedles to provide data on how participants' perception would be affected by the applicator used. Furthermore, the instruments for assessing the treatments, such as, the VAS, MPQ-SF and audio recording were tested in an effort to practice and assess their use prior to the main clinical trial.

The initial pre-clinical testing of 7 applicators [A]-[G] on Volunteer 1 (V1) provided a quick method of testing the audio recording process and selecting 2 of the applicator designs. Though this did not provide any statistical evidence for selecting the inverted-syringe plunger [A] or the foam-tipped rod [E], comments from V1 suggested these 2 designs as the least painful or discomforting (Table 2.1). The inverted-syringe plunger [A] provided

a cheap and effective applicator device, which was readily available in the laboratory and clinic. Based on this availability and less discomforting sensations felt by the 13 volunteers, the inverted-syringe plunger [A] was selected for *ex-vivo* testing. Furthermore, study time for recruitment of volunteers and assessment was limited. Therefore, each of the 7 applicators ([A]-[G]) were not applied on the remaining 12 volunteers, though repeats may have provided more conclusive evidence for which applicator design is most comfortable.

Though microneedles, when attached to an inverted-syringe [A] and applied using the rolling application technique, provided efficient and repeatable penetration to *ex-vivo* human skin, specialist devices may improve the application further. For example, Ding et al (2009) demonstrated effective penetration of an array of 300μm length microneedles using an electric applicator device, whilst Yang et al (2004) reduced penetration force using vibratory actuation.

The microneedles were characterised by SEM and light microscopy, which proved useful methods for assessing the stock of microneedle arrays. However, SEM is an expensive process (Zhou and Wang 2007), thus cheaper light microscopy was used to image and catalogue each microneedle array, whether consisting of 180μm length or 280μm length microneedles (Bal et al. 2008; Jin et al. 2009; Pearton et al. 2008; Verbaan et al. 2007). Thereby ensuring that the microneedles used in *ex-vivo* testing were morphologically

intact and identical in their layout, which reduced the potential for microneedles to break during application due to defects in their structure.

Pyramidal shaped silicon microneedles produced by wet-etching have previously been demonstrated to be morphologically identical (Wilke and Morrissey 2007; Wilke et al. 2005b) and puncture skin efficiently by rolling an applicator mounted microneedle array across the skin surface (Coulman et al. 2006; Pearton et al. 2008). Future investigations, using SEM and light microscopy to observe microneedle applications, could determine optimum microneedle design for successful penetration. Novel techniques using optical coherence tomography could allow observation of microneedles penetrating the skin in real-time and characterise the effect of applying microneedles to the stratum corneum and epidermis (Coulman et al. 2010; Kim et al. 2009a)

Applying microneedle arrays to the *ex-vivo* skin successfully required practice. Initial applications resulted in the force required to push microneedles into the skin being dissipated through the base of the applicator resulting in reduced microneedle penetration. TEWL provided a secondary measure of skin integrity and permeability. When applying a large force to the skin surface, the skin deforms and releases moisture from pores and follicular ducts (Bal et al. 2008), therefore, greater force does not necessarily represent an improvement in penetration by the microneedles on an array (Ayittey et al. 2009).

Using the rolling application technique possibly reduced the "bed of nails" effect as each row of needles on the array came into contact with the skin in

phases rather than simultaneously (Sivamani et al. 2007; Teo et al. 2005; Yang and Zahn 2004). Therefore, first the microneedles closest puncture the skin, then the adjacent row and so on until the whole array is being evenly pushed into the skin (when the applicator is in the vertical position). The forces induced by massaging the applicator through the vertical position for 10 second increases pressure at the microneedle tips, thereby improving puncture efficiency. Though the rolling technique remains a crude method of application, it was well rehearsed and practised to ensure repeatable successful puncture of the skin. Future studies, using pressure sensors mounted to the end of the microneedle applicator, could determine the mechanical forces needed for microneedles to puncture the skin (Davis et al. 2004; McAllister et al. 2003). Whilst further development of the applicator design could incorporate a mechanism on the applicator so that excessive force is not used, as this may cause greater pain and negative sensation for the patient.

Oral commentary by volunteers acted as a spoken diary of the complete application process (Clarke 2009; Lewandowski et al. 2009) and highlighted key descriptors, 'pressing', 'pricking' and 'cold', which were absent from the MPQ-SF. These words are present in the long-form MPQ (Melzack 1975), however and may benefit from being included in the MPQ-SF for better understanding the intensity of these specific sensations, thus the MPQ-SF was adapted for use in the clinical study (Chapter 3) by including 'pressing', 'prickling' and 'cold' as descriptors. The oral commentary provided supportive evidence by highlighting the exact perceptions with key details and analogies given by the participants (Arber 2007; Forbes et al. 2000; van Tilburg et al.

2009). For example, the metal applicator rod design was scored as more painful using the MPQ-SF. However, the audio commentary enabled the participants to express exactly which part of the application process attributed to perceptions of pain and sensation. Thus, the adapted MPQ-SF in conjunction with the audio recording would provide a more complete picture of an individual's assessment of pain and sensation caused by the microneedles themselves during the clinical study (Chapter 3). Oral commentary has not been used in any previous study of microneedle design, however V1's comments in Table 2.1 demonstrate that audio recording provided a suitable method of real-time data collection.

2.4.1 Conclusions

The pre-clinical studies of applicator design provided evidence that a softer material, such as the smooth plastic of an inverted syringe provide a cheap, simple and effective applicator, which could be applied in a clinically acceptable manner by rotating the applicator over 45° degree after applying at a 45° angle to the skin surface for efficient and successful penetration of the stratum corneum. Testing the standard MPQ-SF highlighted that whilst it remains a useful tool for comparing the perception of applicators, the addition of three descriptors may enhance the data collected. Furthermore, audio recording provided an added dimension of understanding to the types of pain and sensations felt during the administration of different applicators to volunteers.

Chapter 3

Assessing the pain and sensation caused by the application of microneedle devices and a hypodermic needle to 12 healthy volunteers

3.1 Introduction

Chapter 2 explained the pre-clinical developmental and evaluation stage in which the instruments to measure pain and sensation were investigated and tested on human volunteers. The pre-clinical development of data collection instruments, as well as the pre-clinical testing and characterisation of microneedles, helped inform the clinical study reported in this chapter. Conducting a clinical study requires time for recruitment and logistic management to ensure staff and participants can attend (Perri et al. 2006; Raynor et al. 2009), whilst also ensuring instruments for data collection are not confusing to use (Vanichseni et al. 2004). Therefore, Chapter 3 outlines the clinical trial methodology and reports the pain and sensations experienced by volunteers following application of silicon microneedle arrays and hypodermic needles to the buttock region.

3.1.1 Assessing pain perception

As introduced in Chapter 1, microneedle-based applications of drugs or vaccines are being investigated in humans, thus it is important to understand the nature of pain induced by microneedles, whilst also confirming the efficient puncture of skin. Pain has been defined in Section 1.1.4.1 by the International Association for the Study of Pain (2009). The administration of needles for vaccine delivery causes pain due to the needle impacting upon pain receptors residing in the dermal layer of skin (Arendt-Nielsen et al. 2006). Fear associated with needles can also increase the perception of pain (Hanas et al. 2000; Nir et al. 2003). Through better understanding pain, medical interventions can be improved to afford greater comfort to patients, whilst

ensuring successful delivery of a drug. Pain can be described by its intensity, descriptive words and location on the body. The various methods of assessing pain have been discussed in Chapter 1 and in Chapter 2 where visual analogue scale (VAS), short form McGill pain questionnaire (MPQ-SF) and audio recording were tested as assessment methods for establishing intensity of pain and sensations of applicator rods.

The pain and sensation assessments detailed in this chapter only consider the level of penetration caused by the application of microneedles and hypodermic needles, as no substances were administered using the needle devices. As reviewed in Section 1.1.4.6, previous studies did not attempt to use specific instruments to record the intensity of pain, but rather used subjective notes and VAS whilst simultaneously confirming that microneedle penetration was successful *in-vivo* (Gill et al. 2008; Kaushik et al. 2001; Miyano et al. 2005; Sivamani et al. 2005). Therefore the types of pain and sensation were not consistently assessed. Furthermore neither the 180μm nor 280μm length silicon microneedle arrays have previously been tested on human volunteers.

3.1.2 Conducting clinical trials

The Medicines and Healthcare products Regulatory Agency (MHRA) defines a clinical trial as "an investigation in human subjects which is intended to discover or verify the clinical, pharmacological and/or other pharmacodynamic effects of one or more medicinal products, identify any adverse reactions or study the absorption, distribution, metabolism and excretion, with the object of ascertaining the safety and/or efficacy of those products. This definition

includes pharmacokinetic studies" (MHRA, 2010). MHRA regulations only apply to trials of medicinal products where "substances or combinations of substances which either prevent or treat disease in human beings or are administered to human beings with a view to making a medical diagnosis or to restore, correct or modify physiological functions in humans" (MHRA, 2010). Based on the MHRA's definition of what requires a clinical trial authorisation (CTA), the research undertaken within this thesis involving only medical devices (microneedle devices) does not require a CTA, however, as a novel medical device was tested on human volunteers, the terms 'clinical trial' or 'clinical study' are used interchangeably in this thesis. Therefore clinical studies answer 2 major questions: is the new treatment effective in humans and is it safe for use on humans.

Whilst the clinical research in this chapter was not testing any new drugs or therapies in humans, understanding of "Good Clinical Practice" (GCP) guidelines (Dupin-Spriet 2005; EMEA 2002) and conducting the study in accordance with the ethical principles established by the Declaration of Helsinki (WMA, 2008), provided a framework for the conducting the research. Prior to conducting a trial, any risks must be weighed up and the benefits must outweigh and justify the risks for a trial to continue. The safety, legal and ethical rights of all participants should prevail over any scientific interests, thus fully informed consent must be freely given by all participants prior the trial. The study can only begin once an independent ethics committee has reviewed and confirmed compliance of the protocols and associated materials such as the patient information sheet and consent forms. Thus it was essential to develop a clear protocol for the whole trial process, to ensure the

same information was provided to each participant at recruitment and justify the purpose of the study.

A qualified physician must be responsible for any treatment administered during the trial. All information produced from the trial should be recorded in 'case report forms' (CRF) and stored in such a way that allows its accurate reporting, interpretation and verification as well as protect the participants' identities in accordance with regulatory requirements (EMEA 2002; Schmidt and Frewer 2007). Therefore a CRF was produced (Appendix VIII) to include the timetables for each researcher and participant, as well as, the data collection instruments, such as the VAS, adapted MPQ-SF and transepidermal water loss (TEWL) and instruction for the participants.

Ex-vivo tests (Chapter 2) have shown the difficulty of administering 280μm microneedles to human skin. Furthermore, Chapter 2 highlighted the potential problems of applicator choice and application technique that may arise when applying i*n-vivo* to human volunteers. *In-vivo,* different anatomical regions of the skin underlying muscle and adipose tissue alter the rigidity and shape of the skin (Batisse et al. 2002; Montagna et al. 1992; Welzel et al. 2004). Therefore the location of the clinical microneedle and hypodermic needle applications needed to be carefully considered.

The clinical study was conducted to determine whether silicon microneedles of a maximum length of 280μm cause pain when administered to human volunteers. Furthermore, microneedles of this morphology have never been

tested *in-vivo*. Thus, it is important to determine the intensity of pain and sensations induced in relation to a 25G hypodermic needle subcutaneous injection.

3.1.3 Aims and objectives

This chapter aims to show that the pain and sensation elicited by the application of microneedles is less than subcutaneous administration of 25G hypodermic needles to human volunteers.

Objectives:

- To use VAS to assess and quantify the intensity of pain elicited when microneedles of 180μm and 280μm length, and a 25-gauge hypodermic needle, are applied *in-vivo* to human volunteers.
- To identify pain and sensations using an adapted MPQ-SF post-application of microneedles and hypodermic needle.
- To establish any additional benefits of the use of audio recording as a method of recording important descriptions of pain and sensations experienced by participants following the application of 180μm and 280μm length microneedle arrays and a 25-gauge hypodermic needle.

3.2 Materials and Methods

3.2.1 Materials

Sanyo TRC-525M dictaphone (Sanyo, Japan); Digital VAS meter DVAS10 (Cardiff Biometrics Ltd, Wales); methylene blue dye powder (Sigma, UK); 25-gauge hypodermic needles (BD, Oxford, UK); microneedle arrays containing

36 pyramidal microneedles of either 280μm or 180μm length (Tyndall National Institute, Ireland).

3.2.2 Ethical approval and consent

Prior to beginning the study, the study protocol (Appendix III) was submitted for ethical approval from the Gwent Healthcare NHS Trust R&D Committee and the South East Wales Research Ethics Committee (SEWREC). Ethics approval for the study was granted. Informed consent was obtained from each participant in adherence with the Declaration of Helsinki and a written explanation of the study provided before their first visit. At the first visit each participant read and signed consent forms (see Appendix VIII) after the nature of the study had been fully explained. Participants were informed that they could withdraw from the study at any stage without any explanation.

3.2.3 Personnel roles for the clinical study

Three main roles were assigned to ensure successful management of the trial. The lead physician, Dr. Anstey, was responsible for the ethics and medical support of this study. The Study Coordinator, Mohammed Haq, developed the specific details for the study, coordinated the trial activities and monitored adherence to good clinical practice (GCP) and ethical guidelines, together with the lead physician. The assisting physician, Dr. Kalavala, was responsible for clinical applications of needles and biopsy sample extraction from the volunteers, whilst ensuring their medical wellbeing and suitability for the study. In addition, Dr. Birchall and Dr. John supervised the study and assisted in data collection for pain and sensation and Miss. Smith assisted with transepidermal water loss (TEWL) data collection by placing the TEWL probe on female participants.

3.2.4 Clinical application of microneedles

Chapter 2 helped to establish an applicator device that was shown to be of minimal discomfort and acceptable for use in this study. Accordingly, the 180μm and 280μm silicon microneedle arrays were adhered to the flattened base of inverted syringe plungers as detailed in Chapter 2. These were sterilised by soaking in 70% ethanol for 12 hours in an aseptic laminar flow hood and then placed individually into pre-sterilised sealed bags until use. To aid the clinician when applying the devices, each syringe was colour coded to represent the length of microneedles mounted to it, thus the clinician was always aware of which device had been applied. Thus, avoiding the same device accidently being applied twice to a participant.

3.2.4.1 Anatomical location selected for needle device application

Ethics committee approval for biopsy of the puncture sites required an anatomical site that would not cause obvious scaring to the participant. Therefore the buttocks were chosen as the application site for all microneedle and hypodermic testing. The buttock was defined to be any region of the gluteus maximus muscle. However, as the buttock varies in elasticity and sub-dermal fat deposition depending on age, diet and gender, it was important to ensure a consistent location for application between volunteers. Therefore the application sites for each participant was the peripheral buttock region closer to the femur (Figure 3.1). This region of the buttock has less fat deposition and is firmer in muscle (Inan et al. 2005; Schilling and Wechsler 1986), thus the fleshy but less fatty nature is better representative of other anatomical regions where the microneedles may ideally be applied, for example, on the volar forearm or bicep region of the arm. Three sites, A, B and C were marked

on each buttock. Sites A, B and C were each used for only one microneedle or hypodermic needle application depending on which needle device was randomised to the particular site (see Section 3.2.5).

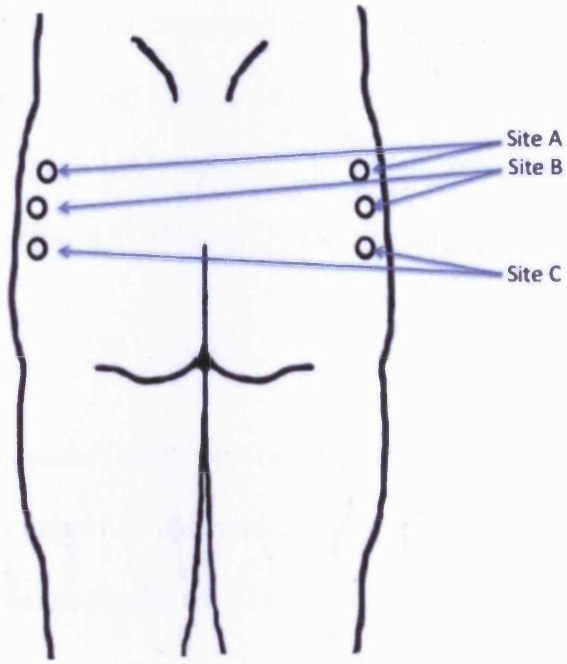


Figure 3.1. Diagram to show the location of each application site on both buttocks.

3.2.5 Trial Randomisation

Randomisation helps to reduce bias, including the effects of hidden variables and uncontrolled factors that might change over the length of the study (Robinson et al. 2005; Schulz et al. 1996; Toroyan et al. 2000). The clinical study was conducted with the 12 participants randomised into 4 groups based on the order of applications and time of biopsy:

Group 1: 3 participants having biopsies at 1 hour.

Group 2: 3 participants having their biopsies at 4 hours.

Group 3: 3 participants having their biopsies at 8 hours.

Group 4: 3 participants having their biopsies at 24 hours.

A randomisation order was generated and tabulated (Table 3.1) to randomise which group each participant belonged to, based on their biopsy time (group 1 - 4); which buttock they will have either the TEWL measurements or biopsy taken from (R = right, L = left); the order in which each needle device will be applied to both buttocks (1^{st}, 2^{nd} or 3^{rd}); and the site of application (A, B or C) in relation to Figure 3.1.

The visual analogue scale (VAS) and perception questionnaire components of this study were conducted in a single-blind manner, whereby the participant did not know the type of application being applied, though the assessor was aware of which applicator was used. Each participant was treated on each buttock with 2 different microneedle arrays consisting of 36 pyramidal microneedles of either 180μm (180 MN) or 280μm (280MN) height, or a 25-gauge hypodermic needle.

3.2.6 Study Design

The trial was conducted in the Dermatology Day Unit at St. Woolos Hospital, Newport. Pain and sensation was assessed using the VAS and adapted short form McGill pain questionnaire (MPQ-SF) after applications of needle devices on either the right or the left buttock. Subsequently, following two 6mm biopsies, one from each microneedle array application site, and one 4mm biopsy from the hypodermic needle application site, wound healing was

Table 3.1. Randomisation table showing the randomisation for each participant in terms of their group and location of each specific needle application (A, B or C in relation to Figure 3.1) and which buttock TEWL is measured on as well as which buttock pain, perception and biopsies were taken from (R = right buttock, L = left buttock in relation to the assessor when facing the buttock). 180 MN = 180μm length microneedle array; 280 MN= 280μm microneedle array; and hypodermic = 25G hypodermic needle.

Key:

Group 1 = Biopsy at 1hr post-application
Group 2 = Biopsy at 4hr post-application
Group 3 = Biopsy at 8hr post-application
Group 4 = Biopsy at 24hrs post-application

Participant No.	Group No.	Biopsy Buttock	Application Order						TEWL Buttock	Application Order					
			1st Site			2nd Site		3rd Site		1st Site			2nd Site		3rd Site
1	1	R	280 MN	B	hypodermic	C	180 MN	A	L	hypodermic	A	180 MN	C	280 MN	B
2	3	R	hypodermic	B	280 MN	C	180 MN	A	L	280 MN	C	180 MN	A	hypodermic	B
3	1	R	hypodermic	A	180 MN	C	280 MN	B	L	hypodermic	B	280 MN	C	180 MN	A
4	2	L	280 MN	B	hypodermic	A	180 MN	C	R	180 MN	C	hypodermic	A	280 MN	B
5	3	L	180 MN	A	hypodermic	B	280 MN	C	R	180 MN	B	hypodermic	C	280 MN	A
6	4	R	280 MN	C	hypodermic	B	180 MN	A	L	280 MN	B	hypodermic	C	180 MN	A
7	4	L	180 MN	A	280 MN	B	hypodermic	C	R	180 MN	B	hypodermic	A	280 MN	C
8	2	L	280 MN	A	180 MN	B	hypodermic	C	R	hypodermic	C	280 MN	A	180 MN	B
9	3	R	180 MN	B	280 MN	C	hypodermic	A	L	280 MN	A	180 MN	B	hypodermic	C
10	2	L	180 MN	C	hypodermic	A	280 MN	B	R	180 MN	A	280 MN	B	hypodermic	C
11	1	L	hypodermic	C	180 MN	A	280 MN	B	R	280 MN	A	180 MN	B	hypodermic	C
12	4	R	280 MN	C	180 MN	A	hypodermic	B	L	hypodermic	C	280 MN	B	180 MN	A

assessed (Chapter 4). The remaining right or left buttock was used to assess TEWL over a 24 hour period, with measurements taken pre-application, and post-application at 3 time points (immediately, 8 and 24 hours). Biopsy, wound healing and TEWL are described in Chapter 4.

3.2.6.1 Recruitment of participants for the study

The recruitment process for this study spanned over 6 weeks. Initially fliers were posted at St. Woolos Hospital. Each enquirer was checked against the recruitment criteria (Appendix III). If all criteria were met, participants were emailed or posted information packs. However, upon contacting the study coordinator for further information, potential participants voiced unease about exposing the buttock region and the biopsies procedure. Therefore, many enquirers withdrew from the trial. Subsequently, the trial coordinator placed recruitment posters in 5 general practice surgeries and 5 dermatology clinics across Cardiff and Newport; and university departments and postgraduate centres were also contacted by e-mail. All volunteers that participated in the trial were offered £120 honoraria plus travel expenses, a figure based upon the inconvenience for spending time at the unit and ratified by the SEWREC.

Records containing the name, contact details and availability of each potential participant were maintained in accordance with data protection legislation and ethical requirements (Earl-Slater 2002; Flather et al. 2001; Gad 2009). Each enquirer was telephoned and participant information packs were sent by email or delivered in person. During all conversations, discussions were limited to only the information contained in the information pack and consent form, including any safety issues. The discussions did not detail the pain and

sensation measurement methods or specifics details of the randomised applications. This ensured that the participants were fully informed but the coordinator did not bias the participants. If after reading the information and considering the answers to any questions the participants were interested, they were provisionally booked into a trial day and e-mailed their specific timetable (Appendix V).

Subsequently, after the initial 9 participants were recruited, the trial dates of Saturday 10th to Monday 12th March 2007 were confirmed with all participants and study personnel. Upon arrival at the Dermatology Day Unit on the trial day, the participant sat in a quiet, comfortable clinic waiting area. Next the physician obtained the participant's informed consent, which was essential before commencing the trial. The participant was reminded that they could withdraw from the study at any stage.

Meticulous planning ensured each participant was guided through the process independently to prevent interaction between multiple participants (Appendix V). Furthermore, the provision of two rooms allowed one participant to be undergoing the pain perception exercise whilst another was undergoing their TEWL evaluations (see Chapter 4). Thereafter each participant swapped rooms to complete their first visit. Participants were informally escorted between rooms, avoiding the waiting area, thereby preventing interaction with other participants to prevent disclosure of the procedure or their perceptions and sensations.

After the first 9 participants had been processed, the trial was delayed for 2 weeks whilst the clinical coordinator recruited and managed the logistics to process the 3 final participants. It was important that these 3 new participants had not, in any way, discussed the trial with any of the previous participants as to bias their opinions or reveal method protocols prior to their participation. Therefore, before providing any further information, or recruitment, potential participants were asked about their knowledge of the trial. Enquirers with detailed knowledge of the trial were not recruited.

3.2.6.2 Assessing pain and sensation during the clinical study

To assess the perception of the needle applications, the participant completed the VAS and adapted MPQ-SF immediately after the application of each needle device. Data was recorded in the case report form (CRF) (Appendix VIII).

Preliminary tests conducted at the WSP with laboratory researchers suggested that applying the devices prior to the participant becoming comfortable and confident with the procedure would lead to inconsistent or minimal vocalisation and commentary on what they felt. Therefore the CRF (Appendix VIII) took into account the need to 'break the ice' with individual participants to ensure they were confident and comfortable with the study environment and the assessors. To ensure consistency during the trial, each instruction and exact question for the assessor to ask the participant was printed in the CRFs. This also prevented the assessor from deviating in their questions or indeed leading the participant.

3.2.6.3 Summary of the first trial day methodology for assessment of pain and sensation to needle devices

Following written informed consent, each participant changed into a long hospital gown and rested on a medical couch on their front to allow access to their buttock. Initially 1 buttock was exposed and 3 areas marked in relation to a participant-specific template (Appendix VI). The participant was asked to relax and general conversation with the assessor enabled the participant to feel more comfortable with the whole process. Two dictaphones, one acting as a backup, were set to record. As the first needle device was applied the participant was asked to speak and explain what they felt using any words and descriptions that they felt to be suitable. Following application, the VAS and questionnaire were completed as per the CRF (Appendix VIII). This was repeated for each device and all additional comments were recorded in the CRF (Appendix VIII) and the dictaphones turned off. The participant was then taken to another room and asked to rest for 15 minutes, following which each needle device was applied to the adjacent buttock and TEWL measurements taken. The order of TEWL and pain measurements were determined by the participants' timetable and which room was available, therefore Participants 3, 7 and 12 had their TEWL measured before the pain and sensation. Further information on the TEWL study and wound healing responses are presented in Chapter 4.

3.2.6.4 Measuring pain using a visual analogue scale (VAS)

At the start of the trial each participant was informed how they would be assessed to measure pain intensity and sensation caused by each needle device. The pain intensity rating was taken immediately after application of each needle using an electronic sliding VAS whereby the participant moved a

slider along a 10cm slide where one end represented 'No Pain = 0cm' and the other end the 'Worst Pain Imaginable = 10cm'. This was done after each subject verbally described sensations as the needles were being applied. The slider was set to 0cm prior to each reading. A digital display, viewed only by the assessor, showed the distance the slider was moved and this result was recorded in the CRF. Each participant received the same instruction at each VAS reading: *"To measure the pain intensity please move the slider to the position that best represents the pain felt during and after the application. The left end of the scale represents no pain and the right end of the scale represents the worst pain imaginable".* VAS is explained in Chapter 1.

3.2.6.4.1 Statistical analysis of VAS data

Non-parametric Wilcoxon signed rank test was performed using Prism GraphPad. Significance was shown when $p<0.05$.

3.2.6.5 Measuring sensation perception with an adapted questionnaire

The perception questionnaire was adapted from the MPQ-SF as explained in Chapter 1 (Melzack 1987). It contains the four main assessment points of the MPQ-SF and three additional words ('pressing', 'pricking' and 'cold') taken from the long form MPQ, which exploratory research within our group (Chapter 2), have shown to be of specific relevance in this study.

To prevent leading the participants, the 'Pain Rating Index' of descriptor words was relabelled the 'Sensation Rating Index' as this provided the opportunity for subjects to consider sensations experienced other than pain. Participants were instructed, as follows, before each adapted MPQ-SF was

completed: *"You will now be shown some descriptive words. As each word is read out to you please state if it describes a sensation you felt during or after the application. Rate it as either none, mild, moderate or severe. If you are unsure of the meaning please ask".*

3.2.6.6 Measuring Pain using the evaluative overall pain index (EOPI)

The EOPI is the penultimate section of the MPQ-SF and is used to rate the overall pain of the process, thus participant have time to reflect on the level of pain felt during the application of the specific device. The participants were asked to *"Looking at the words, which one describes the overall intensity of the total pain you experienced during and after the application?"* and rate on an increasing integer scale from 'no pain = 0', 'mild = 1', 'discomforting = 2', 'distressing = 3', 'horrible = 4' and 'excruciating = 5".

3.2.6.7 Audio recording of participants

Prior to any devices being applied each participant's consent was taken to have the session audio recorded (Appendix VIII). As previously described, 2 dictaphones were set up, unobtrusively but within the direct vicinity of the participant to ensure optimum clarity in the recording. The essential data from the participant's audio commentary was recorded during and after the application of each device. To ensure the participants vocalised fully what they felt during the applications, they were instructed at the start to *"Please describe any sensation you feel during the whole procedure".*

3.2.6.8 Establishing awareness of application site using location diagram

To assess the ability of participants to identify the location of the sensations or pain they felt, participants were asked to mark on a diagram of the buttock region where they felt the application of each device (Appendix VIII).

3.3 Results

3.3.1 Conducting the study

The study was conducted over 7 days in a 4 week period. The results were recorded in a CRF (Appendix VIII) for each participant. Data for each participant is tabulated in Appendix X to show the order of application of the 3 devices; the VAS scores; the sensation words rated as 'moderate' and 'mild', the 'overall evaluative pain index'; the relative discomfort and painful nature of each device deduced from the audio recordings; and finally the tabulated verbatim transcripts for each needle device and overall comments.

3.3.2 Perception of pain and sensation during application

3.3.2.1 VAS score from microneedle application

The VAS scores taken for each device from participants (n=12) show that the 180μm (180 MN) and 280μm (280 MN) length microneedles were significantly less painful than the hypodermic needle, p=0.027 and p=0.0005, respectively (Table 3.2). The 280μm length microneedles were also perceived to be significantly less painful than the 180μm length microneedles, p=0.039 (Figure 3.2).

Table 3.2. VAS scores (cm) for each participant. Pain intensity rated on a scale of 0cm for 'no pain' to 10cm for 'worst pain imaginable'. 180μm (180 MN), 280μm (280 MN) length microneedles were compared to 25G hypodermic needle administered subcutaneously.

	Participant											
Needle Device	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
280 MN	0.14	0	0	0	1.00	0	0.14	0.01	0.88	0.46	0	0
180 MN	0.95	0.60	0	0	2.40	1.44	0.21	0.08	1.08	0.23	0	0.21
Hypodermic	0.25	0.54	1.14	0.11	2.30	2.17	0.28	0.74	2.23	0.92	2.65	1.69

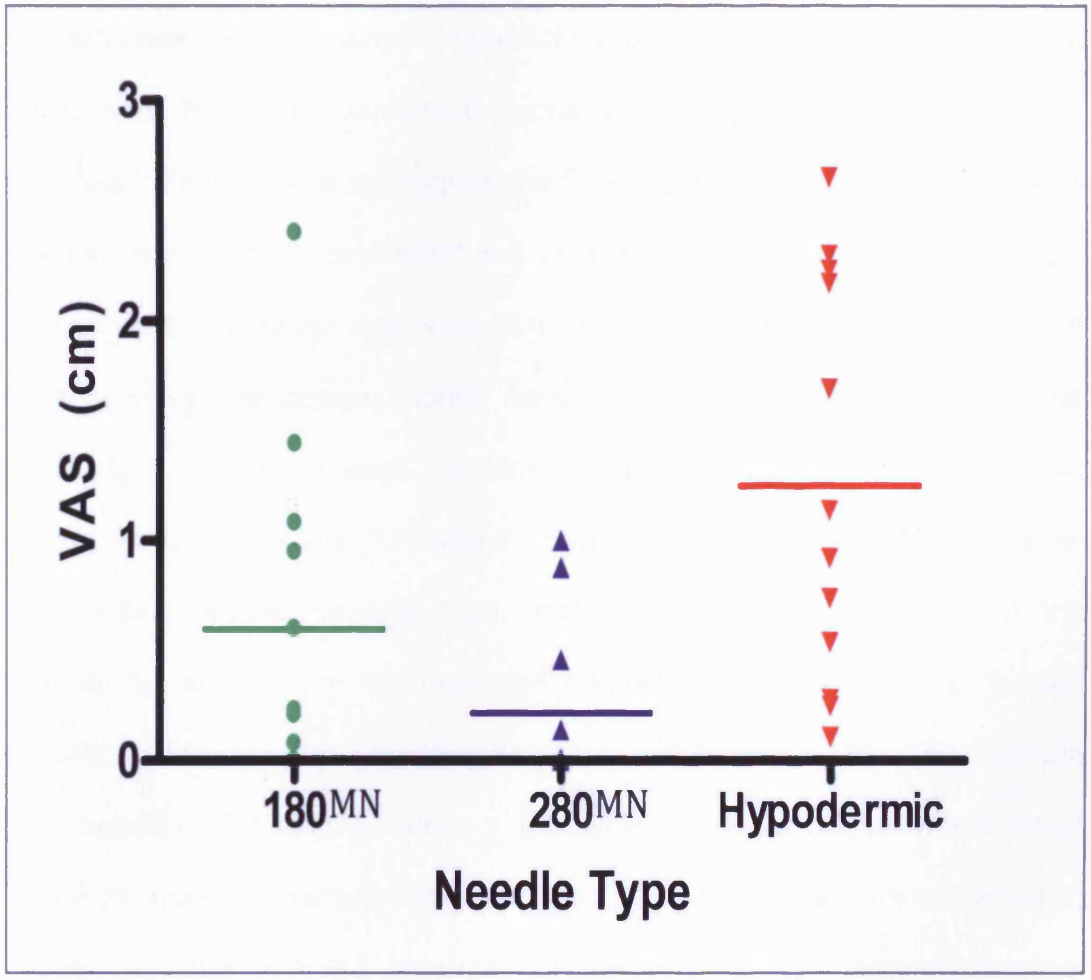


Figure 3.2. Individual and mean VAS score for each needle device: 180μm (180MN) and 280μm (280MN) length microneedle arrays and 25G hypodermic needle. Graph shows the 280μm microneedle array application was the least painful, whilst both microneedle applications were less painful than 25G hypodermic needle.

All 12 participants reported the greater pain intensity for the hypodermic needle than both microneedle devices, though all participants registered the

pain intensity under 3cm on the VAS. Eleven of the 12 participants found the 180μm length microneedle array device to be more painful than the 280μm length microneedle array device. For 6 participants, the 280μm microneedle device registered no pain (0cm), whilst only Participants 5 and 9 reported the pain intensity to be above 0.5cm VAS (Table 3.2).

3.3.2.2 Assessing sensation using an adapted MPQ-SF sensory questionnaire

The adapted MPQ-SF sensory questionnaire consisted of the 15 descriptor terms and the three additional terms ('pressing', 'prickling' and 'cold' appended within a separate page of the CRF (Appendix VIII)). Each word was rated as 'none', 'mild', 'moderate' and 'severe'. Figure 3.3 shows, that overall, greater variety of sensations were perceived for the hypodermic needle than either microneedle device, though none of the participant rated any of the descriptors as being 'severe'. Sensory modalities rated for the hypodermic needle were also more 'moderate' than 'mild', with the term 'moderate' representing greater severity than 'mild'. Five and 3 participants for the hypodermic and 180μm microneedles respectively, perceived mild 'tender' sensation, though no tenderness was experienced for the 280μm microneedles. The top 5 sensory modalities (that is, those words most frequently used by participants) from the adapted MPQ-SF are tabulated in Tables 3.3, 3.4 and 3.5 in order of the decreasing frequency for the hypodermic needle, 180μm microneedle and 280μm microneedles respectively.

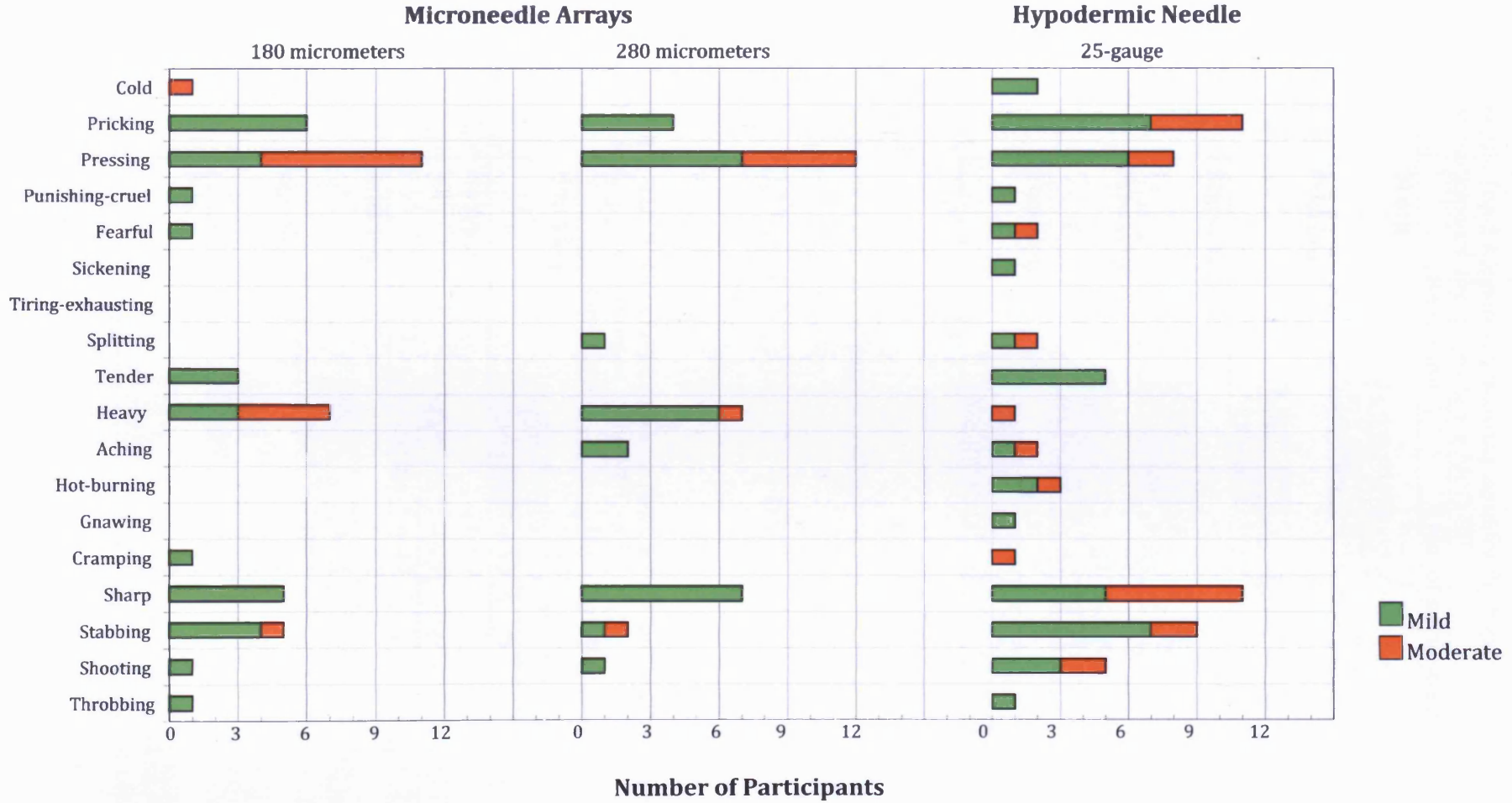


Figure 3.3. Sensory evaluation of each needle application using the adapted MPQ-SF. The adapted MPQ-SF descriptors were rated in order of increasing severity as 'none', 'mild', 'moderate' and 'severe'. None of the participant rated any of the descriptors as being 'severe'. Those sensations not showing a rating were rated as 'none'.

Table 3.3. Top 5 sensory expressions rated for the hypodermic needle as determined by the participants' responses to the MPQ-SF.

Sensation		No. of participants	Participant No.
Sharp		n = 11	
	Mild	n = 5	2, 4, 10, 11, 12
	Moderate	n = 6	1, 3, 5, 7, 8, 9
Pricking		n = 11	
	Mild	n = 7	1, 2, 4, 6, 7, 10, 12
	Moderate	n = 4	3, 5, 8, 9
Stabbing		n = 9	
	Mild	n = 7	1, 2, 4, 5, 6, 8, 11
	Moderate	n = 2	3, 9
Pressing		n = 8	
	Mild	n = 6	2, 3, 6, 7, 9, 12
	Moderate	n = 2	5, 8
Shooting		n = 4	
	Mild	n = 3	3, 5, 6
	Moderate	n = 6	1

Table 3.4. Top 5 sensory expressions rated for 180μm microneedles as determined by the participants' responses to the MPQ-SF.

Sensation		No. of participants	Participant No.
Pressing		n = 11	
	Mild	n = 4	4, 5, 8, 9
	Moderate	n = 7	1, 2, 3, 6, 7, 10, 11
Heavy		n = 7	
	Mild	n = 3	3, 4, 11
	Moderate	n = 4	1, 2, 6, 7
Pricking		n = 6	
	Mild	n = 6	2, 4, 5, 7, 8, 12
	Moderate	n = 0	
Stabbing		n = 5	
	Mild	n = 4	1, 2, 5, 9
	Moderate	n = 1	10
Sharp		n = 5	
	Mild	n = 5	2, 5, 7, 8, 12
	Moderate	n = 0	

Table 3.5. Top 5 sensory expressions rated for 280μm microneedles as determined by the participants' responses to the MPQ-SF.

Sensation		No. of participants	Participant No.
Pressing		n = 12	
	Mild	n = 7	1, 4, 5, 6, 9, 11, 12
	Moderate	n = 5	2, 3, 7, 8, 10
Heavy		n = 7	
	Mild	n = 6	2, 3, 6, 7, 9, 11
	Moderate	n = 1	1
Sharp		n = 7	
	Mild	n = 7	1, 4, 5, 7, 8, 9, 11
	Moderate	n = 0	
Pricking		n = 4	
	Mild	n = 4	4, 5, 7, 8
	Moderate	n = 0	
Stabbing		n = 2	
	Mild	n = 1	5
	Moderate	n = 1	10

3.3.2.3 Audio-recorded oral comments from participants during application

Verbatim-transcribed comments (see Appendix IX) were read for each participant. Table 3.6, shows the pain and discomfort sensations as deduced from audio-recorded participant comments during and after the application of each device. The comments were tabulated based on whether the participant used the words 'pain/painful' or 'discomfort/discomforting' when describing the applications. Each comment from the 3 separate applications were compared, thus, for example Participant 1 comments suggested they felt most pain for the hypodermic needle, whilst the 280μm microneedles were least painful. The 180μm microneedles were not as painful as the hypodermic but more painful than the 280μm microneedles. Table 3.6 also shows the randomised application order for each participant and confirms that every participant found the hypodermic to be the most painful/discomforting of the 3 devices.

Table 3.6. The pain and discomfort sensation as deduced from audio-recorded participant comments during and after the application of each device.

Participant & order of device (1st/2nd/3rd)	**Painful/ discomforting (Deduced from comments)**		
	Least	**Middle**	**Most**
1 (280/hypo/180)	280	180	hypodermic
2 (hypo/280/180)	280	180	hypodermic
3 (hypo/180/280)	180 / 280	---	hypodermic
4 (280/hypo/180)	180	280	hypodermic
5 (180/hypo/280)	280	180	hypodermic
6 (280/hypo/180)	280	180	hypodermic
7 (180/280/hypo)	180 / 280	---	hypodermic
8 (280/180/hypo)	280	180	hypodermic
9 (180/280/hypo)	280 / 180	---	hypodermic
10 (180/hypo/280)	180	280	hypodermic
11 (hypo/180/280)	---	180 / 280	hypodermic
12 (280/180/hypo)	180 / 280	---	hypodermic

The microneedle arrays were perceived as being less painful or discomforting, though 5 participants felt the 180μm microneedle arrays were slightly more discomforting than the 280μm microneedle arrays.

For example, as Participant 5 explained:

"The second one (hypodermic) was the most painful, I could feel it the most. Umm, the third one (280μm) I didn't feel as much, but maybe that's because I had the first two before. So I'm maybe, again 'cause I've had the first two I didn't feel it, but the second one (hypodermic), oh, definitely the most painful. And I felt that one the most stabbing and it prickled it a bit".

3.3.2.3.1 Extracting key descriptions from the audio-recorded oral commentary

Analysis of the verbatim-transcribed, audio-recorded commentary (such a as shown in Appendix X) was used to identify key categories revealed from each participant's transcript for each needle device (Tables 3.7 - 3.9) by calculating the frequency that these descriptors occurred in the audio comments. Square-bracketed words in Tables 3.7, 3.8 and 3.9 are to add clarity to the quotation from the transcripts. For example a participant may have indicated, *"More discomforting than the last",* thus *"the last"* has been substituted with the device to which the individual referred in square brackets.

The oral commentary highlights that all participants found there to be greater sensation of pressure during either microneedle device application (Tables 3.7 and 3.8) compared to the hypodermic needle (Table 3.9).

Table 3.7. Key description categories from oral commentary for the 180μm microneedle device.

	Transcript quotation	Number of participants	Participant number
No Pain (n=2)	No pain	n = 1	12
	Didn't hurt when put in	n = 1	5
Pain (n=4)	Slight pain	n = 1	10
	Dull pain	n = 1	1
	Not excessively painful	n = 1	7
	Hurt more when it was shaken	n = 1	5
Discomforting (n=3)	More discomforting than [280]	n = 2	1, 6
	More discomforting than [hypodermic]	n = 1	6
	Did feel slightly mild discomfort	n = 1	12
Sharp (n=6)	Slightly sharp	n = 2	1, 2
	Slightly sharp towards the centre	n = 1	7
	Prickling	n = 1	5
	Little prick	n = 1	4
	Scratching the area	n = 1	9
Pressure (n=7)	Pressing	n = 2	2, 11
	Big press	n = 1	4
	Firm pressing down	n = 1	6
	Pressure	n = 2	1, 11
	Quite a bit of pressure	n = 1	10
	Quite a lot of pressure	n = 1	10
	Slight pressure	n = 1	3
	Continued pressure [during application]	n = 1	3
	Heavy pressure pushing down	n = 1	6
	Greater feeling of pressure than 280μm array	n = 1	1
Other sensations	Quite comfortable overall	n = 1	4
	Feel it going in	n = 1	6
	Didn't really feel anything	n = 1	5
	Mild	n = 1	1
	Cold	n = 1	2

Table 3.8. Key description categories from oral commentary for the 280μm microneedle device.

	Transcript quotation	Number of participants	Participant number
No Pain (n=4)	No pain	n = 3	2, 10, 12
	Not painful	n = 1	4
	No sharp pain	n = 1	2
Pressure (n=9)	Pressure	n = 3	1, 3, 7
	Dull pressure	n = 1	1
	Bit of pressure	n = 1	8
	Lot of pressure	n = 1	10
	Pushing down	n = 1	7
	Pressing	n = 2	2, 6
	Lot of pressing	n = 1	11
	Slightly heavier touch	n = 1	12
Sharp (n=3) & Pain (n=1)	Sharp	n = 1	5
	Less sharp pain increasing to more sharp pain	n = 1	7
	Tiny prick	n = 1	4
Other sensations	Slight stinging	n = 1	1
	Gripping skin	n = 1	7
	Slightly heavier touch	n = 1	12
	Feels like fingertip	n = 1	8
	Feels like point in centre of circle	n = 1	12
	Barely felt	n = 1	4
	No particular discomfort	n = 1	1
	Hurt less than hypodermic	n = 1	5

Table 3.9. Key description categories from oral commentary for the 25G hypodermic needle.

	Transcript quotation	Number of participants	Participant number
Pain (n=5)	Very mild pain	n = 1	12
	Not a major pain	n = 1	12
	Really painful	n = 1	6
	Shooting pain on puncture	n = 1	12
	Sharp pain	n = 2	1, 9
	A bit more painful [than the 280µm array]	n = 1	4
Sharp (n=5)	Quite sharp	n = 1	6
	Much sharper	n = 1	5
	Sharp scratch	n = 1	2
	Slightly sharper at start	n = 1	7
	Slight prick	n = 1	3
	Sharp prick	n = 1	4
Pressure (n=4)	Slight pressure	n = 1	10
	Pressure initially piercing skin	n = 1	8
	Less pressure [than 280µm array]	n = 1	1
	Pressing on skin	n = 1	8

The hypodermic needle was perceived as 'sharp' and 'painful'. Oral descriptions from participants exemplify the variation in the sensation felt, for example *"sharp"* or *"little prick"*. The hypodermic needle only elicited 3 main descriptions of 'pain' 'sharp' and 'pressure' (Table 3.9) when compared to either microneedle devices, which described using a greater number of other sensations. Comparison between the microneedle devices reveals the 180μm microneedles elicited a greater 'sharp' sensation than the 280μm microneedles. Whilst the 280μm microneedles were described as being less 'discomforting' than the 180μm microneedles.

3.3.2.4 Evaluative overall pain index (EOPI) scoring by participants

The EOPI was the final assessment of the adapted MPQ-SF. This related to the level of pain felt on an increasing integer scale of 'no pain = 0', 'mild = 1', 'discomforting = 2', 'distressing = 3', 'horrible = 4', and 'excruciating = 5'. Table 3.10 collates the data from each participant to show the level of overall pain perceived by the application of each needle device. The hypodermic elicits the greatest level of pain, with 8 participants scoring the pain as 'discomforting' whilst the 280μm microneedle was scored as 'no pain' by 10 participants.

3.3.2.5 Additional comments provided by participants after all the devices were applied

Each participant was asked to comment generally after all 3 needle devices had been applied: *"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*

Table 3.10. EOPI was scored for each needle device at the end of the adapted MPQ-SF by participants.

		Needle device		
		180µm	**280µm**	**Hypodermic**
EOPI Score		**Total number of participants (Participant number)**		
0	**No pain**	6 (3,4,7,8,10,11)	10 (1,2,3,4,6,7,8,10,11,12)	-
1	**Mild**	4 (5,6,9,12)	2 (5,9)	4 (1,2,7,10)
2	**Discomforting**	2 (1,2)		8 (3,4,5,6,8,9,11,12)
3	**Distressing**	-	-	-
4	**Horrible**	-	-	-
5	**Excruciating**	-	-	-

The verbatim transcripts, Table 3.11, show that only Participant 9 did not make any additional comments, whilst some participants commented generally on both microneedle devices as either being similar or when compared together against the hypodermic needle.

Summarising the transcripts of the oral commentary made post-application of each device (Table 3.11) in order of discomfort (Table 3.12) highlights the least discomfort or pain was caused by the 2 microneedle devices, whilst the hypodermic needle caused the greatest pain or discomfort. Four participants found the 180µm and the 280µm microneedle devices to be of similar discomfort, whilst another 5 participants felt the 280µm microneedles were less painful than the 180µm array and the hypodermic.

Table 3.11. Verbatim transcribed oral comments made by each participant immediately post-application of all three needle devices

	"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"		
	280μm microneedle	**180μm microneedle**	**25G hypodermic needle**
1	Erm, the first one (280) was the easiest to tolerate. Erm there is more a feeling of pressure, more like somebody's just pushing onto the skin without actually breaking the skin.	The last (180) umm application felt heavier and more pressure. Same kind of feeling as the first one (280), umm with perhaps just err bit more of err err pressure pain in the middle, perhaps just a sharp in the middle. So like the first one (280) but it felt like perhaps its pushing a bit deeper."	Umm the second one (hypodermic) felt like a sharp needle application, so sort of you know a sub cut injection. Umm it was sharp, and as as uncomfortable as subcut injections normally are. Umm not crazily discomforting.
2	The second one (280) was the least painful, or anything like that	<<No Comment>>	The first one (hypodermic) was the normal needle
3	Umm in terms of the, the second (180) and third (280) applications were similar. Umm there was no pain at all, umm it was just pressing, but I wouldn't say it was a pain sensation. There was just a sensation of pressing down on the skin. So the last two (180, 280) were the most comfortable out of the three."		I thought the first (hypodermic) was hypodermic needle. Erm when it entered the skin there was, there wasn't so much pressing, but there was more of err, there was a slight pain and it continued and umm that lasted until, until it was removed. It was quite cold as well.
4	Err, err, I barely felt the first one (280). But I did feel a tiny pricking sensation (for the 280),	Umm third one (180) perhaps the most comfortable. Out of all the three I would probably prefer the third one (180) for an injection. "Err, I felt no the side effects apart from the odd press in the third one (180) and prickly sensations at times but that's it.	Out of those three I probably thought the second (hypodermic) was the most uncomfortable.
5	Umm, the third one (280) I didn't feel as much, but maybe that's because I had the first 2 before.	<<No Comment>>	The second one (hypodermic) was the most painful, I could feel it the most. second one (hypodermic), oh, definitely the most painful. And I felt most were stabbing and it prickled it a bit
6	the first one (280) was like someone holding onto your arm	Erm, third one (180) felt as if it was being pressed down harder,	Erm, you could tell quite easily the second one (hypodermic) was the needle. That was definitely more uncomfortable than the other two. You could actually feel it in terms of it going into you.

Table 3.11 Continued...

	"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"		
	280μm microneedle	**180μm microneedle**	**25G hypodermic needle**
7	I think the first two (180, 280) felt fine because pressure doesn't feel as bad as this sharp. The first two (180, 280) were fine and not a lot of difference between the two.		Well I suppose you would it as a pain, a sharp pain on the third one (hypodermic) like your skin's being nipped. But I think the third (hypodermic) was the worst.
8	You can sense that are the pricks there. Umm, that's more for the first one.	Umm, yer you felt very different to, the application two (180) felt more, umm, you could feel it more. If that's of any help"... "yer you could tell that more pressure was being applied, umm, and you could feel. I suppose the only way of describing it is if you get like err, like some leaves and they've got little prickly things on the back and you are pressing on them. Like a nettle leaf but without the actual sting in it. So you can feel that its gone in, it feels a bit like that so the more you press on it the more prickly but again it doesn't feel painful. You can sense that are the pricks there.	Well the third application (hypodermic) you could tell was quite different to the other two, umm, and it did feel like pain a needle if I am honest. Umm, so I could feel it going in. felt far more sort of penetrating than the other two
9	<<No Comments>>		
10	The first (180) and the last one (280), they were similar. Couldn't feel any pain, could feel sensation and a lot, a lot of pressure. There was probably actually slightly more pressure on the last one (280)."		The second one (hypodermic) was painful, then slightly painful.
11	The third one (280) was like a mixture between both."	The second one (180) was more intense pressing	First one (hypodermic) was really sharp
12	I would definitely go for one of the first two (280, 180). Umm, err, I can't really remember which one out of the first two was the least actually, umm but I think they were pretty, you know they were quite close by. Umm, yer so the first two (280, 180) were better.		least favourite was the last one (hypodermic), umm which well obviously I didn't see but I am pretty confident that's the standard sort of hypodermic needle, umm. Yer that was my least favourite cause that felt, that was quite. Third one (hypodermic), yer, uncomfortable but not intolerable

Table 3.12. Audio-recording of participants from Table 3.11 summarised depending on which device the participant indicates least discomfort. (280MN = 280μm length microneedles; 180MN = 180μm length microneedles; 25G = 25G hypodermic needle)

Least discomforting	No. of participants	Participant No.
280MN	n = 5	1,2,5,6,8
180MN	n = 2	4, 10
180MN = 280MN	n = 4	3,7,9,12
Less than 25G, but not differentiated between microneedle devices.	n = 1	11

3.4 Discussion

In this clinical study, three main methods of data collection were used to determine the pain and sensation felt by participants by the application of three needle devices to their buttock. The audio commentary complimented the pain questionnaire and gave a more in-depth view of what each participant was experiencing during each application. The level of discomfort felt by each participant corroborated the quantitative visual analogue (VAS) data in identifying the hypodermic needle as the most painful and uncomfortable device. Oral commentary supports this well by detailing the exact perceptions with key details and analogies given by the participants.

Pre-clinical development of the clinical trial methods helped to refine the application of the VAS and MPQ-SF to this clinical study. When testing the applicator designs for the microneedle arrays (Chapter 2) the VAS was conducted using a traditional 10cm horizontal line, however for the clinical trial, an electronic slider with a digital display was used as a simple instrument to make real-time measurements of VAS, rather than measure a marked point on a horizontal line later. The digital VAS meter used during this study is used regularly in clinical practice and is designed to be free moving whilst providing accurate (+/-0.01cm) measurement of the distance the slider moves.

Investigation by Woods et al. (2009), Jamison et al. (2002) and Price et al. (1994) compared different paper-based and computer-based analogue scale methods of collecting VAS data and found these to be valid and functionally interchangeable.

Importantly however, this study confirms that the sensations, including pain, felt from the applications of the silicon microneedle devices were relatively similar, and in each case significantly lower than for the hypodermic needle. VAS can be scored from 0cm to 10cm, though the mean for the hypodermic needle never reached over 2cm. Thus, even the hypodermic needle is not as painful as the pain caused by chronic or extreme acute pain (Choiniere and Amsel 1996). Indeed any pain experienced during this study was of a transient nature and at worst was non-severe in its nature. The VAS illustrates that pain associated to the use of all 3 needle devices in relation to a participant's individual experiences is relatively low. Only Participant 5 rated the 180μm microneedles pain intensity as greater that 2cm, whilst 3 participants (Participants 5, 9 and 11) rated the hypodermic needle to have a pain intensity of over 2cm on the VAS.

As the VAS was taken immediately after each application, prior to any other measurement, the participant was able to register their current feeling in relation to the "Worst Pain Imaginable". Therefore, VAS was an important and highly sensitive way of capturing pain intensity, at the instance of application. Bergh et al. (2009) and Kelly et al. (2001) found the VAS to be a highly sensitive and reliable measure of pain intensity. Kelly et al. (2001) defined all

VAS rating under 30mm as 'mild pain', 70mm or more were defined as 'severe pain' and 31mm to 69mm as 'moderate pain'. Whilst this may help characterise pain intensity it reduces the ability to observe smaller inter-participant variations between needle devices, however it does show, when compared to other types of pain, that the pain from microneedle and hypodermic needles is relatively low.

As all sensation and perception assessments are highly subjective, these scales are of most value when comparing intra-participant changes rather than inter-participant changes. Where small groups of participants are being studied, and a significant treatment effect is found, it is important to consider what proportion of participants were accountable for the significant group affect. This study consisted of only 12 participants, each with their own interpretation and perception of what they felt during application of the devices. Therefore, it is difficult to examine the reproducibility of VAS in terms of inter-participant reliability, however a lack of correspondence between scores may be due to genuine differences in individuals' interpretation of the scale.

The VAS data shows that 5 participants registered more pain for the 180μm microneedles compared the 280μm microneedles, though it may initially seem illogical that the shorter 180μm microneedles caused greater pain than the 280μm microneedles, a simple explanation is that this result is an artifact of the application process. As this study demonstrates (Chapter 3 and 4), the pain responses are based on an application technique that was previously

tested in *ex-vivo* studies (Chapter 2) to ensure sufficient penetration of the stratum corneum to facilitate drug delivery. The amount of application force required to provide reliable skin penetration is analogous with the force used to massage an aching muscle. Extensive *ex-vivo* studies have clearly demonstrated that smaller microneedles, such as 180μm in length, need to be applied more firmly to the skin than the longer 280μm microneedles to ensure satisfactory skin penetration. Therefore the physician in this clinical study was trained to administer the shorter 180μm microneedles with more force. Future research to develop a suitable application device for consistent and reproducible application of microneedles may standardise clinical application.

Microneedle morphology, applicator design and application techniques could be responsible for increased sensations. Adapted MPQ-SF results determine the microneedle applications as being perceived as more 'pressing' and 'heavy' than the 'sharp' and 'pricking' hypodermic needle application. These perceptions may be related to the surface area of the microneedle array. As microneedles are spread over a larger surface area than a single hypodermic needle, they may require more force to penetrate the skin. The hypodermic needle, however, was applied by the clinically accepted subcutaneous technique and though using finer needles could reduce pain, they will still enter deeper into the dermis than the microneedles, thus activate pain receptors. Sensation responses from the adapted MPQ-SF and oral commentary suggest that reducing the force required for microneedles to penetrate the stratum corneum would benefit patients and could be achieved

by improvements in the microneedle array design, application device and technique.

A secondary pain assessment, the evaluative overall pain index (EOPI), was rated by participants relative to their overall pain experience at the end of the adapted MPQ-SF. Only 2 of the 12 participants reported the 180μm microneedles as 'discomforting' compared to the 280μm microneedles. Using the EOPI, 10 participants rated the 280μm microneedles as exhibiting 'no pain'. Even the hypodermic needle was not rated to be greater than 'discomforting'. When compared to VAS, where 5 participants felt the 180μm microneedle device was more painful than the 280μm microneedle device, only 2 of these 5 participants rated the 180μm microneedles as more 'discomforting' than the 280μm microneedles. Though a total of 8 participants also rated the hypodermic needle as 'discomforting'. This difference in VAS and EOPI could be because participants had approximately 5 minutes to consider what they felt as they answered the adapted MPQ-SF.

Furthermore, unlike the VAS, which is a continuous sliding scale, the EOPI is a psychometric response scale, in which a person is asked to select a category label from a list indicating their perception. These labels have integers of 0-5 assigned with increasing pain (Melzack 2005, 2001; Wright et al. 2001). However the words used may be distinguished differently by individual participants, therefore some may feel that a device is more 'discomforting' (integer two) than 'mild' (integer one) despite feeling that the 'mild' device is in fact less painful than the 'discomforting' one. Indeed the

EOPI is more complex and defined measure of pain, which may confuse the respondent, as it requires more thought than VAS (Fabbri et al. 2009; Katz and Melzack 1999; Wright et al. 2001).

The adapted MPQ-SF (Appendix VIII) was used in order to learn more about the types of sensations felt during the application of each microneedle or hypodermic needle device. In normal clinical use, the standard MPQ-SF (Melzack, 1975) is repeated at each clinical examination over the duration of care (Melzack 1987). Each level of pain or sensation intensities are used to compare between clinical assessments. Each clinical assessment is used to measure improvements or worsening of the medical condition and helps to determine the effectiveness of any intervention (Melzack 2005; Mystakidou et al. 2002; Wright et al. 2001). However, measuring the pain and sensation of three needle devices required one-off immediate responses post-application of the devices, thus multiple intra-participant comparisons of MPQ-SF scores, such those in Chapter 2 (Melzack 1987; Wright et al. 2001) were not required. Moreover, pain and sensations felt by the application of both the microneedle devices and the hypodermic needle were measured using the adapted MPQ-SF.

The adapted MPQ-SF included three key adjectives that were mentioned by participants in the pre-clinical applicator design testing (Chapter 2) and are also present in the MPQ (Melzack 1975). Therefore, 'pressing', 'pricking' and 'cold' were included as a subsection of the adapted MPQ-SF in the CRF (Appendix VIII). These words were not originally tested by Melzack (1987) for the MPQ-SF, thus it would be invalid and unreliable to assign them to either

the "sensory" or "affective" categories. Hence, quantified scoring would not have been validated for the context in which the adapted MPQ-SF was applied in this study.

Subsequent transient pain studies should test the adapted MPQ-SF further; possibly redevelop a new questionnaire for transient pain based on the MPQ-SF but with other key adjectives, which could be determined from a survey of transient pain. This would require extensive testing and validation to ensure reliability for use across varying sources of transient pain (Leonard et al. 2009; Macmillan et al. 2008; Mystakidou et al. 2002). Validation of such a questionnaire may be excessive considering the pain and sensations measured would be transient in their nature and potentially with no long-term effects.

As detailed in Section 3.2.6, the order of sensory assessment was VAS followed by the adapted MPQ-SF, whilst audio-recorded oral comments, were recorded real-time during each needle device application. The researcher has then summarised these comments into an order of pain or discomfort depending on what was said by the participants (Table 3.6), though care must be taken to ensure over analysis of the commentary does not occur as this may lead to the assessor incorrectly interpreting the participants' comments.

Analysis of the oral commentary, where only one participant made a specific comment, cannot be used to evidence generalised conclusions. However, audio recording did increase the level of detail of the type of pain and sensations experienced by participants during applications of the needle

devices. These comments increased the integrity of the data (Werner and Benrimoj 2008) and correlated well to the adapted MPQ-SF as key sensory modalities such as 'stabbing', 'sharp', and 'pressing' were spoken by the participants during application of the devices and later marked on their questionnaire. As participants were exposed to the questionnaire after the first application, it is possible that they recalled some of the adjectives at the second and third applications, and therefore used the same or similar words to orally describe the application processes (Fabbri et al. 2009; Fischer et al. 2009).

Each participant was audio recorded throughout the entire application process. The oral commentary represents the equivalent of a verbal diary for the duration of the application processes (Munyard et al. 1994). The audio recordings taken during the applications describe the timeline and real-time sensations as felt and vocalised by the participants. The depth of these descriptions is based solely on the parts of the application experience the participant chose to vocalise, as they were not prompted. Participants were instructed to say if they felt "*nothing*" for every stage of the applications and MPQ-SF, some might have refrained from speaking it due to experiencing some sensation that they were unable specifically describe orally. Differences in oral comments will have also depended on the participants' oral ability and their depth of vocabulary (Arber 2007; Sokunbi et al. 2008; Zollo et al. 2009). Thus care needs to be taken not to 'over-analyse' the audio-recorded commentary.

Despite audio recording throughout the whole procedure, from the applications of devices through the adapted MPQ-SF to the final comments, it is important to note that the participants were not instructed to provide a commentary at any stage other than during the application of each needle device and to comment after all 3 devices had been applied. Indeed, Participants 3, 5, 6, 8, 11 did not make oral comment (Appendix X) during the VAS or MPQ-SF assessments. Therefore, audio-recorded data at any stage other than the application or final comments cannot be used for inferring any differences in the inter-participant understanding of VAS or MPQ-SF as instruments for measuring pain and sensation. Further studies should instruct participants to comment at each stage. If they have no comment, they should be reminded to say "*no comment*".

Each participant will have had differing experiences of pain, though past needle-related history was checked only to ensure they were not needle phobic. None of the participants admitted to being needle phobic, however, psychological factors may have increased the 'fear factor' to hypodermic needles (Deacon and Abramowitz 2006; Fassler 1985; Kettwich et al. 2007; Nir et al. 2003). Pain is subjective and how the participants' perceived the needle devices depended on how much pain they had experienced in the past and also how much pain they could imagine. Therefore, despite single-blind application of each device to ensure the participant was uncertain of application order, participants may have been able to distinguish between the microneedle devices and the hypodermic needle at the very early stage of application, which may have reduced or increased tension during the subsequent application stages. This is a limitation of pain assessment as it

involves qualifying or quantifying an individual's unique personal perception. Despite this, the variability of inter-participants VAS scores post-application of needle devices was minimal. However, importantly, this research used intra-participant comparisons to establish relative pain of each needle device.

Chapter 2 assessed applicator design on the volar forearm, whilst the clinical application of microneedle arrays was conducted on the buttock. These 2 areas of the body vary in their adiposity whereby the buttock had greater fat tissue and skin flexibility on young adults (Schilling and Wechsler 1986), whilst the volar forearm may have lower follicular concentration (Boles et al. 2000). Ideally any future testing of applications and applicators should be conducted at the same anatomical site to maintain consistency.

A further limitation of this study was that in clinical use, microneedles and the hypodermic needle would be used to administer an active ingredient. If a substance or combination of substances were to be delivered via microneedles, then pre-clinical animal and *ex-vivo* studies would have to be conducted to repeatedly show the proposed treatment to be safe and effective for use with humans (HMSO 2004). The human body processes and reacts to medicaments and medical devices in different ways to cells, excised tissues and animal models (Furuzawa-Carballeda et al. 2009; Gonzalez et al. 1999; Hwang et al. 2009; Kraemer et al. 2009; Swindle et al. 2003). When a hypodermic needle injects a solution into the epidermal and dermal tissues, which are forcibly displaced by the mechanical pressures and chemical properties of the solution injected. This can cause an increase in sensory activations; therefore, future studies should look at the overall scenario of a

medicament being delivered via needle or microneedle injection in clinical use.

This pilot study has shown that even the pain caused by a 25G hypodermic needle is less than a VAS score of 3cm for each participant (Figure 3.2). Therefore future studies should establish what other factors, regardless of pain, affect vaccination delivery and uptake, and how microneedles can play a pivotal role in vaccination delivery. Future clinical studies assessing the administration of a drug using microneedles should consider incorporating a sensation questionnaire and audio recording of sensations during and after the administration process. This can serve to highlight the differences in sensations when a substance is administered transdermally in relation to the specific application technique. Focus group research to establish the detrimental and beneficial effects of specific sensations in patient groups could improve microneedle and applicator device design. This in turn may increase availability, acceptance and use of microneedles by clinicians and patient.

3.4.1 Conclusions

This study has shown that hypodermic injections are perceived as significantly more painful than microneedles, though currently microneedles still cause some pain and discomfort due in part to the application technique, which needs to be developed further for consistent applications. Whilst pain and sensation data acquired by audio recording complimented the pain questionnaire data, key descriptors, of 'pressing' and 'heavy' emerged as

areas where the applicator design and application technique of microneedles could be improved to reduce discomforting sensations.

Chapter 4

The physical and biochemical effects of *in-vivo* microneedle administration on human volunteers during a clinical study.

4.1 Introduction

Chapter 3 introduced the element of the clinical study focussing on pain and other sensory modalities caused by clinical microneedle administration. Chapter 4 aims to further assess microneedle efficacy and safety in relation to the nature of the punctures created *in-vivo* by 180μm and 280μm microneedles versus those created by 25G hypodermic needles. Microscopy and transepidermal water loss (TEWL) will enable observation of the physical changes in skin barrier caused by needle puncture (Badran et al. 2009; Bal et al. 2008; Verbaan et al. 2007). Simultaneously biochemical evaluation using immunohistochemistry (IHC) will highlight changes in inflammatory and proliferation markers (Freedberg et al. 2001; Hakvoort et al. 2000; Patel et al. 2005).

Despite microneedles penetrating the skin, previous studies have shown that microneedle application to skin does not cause significant pain (Bal et al. 2008; Gill et al. 2008; Sivamani et al. 2005). Information on the extent and nature of the wound responses resulting from administration of these devices is however lacking. Given that microneedles are being developed to deliver therapeutics and vaccines cutaneously (Birchall et al. 2005; Ding et al. 2009; Hantash et al. 2009; Pearton et al. 2008), it is essential to establish the skin's response to the injury, especially as any alteration in the integrity of the skin might lead to pathogenic invasion or release arising from the cellular damage (Elias and Choi 2005; Pedersen and Jemec 2006; Poindexter et al. 2006; Sorensen et al. 2003). Therefore, it is important to compare the microneedle wounds to conventional hypodermic needle wounds from a physical and

biological perspective to distinguish between the localised wound responses at puncture sites for each needle device.

4.1.1 Assessing skin puncture using Transepidermal Water Loss (TEWL), Methylene blue and haematoxylin and eosin (H & E) staining

As well as investigating wound healing post-application of microneedles, a measure of biological disruption, the skin will also be assessed to establish whether the microneedles have successfully penetrated the skin surface during clinical application, a measure of physical disruption. TEWL has been described in Section 1.1.2 and used to assess skin barrier disruption caused by the application of microneedles to human volunteers (Bal et al. 2008). TEWL combined with topical staining, using a blue dye, immediately post-application of microneedles has also been used to determine skin penetration by microneedles *ex-vivo* (Ding et al. 2009). Chapter 4 aims to use TEWL measurements and external dye staining to confirm that microneedles of 280μm and 180μm length puncture the skin of human volunteers.

Methylene blue is a hydrophilic, tricyclic phenothiazine dye with the chemical formula $C_6H_{18}ClN_3S$ and molecular weight of 319.85. Once in solution methylene blue forms a deep blue colour; the solubility of methylene blue is 3.55g/100ml of water and 1.5g/100ml of alcohol. In aqueous solutions, methylene blue is present as a monomer and dimer, thus has two absorption peaks at 668nm and 609nm. When applied topically to skin, methylene blue does not diffuse through the hydrophobic lipid-rich corneocytes that comprise the stratum corneum (SC). Once methylene blue has penetrated the SC, methylene blue ions interact with proteins present in cellular membranes and

interstitial fluid (Clifton and Leikin 2003; Genina et al. 2008; Little 1990; Mordon et al. 2003; Simmons et al. 2001; Talreja et al. 2001). Methylene blue has been extensively used as a topically applied stain to visualise the cellular layers of the stratum corneum (Clifton and Leikin 2003; Dykes et al. 2001; Zhai and Maibach 1996); highlighting cancers and lymph nodes (Simmons et al. 2001; Yaroslavsky et al. 2005) and surgical skin marking (Granick et al. 1987). Methylene blue has also used in the laboratory setting to monitor the affect of electric-pulse skin penetration (Johnson et al. 1998) and identify skin microconduits created by radio frequency wave ablation (Levin et al. 2005), microinjection (Noori et al. 2009), and microneedles (Coulman et al. 2010; Escobar-Chavez et al. 2010; Fernandez and et al. 2009; Gill and Prausnitz 2007; Kolli and Banga 2008; Lee et al. 2011; Li et al. 2008; McAllister et al. 2003; Mikolajewska et al. 2010; Pearton et al. 2010).

Tissue sectioning and counterstaining is used to highlight changes in skin strata and cells following puncture (Coleman 2006; Titford 2005). Haematoxylin (H) has an affinity for the nucleic acids of the cell nucleus whilst eosin (E) highlights the cytoplasm of cells. H & E staining therefore permits visualisation of morphological and numerical changes in viable cell populations. H & E can be applied by either leaving slides in a solution for a set time and then washing through solutions of acid-alcohol, or progressively by dipping the slides into stain solution until the required intensity is obtained (Coleman 2006; de Boer et al. 2007; Titford 2005; Tuli et al. 2006). Adjusting incubation times during joint staining enables variation in the contrast between cell nuclei and cytoplasm to enable clear visualisation.

4.1.2 Wound healing processes due to minor injury in the skin

As detailed in Section 1.1.2, wound healing involves numerous complex processes involving cytokine and growth factor driven migration of keratinocytes (Diegelmann and Evans 2004; Kiritsy and Lynch 1993; Kondo 2007). As described in Chapter 1 the skin is comprised of several separate layers. The most prevalent skin cell, the keratinocytes, interact with fibroblasts to coordinate a normal wound healing response that leads to scar formation. (Diegelmann and Evans 2004; Ejaz et al. 2009; Suter et al. 2009).

Skin healing is modulated by a plethora of cytokines and chemokines, which initiate and drive inflammation, proliferation and remodelling, to heal the skin (Barrientos et al. 2008; Freedberg et al. 2001; Spiekstra et al. 2007; Yager et al. 2007). During the clinical study (as described in Chapter 2) skin biopsies were taken from participants over a 24-hour period post-penetration by the three different needle devices. The wound healing responses investigated in this chapter will be inflammation and keratinocyte proliferation, which have both been shown to occur within the first 24 hours after wounding (Bacci et al. 2006; Gillitzer and Goebeler 2001; Patel et al. 2005).

Inflammation occurs almost immediately after skin wounding. Cytokines such as TNF-α, IL-1β and TGF-β1 are released from epidermal cells to act as key inflammatory mediators (Barrientos et al. 2008; Gordon et al. 2009; Kondo 2007; Li et al. 2007). These cytokines manipulate fibroblast and keratinocyte functions thereby controlling the proliferation and migration of keratinocytes to the wound bed (Barrientos et al. 2008; Spiekstra et al. 2007; Werner et al. 2007). If any vasculature is damaged, neutrophils released from the leaking

capillaries express TNF-α soon after wounding and induce other chemokines (Barrientos et al. 2008; Gillitzer and Goebeler 2001; Hertle et al. 1995; Sarret et al. 1992; Suter et al. 2009). During the later stages of wound healing, macrophages also express TNF-α (Paladini et al. 1996; Smith 2003; Smoller et al. 1989). Therefore given its intimate involvement in the inflammatory response, TNF-α provides a suitable marker for observing inflammation post-wounding by microneedles or hypodermic needle.

Pro-inflammatory cytokines and growth factors also affect the epidermal cells, activating keratinocytes above the basement membrane through altering keratin expression (Coulombe 2003; Coulombe et al. 1998; Hesse et al. 2000). Keratins are either type I or II intermediate filament proteins which are expressed in pairs to form cytoskeletal structures within epithelial cells (Moll et al. 1982). Type I and type II keratin proteins are encoded by a multigene family (425 members each) and heteropolymerise with one another to produce intermediate-sized filaments in the cytoplasm of epithelial cells (Fuchs and Weber 1994). Type I keratins (K9-K21) range from 40 to 63kDa and are more acidic, whereas type II keratins (K1-K8) are larger (53-67kDa) and more basic (Paladini et al. 1996; Wawersik et al. 1997).

In normal healthy skin, K5 and K14 complexes are constitutively expressed in all keratinotcytes, however, only inactive epidermal keratinocytes express K1 and K10 complexes (Cheng et al. 2008; Coulombe 1997; Suter et al. 2009). Shortly after wounding, keratinocytes proximal to the wound edge undergo cytoskeletal and morphological changes characterised by the down-regulation of K1 and K10 expression and increased expression of K16 and K17

(Freedberg et al. 2001). Human cytokeratin 16 (K16; 48kDa) is of particular interest as it is constitutively expressed in keratinocytes in a variety of epithelial tissues and known to be upregulated during hyperproliferation or abnormal differentiation of epithelial cells (Freedberg et al. 2001; Paladini et al. 1996; Usui et al. 2008). Within 4 to 6 hours post-wounding, activated keratinocytes drive hyperproliferation of inactive keratinocytes at the wound edge by autocrine release of cytokines (Coulombe 1997). As the expression of K16 and K17 increases, between 6 to 24 hours post-wounding, the activated keratinocytes are less adhesive and migrate into the wound site (Freedberg et al. 2001; Usui et al. 2008). Subsequently, the wound site is populated by newly differentiated keratinocytes which return to their stable state expressing keratins K1 and K10, thereby restoring the skin's protective barrier function (Freedberg et al. 2001; Paladini et al. 1996; Usui et al. 2008). In this chapter we aim to identify changes in K16 expression to explore the activation status of keratinocytes within the first 24 hours after application of microneedle devices and hypodermic needles in human volunteers.

4.1.3 Aims and Objectives

This study aims to characterise any wound healing repair responses apparent during the first 24 hours after microneedle or hypodermic needle application to human volunteers.

Objectives:

- To observe the penetration and closing of microneedle and hypodermic needle punctures up to 24 hours post-application using skin surface staining and transepidermal water loss (TEWL).

- To assess and characterise the architecture of the skin post-needle application using H & E staining.
- To establish if the known wound healing marker, K16, is up-regulated during the initial 24 hours post-application.
- To investigate the inflammatory response caused by microneedles and hypodermic needles by assessing the presence of TNF-α.
- To assess the impact of the microneedle or hypodermic puncture on activation and migration of dendritic cells.
- To consider the structural integrity of the microneedles after a single application to human skin.

4.2 Materials and methods

4.2.1 Materials

Acetone; Dry Ice; Ethanol 99.8+% absolute duty free for HPLC CertiFied HPLC; Phosphate buffered saline (PBS); CD68 Dako Clone EBM11, Anti-Human Monoclonal Mouse, Dako (Ely, UK); K16 antibody Dako staining kit, Dako (Ely, UK); Canon IXUS 500 digital camera (Canon, UK), Dermatoscope, Methylene blue dye, propranolol hydrochloride (Fisher Scientific UK Ltd, UK), 25-gauge hypodermic needles; 2% lignocaine with adrenaline local anaesthetic (Fisher Scientific UK Ltd, UK); 280μm and 180μm length 36-pyramidal solid microneedle arrays (Tyndall National Institute, Ireland). Tewameter, Courage & Khazaka GmbH (Koln, Germany).

All reagents were obtained from Fisher Scientific UK (Loughborough, UK) unless otherwise stated.

All materials for IHC were obtained from RA Lamb Limited (Eastborne, UK), these include Optimal cutting tissue compound embedding media (OCT); Histobond adhesive microscope slide. Haematoxylin Gurrs' Eosin aqueous solution 1%; Histomount, xylene (low sulphur) were obtained from Lab 3 (Bristol, UK).

4.2.2 Ethical and consent approval

Prior to beginning the study, the study protocol (Appendix III) was submitted for ethical approval from the Gwent Healthcare NHS Trust R&D Committee and the South East Wales Research Ethics Committee (SEWREC). Ethics approval for the study was granted and Informed consent was obtained from each participant as in adherence with the Declaration of Helsinki. A written explanation of the study was provided before each participant's first visit. Participants signed consent forms (see Appendix VIII) at the first visit after the nature of the study had been fully explained and were informed that they could withdraw from the study at any stage without any explanation.

4.2.3 Summary of the trial day methodology for transepidermal water loss (TEWL) measurement and skin biopsy

Participants visited St. Woolos Hospital on three separate occasions for TEWL measurements, whereby a TEWL reading was taken from the three sites of needle device application. A fourth visit was required for biopsies to be taken of the 3 application sites.

TEWL was measured using a Tewameter (Courage & Khazaka) (Figure 4.1) at 4 time points: immediately baseline, immediately post-application and at 8 and 24 hours post-application.

The Tewameter TEWL probe is a delicate measuring device, which must be protected from shock, dirt and liquids at all times, and therefore was kept covered by a cap at all times when not in use. The sensors were never touched and care was taken to only hold the handle at the farthest point from the sensory end. Air-conditioning maintained the room temperature at 22°C and relative humidity of 45 ± 5%.

Figure 4.1. Tewameter® TM 210 was used for measuring TEWL.

The participant's buttock was not exposed to strong sunlight, heat sources or air circulation, as these may have effected with the transepidermal water loss rate. Each participant was asked to not bathe or wash those areas of their buttock for the 24 hour period.

4.2.3.1 Participant preparation to measure TEWL

The participant was asked to lie comfortably and relax on their front or side with their marked buttock region exposed for 15 minutes prior to any readings being taken. The Tewameter probe was placed horizontal onto the skin

surface and a clamp was used to secure the probe onto the skin. This prevented any interferences arising from hand movement or heating of the probe by the assessor's hand.

4.2.3.2 Measurement of TEWL

Once the participant was comfortable the probe was positioned over the skin region to be measured. Using the clamp the probe was lowered onto the surface of the skin so the cylinder on the sensory end of the probe was vertical and resting gently on the skin surface. Throughout the measurement time, talking was not permitted and a notice was placed on the door to prevent entry, which would upset the airflow around the probe.

The measurement was performed for three minutes. The average TEWL of the previous 20 seconds and the standard deviation were recorded in the case report form (CRF) (Appendix VIII). The units of measurement are gm H_2O/sq.m/hour.

If there were any uncharacteristic spikes during the last 20 seconds of the 3 minutes, the cursor was used to move to a more representative 20 seconds of the overall 3 minute duration. The probe was then recovered and moved to an undisturbed part of the room and left for 2 minutes before the next measurement was taken.

TEWL was measured at 4 time points: immediately baseline, immediately post-application and at 8 and 24 hours post-application as described in Chapter 2.

4.2.4 Methylene blue application, imaging and biopsy of application sites

Depending on which group the participant was assigned to they returned at either 1, 4, 8 or 24 hours after the initial applications for visual assessment of skin punctures and skin biopsies.

4.2.4.1 Visual assessment of skin puncture

Following application of the microneedles and hypodermic needle, external staining of the skin with methylene blue was used to monitor microchannel healing and re-sealing. Using a cotton bud, methylene blue stain was only applied to each of the 3 application sites on the buttock region, which were then biopsied. The stain was allowed 10 minutes to dry before excess stain was removed using ethanol wipes. Subsequently the sites were visually assessed through the dermatoscope and photographed to determine whether the microconduits in the skin were visible.

4.2.4.2 Biopsy of application sites and cryo-transportation of skin samples

The primary aim of the clinical trial was to establish if there is any wound healing response within the epidermis and dermis of the skin after puncture by microneedles and hypodermic needles. Thus it was crucial to fix the skin immediately after biopsy to ensure that any healing response due to needle puncture, were not interfered with or altered by the biopsy process. The fixing and transfer of samples also needed to be considered to ensure all health and safety issues were dealt with. For these reasons a mock run-through of the protocol using ethically obtained excised human skin was conducted with Dr. Rebecca Porter at her Dermatology laboratory in The University Hospital of

Wales, Cardiff. For further details of ethical and consented use of excised skin see Chapter 3.

4.2.4.2.1 Biopsying the application sites

Cryotubes were labelled with date, patient number and sample number. Corkboard was cut to ensure it could easily fit inside the cryotubes. Dry ice was added to a polystyrene icebox into which the labelled cryotubes were placed to be cooled. On a secure surface/bench near the participant, the fixing equipment (Figure 4.2) was prepared, ensuring it was suitably positioned for easy access and satisfying health and safety requirements.

The 3 application sites to be biopsied were injected with 2% lignocaine with adrenaline as local anaesthetic. The 2 microneedle-device sites were excised with 6mm diameter punch biopsies and closed with 2 Ethilon sutures. The hypodermic needle site was excised with a 4mm diameter punch biopsy and closed with a single Ethilon suture (Figure 4.3).

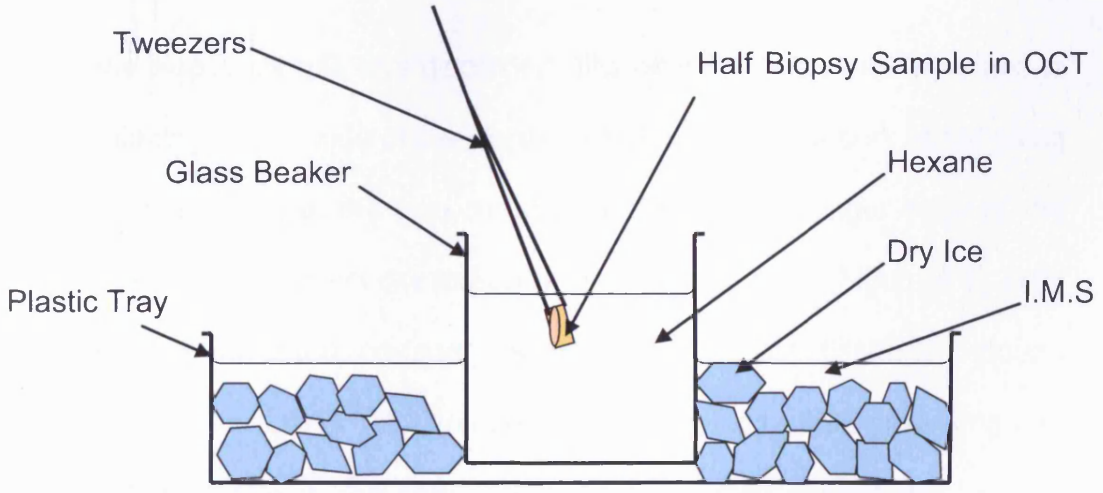


Figure 4.2. Diagram to show the snap-freezing fixing method for freshly biopsied skin.

Immediately after the biopsy, to ease mounting onto the cryostat, the circular skin sample was placed on a cutting board and cut down the middle in between the rows of punctures (Figure 4.4). To reduce damaging the microchannels, the biopsy samples were cut in between the rows of microneedle punctures, which were highlighted by methylene blue.

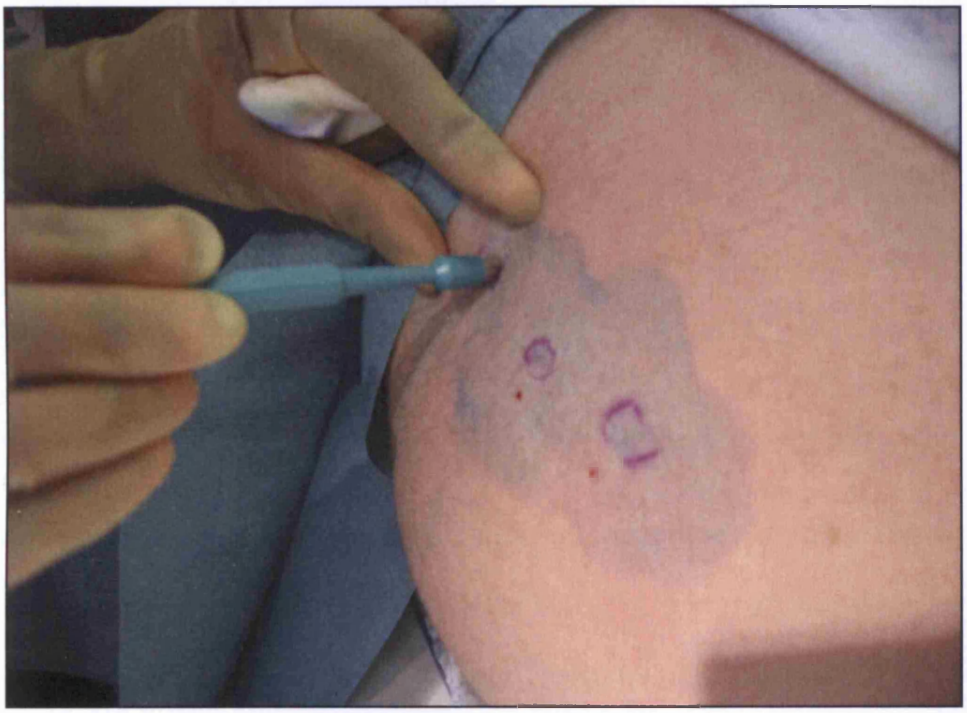


Figure 4.3. *In-vivo* example of the biopsy sites

Half of the biopsy sample was discarded. The other half was then mounted to cork by placing the flat side of the biopsy sample against the cork board using tweezers, ensuring that the skin surface lined up to the longer edge of the rectangular cork for correct orientation when cryosectioning (Figure 4.4). Skin samples were mounted on cork using optimal cutting tissue compound embedding media (OCT). OCT freezes over the sample, thus protecting and securing the sample to the cork mount. The mounted sample was quickly placed into a hexane beaker as shown in Figure 4.2 to freeze the OCT. Finally

the sample was placed into an appropriately labelled cryotube, which was stored in dry ice ready for transport. Until they were analysed, the cryotubes were stored at -80°C at The University Hospital of Wales, Cardiff.

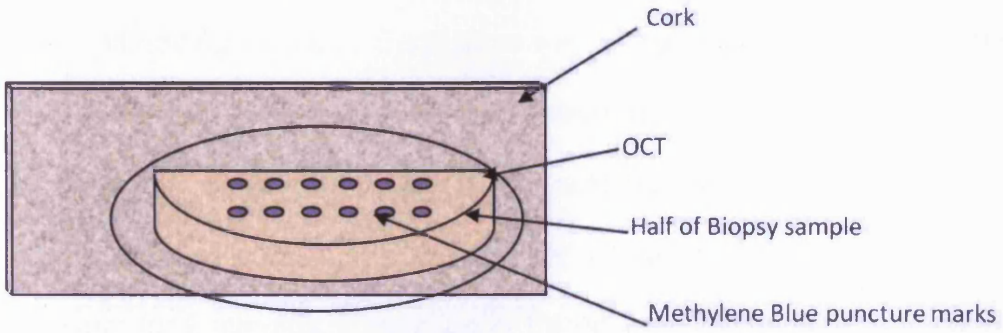


Figure 4.4. Diagram to show orientation and mounting of biopsied skin prior to snap freezing.

4.2.5 Immunohistochemistry (IHC) of sectioned biopsy samples for wound healing analysis

The participant's initials, biopsy site and the unique study number were used to identify each skin sample. Frozen sections were cryosectioned jointly by Dr Rebecca Porter at The University Hospital of Wales, Cardiff and Mohammed Haq at Welsh School of Pharmacy, Cardiff and prepared for IHC assessment of the wound healing response by identifying the up-regulation, stasis or down-regulation of specific markers for 'repair and stress responses'. The frozen biopsy samples were mounted in the cryostat so as to section across the length of the channels. The sections were mounted onto microscope slides and stained with antibodies to keratins K16 to investigate the degree of tissue damage, whilst an antibody to the dendritic marker, CD68 and inflammatory marker, TNFα was used to identify any enhanced immunological and inflammatory reaction to the needle puncture.

4.2.5.1 Preparation of cryosectioned biopsy samples taken post-application of microneedles and hypodermic needle.

The skin samples were then frozen in OCT embedding medium in a cold ethanol bath on solid CO_2 and cryosectioned at a thickness of 10μm using the Leica CM3050S cryostat. Cryosections were washed in PBS to remove the OCT embedding medium. Each slide was immersed in Harris' haematoxylin (H) solution for 10 minutes for progressive H staining. Subsequently, after rinsing under running tap water for 1 minutes, differentiation in 0.3% acid alcohol (ethanol containing 0.3%HCl) for 10 seconds, rinsing under running tap water for 2 minutes, immersion in 1% eosin (E) solution for 1 second, and rinsing under running tap water for 2 minutes, the H & E-stained cryosections were permanently mounted with Histomount™ and a coverslip applied.

4.2.5.2 K16, TNFα and CD68 staining protocol

Unfixed cryosectioned samples mounted on slides were stored at -80°C until use. Initially the sample slides were placed in a staining trough. This was filled with acetone and left to stand for 15 minutes to fix the slides. The slides were then removed and left for 5 minutes to air dry. The slides were then placed into a clean staining trough, filled with 10% PBS for 5 minutes to remove any fixation deposits. This was repeated 3 times after which the excess was tapped off, and the slides carefully wiped using tissue around the samples to form a wet area over the sample. A wax pen was also used to form a barrier around the samples to keep them moist. The samples were placed in an incubation chamber and covered liberally in peroxidase for 5 minutes.

Following peroxidase blocking, the slides were rinsed in PBS and primary antibody solution was pipetted onto each slide. Dilutions for the primary

antibodies were optimising by staining a range from 1:100 to 1:500 and altering incubation time from 1 hour to 24 hours. K16 was found to work optimally at 1:500 dilution with an incubation of 24 hours at 3°C, whilst K14, CD68 and TNFα were diluted 1:100 and incubated for 1 hour at room temperature.

After the primary antibody incubation, the slides were all washed twice in 2 separate 10% PBS washes. The excess was tapped off and slides incubated with secondary antibody (anti-mouse monoclonal) for 30 minutes in the incubation chamber at room temperature.

Finally, the slides were rinsed in 10% PBS for five minutes, excess tapped off and chromogen (DAB) applied for a maximum of 5 minutes with continuous observation under microscope to prevent over-staining. To quench the staining, slides were placed in a PBS bath. Thereafter the slides were imaged through a microscope, counterstained with haematoxylin for 2 minutes, and quenched in a cold-water bath.

The slides were then dehydrated through an ethanol gradient from 30%, 50%, 70%, 90% for 3 to 5 minutes in each. Subsequently the slides were transferred to a xylene gradient from 30%, 50%, 70%, 90% for 2 minutes in each. Finally, the slides were covered in Histomount and a cover slip applied. The slides were left to set and imaged under microscope before being stored.

4.2.6 Imaging stained slides

The samples were now mounted on slides and protected by cover slips. Each slide was viewed and photographed under a microscope at 20x magnification. A graticule was used to establish scale to make suitable measurements of the images.

4.2.7 Microneedle deformation

To facilitate application during *ex-vivo* and *in-vivo* treatments, the microneedle arrays are adhered to the solid base of an applicator. During the clinical trial, an inverted-syringe plunger was used as the applicator, the plastic affording some softness and flexibility during application whilst still enabling microneedle puncture of the skin. Stereomicroscopy and SEM imaging of the microneedles post-application was used to assess any surface damage to the microneedles or the arrays used during the study.

4.3 Results

The microneedles and hypodermic needle were applied to human volunteers. Subsequently the participants returned at 1, 4, 8 and 24 hours post-application for topical staining and punch biopsy of the application sites. Furthermore the biopsy samples were snap-frozen on site, transported to secure storage and subsequently sectioned and analysed by immunohistochemistry to observe any wound healing or immunological responses.

4.3.1 Topical staining of needle punctures visualised dermatoscopically

The participants all returned at their required time for methylene blue application and photography prior to the punch biopsy. Dermatoscopy allowed

a magnified view of the application site to be photographed. At the 1 hour time point the microneedle punctures were prominently stained and visualised as a defined and evenly spaced grid of 6 by 6 microneedle punctures. However, not all the microneedles of either 180μm or 280μm appeared to have punctured the skin surface, as no methylene blue stain was apparent as some of the points of the 6 by 6 array (Figure 4.5-4.8).

At 1 hour post-application, the microneedle and hypodermic channels would be expected to be most visible and this time point therefore best represents the puncture efficiency of each needle type. Figure 4.5 shows that the 180μm microneedle arrays did not penetrate the skin with every microneedle. However, of the 3 participants stained 1 hour post-application, the 280μm microneedles appeared to puncture the skin better than the 180μm microneedles, as both participant 1 and 3 have 100% punctures visible for the 280μm microneedle application sites, but not for the 180μm microneedle sites (Figure 4.5).

The microneedle punctures are more prominent at 1 hour after application than at 24 hours after application. However at each time point there are clear differences in puncture staining between different participants suggesting the microneedles either did not penetrate as efficiently or the skin of each participant reacted differently to the application thus less methylene blue diffused through each microneedle induced puncture.

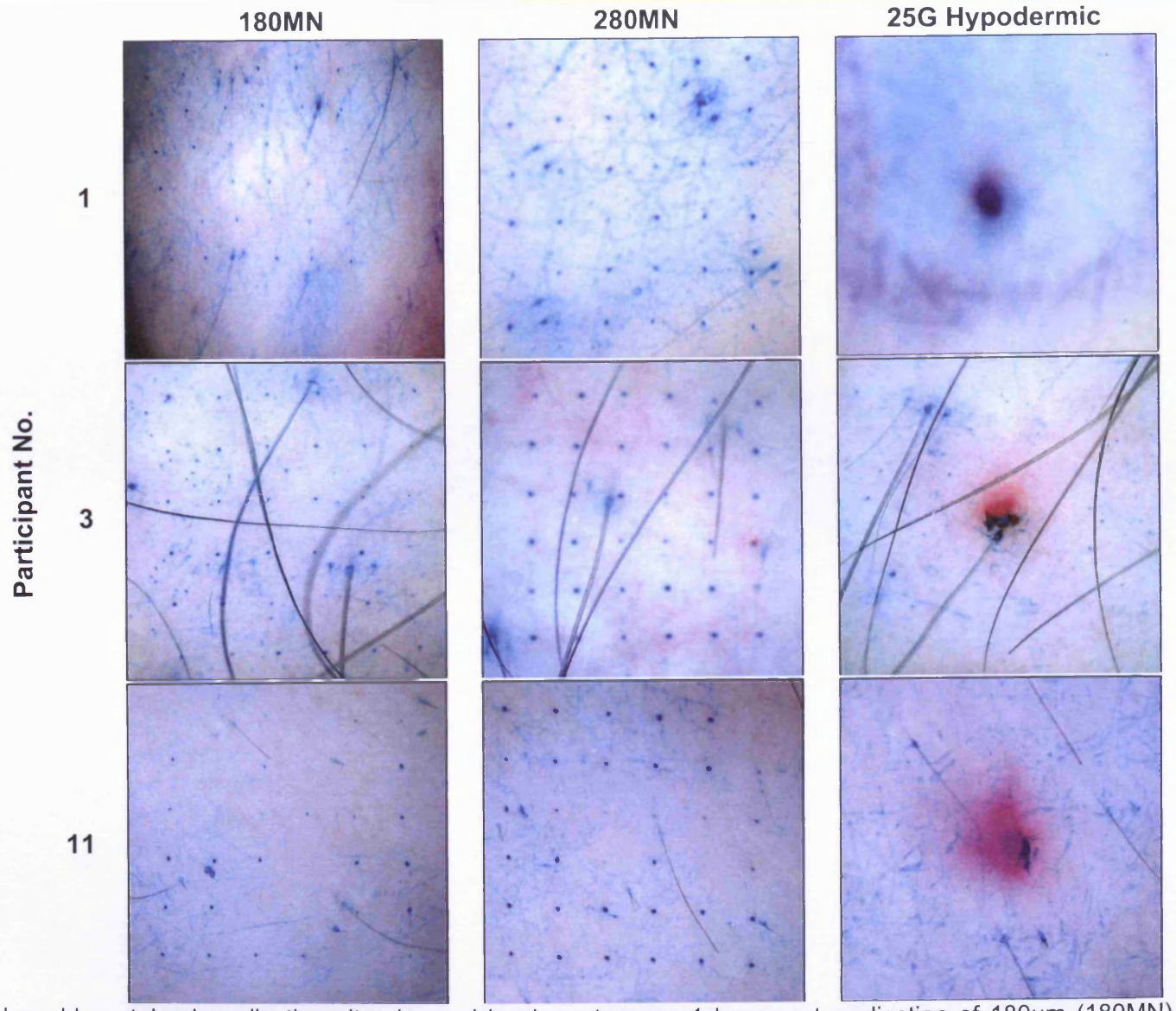


Figure 4.5. Methylene blue stained application sites imaged by dermatoscopy 1 hour post-application of 180µm (180MN), 280µm (280MN) microneedles and 25G hypodermic needle.

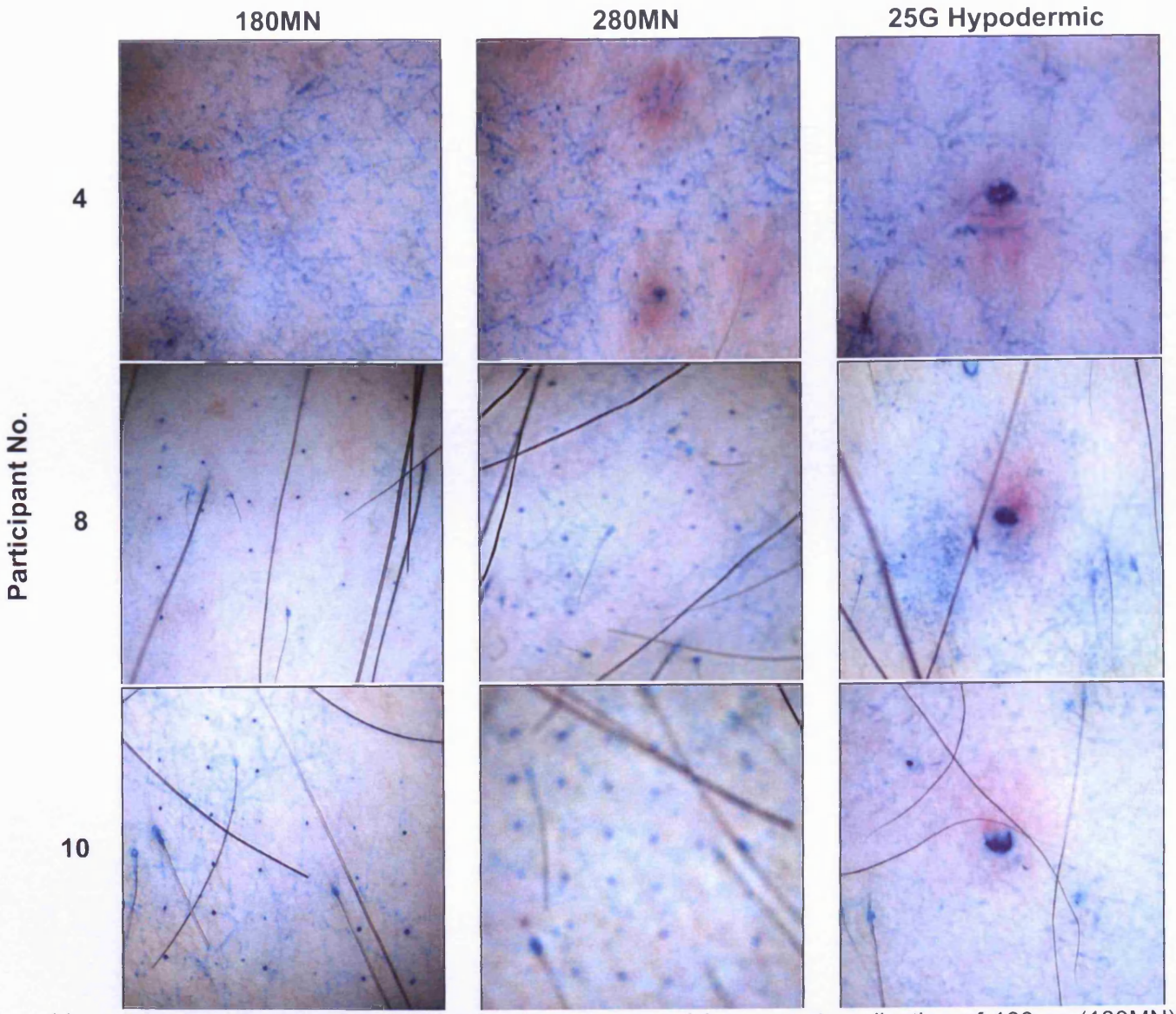


Figure 4.6. Methylene blue stained application sites imaged by dermatoscopy 4 hours post-application of 180µm (180MN), 280µm (280MN) microneedles and 25G hypodermic needle.

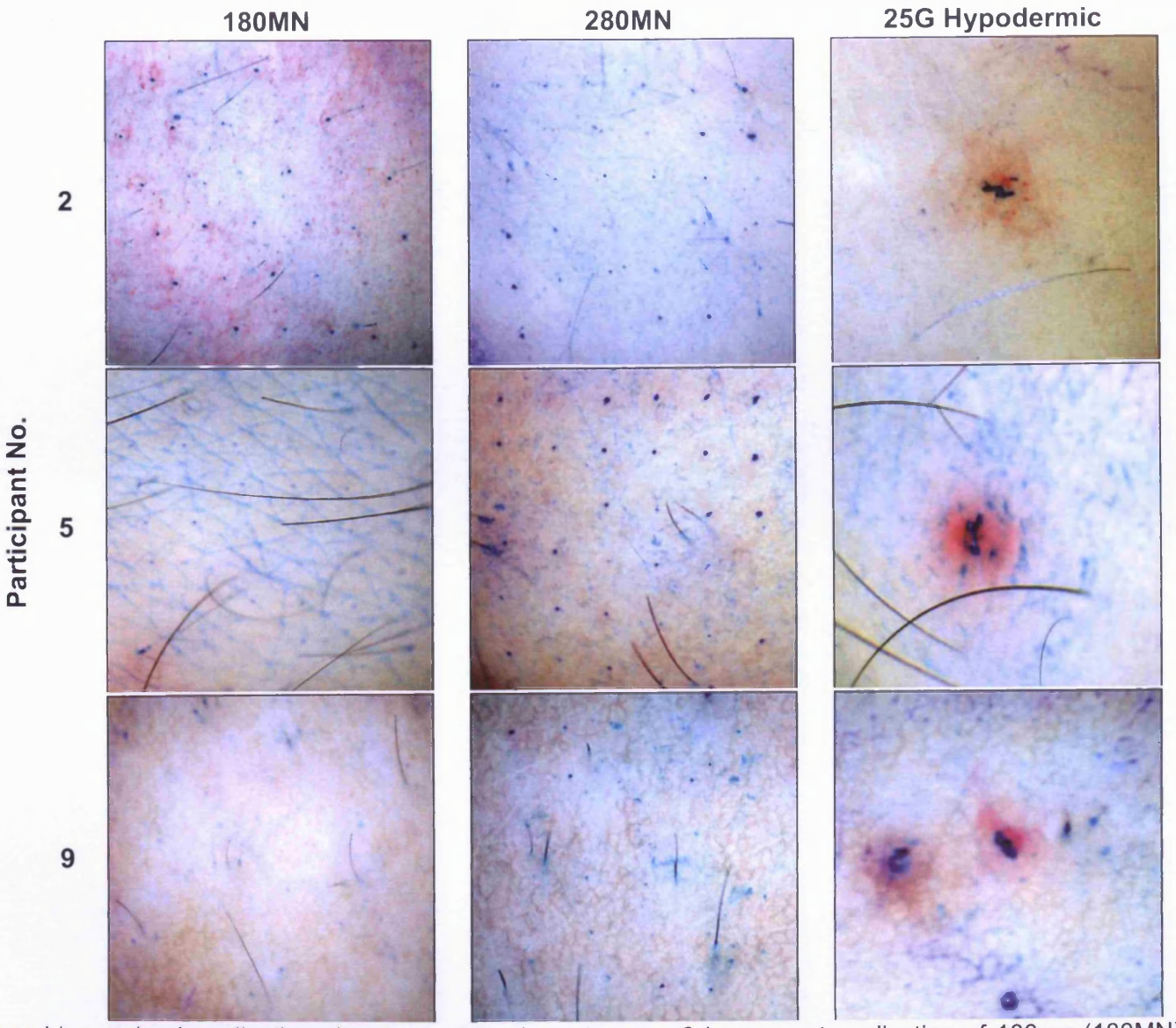


Figure 4.7. Methylene blue stained application sites imaged by dermatoscopy 8 hours post-application of 180μm (180MN), 280μm (280MN) microneedles and 25G hypodermic needle.

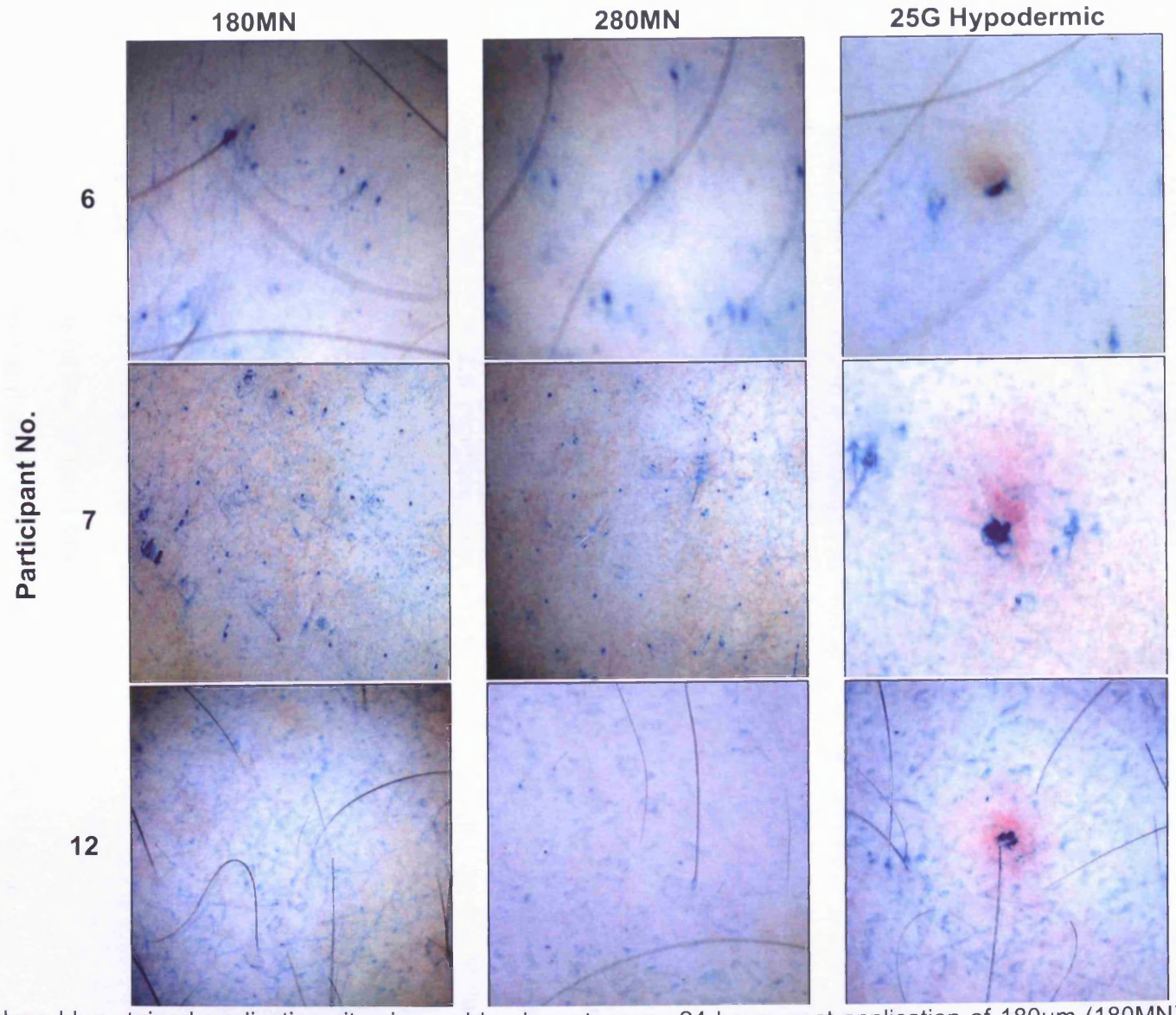


Figure 4.8. Methylene blue stained application sites imaged by dermatoscopy 24 hours post-application of 180μm (180MN), 280μm (280MN) microneedles and 25G hypodermic needle.

Comparison of the microneedle applications to the hypodermic needle shows significant differences caused by the size of needle penetrating the skin: At each time point the hypodermic needle has not only caused extensive trauma, but the skin tone has altered due to the erythematic reaction in the skin caused by the damage to subcutaneous and intra-dermal vasculature and the subsequent, inflammation, clotting and healing around the puncture site.

The shorter 180μm microneedle appears to penetrate less effectively or indeed the punctures are shallow enough to reseal quickly thereby preventing methylene blue to penetrate into the puncture. Figure 2 4.5-4.8 also show the microneedle channels in relation to hair follicles.

Regarding resealing of the observed microchannels, methylene blue staining showed skin repair appearing between 8 to 24 hours post-application. Photographs taken of the puncture sites show that the 280μm device initially made the greatest number of puncture marks, with a mean of 96% of the 36 pyramidal needles puncturing the skin (Table 4.1). Whilst the 180μm was less penetrating, both the microneedle array puncture marks appear to be closing as there is a reduction over the 24 hour period in the staining of punctures (Figure 4.9).

4.3.2 Transepidermal Water Loss

TEWL measurements were taken over a period of 24 hours. Post-application of the needle devices TEWL measurements further demonstrated perturbation of the SC barrier with water loss increasing post application of each device. Mean TEWL increased immediately post-application for the hypodermic needle

from 3.4 (range 13.6) - 8.7 (range 17.4) gm H_2O/sq.m/hour; from 4.85 (range 13.9) -8.7 (range 8.8) for 180µm; and from 4.1 (range 11.1) - 6.1 (range 49.1) for the 280µm array. Each recovered to baseline value by 24 hours.

Table 4.1. Percentage of puncture marks (out of a maximum of 36 channels) as observed by dermatoscopy for both the 180µm (180MN) and 280µm (280MN) microneedle arrays

Needle type	Time (hours)			
	1	4	8	24
180MN	89%	39%	50%	6%
	52%	72%	19%	58%
	61%	86%	75%	3%
Mean	**67%**	**66%**	**48%**	**22%**
280MN	97%	67%	97%	6%
	100%	78%	42%	83%
	92%	72%	89%	6%
Mean	**96%**	**72%**	**76%**	**31%**

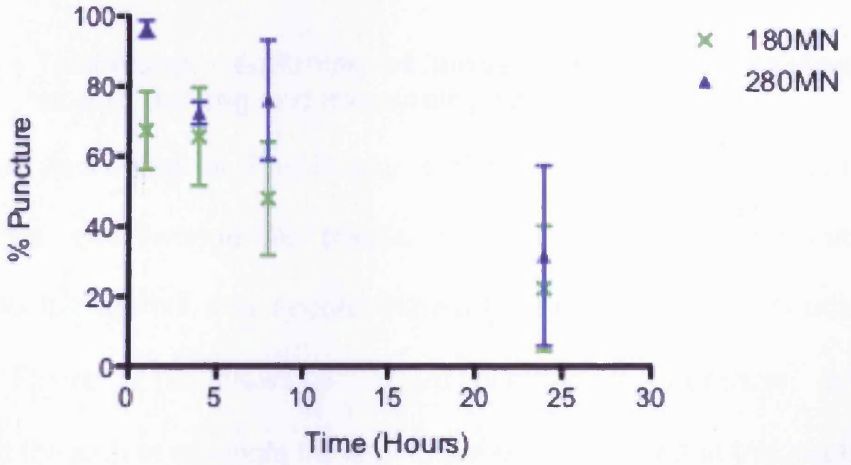


Figure 4.9. Mean percentage of punctures marks observed through dermatoscopy at each biopsy time (1, 4, 8 and 24 hours post-application). Varying numbers of puncture marks were observed at the different time points over the 24 hour period. Puncture marks appear to reduce over the 24 hour period. The 180µm array appears to have penetrated less efficiently than the 280µm array and these punctures are potentially closing at a faster rate than those created by the 280µm array.

Figure 4.10 shows the increase in mean TEWL immediately post-application and the TEWL normalising over the 24 hour period. The initial increase in TEWL immediately post-application (pre-application v 1hr) shows significant change for the 280µm (5.7-10.3gmH2O/sq.m/hour) and hypodermic devices (5.1-

8.7gmH_2O/sq.m/hour) with p=0.0093 and p=0.0024 respectively, whilst p=0.34 for the 180 (5.9-7.9gmH_2O/sq.m/hour) was insignificant. At 8 hours post-application, the significance reduced to p=0.0049 (280μm), p=0.021 (hypodermic) and p=0.15 (180μm). By 24 hours the TEWL had returned to a normalised level with their being no significance for any device when compared to control: 180 (p=0.47), 280 (p=0.41), Hypodermic (p=0.79).

Figure 4.11 compares the visible puncture marks, as observed using methylene blue staining, to the TEWL measurements. Both the numbers of puncture marks and increases in TEWL were more pronounced for the 280μm microneedle compared with the 180μm microneedles. As the mean TEWL normalises over 24 hours the mean puncture marks observed reduce too.

4.3.3 Transverse sectioning of biopsy samples for assessment of wound healing and immunological responses

Transverse sectioning of the biopsy samples enabled the punctures from microneedles and hypodermic needle to be observed through the stratum corneum to the dermis and deeper dermis for the hypodermic needle (Figure 4.12-14). Figure 4.12 shows a 180μm microneedle puncture, which has penetrated the skin at an angle through to the epidermis, yet at this section of the microchannel, the stratum corneum (SC) appears intact. Certain sections, such as Figure 4.13A, revealed how the multiple 280μm microneedles along the array penetrated the skin, whilst the longer 280μm microneedles also penetrated deeper through the epidermis to the basement membrane (Figure 4.13C).

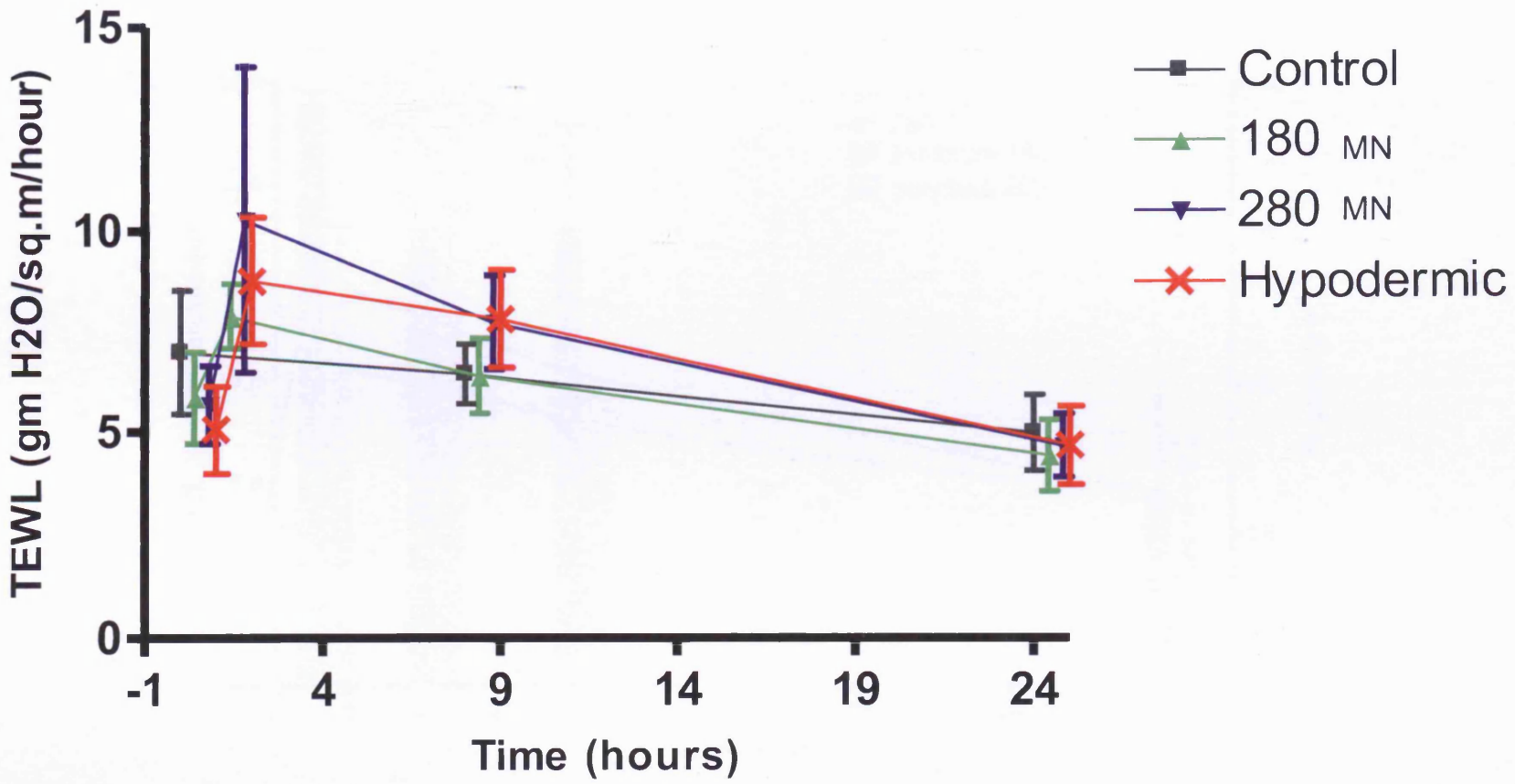


Figure 4.10. Changes in mean TEWL over the 24 hour period post-application of 180µm, 280µm microneedles and hypodermic needle. TEWL readings were taken pre-application of device, immediately post-application and at 8 hours and 24 hours post-application. Graph shows the sudden increase post-application of each needle type, which then normalised over the 24-hour period. The mean TEWL and standard error of the mean are plotted. *time points have been offset by 20 minutes to enable the data points to be easily read.

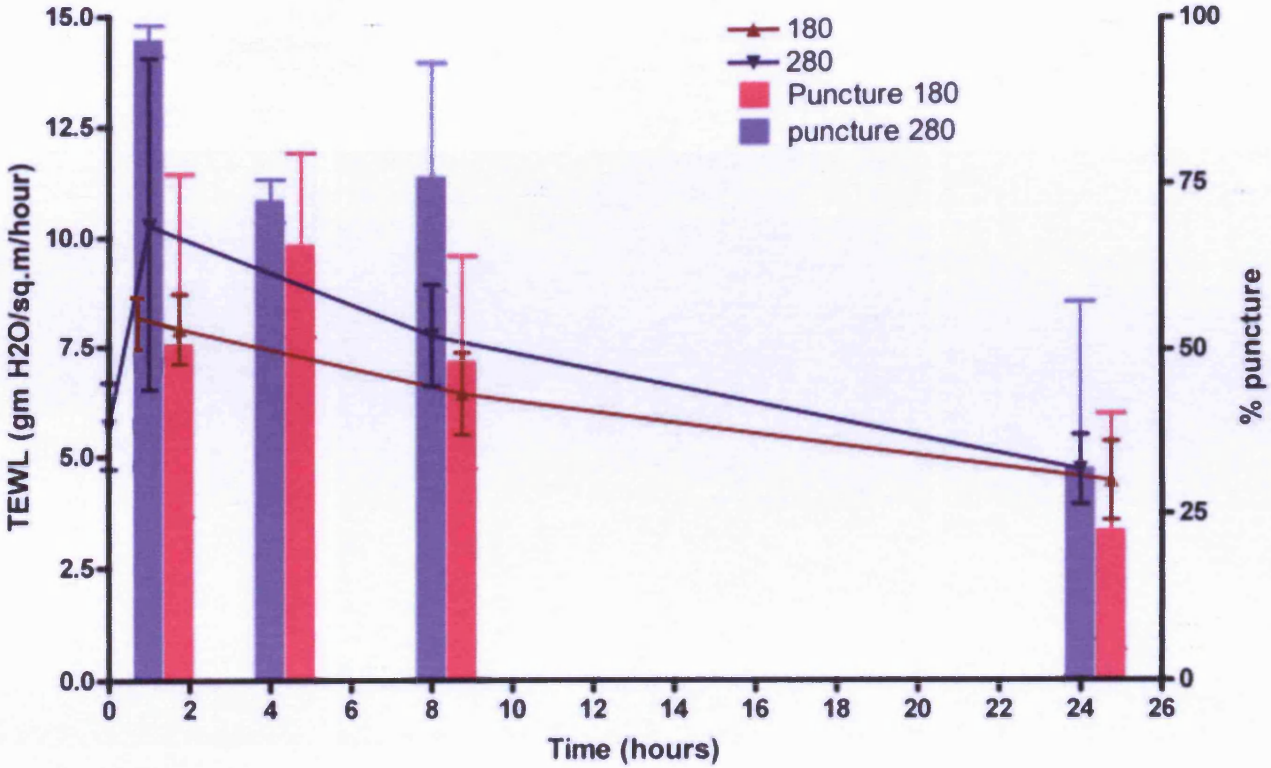


Figure 4.11. Comparison of mean TEWL with percentage puncture as observed by dermatoscopy over the 24 hour period post-application of 180µm and 280µm microneedle arrays. N.B. Time points have been offset by 20 minutes to enable the data points to be easily read.

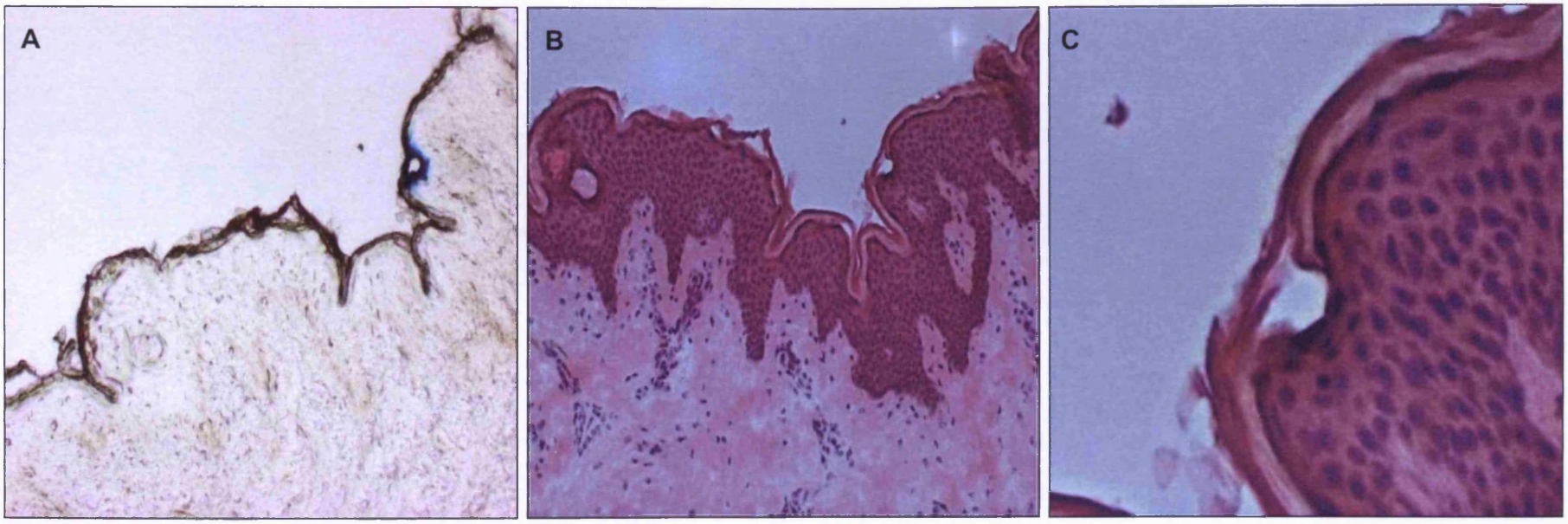


Figure 4.12. Transverse sections of biopsy samples taken post-methylene blue staining from Participant 11 at 1 hour post-application of 180µm microneedle array. [A] 180µm microneedle puncture at x20 magnification; [B] x20 magnification haematoxylin stained; [C] x40 magnification of individual puncture.

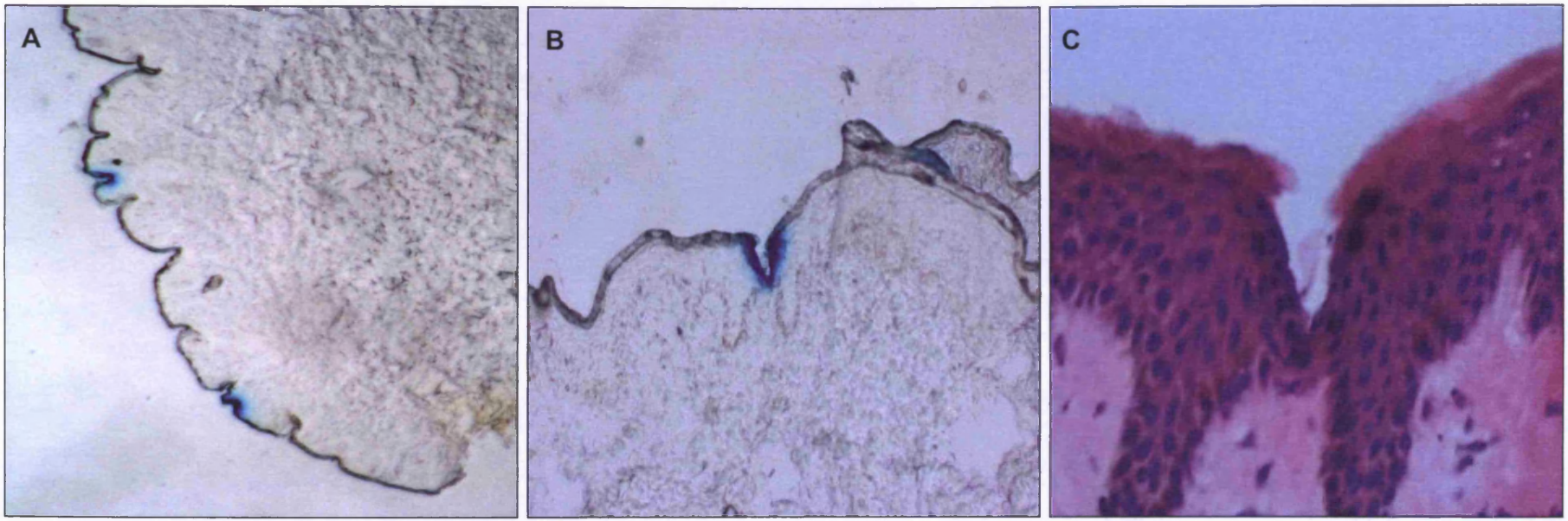


Figure 4.13. Transverse sections of biopsy samples taken post-methylene blue staining from Participant 3 at 1 hour post-application of 280μm microneedle array. [A] 280μm microneedle puncture pre-stained x10 shows 2 neighbouring microneedle punctures; [B] 280μm microneedle puncture at x20 magnification; [C] 280μm microneedle puncture counterstained with haematoxylin at x40 magnification.

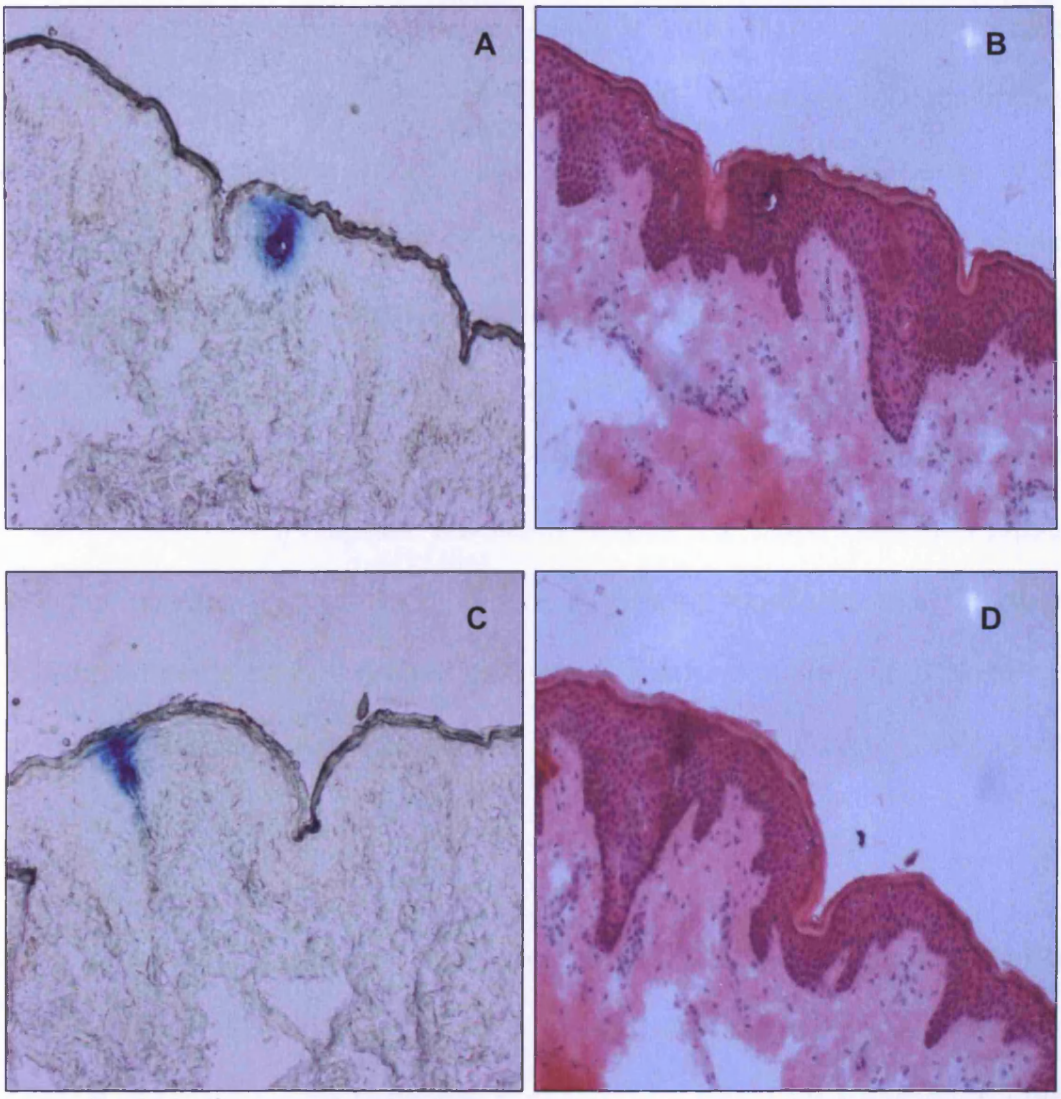


Figure 4.14. Transverse sections of biopsy samples taken post-methylene blue staining from Participant 11 at 1 hour post-application of 280μm microneedle array highlight microneedle penetration is not perpendicular to the skin surface. Images [A]–[D] show the 280μm microneedle penetrated the skin at an angle thereby puncturing through some of the skin valleys, thus not all sections had breaks in their stratum corneum but did have microneedle punctures visible.

Figure 4.13A also demonstrates the size of 280μm microneedle punctures in relation to the ridges and troughs in the skin surface.

Microneedles do not penetrate the skin along a single plane as they are rolled onto the skin using the applicator rod (Figure 4.14). Therefore, sections showed signs of conduits passing through the epidermis but without evidence of SC damage, though higher intensity of haematoxylin and eosin (H & E) staining around the puncture site suggests rupture and compression of surrounding cells. Without visible methylene blue, it was very difficult to distinguish punctures from skin ridges. The hypodermic needle, however, created a large diameter wound through the deeper dermis, thus associated debris and blood clotting is present within the conduit (Figure 4.15). Figure 4.15 also highlights that whilst the hypodermic needle causes greater damage, the wound is starting to close by 8 hours post-application of needle and the methylene blue has diffused through the epidermis.

Immunohistochemical analysis of biopsy samples showed up-regulation of the wound healing K16 marker (Figure 4.16A). K16 up-regulation was localised to the site of puncture by the hypodermic needle at 8 hours for Participant 2, with reduced up-regulation further from the puncture site and at the periphery of the sample. Contrast analysis of the image highlights the K16 intensity (Figure 4.16B). K16 was shown to be up-regulated around the 280μm microneedle punctures at 24 hours post-application (Figure 4.16C) although this was not as clear as for hypodermic punctures. K16 was not up-regulated over the 24 hours period following 180μm microneedle applications (not shown). The K14 positive

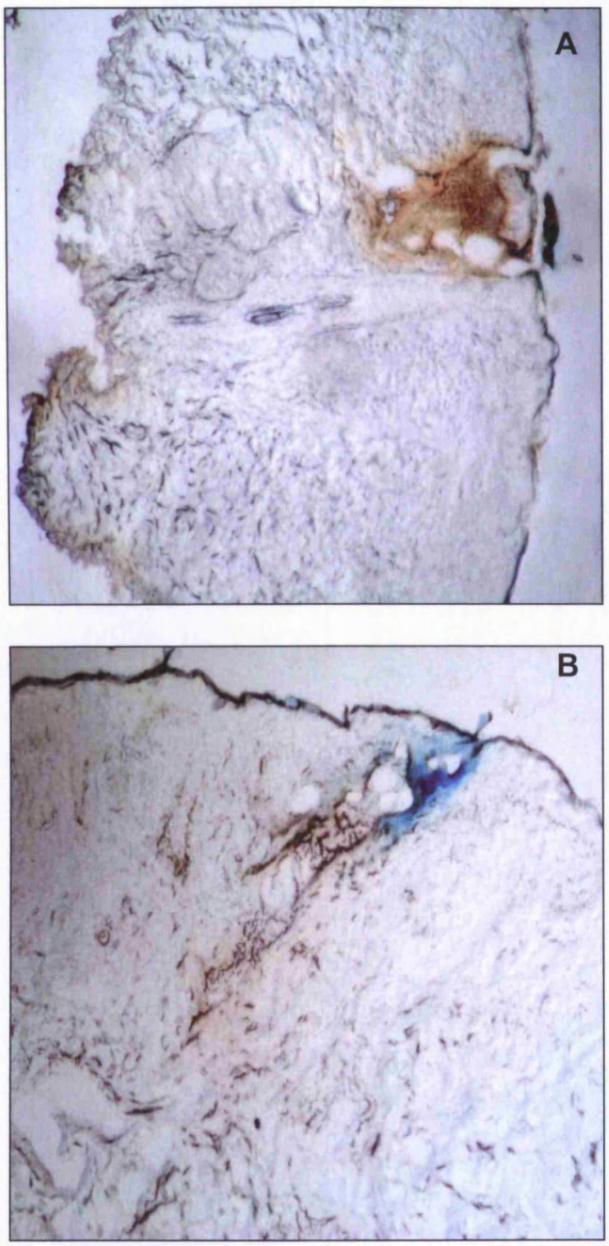


Figure 4.15. 25G hypodermic needle puncture disrupts dermal vasculature and elicits clotting. [A] The hypodermic needle applied to Participant 1 imaged at x10 magnification. The needle penetrated to the deeper dermis and through the biopsy sample. Signs of clotting and the magnitude of the wound are overt at 1 hour post-application; [B] Participant 5 was biopsied at 8 hours post application. At x20 magnification the wound created is healing and resealing. The methylene blue has diffused through the epidermis.

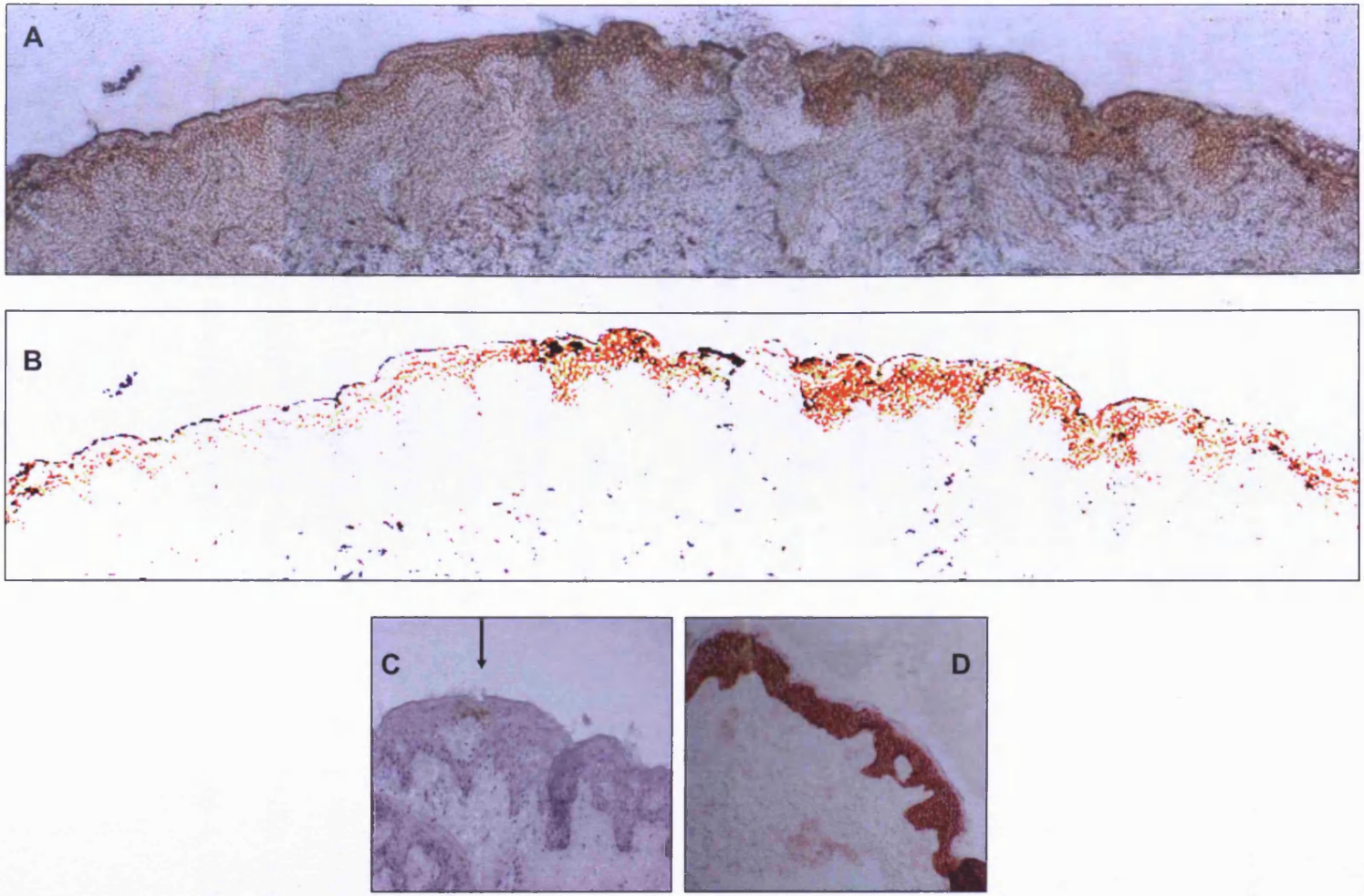


Figure 4.16. Transverse sections of hypodermic and microneedle punctured biopsy samples stained for K16. [A] Up-regulation of K16 in the epidermis around the site of puncture at 4 hours post-application; [B] altering the contrast highlights the localisation of K16 is more intense around the puncture site. [C] K16 was only shown to be up-regulated around the 280μm microneedle puncture site at 24 hours post-application of Participant 12; [D] K14 positive control.

control, Figure 4.16D, showed how the samples would look if the markers are present throughout the epidermis as K14 is expressed on the surface of all keratinocytes in the epidermis.

Figure 4.17 displays images stained for the immunological marker CD68. Up-regulation of CD68 was present around the hypodermic insertion site as early as 4hrs post-application, suggesting a migration of maturing dendritic cells to the point of injury (Figure 4.17C). However, from 1 hour to 24 hours CD68 was not up-regulated or localised at either the 180μm microneedle (Figure 4.17D) or 280μm microneedle (Figure 4.17E) puncture sites. Other immunological marker, Langerin, was applied to transverse sections to establish the presence and location of Langerhans' cells and whether they migrate in response to needle puncture. Figure 4.17F highlights the presence of Langerhans' cells in the epidermis of Participant 6 (Figure 4.17F).

TNFα was chosen as a marker for inflammation as it is released from dendritic cells and blood cells after trauma. The hypodermic needle instigated an immediate inflammatory response with TNFα up-regulation seen at one hour post-application (Figure 4.18A). TNFα remained present at 4 hours post-application of the hypodermic needle (Figure 4.18B), though no evidence of TNFα up-regulation was found for either the 180μm (Figure 4.18C) or 280μm (Figure 4.18D) microneedle applications.

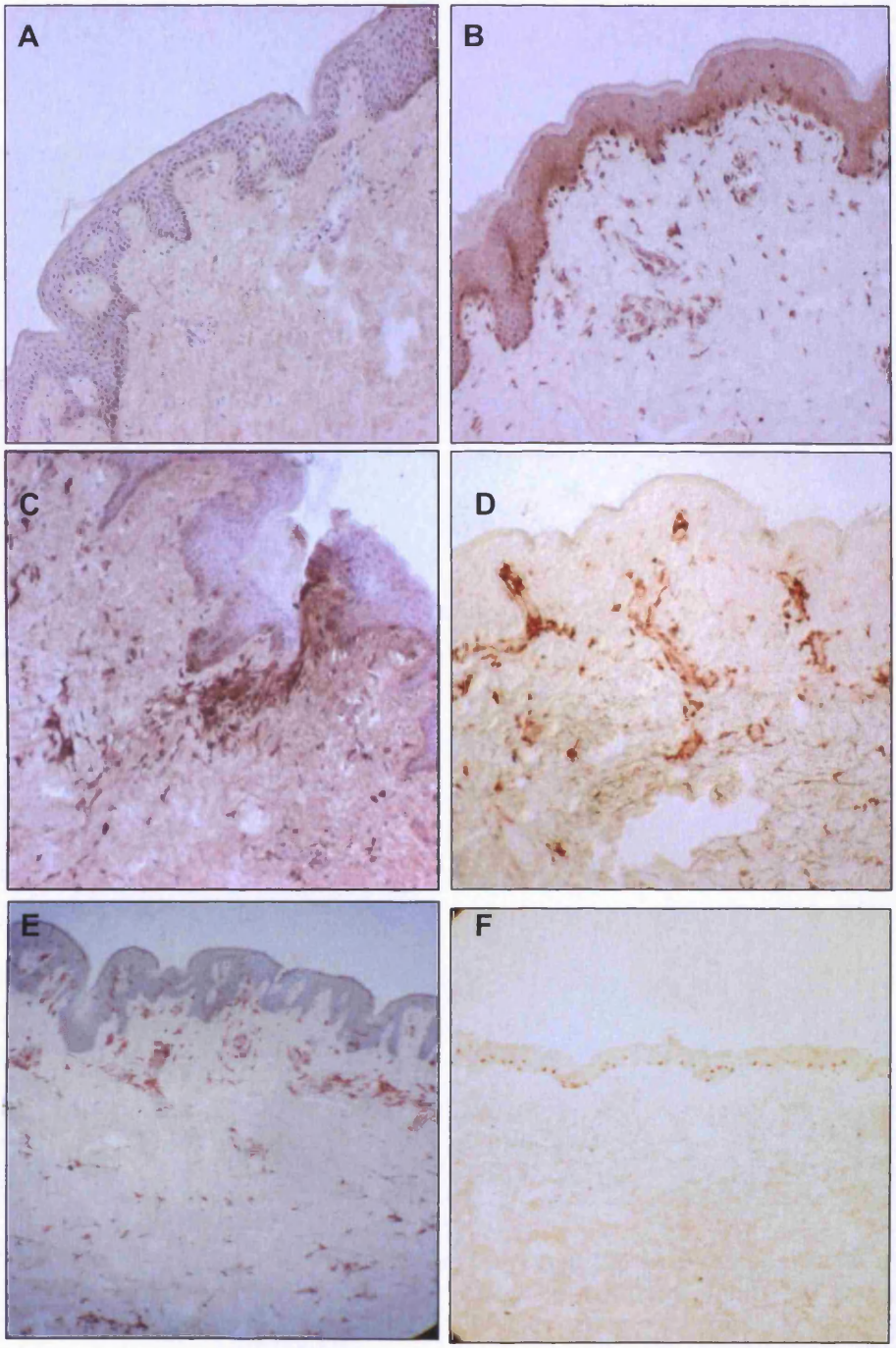


Figure 4.17. IHC staining for immune dendritic cell markers CD68 and Langerin for the 180µm and 280µm microneedles and hypodermic needle. [A] Haematoxylin counter stained control sample. [B] CD68 positive control with no needle puncture; [C] CD68 localisation around the hypodermic site at 4 hours post-application; [D] CD68 distribution in sample punctured by 180µm microneedle shows no localisation up to 24 hours post-application; [E] Haematoxylin counterstained 280µm microneedle puncture has no CD68 localisation at 1 hour post-application. [F] Langerin stained sample with no puncture from Participant 6 highlights the presence of Langerhans'' cells in the epidermis.

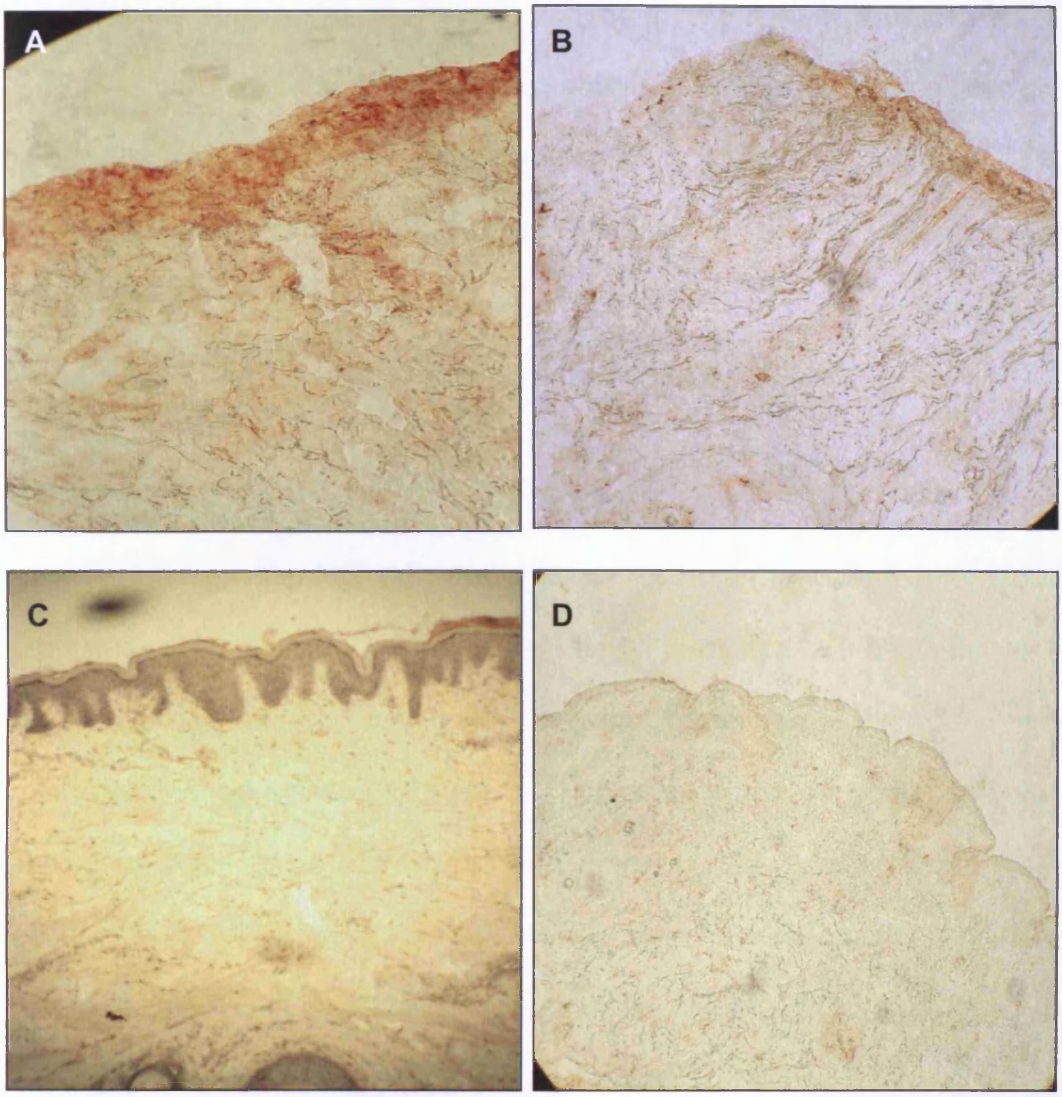


Figure 4.18. TNFα stained biopsy samples from hypodermic needle [A-B] show up-regulation of TNFα but not for microneedle array applications [C-D]. [A] Sample from Participant 11 biopsy taken at 1 hour post application of Hypodermic needle; [B] sample from Participant 8, taken 4 hours post-application of hypodermic needle; [C] sample from Participant 8, post-application of 180μm microneedle array (counterstained with haematoxylin); [D] sample from Participant 9, taken 8 hours post-application of 280μm microneedle array.

4.3.4 Characterisation of Microneedle Arrays

All the 280μm and 180μm microneedle arrays to be used in the clinical trial where checked for damage using SEM and stereomicroscopy (details in Chapter 2). Any damaged arrays were removed from the stock that was used in the clinical trial. All the microneedle arrays were re-characterised post-application *ex-vivo* and following clinical application to human volunteers. Each of the 36 microneedles on the 12 arrays remained morphologically intact after single application to human volunteers. Figure 4.19 shows the post-application debris of human skin left on the array and microneedle surfaces, respectively.

4.4 Discussion

Basic visual assessment of skin puncture caused by the 180μm and 280μm microneedles and the 25G hypodermic needle provided an estimate of the physical damage caused by these needles penetrating the skin. If all 36 microneedles penetrated the skin, a calculation of the cellular volume displaced by each array can be made. Comparing this displacement to that potentially caused by a fully inserted 25G hypodermic needle gives and idea of the different volumes of displacement between the different needle devices.

Assuming each microneedle has a perfect octagonal base, whereby each of outer lengths of the 8 sides is of equal dimensions. The octagonal base contains 8 isosceles triangles (Figure 4.20). The area of each triangle can be calculated, whereby the perpendicular length from the base of each triangle is half of 150μm.

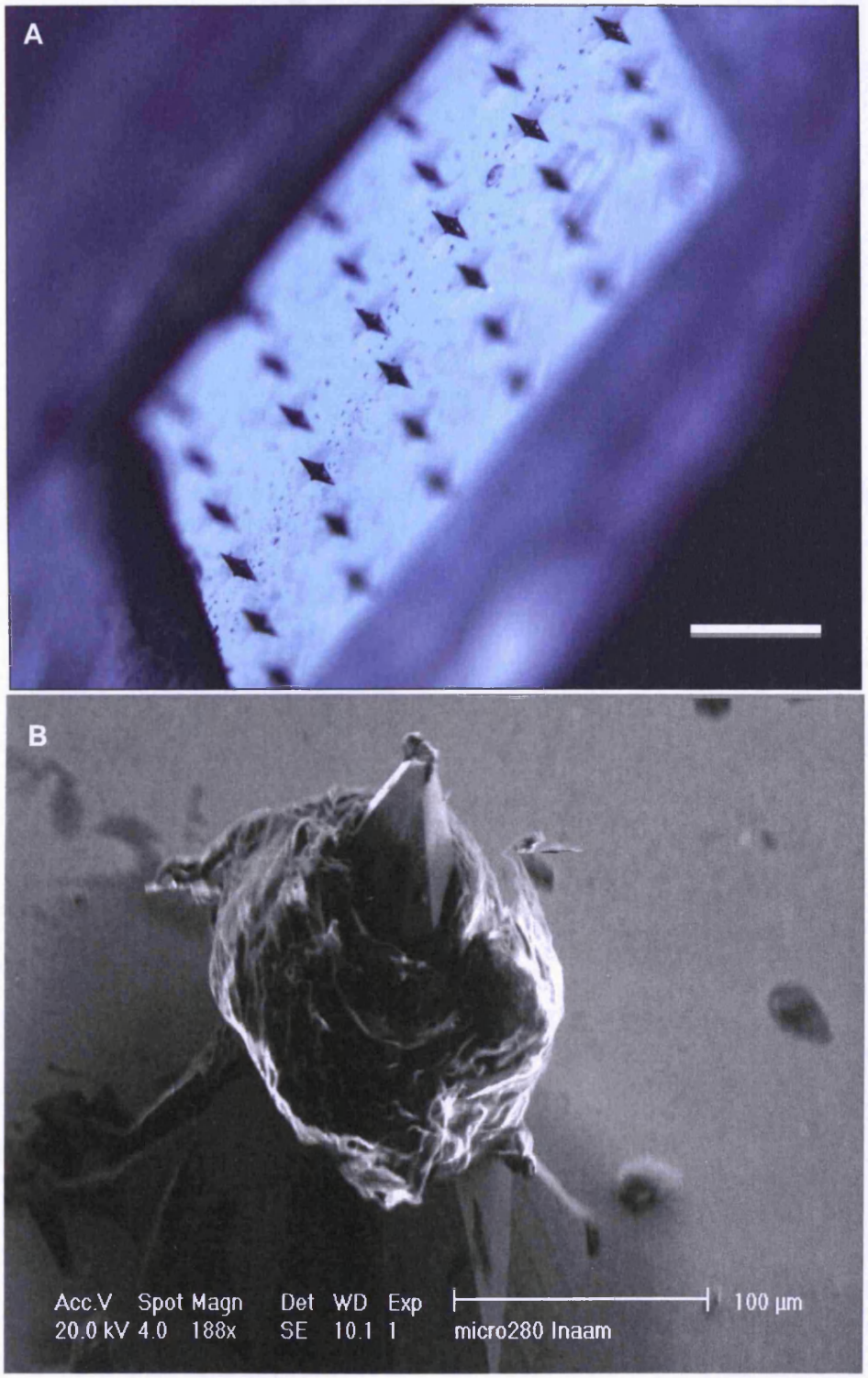


Figure 4.19. Example images from stereomicroscopy and SEM of 280µm array post-application *in-vivo*. Stereomicroscopy image [A] shows intact 280µm microneedle array with its surface covered in fine skin debris (Bar = 1mm); and SEM micrographs [B] showing skin from the participant wrapped around a single microneedle.

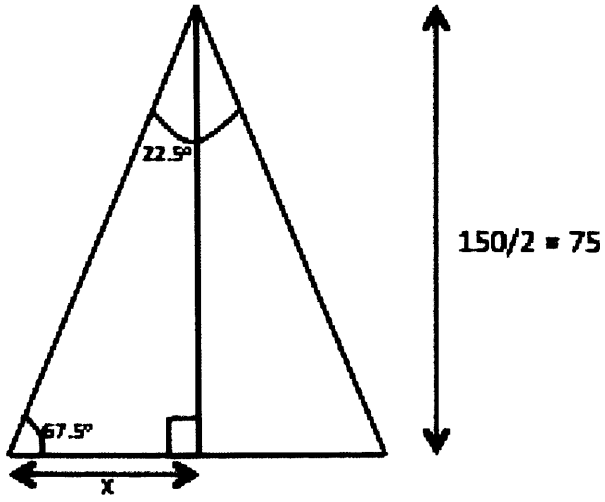


Figure 4.20. Isosceles triangle depicting a one eighth segment of the cross section of the hilt of a single silicon microneedle.

Therefore, using trigonometry, the base–length of each triangle can be calculated:

X = 75 tan 22.5 = 31.06

The area of the isosceles triangle **= ½(base)(height)**

= 0.5 x 62.12 x 75 = 2329.5μm^2

The total area of the octagonal base **= 8 x 2329.5 = 18636μm^2**

Volume of pyramid **= 1/3(base area)(height)**

Volume of a single 180μm length microneedle:

= 1/3 x 18636 x 180 = 1.12 x 10^{-6}μm^3

Therefore, a 180μm-length 36-microneedle array will displace approximately 0.040mm^3, whilst a 280μm-length 36-microneedle array will displace

approximately 0.06mm^3. By comparison, if a 25G hypodermic needle penetrated the skin by the same length as the 280μm microneedle, it would cause a volume displacement at least 10 times that of a 280μm microneedle array. The tip of a hypodermic needle is bevelled and not cylindrical, thus the volume would be less than the approximate calculation below:

$$\textbf{Volume of cylinder} = \pi(\textbf{radius}^2)(\textbf{length})$$

$$= \pi\ (0.5144/2)^2 \times 0.280 = 0.58\text{mm}^3$$

Therefore, the hypodermic needle would cause greater morphological damage to the dermis and epidermis than either of the microneedle arrays. The reduced displaced volume by the microneedles leaves a smaller microconduit for the entry of medicaments; subsequently dosages and concentrations of any medicament may need to be modified for delivery by microneedles (Badran et al. 2009; Chen et al. 2009a; Coulman et al. 2009; Gupta et al. 2009; Hafeli et al. 2009; Ito et al. 2006a; Katikaneni et al. 2009; Rizwan et al. 2009). However, due the reduced puncture depth and volume, there might be limited physiological response and chances of pathogen entry will be greatly reduced.

Topically applied methylene blue dye was used to visualise needle puncture sites with *en-face* imaging. Whilst this provided a method to assess the level of puncture, subsequent transverse sectioning also highlighted the difficulties in finding microneedle punctures. As the time points increase post-application,

the punctures can no longer be visualised, methylene blue staining therefore highlights the number of microconduits present at that time post-application (Figure 4.5-4.7).

The topical puncture staining at 1, 4, 8 and 24 hours after initial application collate with the increase in skin water loss over the 24 hour period suggesting the microneedles penetrate skin efficiently. The 280μm microneedles were more efficient at skin penetration, being as high as 90% effective at one hour post-application. These microconduits started to seal over the 24 hour period. The 180μm microneedle array did not cause as much disturbance to the skin barrier properties, whereas the 280μm array increased transepidermal water loss (TEWL) in a similar manner to the hypodermic needle. It therefore may be preferable to use the 280μm array over the 180μm array to ensure sufficient penetration through the stratum corneum (SC). However, it is acknowledged that TEWL is a very sensitive measurement of skin permeability. It is possible that TEWL can be affected by the participant's movements, diet and atmosphere over the 24 hours despite taking precautions during the clinical study (Badran et al. 2009; Bal et al. 2008; Kolli and Banga 2008; Lanke et al. 2009; Netzlaff et al. 2006; Rosado et al. 2005b; Shimada et al. 2009; Verbaan et al. 2007; Zhai et al. 2007).

Microneedles puncture through the uppermost layer of the skin, the stratum corneum, and into the epidermis whilst the hypodermic penetrates into the deeper dermis. The keratinocytes in the epidermis mature from the basal and

germinative layer where they undergo mitosis and proliferate. These then differentiate into the stratum spinosum where the morphology changes with the production of tonofilaments. Subsequently, the keratinocytes mature to keratohyalin granules and migrate to the outer stratum granulosum layer where programmed cell death occurs. These cells finally desquamate from the skin SC (Amjad et al. 2007; Colwell et al. 2007; Girardeau et al. 2009; Pappas 2009; Scardina et al. 2009; Suter et al. 2009; Usui et al. 2008).

The epidermis also contains Langerhans' cells that reside suprabasally to provide immunological defence for the skin (Glenn and Kenney 2006; Kubo et al. 2009; Kurban and Bhawan 1990; Pickard et al. 2009; Wang et al. 2005a). Upon foreign antigen interaction, these cells migrate to lymphatic nodes thus causing coetaneous inflammation. The dermis contains vasculature, lymphatics and nerves as well as dermal fibroblasts, which provide intercellular support matrices and are implicated in wound healing. Macrophages, melanocytes and mast cells are also present in the dermis (Barrientos et al. 2008; Gratchev et al. 2008; Nishio et al. 2009; Park et al. 2009; Sindrilaru et al. 2009).

When a hypodermic needle penetrates the skin it causes damage to the dermal vasculature and impacts on sensory receptors, which causes bleeding and pain, as well as a whole host of complex cellular processes to be activated. The details of how growth factors, cytokines, matrix-cell and cell-cell interactions influence proliferation, differentiation and migration of cells in

response to a wound is somewhat understood. Thus the injury caused by the hypodermic needle causes leakage of blood from the vessels which contains platelets and fibrinogen (Barrientos et al. 2008; Frechette et al. 2005; Martin and Leibovich 2005; Valeri et al. 2006). This produces a clot, to close the open wound site, allowing aggregation of injured cells and degrading platelets releasing chemotactic cytokines and growth factors. These proteins act as signalling molecule for inflammatory leukocytes, which are present in capillaries and migrate towards the puncture site (Gillitzer and Goebeler 2001; Martin 1997). In conjunction to this, the macrophages, mast cells, lymphocytes and neutrophils clear away the damaged cells and any pathogens and cellular debris (Martin 1997; Ravanti et al. 1999).

Therefore the underlying histological, biological and immunological effects of puncture from the formation and subsequent closure of microneedle and hypodermic needle punctures was studied. The histological disturbance to the skin due to microneedle and hypodermic needle was observed by transverse section. For example, in Figure 4.13C the broken SC and epidermal cells were pushed into the dermis region, which is highlighted by the intense regions of H&E stain along the side of the microconduit. Often the puncture did not penetrate through the basement membrane, potentially due the angle of puncture. However, the hypodermic needle ruptured a much larger volume of skin tissue (Figure 4.15B) and also penetrated blood vessels (Gill et al. 2008; Papanastasiou and Hart 1995).

Safety of a device is a major factor to consider when introducing anything into the skin. Methylene blue staining showed that the microchannels were very small in their nature, in some cases similar to the diameter of hair follicles, whilst TEWL returned to normal over a 24 hour period. Thus microneedles appear to not cause any long-term problems with the integrity of the skin, which responds to close the channels within 24 hours, thereby reducing the likelihood of infection. Furthermore, no significant immunological or wound healing responses were observed over the 24 hour period post-application of the microneedle devices, unlike the hypodermic needle where K16 and CD68 were both up-regulated. This suggests that microneedle insult causes minimal injury to the skin. The small nature of the microconduits and the skin's natural propensity to heal may prevent any passive pathogen entry through the microconduits.

The release of cytokines from the ruptured cells, the subsequent presence of blood cells and platelets, elicit a cascade of inflammatory responses due by the up-regulation of TNF. Therefore inflammation drives the wound healing response, whilst the damaged stratified epithelia causes a strong induction of K6 and K16 in post-mitotic keratinocytes located at the wound edge (Philipp et al. 2004; Roh and Lyle 2006; Simon R. Myers 2007; Suter et al. 2009; Usui et al. 2008). Paladini et al. (1996) concluded that induction of K6 and K16 occurs within six hours after injury to human epidermis. *In-vitro* studies in cultured cells have determined K6 and K16 filaments accumulate post-injury, whilst the keratin pair K5 and K14 remains constitutively expressed in epidermis.

Further *in-vivo* work to assess mRNA might provide greater sensitivity to wound healing markers, whilst a larger sample store would enable immunohistochemistry (IHC) for both K6 and K16 markers.

In minor wounds, the innate inflammatory and immune responses are connected, but are two distinct processes. The inflammatory response is designed to neutralise pathogens and remove necrotic tissue, and subsequently initiate early regeneration and repair by formation of granulation tissue and epidermal migration by factors such as TNFα.

TNFα is a mediator that affects the up-regulation of proinflammatory cytokines. Ashcroft and Mills (2002) investigated TNFα levels in wounds of castrated and intact male mice. Five and 21 days after wounding castrated mice, expression of TNFα was lower than when compared to intact mice. TNFα cytokine is released by macrophages and epidermal cells, therefore, increasing the production of chemokines that attract neutrophils.

Dedicated *in-vivo* wound healing studies for microneedle and hypodermic needles would require extensive ethical approval for biopsies, however if sufficient samples were present, IHC for accumulation of K6, K16, and K17 would correlate with major changes in epithelial architecture (Coulombe 2003; Coulombe et al. 1998; Paladini et al. 1996). Paladini et al (1996) and establish that K6, K16, and K17 proteins are all induced in epidermis at the proximal edge of the wound within hours after acute injury to mouse and human skin.

Early in wound healing as suprabasal keratinocytes begin to differentiate with an increased expression of K16 (Coulombe et al. 1998; Paladini and Coulombe 1998; Paladini et al. 1995). As differentiation continues, the expression of K16 and K6 spreads distally from the edge of the wound but stops when reepithelialisation is completed (McGowan and Coulombe 1998; Wawersik et al. 2001). Activated keratinocyte generate cytoplasmic processes to aid cell migration by producing keratin intermediate filament networks (Coulombe et al. 1998; Usui et al. 2005). Approximately 48 hours after acute wounding, down-regulation of keratins 1, 10 and 2 occurs whilst K6 and K16 remain expressed until wound closure which occurs between 24 and 72 hours post-injury (Garlick and Taichman 1994a; Usui et al. 2008). Macrophages reach their highest number in the normal acute human wound after 48 hours of injury (Barrientos et al. 2008; DiPietro 1995; Gillitzer and Goebeler 2001; Hays et al. 2008). CD68 is expressed throughout monocyte differentiation, usually more intense in macrophages than monocytes. Granulocytic precursors and mast cells may also exhibit CD68 positivity. Therefore further studies using samples of skin taken between 24 and 72 hours post-injury would ascertain the difference in wound closure between microneedles and hypodermic needles.

Further study into the biochemical aspects of wound healing caused by microneedle and hypodermic penetration, may be conducted by measuring mRNA of early markers. This would enable us to better understand the

biochemical cascades and effects of skin injury. This could improve understanding of immunological responses to skin injury and thus improve the use of microneedle formulations for efficient and efficacious medicament delivery.

4.4.1 Conclusions

TEWL measurements, en face imaging and histological sections demonstrated that microneedles effectively puncture human skin in a superficial and transient manner. The inflammatory marker TNFα and wound healing marker K16 are up-regulated locally to the hypodermic needle puncture by 4 hours post-application. Whilst the 180μm microneedle punctures did not exhibit any such response, the 280μm punctures did elicit a small but localised K16 response 8 hours post-application.

Chapter 5

Developing a method of consistent application for polycarbonate microneedle arrays

5.1 Introduction

To date microneedles have been developed using a variety of manufacturing techniques on different materials. Etching of silicon wafers was used to form the initial microneedle designs. For example, Tyndall National Institute in Ireland developed a manufacturing process that creates microneedles by wet-etching a silicon wafer to form the needle projections, which are coated in an angstrom-thickness layer of platinum (details in Chapter 2). However, whilst this manufacturing technique is extensively used in the development of silicon microchips for the microelectronics industry, the financial and environmental implications of bulk producing silicon microneedle arrays may be prohibitive for mass-market distribution. Recently our group has acquisitioned polycarbonate microneedle arrays. Polycarbonate microneedle arrays are manufactured using common and cheap plastic injection moulding techniques using a stainless steel laser-etched mould.

The polycarbonate microneedle arrays used in this study comprised a circular-based array with microneedles of 3 different heights with different morphology and pattern to the wet-etched silicon-based arrays, which has equally spaced microneedles across the square base of the array. Due to differences in material composition and manufacture process, the silicon and polycarbonate microneedles differ considerably in their morphology. The silicon microneedles

have an 8-sided pyramidal structure, whereas the polycarbonate microneedles are single cylindrical protrusions from a 4mm deep polycarbonate base.

Whilst the silicon microneedles have been extensively tested within our research group, these polycarbonate microneedles require characterisation, proof of concept and refinement of their application method to establish whether they represent a viable alternative to wet-etched microneedle arrays.

During the clinical study (Chapters 3 and 4) the silicon 36-microneedle arrays were applied to human volunteers for pain and sensation testing and the depth of penetration and associated wound healing investigated. Prior to this study a preliminary applicator study was also conducted to establish a simple rod-based rolling application technique to apply the solid microneedles (see Chapter 2). At the time the clinical study showed that, for clinical application, it was better to have a circular shaped applicator to avoid sharp corners digging into the patient. Also the applicators with a softer surface were found to be more comfortable in their use than a metallic rod (Chapter 2). Therefore an inverted 2ml syringe plunger was used as the applicator rod for the microneedles during the clinical study.

Though the inverted-syringe plunger worked as an applicator, the clinician was required to undertake training to ensure consistent punctures from each of the 36 microneedles on an array. It was evident that the forces of application may have had an effect on the level of pain or sensations felt by participants in the

study. The application method may then limit the use of microneedles until such a stage where each application is consistent to enable accurate dose delivery.

Consistent application is essential for any new drug delivery method, to ensure correct dose delivery and drug efficacy. Commonly, hypodermic needles are applied by attaching to the end of a syringe through which the drug is then administered after inserting the needle into the skin or muscle. Even the smallest hypodermic needles 31G, which are 5-6mm in length, are attached to pen-like cylinders to deliver insulin for diabetics. Wang et al (2006) used a rotary drilling device for inserting single microneedles into skin. This reduced skin deformation, thus providing greater control of penetration depth. However this study also conceded that the skin did still deform and the insertion depth remained only proportional and not equal to needle length. Also for this technique to work on a multi-microneedle array, such as the polymer or wet-etched silicon ones, each microneedle would have to separately spin to allow it to drill.

Yang and Zahn (2004) reported that vibration of the microneedles reduced the required insertion force. This would over complicate the design for mass manufacture of an efficiently produced applicator. Others have used patch-based delivery systems to apply the microneedles to skin for a sustained drug release (Cormier et al. 2004; Nordquist et al. 2007; Widera et al. 2006b). Sivamani et al (2005) glued their hollow microneedle array to a syringe and

pushed a volume of drug solution through the array. Despite various techniques being tested there are also many which are patented or patent-pending which involve more complex electrical or mechanical design features to apply either hollow or solid microneedles. Therefore it is important that both the microneedle design and method of application is developed simultaneously to ensure effective and efficient drug delivery.

5.1.1 Aims and Objectives

This chapter aims to develop a simple, reliable and clinically suitable method of application for polycarbonate microneedles.

Objectives:

- To assess and characterise the morphology of polycarbonate microneedles.
- To test the *ex-vivo* human skin penetration ability of polycarbonate microneedles using methylene blue staining methods.
- To use TEWL to assess the level of human skin barrier disruption caused by polycarbonate microneedles.
- Design and test simple applicators for applying polycarbonate microneedles to human skin.
- Establish a prototype design for reproducibly successful microneedle application.

5.2 Materials and methods

5.2.1 Materials

Aluminium rods (Cardiff School of Engineering, Cardiff University); Araldite (Bostik Findley LTD, UK); Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, UK); Double-sided sticky tape (3M, UK); Hand-held drilling and cutting tool; 2ml Plastipak syringe and syringe plunger (Becton Dickinson, Spain); Polycarbonate microneedles (Cardiff School of Engineering, Cardiff University); Polycaprolactone (PCL) (Sigma-Aldrich, USA); 5ml Round-bottom Falcon tubes 12mm x 75mm (Becton Dickinson, USA); Rubber stoppers (Washer for ¾ Tap Jumper Assembly, Robimatic, UK); Silicone Elastomer Base and Curing Agent, Polydimethylsiloxane (PDMS) (Sylgard, UK); Springs (Airedale Springs LTD, UK); wet-etched microneedles (Tyndall National Institute, Cork, Ireland); Gold sputter coater (EM Scope, Kent, UK); Philips XL-20 Scanning Electron Microscope (Philips, Eindhoven, Netherlands)

5.2.2 Characterisation of polycarbonate microneedles

The manufacturer provided basic information on the polycarbonate microneedles. The microneedles are injection moulded on a circular array base to provide 13 cylindrical projections forming a star pattern with varying length needles at different points from the centre to the outer edge of the array. Two distinct arrays are joined by a polycarbonate T-bar, whereby one array has the longest microneedle (approximately 700μm) in the centre, and the other array has the shortest (approximately 300μm) microneedle in the centre. On both arrays the centre and outer most microneedles are separate by a circular row of microneedles of approximately 400μm length.

5.2.2.1 Light stereomicroscopy imaging of microneedles

Each polycarbonate microneedle array was photographed systematically to assess the overall design and morphology of the microneedles (for further details of light microscopy see Chapter 2.2).

5.2.2.2 Scanning electron microscopy (SEM) of microneedles

A sample of polycarbonate arrays were examined under high resolution SEM. As the polycarbonate arrays were not metal coated like the wet-etched platinum-coated silicon microneedles, each was mounted to an aluminium stub and gold sputter coated prior to SEM analysis (Chapter 2.2).

5.2.3 Preparation of *ex-vivo* human skin samples

Both microneedle arrays were applied in triplicate using each application method on excised and fully defrosted full thickness mastectomy skin. The skin samples, taken from consenting female patients with full ethical approval, were transported and stored in DMEM media, transferred to a -18°C freezer until use at which point the samples were defrosted and stretched and pinned to a cork board. No subcutaneous fat was removed from the samples. Further details of this procedure are described in Section 2.2.3.

5.2.4 Application of unmounted polycarbonate microneedles to *ex-vivo* skin

Polycarbonate microneedle arrays were initially left attached to the T-bar skeleton and rolled into the skin so that the microneedles would push into the skin with sufficient force and angle to pierce, Figure 5.1i. The application technique was varied using 2 other methods: A detached applicator rod push onto the array in a stabbing fashion and holding the array depressed into

the skin for 10 seconds, Figure 5.1ii; and rolling the detached applicator rod over the surface of the back of the array with the needles seated on the skin surface, Figure 5.1iii.

5.2.5 Development of an applicator rod for microneedle application to *ex-vivo* skin

Each polycarbonate microneedle array was mounted on the rubber bung end of a 1ml syringe plunger. The plunger has a diameter of 4mm, which is similar to the diameter of the microneedle array (3mm). The arrays were araldite-glued onto 2 plungers that were labelled to distinguish between the 2 types of arrays, [A] and [B].

Another applicator rod was made from polycaprolactone (PCL). The cylindrical 5mm diameter rod was cast in a polydimethylsiloxane (PDMS) mould. The PDMS mould was formed by adding 10 parts PDMS ($10cm^3$) with 1 part catalyst and mixing vigorously to give a homogenous viscous solution in a Falcon tube. A glass rod was then inserted into the tube and carefully positioned in the centre line but 2cm from the base of the tube. This was clamped into place whilst the tube containing the mixture was clamped vertically and heated in a water bath at 60°C to partially set. Subsequently this was left to set for a minimum of 18 hours at 40°C under vacuum to remove all moisture and air bubbles from the hardening PDMS mould.

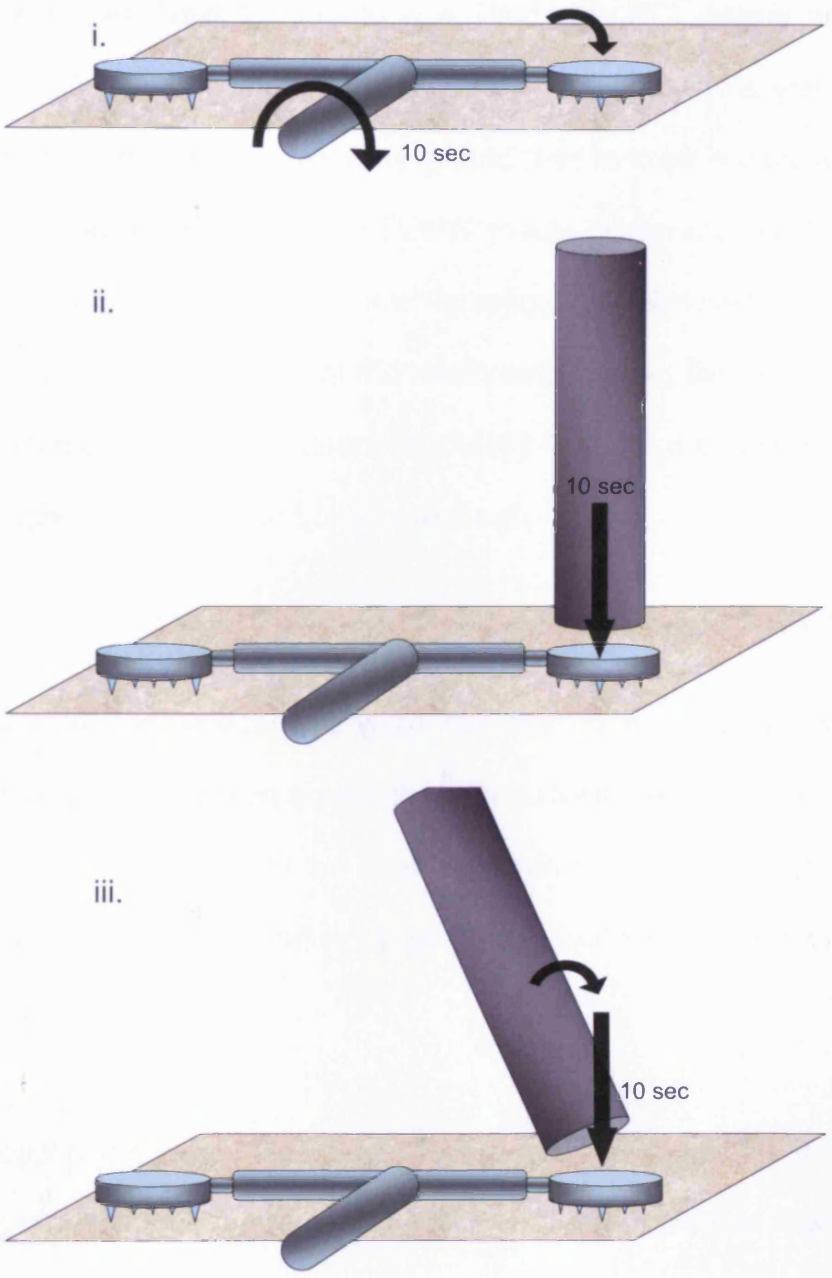


Figure 5.1. Application of unmounted polycarbonate microneedles. i) Application techniques tested without applicator rod; ii) stabbing aluminium applicator rod; and iii) rolling applicator rod. Arrows delineate the direction of force being applied. All forces were qualitatively maintained as constant by the researcher and applied for 10 seconds.

After 18 hours the clamps were released and the glass rod carefully removed from the mould. Next the mould was filled with PCL beads and heated to 100°C for 2 hours to melt the PCL into the mould. This was done under vacuum to extract the air from the mould and remove moisture to ensure a solid PCL rod would form. The PDMS mould containing the PCL was then cooled at 4°C. The PCL rod was subsequently liberated from the mould. Instead of using glue to mount the microneedle array, the end of the PCL rod was heated on a hot plate to approximately 60°C till it became tacky and the array pushed onto it and left to cool and set.

5.2.6 Application techniques for microneedle arrays mounted on applicator rods and rubber stoppers

Once the microneedle arrays were mounted to the applicators these were applied in a rolling fashion across the skin surface. The skin was stretched and pinned to a corkboard and the local application sites were stretched between the fingertips as representative of the clinical method for inserting hypodermic needles.

Each applicator was held in a pen-like fashion (between the thumb and forefinger) and rolled over the skin. Each application method repeated 3 times. The applicator rods were held at three different heights from the base, 2.5cm, 5cm and 7.5cm. Initially the syringe plunger applicator (Figure 5.2), array [A], was applied 9 times, i.e. triplicate for each height. Followed by plunger mounted with array [B] and finally PCL rods with arrays [A] and [B] were

tested. The aim was to establish if the applicator rod length affected the efficiency of microneedle puncture.

The applicators were also compared to other potential applicators that might be readily available. The local DIY store (Homebase Ltd, Cardiff) had a few different size rubber stoppers, one of which had a stalk with the diameter only 2mm larger than the diameter of the microneedle array. This was tested as an applicator as it is easy to hold and apply by rolling across the skin and also can be pushed down into the skin during the rolling in an effort to improve puncture efficiency (Figure 5.2).

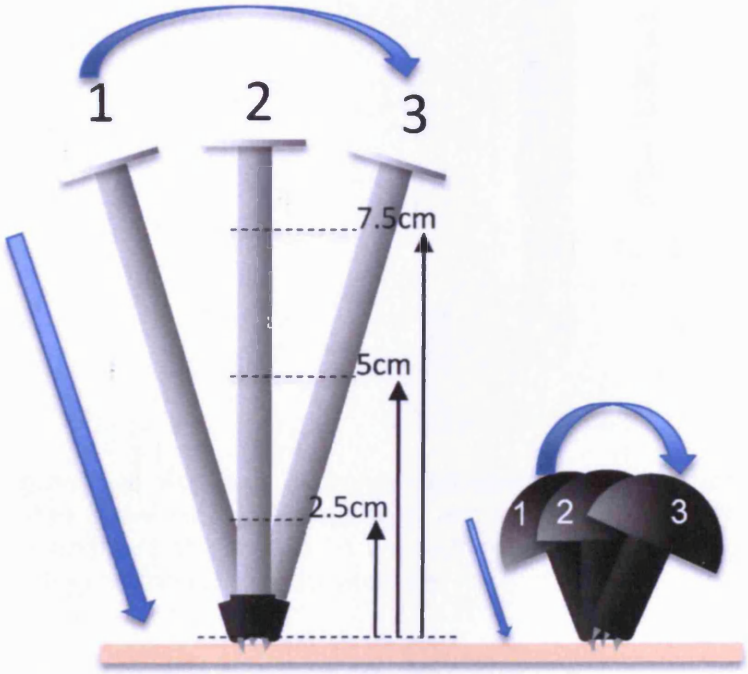


Figure 5.2. Applying microneedles using a syringe plunger and a rubber stopper. A microneedle array was adhered to the rubber bung end of a 2ml syringe plunger and the rubber stopper. The plunger was held in a pen-like fashion at 3 different heights from the base and rolled over the skin, whilst the stopper was held at the round top. Force applied downwards and throughout the massaging motion (1-3), shown by blue arrows.

5.2.7 Developing spring-based applicators for microneedle administration to *ex-vivo* human skin

From all previous laboratory and clinical tests, microneedle arrays have been able to penetrate the skin consistently when rapidly 'jabbed' into the skin (Figure 5.3). Therefore to standardise this action of administration, a rudimentary mechanised applicator was developed. This entailed using various resources available in our laboratory, i.e. Falcon tubes, syringes, pens and paperclips. These were disassembled, cut and tooled using a small electric hand-held modelling tool to form an initial design for a spring actuated microneedle array applicator.

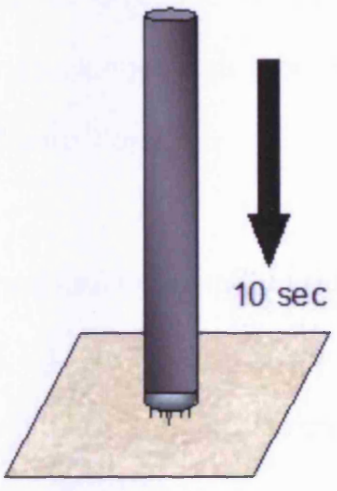


Figure 5.3. Applying microneedles using an aluminium rod. Application technique tested previously, Jabbing metal applicator rod with microneedle array mounted on the end into the skin from a height of approximately 5cm and pushing down and holding on the skin for 10 seconds.

An initial crude spring-loaded applicator was fabricated by trimming a 5ml round-bottomed Falcon tube to a length of 8cm and drilling a 2ml diameter hole through the bottom. A 2ml syringe plunger has a thumb press at one end and a rubber bung at the lower end where it would normally contact the fluid in

the syringe. However, removing the flexible rubber bung left a round plastic stub that was cut off. Through this plastic stub a paperclip was inserted, folded and twisted. A spring from a ballpoint pen was placed over the paperclip wire before pushing the wire through the hole at the bottom of the Falcon tube. Finally a polycarbonate microneedle array [B] was attached and tested to puncture *ex-vivo* human skin. This device was compared to the rubber plug applicator with another array [B] araldite glued to the end of the bung.

Further evolutions of the design reduced the number of components by removing the paperclip wire and using a trimmed syringe plunger. The open end of the Falcon tube was cut to reduce the overall length of the tube from 75mm to 70mm. The syringe plunger was protruding from the end by 3mm and the array was mounted onto the end.

The applicator was developed further using by using springs with known rating values (Airedale Springs LTD, UK). This allowed for better optimisation by testing different springs in the tube to ensure consistent microneedle puncturing of the skin.

5.2.8 Application technique used for prototype spring-based applicator device

The prototype applicator device (Figure 5.4) was used to apply the microneedle array. The cap was pulled back to compress the spring and raise

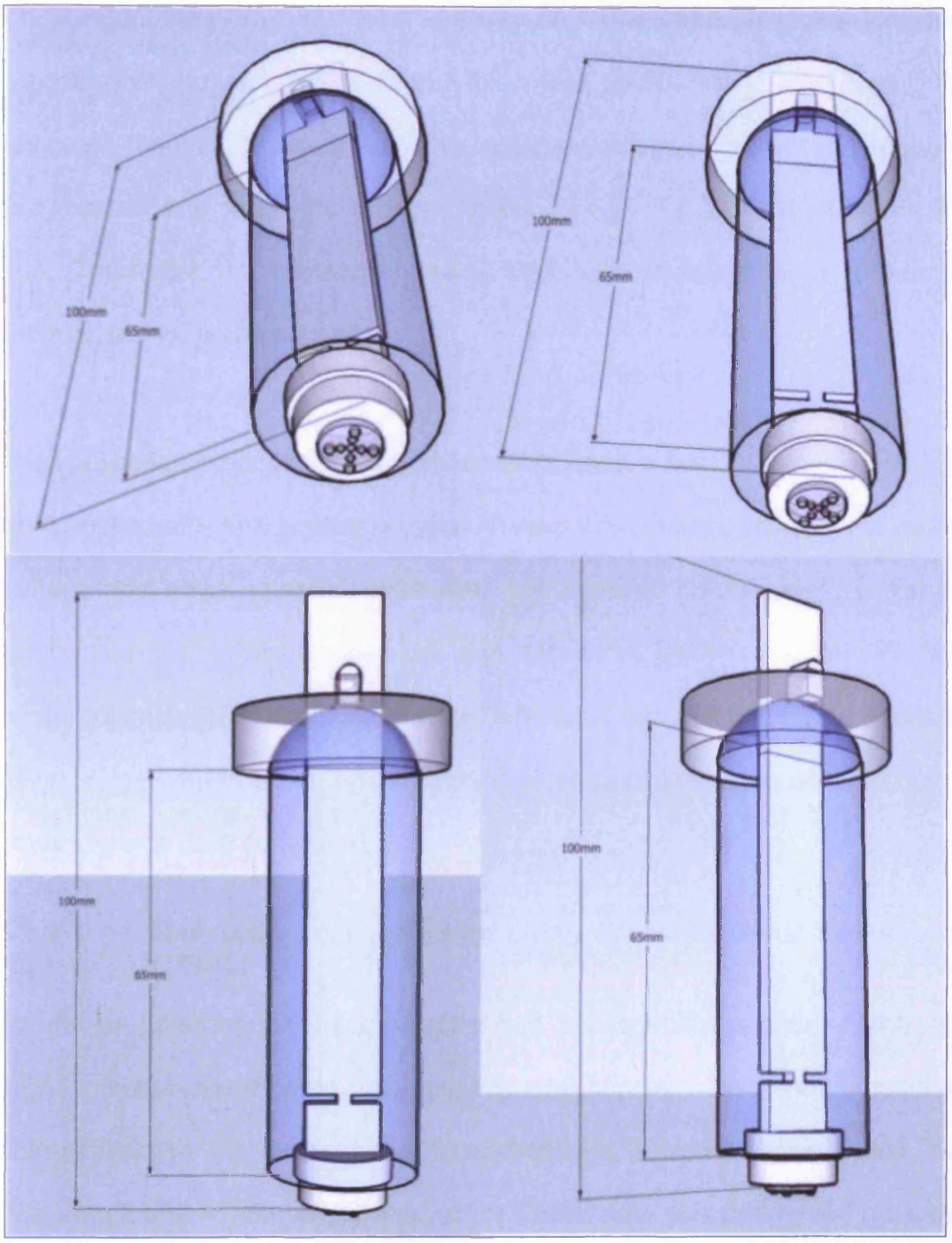


Figure 5.4. Prototype spring-loaded applicator device. Microneedle applicator device was produced using only 4 separate components and assembled with a spring of known rating being inserted over the shaft of the syringe plunger (spring not shown), which was inserted into the Falcon tube. A microneedle array [B] was mounted to the end with double-sided sticky tape.

the plunger, then the tube held vertically and perpendicular to the skin and lowered onto the skin. No additional force was applied other than resting the device on the skin. Subsequently the cap was released, causing the spring to decompress and force the plunger down, driving the microneedles into the skin. Thereafter the prototype device was held in place for a further 10 seconds before being removed.

The application process was performed in triplicate with topical methylene blue stain applied pre- and post-application. A negative control using just the device without any array attached was also administered to the skin, whilst for comparison the rubber stopper was also applied by jabbing into the skin from an approximate height of 5cm and held with force into the skin for 10 seconds. At no stage was the skin heavily stretched though pins were used to hold it firmly in place on a corkboard.

5.2.9 Measuring skin puncture using Transepidermal Water Loss (TEWL)

To gauge whether the microneedles had successfully punctured the skin TEWL measurements were taken prior to applying the arrays and immediately post-application for each of the 18 applications. For details on TEWL see Chapters 2 and 4. Statistical analysis of TEWL data was conducted by 2-way ANOVA with Bonferroni post-tests analysis.

5.2.10 Staining and imaging of *ex-vivo* skin post-application of microneedles

Methylene blue (10µl of a 5%w/v solution) was applied topically pre- or post-application to each site and allowed to air-dry for 10 minutes. Excess was wiped off using 70% alcohol wipes. The skin was finally examined by microscopy and photographed.

5.2.11 Transverse sectioning and staining of *ex-vivo* skin post-application of polycarbonate microneedles

Post-application, the punctured *ex-vivo* skin samples were placed in formalin for 24 hours. Subsequently the samples were dehydrated for 3 hours through an ethanol gradient and transferred to molten paraffin wax for 18 hours. Thereafter the samples were orientated and set in moulds of wax before being sectioned using a microtome. The sections produced were mounted on slides and counterstained in haematoxylin. Further details of sectioning and staining can be found in Section 4.2.5.2.

5.3 Results

5.3.1 Characterisation of the polycarbonate microneedles

Each microneedle array contains 13 microneedles. There are 2 types of array attached to each 'T-bar'. Figure 5.5 highlights the small size of each array in comparison to a 1p piece. The arrays are overhanging at each end and array [A] has the longest microneedle in the centre of the array and [B] has the shortest microneedle in the centre of the array.

The microneedle heights of array [A] vary in 3 tiers, from 673µm microneedle length in the centre, to 524µm and the outermost microneedle needles are 335µm in length, whilst array [B] has the shortest microneedle of 335µm at the centre and the longest microneedles of 673µm at the periphery (Figure 5.6).

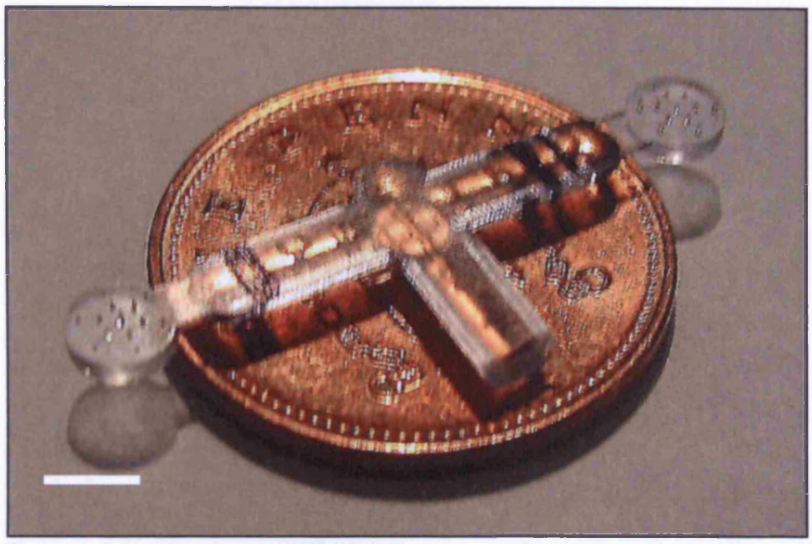


Figure 5.5. Polycarbonate arrays attached to manufacturing T skeleton. Left array is marked with a single stripe, whilst the right is marked with two stripes. (Bar = 5mm)

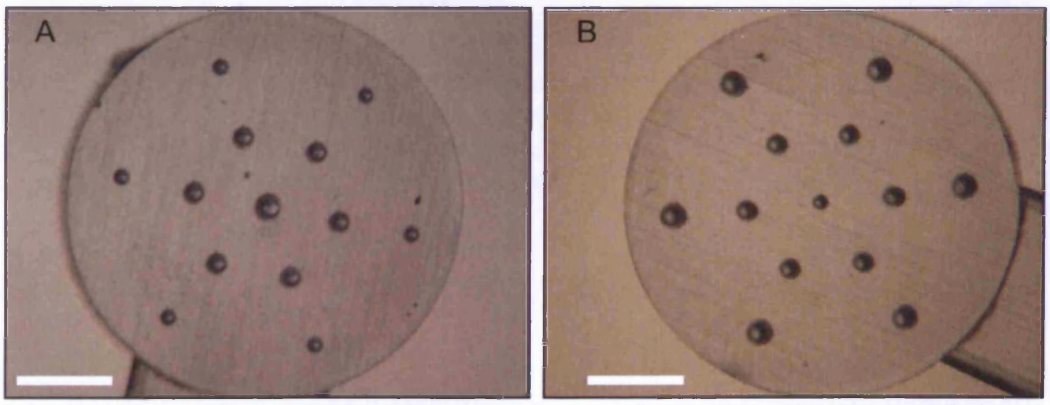


Figure 5.6. Two different polycarbonate microneedle arrays. [A] Array with longest microneedle in the centre decreasing in diameter to the shortest on the periphery. [B] Array with shortest microneedle in centre increasing in diameter to the longest on the periphery. (Bar = 1mm)

On both arrays, as the needles span outwards along the diameter of the array, the distance between microneedles also increases. Though the distance between a microneedle at one diameter and its neighbour at the next diameter is identical when measuring from the centre of the base of each microneedle.

Closer microscopic examination of the polycarbonate arrays (Figure 5.7) reveals the microneedles on array A appear to have a sharper and more defined point that those of B. Though this is not necessarily representative of all the arrays in this batch as similar images of a sister 'T-bar' shows 2 arrays with equally pointed tips for each of the 13 microneedles on each array.

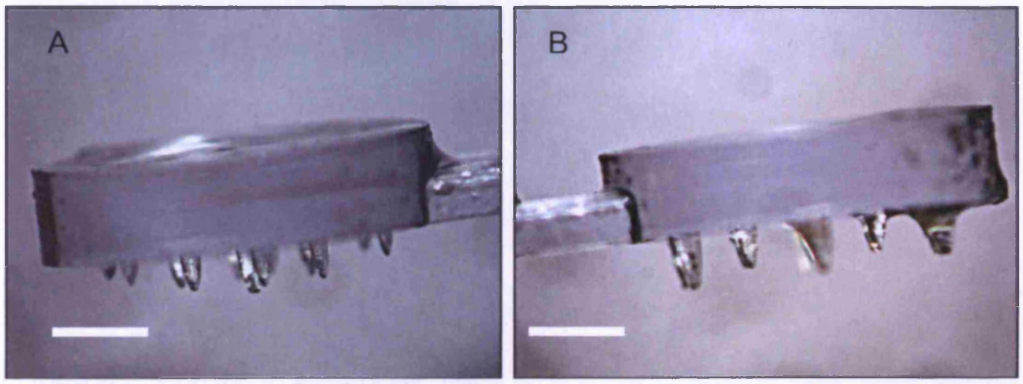


Figure 5.7. Lateral view of polycarbonate microneedles. Stereomicroscope images of arrays [A] and [B] shows differences in microneedle length at varying points along the diameter of the base. Signs of production defects are especially visible in this particular array (microneedle on far right of image) [B]. (Bar = 1mm)

SEM images (Figure 5.8) show the cylindrical nature of the microneedles, which project from the polycarbonate base as rounded needles and not angular or pyramidal structures. Figure 5.8 also highlights the imperfections on the microneedle surface.

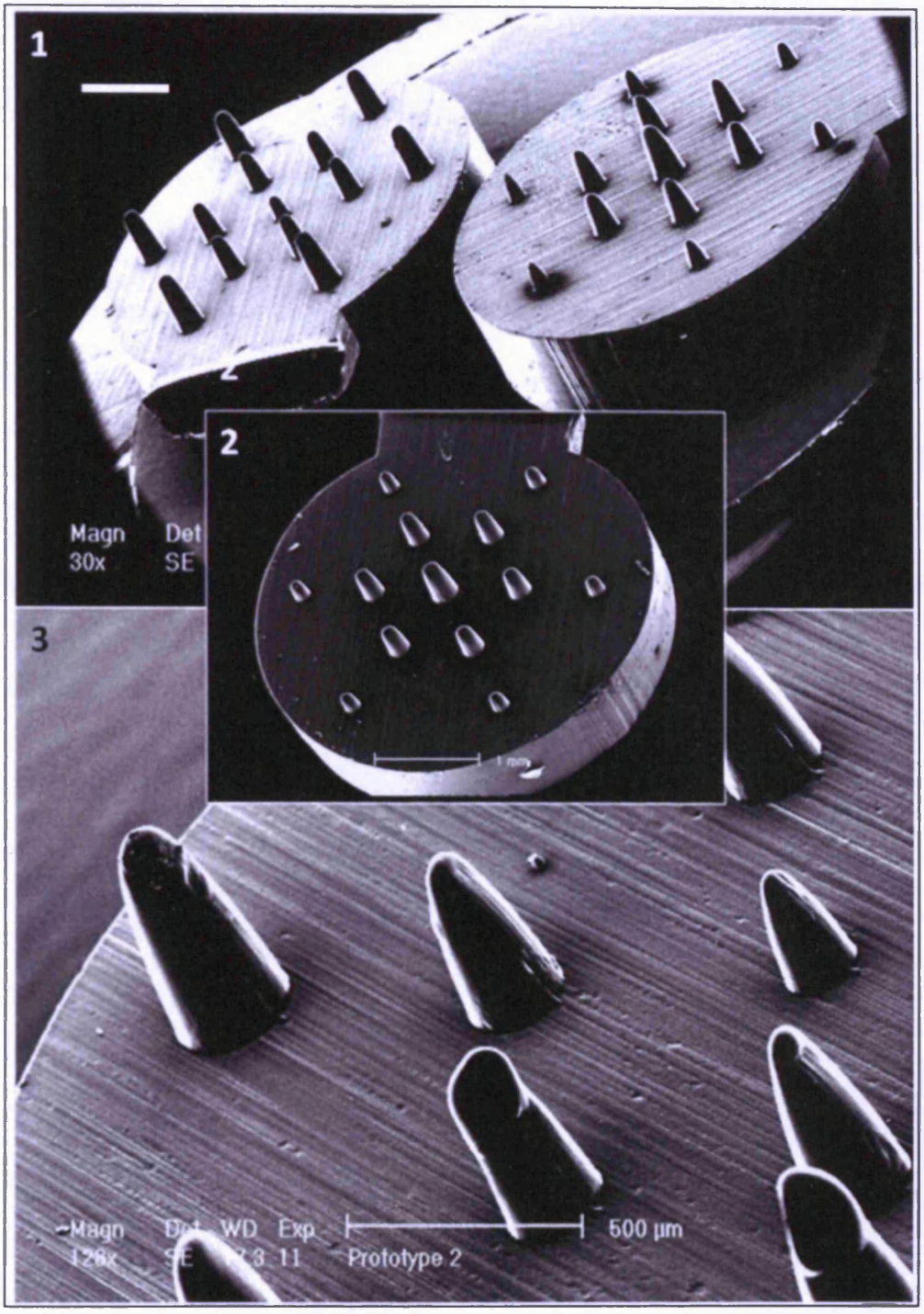


Figure 5.8. SEM images of polycarbonate microneedle arrays. 1) Two different arrays with reversed microneedle length gradients. (Bar = 0.5mm) Image highlights differences in microneedle length and girth across the array. 3) Microneedles are almost cylindrical, some less pointed than others and a few contain surface deformities at production.

5.3.2 Application of unmounted polycarbonate microneedle arrays to *ex-vivo* skin

The *en-face* photograph of methylene blue stained skin post-application of microneedle arrays (Figure 5.9) exemplifies the problem of microneedles not penetrating the skin when applied with the 3 application methods using unmounted microneedles (Figure 5.1). The stratum corneum has not been pierced by any of the microneedles. This is further substantiated by the TEWL data (Figure 5.10), which shows no substantial increase in water loss between pre- and post-application at any of the 9 application sites. Significant differences in TEWL were noted between the 3 methods (P=0.337) or the 2 different arrays (P=0.580).

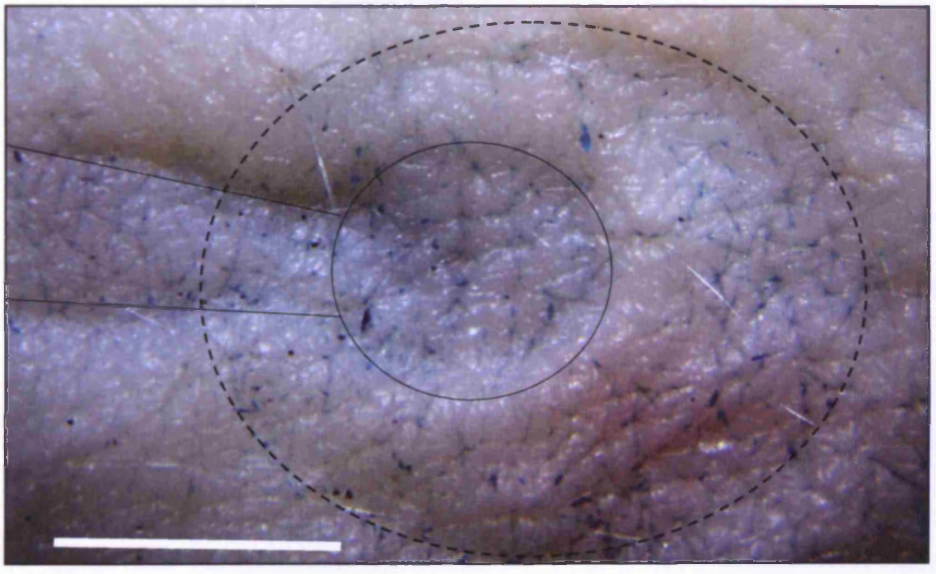


Figure 5.9. Magnified image of polycarbonate microneedle array applied to skin using an aluminium rod to which the array was not mounted. Polycarbonate array and T-bar skeleton leave indent (solid line) after 10 second application to skin using applicator rod. Applicator rod indent is shown with broken line. Skin has been stained with 10µl of 5% methylene blue stain for 10 minutes. However, it is unclear if any microneedles punctured the skin, as no characteristic microconduit staining is visible. (Bar = 5mm)

Figure 5.9 also shows that the array failed to penetrate despite the array being pushed into the skin with sufficient force to leave a temporary indent of array and T-bar in the skin surface. Thus at this stage, the force of pushing the array onto the skin is not the main concern but the application method by which this force is exerted needs to be reconsidered.

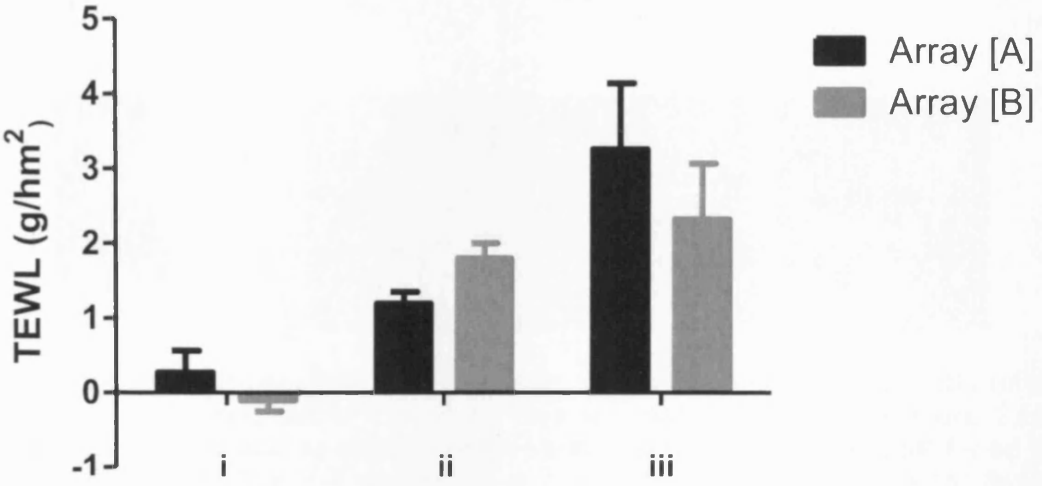


Figure 5.10. Assessing changes in skin barrier function using TEWL when applying unmounted microneedle arrays [A] and [B]. Application methods are defined in Figure 5.1. TEWL increase was significantly affected (P>0.05) by the array type (P=0.580); the array type did significantly have the same effect at the different application methods (P=0.337) (2-way ANOVA, Bonferroni post-test).

5.3.3 Comparing application of microneedles to skin when mounted to applicator rods

Polycarbonate microneedles were attached to the end of different applicator rods made from PCL (Figure 5.11) and syringe plungers (Figure 5.12). Subsequently the applicators were rolled over skin and the skin stained with methylene blue allowing the puncture marks (maximum of 13) to be counted.

Figure 5.11. PCL rod formed from PDMS mould of a glass rod. Polycarbonate microneedle array was mounted on the end (right-hand side of photo). The rod was made to a length of 7.5cm and cut down to 5cm and 2.5cm to assess the length of leverage on microneedle puncture.

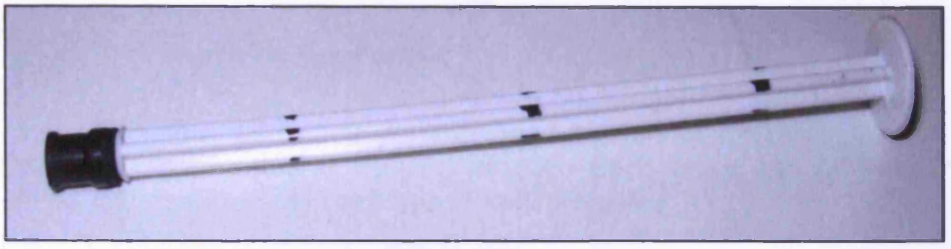


Figure 5.12. Syringe plunger as an applicator. The array was adhered to the rubber bung on the right hand side of the image. This was marked at 7.5cm, 5cm and 2.5cm along the shaft and held at each point for various applications. The shaft flexed too much at 7.5cm and 5cm, but was stable at 2.5cm. The rubber bung was too flexible on the end and the needles gripped the skin but appeared not to penetrate.

Figure 5.13 shows that the puncture efficiency of the arrays is not significantly (P=0.0041) affected by the heights at which the applicators are held. Further, there is significant difference (P=0.29) in efficiency between the 2 different microneedle arrays. The longer applicators suffered from too much flex, though the PCL was still solid at the base and the microneedles appeared to penetrate the skin. However, the syringe plunger not only flexed, but due to the array being mounted on the rubber end, the level of control and direction of the forces onto the skin were difficult to control when the rod was held at a higher point.

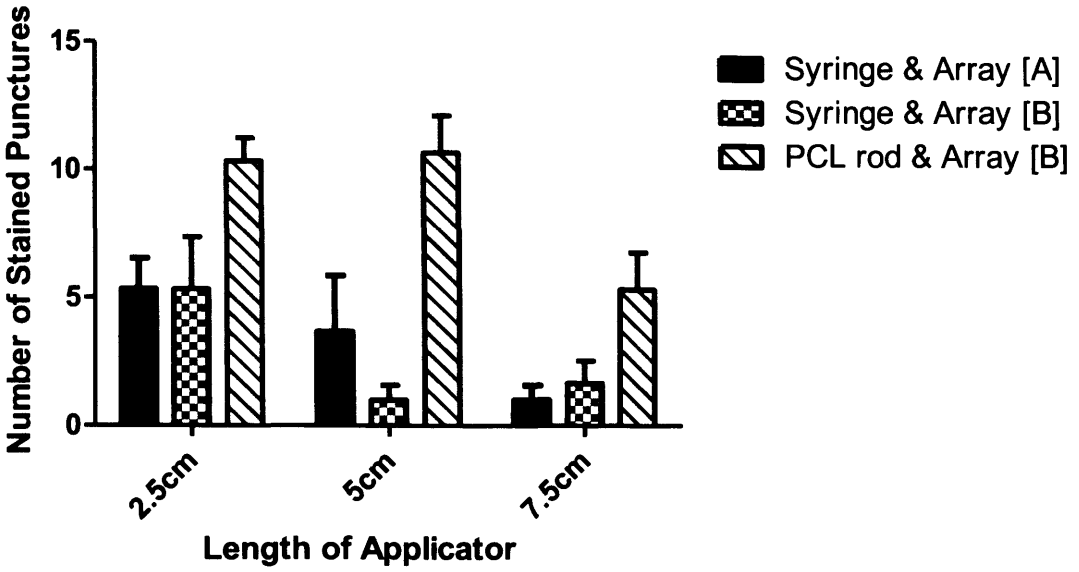


Figure 5.13. Puncture efficiency as visualised by methylene blue staining post-application using applicators of varying lengths. Each array can only puncture a maximum 13 microconduits as each has 13 microneedles.

Initial testing using array [A] mounted onto the end of a PCL rod caused microneedles to collapse onto the base of the array, thus array [A] was excluded from the final TEWL study. Therefore, the final study to compare TEWL was conducted using the PCL and inverted syringe applicators held in a pen-like fashion, at only 2.5cm height from the base of the applicator, before being rolled over the skin. The PCL rod was cut to size, which also gave the advantage that pressure could be applied down along the shaft by pushing on the top of the rod during rolling. TEWL data (Figure 5.14) was tested by one-way ANOVA and the PCL rod with array [B] was significantly (P=0.263) better at skin barrier disruption than either syringe plunger with array [A] or array [B].

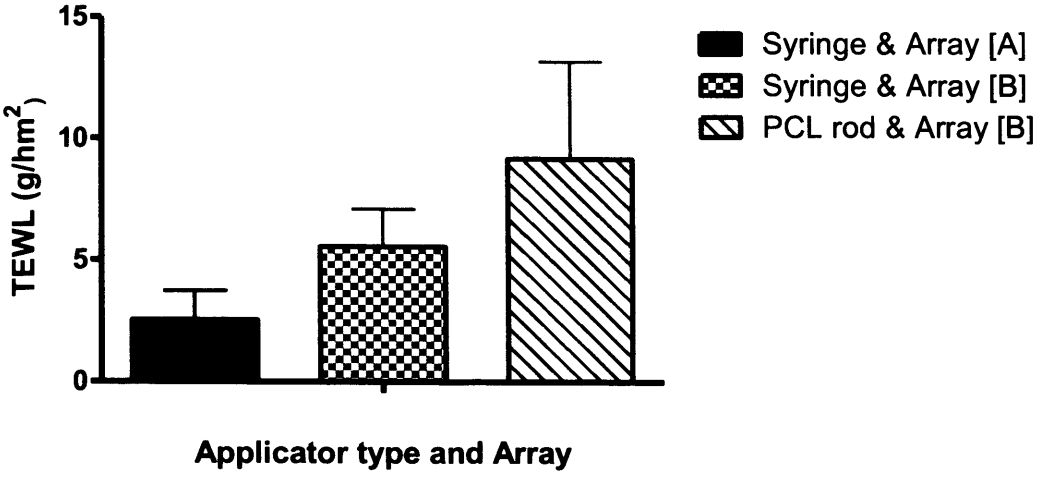


Figure 5.14. TEWL assessment of skin barrier function post-applications of microneedle arrays [A] and [B] when mounted to applicator syringe and PCL rod. Mean TEWL increases when the PCL rod with array [B] is applied to the skin, one-way ANOVA with post hoc test highlights significance (P=0.263) in between the three devices.

Of the all applicators currently tested, the rubber stopper (Figure 5.15) applicator worked the best as most of the microneedles impacted on the skin, and the outer microneedles, which are the longest on this array, penetrated the skin (Figure 5.16). Microneedle array [A], which has the longest needle in the middle, appeared to consistently only puncture in the middle, although sometimes the middle length microneedles would penetrate, but not reproducibly.

5.3.4 Microneedle stability post-application using applicator rods

Microneedle arrays of [A] and [B] design were mounted on the different applicators and applied to assess their morphology and stability after ten applications. Array [A], remained relatively intact with only the longest central microneedle deforming after 10 applications. However, array [B], which has

Figure 5.15. Rubber stopper used as applicator. The larger top surface provides area for grip whilst the short cylindrical stem is firm enough to not bend during rolling application. Microneedle array [B] mounted on the end.

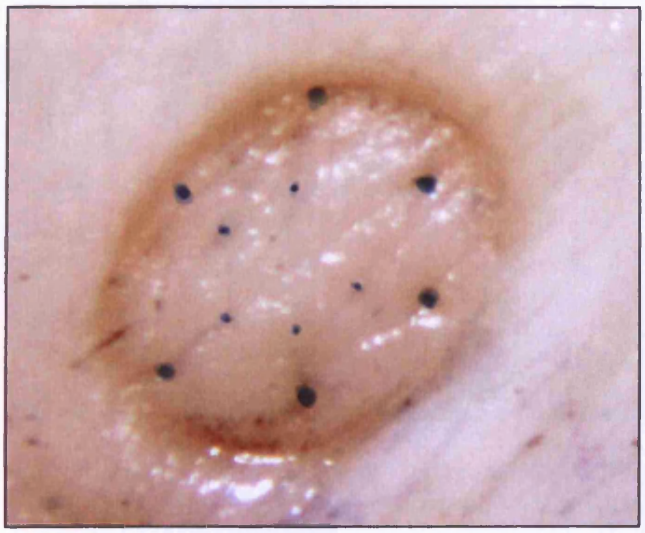


Figure 5.16. Rubber stopper applicator was used to apply microneedle array [B]. *En-face* imaging shows clear impression and punctures into the skin from most of the microneedles. This is highlighted by methylene blue staining post-application. All 6 of the longest (~600μm) microneedles on the outer edge have penetrated, but the only 5 of the ~400μm left punctures, whilst the shortest microneedle (~200μm) in the centre has not punctured at all.

the longest microneedles at periphery, was heavily deformed whereby all of the tallest microneedles collapsed inwards during the applications using the PCL or the rubber stopper applicators (Figure 5.17). However, the syringe plunger did not cause either array [A] or [B] to deform (Figure 5.18).

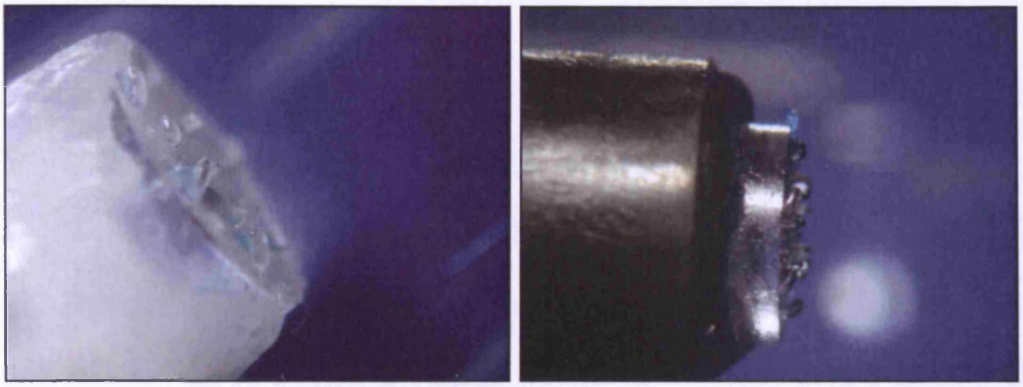


Figure 5.17. Deformation of microneedle array mounted PCL rod and rubber stopper. Microneedle array [B] is deformed after a ten applications using the rolling technique.

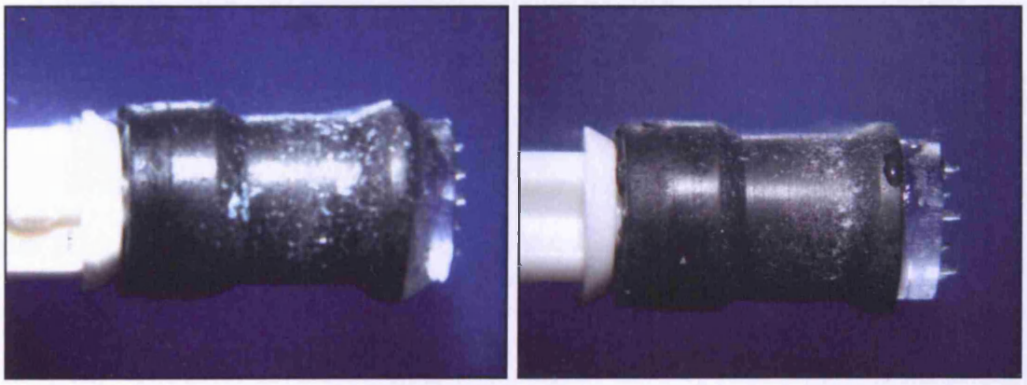


Figure 5.18. Deformation of microneedle array mounted to rubber bung of syringe plunger. Microneedle array [A] and [B] are not deformed when applied 10 times using the syringe plunger, however these applications did not yield more than 1-3 puncture marks with methylene blue staining.

5.3.5 Developing the initial spring-based applicator prototype

Having tried syringe plungers and rubber stoppers as applicators for rolling the microneedle arrays over skin, it was clear that this application often caused

deformation or complete collapse of the microneedle arrays whilst still not providing efficient or regular puncture of the skin. Thus it was determined the best way to administer such microneedles is for them to be rapidly administered perpendicular to the skin, thereby giving a straight trajectory for successful penetration.

The initial spring-loaded applicator had a weak spring in it, which meant that once the metal wire was pulled up and released, the plunger did not move smoothly within the piston-like design. Thus the skin was not penetrated at all. A rubber washer was added to the end to cushion the device on the skin, though this did not improve skin puncturing by the microneedles (Figure 5.19).

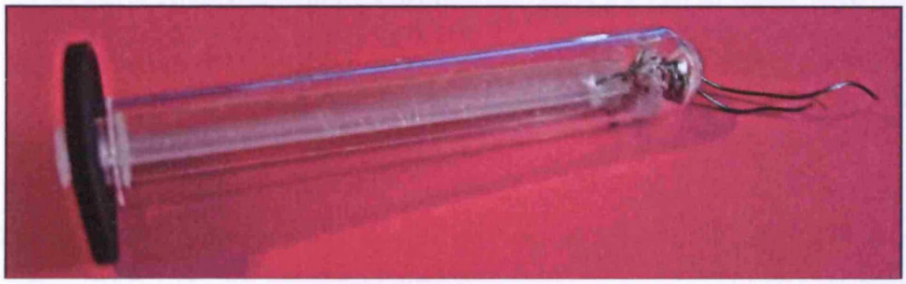


Figure 5.19. Stage 1 spring-loaded applicator. Applicator designed originally to have the open end with the rubber seal placed on the surface of the skin, the spring compressed by pulling up on the wires and then releasing. This is essentially a valve design with the plunger moving inside the outer casing.

Subsequently this device was improved using a more substantial spring from a ballpoint pen. This spring was 2cm long when uncompressed, however, the spring would deform and not coil evenly when compressed (Figure 5.20). Thus the syringe plunger was modified by narrowing the top end to make it thinner so as to be inserted into the spring, thereby acting as a guide and support for the spring. This ensured that when the spring was compressed it was

straighter along the line of travel in relation to the plunger. This should ensure more of the force from the spring is efficiently delivered to the plunger.

Figure 5.20. Stage 2 spring-loaded applicator. The syringe plunger has been cut to slide inside the spring, thus when the plastic cap (right) is pulled back, the spring coils over the plunger and compresses straight. The black rubber stopper was needed to prevent this spring from popping out of the hole at the round-bottom end of the Falcon tube. This spring-loaded applicator with microneedle array mounted on the end was used to apply the microneedles into the skin from a vertical position perpendicular to the skin.

When the spring-loaded applicator is placed on the skin surface, a suction effect causes the skin to be raised into the open end of the applicator valve (Figure 5.21). Therefore, as the spring is released, the microneedles penetrate the skin and remain *in-situ* with the force from the uncompressed spring against the raised skin. Skin was pinned to the corkboard (Figure 5.22) and the type [B] microneedle arrays applied in comparison to a negative control by rolling of the microneedles (Figure 5.1i) and rubber stopper mounted microneedles.

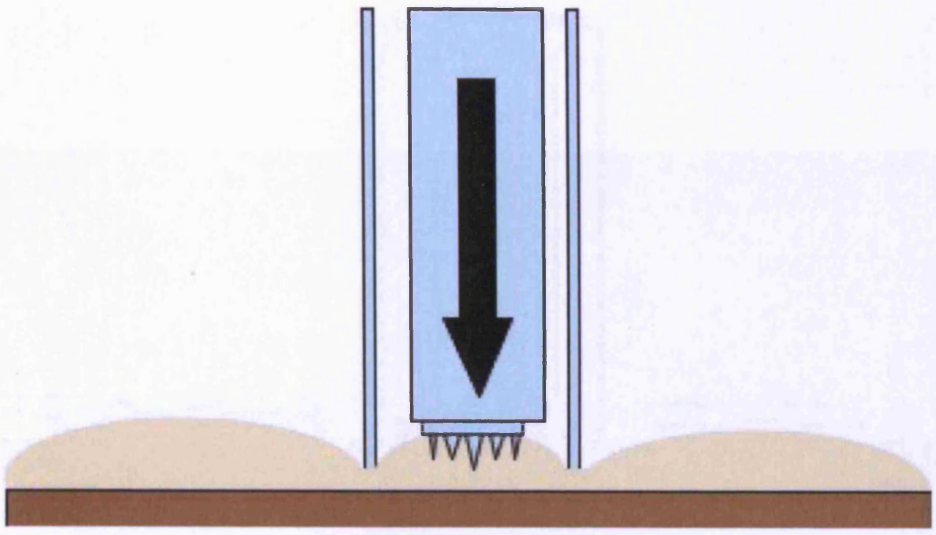


Figure 5.21. Meniscus-like effect is formed inside the outer tube of the application device. As the skin is raised into the tube when it is rested into the skin, the spring is unable to fully uncompress. Therefore the spring continues to apply a constant force onto the skin during the ten-second application.

Closer examination of the applications was conducted under light microscopy (Figure 5.23). Methylene blue dye applied post-application and pre-application had penetrated into the skin through microconduit punctures created by the microneedles. There was no apparent puncture when only the applicator was rested and pushed onto the skin without firing the microneedles with any force. When the microneedles were released forcefully into the skin, the shortest microneedle in the centre appears to have not punctured, whilst the longest periphery ones have punctured effectively. Furthermore, the raised bleb around the needle application site distinctly marks where the applicator rested on the skin surface causing a level of suction to raise the surface of the skin into the opening of the applicator.

The impact of the resting outer tube formed around the microneedle punctures whilst the indent due to the outer edge of the array was also visible.

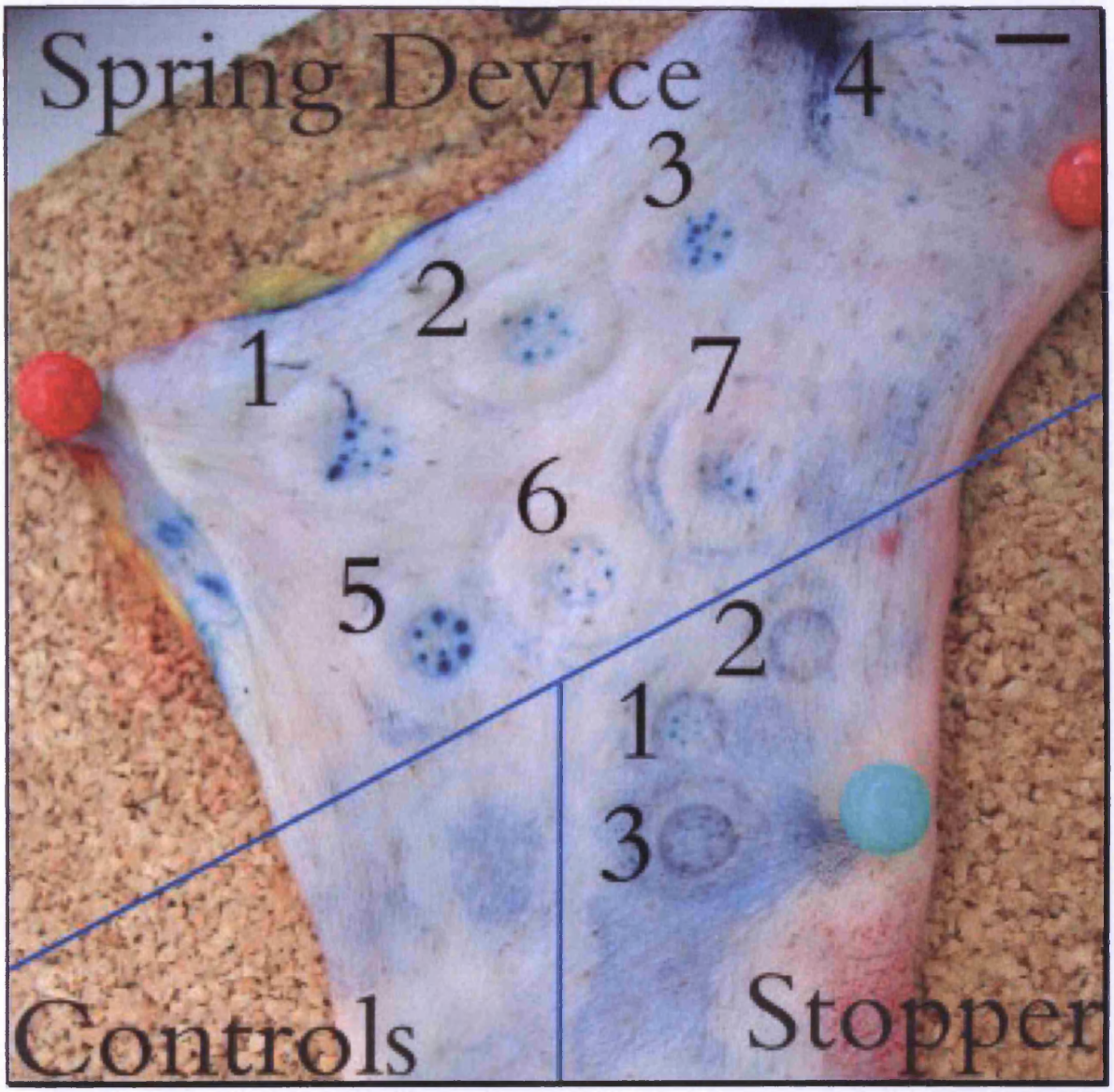


Figure 5.22. Testing of spring-loaded applicator on an *ex-vivo* skin sample. 1-4: applications using the spring device stained post-application, 5-7: applications using the spring device stained pre-application. 1-3 and 5-7 were applications with the cap fully raised and spring fully compressed before release on the skin surface. However, application 4 was with the spring not compressed and just pushing the device into the skin. The stopper applicator was raised to a vertical height of 5cm and then jabbed into the skin with the same researcher attempting to maintain similar pressure, force and trajectory. The controls show a larger imprint of the spring device only, with no microneedle array attached. (Bar = 5mm)

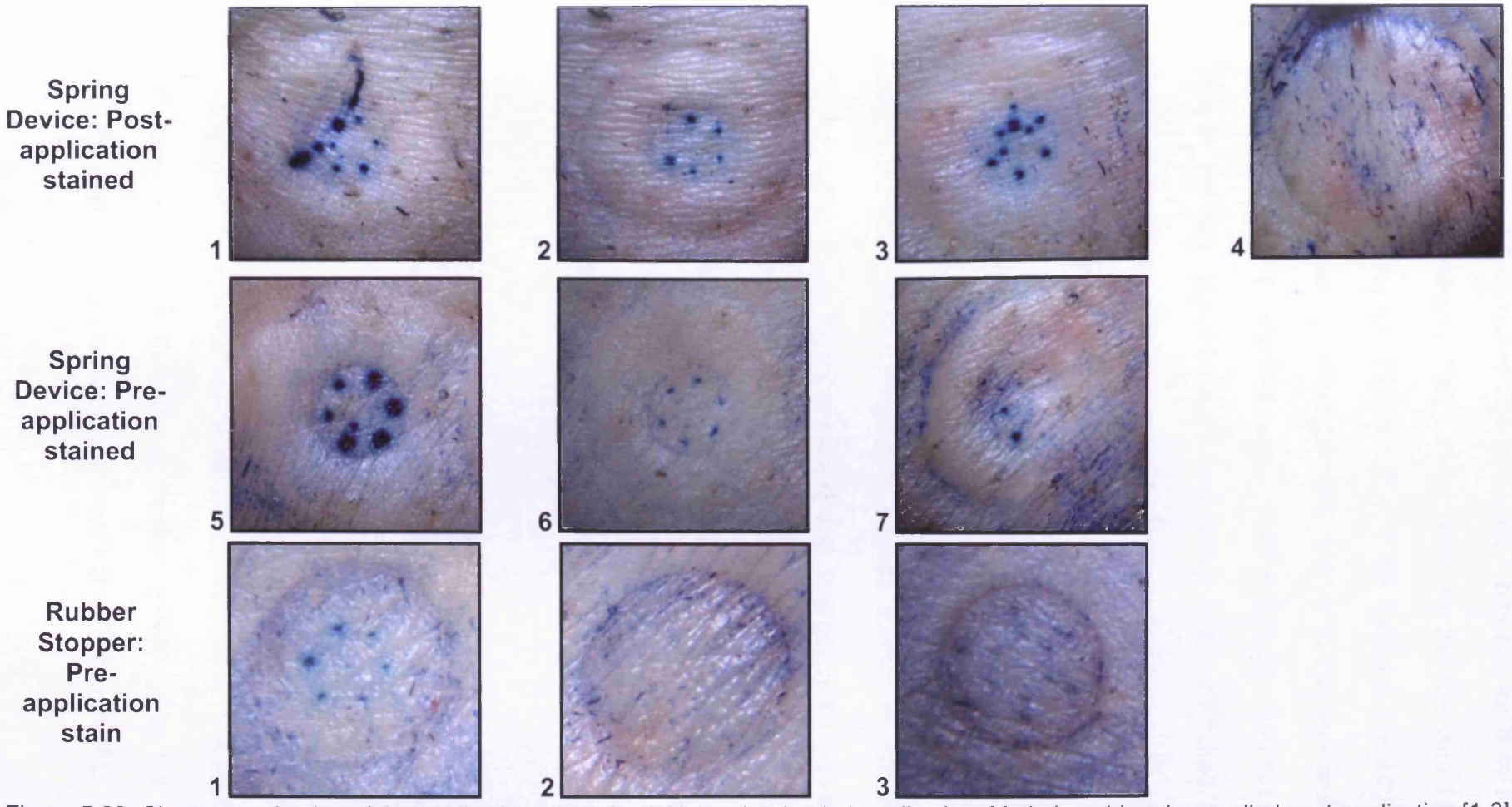


Figure 5.23. Closer examination of the application sites after initial spring-loaded application. Methylene blue dye applied post-application [1-3] and pre-application [5-7] has penetrated into the skin through microconduits created by the microneedles. Simply resting and pushing the applicator onto the skin shows no apparent puncture [4]. The shortest microneedle in the centre appears to have not punctured, whilst the longest peripheral ones have punctured effectively. The raised bleb around the needle application site distinctly marks where applicator rested on the skin surface causing a level of suction to raise the surface of the skin into the opening of the application.

The skin did stretch during the application process for both the spring and rubber stopper devices. This can be seen by the rise and fall of the skin surface after application (Figure 5.23). If the spring is not fully compressed, rather the applicator is rested perpendicular onto the skin and pressed, the force exerted by the microneedles is insufficient to puncture the skin. Previous work in the laboratory (unpublished data), has established that an initial driving force is needed to penetrate the stratum corneum after which the device can have gentle pressure applied to maintain its position in the skin.

5.3.6 Developing the final prototype spring-based applicator

To further improve the efficiency and consistency of application, Airedale Springs Ltd (UK) were contacted to provide springs of different but known characteristics. Four identical closed and ungrounded compression springs (Table 5.1) were provided for use in an updated applicator device constructed to fit these springs (Figure 5.24). Table 5.1 highlights key details of the springs used in the final applicator. 'Spring 1' was used in the prototype applicator to successfully puncture the skin.

Table 5.1. Details of spring provided by Airedale Springs Ltd

	Total No. of Coils	Total Dead Coils	Wire Diameter (mm)	Outside Diameter of Spring (mm)	Spring Rate (N/mm)	Free Length (mm)
Spring 1	12.56	2.00	0.610	7.80	0.310	30.00

Rated springs used in the prototype applicator appeared to provide a more consistent application method on *ex-vivo* skin samples. The *en-face* images of methylene blue show that the applicator worked using the polycarbonate array [B] (Figure 5.25) and silicon microneedles (Figure 5.26).

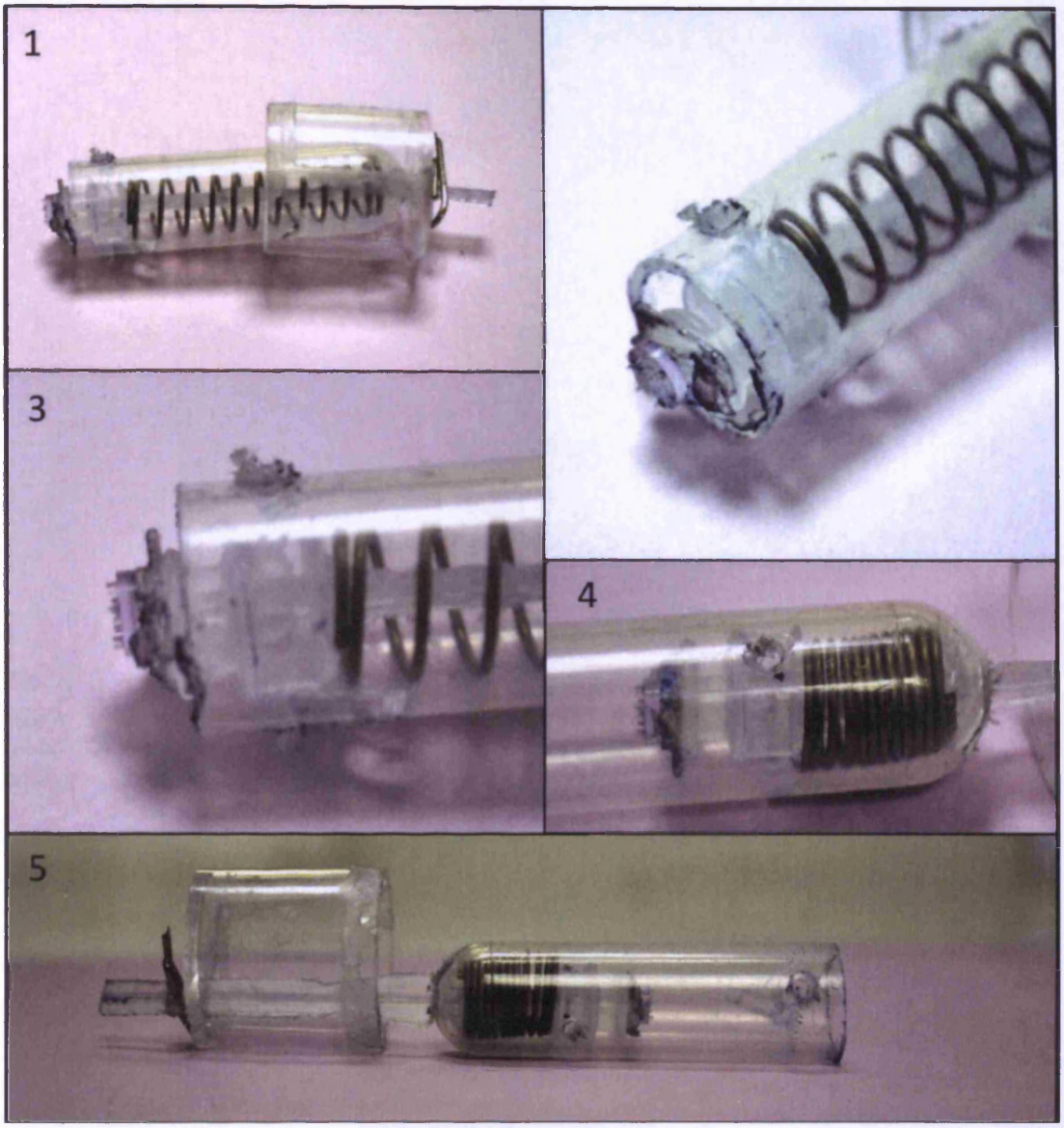


Figure 5.24. Prototype spring-loaded applicator device. 1) Applicator with compression spring released. 2) Microneedle array [B] mounted on end of syringe plunger using double-sided sticky tape. 3) Microneedle array [B] remains intact after use. 4) Applicator loaded with spring compressed in chamber. 5) Applicator ready for use by placing on skin surface and releasing the spring by rotating the upper plastic holder (left of image).

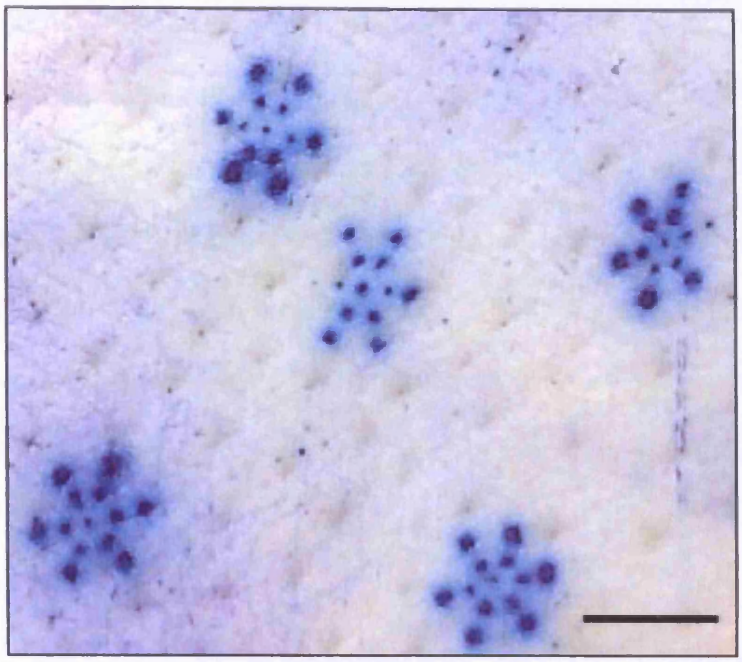


Figure 5.25. *En-face* image of skin punctured by polycarbonate microneedle array [B] applied using final prototype applicator. Polycarbonate array [B] applied five consecutive times whilst attached to the spring–loaded applicator. This applicator contained spring 1. (Bar = 5mm)

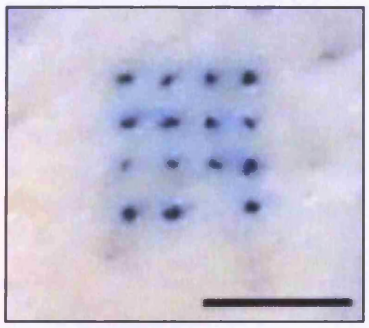


Figure 5.26. *En-face* image of skin puncture by wet-etched silicon microneedle array. Wet-etched silicon 250μm length microneedles on a on a 16-microneedle array also punctured the skin when applied using the prototype applicator, though the array shattered at first use. This applicator contained spring 1. (Bar = 5mm)

5.4 Discussion

This chapter aimed to develop a suitable method of application for newly acquired polycarbonate microneedles. The microneedles were initially imaged by scanning electron microscopy (SEM) and light microscopy, which

highlighted some manufacturing deficiencies when comparing different microneedle arrays. Whilst the injection moulding process is relatively simple and cheap, the moulds that are used to form such intricate microneedles need to be prepared carefully to ensure a hundred percent reproducibility between arrays in the length of the microneedles and their surface texture. This will prevent differences in the level of puncture or indeed discrepancies in the microneedles ability to be coated, hold and release defined dosages of drug particles into the skin.

Weakness in polycarbonate microneedles is probably due to their design, where the base of each microneedle is neither wide nor supportive enough to limit the needles bending and flexing during application. Indeed, this might be why generally these polycarbonate microneedles do not penetrate the skin as easily as the previously studied silicon microneedles. Wang et al. (2009) developed hollow polymer microneedles using photolithography processes combined with micromoulding and developed a method for batch production of morphologically identical microneedles with a precise lumen and outer diameter. Meanwhile Gittard et al. (2009) used two-photon polymerization and polydimethylsiloxane (PDMS) micromoulding processes to create microneedles with suitable structural, mechanical, and biological properties for the delivery of protein-based agents, such as insulin. Future collaboration with engineers will enable polycarbonate microneedles to be batch produced within precise structural and mechanical tolerances for consistent drug delivery applications.

Unmounted microneedle application techniques showed a slight, but not significant, increase in transepidermal water loss (TEWL) in skin. Further increases in TEWL were possible when additional forces were exerted by the use of the applicator rod. This rod was 10mm in diameter at the base and would have disturbed a larger area of the skin surface than the 4mm diameter array. The pressure on the skin surface from the array and applicator rod could have caused greater water loss through skin pores, sweat ducts and hair follicles.

As discussed in Chapter 2, TEWL is very sensitive to environment temperature and humidity and is therefore not ideally suited to measuring small changes. *En-face* staining and imaging assists in establishing the location and quantity of potential puncture in the skin (Bal et al. 2008; Lanke et al. 2009; Pearton et al. 2008), however, each stained puncture may not represent complete penetration and just highlight a deep indent in the skin surface caused by the microneedle. Thus, subsequent delivery experiments would be required to determine the level of penetration. Further repeats of TEWL would increase the power of the statistical data.

Though the microneedles did not penetrate the skin by simply pushing or rolling onto the skin surface, using syringe plungers as rods with the arrays attached to the black rubber end highlighted the need for a solid base to the applicator to preventing flexing and cushioning when push against the skin. The syringe plunger with the rubber bung is definitely not suited to sustained application. When the rubber bung is removed and the plastic of the plunger cut flat to mount the array onto directly, the flex in the plunger shaft remains a

problem. Therefore, a more solid rod applicator, such as a stronger plastic rod or polycaprolactone (PCL) applicator was easy to hold and rotate without having any flexible link to the array. However after multiple applications or if too much force was applied, the PCL applicator also flexed and bent.

Hafeli et al. (2009) fabricated miniature syringes from a silicon plate with an array of hollow out-of-plane needles and a PDMS reservoir attached to the back of the plate to hold drug solution. Though Hafeli et al. (2009) conduced *in-vivo* testing on mice to demonstrate successful albumin delivery, it is unclear how reproducible or fragile the microneedle syringe and reservoir would be for clinical distribution and use on humans.

With some practice, using the rolling method (Figure 5.2) with the array mounted on the PCL rod, all 13 microneedles on arrays [A] and [B] did efficiently puncture the skin. However, microscopic inspection reveals that after 9 applications the microneedles on arrays [A] and [B] start to collapse and no longer make clear puncture indents, rather they just scratch the skin surface. This could potentially be due to design of the polycarbonate microneedles, which lack of pyramidal morphology that makes the silicon microneedles more rigid (Jin et al. 2009; Rizwan et al. 2009; Sivamani et al. 2009).

Array [A] with the longest microneedle in the middle appeared to be more susceptible to damage and collapse during application. Array [B] however, did not suffer the same level of collapse. This could be due to the longest peripheral microneedles puncturing the skin and then as it rolls over the skin,

the central ones are pushed into the skin surface while the peripheral microneedles are still gripping the skin. Further study using array [A] could establish whether the collapse is because only the centre microneedle penetrates the skin at initial impact, whilst the peripheral ones are too short to grip the skin. Thus, whilst rolling across the skin, the middle needle collapses whereas the smaller peripheral microneedles hit the skin surface at a shallow angle and collapse.

However, simply attaching a microneedle array onto the end of a metal rod and stabbing it into the skin from a vertical height was far from simple, or indeed accurate, in terms of puncture efficiency and damage to the microneedles and the skin. Repeating such a procedure in clinical practice may cause severe bruising and discomfort to the patient, if the application was performed with too much force. Applicators for mass distribution, for example for vaccine delivery, should be cost effective in their manufacture using few components and suitable environmental and aesthetic properties (Abbas et al. 2005; Doab et al. 2009; Hafeli et al. 2009; Latkin et al. 2008). The applicator developed in our study is simple in design and each component can be manufactured using existing techniques and materials, including environmentally friendly plastics. This new spring-loaded application method is much more consistent at facilitating microneedle puncture of skin, however it still needs perfecting. As with other applicators the type [B] microneedle array punctures the skin more efficiently, but the shorter needles do not always penetrate the skin. This test needs to be repeated using the type [A] microneedle array, as these needles exhibit different characteristics. The forces and deflection of the array caused by the spring mechanism are all

unknown and future studies are needed to quantify these attributes. Also, spring samples of varying sizes but known characteristics need to be tested to optimise the applicator design.

The final spring-loaded applicator was tooled so that, when the spring was unloaded, the microneedle array protruded from the opening of the device. Therefore as the spring was released from a compressed state the microneedles punctured the skin and the skin reacted by exerting a force on the microneedles causing the skin to deflect as the microneedles came to rest on the skin surface. At this point the spring was no longer fully uncompressed, rather the spring was exerting a small force to help maintain the microneedles in the created micro-conduits. We have shown that a spring with a rating of 0.310N/mm and free length of 30mm is adequate for replicable skin puncture. Collaboration with engineers would provide expertise to measure the weight and force applied onto the skin by initial decompression of the spring and the subsequent force by the incomplete decompression into the skin.

The outer tube could be altered in diameter as well, however, currently it may be better to retain one size for easier development and only alter the length. Also the narrow diameter may be of benefit as it causes a meniscus-like effect on the skin when the applicator is rested on the skin surface. The device could be made very small with the correct spring, tube length and diameter, though this might make it harder to hold and use.

Further investigations in the use of polymeric materials to form microneedles will provide for better structurally stable and efficiently puncturing

microneedles. Tightening manufacturing tolerances will reduce the differences in between microneedle array morphologies and improve their capacity to be coated and release drugs, for example, manufacturers can use hydrogels to establish the elasticity effects on microneedles and forces during drug delivery in an effort to standardise and optimize microneedle design (Chippada et al. 2009). In addition, testing devices of different sizes, materials and spring ratings will ensure the development of a more compact, user-friendly applicator for optimum drug delivery by the microneedles.

5.4.1 Conclusions

To conclude, this research has characterised solid polycarbonate microneedles and progressed the development of a simple applicator device for the successful administration of these microneedles to human skin using cheap production methods and minimal parts.

Chapter 6

General Discussion

6.1 General Discussion

Microneedles have been manufactured using technologies developed through the growth of the silicon semiconductor industry. Prausnitz group's publication of the late 90's (Henry et al. 1998) spurred researchers to consider the clinical benefits of microneedles as a less invasive and pain free method for drug delivery through the stratum corneum. Despite considerable progress in developing microneedles for therapeutic use, microneedles have yet to make the transition from laboratory to clinic. The clinical study presented in this thesis provided a small but important step forward in microneedle research. For the first time microneedles were shown to be successful at penetrating the skin of human volunteers whilst simultaneously proving to be minimally discomforting and preferable compared with hypodermic injection. This project also considered cutaneous wound healing responses post-application of a 25G hypodermic needle and microneedles in human volunteers and progressed the development of a suitable microneedle applicator device for polymer microneedles.

The main study presented in this thesis involved measuring sensory perceptions and biological responses to silicon microneedles being applied to human volunteers. Therefore, prior to clinical applications, preliminary research was required to consider the organisation, management and delivery of the study. This included producing a suitable protocol (Appendix III) and establishing techniques for the appropriate measurement of pain, sensation and biological response during and post-application of the microneedles. Previously, silicon microneedles have been applied using fingertips (Bal et al.

2008; Henry et al. 1998; McAllister et al. 2003) or metallic rods (as tested in Chapter 2). Pre-clinical testing revealed the fragility of silicon-based microneedles when applied without applicators, as the base of the array was not supported firmly and thus the mechanical forces during impact with the skin caused the array to shatter. Evaluation of simple applicator designs identified inverted syringe plungers as practical, simple, yet effective applicators for administering silicon microneedles in a clinical setting (Chapter 2). The rudimentary applicator provided consistent levels of skin penetration, although improper use of these applicators could cause microneedles to graze the skin causing wounding, or minimal puncture thus reducing medicament uptake into the skin.

Transepidermal water loss (TEWL) measurement and topical staining of the application site were used to assess the ability of microneedles to puncture the skin when applied using an applicator device. Methylene blue does not actively diffuse through the stratum corneum (SC). However if the SC is compromised, methylene blue diffuses through consequently highlighting those areas of skin damage. Previous (Coulman et al. 2006; Kolli and Banga 2008) and recent (Kalluri and Banga 2011; Li et al. 2010; Li et al. 2009) microneedle studies have demonstrated that methylene blue reliably highlights microneedle puncture. Methylene blue is also used as a histological stain to highlight cell membranes (Yaroslavsky et al. 2005). During the research in this thesis methylene blue was applied topically during application of microneedle devices (silicon or polycarbonate), thus any passive diffusion of methylene blue stained the microneedle-formed conduits, as well as any damage caused by the base of the arrays (Sections 2.3.2 and 5.3).

Methylene blue staining highlighted the microneedle punctures whilst affirming that the base of the arrays did not cause any damage to the SC. However, when rolled onto the skin, the sharp edge and corners of the base of the silicon microneedle arrays caused SC damage, as visualised by microneedle staining. Concurrently TEWL measurement also demonstrated that microneedles had breached the SC both *ex-vivo* (Section 2.32) and *in-vivo* (Sections 4.3.2). As the microneedle punctures are a theoretical maximum of 150μm in width and 280μm in depth, rigorous precautions at the time of measurement were taken as TEWL is acknowledged as an extremely sensitive measurement of skin permeability (Fluhr et al. 2006; Shah et al. 2005; Suehiro et al. 2004) and is likely affected by the participant's movements, diet and atmosphere over the 24 hour study period. TEWL data correlated with the methylene blue skin staining, showing an increase in TEWL immediately post-application of microneedle device, which diminished to baseline over the 24 hour study period (Chapter 4). The TEWL values also reflect the relative efficiency of skin puncture with significant differences in TEWL observed when using 280μm microneedles compared with 180μm microneedles (Chapter 4).

The encouraging skin puncture results were obtained using silicon microneedle arrays, 36 microneedles of length 180μm or 280μm, that had never previously been applied clinically and are substantially shorter than those used by other researchers (Bal et al. 2008; Ding et al. 2009; Kaushik et al. 2001; Sivamani et al. 2005). It was anticipated that the length of the microneedles would pose a challenge for effective clinical application, with

their proximity to each other on the array confounding the problem of reproducible and complete insertion due to the "bed of nails" effect (Sivamani et al. 2007; Teo et al. 2006). Whilst silicon microneedles of this length proved to effectively puncture human skin further work continues to enhance and optimise the production techniques and materials used to make microneedles, with particular focus on metal and polymer microneedles of different morphologies (Ayittey et al. 2009; Chippada et al. 2009).

To ensure appropriate sensory modality measurements were made during the clinical study (Chapter 3), suitable pain and sensory-description measurement instruments, the short form McGill pain questionnaire (MPQ-SF) and audio recordings were tested alongside the applicator devices (Chapter 2). Extensive literature searches highlighted the lack of any suitable transient pain measurement instrument. However, the MPQ-SF has been used for acute and chronic pain studies using the visual analogue scale (VAS) for initial assessment of pain intensity (Gubin et al. 2009; Titler et al. 2009). Thus, the MPQ-SF proved a suitable instrument to assess the level of sensations felt by the participants during the application of needle devices. Audio recording is most commonly used during focus groups and interviews. In this study audio recording created a verbal diary for capturing the pain and sensations experienced real-time during microneedle and hypodermic needle application.

Following the pre-clinical development of the microneedle applicator, silicon microneedles were applied to human volunteers (Chapter 3) to establish the sensory perception of each microneedle device compared to a

25G hypodermic needle, whilst still evidencing the reproducibility of transcutaneous punctures created by each microneedle on the arrays. VAS scores identified the hypodermic needle to be significantly more painful on insertion than either 180μm or 280μm microneedle arrays. Audio recording of each participant's comments captured further details of sensory modalities allowing for cross-reference against VAS data. For example, initial VAS results showed the shorter 180μm microneedles to be more painful than the longer 280μm microneedles, however, audio comments from participants describe the main difference in sensation during the application of each device being attributed to the level of 'pressing' which is understandable given the method of application. It is therefore not so surprising that the shorter 180μm microneedles caused more 'pain' than the longer 280μm microneedles given that the administering clinician may well be applying greater force during application of the shorter microneedles to ensure skin penetration.

Other pain studies have used VAS scores to determine that microneedles cause significantly less pain than hypodermic needles (Bal et al. 2008; Gill et al. 2008). Gill et al. (2008) determined that increasing microneedle length from 480μm to 1450μm caused a significant increase in pain, as shown by the mean VAS scores of 2±2mm (mean ± standard deviation) for the 480μm to 15±17mm for the 1450μm microneedles, respectively. Gill et al. (2008) recorded the hypodermic needle to be significantly more painful than any microneedle device with a mean VAS score of 24±16mm. Whilst these other studies provide context, in our study the 180μm and 280μm length microneedles were of much smaller dimensions.

Skin staining has been used to illustrate successful penetration through the SC into the epidermis in various *ex-vivo* human skin studies (Birchall et al. 2005; McAllister et al. 2003; Park et al. 2005; Pearton et al. 2008; Pearton et al. 2010). However, our clinical study used methylene blue staining of the human skin surface following *in-vivo* needle insertion. Staining showed the intensity of punctures reduced over a 24 hour period post-application of microneedle devices, suggesting the transient microconduits formed by microneedle penetration were resealing (Section 4.3.1). Physical resealing of skin channels and wound healing responses post-application of 280μm and 180μm length microneedles and a 25G hypodermic needle were further explored in Chapter 4. These studies highlighted the greater cellular damage caused by the subcutaneous insertion of a hypodermic needle in relation to intra-epidermal insertion of microneedles, which can be indistinguishable from the natural troughs in between the ridges of the skin surface (Section 4.3.3). Transverse sections of *in-vivo* treated human skin demonstrated the ability of skin to deform around the microneedles. Consequently, some microneedles only penetrated the SC and not deeper into the epidermis. Previously, no other *in-vivo* penetration studies on human skin, involving biopsy of the application site, have been conducted. Although, puncture and resealing of microneedle punctures have been assessed by biopsy and transverse sectioning of *in-vivo* treated animal models. For example, 559μm length maltose microneedles created microchannels of 160μm depth in rabbit skin which resealed within 15 hours post-application (Kalluri and Banga 2011).

Regarding *in-vivo* human studies not involving biopsies, estimations of penetration depth have been based on the corneocytes debris on microneedles. For example, microneedles of 500μm length penetrated to a depth of 120μm when being applied to the human skin (Shirkhanzadeh 2005). As new non-destructive optical imaging techniques become available, the need for biopsying samples to measure mechanical interaction between microneedles and skin diminishes. Real-time interferometric imaging by optical coherence tomography (OCT) can resolve the skin's stratified architecture *in-vivo*. Therefore, OCT has been used to observe and measure the morphological alterations in skin during and after microneedle penetration (Coulman et al. 2010; Donnelly et al. 2011; Enfield et al. 2010). For example, both Donnelly et al. (2011) and Coulman et al. (2010) demonstrated that microneedles do not penetrate the skin their full length. Furthermore, Coulman et al. (2010) suggested that microneedles of varying materials and dimensions did not penetrate as greatly as estimated measurements from histological techniques. Notably, all microconduits collapsed upon needle removal. Even when a 26G hypodermic needle was administered and removed, the skin contracted forming a microconduit that was wider in the epidermis and narrowing at the SC.

Other *in-vivo* studies, have measured systemic plasma concentrations of molecules such as nicotine and insulin that were administered by microneedle insertions (Martanto et al. 2004; Sivamani et al. 2005). However, Teo et al. (2005) unsuccessfully attempted to deliver insulin through hollow

microneedle, concluding that the design of the microneedles needed improving for systemic delivery. Conversely, influenza virus-like particles (VLPs) did elicit systemically measurable immune responses when microneedles coated with VLPs were administered to mice (Song et al. 2010).

Chapter 4 aimed to investigate the wound healing responses within the first 24 hours after microneedle application to human volunteers. However the breadth of biochemical and physiological healing responses that occur after cutaneous wounding (as explained in Sections 1.1.2 and 4.1.2), required greater sample numbers and resources than permitted during this postgraduate research. Investigations into wound healing responses following puncture by 180μm microneedles showed no increase in K16 expression within the 24 hour study period (Section 4.3.3). However, at 24 hours post-puncture by 280μm microneedles, there was some evidence of K16 expression localised to the microconduit. This upregulation was minimal when compared to the K16 upregulation elicited after only 8 hours by the hypodermic needle puncture. The hypodermic needle insult initiates an immediate response of inflammatory markers and platelet release from damaged underlying vasculature (Hussein et al. 2007; Jeremy et al. 2003; Ko and Marinkovich 2010; Koch et al. 2006; Tamariz-Dominguez et al. 2002).

Research into wound healing associated to microneedle puncture forms a small part of the larger picture of skin responses. Many studies have investigated the variations in skin based on the sex, age and exposure of the skin. However, for a microneedle to deliver efficaciously, the underlying

immune responses and pharmacokinetics of the delivered drug formulation need to be thoroughly researched in an effort to optimise targeted delivery using microneedles (Fu et al. 2007; Girardeau et al. 2009; Mine et al. 2008; Querleux et al. 2009; Rees 2004; Stamatas et al. 2009). Solid microneedle structures developed from silicon, polymers or metals are likely be the first commercially available devices as they exploit well established production processes. Coating of solid microneedles will enable a suitable drug dosage to be loaded and delivered to target cells under the skin surface (Gill and Prausnitz 2007; Prausnitz et al. 2009; Quan et al. 2009).

Chapter 5 aimed to optimise an application device for administering polycarbonate microneedle arrays to human skin. Microneedle application requires a suitable force and velocity to be applied during skin insertion to ensure reproducible and efficient piercing of the SC. Patent searches reveal the use of impact insertion devices for the application of microneedles (Rizwan et al. 2009; Singh et al. 2011; Verbaan et al. 2008). Verbaan et al. (2008) mounted the microneedle array to a metal rod and inserted the rod into a 20cm long device through a metal coil. An electrical current was applied to the coil to create a magnetic field ejecting the metal rod at a calibrated velocity into the skin. The applicator provided reproducible and consistent skin punctures. To simplify applicator design, spring-loaded devices have also being designed and tested (Frederickson 2010; Singh et al. 2011). BD Soluvia^{TM} (BD, New Jersey, USA) is a commercially available microinjection system which uses a centrally mounted spring to insertion of a single microneedle (Laurent et al. 2007). 3M (Minnesota, USA) are currently recruiting participants for a clinical trial to test their microneedle arrays using

spring-loaded applicator devices (ClinicalTrials.gov 2010b). Whilst also characterising a new type of polymer microneedle array, Chapter 5 aimed to investigate similar methods for applying these microneedles to skin in a simple and reproducible manner.

A spring-loaded device was manufactured using readily available plastics from the laboratory (Section 5.3.5). The device contained parts machined to fit into a simple cylindrical tube containing a spring to apply a rapid downward force to the microneedle array. Whilst silicon forms solid microneedle structures, the array base was shown to be fragile when insufficiently supported by the applicator, causing the array to fracture at its corners during application. Indeed, when the array was placed on the skin prior to insertion using by the spring-loaded applicator, the silicon array shattered. The polycarbonate microneedles deformed when applied using a rolling application method. However, design of the spring-loaded applicator enabled the device to rest on the skin surface with the microneedles raised inside the chamber. This ensured that upon release of the spring, the microneedles impacted perpendicular to the skin surface. Further development using specially manufactured steel springs resulted in an applicator that provided a reproducible method for applying polycarbonate microneedles (Section 5.3.6), i.e. all the microneedles on the array penetrated *ex-vivo* human skin and did not deform on impact as with the rolling application method.

6.2 Limitations and further work

Throughout this thesis, research was undertaken on human skin. Prior to clinical testing, medical devices and investigational drugs would normally

undergo *in-vivo* animal testing. However microneedle dimensions would need to be scaled down for *in-vivo* animal administration, altering the drug loading capacity, release kinetics and possibly the microneedle manufacture process. Furthermore animal skin models vary considerably in follicular density, skin thickness, subcutaneous fat and muscle density, which alter the depth and penetration characteristics of microneedles. Whilst *ex-vivo* human skin testing is therefore relevant and important it is not without limitations. *Ex-vivo* human skin samples are sourced from surgical procedures such as mastectomy and breast reduction. These samples can be unpredictable in size and quality and are generally only obtained from older patient groups. Thus limitations in sourcing skin samples prevents microneedle testing at different anatomical locations, such as the upper arm, which may be more relevant for clinical application.

Research into healing of the skin post-application of microneedles was limited due to the small sample size, whereby only 12 participants had microneedles applied. Twelve participants are a low number for any study, but the low sample number was compounded further as biopsy sites were limited to one application of each device per person per time point. The snap freezing process in the clinical study required the biopsy sample to be orientated quickly and mounted to cork for cryosectioning. This led to half of each biopsy sample being discarded, thereby halving the number of potential microneedle punctures for analysis on each sample. Furthermore, immunohistochemistry (IHC) of sectioned samples is wasteful, as each sample has to be carefully mounted within the cryostat and sectioning settings optimised for transverse sectioning to be collected onto a microscope slide (Webster and Webster

2007). Therefore, during the storage, sectioning, optimising and collection, the samples are affected by temperature changes and humidity causing ice crystals to form and tearing or curling of sections. Though each process was carefully rehearsed, future work, using a larger collection of samples would increase the chances of collecting complete sections with the whole array pattern of microneedle conduits visible and permit more thorough evaluation of wound responses to the needle insertions.

Uniquely, this study focused on the pain and sensations experienced to microneedle use when they were applied in such a way as to ensure sufficient penetration of the skin's outer layer. Though participants in this study found microneedle treatment to be acceptable, further research is needed to develop the microneedle devices for transition from the laboratory to the clinic. To this end, establishing social science studies to investigate the clinician and patient needs and requirements will improve microneedle device designs, whilst market research will ensure microneedle design, manufacture and marketing is directed for suitable medicinal or cosmetic purposes. Studies of this nature may also help to inform improved patient information leaflets and determine the self-administrable nature of microneedle devices.

Due to skin's elastic properties, the forces required to consistently puncture the skin with microneedles may vary and were not quantitatively controlled during the clinical trial (Chapter 3). It was, however, evident that a better application technique or device was required for microneedles to be delivered consistently and reproducibly.

Microneedle penetration did not appear to cause TNFα upregulation. Limited K16 upregulation was observed only for the 280μm microneedles. Further optimisation of the IHC procedure may improve the visible staining of K16 or TNFα. However, as the wound created by microneedles is so small, more sensitive analysis using polymerase chain reaction (PCR) could amplify the mRNA signal of a specific marker protein, such as mRNA for K16. Thus, analysis of nucleic acid may reveal differences in precursors to inflammatory and wound healing markers (Smith et al. 1999; Wu et al. 2009). Performing PCR would require the biopsy sample to be homogenised and thus reduce sample stock further (Tuli et al. 2006; Zampetti et al. 2009). PCR, and IHC analysis of skin punctured with microneedles when a drug or gene is delivered would improve understanding of the effects of formulation and penetration depth for a required biological response. Whilst these studies have been undertaken on *ex-vivo* tissue, *in-vivo* investigations are required. Furthermore, due to ethical considerations, control samples were not biopsied to establish the 'normal' biochemical activity in each participant. During future clinical studies, biopsy samples for each participant with no devices applied would act as control samples and improve comparisons post-application of devices.

Delivering an active ingredient or gene would enable measurements of plasma concentrations of the drug or visualisation of the gene product following microneedle application (Ito et al. 2006a; Pearton et al. 2008; Prausnitz et al. 2009; So et al. 2009). This would enable different size arrays with various microneedle shapes and numbers to be tested in parallel, thereby verifying the pharmacokinetics of a drug and optimising the level of

penetration needed for successful delivery. Measuring the therapeutic responses would move the trial into a new phase of clinical research.

Whilst microneedles can be applied once and removed, microneedle patches are also being considered for long-term dosage release of coated drug (Cormier et al. 2004; Jin et al. 2009; Nordquist et al. 2007). Further clinical studies will need to show the effects of long-term placement of microneedles in the skin and this may alter the wound healing and immunological responses to having microneedles of various materials *in-situ* in the body. Thus further safety studies will determine the clinical use and duration of applications of different microneedle devices.

Despite the ability to apply microneedles into the skin, another vital requirement is becoming ever apparent. Increasingly microneedles are being designed to incorporate, or be coated by, the drug or vaccine. Thus once applied to the skin, the microneedle is required to remain *in-situ* until the skin has absorbed the dosage. Consequently, to enable the microneedles to remain *in-situ*, any applicator must be detachable from the microneedle arrays. Furthermore, depending on the release kinetics of any molecule coated on, or contained within, the microneedles, a suitable method may be required to hold the microneedle in place. It is important therefore that microneedle design, applicator design and application technique, and drug formulation must all be considered in unison to optimise molecular delivery into the skin.

Unfortunately, due to constraints in engineering resources, it was not possible to test different applicator designs with various types of microneedles. Collaboration with biomechanical engineers will improve understanding of the mechanical forces on the skin to optimise microneedle penetration (Davis et al. 2004), thereby improving spring-loaded application device design for subsequent laboratory and possibly clinical use. This could also enhance the safety of microneedle use and reduce needle-prick incidences by offering a single use pre-fabricated and packaged microneedle application device (Dumas et al. 2006; Jin et al. 2009; Pandit and Choudhary 2008; Van Damme et al. 2009). Regarding economical considerations, a small device, such as the one developed in Chapter 5, may prove to be relatively inexpensive as it utilises only 5 components, is small in size and would be cheaper to transport and store than conventional vials and hypodermic needle and syringes.

6.3 Conclusions

Silicon microneedles of 180μm and 280μm length were administered successfully to *ex-vivo* and *in-vivo* human skin when applied using an inverted-syringe applicator. Methylene blue staining and TEWL measurements determined the consistency of skin puncture. Pre-clinical testing determined that VAS, MPQ-SF and audio recording were suitable instruments for the measure of sensory modalities including pain. Assessment of pain using VAS proved that the 180μm and 280μm microneedles were significantly less painful than a 25G hypodermic needle. The adapted MPQ-SF and audio recording determined that the microneedle devices inducing minimal discomfort and highlighting specific sensations which may be

addressed in future by optimising the design and application methods of microneedles.

Sectioning of human skin biopsies showed that the physical trauma caused by application of the microneedles was significantly less than that caused by the 25G hypodermic needle. As a corollary, the wound healing response was greater for the hypodermic needle as shown by upregulation of K16 wound healing marker. Preliminary testing of a new microneedle design used in conjunction with a trajectory-based applicator device showed successful and reproducible penetration of *ex-vivo* human skin. These studies aim to help facilitate the transition of microneedles to the clinical setting.

REFERENCES

2009. *Oxford English dictionary [electronic resource].* Available at: <URL: http://www.askoxford.com/dictionaries/?view=uk> [Accessed: 3rd September 2009].

Abbas, S. Q., Yeldham, M. and Bell, S. 2005. The use of metal or plastic needles in continuous subcutaneous infusion in a hospice setting. *Am J Hosp Palliat Care* 22(2) pp. 134-138.

Abdel-Naser, M. B., Zaki, M. S., Mousa, M. H., Hamed, H., Rizk, M. S., Mohamed, H. E. and Habib, M. A. 2007. Cutaneous mononuclear cells and eosinophils are significantly increased after warm water challenge in pruritic areas of polycythemia vera. *J Cutan Pathol* 34(12), pp. 924-929.

Abe, Y., Inagaki, K., Fujiwara, A. and Kuriyama, K. 2000. Wound healing acceleration of a novel transforming growth factor-beta inducer, SEK-1005. *Eur J Pharmacol* 408(2), pp. 213-218.

Abrams, J. 1984. Nitrate delivery systems in perspective. A decade of progress. *Am J Med* 76(6A), pp. 38-46.

Ahn, S., Yoon, H., Kim, G., Kim, Y., Lee, S. and Chun, W. 2009. Designed Three-Dimensional Collagen Scaffolds for Skin Tissue Regeneration. *Tissue Eng Part C Methods* 16(5), pp. 813-820.

Akimoto, S., Ishikawa, O., Igarashi, Y., Kurosawa, M. and Miyachi, Y. 1998. Dermal mast cells in scleroderma: their skin density, tryptase/chymase phenotypes and degranulation. *Br J Dermatol* 138(3), pp. 399-406.

Al Jarad, N., Empey, D. W. and Duckworth, G. 1999. Administration of the BCG vaccination using the multipuncture method in schoolchildren: a comparison with the intradermal method. *Thorax* 54(9), pp. 762-764.

Al-Qallaf, B. and Das, D. B. 2009. Optimizing microneedle arrays for transdermal drug delivery: Extension to non-square distribution of microneedles. *Journal of Drug Targeting* 17(2), pp. 108-122.

Alarcon, J. B., Hartley, A. W., Harvey, N. G. and Mikszta, J. A. 2007. Preclinical evaluation of microneedle technology for intradermal delivery of influenza vaccines. *Clin Vaccine Immunol* 14(4), pp. 375-381.

Alonso, A., Meirelles, N. C., Yushmanov, V. E. and Tabak, M. 1996. Water increases the fluidity of intercellular membranes of stratum corneum: Correlation with water permeability, elastic, and electrical resistance properties. *Journal of Investigative Dermatology* 106(5), pp. 1058-1063.

Alvarez-Román R., Merino G., Kaila Y.N., Naik A., amd Guy R.H. 2003. Skin permeability enhancement by low frequency sonophoresis: Lipid extraction and transport pathways. *J Pharm Sci* 92(6), pp. 1138-1146.

Alves, B., Zakka, T., Teixeira, M. J., Siqueira, J. T. and Siqueira, S. R. 2009. Evaluation of pain before and after vaginal delivery. *Clin Exp Obstet Gynecol* 36(4), pp. 241-244.

Amento, E. P. and Beck, L. S. 1991. TGF-beta and wound healing. *Ciba Found Symp* 157, pp. 115-123; discussion 123-119.

Amjad, S. B., Carachi, R. and Edward, M. 2007. Keratinocyte regulation of TGF-beta and connective tissue growth factor expression: a role in suppression of scar tissue formation. *Wound Repair Regen* 15(5), pp. 748-755.

Anderson, R. L. and Cassidy, J. M. 1973. Variation in physical dimensions and chemical composition of human stratum corneum. *J Invest Dermatol* 61(1), pp. 30-32.

Ansari, M., Kazemipour, M. and Aklamli, M. 2006. The study of drug permeation through natural membranes. *Int J Pharm* 327(1-2), pp. 6-11.

Apetrei, C., Becker, J., Metzger, M., Gautam, R., Engle, J., Wales, A. K., Eyong, M., Enyong, P., Sama, M., Foley, B. T., Drucker, E. and Marx, P. A. 2006. Potential for HIV transmission through unsafe injections. *Aids* 20(7), pp. 1074-1076.

Apolone, G., Corli, O., Negri, E., Mangano, S., Montanari, M., Greco, M. T., Bertetto, O., Caraceni, A., De Conno, F., Labianca, R., Maltoni, M., Nicora Maria, F., Torri, V. and Zucco, F. 2009. Effects of transdermal buprenorphine on patients-reported outcomes in cancer patients: results from the Cancer Pain Outcome Research (CPOR) Study Group. *Clin J Pain* 25(8), pp. 671-682.

Aramaki, J., Kawana, S., Effendy, I., Happle, R. and Loffler, H. 2002. Differences of skin irritation between Japanese and European women. *Br J Dermatol* 146(6), pp. 1052-1056.

Arber, A. 2007. "Pain talk" in hospice and palliative care team meetings: An ethnography. *International Journal of Nursing Studies* 44(6), pp. 916-926.

Arendt-Nielsen, L., Egekvist, H. and Bjerring, P. 2006. Pain following controlled cutaneous insertion of needles with different diameters. *Somatosens Mot Res* 23(1-2), pp. 37 - 43.

Arnett, H. A., Mason, J., Marino, M., Suzuki, K., Matsushima, G. K. and Ting, J. P. 2001. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat Neurosci* 4, pp. 1116 - 1122.

Asbill, C. S., El-Kattan, A. F. and Michniak, B. 2000. Enhancement of transdermal drug delivery: chemical and physical approaches. *Crit Rev Ther Drug Carrier Syst* 17(6), pp. 621-658.

Ashcroft, G. S. and Mills, S. J. 2002. Androgen receptor-mediated inhibition of cutaneous wound healing. *J Clin Invest* 110(5), pp. 615-624.

Ashraf, M. W., Tayyaba, S., Nisar, A., Afzulpurkar, N., Bodhale, D. W., Lomas, T., Poyai, A. and Tuantranont, A. 2010. Design, fabrication and analysis of silicon hollow microneedles for transdermal drug delivery system for treatment of hemodynamic dysfunctions. *Cardiovasc Eng* 10(3), pp. 91-108.

Atrux-Tallau, N., Romagny, C., Padois, K., Denis, A., Haftek, M., Falson, F., Pirot, F. and Maibach, H. I. 2009. Effects of glycerol on human skin damaged by acute sodium lauryl sulphate treatment. *Arch Dermatol Res* 302(6), pp. 435-41.

Ayittey, P., Walker, J., Rice, J. and de Tombe, P. 2009. Glass microneedles for force measurements: a finite-element analysis model. *Pflügers Archiv European Journal of Physiology* 457(6), pp. 1415-1422.

Bacci, S., Romagnoli, P., Norelli, G., Forestieri, A. and Bonelli, A. 2006. Early increase in TNF-alpha-containing mast cells in skin lesions. *International Journal of Legal Medicine* 120(3), pp. 138-142.

Badran, M. M., Kuntsche, J. and Fahr, A. 2009. Skin penetration enhancement by a microneedle device (Dermaroller) in vitro: dependency on needle size and applied formulation. *Eur J Pharm Sci* 36(4-5), pp. 511-523.

Bae, J. S., Lee, S. H., Kim, J. E., Choi, J. Y., Park, R. W., Yong Park, J., Park, H. S., Sohn, Y. S., Lee, D. S., Bae Lee, E. and Kim, I. S. 2002. Betaig-h3 supports keratinocyte adhesion, migration, and proliferation through alpha3beta1 integrin. *Biochem Biophys Res Commun* 294(5), pp. 940-948.

Bal, S. M., Caussin, J., Pavel, S. and Bouwstra, J. A. 2008. In vivo assessment of safety of microneedle arrays in human skin. *European Journal of Pharmaceutical Sciences* 35(3), pp. 193-202.

Balbierz, J. M., Petajan, J. H., Alder, S. C. and Vlach, S. A. 2006. Differences in Pain Perception in Women Using Concentric and Monopolar Needles. *Archives of Physical Medicine and Rehabilitation* 87(10), pp. 1403-1406.

Banks, S. L., Paudel, K. S., Brogden, N. K., Loftin, C. D. and Stinchcomb, A. L. 2011. Diclofenac Enables Prolonged Delivery of Naltrexone Through Microneedle-Treated Skin. *Pharm Res* Feb 8, [Epub ahead of print].

Banks, S. L., Pinninti, R. R., Gill, H. S., Crooks, P. A., Prausnitz, M. R. and Stinchcomb, A. L. 2008. Flux across [corrected] microneedle-treated skin is increased by increasing charge of naltrexone and naltrexol in vitro. *Pharm Res* 25(7), pp. 1677-1685.

Barakatt, E. T., Romano, P. S., Riddle, D. L., Beckett, L. A. and Kravitz, R. 2009. An Exploration of Maitland's Concept of Pain Irritability in Patients with Low Back Pain. *J Man Manip Ther* 17(4), pp. 196-205.

Barbero, A. M. and Frasch, H. F. 2005. Modeling of diffusion with partitioning in stratum corneum using a finite element model. *Ann Biomed Eng* 33(9), pp. 1281-1292.

Barbero, A. M. and Frasch, H. F. 2006. Transcellular route of diffusion through stratum corneum: Results from finite element models. *Journal of Pharmaceutical Sciences* 95(10), pp. 2186-2194.

Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H. and Tomic-Canic, M. 2008. Growth factors and cytokines in wound healing. *Wound Repair Regen* 16(5), pp. 585-601.

Barry, B. W. 1999. Reflections on transdermal drug delivery. *Pharm Sci Technolo Today* 2(2), pp. 41-43.

Bartell, J. C., Roberts, K. A., Schutte, N. J., Sherman, K. C., Muller, D. and Hayney, M. S. 2008. Needle temperature effect on pain ratings after injection. *Clin J Pain* 24(3), pp. 260-264.

Bashir, S. J., Chew, A. L., Anigbogu, A., Dreher, F. and Maibach, H. I. 2001. Physical and physiological effects of stratum corneum tape stripping. *Skin Res Technol* 7(1), pp. 40-48.

Batisse, D., Bazin, R., Baldeweck, T., Querleux, B. and Leveque, J. L. 2002. Influence of age on the wrinkling capacities of skin. *Skin Res Technol* 8(3), pp. 148-154.

Baxter, L. L., Loftus, S. K. and Pavan, W. J. 2009. Networks and pathways in pigmentation, health, and disease. *Wiley Interdiscip Rev Syst Biol Med* 1(3), pp. 359-371.

Benoliel, R., Eliav, E. and Sharav, Y. 2009. Self-reports of pain-related awakenings in persistent orofacial pain patients. *J Orofac Pain* 23(4), pp. 330-338.

Berardesca, E. and Distante, F. 1994. The modulation of skin irritation. *Contact Dermatitis* 31(5), pp. 281-287.

Bergh, I., Jakobsson, E. and Sjostrom, B. 2008. Worst experiences of pain and conceptions of worst pain imaginable among nursing students. *J Adv Nurs* 61(5), pp. 484-491.

Bergh, I. H., Stener-Victorin, E., Wallin, G. and Martensson, L. 2009. Comparison of the PainMatcher and the Visual Analogue Scale for assessment of labour pain following administered pain relief treatment. *Midwifery* 27(1), pp. 134-139.

Berner, B. and John, V. A. 1994. Pharmacokinetic characterisation of transdermal delivery systems. *Clin Pharmacokinet* 26(2), pp. 121-134.

Bessel, C. A., Denison, G. M., DeSimone, J. M., DeYoung, J., Gross, S., Schauer, C. K. and Visintin, P. M. 2003. Etchant solutions for the removal of Cu(0) in a supercritical CO2-based "dry" chemical mechanical planarization process for device fabrication. *J Am Chem Soc* 125(17), pp. 4980-4981.

Bimstein, E., Azari, A. F., Sotto, J. J. and Riley, J. L., III 2009. Students' Perceptions About Pediatric Dental Behavior Guidance in an Undergraduate Four-Year Dental Curriculum. *J Dent Educ.* 73(12), pp. 1366-1371.

Birchall, J., Coulman, S., Anstey, A., Gateley, C., Sweetland, H., Gershonowitz, A., Neville, L. and Levin, G. 2006. Cutaneous gene expression of plasmid DNA in excised human skin following delivery via microchannels created by radio frequency ablation. *Int J Pharm* 312(1-2), pp. 15-23.

Birchall, J., Coulman, S., Pearton, M., Allender, C., Brain, K., Anstey, A., Gateley, C., Wilke, N. and Morrissey, A. 2005. Cutaneous DNA delivery and gene expression in ex vivo human skin explants via wet-etch micro-fabricated micro-needles. *J Drug Target* 13(7), pp. 415-421.

Bjorklund, S., Engblom, J., Thuresson, K. and Sparr, E. 2010. A water gradient can be used to regulate drug transport across skin. *J Control Release* 143(2), pp. 191-200.

Blair, C. 1968. Morphology and thickness of the human stratum corneum. *Br J Dermatol* 80(7), pp. 430-436.

Blanpain, C. and Fuchs, E. 2006. Epidermal stem cells of the skin. *Annu Rev Cell Dev Biol* 22, pp. 339-373.

Blomkalns, A. L., Kalokhe, G. P. and Lindsell, C. J. 2005. Sex Differences in Pain Perception and Description. *Behaviour Sci* 5(3), pp. 84-90.

Boles, C. A., Kannam, S. and Cardwell, A. B. 2000. The forearm: anatomy of muscle compartments and nerves. *AJR Am J Roentgenol* 174(1), pp. 151-159.

Bolyard, E. A., Tablan, O. C., Williams, W. W., Pearson, M. L., Shapiro, C. N. and Deitchmann, S. D. 1998. Guideline for infection control in healthcare personnel, 1998. Hospital Infection Control Practices Advisory Committee. *Infect Control Hosp Epidemiol* 19(6), pp. 407-463.

Bonte, F., Saunois, A., Pinguet, P. and Meybeck, A. 1997. Existence of a lipid gradient in the upper stratum corneum and its possible biological significance. *Arch Dermatol Res* 289(2), pp. 78-82.

Bornkessel, A., Flach, M., Arens-Corell, M., Elsner, P. and Fluhr, J. W. 2005. Functional assessment of a washing emulsion for sensitive skin: mild impairment of stratum corneum hydration, pH, barrier function, lipid content, integrity and cohesion in a controlled washing test. *Skin Res Technol* 11(1), pp. 53-60.

Bos, J. D. and Meinardi, M. M. 2000. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Exp Dermatol* 9(3), pp. 165-169.

Brand, C. U., Hunziker, T. and Braathen, L. R. 1992. Studies on human skin lymph containing Langerhans cells from sodium lauryl sulphate contact dermatitis. *J Invest Dermatol* 99(5), pp. 109S-110S.

Branzan, A. L., Landthaler, M. and Szeimies, R. M. 2007. In vivo confocal scanning laser microscopy in dermatology. *Lasers Med Sci* 22(2), pp. 73-82.

Braverman, I. M. 1983. The role of blood vessels and lymphatics in cutaneous inflammatory processes: an overview. *Br J Dermatol* 109 Suppl 25, pp. 89-98.

Bridges, C. B., Thompson, W. W., Meltzer, M. I., Reeve, G. R., Talamonti, W. J., Cox, N. J., Lilac, H. A., Hall, H., Klimov, A. and Fukuda, K. 2000. Effectiveness and cost-benefit of influenza vaccination of healthy working adults: A randomized controlled trial. *Jama* 284(13), pp. 1655-1663.

Briggaman, R. A. 1982. Epidermal-dermal interactions in adult skin. *J Invest Dermatol* 79 Suppl 1, pp. 21s-24s.

Briggaman, R. A. and Wheeler, C. E., Jr. 1968. Epidermal-dermal interactions in adult human skin: role of dermis in epidermal maintenance. *J Invest Dermatol* 51(6), pp. 454-465.

Bringuier, S., Picot, M. C., Dadure, C., Rochette, A., Raux, O., Boulhais, M. and Capdevila, X. 2009. A prospective comparison of post-surgical behavioral pain scales in preschoolers highlighting the risk of false evaluations. *Pain* 145(1-2), pp. 60-68.

Brody, I. 1989. A light and electron microscopy study of normal human stratum corneum with particular reference to the intercellular space. *Ups J Med Sci* 94(1), pp. 29-45.

Bronaugh, R. L. and Maibach, H. I. 1999. *Percutaneous absorption : drugs-cosmetics-mechanisms-methodology*. 3rd ed., rev. and expanded. ed. New York: Dekker.

Bronaugh, R. L. and Maibach, H. I. 2002. *Topical absorption of dermatological products*. New York: Dekker.

Brown, M. B., Martin, G. P., Jones, S. A. and Akomeah, F. K. 2006. Dermal and transdermal drug delivery systems: current and future prospects. *Drug delivery* 13(3), pp. 175-187.

Brysk, M. M. and Rajaraman, S. 1992. Cohesion and desquamation of epidermal stratum corneum. *Prog Histochem Cytochem* 25(1), pp. 1-53.

Bulat, V., Mihic, L. L., Situm, M., Buljan, M., Blajic, I. and Pusic, J. 2009. Most common clinical presentations of cutaneous mastocytosis. *Acta Clin Croat* 48(1), pp. 59-64.

Busam, K. J., Charles, C., Lee, G. and Halpern, A. C. 2001. Morphologic Features of Melanocytes, Pigmented Keratinocytes, and Melanophages by In Vivo Confocal Scanning Laser Microscopy. *Mod Pathol* 14(9), pp. 862-868.

Busse, K. L. and Maibach, H. I. 2011. Transdermal estradiol and testosterone transfer in man: existence, models, and strategies for prevention. *Skin Pharmacol Physiol* 24(2), pp. 57-66.

Cakmak, B. 2002. Fabrication and characterization of dry and wet etched InGaAs/InGaAsP/InP long wavelength semiconductor lasers. *Opt Express* 10(13), pp. 530-535.

Cameron, A. L., Kirby, B., Fei, W. and Griffiths, C. E. 2002. Natural killer and natural killer-T cells in psoriasis. *Arch Dermatol Res* 294(8), pp. 363-369.

Caraceni, A., Zecca, E., Martini, C., Brunelli, C., Pigni, A., Gorni, G., Galbiati, A., Ibazeta, M., Hjermstad, M. and Kaasa, S. 2009. The validity of average 8-h pain intensity assessment in cancer patients. *Eur J Pain* 14(4), pp. 441-445.

Carnes, D. 2006. "Understanding and measuring chronic musculoskeletal pain in the community using self completed pain drawings", Thesis, University of London.

Carruthers, J. and Carruthers, A. 2007. The evolution of botulinum neurotoxin type A for cosmetic applications. *J Cosmet Laser Ther* 9(3), pp. 186-192.

Caspers, P. J., Lucassen, G. W. and Puppels, G. J. 2003. Combined In Vivo Confocal Raman Spectroscopy and Confocal Microscopy of Human Skin. *Biophys J* 85(1), pp. 572-580.

Catanzaro, J. N., Makaryus, A. N., Rosman, D. and Jadonath, R. 2006. Emotion-triggered cardiac asystole-inducing neurocardiogenic syncope. *Pacing Clin Electrophysiol* 29(5), pp. 553-556.

Cerio, R., Griffiths, C. E., Cooper, K. D., Nickoloff, B. J. and Headington, J. T. 1989. Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. *Br J Dermatol* 121(4), pp. 421-431.

Chabri, F., Bouris, K., Jones, T., Barrow, D., Hann, A., Allender, C., Brain, K. and Birchall, J. 2004. Microfabricated silicon microneedles for nonviral cutaneous gene delivery. *Br J Dermatol* 150(5), pp. 869-877.

Chambers, C. T., Taddio, A., Uman, L. S. and McMurtry, C. M. 2009. Psychological interventions for reducing pain and distress during routine childhood immunizations: A systematic review. *Clinical Therapeutics* 31(Supplement 2), pp. S77-S103.

Chekurov, N., Grigoras, K., Peltonen, A., Franssila, S. and Tittonen, I. 2009. The fabrication of silicon nanostructures by local gallium implantation and cryogenic deep reactive ion etching. *Nanotechnology* 20(6), p. 065307.

Chen, H., Zhu, H., Zheng, J., Moua, D., Wan, J., Zhang, J., Shi, T., Zhao, Y., Xu, H. and Yang, X. 2009a. Iontophoresis-driven penetration of nanovesicles through microneedle-induced skin microchannels for enhancing transdermal delivery of insulin. *J Control Release* 139(1), pp. 63-72.

Chen, J. D., Kim, J. P., Zhang, K., Sarret, Y., Wynn, K. C., Kramer, R. H. and Woodley, D. T. 1993. Epidermal growth factor (EGF) promotes human keratinocyte locomotion on collagen by increasing the alpha 2 integrin subunit. *Exp Cell Res* 209(2), pp. 216-223.

Chen, K. M., Tsai, H. C., Sy, C. L., Lee, S. S., Liu, Y. C., Wann, S. R., Wang, Y. H., Mai, M. H., Chen, J. K., Wu, K. S., Chen, Y. J. and Chen, Y. S. 2009b. Clinical manifestations of Japanese encephalitis in southern Taiwan. *J Microbiol Immunol Infect* 42(4), pp. 296-302.

Cheng, C. F., Fan, J., Bandyopahdhay, B., Mock, D., Guan, S., Chen, M., Woodley, D. T. and Li, W. 2008. Profiling motility signal-specific genes in primary human keratinocytes. *J Invest Dermatol* 128(8), pp. 1981-1990.

Chien, Y. W. 1984. Pharmaceutical considerations of transdermal nitroglycerin delivery: the various approaches. *Am Heart J* 108(1), pp. 207-216.

Chippada, U., Yurke, B., Georges, P. C. and Langrana, N. A. 2009. A nonintrusive method of measuring the local mechanical properties of soft hydrogels using magnetic microneedles. *J Biomech Eng.* 131(2), p. 021014.

Choi, Y. and Fuchs, E. 1990. TGF-beta and retinoic acid: regulators of growth and modifiers of differentiation in human epidermal cells. *Cell Regul* 1(11), pp. 791-809.

Choiniere, M. and Amsel, R. 1996. A visual analogue thermometer for measuring pain intensity. *J Pain Symptom Manage* 11(5), pp. 299-311.

Clark, M. E., Gironda, R. J. and Young, R. W. 2003. Development and validation of the Pain Outcomes Questionnaire-VA. *J Rehabil Res Dev* 40(5), pp. 381-395.

Clarke, K. A. 2009. Uses of a research diary: learning reflectively, developing understanding and establishing transparency. *Nurse Res* 17(1), pp. 68-76.

Clever, L. H. and Omenn, G. S. 1988. Hazards for Health Care Workers. *Annual Review of Public Health* 9(1), pp. 273-303.

Clifton, J., 2nd and Leikin, J. B. 2003. Methylene blue. *Am J Ther* 10(4), pp. 289-291.

ClinicalTrials.gov. 2010a. *Dose Sparing Intradermal S-OIV H1N1 Influenza Vaccination Device.* U.S. National Institutes of Health. Available at: <URL: http://www.clinicaltrials.gov/ct2/show/NCT01049490> [Accessed: 7/2/2011].

ClinicalTrials.gov. 2010b. *Tolerability Study of the Application of a 3M Microstructure Transdermal System.* U.S. National Institutes of Health. Available at: <URL: http://www.clinicaltrials.gov/ct2/show/NCT01257763> [Accessed: 7/02/2011].

Cohen, J. C., Hartman, D. G., Garofalo, M. J., Basehoar, A., Raynor, B., Ashbrenner, E. and Akin, F. J. 2009a. Comparison of closed chamber and open chamber evaporimetry. *Skin Res Technol* 15(1), pp. 51-54.

Cohen, L. A., Bonito, A. J., Akin, D. R., Manski, R. J., Macek, M. D., Edwards, R. R. and Cornelius, L. J. 2009b. Toothache pain: behavioral impact and self-care strategies. *Spec Care Dentist* 29(2), pp. 85-95.

Coleman, R. 2006. The long-term contribution of dyes and stains to histology and histopathology. *Acta Histochemica* 108(2), pp. 81-83.

Colwell, A. S., Yun, R., Krummel, T. M., Longaker, M. T. and Lorenz, H. P. 2007. Keratinocytes modulate fetal and postnatal fibroblast transforming growth factor-beta and Smad expression in co-culture. *Plast Reconstr Surg* 119(5), pp. 1440-1445.

Corcuff, P., Fiat, F. and Minondo, A. M. 2001. Ultrastructure of the human stratum corneum. *Skin Pharmacol Appl Skin Physiol* 14 Suppl 1, pp. 4-9.

Cormier, M., Johnson, B., Ameri, M., Nyam, K., Libiran, L., Zhang, D. D. and Daddona, P. 2004. Transdermal delivery of desmopressin using a coated microneedle array patch system. *J Control Release* 97(3), pp. 503-511.

Coulman, S. A., Anstey, A., Gateley, C., Morrissey, A., McLoughlin, P., Allender, C. and Birchall, J. C. 2009. Microneedle mediated delivery of nanoparticles into human skin. *International Journal of Pharmaceutics* 366(1-2), pp. 190-200.

Coulman, S. A., Barrow, D., Anstey, A., Gateley, C., Morrissey, A., Wilke, N., Allender, C., Brain, K. and Birchall, J. C. 2006. Minimally invasive cutaneous

delivery of macromolecules and plasmid DNA via microneedles. *Curr Drug Deliv* 3(1), pp. 65-75.

Coulman, S. A., Birchall, J. C., Alex, A., Pearton, M., Hofer, B., O'Mahony, C., Drexler, W. and Povazay, B. 2010. In Vivo, In Situ Imaging of Microneedle Insertion into the Skin of Human Volunteers Using Optical Coherence Tomography. *Pharm Res* 28(1), pp. 68-81.

Coulombe, P. A. 1997. Towards a Molecular Definition of Keratinocyte Activation after Acute Injury to Stratified Epithelia. *Biochemical and Biophysical Research Communications* 236(2), pp. 231-238.

Coulombe, P. A. 2003. Wound epithelialization: accelerating the pace of discovery. *J Invest Dermatol* 121(2), pp. 219-230.

Coulombe, P. A., Wawersik, M., Paladini, R. D. and Noensie, E. 1998. Type I Keratin 16 Forms Relatively Unstable Tetrameric Assembly Subunits With Various Type II Keratin Partners: Biochemical Basis and Functional Implications. *Biol Bull 194(3),* pp. 364-366.

Cox, B. J., Parker, J. D. A. and Swinson, R. P. 1996. Confirmatory factor analysis of the fear questionnaire with social phobia patients. *The British Journal of Psychiatry.* 168(4), p. 497.

Cutts, F. T., Glik, D. C., Gordon, A., Parker, K., Diallo, S., Haba, F. and Stone, R. 1990. Application of multiple methods to study the immunization programme in an urban area of Guinea. *Bull World Health Organ* 68(6), pp. 769-776.

Davis, S. P., Landis, B. J., Adams, Z. H., Allen, M. G. and Prausnitz, M. R. 2004. Insertion of microneedles into skin: measurement and prediction of insertion force and needle fracture force. *J Biomech* 37(8), pp. 1155-1163.

de Boer, A. G., van Lanschot, J. J., Stalmeier, P. F., van Sandick, J. W., Hulscher, J. B., de Haes, J. C. and Sprangers, M. A. 2004. Is a single-item visual analogue scale as valid, reliable and responsive as multi-item scales in measuring quality of life? *Qual Life Res* 13(2), pp. 311-320.

de Boer, O. J., van der Loos, C. M., Teeling, P., van der Wal, A. C. and Teunissen, M. B. 2007. Immunohistochemical analysis of regulatory T cell markers FOXP3 and GITR on CD4+CD25+ T cells in normal skin and inflammatory dermatoses. *J Histochem Cytochem* 55(9), pp. 891-898.

De Paepe, K., Houben, E., Adam, R., Wiesemann, F. and Rogiers, V. 2005. Validation of the VapoMeter, a closed unventilated chamber system to assess transepidermal water loss vs. the open chamber Tewameter&reg. pp. 61-69.

de Salis, I., Tomlin, Z., Toerien, M. and Donovan, J. 2008. Qualitative research to improve RCT recruitment: Issues arising in establishing research collaborations. *Contemporary Clinical Trials* 29(5), pp. 663-670.

Deacon, B. and Abramowitz, J. 2006. Fear of needles and vasovagal reactions among phlebotomy patients. *Journal of Anxiety Disorders* 20(7), pp. 946-960.

Degim, I. T. 2006. New tools and approaches for predicting skin permeability. *Drug Discov Today* 11(11-12), pp. 517-523.

Delobelle, P., Rawlinson, J. L., Ntuli, S., Malatsi, I., Decock, R. and Depoorter, A. M. 2009. HIV/AIDS knowledge, attitudes, practices and perceptions of rural nurses in South Africa. *J Adv Nurs* 65(5), pp. 1061-1073.

Devine, C., Brennan, G. P., Lanusse, C. E., Alvarez, L. I., Trudgett, A., Hoey, E. and Fairweather, I. 2009. Inhibition of cytochrome P450-mediated metabolism enhances ex vivo susceptibility of Fasciola hepatica to triclabendazole. *Parasitology*, pp. 1-10.

Diegelmann, R. F. and Evans, M. C. 2004. Wound healing: an overview of acute, fibrotic and delayed healing. *Frontiers in Bioscience* 9, pp. 283-289.

Ding, Z., Verbaan, F. J., Bivas-Benita, M., Bungener, L., Huckriede, A., van den Berg, D. J., Kersten, G. and Bouwstra, J. A. 2009. Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice. *J Control Release* 136(1), pp. 71-78.

DiPietro, L. A. 1995. Wound healing: the role of the macrophage and other immune cells. *Shock* 4(4), pp. 233-240.

Doab, A., Topp, L., Day, C. A., Dore, G. J. and Maher, L. 2009. Clinical trial literacy among injecting drug users in Sydney, Australia: A pilot study. *Contemporary Clinical Trials* 30(5), pp. 431-435.

Dodoo, A. N., Renner, L., van Grootheest, A. C., Labadie, J., Antwi-Agyei, K. O., Hayibor, S., Addison, J., Pappoe, V. and Appiah-Danquah, A. 2007. Safety monitoring of a new pentavalent vaccine in the expanded programme on immunisation in Ghana. *Drug Saf* 30(4), pp. 347-356.

Donnelly, R. F., Majithiya, R., Singh, T. R., Morrow, D. I., Garland, M. J., Demir, Y. K., Migalska, K., Ryan, E., Gillen, D., Scott, C. J. and Woolfson, A. D. 2011. Design, optimization and characterisation of polymeric microneedle arrays prepared by a novel laser-based micromoulding technique. *Pharm Res* 28(1), pp. 41-57.

Donnelly, R. F., Morrow, D. I., Singh, T. R., Migalska, K., McCarron, P. A., O'Mahony, C. and Woolfson, A. D. 2009. Processing difficulties and instability of carbohydrate microneedle arrays. *Drug Dev Ind Pharm* 35(10), pp. 1242-1254.

Donnelly, R. F., Raj Singh, T. R. and Woolfson, A. D. 2010. Microneedle-based drug delivery systems: microfabrication, drug delivery, and safety. *Drug Deliv* 17(4), pp. 187-207.

Dumas, H., Panayiotopoulos, P., Parker, D. and Pongpairochana, V. 2006. Understanding and meeting the needs of those using growth hormone injection devices. *BMC Endocr Disord* 6, p. 5.

Dunwoody, C. J., Krenzischek, D. A., Pasero, C., Rathmell, J. P. and Polomano, R. C. 2008. Assessment, Physiological Monitoring, and Consequences of Inadequately Treated Acute Pain. *Journal of PeriAnesthesia Nursing* 23(1, Supplement 1), pp. S15-S27.

Dupin-Spriet, A. S. T. 2005. *Good practice of clinical drug trials*. Libraire Luginbuhl, p. 273.

Dutton, J. J., Gayre, G. S. and Proia, A. D. 2007. *Diagnostic atlas of common eyelid diseases*. New York: Informa Healthcare ; London : Taylor & Francis.

Dykes, P. J., Heggie, R. and Hill, S. A. 2001. Effects of adhesive dressings on the stratum corneum of the skin. *J Wound Care* 10(2), pp. 7-10.

Earl-Slater, A. 2002. *The handbook of clinical trials and other research*. Abingdon: Radcliffe Medical.

Ejaz, S., Ashraf, M., Nawaz, M. and Lim, C. W. 2009. Total particulate matter and wound healing: an in vivo study with histological insights. *Biomed Environ Sci* 22(4), pp. 278-287.

El Gammal, S., El Gammal, C., Kaspar, K., Pieck, C., Altmeyer, P., Vogt, M. and Ermert, H. 1999. Sonography of the skin at 100 MHz enables in vivo visualization of stratum corneum and viable epidermis in palmar skin and psoriatic plaques. *J Invest Dermatol* 113(5), pp. 821-829.

Elias, P. M. 2005. Stratum corneum defensive functions: an integrated view. *J Invest Dermatol* 125(2), pp. 183-200.

Elias, P. M. and Choi, E. H. 2005. Interactions among stratum corneum defensive functions. *Exp Dermatol* 14(10), pp. 719-726.

Elias, P. M., McNutt, N. S. and Friend, D. S. 1977. Membrane alterations during cornification of mammalian squamous epithelia: a freeze-fracture, tracer, and thin-section study. *Anat Rec* 189(4), pp. 577-594.

EMEA 2002. *Guideline for Good Clinical Practice*. London: European Medicines Agency.

Enfield, J., O'Connell, M. L., Lawlor, K., Jonathan, E., O'Mahony, C. and Leahy, M. 2010. In-vivo dynamic characterization of microneedle skin

penetration using optical coherence tomography. *J Biomed Opt* 15(4), p. 046001.

Epstein, F. H., Singer, A. J. and Clark, R. A. F. 1999. Cutaneous Wound Healing. *New England Journal of Medicine* 341(10), pp. 738-746.

Escobar-Chavez, J. J., Bonilla-Martinez, D., Villegas-Gonzalez, M. A., Molina-Trinidad, E., Casas-Alancaster, N. and Revilla-Vazquez, A. L. 2010. Microneedles: A Valuable Physical Enhancer to Increase Transdermal Drug Delivery. *The Journal of Clinical Pharmacology* [Epub ahead of print].

Escobar-Chavez, J. J., Bonilla-Martinez, D., Villegas-Gonzalez, M. A. and Revilla-Vazquez, A. L. 2009a. Electroporation as an efficient physical enhancer for skin drug delivery. *J Clin Pharmacol* 49(11), pp. 1262-1283.

Escobar-Chavez, J. J., Bonilla-Martinez, D., Villegas-Gonzalez, M. A., Rodriguez-Cruz, I. M. and Dominguez-Delgado, C. L. 2009b. The use of sonophoresis in the administration of drugs throughout the skin. *J Pharm Pharm Sci* 12(1), pp. 88-115.

Evers, L. H., Bhavsar, D. and Mailander, P. 2010. The biology of burn injury. *Exp Dermatol* 19(9), pp. 777-783.

Fabbri, E., Villa, G., Mabrouk, M., Guerrini, M., Montanari, G., Paradisi, R., Venturoli, S. and Seracchioli, R. 2009. McGill Pain Questionnaire: a multi-dimensional verbal scale assessing postoperative changes in pain symptoms associated with severe endometriosis. *J Obstet Gynaecol Res* 35(4), pp. 753-760.

Falanga, V. 2005. Wound healing and its impairment in the diabetic foot. *The Lancet* 366(9498), pp. 1736-1743.

Fassler, D. 1985. The fear of needles in children. *Am J Orthopsychiatry* 55(3), pp. 371-377.

Fauconnier, A., Dallongeville, E., Huchon, C., Ville, Y. and Falissard, B. 2009. Measurement of acute pelvic pain intensity in gynecology: a comparison of five methods. *Obstet Gynecol* 113(2 Pt 1), pp. 260-269.

Faurschou, A., Wiegell, S. R. and Wulf, H. C. 2007. Transepidermal water loss after photodynamic therapy, UVB radiation and topical corticosteroid is independent of inflammation. *Skin Res Technol* 13(2), pp. 202-206.

Fenske, N. A. and Lober, C. W. 1986. Structural and functional changes of normal aging skin. *J Am Acad Dermatol* 15(4 Pt 1), pp. 571-585.

Fernandez and et al. 2009. Study of functional viability of SU-8-based microneedles for neural applications. *Journal of Micromechanics and Microengineering* 19(2), p. 025007.

Fischer, D. J., Villines, D., Kim, Y. O., Epstein, J. B. and Wilkie, D. J. 2009. Anxiety, depression, and pain: differences by primary cancer. *Support Care Cancer* 18(7), pp. 801-810.

Flaskerud, J. H. and Nyamathi, A. M. 1996. Home medication injection among Latina women in Los Angeles: implications for health education and prevention. *AIDS Care* 8(1), pp. 95-102.

Flather, M., Aston, H. and Stables, R. 2001. *Handbook of clinical trials.* London: ReMedica.

Fluhr, J. W., Feingold, K. R. and Elias, P. M. 2006. Transepidermal water loss reflects permeability barrier status: validation in human and rodent in vivo and ex vivo models. *Exp Dermatol* 15(7), pp. 483-492.

Foege, W. H. and Eddins, D. L. 1973. Mass vaccination programs in developing countries. *Prog Med Virol* 15, pp. 205-243.

Forbes, A., MacAuley, S. and Chiotakakou-Faliakou, E. 2000. Hypnotherapy and therapeutic audiotape: effective in previously unsuccessfully treated irritable bowel syndrome? *Int J Colorectal Dis* 15(5-6), pp. 328-334.

Frankel, A. S. and Markarian, A. 2007. Cosmetic treatments and strategies for the upper face. *Facial Plast Surg Clin North Am* 15(1), pp. 31-39.

Frechette, J. P., Martineau, I. and Gagnon, G. 2005. Platelet-rich plasmas: growth factor content and roles in wound healing. *J Dent Res* 84(5), pp. 434-439.

Frederickson, F. L. W. B. L., (MN, US), Johnson, Michael D. (College Station, TX, US) 2010. Microneedle Array Applicator Device and Method of Array Application. United States.

Fredrikson, M., Annas, P., Fischer, H. and Wik, G. 1996. Gender and age differences in the prevalence of specific fears and phobias. *Behaviour Research and Therapy* 34(1), pp. 33-39.

Freedberg, I. M., Tomic-Canic, M., Komine, M. and Blumenberg, M. 2001. Keratins and the keratinocyte activation cycle. *J Invest Dermatol* 116(5), pp. 633-640.

Fu, X., Fang, L., Li, H., Li, X., Cheng, B. and Sheng, Z. 2007. Adipose tissue extract enhances skin wound healing. *Wound Repair Regen* 15(4), pp. 540-548.

Fuchs, E. 2007. Scratching the surface of skin development. *Nature* 445(7130), pp. 834-842.

Fuchs, E. 2008. Skin stem cells: rising to the surface. *J Bell Biol* 180(2), pp. 273-284.

Fuchs, E. and Horsley, V. 2008. More than one way to skin. *Genes Dev* 15(22), pp. 976-985.

Fuchs, E. and Weber, K. 1994. Intermediate Filaments: Structure, Dynamics, Function and Disease. *Annual Review of Biochemistry* 63(1), pp. 345-382.

Furuzawa-Carballeda, J., Munoz-Chable, O. A., Macias-Hernandez, S. I. and Agualimpia-Janning, A. 2009. Effect of polymerized-type I collagen in knee osteoarthritis. II. In vivo study. *Eur J Clin Invest* 39(7), pp. 598-606.

Gabbanini, S., Lucchi, E., Carli, M., Berlini, E., Minghetti, A. and Valgimigli, L. 2009. In vitro evaluation of the permeation through reconstructed human epidermis of essentials oils from cosmetic formulations. *J Pharm Biomed Anal* 50(3), pp. 370-376.

Gabrielli, A., Di Loreto, C., Taborro, R., Candela, M., Sambo, P., Nitti, C., Danieli, M. G., DeLustro, F., Dasch, J. R. and Danieli, G. 1993. Immunohistochemical localization of intracellular and extracellular associated TGF beta in the skin of patients with systemic sclerosis (scleroderma) and primary Raynaud's phenomenon. *Clin Immunol Immunopathol* 68(3), pp. 340-349.

Gad, S. C. 2009. *Clinical trials handbook*. Hoboken, N.J.: Wiley.

Garcia Bartels, N., Mleczko, A., Schink, T., Proquitte, H., Wauer, R. R. and Blume-Peytavi, U. 2009. Influence of bathing or washing on skin barrier function in newborns during the first four weeks of life. *Skin Pharmacol Physiol* 22(5), pp. 248-257.

Garlick, J. A. and Taichman, L. B. 1994a. Effect of TGF-beta 1 on re-epithelialization of human keratinocytes in vitro: an organotypic model. *J Invest Dermatol* 103(4), pp. 554-559.

Garlick, J. A. and Taichman, L. B. 1994b. Fate of human keratinocytes during reepithelialization in an organotypic culture model. *Lab Invest* 70(6), pp. 916-924.

Genina, E. A., Bashkatov, A. N. and Tuchin, V. V. 2008. Effect of ethanol on the transport of methylene blue through stratum corneum. *Medical Laser Application* 23(1), pp. 31-38.

Giangreco, A., Qin, M., Pintar, J. E. and Watt, F. M. 2008. Epidermal stem cells are retained in vivo throughout skin aging. pp. 250-259.

Gibaldi, M., Lee, M., Desai, A. and American Society of Health-System Pharmacists. 2007. *Gibaldi's drug delivery systems in pharmaceutical care*. Bethesda, USA: American Society of Health-System Pharmacists.

Gill, H. S., Denson, D. D., Burris, B. A. and Prausnitz, M. R. 2008. Effect of microneedle design on pain in human volunteers. *Clin J Pain* 24(7), pp. 585-594.

Gill, H. S. and Prausnitz, M. R. 2007. Coated microneedles for transdermal delivery. *Journal of Controlled Release* 117(2), pp. 227-237.

Gillitzer, R. and Goebeler, M. 2001. Chemokines in cutaneous wound healing. *J Leukoc Biol* 69(4), pp. 513-521.

Girardeau, S., Mine, S., Pageon, H. and Asselineau, D. 2009. The Caucasian and African skin types differ morphologically and functionally in their dermal component. *Exp Dermatol* 18(8), pp. 704-711.

Giske, L., Bautz-Holter, E., Sandvik, L. and Roe, C. 2009. Relationship between pain and neuropathic symptoms in chronic musculoskeletal pain. *Pain Med* 10(5), pp. 910-917.

Gisselquist, D. 2009. Double standards in research ethics, health-care safety, and scientific rigour allowed Africa's HIV/AIDS epidemic disasters. *Int J STD AIDS* 20(12), pp. 839-845.

Gittard, S. D., Ovsianikov, A., Monteiro-Riviere, N. A., Lusk, J., Morel, P., Minghetti, P., Lenardi, C., Chichkov, B. N. and Narayan, R. J. 2009. Fabrication of polymer microneedles using a two-photon polymerization and micromolding process. *J Diabetes Sci Technol* 3(2), pp. 304-311.

Glenn, G. M. and Kenney, R. T. 2006. Mass vaccination: solutions in the skin. *Curr Top Microbiol Immunol* 304, pp. 247-268.

Goldsmith, L. A. 1991. *Physiology, biochemistry, and molecular biology of the skin*. 2nd ed. ed. Oxford University Press.

Gonzalez, S., Rajadhyaksha, M., Rubinstein, G. and Anderson, R. R. 1999. Characterization of psoriasis in vivo by reflectance confocal microscopy. *J Med* 30(5-6), pp. 337-356.

Gordon, G. M., Ledee, D. R., Feuer, W. J. and Fini, M. E. 2009. Cytokines and signaling pathways regulating matrix metalloproteinase-9 (MMP-9) expression in corneal epithelial cells. *J Cell Physiol* 221(2), pp. 402-411.

Granick, M. S., Heckler, F. R. and Jones, E. W. 1987. Surgical skin-marking techniques. *Plast Reconstr Surg* 79(4), pp. 573-580.

Gratchev, A., Kzhyshkowska, J., Kannookadan, S., Ochsenreiter, M., Popova, A., Yu, X., Mamidi, S., Stonehouse-Usselmann, E., Muller-Molinet, I., Gooi, L. and Goerdt, S. 2008. Activation of a TGF-beta-specific multistep gene expression program in mature macrophages requires glucocorticoid-mediated surface expression of TGF-beta receptor II. *J Immunol* 180(10), pp. 6553-6565.

Gray, H. and Williams, P. L. 1974. *Gray's anatomy*. New York, USA: Running Press.

Greenwald, H. P. 1991. Interethnic differences in pain perception. *Pain* 44(2), pp. 157-163.

Grimstad, Ø., Sandanger, Ø., Ryan, L., Otterdal, K., Damaas, J. K., Pukstad, B. and Espevik, T. 2011. Cellular sources and inducers of cytokines present in acute wound fluid. *Wound Repair and Regeneration* 19(3), pp. 337-347.

Grob, D. and Mannion, A. F. 2009. The patient's perspective on complications after spine surgery. *Eur Spine J* 18 Suppl 3, pp. 380-385.

Grond, S., Radbruch, L. and Lehmann, K. A. 2000. Clinical pharmacokinetics of transdermal opioids: focus on transdermal fentanyl. *Clin Pharmacokinet* 38(1), pp. 59-89.

Groneberg, D. A., Bester, C., Grutzkau, A., Serowka, F., Fischer, A., Henz, B. M. and Welker, P. 2005. Mast cells and vasculature in atopic dermatitis--potential stimulus of neoangiogenesis. *Allergy* 60(1), pp. 90-97.

Grotle, M., Brox, J. I. and Vollestad, N. K. 2004. Concurrent comparison of responsiveness in pain and functional status measurements used for patients with low back pain. *Spine* 29(21), pp. E492-501.

Gruschwitz, M., Sepp, N., Kofler, H. and Wick, G. 1991. Expression of class II-MHC antigens in the dermis of patients with progressive systemic sclerosis. *Immunobiology* 182(3-4), pp. 234-255.

Gruson, L. M. and Cohen, D. E. 2004. Dermatomyositis. *Dermatol Online J* 10(3), p. 7.

Gubin, A. V., Ulrich, E. V., Taschilkin, A. I. and Yalfimov, A. N. 2009. Etiology of child acute stiff neck. *Spine (Phila Pa 1976)* 34(18), pp. 1906-1909.

Gupta, A. K., Baadsgaard, O., Ellis, C. N., Voorhees, J. J. and Cooper, K. D. 1989. Lymphocytes and macrophages of the epidermis and dermis in lesional psoriatic skin, but not epidermal Langerhans cells, are depleted by treatment with cyclosporin A. *Arch Dermatol Res* 281(4), pp. 219-226.

Gupta, J., Felner, E. I. and Prausnitz, M. R. 2009. Minimally invasive insulin delivery in subjects with type 1 diabetes using hollow microneedles. *Diabetes Technol Ther* 11(6), pp. 329-337.

Guy, R. H. and Hadgraft, J. 2003. *Transdermal drug delivery*. 2nd ed., rev. and expanded / edited by Richard H. Guy, Jonathan Hadgraft. ed. New York: M. Dekker, pp. viii, 383 p.

Guy, R. H., Hadgraft, J. and Bucks, D. A. W. 1987. Transdermal drug delivery and cutaneous metabolism. *Xenobiotica* 17(3), pp. 325-343.

Guyatt, G. H., Townsend, M., Berman, L. B. and Keller, J. L. 1987. A comparison of Likert and visual analogue scales for measuring change in function. *J Chronic Dis* 40(12), pp. 1129-1133.

Hackam, D. J. and Ford, H. R. 2002. Cellular, Biochemical, and Clinical Aspects of Wound Healing. *Surgical Infections* 3(s1), pp. s23-s35.

Hadgraft, J. 2001a. Modulation of the barrier function of the skin. *Skin Pharmacol Appl Skin Physiol* 14 Suppl 1, pp. 72-81.

Hadgraft, J. 2001b. Skin, the final frontier. *Int J Pharm* 224(1-2), pp. 1-18.

Hadgraft, J. and Lane, M. E. 2005. Skin permeation: The years of enlightenment. *International Journal of Pharmaceutics* 305(1-2), pp. 2-12.

Hadgraft, J. and Valenta, C. 2000. pH, pK(a) and dermal delivery. *Int J Pharm* 200(2), pp. 243-247.

Hafeli, U. O., Mokhtari, A., Liepmann, D. and Stoeber, B. 2009. In vivo evaluation of a microneedle-based miniature syringe for intradermal drug delivery. *Biomed Microdevices* [Epub ahead of print].

Hakvoort, T., Altun, V., van Zuijlen, P. P., de Boer, W. I., van Schadewij, W. A. and van der Kwast, T. H. 2000. Transforming growth factor-beta(1), -beta(2), -beta(3), basic fibroblast growth factor and vascular endothelial growth factor expression in keratinocytes of burn scars. *Eur Cytokine Netw* 11(2), pp. 233-239.

Hanas, R., Lytzen, L. and Ludvigsson, J. 2000. Thinner needles do not influence injection pain, insulin leakage or bleeding in children and adolescents with type 1 diabetes. *Pediatr Diabetes* 1(3), pp. 142-149.

Hanley, M. A., Ehde, D. M., Jensen, M., Czerniecki, J., Smith, D. G. and Robinson, L. R. 2009. Chronic pain associated with upper-limb loss. *Am J Phys Med Rehabil* 88(9), pp. 742-779.

Hantash, B. M., Renton, B., Berkowitz, R. L., Stridde, B. C. and Newman, J. 2009. Pilot clinical study of a novel minimally invasive bipolar microneedle radiofrequency device. *Lasers Surg Med* 41(2), pp. 87-95.

Harding, C. R. 2004. The stratum corneum: structure and function in health and disease. *Dermatol Ther* 17 Suppl 1, pp. 6-15.

Harding, G., Coyne, K., Barrett, R. J. and Pixton, G. C. 2009. Modified visual analog scale symptom-intensity and overall-bother measures for the assessment of symptoms in studies of pharmacologic stress agents. *Clin Ther* 31(4), pp. 889-901.

Hart, P. S. and Yanny, W. 1998. Needle phobia and malignant vasovagal syndrome. *Anaesthesia* 53(10), pp. 1002-1004.

Harvima, I. T., Viinamaki, H., Naukkarinen, A., Paukkonen, K., Neittaanmaki, H., Harvima, R. J. and Horsmanheimo, M. 1993. Association of cutaneous mast cells and sensory nerves with psychic stress in psoriasis. *Psychother Psychosom* 60(3-4), pp. 168-176.

Hays, P. L., Kawamura, S., Deng, X. H., Dagher, E., Mithoefer, K., Ying, L. and Rodeo, S. A. 2008. The role of macrophages in early healing of a tendon graft in a bone tunnel. *J Bone Joint Surg Am* 90(3), pp. 565-579.

Health, U. S. N. I. o. 2011. *ClinicalTrails.gov.* Available at: <URL: http://www.clinicaltrials.gov/ct2/results?term=microneedle> [Accessed: 1/2/2011].

Heisig, M., Lieckfeldt, R., Wittum, G., Mazurkevich, G. and Lee, G. 1996. Non steady-state descriptions of drug permeation through stratum corneum. I. The biphasic brick-and-mortar model. *Pharm Res* 13(3), pp. 421-426.

Henning, A., Neumann, D., Kostka, K. H., Lehr, C. M. and Schaefer, U. F. 2008. Influence of human skin specimens consisting of different skin layers on the result of in vitro permeation experiments. *Skin Pharmacol Physiol* 21(2), pp. 81-88.

Henry, S., McAllister, D. V., Allen, M. G. and Prausnitz, M. R. 1998. Microfabricated microneedles: a novel approach to transdermal drug delivery. *J Pharm Sci* 87(8), pp. 922-925.

Hertle, M. D., Jones, P. H., Groves, R. W., Hudson, D. L. and Watt, F. M. 1995. Integrin expression by human epidermal keratinocytes can be modulated by interferon-gamma, transforming growth factor-beta, tumor necrosis factor-alpha, and culture on a dermal equivalent. *J Invest Dermatol* 104(2), pp. 260-265.

Hesse, M., Franz, T., Tamai, Y., Taketo, M. M. and Magin, T. M. 2000. Targeted deletion of keratins 18 and 19 leads to trophoblast fragility and early embryonic lethality. *EMBO J* 19(19), pp. 5060-5070.

Hirsh, A. T., George, S. Z. and Robinson, M. E. 2009. Pain assessment and treatment disparities: a virtual human technology investigation. *Pain* 143(1-2), pp. 106-113.

HMSO 2004. The Medicines for Human Use (Clinical Trials) Regulations 2004. In: HMSO ed. *1031.* The Stationery Office Limited.

Ho, K., Spence, J. and Murphy, M. F. 1996. Review of Pain-Measurement Tools. *Annals of Emergency Medicine* 27(4), pp. 427-432.

Hogan, M. 2006. Problems in medical injection study. *Aids* 20(1), pp. 133-135.

Holbrook, K. A., Byers, P. H. and Pinnell, S. R. 1982. The structure and function of dermal connective tissue in normal individuals and patients with inherited connective tissue disorders. *Scan Electron Microsc* (Pt 4), pp. 1731-1744.

Holbrook, K. A. and Odland, G. F. 1974. Regional differences in the thickness (cell layers) of the human stratum corneum: an ultrastructural analysis. *J Invest Dermatol* 62(4), pp. 415-422.

Holbrook, K. A. and Odland, G. F. 1975. The fine structure of developing human epidermis: light, scanning, and transmission electron microscopy of the periderm. *J Invest Dermatol* 65(1), pp. 16-38.

Holtan, A. and Kongsgaard, U. E. 2009. The use of pain descriptors in cancer patients. *J Pain Symptom Manage* 38(2), pp. 208-215.

Hristakieva, E., Lazarova, R., Lazarov, N., Stanimirovic, A. and Shani, J. 2000. Markers for vitiligo related neuropeptides in human skin nerve fibers. *Acta Med Croatica* 54(2), pp. 53-57.

Huang, Y. B., Chang, J. S., Liu, J. C., Tsai, M. J., Tsai, Y. H. and Wu, P. C. 2004. The influence of anti-irritants on captopril hydrophilic gel. *Drug Dev Ind Pharm* 30(2), pp. 163-169.

Hussein, M. R., Ali, F. M. and Omar, A. E. 2007. Immunohistological analysis of immune cells in blistering skin lesions. *J Clin Pathol* 60(1), pp. 62-71.

Hutin, Y. J., Hauri, A. M. and Armstrong, G. L. 2003. Use of injections in healthcare settings worldwide, 2000: literature review and regional estimates. *BMJ* 327(7423), p. 1075.

Hwang, J. H., Wang, Y. N., Warren, C., Upton, M. P., Starr, F., Zhou, Y. and Mitchell, S. B. 2009. Preclinical in vivo evaluation of an extracorporeal HIFU device for ablation of pancreatic tumors. *Ultrasound Med Biol* 35(6), pp. 967-975.

Hwang, K. and Baik, S. H. 1997. Distribution of hairs and sweat glands on the bodies of Korean adults: a morphometric study. *Acta Anat (Basel)* 158(2), pp. 112-120.

Inan, M., Alkan, A., Harma, A. and Ertem, K. 2005. Evaluation of the gluteus medius muscle after a pelvic support osteotomy to treat congenital dislocation of the hip. *J Bone Joint Surg Am* 87(10), pp. 2246-2252.

Ipp, M., Parkin, P. C., Lear, N., Goldbach, M. and Taddio, A. 2009. Order of vaccine injection and infant pain response. *Arch Pediatr Adolesc Med* 163(5), pp. 469-472.

Ipp, M., Taddio, A., Sam, J., Gladbach, M. and Parkin, P. C. 2007. Vaccine-related pain: randomised controlled trial of two injection techniques. *Arch Dis Child,* pp. 1105-1108.

Ito, Y., Hagiwara, E., Saeki, A., Sugioka, N. and Takada, K. 2006a. Feasibility of microneedles for percutaneous absorption of insulin. *European Journal of Pharmaceutical Sciences* 29(1), pp. 82-88.

Ito, Y., Yoshimitsu, J., Shiroyama, K., Sugioka, N. and Takada, K. 2006b. Self-dissolving microneedles for the percutaneous absorption of EPO in mice. *J Drug Target* 14(5), pp. 255-261.

Ivanusic, J. J., Sahai, V. and Mahns, D. A. 2009. The cortical representation of sensory inputs arising from bone. *Brain Res* 1269, pp. 47-53.

Jagger, J., Hunt, E. H., Brand-Elnaggar, J. and Pearson, R. D. 1988. Rates of Needle-Stick Injury Caused by Various Devices in a University Hospital. *New England Journal of Medicine* 319(5), pp. 284-288.

Jamison, R. N. 1996. Psychological factors in chronic pain: assessment and treatment issues. *Journal of Back and Musculoskeletal Rehabilitation* 7, pp. 79-95.

Jamison, R. N., Gracely, R. H., Raymond, S. A., Levine, J. G., Marino, B., Herrmann, T. J., Daly, M., Fram, D. and Katz, N. P. 2002. Comparative study of electronic vs. paper VAS ratings: a randomized, crossover trial using healthy volunteers. *Pain* 99(1-2), pp. 341-347.

Jamison, R. N., Link, C. L. and Marceau, L. D. 2009. Do pain patients at high risk for substance misuse experience more pain? A longitudinal outcomes study. *Pain Med* 10(6), pp. 1084-1094.

Janjua, N. Z., Akhtar, S. and Hutin, Y. J. F. 2005. Injection use in two districts of Pakistan: implications for disease prevention. *Int J Qual Health Care,* pp. 401-408.

Jarvikallio, A., Harvima, I. T. and Naukkarinen, A. 2003. Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. *Arch Dermatol Res* 295(1), pp. 2-7.

Jeba, J., George, R., Thangakunam, B. and Christopher, D. J. 2009. Pain assessment and analgesic prescription for cancer patients in a medical ward: the influence of an educational intervention. *Natl Med J India* 22(4), pp. 177-180.

Jennings, P. A., Cameron, P. and Bernard, S. 2009. Measuring acute pain in the prehospital setting. *Emerg Med J* 26(8), pp. 552-555.

Jensen, J. M. and Proksch, E. 2009. The skin's barrier. *G Ital Dermatol Venereol* 144(6), pp. 689-700.

Jensen, M. P., Karoly, P., O'Riordan, E. F., Bland, F., Jr. and Burns, R. S. 1989. The subjective experience of acute pain. An assessment of the utility of 10 indices. *Clin J Pain* 5(2), pp. 153-159.

Jeremy, A. H., Holland, D. B., Roberts, S. G., Thomson, K. F. and Cunliffe, W. J. 2003. Inflammatory events are involved in acne lesion initiation. *J Invest Dermatol* 121(1), pp. 20-27.

Jin, C., Han, M., Lee, S. and Choi, Y. 2009. Mass producible and biocompatible microneedle patch and functional verification of its usefulness for transdermal drug delivery. *Biomedical Microdevices* 11(6), pp. 1195-1203.

Jin, H., Xiao, C., Geng, S., Hu, Y., She, R., Yu, Y., Kang, Y. and Wang, B. 2008. Protein/DNA vaccine-induced antigen-specific Treg confer protection against asthma. *Eur J Immunol* 38(9), pp. 2451-2463.

Johnson, M. E., Blankschtein, D. and Langer, R. 1997. Evaluation of solute permeation through the stratum corneum: Lateral bilayer diffusion as the primary transport mechanism. *Journal of Pharmaceutical Sciences* 86(10), pp. 1162-1172.

Johnson, P. G., Gallo, S. A., Hui, S. W. and Oseroff, A. R. 1998. A pulsed electric field enhances cutaneous delivery of methylene blue in excised full-thickness porcine skin. *J Invest Dermatol* 111(3), pp. 457-463.

Kalluri, H. and Banga, A. K. 2011. Formation and closure of microchannels in skin following microporation. *Pharm Res* 28(1), pp. 82-94.

Kane, A., Lloyd, J., Zaffran, M., Simonsen, L. and Kane, M. 1999. Transmission of hepatitis B, hepatitis C and human immunodeficiency viruses through unsafe injections in the developing world: model-based regional estimates. *Bull World Health Organ* 77(10), pp. 801-807.

Karande, P., Jain, A., Ergun, K., Kispersky, V. and Mitragotri, S. 2005. Design principles of chemical penetration enhancers for transdermal drug delivery. *Proc Natl Acad Sci USA* 102(13), pp. 4688-4693.

Katikaneni, S., Badkar, A., Nema, S. and Banga, A. K. 2009. Molecular charge mediated transport of a 13kD protein across microporated skin. *Int J Pharm.*

Katz, I., Schneider, H., Shezi, Z., Mdleleni, G., Gerntholtz, T., Butler, O., Manderson, L. and Naicker, S. 2009. Managing type 2 diabetes in Soweto-The South African Chronic Disease Outreach Program experience. *Prim Care Diabetes* 3(3), pp. 157-164.

Katz, J. and Melzack, R. 1999. Measurement of pain. *Surg Clin North Am* 79(2), pp. 231-252.

Kaushik, S., Hord, A. H., Denson, D. D., McAllister, D. V., Smitra, S., Allen, M. G. and Prausnitz, M. R. 2001. Lack of Pain Associated with Microfabricated Microneedles. *Anesth Analg,* pp. 502-504.

Kelly, A. M. 2001. The minimum clinically significant difference in visual analogue scale pain score does not differ with severity of pain. *Emerg Med J,* pp. 205-207.

Kermode, M. 2004. Unsafe injections in low-income country health settings: need for injection safety promotion to prevent the spread of blood-borne viruses. *Health Promot Int* 19(1), pp. 95-103.

Kettwich, S. C., Sibbitt, W. L., Jr., Brandt, J. R., Johnson, C. R., Wong, C. S. and Bankhurst, A. D. 2007. Needle Phobia and Stress-Reducing Medical Devices in Pediatric and Adult Chemotherapy Patients. *J Pediatr Oncol Nurs,* pp. 20-28.

Khan, W. A., Park, S. S., Gelboin, H. V., Bickers, D. R. and Mukhtar, H. 1989. Epidermal cytochrome P-450: immunochemical characterization of isoform induced by topical application of 3-methylcholanthrene to neonatal rat. *J Pharmacol Exp Ther* 249(3), pp. 921-927.

Kim, C. S., Wilder-Smith, P., Ahn, Y. C., Liaw, L. H., Chen, Z. and Kwon, Y. J. 2009a. Enhanced detection of early-stage oral cancer in vivo by optical coherence tomography using multimodal delivery of gold nanoparticles. *J Biomed Opt* 14(3), p. 034008.

Kim, H. and Colton, J. S. 2005. Fabrication and analysis of plastic hypodermic needles. *J Med Eng Technol* 29(4), pp. 181-186.

Kim, M. H., Liu, W., Borjesson, D. L., Curry, F. R., Miller, L. S., Cheung, A. L., Liu, F. T., Isseroff, R. R. and Simon, S. I. 2008. Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging. *The Journal of investigative dermatology* 128(7), pp. 1812-1820.

Kim, Y.-C., Quan, F.-S., Yoo, D.-G., Compans, R. W., Kang, S.-M. and Prausnitz, M. R. 2009b. Improved influenza vaccination in the skin using vaccine coated microneedles. *Vaccine* 27(49), pp. 6932-6938.

Kiritsy, C. P. and Lynch, S. E. 1993. Role of Growth Factors in Cutaneous Wound Healing: A Review. *Critical Reviews in Oral Biology & Medicine* 4(5), pp. 729-760.

Kirschner, N., Bohner, C., Rachow, S. and Brandner, J. M. 2010. Tight junctions: is there a role in dermatology? *Arch Dermatol Res* 302(7), pp. 483-493.

Kleinknecht, R. A. 1994. Acquisition of blood, injury, and needle fears and phobias. *Behav Res Ther* 32(8), pp. 817-823.

Kligman, A. M. 1984. Skin permeability: dermatologic aspects of transdermal drug delivery. *Am Heart J* 108(1), pp. 200-206.

Knepp, V. M., Hadgraft, J. and Guy, R. H. 1987. Transdermal drug delivery: problems and possibilities. *Crit Rev Ther Drug Carrier Syst* 4(1), pp. 13-37.

Knip, M. 2008. Should we screen for risk of type 1 diabetes? *Diabetes Care* 31(3), pp. 622-623.

Ko, M. S. and Marinkovich, M. P. 2010. Role of dermal-epidermal basement membrane zone in skin, cancer, and developmental disorders. *Dermatol Clin* 28(1), pp. 1-16.

Kobayashi, Y., Kurata, J., Sekiguchi, M., Kokubun, M., Akaishizawa, T., Chiba, Y., Konno, S. and Kikuchi, S. 2009. Augmented cerebral activation by lumbar mechanical stimulus in chronic low back pain patients: an FMRI study. *Spine (Phila Pa 1976)* 34(22), pp. 2431-2436.

Koch, S., Kohl, K., Klein, E., von Bubnoff, D. and Bieber, T. 2006. Skin homing of Langerhans cell precursors: Adhesion, chemotaxis, and migration. *Journal of Allergy and Clinical Immunology* 117(1), pp. 163-168.

Kolli, C. S. and Banga, A. K. 2008. Characterization of Solid Maltose Microneedles and their Use for Transdermal Delivery. *Pharm Res*, pp. 104-113.

Kondo, T. 2007. Timing of skin wounds. *Legal Medicine* 9(2), pp. 109-114.

Koutsonanos, D. G., del Pilar Martin, M., Zarnitsyn, V. G., Sullivan, S. P., Compans, R. W., Prausnitz, M. R. and Skountzou, I. 2009. Transdermal influenza immunization with vaccine-coated microneedle arrays. *PLoS One* 4(3), p. e4773.

Kraemer, H. C., Glick, I. D. and Klein, D. F. 2009. Clinical Trials Design Lessons From the CATIE Study. *Am J Psychiatry* 166(11), pp. 1222-1228.

Kubo, A., Nagao, K., Yokouchi, M., Sasaki, H. and Amagai, M. 2009. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. *J Exp Med* 206(13), pp. 2937-2946.

Kurban, R. S. and Bhawan, J. 1990. Histologic changes in skin associated with aging. *J Dermatol Surg Oncol* 16(10), pp. 908-914.

Lademann, J., Otberg, N., Jacobi, U., Hoffman, R. M. and Blume-Peytavi, U. 2005. Follicular penetration and targeting. *J Investig Dermatol Symp Proc* 10(3), pp. 301-303.

LaDuca, J. R. and Gaspari, A. A. 2001. Targeting tumor necrosis factor alpha. New drugs used to modulate inflammatory diseases. *Dermatol Clin* 19(4), pp. 617-635.

Lane, B. R., Ausmundson, S. J., Butler, R. S., Zippe, C. D., Jones, J. S. and Lakin, M. M. 2005. Use of a visual analog scale to assess pain of injection with intracavernous injection therapy. *J Sex Med* 2(3), pp. 428-431.

Lanke, S. S. S., Kolli, C. S., Strom, J. G. and Banga, A. K. 2009. Enhanced transdermal delivery of low molecular weight heparin by barrier perturbation. *International Journal of Pharmaceutics* 365(1-2), pp. 26-33.

Larjava, H., Salo, T., Haapasalmi, K., Kramer, R. H. and Heino, J. 1993. Expression of integrins and basement membrane components by wound keratinocytes. *J Clin Invest* 92(3), pp. 1425-1435.

Latkin, C. A., Buchanan, A. S., Metsch, L. R., Knight, K., Latka, M. H., Mizuno, Y. and Knowlton, A. R. 2008. Predictors of Sharing Injection Equipment by HIV-Seropositive Injection Drug Users. *J Acquir Immune Defic Syndr*, pp. 447-450.

Laurent, P. E., Bonnet, S., Alchas, P., Regolini, P., Mikszta, J. A., Pettis, R. and Harvey, N. G. 2007. Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system. *Vaccine* 25(52), pp. 8833-8842.

Lawes, C., Sawyer, L., Amos, S., Kandiah, M., Pearce, L. and Symons, J. 2008. Impact of an education programme for staff working with children undergoing painful procedures. *Paediatr Nurs* 20(2), pp. 33-37.

Lawrence, D. A. 1996. Transforming growth factor-beta: a general review. *Eur Cytokine Netw* 7(3), pp. 363-374.

Lazarova, R., Hristakieva, E., Lazarov, N. and Shani, J. 2000. Vitiligo-related neuropeptides in nerve fibers of the skin. *Arch Physiol Biochem* 108(3), pp. 262-267.

Lee, J. W., Park, J. H. and Prausnitz, M. R. 2008a. Dissolving microneedles for transdermal drug delivery. *Biomaterials* 29(13), pp. 2113-2124.

Lee, K., Lee, C. Y. and Jung, H. 2011. Dissolving microneedles for transdermal drug administration prepared by stepwise controlled drawing of maltose. *Biomaterials* 32(11), pp. 3134-3140.

Lee, P. W., Peng, S. F., Su, C. J., Mi, F. L., Chen, H. L., Wei, M. C., Lin, H. J. and Sung, H. W. 2008b. The use of biodegradable polymeric nanoparticles in combination with a low-pressure gene gun for transdermal DNA delivery. *Biomaterials* 29(6), pp. 742-751.

Lee, S., Jeong, W. and Beebe, D. J. 2003. Microfluidic valve with cored glass microneedle for microinjection. *Lab Chip* 3(3), pp. 164-167.

Lee, S. E., Choi, K. J., Menon, G. K., Kim, H. J., Choi, E. H., Ahn, S. K. and Lee, S. H. 2009. Penetration Pathways Induced by Low-Frequency Sonophoresis with Physical and Chemical Enhancers: Iron Oxide Nanoparticles versus Lanthanum Nitrates. *J Invest Dermatol* 130(4), pp.1063-1072.

Leonard, J. H., Choo, C. P., Manaf, M. R., Md Isa, Z., Mohd Nordin, N. A. and Das, S. 2009. Development and evaluation of 'neck pain and functional limitation scale': A validation study in the Asian context. *Indian J Med Sci* 63(10), pp. 445-454.

Leppert, W. 2009. Tramadol as an analgesic for mild to moderate cancer pain. *Pharmacol Rep* 61(6), pp. 978-992.

Levi, K., Weber, R. J., Do, J. Q. and Dauskardt, R. H. 2009. Drying stress and damage processes in human stratum corneum. *Int J Cosmet Sci* 32(4), pp. 276-293.

Levin, G., Gershonowitz, A., Sacks, H., Stern, M., Sherman, A., Rudaev, S., Zivin, I. and Phillip, M. 2005. Transdermal Delivery of Human Growth Hormone Through RF-Microchannels. *Pharmaceutical Research* 22(4), pp. 550-555.

Lewandowski, A. S., Palermo, T. M., Kirchner, H. L. and Drotar, D. 2009. Comparing diary and retrospective reports of pain and activity restriction in children and adolescents with chronic pain conditions. *Clin J Pain* 25(4), pp. 299-306.

Li, G., Badkar, A., Kalluri, H. and Banga, A. K. 2010. Microchannels created by sugar and metal microneedles: characterization by microscopy, macromolecular flux and other techniques. *J Pharm Sci* 99(4), pp. 1931-1941.

Li, G., Badkar, A., Nema, S., Kolli, C. S. and Banga, A. K. 2008. In vitro transdermal delivery of therapeutic antibodies using maltose microneedles. *Int J Pharm* 19, p. 19.

Li, G., Badkar, A., Nema, S., Kolli, C. S. and Banga, A. K. 2009. In vitro transdermal delivery of therapeutic antibodies using maltose microneedles. *Int J Pharm* 368(1-2), pp. 109-115.

Li, J., Chen, J. and Kirsner, R. 2007. Pathophysiology of acute wound healing. *Clin Dermatol* 25(1), pp. 9-18.

Li, W., Dasgeb, B., Phillips, T., Li, Y., Chen, M., Garner, W. and Woodley, D. T. 2005. Wound-healing perspectives. *Dermatol Clin* 23(2), pp. 181-192.

Lin, J. Y. and Fisher, D. E. 2007. Melanocyte biology and skin pigmentation. *Nature* 445(7130), pp. 843-850.

Lindberger, M., Schroder, H. D., Schultzberg, M., Kristensson, K., Persson, A., Ostman, J. and Link, H. 1989. Nerve fibre studies in skin biopsies in peripheral neuropathies. I. Immunohistochemical analysis of neuropeptides in diabetes mellitus. *J Neurol Sci* 93(2-3), pp. 289-296.

Little. 1990. *Methylene Blue.* National Toxicology Program. Available at: <URL: http://ntp.niehs.nih.gov/go/8170> [Accessed: 22/02/2011].

Liu, Y. 2006. Performance studies of particle acceleration for transdermal drug delivery. *Med Biol Eng Comput* 44(7), pp. 551-559.

Loden, M. 2003. Role of topical emollients and moisturizers in the treatment of dry skin barrier disorders. *Am J Clin Dermatol* 4(11), pp. 771-788.

Lundqvist, C., Benth, J. S., Grande, R. B., Aaseth, K. and Russell, M. B. 2009. A vertical VAS is a valid instrument for monitoring headache pain intensity. *Cephalalgia* 29(10), pp. 1034-1041.

Macmillan, A. K., Merrie, A. E., Marshall, R. J. and Parry, B. R. 2008. Design and validation of a comprehensive fecal incontinence questionnaire. *Dis Colon Rectum* 51(10), pp. 1502-1522.

Madison, K. C. 2003. Barrier function of the skin: "la raison d'etre" of the epidermis. *J Invest Dermatol* 121(2), pp. 231-241.

Madou, M. J. 2001. *Fundamentals of microfabrication : the science of miniaturization.* 2nd ed. ed. Boca Raton, FL: CRC Press.

Maikler, V. E. 1991. Effects of a skin refrigerant/anesthetic and age on the pain responses of infants receiving immunizations. *Res Nurs Health* 14(6), pp. 397-403.

Mancini, A. J. 2004. Skin. *Pediatrics* 113(4 Suppl), pp. 1114-1119.

Margolis, R. B., Tait, R. C. and Krause, S. J. 1986. A rating system for use with patient pain drawings. *Pain* 24(1), pp. 57-65.

Marilyn, G. 2002. Clinical problems in diabetes/European study of insulin injections. *Pract Diabetes Int* 19, p. 149.

Marks, I. 1988. Blood-injury phobia: a review. *Am J Psychiatry* 145(10), pp. 1207-1213.

Martanto, W., Davis, S. P., Holiday, N. R., Wang, J., Gill, H. S. and Prausnitz, M. R. 2004. Transdermal delivery of insulin using microneedles in vivo. *Pharm Res* 21(6), pp. 947-952.

Martanto, W., Moore, J. S., Kashlan, O., Kamath, R., Wang, P. M., O'Neal, J. M. and Prausnitz, M. R. 2006. Microinfusion using hollow microneedles. *Pharm Res* 23(1), pp. 104-113.

Martin, P. 1997. Wound healing--aiming for perfect skin regeneration. *Science* 276(5309), pp. 75-81.

Martin, P. and Leibovich, S. J. 2005. Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends Cell Biol* 15(11), pp. 599-607.

Martinez-Ferrer, M., Afshar-Sherif, A. R., Uwamariya, C., de Crombrugghe, B., Davidson, J. M. and Bhowmick, N. A. 2009. Dermal transforming growth factor-beta responsiveness mediates wound contraction and epithelial closure. *Am J Pathol* 176(1), pp. 98-107.

Mathur, V., Satrawala, Y. and Rajput, M. 2010. Physical and chemical penetration enhancers in transdermal drug delivery system. *Asian J Pharm* 4, pp. 173-183.

Mazer, N., Bell, D., Wu, J., Fischer, J., Cosgrove, M. and Eilers, B. 2005. Comparison of the steady-state pharmacokinetics, metabolism, and variability of a transdermal testosterone patch versus a transdermal testosterone gel in hypogonadal men. *J Sex Med* 2(2), pp. 213-226.

McAllister, D. V., Allen, M. G. and Prausnitz, M. R. 2000. Microfabricated microneedles for gene and drug delivery. *Annu Rev Biomed Eng* 2, pp. 289-313.

McAllister, D. V., Wang, P. M., Davis, S. P., Park, J. H., Canatella, P. J., Allen, M. G. and Prausnitz, M. R. 2003. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. *Proc Natl Acad Sci U S A* 100(24), pp. 13755-13760.

McCrory, C. and Fitzgerald, D. 2004. Spinal prostaglandin formation and pain perception following thoracotomy: a role for cyclooxygenase-2. *Chest* 125(4), pp. 1321-1327.

McGowan, K. M. and Coulombe, P. A. 1998. Onset of keratin 17 expression coincides with the definition of major epithelial lineages during skin development. *Journal of Cell Biology* 143(2), pp. 469-486.

McLellan, A. D., Heiser, A., Sorg, R. V., Fearnley, D. B. and Hart, D. N. J. 1998. Dermal Dendritic Cells Associated with T Lymphocytes in Normal Human Skin Display an Activated Phenotype. 111(5), pp. 841-849.

McNeil, J. J., Piccenna, L. and Ioannides-Demos, L. L. 2010. Smoking cessation-recent advances. *Cardiovasc Drugs Ther* 24(4), pp. 359-367.

Meijer-Jorna, L. B., Mekkes, J. R. and van der Wal, A. C. 2002. Platelet involvement in cutaneous small vessel vasculitis. *J Cutan Pathol* 29(3), pp. 176-180.

Meit, S. S., Yasek, V. A. N., Shannon, C. K., Hickman, D. and Williams, D. 2004. Techniques for reducing anesthetic injection pain: An interdisciplinary survey of knowledge and application. *J Am Dent Assoc* 135(9), pp. 1243-1250.

Melzack, R. 1975. The McGill Pain Questionnaire: Major properties and scoring methods. *Pain* 1(3), pp. 277-299.

Melzack, R. 1987. The short-form McGill Pain Questionnaire. *Pain* 30(2), pp. 191-197.

Melzack, R. 2005. The McGill pain questionnaire: from description to measurement. *Anesthesiology* 103(1), pp. 199-202.

Melzack, R., Katz, J. 2001. The McGill Pain Questionnaire: Appraisal and Current Status. In: Turk, D.C., Ronald Melzack ed. *Handbook of Pain Assessement*. The Guilford Press, pp. 35-52.

Melzack R, K. J. 1994. Pain measurement in persons in pain. In: Wall P D, M.R. ed. *Textbook of pain*. 3rd ed. Churchill Livingstone, New York, pp. 337–351.

Merz, K. M., Pfau, M., Blumenstock, G., Tenenhaus, M., Schaller, H. E. and Rennekampff, H. O. 2010. Cutaneous microcirculatory assessment of the burn wound is associated with depth of injury and predicts healing time. *Burns* 36(4), pp. 477-482.

Meunier, L., Bata-Csorgo, Z. and Cooper, K. D. 1995. In human dermis, ultraviolet radiation induces expansion of a CD36+ CD11b+ CD1- macrophage subset by infiltration and proliferation; CD1+ Langerhans-like dendritic antigen-presenting cells are concomitantly depleted. *J Invest Dermatol* 105(6), pp. 782-788.

MHRA, 2007, *Clinical trial authorisations: Is a clinical trial authorisation required?* Available at: <URL: http://www.mhra.gov.uk/Howweregulate/Medicines/Licensingofmedicines/Clinicaltrials/Isaclinicaltrialauthorisationrequired/index.htm> [Accessed 15/01/2009.]

Michaels, A. S., Chandrasekaran, S. K. and Shaw, J. E. 1975. Drug permeation through human skin: Theory and invitro experimental measurement. *AIChE Journal* 21(5), pp. 985-996.

Midwood, K. S., Williams, L. V. and Schwarzbauer, J. E. 2004. Tissue repair and the dynamics of the extracellular matrix. *Int J Biochem Cell Biol* 36(6), pp. 1031-1037.

Mikolajewska, P., Donnelly, R. F., Garland, M. J., Morrow, D. I., Singh, T. R., Iani, V., Moan, J. and Juzeniene, A. 2010. Microneedle pre-treatment of human skin improves 5-aminolevulininc acid (ALA)- and 5-aminolevulinic acid

methyl ester (MAL)-induced PpIX production for topical photodynamic therapy without increase in pain or erythema. *Pharm Res* 27(10), pp. 2213-2220.

Milgrom, P., Coldwell, S. E., Getz, T., Weinstein, P. and Ramsay, D. S. 1997. Four dimensions of fear of dental injections. *J Am Dent Assoc* 128(6), pp. 756-766.

Miller, M. C. and Nanchahal, J. 2005. Advances in the modulation of cutaneous wound healing and scarring. *BioDrugs* 19(6), pp. 363-381.

Mine, S., Fortunel, N. O., Pageon, H. and Asselineau, D. 2008. Aging alters functionally human dermal papillary fibroblasts but not reticular fibroblasts: a new view of skin morphogenesis and aging. *PLoS One* 3(12), p. e4066.

Miteva, M., Richter, S., Elsner, P. and Fluhr, J. W. 2006. Approaches for optimizing the calibration standard of Tewameter TM 300. *Exp Dermatol* 15(11), pp. 904-912.

Miyano, T., Tobinaga, Y., Kanno, T., Matsuzaki, Y., Takeda, H., Wakui, M. and Hanada, K. 2005. Sugar micro needles as transdermic drug delivery system. *Biomed Microdevices* 7(3), pp. 185-188.

Moll, R., Franke, W. W., Schiller, D. L., Geiger, B. and Krepler, R. 1982. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 31(1), pp. 11-24.

Montagna, W. and Carlisle, K. 1979. Structural changes in aging human skin. *J Invest Dermatol* 73(1), pp. 47-53.

Montagna, W., Kligman, A. M. and Carlisle, K. S. 1992. *Atlas of normal human skin*. Springer-Verlag.

Montagna, W. and Parakkal, P. F. 1974. *The structure and function of skin*. 3rd ed. ed. New York ; London: Academic Press.

Montgomery, B. S., Borwell, J. P. and Higgins, D. M. 2005. Does needle size matter? Patient experience of luteinising hormone-releasing hormone analogue injection. *Prostate Cancer Prostatic Dis* 8(1), pp. 66-68.

Moore, K. T. 2009. X-ray and electron microscopy of actinide materials. *Micron* 41(4), pp. 336-358.

Moore, S. J. and Munger, B. L. 1989. The early ontogeny of the afferent nerves and papillary ridges in human digital glabrous skin. *Brain Res Dev Brain Res* 48(1), pp. 119-141.

Mordon, S., Sumian, C. and Devoisselle, J. M. 2003. Site-specific methylene blue delivery to pilosebaceous structures using highly porous nylon microspheres: An experimental evaluation. *Lasers in Surgery and Medicine* 33(2), pp. 119-125.

Morgan, C. J., Renwick, A. G. and Friedmann, P. S. 2003. The role of stratum corneum and dermal microvascular perfusion in penetration and tissue levels of water-soluble drugs investigated by microdialysis. *Br J Dermatol* 148(3), pp. 434-443.

Morita, K. and Miyachi, Y. 2003. Tight junctions in the skin. *J Dermatol Sci* 31(2), pp. 81-89.

Moser, K., Kriwet, K., Naik, A., Kalia, Y. N. and Guy, R. H. 2001. Passive skin penetration enhancement and its quantification in vitro. *European Journal of Pharmaceutics and Biopharmaceutics* 52(2), pp. 103-112.

Munyard, P., Busst, C., Logan-Sinclair, R. and Bush, A. 1994. A new device for ambulatory cough recording. *Pediatr Pulmonol* 18(3), pp. 178-186.

Myers, S. R., Leigh, I. M. and Navsaria, H. 2007. Epidermal repair results from activation of follicular and epidermal progenitor keratinocytes mediated by a growth factor cascade. *Wound Repair Regen* 15(5), pp. 693-701.

Mystakidou, K., Parpa, E., Tsilika, E., Kalaidopoulou, O., Georgaki, S., Galanos, A. and Vlahos, L. 2002. Greek McGill Pain Questionnaire: Validation and Utility in Cancer Patients. *Journal of Pain and Symptom Management* 24(4), pp. 379-387.

Naik, A., Kalia, Y. N. and Guy, R. H. 2000. Transdermal drug delivery: overcoming the skin's barrier function. *Pharmaceutical Science & Technology Today* 3(9), pp. 318-326.

Nandedkar, T. D. 2009. Nanovaccines: recent developments in vaccination. *J Biosci* 34(6), pp. 995-1003.

Ndinya-achola, J. O., Plummer, F. A., Ronald, A. R. and Piot, P. 1986. Acquired immunodeficiency syndrome: epidemiology in Africa and its implications for health services. *Afr J Sex Transmi Dis* 2(2), pp. 77-80.

Nedelec, B., Ghahary, A., Scott, P. G. and Tredget, E. E. 2000. Control of wound contraction. Basic and clinical features. *Hand Clin* 16(2), pp. 289-302.

Netzlaff, F., Kostka, K.-H., Lehr, C.-M. and Schaefer, U. F. 2006. TEWL measurements as a routine method for evaluating the integrity of epidermis sheets in static Franz type diffusion cells in vitro. Limitations shown by transport data testing. *European Journal of Pharmaceutics and Biopharmaceutics* 63(1), pp. 44-50.

Newman, P. A., Duan, N., Rudy, E. T. and Johnston-Roberts, K. 2004. HIV risk and prevention in a post-vaccine context. *Vaccine* 22(15-16), pp. 1954-1963.

NHS. 2008. *Routine childhoood immunisation programme.* Available at: <URL: http://www.immunisation.nhs.uk/files/schedule_oct08.pdf> [Accessed: 20/4/2010].

Nijrolder, I., van der Windt, D. and van der Horst, H. 2009. Prediction of outcome in patients presenting with fatigue in primary care. *Br J Gen Pract* 59(561), pp. e101-109.

Nir, Y., Paz, A., Sabo, E. and Potasman, I. 2003. Fear of injections in young adults: prevalence and associations. *Am J Trop Med Hyg* 68(3), pp. 341-344.

Nishio, N., Ito, S., Suzuki, H. and Isobe, K. 2009. Antibodies to wounded tissue enhance cutaneous wound healing. *Immunology* 128(3), pp. 369-380.

Noori, A., Selvaganapathy, P. R. and Wilson, J. 2009. Microinjection in a microfluidic format using flexible and compliant channels and electroosmotic dosage control. *Lab Chip* 9(22), pp. 3202-3211.

Nordquist, L., Roxhed, N., Griss, P. and Stemme, G. 2007. Novel Microneedle Patches for Active Insulin Delivery are Efficient in Maintaining Glycaemic Control: An Initial Comparison with Subcutaneous Administration. *Pharm Res* 24(7), pp. 1381-1388.

Nouveau-Richard, S., Monot, M., Bastien, P. and de Lacharriere, O. 2004. In vivo epidermal thickness measurement: ultrasound vs. confocal imaging. *Skin Res Technol* 10(2), pp. 136-140.

Nuutinen, J., Alanen, E., Autio, P., Lahtinen, M.-R., Harvima, I. and Lahtinen, T. 2003. A closed unventilated chamber for the measurement of transepidermal water loss. *Skin Res Technol* 9(2), pp. 85-89.

Oh, J. H., Park, H. H., Do, K. Y., Han, M., Hyun, D. H., Kim, C. G., Kim, C. H., Lee, S. S., Hwang, S. J., Shin, S. C. and Cho, C. W. 2008. Influence of the delivery systems using a microneedle array on the permeation of a hydrophilic molecule, calcein. *Eur J Pharm Biopharm* 69(3), pp. 1040-1045.

Okawa, K., Ichinohe, T. and Kaneko, Y. 2005. Anxiety may enhance pain during dental treatment. *Bull Tokyo Dent Coll* 46(3), pp. 51-58.

Ost, L. G. 1992. Blood and injection phobia: background and cognitive, physiological, and behavioral variables. *J Abnorm Psychol* 101(1), pp. 68-74.

Paladini, R. D., Takahashi, K., Bravo, N. S. and Coulombe, P. A. 1996. Onset of re-epithelialization after skin injury correlates with a reorganization of keratin filaments in wound edge keratinocytes: defining a potential role for keratin 16. *Journal of Cell Biology* 132(3), pp. 381-397.

Panchagnula, R., Bokalial, R., Sharma, P. and Khandavilli, S. 2005. Transdermal delivery of naloxone: skin permeation, pharmacokinetic, irritancy and stability studies. *Int J Pharm* 293(1-2), pp. 213-223.

Pandit, N. B. and Choudhary, S. K. 2008. Unsafe injection practices in Gujarat, India. *Singapore Med J* 49(11), pp. 936-939.

Papanastasiou, S. and Hart, N. B. 1995. Multiple uses of the hypodermic needle. *British Journal of Plastic Surgery* 48(5), pp. 338-338.

Pappas, A. 2009. Epidermal surface lipids. *Dermatoendocrinol* 1(2), pp. 72-76.

Park, J. H., Allen, M. G. and Prausnitz, M. R. 2005. Biodegradable polymer microneedles: fabrication, mechanics and transdermal drug delivery. *J Control Release* 104(1), pp. 51-66.

Park, J. H., Allen, M. G. and Prausnitz, M. R. 2006. Polymer microneedles for controlled-release drug delivery. *Pharm Res* 23(5), pp. 1008-1019.

Park, S. K., Cho, M. K., Park, H. K., Lee, K. H., Lee, S. J., Choi, S. H., Ock, M. S., Jeong, H. J., Lee, M. H. and Yu, H. S. 2009. Macrophage migration inhibitory factor homologs of anisakis simplex suppress Th2 response in allergic airway inflammation model via CD4+CD25+Foxp3+ T cell recruitment. *J Immunol* 182(11), pp. 6907-6914.

Parker, H., Wood, P. L. and Main, C. J. 1995. The use of the pain drawing as a screening measure to predict psychological distress in chronic low back pain. *Spine* 20(2), pp. 236-243.

Patel, G. K., Wilson, C. H., Harding, K. G., Finlay, A. Y. and Bowden, P. E. 2005. Numerous Keratinocyte Subtypes Involved in Wound Re-Epithelialization. *J Invest Dermatol* 126(2), pp. 497-502.

Pearton, M., Allender, C., Brain, K., Anstey, A., Gateley, C., Wilke, N., Morrissey, A. and Birchall, J. 2007. Gene Delivery to the Epidermal Cells of Human Skin Explants Using Microfabricated Microneedles and Hydrogel Formulations. *Pharm Res* 25(2), pp. 407-416.

Pearton, M., Allender, C., Brain, K., Anstey, A., Gateley, C., Wilke, N., Morrissey, A. and Birchall, J. 2008. Gene delivery to the epidermal cells of human skin explants using microfabricated microneedles and hydrogel formulations. *Pharm Res* 25(2), pp. 407-416.

Pearton, M., Kang, S.-M., Song, J.-M., Kim, Y.-C., Quan, F.-S., Anstey, A., Ivory, M., Prausnitz, M. R., Compans, R. W. and Birchall, J. C. 2010. Influenza virus-like particles coated onto microneedles can elicit stimulatory effects on Langerhans cells in human skin. *Vaccine* 28(37), pp. 6104-6113.

Pedersen, L. and Jemec, G. B. 2006. Mechanical properties and barrier function of healthy human skin. *Acta Derm Venereol* 86(4), pp. 308-311.

Perri, R., Wollin, S., Drolet, N., Mai, S., Awad, M. and Feine, J. 2006. Monitoring recruitment success and cost in a randomized clinical trial. *Eur J Prosthodont Restor Dent* 14(3), pp. 126-130.

Philipp, K., Riedel, F., Sauerbier, M., Hormann, K. and Germann, G. 2004. Targeting TGF-beta in human keratinocytes and its potential role in wound healing. *Int J Mol Med* 14(4), pp. 589-593.

Pickard, C., Louafi, F., McGuire, C., Lowings, K., Kumar, P., Cooper, H., Dearman, R. J., Cumberbatch, M., Kimber, I., Healy, E. and Friedmann, P. S. 2009. The cutaneous biochemical redox barrier: a component of the innate immune defenses against sensitization by highly reactive environmental xenobiotics. *J Immunol* 183(11), pp. 7576-7584.

Pierard, G. E., Goffin, V., Hermanns-Le, T. and Pierard-Franchimont, C. 2000. Corneocyte desquamation. *Int J Mol Med* 6(2), pp. 217-221.

Pinnagoda, J., Tupkek, R. A., Agner, T. and Serup, J. 1990. Guidelines for transepidermal water loss (TEWL) measurement. *Contact Dermatitis* 22(3), pp. 164-178.

Poindexter, B. J., Bhat, S., Buja, L. M., Bick, R. J. and Milner, S. M. 2006. Localization of antimicrobial peptides in normal and burned skin. *Burns* 32(4), pp. 402-407.

Potts, R. O. and Francoeur, M. L. 1991. The influence of stratum corneum morphology on water permeability. *J Invest Dermatol* 96(4), pp. 495-499.

Pradhan, L., Cai, X., Wu, S., Andersen, N. D., Martin, M., Malek, J., Guthrie, P., Veves, A. and Logerfo, F. W. 2009. Gene Expression of Pro-Inflammatory Cytokines and Neuropeptides in Diabetic Wound Healing. *J Surg Res* 167(2), pp. 336-342.

Prausnitz, M. R. 2004. Microneedles for transdermal drug delivery. *Adv Drug Deliv Rev* 56(5), pp. 581-587.

Prausnitz, M. R. and Langer, R. 2008. Transdermal drug delivery. *Nat Biotech* 26(11), pp. 1261-1268.

Prausnitz, M. R., Mikszta, J. A., Cormier, M. and Andrianov, A. K. 2009. Microneedle-based vaccines. *Curr Top Microbiol Immunol* 333, pp. 369-393.

Price, D. D., Bush, F. M., Long, S. and Harkins, S. W. 1994. A comparison of pain measurement characteristics of mechanical visual analogue and simple numerical rating scales. *Pain* 56(2), pp. 217-226.

Price, K. M., Williams, Z. Y. and Woodward, J. A. 2009. Needle Preference in Patients Receiving Cosmetic Botulinum Toxin Type A. *Dermatol Surg* 36(1), pp. 109-112.

Priddy, F., Tesfaye, F., Mengistu, Y., Rothenberg, R., Fitzmaurice, D., Mariam, D. H., del Rio, C., Oli, K. and Worku, A. 2005. Potential for medical transmission of HIV in Ethiopia. *Aids* 19(3), pp. 348-350.

Proksch, E., Brandner, J. M. and Jensen, J. M. 2008. The skin: an indispensable barrier. *Exp Dermatol* 17(12), pp. 1063-1072.

Quan, F. S., Kim, Y. C., Yoo, D. G., Compans, R. W., Prausnitz, M. R. and Kang, S. M. 2009. Stabilization of influenza vaccine enhances protection by microneedle delivery in the mouse skin. *PLoS One* 4(9), p. e7152.

Querleux, B., Baldeweck, T., Diridollou, S., de Rigal, J., Huguet, E., Leroy, F. and Holloway Barbosa, V. 2009. Skin from various ethnic origins and aging: an in vivo cross-sectional multimodality imaging study. *Skin Res Technol* 15(3), pp. 306-313.

Quevedo, A. S. and Coghill, R. C. 2009. Filling-in, spatial summation, and radiation of pain: evidence for a neural population code in the nociceptive system. *J Neurophysiol* 102(6), pp. 3544-3553.

Raja, Sivamani, K., Garcia, M. S. and Isseroff, R. R. 2007. Wound re-epithelialization: modulating keratinocyte migration in wound healing. *Frontiers in Bioscience* 12, pp. 2849-2868.

Ravanti, L., Hakkinen, L., Larjava, H., Saarialho-Kere, U., Foschi, M., Han, J. and Kahari, V. M. 1999. Transforming growth factor-beta induces collagenase-3 expression by human gingival fibroblasts via p38 mitogen-activated protein kinase. *J Biol Chem* 274(52), pp. 37292-37300.

Raynor, H. A., Osterholt, K. M., Hart, C. N., Jelalian, E., Vivier, P. and Wing, R. R. 2009. Evaluation of active and passive recruitment methods used in randomized controlled trials targeting pediatric obesity. *Int J Pediatr Obes* 4(4), pp. 224-232.

Reeler, A. V. 1990. Injections: a fatal attraction? *Soc Sci Med* 31(10), pp. 1119-1125.

Rees, J. L. 2004. The genetics of sun sensitivity in humans. *Am J Hum Genet* 75(5), pp. 739-751.

Reid, S. 2009. Increase in clinical prevalence of AIDS implies increase in unsafe medical injections. *Int J STD AIDS* 20(5), pp. 295-299.

Richards, G. M., Oresajo, C. O. and Halder, R. M. 2003. Structure and function of ethnic skin and hair. *Dermatol Clin* 21(4), pp. 595-600.

Rizwan, M., Aqil, M., Talegaonkar, S., Azeem, A., Sultana, Y. and Ali, A. 2009. Enhanced transdermal drug delivery techniques: an extensive review of patents. *Recent Pat Drug Deliv Formul* 3(2), pp. 105-124.

Robinson, E. J., Kerr, C. E., Stevens, A. J., Lilford, R. J., Braunholtz, D. A., Edwards, S. J., Beck, S. R. and Rowley, M. G. 2005. Lay public's understanding of equipoise and randomisation in randomised controlled trials. *Health Technol Assess* 9(8), pp. 1-192.

Robinson, M. K. and Perkins, M. A. 2002. A strategy for skin irritation testing. *Am J Contact Dermat* 13(1), pp. 21-29.

Rodero, M. P. and Khosrotehrani, K. 2010. Skin wound healing modulation by macrophages. *International journal of clinical and experimental pathology* 3(7), pp. 643-653.

Rodrigues, L. M., Pinto, P. C., Magro, J. M., Fernandes, M. and Alves, J. 2004. Exploring the influence of skin perfusion on transepidermal water loss. *Skin Res Technol* 10(4), pp. 257-262.

Roh, C. and Lyle, S. 2006. Cutaneous stem cells and wound healing. *Pediatr Res* 59(4 Pt 2), pp. 100R-103R.

Rook, A. and Burns, T. 2004. *Rook's textbook of dermatology*. 8th ed. Chichester, West Sussex, UK ; Hoboken, NJ: Wiley-Blackwell.

Rosado, C., Pinto, P. and Rodrigues, L. M. 2005a. Comparative assessment of the performance of two generations of Tewameter®: TM210 and TM300. *Int J Cosmet Sci* 27(4), pp. 237-241.

Rosado, C., Pinto, P. and Rodrigues, L. M. 2005b. Modeling TEWL-desorption curves: a new practical approach for the quantitative in vivo assessment of skin barrier. *Exp Dematol* 14(5), pp. 386-390.

Roskos, K. V. and Guy, R. H. 1989. Assessment of Skin Barrier Function Using Transepidermal Water Loss: Effect of Age. *Pharmaceutical Research* 6(11), pp. 949-953.

Ross, M. W., McCurdy, S. A., Kilonzo, G. P., Williams, M. L. and Leshabari, M. T. 2008. Drug use careers and blood-borne pathogen risk behavior in male and female Tanzanian heroin injectors. *Am J Trop Med Hyg* 79(3), pp. 338-343.

Rutledge, R., Dorghazi, P. and Peralgie, C. 2006. Efficacy of estradiol topical patch in the treatment of symptoms of depression following mini-gastric bypass in women. *Obes Surg* 16(9), pp. 1221-1226.

Sarret, Y., Woodley, D. T., Grigsby, K., Wynn, K. and O'Keefe, E. J. 1992. Human keratinocyte locomotion: the effect of selected cytokines. *J Invest Dermatol* 98(1), pp. 12-16.

Sasaki, G. H., Travis, H. M. and Tucker, B. 2009. Fractional CO2 laser resurfacing of photoaged facial and non-facial skin: histologic and clinical results and side effects. *J Cosmet Laser Ther* 11(4), pp. 190-201.

Scardina, G. A., Ruggieri, A. and Messina, P. 2009. Correlation of " in vivo" microcirculation and degeneration of the basal cells of the epithelium in lingual lichen planus. *Indian J Dent Res* 20(4), pp. 407-411.

Schaefer, H. and Lademann, J. 2001. The role of follicular penetration. A differential view. *Skin Pharmacol Appl Skin Physiol* 14 Suppl 1, pp. 23-27.

Schellander, F. A. and Headington, J. T. 1974. The stratum corneum--some structural and functional correlates. *Br J Dermatol* 91(5), pp. 507-515.

Scheuplein, R. J. 1967. Mechanism of percutaneous absorption. II. Transient diffusion and the relative importance of various routes of skin penetration. *J Invest Dermatol* 48(1), pp. 79-88.

Schiech, L. 2002. Malignant cutaneous wounds. *Clin J Oncol Nurs* 6(5), pp. 305-309.

Schilling, J. F. and Wechsler, R. J. 1986. Computed tomographic anatomy of the buttock. *Skeletal Radiol* 15(8), pp. 613-618.

Schmid, K., Broding, H. C., Uter, W. and Drexler, H. 2005. Transepidermal water loss and incidence of hand dermatitis in a prospectively followed cohort of apprentice nurses. *Dermatitis* 52(5), pp. 247-253.

Schmid, P., Cox, D., Bilbe, G., McMaster, G., Morrison, C., Stahelin, H., Luscher, N. and Seiler, W. 1993. TGF-beta s and TGF-beta type II receptor in human epidermis: differential expression in acute and chronic skin wounds. *J Pathol* 171(3), pp. 191-197.

Schmidt, U. and Frewer, A. 2007. *History and theory of human experimentation : the declaration of Helsinki and modern medical ethics*. Stuttgart: Steiner, p. 364

Schnoll, R. A., Patterson, F., Wileyto, E. P., Tyndale, R. F., Benowitz, N. and Lerman, C. 2009. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. *Pharmacol Biochem Behav* 92(1), pp. 6-11.

Schorr, M. R. 1966. Needles some points to think about part 1. *Anesthesia & Analgesia* 45(4), pp. 509-513.

Schulz, K. F., Grimes, D. A., Altman, D. G. and Hayes, R. J. 1996. Blinding and exclusions after allocation in randomised controlled trials: survey of published parallel group trials in obstetrics and gynaecology. *BMJ* 312(7033), pp. 742-744.

Schwindt, D. A., Wilhelm, K. P. and Maibach, H. I. 1998. Water diffusion characteristics of human stratum corneum at different anatomical sites in vivo. *J Invest Dermatol* 111(3), pp. 385-389.

Sellheyer, K. and Krahl, D. 2009. Cutaneous mesenchymal stem cells: status of current knowledge, implications for dermatopathology. *J Cutan Pathol* 37(6), pp. 624-634.

Sepehr, A., Chauhan, N., Alexander, A. J. and Adamson, P. A. 2010. Botulinum toxin type a for facial rejuvenation: treatment evolution and patient satisfaction. *Aesthetic Plast Surg* 34(5), pp. 583-586.

Serpell, M. G. 2002. Gabapentin in neuropathic pain syndromes: a randomised, double-blind, placebo-controlled trial. *Pain* 99(3), pp. 557-566.

Servold, S. A. 1991. Growth factor impact on wound healing. *Clin Podiatr Med Surg* 8(4), pp. 937-953.

Shah, J. H., Zhai, H. and Maibach, H. I. 2005. Comparative evaporimetry in man. *Skin Res Technol* 11(3), pp. 205-208.

Shah, V., Taddio, A. and Rieder, M. J. 2009. Effectiveness and tolerability of pharmacologic and combined interventions for reducing injection pain during routine childhood immunizations: Systematic review and meta-analyses. *Clinical Therapeutics* 31(Supplement 2), pp. S104-S151.

Shiffman, S., Sweeney, C. T., Ferguson, S. G., Sembower, M. A. and Gitchell, J. G. 2008. Relationship between adherence to daily nicotine patch use and treatment efficacy: secondary analysis of a 10-week randomized, double-blind, placebo-controlled clinical trial simulating over-the-counter use in adult smokers. *Clin Ther* 30(10), pp. 1852-1858.

Shimada, K., Yoon, J. S., Yoshihara, T., Iwasaki, T. and Nishifuji, K. 2009. Increased transepidermal water loss and decreased ceramide content in lesional and non-lesional skin of dogs with atopic dermatitis. *Vet Dermatol* 20(5-6), pp. 541-546.

Shirkhanzadeh, M. 2005. Microneedles coated with porous calcium phosphate ceramics: effective vehicles for transdermal delivery of solid trehalose. *J Mater Sci Mater Med* 16(1), pp. 37-45.

Sim, J. and Waterfield, J. 1997. Validity, reliability and responsiveness in the assessment of pain. *Physiotherapy Theory and Practice* 13(1), pp. 23-37.

Simmons, R. M., Smith, S. M. and Osborne, M. P. 2001. Methylene blue dye as an alternative to isosulfan blue dye for sentinel lymph node localization. *Breast J* 7(3), pp. 181-183.

Simonsen, L., Kane, A., Lloyd, J., Zaffran, M. and Kane, M. 1999. Unsafe injections in the developing world and transmission of bloodborne pathogens: a review. *Bull World Health Organ* 77(10), pp. 789-800.

Sindrilaru, A., Peters, T., Schymeinsky, J., Oreshkova, T., Wang, H., Gompf, A., Mannella, F., Wlaschek, M., Sunderkotter, C., Rudolph, K. L., Walzog, B., Bustelo, X. R., Fischer, K. D. and Scharffetter-Kochanek, K. 2009. Wound healing defect of Vav3-/- mice due to impaired {beta}2-integrin-dependent macrophage phagocytosis of apoptotic neutrophils. *Blood* 113(21), pp. 5266-5276.

Singh, J., Gross, M., Sage, B., Davis, H. T. and Maibach, H. I. 2001. Regional variations in skin barrier function and cutaneous irritation due to iontophoresis in human subjects. *Food Chem Toxicol* 39(11), pp. 1079-1086.

Singh, T. R., Dunne, N. J., Cunningham, E. and Donnelly, R. F. 2011. Review of patents on microneedle applicators. *Recent Pat Drug Deliv Formul* 5(1), pp. 11-23.

Sivamani, R. K., Liepmann, D. and Maibach, H. I. 2007. Microneedles and transdermal applications. *Expert Opin Drug Deliv* 4(1), pp. 19-25.

Sivamani, R. K., Stoeber, B., Liepmann, D. and Maibach, H. I. 2009. Microneedle penetration and injection past the stratum corneum in humans. *Journal of Dermatological Treatment* 20(3), pp. 156-159.

Sivamani, R. K., Stoeber, B., Wu, G. C., Zhai, H., Liepmann, D. and Maibach, H. 2005. Clinical microneedle injection of methyl nicotinate: stratum corneum penetration. *Skin Res Technol* 11(2), pp. 152-156.

Sloan, K. B., Wasdo, S. C. and Rautio, J. 2006. Design for optimized topical delivery: Prodrugs and a paradigm change. *Pharm Res* 23(12), pp. 2729-2747.

Smith, F. 2003. The molecular genetics of keratin disorders. *Am J Clin Dermatol* 4(5), pp. 347-364.

Smith, F. J., McKenna, K. E., Irvine, A. D., Bingham, E. A., Coleman, C. M., Uitto, J. and McLean, W. H. 1999. A mutation detection strategy for the human keratin 6A gene and novel missense mutations in two cases of pachyonychia congenita type 1. *Exp Dermatol* 8(2), pp. 109-114.

Smith, L. T. and Holbrook, K. A. 1982. Development of dermal connective tissue in human embryonic and fetal skin. *Scan Electron Microsc* (Pt 4), pp. 1745-1751.

Smoller, B. R., Dover, J. S. and Hsu, A. 1989. Keratinocyte protein expression in rapidly regenerating epidermis following laser-induced thermal injury. *Lasers Surg Med* 9(3), pp. 264-270.

Snow, A. L., Chandler, J. F., Kunik, M. E., Davila, J. A., Balasubramanyam, V., Steele, A. B. and Morgan, R. O. 2009. Self-reported pain in persons with dementia predicts subsequent decreased psychosocial functioning. *Am J Geriatr Psychiatry* 17(10), pp. 873-880.

Stamatas, G. N., Nikolovski, J., Luedtke, M. A., Kollias, N. and Wiegand, B. C. 2009. Infant Skin Microstructure Assessed In Vivo Differs from Adult Skin in Organization and at the Cellular Level. *Pediatr Dermatol* 27(2), pp. 125-131.

So, J. W., Park, H. H., Lee, S. S., Kim, D. C., Shin, S. C. and Cho, C. W. 2009. Effect of microneedle on the pharmacokinetics of ketoprofen from its transdermal formulations. *Drug Deliv* 16(1), pp. 52-56.

Sokunbi, O., Watt, P. and Moore, A. 2008. Experiences of patients with chronic low back disorder of the use of spinal stabilisation exercises--a qualitative study. *Nig Q J Hosp Med* 18(4), pp. 231-243.

Song, J. M., Kim, Y. C., Lipatov, A. S., Pearton, M., Davis, C. T., Yoo, D. G., Park, K. M., Chen, L. M., Quan, F. S., Birchall, J. C., Donis, R. O., Prausnitz, M. R., Compans, R. W. and Kang, S. M. 2010. Microneedle delivery of H5N1 influenza virus-like particles to the skin induces long-lasting B- and T-cell responses in mice. *Clin Vaccine Immunol* 17(9), pp. 1381-1389.

Sorensen, O. E., Cowland, J. B., Theilgaard-Monch, K., Liu, L., Ganz, T. and Borregaard, N. 2003. Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. *J Immunol* 170(11), pp. 5583-5589.

Sorrell, J. M., Baber, M. A. and Caplan, A. I. 2007. Clonal characterization of fibroblasts in the superficial layer of the adult human dermis. *Cell Tissue Res* 327(3), pp. 499-510.

Sorrell, J. M., Baber, M. A. and Caplan, A. I. 2008. Human dermal fibroblast subpopulations; differential interactions with vascular endothelial cells in coculture: nonsoluble factors in the extracellular matrix influence interactions. *Wound Repair Regen* 16(2), pp. 300-309.

Spiekstra, S. W., Breetveld, M., Rustemeyer, T., Scheper, R. J. and Gibbs, S. 2007. Wound-healing factors secreted by epidermal keratinocytes and dermal fibroblasts in skin substitutes. *Wound Reapir Regen* 15(5), pp. 708-717.

Spielberg, F., Branson, B. M., Goldbaum, G. M., Lockhart, D., Kurth, A., Celum, C. L., Rossini, A., Critchlow, C. W. and Wood, R. W. 2003. Overcoming barriers to HIV testing: preferences for new strategies among clients of a needle exchange, a sexually transmitted disease clinic, and sex venues for men who have sex with men. *J Acquir Immune Defic Syndr* 32(3), pp. 318-327.

Sprung, J., Abdelmalak, B. and Schoenwald, P. K. 1998. Vasovagal cardiac arrest during the insertion of an epidural catheter and before the administration of epidural medication. *Anesth Analg* 86(6), pp. 1263-1265.

Steffensen, B., Hakkinen, L. and Larjava, H. 2001. Proteolytic events of wound-healing--coordinated interactions among matrix metalloproteinases

(MMPs), integrins, and extracellular matrix molecules. *Crit Rev Oral Biol Med* 12(5), pp. 373-398.

Stocks, J. M., Taylor, N. A., Tipton, M. J. and Greenleaf, J. E. 2004. Human physiological responses to cold exposure. *Aviat Space Environ Med* 75(5), pp. 444-457.

Strand, L. I., Ljunggren, A. E., Bogen, B., Ask, T. and Johnsen, T. B. 2008. The Short-Form McGill Pain Questionnaire as an outcome measure: Test-retest reliability and responsiveness to change. *Eur J Pain* 12(7), pp. 917-925.

Strodtbeck 2001. Physiology of wound healing. *Newborn & Infant Nursing* 1(1), pp. 43-52.

Suehiro, M., Hirano, S., Ikenaga, K., Katoh, N., Yasuno, H. and Kishimoto, S. 2004. Characteristics of skin surface morphology and transepidermal water loss in clinically normal-appearing skin of patients with atopic dermatitis: a video-microscopy study. *J Dermatol* 31(2), pp. 78-85.

Sugibayashi, K., Nakayama, S., Seki, T., Hosoya, K. and Morimoto, Y. 1992. Mechanism of skin penetration-enhancing effect by laurocapram. *J Pharm Sci* 81(1), pp. 58-64.

Sugino, M., Todo, H. and Sugibayashi, K. 2009. [Skin permeation and transdermal delivery systems of drugs: history to overcome barrier function in the stratum corneum]. *Yakugaku Zasshi* 129(12), pp. 1453-1458.

Sulaimon, S. S. and Kitchell, B. E. 2003. The biology of melanocytes. *Veterinary Dermatology* 14(2), pp. 57-65.

Sullivan, S. P., Koutsonanos, D. G., Del Pilar Martin, M., Lee, J. W., Zarnitsyn, V., Choi, S. O., Murthy, N., Compans, R. W., Skountzou, I. and Prausnitz, M. R. 2010. Dissolving polymer microneedle patches for influenza vaccination. *Nat Med* 16(8), pp. 915-920.

Suter, M. M., Schulze, K., Bergman, W., Welle, M., Roosje, P. and Muller, E. J. 2009. The keratinocyte in epidermal renewal and defence. *Vet Dermatol* 20(5-6), pp. 515-532.

Swindle, L. D., Thomas, S. G., Freeman, M. and Delaney, P. M. 2003. View of normal human skin in vivo as observed using fluorescent fiber-optic confocal microscopic imaging. *J Invest Dermatol* 121(4), pp. 706-712.

Taddio, A., Ilersich, A. L., Ipp, M., Kikuta, A. and Shah, V. 2009. Physical interventions and injection techniques for reducing injection pain during routine childhood immunizations: Systematic review of randomized controlled trials and quasi-randomized controlled trials. *Clinical Therapeutics* 31(Supplement 2), pp. S48-S76.

Tadokoro, T., Yamaguchi, Y., Batzer, J., Coelho, S. G., Zmudzka, B. Z., Miller, S. A., Wolber, R., Beer, J. Z. and Hearing, V. J. 2005. Mechanisms of skin tanning in different racial/ethnic groups in response to ultraviolet radiation. *J Invest Dermatol* 124(6), pp. 1326-1332.

Tagami, H., Kobayashi, H. and Kikuchi, K. 2002. A portable device using a closed chamber system for measuring transepidermal water loss: comparison with the conventional method. *Skin Res Technol* 8(1), pp. 7-12.

Talreja, P., Kleene, N. K., Pickens, W. L., Wang, T. F. and Kasting, G. B. 2001. Visualization of the lipid barrier and measurement of lipid pathlength in human stratum corneum. *AAPS PharmSci* 3(2), p. E13.

Tamariz-Dominguez, E., Castro-Munozledo, F. and Kuri-Harcuch, W. 2002. Growth factors and extracellular matrix proteins during wound healing promoted with frozen cultured sheets of human epidermal keratinocytes. *Cell Tissue Res* 307(1), pp. 79-89.

Teo, A. L., Shearwood, C., Ng, K. C., Lu, J. and Moochhala, S. 2006. Transdermal microneedles for drug delivery applications. *Materials Science and Engineering: B* 132(1-2), pp. 151-154.

Teo, M. A., Shearwood, C., Ng, K. C., Lu, J. and Moochhala, S. 2005. In vitro and in vivo characterization of MEMS microneedles. *Biomed Microdevices* 7(1), pp. 47-52.

Titford, M. 2005. The long history of hematoxylin. *Biotech Histochem* 80(2), pp. 73-78.

Titler, M. G., Herr, K., Brooks, J. M., Xie, X. J., Ardery, G., Schilling, M. L., Marsh, J. L., Everett, L. Q. and Clarke, W. R. 2009. Translating research into practice intervention improves management of acute pain in older hip fracture patients. *Health Serv Res* 44(1), pp. 264-287.

Todd, P. A., Goa, K. L. and Langtry, H. D. 1990. Transdermal nitroglycerin (glyceryl trinitrate). A review of its pharmacology and therapeutic use. *Drugs* 40(6), pp. 880-902.

Toroyan, T., Roberts, I. and Oakley, A. 2000. Randomisation and resource allocation: a missed opportunity for evaluating health care and social interventions. *J Med Ethics* 26(5), pp. 319-322.

Tuli, S. S., Liu, R., Chen, C., Blalock, T. D., Goldstein, M. and Schultz, G. S. 2006. Immunohistochemical localization of EGF, TGF-alpha, TGF-beta, and their receptors in rat corneas during healing of excimer laser ablation. *Curr Eye Res* 31(9), pp. 709-719.

Tunks, E. R., Weir, R. and Crook, J. 2008. Epidemiologic perspective on chronic pain treatment. *Can J Psychiatry* 53(4), pp. 235-242.

Turner, J., Larsen, M., Tarassenko, L., Neil, A. and Farmer, A. 2009. Implementation of telehealth support for patients with type 2 diabetes using insulin treatment: an exploratory study. *Inform Prim Care* 17(1), pp. 47-53.

Uitto, J. and Pulkkinen, L. 1996. Molecular complexity of the cutaneous basement membrane zone. *Mol Biol Rep* 23(1), pp. 35-46.

Uman, L. S., Chambers, C. T., McGrath, P. J. and Kisely, S. 2006. Psychological interventions for needle-related procedural pain and distress in children and adolescents. *Cochrane Database Syst Rev* (4), p. CD005179.

Usui, M. L., Mansbridge, J. N., Carter, W. G., Fujita, M. and Olerud, J. E. 2008. Keratinocyte Migration, Proliferation, and Differentiation in Chronic Ulcers From Patients With Diabetes and Normal Wounds. *J Histochem Cytochem* 56(7), pp. 687-696.

Usui, M. L., Underwood, R. A., Mansbridge, J. N., Muffley, L. A., Carter, W. G. and Olerud, J. E. 2005. Morphological evidence for the role of suprabasal keratinocytes in wound reepithelialization. *Wound Repair Regen* 13(5), pp. 468-479.

Uthaikhup, S., Sterling, M. and Jull, G. 2009. Psychological, cognitive and quality of life features in the elderly with chronic headache. *Gerontology* 55(6), pp. 683-693.

Valeri, C. R., Saleem, B. and Ragno, G. 2006. Release of platelet-derived growth factors and proliferation of fibroblasts in the releasates from platelets stored in the liquid state at 22 degrees C after stimulation with agonists. *Transfusion* 46(2), pp. 225-229.

Van Damme, P., Oosterhuis-Kafeja, F., Van der Wielen, M., Almagor, Y., Sharon, O. and Levin, Y. 2009. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults. *Vaccine* 27(3), pp. 454-459.

Van Hal, D. A., Jeremiasse, E., Junginger, H. E., Spies, F. and Bouwstra, J. A. 1996. Structure of fully hydrated human stratum corneum: a freeze-fracture electron microscopy study. *J Invest Dermatol* 106(1), pp. 89-95.

van Tilburg, M. A., Chitkara, D. K., Palsson, O. S., Turner, M., Blois-Martin, N., Ulshen, M. and Whitehead, W. E. 2009. Audio-recorded guided imagery treatment reduces functional abdominal pain in children: a pilot study. *Pediatrics* 124(5), pp. e890-897.

Vanichseni, S., Tappero, J. W., Pitisuttithum, P., Kitayaporn, D., Mastro, T. D., Vimutisunthorn, E., van Griensvan, F., Heyward, W. L., Francis, D. P. and Choopanya, K. 2004. Recruitment, screening and characteristics of injection drug users participating in the AIDSVAX B/E HIV vaccine trial, Bangkok, Thailand. *Aids* 18(2), pp. 311-316.

Varvel, J. R., Shafer, S. L., Hwang, S. S., Coen, P. A. and Stanski, D. R. 1989. Absorption characteristics of transdermally administered fentanyl. *Anesthesiology* 70(6), pp. 928-934.

Verbaan, F. J., Bal, S. M., van den Berg, D. J., Dijksman, J. A., van Hecke, M., Verpoorten, H., van den Berg, A., Luttge, R. and Bouwstra, J. A. 2008. Improved piercing of microneedle arrays in dermatomed human skin by an impact insertion method. *Journal of Controlled Release* 128(1), pp. 80-88.

Verbaan, F. J., Bal, S. M., van den Berg, D. J., Groenink, W. H., Verpoorten, H., Luttge, R. and Bouwstra, J. A. 2007. Assembled microneedle arrays enhance the transport of compounds varying over a large range of molecular weight across human dermatomed skin. *J Control Release* 117(2), pp. 238-245.

Veresciagina, K., Ambrozaitis, K. V. and Spakauskas, B. 2009. The measurements of health-related quality-of-life and pain assessment in the preoperative patients with low back pain. *Medicina (Kaunas)* 45(2), pp. 111-122.

Vieira, A. P. G. F., Lawrence, H. P., Limeback, H., Sampaio, F. C. and Grynpas, M. 2005. A visual analog scale for measuring dental fluorosis severity. *J Am Dent Assoc* 136(7), pp. 895-901.

Vierck, C. J., Hansson, P. T. and Yezierski, R. P. 2008. Clinical and pre-clinical pain assessment: Are we measuring the same thing. *Pain* 135(1-2), pp. 7-10.

Voorhies, R. M., Jiang, X. and Thomas, N. 2007. Predicting outcome in the surgical treatment of lumbar radiculopathy using the Pain Drawing Score, McGill Short Form Pain Questionnaire, and risk factors including psychosocial issues and axial joint pain. *Spine J* 7(5), pp. 516-524.

Vyas, P. M., Roychowdhury, S., Khan, F. D., Prisinzano, T. E., Lamba, J., Schuetz, E. G., Blaisdell, J., Goldstein, J. A., Munson, K. L., Hines, R. N. and Svensson, C. K. 2006. Enzyme-mediated protein haptenation of dapsone and sulfamethoxazole in human keratinocytes: I. Expression and role of cytochromes P450. *J Pharmacol Exp Ther* 319(1), pp. 488-496.

Wang, H., Gao, X. H., Wang, Y. K., Li, P., He, C. D., Xie, Y. and Chen, H. D. 2005a. Expression of haptoglobin in human keratinocytes and Langerhans cells. *Br J Dermatol* 153(5), pp. 894-899.

Wang, P. C., Wester, B. A., Rajaraman, S., Paik, S. J., Kim, S. H. and Allen, M. G. 2009. Hollow polymer microneedle array fabricated by photolithography process combined with micromolding technique. *Conf Proc IEEE Eng Med Biol Soc* 1, pp. 7026-7029.

Wang, P. M., Cornwell, M., Hill, J. and Prausnitz, M. R. 2006. Precise microinjection into skin using hollow microneedles. *J Invest Dermatol* 126(5), pp. 1080-1087.

Wang, P. M., Cornwell, M. and Prausnitz, M. R. 2005b. Minimally invasive extraction of dermal interstitial fluid for glucose monitoring using microneedles. *Diabetes Technol Ther* 7(1), pp. 131-141.

Waters, A. and Bull, V. 2006. *Oxford basic English dictionary*. 3rd ed. ed. Oxford: Oxford University Press.

Wawersik, M., Paladini, R. D., Noensie, E. and Coulombe, P. A. 1997. A Proline Residue in the alpha -Helical Rod Domain of Type I Keratin 16 Destabilizes Keratin Heterotetramers. *J Biol Chem* 272(51), pp. 32557-32565.

Wawersik, M. J., Mazzalupo, S., Nguyen, D. and Coulombe, P. A. 2001. Increased Levels of Keratin 16 Alter Epithelialization Potential of Mouse Skin Keratinocytes In Vivo and Ex Vivo. *Mol Biol Cell 12(11),* pp. 3439-3450.

Weber-Matthiesen, K. and Sterry, W. 1990. Organization of the monocyte/macrophage system of normal human skin. *J Invest Dermatol* 95(1), pp. 83-89.

Webster, P. and Webster, A. 2007. Cryosectioning fixed and cryoprotected biological material for immunocytochemistry. *Methods Mol Biol* 369, pp. 257-289.

Welzel, J., Reinhardt, C., Lankenau, E., Winter, C. and Wolff, H. H. 2004. Changes in function and morphology of normal human skin: evaluation using optical coherence tomography. *Br J Dermatol* 150(2), pp. 220-225.

Wenngren, A. and Stalnacke, B. M. 2009. Computerized assessment of pain drawing area: A pilot study. *Neuropsychiatr Dis Treat* 5, pp. 451-456.

Wermeling, D. P., Banks, S. L., Hudson, D. A., Gill, H. S., Gupta, J., Prausnitz, M. R. and Stinchcomb, A. L. 2008. Microneedles permit transdermal delivery of a skin-impermeant medication to humans. *Proc Natl Acad Sci U S A* 105(6), pp. 2058-2063.

Werner, J. B. and Benrimoj, S. I. 2008. Audio taping simulated patient encounters in community pharmacy to enhance the reliability of assessments. *Am J Pharm Educ* 72(6), p. 136.

Werner, S. and Grose, R. 2003. Regulation of wound healing by growth factors and cytokines. *Physiol Rev* 83(3), pp. 835-870.

Werner, S., Krieg, T. and Smola, H. 2007. Keratinocyte-fibroblast interactions in wound healing. *J Invest Dermatol* 127(5), pp. 998-1008.

Whitworth, J. A., Biraro, S., Shafer, L. A., Morison, L. A., Quigley, M., White, R. G., Mayanja, B. N., Ruberantwari, A. and Van der Paal, L. 2007. HIV incidence and recent injections among adults in rural southwestern Uganda. *Aids* 21(8), pp. 1056-1058.

WHO. 1999. *Safety of injections : questions and answers.* World Health Organization. Available at: <URL: http://whqlibdoc.who.int/fact_sheet/1999/FS_234.pdf> [Accessed: 06/12/2007].

WHO. 2004. *Safety of Injections.* Secretariat of the Safe Injection Global Network, Department of Essential Health Technologies, World Health Organization. Available at: <URL: http://www.who.int/injection_safety/about/resources/en/FactAndFiguresInjectionSafety.pdf> [Accessed: 06/12/2007].

Widera, G., Johnson, J., Kim, L., Libiran, L., Nyam, K., Daddona, P. E. and Cormier, M. 2006a. Effect of delivery parameters on immunization to ovalbumin following intracutaneous administration by a coated microneedle array patch system. *Vaccine* 24(10), pp. 1653-1664.

Widera, G., Johnson, J., Kim, L., Libiran, L., Nyam, K., Daddona, P. E. and Cormier, M. 2006b. Effect of delivery parameters on immunization to ovalbumin following intracutaneous administration by a coated microneedle array patch system. *Vaccine* 24(10), pp. 1653-1664.

Wilke, N., Hibert, C., O'Brien, J. and Morrissey, A. 2005a. Silicon microneedle electrode array with temperature monitoring for electroporation. *Sensors and Actuators A: Physical* 123-124, pp. 319-325.

Wilke, N. and Morrissey, A. 2007. Silicon microneedle formation using modified mask designs based on convex corner undercut. *Journal of Micromechanics and Microengineering* 17(2), p. 238.

Wilke, N., Mulcahy, A., Ye, S. R. and Morrissey, A. 2005b. Process optimization and characterization of silicon microneedles fabricated by wet etch technology. *Microelectronics Journal* 36(7), pp. 650-656.

Wilkinson, C. D. and Rahman, M. 2004. Dry etching and sputtering. *Philos Transact A Math Phys Eng Sci* 362(1814), pp. 125-138.

Wille, J. J. 2006. *Skin delivery systems : transdermals, dermatologicals, and cosmetic actives.* 1st ed. ed. Ames, Iowa ; Oxford: Blackwell.

World Medical Association (WMA), 2008, *Declaration of Helsinki, Ethical Principles for Medical Research involving human subjects.* [WWW] <URL:http://www.wma.net/e/policy/b3.htm> [Accessed on 20/02/2010.]

Wong, T. W., Zhao, Y. L., Sen, A. and Hui, S. W. 2005. Pilot study of topical delivery of methotrexate by electroporation. *Br J Dermatol* 152(3), pp. 524-530.

Wonglertnirant, N., Todo, H., Opanasopit, P., Ngawhirunpat, T. and Sugibayashi, K. 2010. Macromolecular delivery into skin using a hollow microneedle. *Biol Pharm Bull* 33(12), pp. 1988-1993.

Woods, C. A. and Cumming, B. 2009. The Impact of Test Medium on Use of Visual Analogue Scales. *Eye & Contact Lens* 35(1), pp. 6-10.

Wright, K. D., Asmundson, G. J. G. and McCreary, D. R. 2001. Factorial validity of the short-form McGill pain questionnaire (SF-MPQ). *European Journal of Pain* 5(3), pp. 279-284.

Wu, C., Luan, Q., Li, C. and Zheng, Z. 2009. Effects of antikeratin 16 antibodies on the expression of Toll-like receptors 2 and 4 in keratinocytes. *Clin Exp Dermatol* 34(2), pp. 236-239.

Wynne, A., Whitefield, M., Dixon, A. J. and Anderson, S. 2002. An effective, cosmetically acceptable, novel hydro-gel emollient for the management of dry skin conditions. *J Dermatolog Treat* 13(2), pp. 61-66.

Xing, M. M., Hui, X., Zhong, W., Pan, N., Yaghmaie, F. and Maibach, H. I. 2009. In vitro human topical bioactive drug transdermal absorption: estradiol. *Cutan Ocul Toxicol* 28(4), pp. 171-175.

Xiong, H., Kim, J., Kim, E. and Amemiya, S. 2009. Scanning Electrochemical Microscopy of One-Dimensional Nanostructure: Effects of Nanostructure Dimensions on the Tip Feedback Current under Unbiased Conditions. *J Electroanal Chem (Lausanne Switz)* 629(1-2), pp. 78-86.

Yager, D. R., Kulina, R. A. and Gilman, L. A. 2007. Wound fluids: a window into the wound environment? *Int J Low Extrem Wounds* 6(4), pp. 262-272.

Yan, G., Warner, K. S., Zhang, J., Sharma, S. and Gale, B. K. 2010. Evaluation needle length and density of microneedle arrays in the pretreatment of skin for transdermal drug delivery. *Int J Pharm* 391(1-2), pp. 7-12.

Yang, M. and Zahn, J. D. 2004. Microneedle insertion force reduction using vibratory actuation. *Biomed Microdevices* 6(3), pp. 177-182.

Yang, N. S., Burkholder, J., McCabe, D., Neumann, V. and Fuller, D. 2001. Particle-mediated gene delivery in vivo and in vitro. *Curr Protoc Hum Genet* Chapter 12, p. Unit 12 16.

Yaroslavsky, A. N., Barbosa, J., Neel, V., DiMarzio, C. and Anderson, R. R. 2005. Combining multispectral polarized light imaging and confocal microscopy for localization of nonmelanoma skin cancer. *J Biomed Opt* 10(1), p. 14011.

Yeshurun, Y. H., IL), De Boer, Meint (Enschede, NL), Berenschot J. W. (Winterswijk, NL) 2010. Method for Producing Microneedle Structures Employing one-sided Processing.United States: NanoPass Technologies Ltd.

Yomtoob, D. E., Dewan, M. A., Lee, M. S. and Harrison, A. R. 2009. Comparison of pain scores with 30-gauge and 32-gauge needles for periocular botulinum toxin type a injections. *Ophthal Plast Reconstr Surg* 25(5), pp. 376-377.

Yonei, N., Kanazawa, N., Ohtani, T., Furukawa, F. and Yamamoto, Y. 2007. Induction of PDGF-B in TCA-treated epidermal keratinocytes. *Arch Dermatol Res* 299(9), pp. 433-440.

Zaba, L. C., Fuentes-Duculan, J., Steinman, R. M., Krueger, J. G. and Lowes, M. A. 2007. Normal human dermis contains distinct populations of CD11c+BDCA-1+ dendritic cells and CD163+FXIIIA+ macrophages. *J Clin Invest* 117(9), pp. 2517-2525.

Zampetti, A., Mastrofrancesco, A., Flori, E., Maresca, V., Picardo, M., Amerio, P. and Feliciani, C. 2009. Proinflammatory cytokine production in HaCaT cells treated by eosin: implications for the topical treatment of psoriasis. *Int J Immunopathol Pharmacol* 22(4), pp. 1067-1075.

Zhai, H., Dika, E., Goldovsky, M. and Maibach, H. I. 2007. Tape-stripping method in man: comparison of evaporimetric methods. *Skin Res Technol* 13(2), pp. 207-210.

Zhai, H. and Maibach, H. I. 1996. Effect of barrier creams: human skin in vivo. *Contact Dermatitis* 35(2), pp. 92-96.

Zhou, W. and Wang, Z. L. 2007. *Scanning microscopy for nanotechnology : techniques and applications*. New York ; London: Springer.

Zhu, Q., Zarnitsyn, V. G., Ye, L., Wen, Z., Gao, Y., Pan, L., Skountzou, I., Gill, H. S., Prausnitz, M. R., Yang, C. and Compans, R. W. 2009. Immunization by vaccine-coated microneedle arrays protects against lethal influenza virus challenge. *Proc Natl Acad Sci U S A* 106(19), pp. 7968-7973.

Zollo, R. A., Lurie, S. J., Epstein, R. and Ward, D. S. 2009. Patterns of communication during the preanesthesia visit. *Anesthesiology* 111(5), pp. 971-978.

APPENDIX I

Applicator testing information sheet and consent form

VOLUNTEER INFORMATION SHEET
An Assessment of Pain Perception of Potential Applicators for Microneedle Arrays

Aim of the Study

To assess the intensity of pain caused by four potential applicators for microneedle arrays when applied to the arm. There will be no microneedles attached to the applicators.

What the Study Involves

The study will be split into two sessions. At the first session the first applicator will be applied to either your left or right forearm. After the application you will be asked to complete a short questionnaire that will assess any pain you felt. Then the second applicator will be applied to your other forearm and again you will be asked to complete the same short questionnaire. This should all take around 20 minutes.

Time will then be given for recovery of the application sites before the second session.

At the second session the third applicator will be applied to either your right or left forearm. After the application you will be asked to complete the same short questionnaire as previously. Then the fourth applicator will be applied to your other forearm and again you will be asked to complete the same short questionnaire. Again this should take around 20 minutes.

Your participation in the study will then be complete.

Thank you for your time.

VOLUNTEER SUBJECT CONSENT FORM

An Assessment of Pain Perception of Potential Applicators for Microneedle Arrays

Please initial each box to indicate that you have read and agree to each statement.

1. I confirm that I have read and understand the information sheet for this study, that I have had the opportunity to ask questions and that I have received satisfactory answers to the questions I have asked. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that data collected for this study may be used in publications about the study. ☐
4. I agree to take part in the above study. ☐

_____	_____	_____
NAME OF SUBJECT	**DATE**	**SIGNATURE**

(Please print your name and date your own signature)

_____	_____	_____
Name of Person taking consent	Date	Signature

(Investigator)

APPENDIX II

Standard short form McGill Pain Questionnaire (MPQ-SF) for applicator testing

Participant Number		Application Number	

An Assessment of Pain Perception of Potential Applicators for Microneedle Arrays

Pain Perception Questionnaire

1) Visual Analogue Scale

"To measure the pain intensity please mark on the line below with a vertical line where you feel best represents the pain felt during and after the application. The left end of the scale represents no pain and the right end of the scale represents the worst pain imaginable."

No pain _____ Worst pain imaginable

2) Key Descriptive Words

"You will now be shown some descriptive words. As each word is read out to you please state if it describes a sensation you felt during or after the application. Rate it as either none, mild, moderate or severe."

		None		Mild		Moderate		Severe
Throbbing	0		1		2		3	
Shooting	0		1		2		3	
Stabbing	0		1		2		3	
Sharp	0		1		2		3	
Cramping	0		1		2		3	
Gnawing	0		1		2		3	
Hot burning	0		1		2		3	
Aching	0		1		2		3	
Heavy	0		1		2		3	
Tender	0		1		2		3	
Splitting	0		1		2		3	
Tiring-exhausting	0		1		2		3	
Sickening	0		1		2		3	
Fearful	0		1		2		3	
Punishing-cruel	0		1		2		3	

3) Present Pain Index

"Looking at the words, which one describes the overall intensity of the total pain you experienced during and after the application?"

0	**No pain**	
1	**Mild**	
2	**Discomforting**	
3	**Distressing**	
4	**Horrible**	
5	**Excruciating**	

APPENDIX III

Clinical Trial Protocol

PROTOCOL 11

AN ASSESSMENT OF PAIN, PERCEPTION AND WOUND HEALING FOLLOWING APPLICATION OF SILICON MICRONEEDLE ARRAYS

Dr A V Anstey, MD FRCP, Consultant Dermatologist, Royal Gwent Hospital, Newport.

J Birchall, PhD, Lecturer in Drug Delivery, Welsh School of Pharmacy, Cardiff University.

R M Porter, PhD, Lecturer of Cell & Molecular Biology, Wales College of Medicine, Cardiff University.

Dr M Kalavala, MD MRCP, Specialist Registrar in Dermatology, Royal Gwent Hospital, Newport.

CONTACT INFORMATION

Dr. A V Anstey, MD, FRCP
Consultant Dermatologist
Royal Gwent Hospital
Cardiff Road, Newport, NP20 2UB
Tel: 01633 234450, Fax: 01633 215729
e-mail: alex.anstey@gwent.wales.nhs.uk

J Birchall, PhD
Lecturer in Drug Delivery
Welsh School of Pharmacy
Redwood Building, King Edward VII Avenue
Cardiff University. CF10 3XF
Tel: 029 2087 5815, fax: 029 2087 4149
e-mail: BirchallJC@cf.ac.uk

Dr Dai John
Senior Lecturer
Welsh School of Pharmacy,
Redwood, King Edward VII Ave,
Cardiff, CF10 3XF
Tel: 029 20875804 Fax: 029 20874535
Email: JohnDN@cf.ac.uk

Mohammed Haq
Postgraduate Researcher
Welsh School of Pharmacy,
Redwood, King Edward VII Ave,
Cardiff, CF10 3XF
Tel: 029 20875804 Fax: 029 20874535
Email: HaqMI@cf.ac.uk

R M Porter, PhD
Lecturer of Cell & Molecular Biology,
Department of Dermatology, Wales College of Medicine,
Cardiff University. CF14 4XN
Tel: 029 2074 4369, fax: 029 2074 4312
e-mail: PorterRM@cf.ac.uk

M Kalavala MRCP, MD
Specialist Registrar in Dermatology
Royal Gwent Hospital
Cardiff Road, Newport, NP20 2UB
Tel: 01633 234234, Fax: 01633 215729
e-mail: manjuk04@yahoo.com

TABLE OF CONTENTS

1 PROTOCOL SUMMARY

AN ASSESSMENT OF PAIN, PERCEPTION AND WOUND HEALING FOLLOWING APPLICATION OF SILICON MICRONEEDLE ARRAYS.

Investigators:

Dr Anstey, Dr Birchall, Dr John, MI Haq, Dr Porter, Dr Kalavala.

Lay Summary:

Tiny microneedles, which are able to pierce the outermost barrier layer of skin without stimulating the underlying pain receptors or blood vessels, have been developed and exploited at the Welsh School of Pharmacy as a new method of delivering medicaments and vaccines through the skin. However, these needle arrays may still produce sensations in the skin and cause some damage to the skin's outer layers. This study will investigate the pain and perception of microneedle application in 12 volunteers and gain further unique information regarding the rate and extent of healing of the resulting damage. Pain and perception will be measured by Visual analogue scale (VAS) and recorded comments; skin barrier function will be measured by dye staining and trans-epidermal water loss (TEWL), and the nature of the wound healing response will be measured by assessment of skin samples using fluorescently labelled antibodies, following excision of three injection sites using the punch biopsy technique.

Objectives:

- To determine the pain and perception of microneedle application.
- To determine the damage to skin barrier function after microneedle application.
- To determine and compare the wound healing process of skin sites after the application of microneedles.

Study population:

12 healthy human volunteers, male or female, aged 18 to 65 years. Suitability will be determined by a full medical history and lack of significant abnormalities.

Study Site:

All visits and assessments will be carried out in the Dermatology Day Unit at St Woolos Hospital, Newport.

Number of Visits:

Each subject will be asked to attend for up to 5 visits.

Design

This will be an open randomised study in 12 subjects. 3 volunteer subjects will be randomised to each of 4 groups attending for a maximum of 5 visits. Treatment sites will be on the buttock area. The subjects will be treated with two types of

microneedle device (180μm and 280μm microneedle length) and a hypodermic needle. Subjects will have two sets of 3 needle applications followed by 3 biopsies (2x 6mm, 1x 4mm) at either 1, 4, 8 or 24 hours after application. Visual analogue scales and audio-recorded descriptions by the subjects will be used to compare pain, discomfort and perception of microneedles and hypodermic needle. Microchannel healing and re-sealing following application of the microneedles will be monitored by dermatoscope and external staining. TEWL measurements will be taken, immediately after injection, before biopsy and at 8 and 24 hours. The wound healing response will be measured by immunohistochemistry assessment of skin samples using fluorescently labelled antibodies.

Methodology

Baseline Visit Written consent will be taken. 6 areas will be marked out on the buttocks using a template (3 on each buttock). The subjects will be asked to rest for 15 minutes. Six needle applications will then be administered, 1 each of 2 different microneedle arrays and a standard hypodermic injection to each buttock. VAS scores and comments on pain and perception will be recorded. TEWL measurements will be taken at 3 sites and a control site on one buttock.

Biopsy Visit At either 1, 4, 8 or 24 (visit 3) hours after needle applications, TEWL measurements will be repeated on one buttock. The 3 sites on the other buttock will be assessed by dermatoscope then stained with methylene blue stain and photographed before biopsies are taken.

+8 hours after needle applications. Subjects in groups 1, 2 and 4 (visit 2) will be asked to rest for 15 minutes before TEWL measurements are taken at the non-biopsy and control sites.

+24 hours after needle applications. Subjects in groups 1, 2 and 3 will be asked to rest for 15 minutes before TEWL measurements are taken at the non-biopsy and control sites.

Visit 5. Removal of sutures.

Needles:

Two sizes of 36-microneedle arrays, 180μm and 280μm in length, and a hypodermic needle 0.5 x 16mm (orange) will be used.

Pain Perception:

The subjects will be asked to score pain or discomfort on a 10 cm VAS meter for each injection and their verbal comments will be audio-recorded, then transcribed.

TEWL:

TEWL will be carried out using a Tewameter (Courage & Khazaka) in a room with a controlled atmosphere (22 °C, relative humidity 45 ± 5%).

Dermatoscope

Assessment of skin channels using a dermatoscope.

Skin Staining:

Microchannel healing and re-sealing following application of the microneedles will be monitored by external staining of the skin with methylene blue stain.

Skin Biopsies:

The 3 sites to be biopsied will be injected with 2% lignocaine with adrenaline, local anaesthetic. The 2 microneedle sites will be excised with 6mm punch biopsies and closed with 2 Ethilon sutures. The standard needle site will be excised with a 4mm punch biopsy and closed with a single suture. Skin samples will be snap frozen in OCT and placed in separate, labelled, vials for transportation to Cardiff University to be processed for immunohistochemistry assessments.

Immunohistochemistry:

The skin samples will be processed to provide frozen sections for immunohistochemistry assessment of the wound healing response by identifying the up-regulation, stasis or down-regulation of specific markers for 'repair and stress responses'. Antibodies to keratins K6, K16 and K17 will be used to investigate the degree of tissue damage and an antibody to the proliferation marker, Ki67, will be used to identify any enhanced cellular proliferation that may occur.

Statistical analysis:

As this is a pilot study with small numbers, descriptive analysis will be used initially. Suitable statistical analysis may be applied to TEWL data if appropriate.

2 INTRODUCTION

2.1 BACKGROUND

The skin represents an appropriate and convenient target organ for the localised and systemic delivery of conventional drugs, gene therapies and vaccines. The skin, however, contains a protective barrier layer and therefore most drugs and larger molecules cannot permeate. Microfabricated microneedle arrays, i.e. plates of tiny needles, are able to pierce the skin barrier layer, the stratum corneum, in a minimally invasive manner to provide a series of transient pathways for the delivery of therapeutics to the underlying skin epidermis. The needles are designed to be of sufficient length to pierce the outermost barrier layer without impinging on the underlying pain receptors or blood vessels and therefore microneedle application, as opposed to conventional needle injection, is potentially pain-free and does not cause bleeding. Over the past five years the Welsh School of Pharmacy has become established as the leading UK institution for the exploitation of microfabricated microneedles for transcutaneous drug and gene delivery and were the first group worldwide to demonstrate the ability of microneedles to facilitate gene delivery and expression in excised human skin[1]. In this study the researchers involved in developing microneedle technology will collaborate with dermatology scientists in The School of Medicine, Cardiff University and clinicians at Gwent Healthcare NHS Trust to address a range of important questions relating to microneedle use in clinical practice. The lack of pain associated with the use of prototype silicon microneedles has been previously determined and reported as analogous with placebo control (no microneedles).[2,3] Preliminary studies have already identified the morphology of microneedles for efficient skin penetration.[1,4,5] Fresh human skin from cosmetic surgery has been used in previous studies to investigate the relationship between microneedle geometry and applied force and the size and shape of the resultant microchannels.

2.2 RATIONALE

Funding has recently been secured to manufacture up to 200 design-specific microneedle arrays from our principal engineering collaborators. In this project we will use our novel microneedles to test pain and perception of microneedle application in volunteers and gain further unique information regarding the rate and extent of healing of the resulting skin microchannels.

2.3 SAMPLE SIZE CALCULATION

This is a small pilot study and experience from previous studies has informed the sample size chosen.

3 OBJECTIVES

- To determine the pain and perception of microneedle application.
- To determine the damage to skin barrier function after microneedle application.
- To determine and compare the wound healing process of skin sites after application of microneedles.

4 STUDY DESIGN

4.1 SUMMARY

This will be an open randomised study in 12 subjects attending for up to 5 visits. 3 volunteer subjects will be randomised to each of 4 groups. Treatment sites will be on the buttock area. The subjects will be treated with two types of microneedle device (180μm and 280μm microneedle length) and a hypodermic needle. Subjects will have two sets of 3 needle applications followed by 3 biopsies (2x 6mm, 1x 4mm) at either 1, 4, 8 or 24 hours after application. Visual analogue scales and audio-recorded descriptions by the subjects will be used to compare pain, discomfort and perception of microneedles and hypodermic needle versus placebo. Microchannel healing and re-sealing following application of the microneedles will be monitored by external staining prior to biopsy. TEWL measurements will be taken before needle application, immediately after application and at 8 and 24 hours after application. The wound healing response will be measured by immunohistochemistry assessment of skin samples using fluorescently labelled antibodies.

4.2 RECRUITMENT

12 healthy human volunteers, male or female and aged between 18 and 65 years, will be recruited and randomised to one of four groups. Suitability will be determined by a full medical history and lack of significant abnormalities.

4.3 STUDY DURATION

It is anticipated that the study will take six months to complete and will start as soon as all ethical requirements are satisfied.

4.4 RANDOMISATION

The 12 subjects will be randomised to four groups of 3 subjects:

Group 1 will have 3 sites biopsied at 1 hour after needle applications.

Group 2 will have 3 sites biopsied at 4 hours after needle applications.

Group 3 will have 3 sites biopsied at 8 hours after needle applications.

Group 4 will have 3 sites biopsied at 24 hours after needle applications.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

(i) 18 – 65 years, male or female.

(ii) No significant clinical or skin abnormalities.

(iii) The patient has signed the consent form after the nature of the study has been fully explained.

5.2 EXCLUSION CRITERIA

(i) Patients with conditions likely to interfere with the study results.

(ii) Use of experimental drug within the previous 30 days.

(iii) Patients unable or unwilling to give consent to participate.

6 OUTCOME MEASURES

6.1 PRIMARY OUTCOME MEASURE

Assessment of the wound healing response by identifying the up-regulation, stasis or down-regulation of specific markers for 'repair and stress responses', specifically antibodies to keratins K6 K16 and K17 to assess the degree of tissue damage and Ki67 to identify any enhanced cellular proliferation that may occur.[6,7]

6.2 SECONDARY OUTCOME MEASURES

Damage to skin barrier function and time taken for skin to repair measured by trans-epidermal water loss[8] and skin staining.

Pain and perception of the different needle type injections measured by VAS and verbal comments.

7 METHODOLOGY

7.1 SUBJECT VISITS

Group 1.

Visit 1 Written consent will be taken. 6 areas will be marked out on the buttocks using a template (3 on each buttock). The subjects will be asked to rest for 15 minutes. Six needle applications will then be administered, 1 each of 180μm and 280μm 36-microneedle arrays and a standard subcutaneous injection to each buttock. VAS scores and comments on pain and perception will be recorded. TEWL measurements will be taken at 3 sites and a control site on one buttock. 50 minutes after needle applications TEWL measurements will be repeated on one buttock. The 3 sites on the other buttock will be stained and assessed before being biopsied.

Visit 2 8 hours after injection subjects will be asked to rest for 15 minutes before TEWL measurements are taken at the non-biopsy and control sites.

Visit 3 24 hours after injection subjects will be asked to rest for 15 minutes before TEWL measurements are taken at the non-biopsy and control sites.

Visit 4 10-14 days after biopsy the sutures will be removed.

Group 2.

Visit 1 Written consent will be taken. 6 areas will be marked out on the buttocks using a template (3 on each buttock). The subjects will be asked to rest for 15 minutes. Six needle applications will then be administered, 1 each of 180μm and 280μm 49-microneedle arrays and a standard subcutaneous injection to each buttock. VAS scores and comments on pain and perception will be recorded. TEWL measurements will be taken at 3 sites and a control site on one buttock.

Visit 2 3½ hours after needle applications TEWL measurements will be repeated on one buttock. The 3 sites on the other buttock will be stained and assessed before being biopsied.

Visit 3 8 hours after injection subjects will be asked to rest for 15 minutes before TEWL measurements are taken at the non-biopsy and control sites.

Visit 4 24 hours after injection subjects will be asked to rest for 15 minutes before TEWL measurements are taken at the non-biopsy and control sites.

Visit 5 10-14 days after biopsy the sutures will be removed.

Group 3.

Visit 1 Written consent will be taken. 6 areas will be marked out on the buttocks using a template (3 on each buttock). The subjects will be asked to rest for 15 minutes. Six needle applications will then be administered, 1 each of 180μm and 280μm 36-microneedle arrays and a standard subcutaneous injection to each buttock. VAS scores and comments on pain and perception will be recorded. TEWL measurements will be taken at 3 sites and a control site on one buttock.

Visit 2 7½ hours after needle applications TEWL measurements will be repeated on one buttock. The 3 sites on the other buttock will be stained and assessed before being biopsied.

Visit 3 24 hours after injection subjects will be asked to rest for 15 minutes before TEWL measurements are taken at the non-biopsy and control sites.

Visit 4 10-14 days after biopsy the sutures will be removed.

Group 4.

Visit 1 Written consent will be taken. 6 areas will be marked out on the buttocks using a template (3 on each buttock). The subjects will be asked to rest for 15 minutes. Six needle applications will then be administered: 1 each of 180μm and 280μm 36-microneedle arrays and a standard subcutaneous injection to each buttock. VAS scores and comments on pain and perception will be recorded. TEWL measurements will be taken at 3 sites and a control site on one buttock.

Visit 2 8 hours after injection subjects will be asked to rest for 15 minutes before TEWL measurements are taken at the non-biopsy and control sites.

Visit 3 23½ hours after needle applications TEWL measurements will be repeated on one buttock. The 3 sites on the other buttock will be stained and assessed before being biopsied.

Visit 4 10-14 days after biopsy the sutures will be removed.

7.1.1 BIOPSIES

The 3 sites to be biopsied will be injected with 2% lignocaine with adrenaline, local anaesthetic. The 2 microneedle sites will be excised with 6mm punch biopsies and closed with 2 Ethilon sutures. The standard needle site will be excised with a 4mm punch biopsy and closed with a single suture. Skin samples will be snap frozen in OCT and placed in separate, labelled, vials.

7.2 ASSESSMENTS

7.2.1 PAIN AND PERCEPTION

The subjects will be asked to score pain or discomfort using validated assessment tools such as a 10 cm VAS meter for each injection where 0 represents no pain and 10 cm represents the worst pain imaginable. Their verbal comments will be audio-recorded and then transcribed.

7.2.2 DERMATOSCOPE

The application sites will be assessed using a hand-held Dermatoscope to confirm the incidence of micro-channels immediately after application. In the unlikely event that there is no evidence of any microchannels, the biopsies will not be taken and the subject will be withdrawn from the study.

7.2.3 SKIN STAINING

Microchannel healing and re-sealing following application of the microneedles and hypodermic needle will be monitored by external staining of the skin with methylene blue stain just prior to the sites being biopsied. A standard volume of stain will be applied to each site. The site will then be swabbed to remove the stain from the surface. The sites will be visually assessed and photographed to determine whether the microchannels are still open or have re-sealed.

7.2.4 TEWL

Assessments will be made using a Tewameter (Courage & Khazaka) and will be carried out in a room with a controlled atmosphere (22 °C, relative humidity 45 ± 5%).[8]

Assessments will be made, immediately after injection prior to biopsies for groups 1 & 2, and at 8 and 24 hours after injection.

7.2.5 IMMUNOHISTOCHEMISTRY

Skin samples will be identified by subject initials, biopsy site and the subject's unique study number. Samples will snap frozen in OCT and placed in separate labelled vials for transportation to the Department of Dermatology, Wales College of Medicine, Cardiff University to be processed for immunohistochemistry assessment.

Frozen sections will be prepared for immunohistochemistry assessment of the wound healing

response by identifying the up-regulation, stasis or down-regulation of specific markers for 'repair and stress responses'. Antibodies to keratins K6, K16 and K17 will be used to investigate the degree of tissue damage and an antibody to the proliferation marker, Ki67, will be used to identify any enhanced cellular proliferation that may occur.[6,7]

8 MONITORING AND DATA HANDLING

All study documentation may be examined by regulatory authorities for monitoring the quality of the research during the course of the study.

All Case Report Forms (CRFs) and skin samples will be identified only by subject initials, date of birth, and their unique study number. All original consent forms and CRFs will be kept in a secure location.

The results of this study will be confidential and any report or publication arising from it will not reveal the subject's identity. All handling and processing of data will comply with the Data Protection Act, 1998.

9 STATISTICAL ANALYSIS

As this is a pilot study with small numbers descriptive analysis will be used initially. Suitable statistical analysis may be applied to TEWL data if appropriate.

10 ETHICAL CONSIDERATIONS

10.1 ETHICS COMMITTEES APPROVAL

Before starting the study, ethical approval will be obtained from the Local Research Ethics Committee. Approval will also be sought from Gwent Healthcare NHS Trust Scrutiny and Risk Review Committees.

10.2 INFORMED CONSENT

The principles of the Declaration of Helsinki will be adhered to. A written explanation of the study will be given to all volunteer subjects at least one week before their first study visit.

At the first study visit all subjects will be required to give their written consent before starting the study and after the nature of the study has been fully explained. The subjects will be informed that they are able to withdraw from the study at any stage without being required to state a reason and without prejudice to any future care.

11 SIGNATURES

Study Protocol Approved by:

Principal Investigator:

..... Date: .....

Dr. A Anstey, Consultant Dermatologist, Royal Gwent Hospital, Newport

Co-investigators:

..... Date: .....

Dr J. Birchall, Lecturer in Drug Delivery, Welsh School of Pharmacy, Cardiff. (Joint supervisor)

..... Date: .....

Dr D John, Senior Lecturer, Welsh School of Pharmacy, Cardiff. (Joint supervisor)

..... Date: .....

M I Haq, Postgraduate researcher, Welsh School of Pharmacy, Cardiff. (PhD Student)

..... Date: .....

Dr R Porter, Lecturer of Cell & Molecular Biology, College of Medicine, Cardiff University

12 REFERENCES

1 Birchall J, Coulman S, Pearton M, Allender C, Brain K, Anstey A, Gateley C, Willke N and Morrissey A. *Cutaneous DNA delivery and gene expression in ex vivo human skin explants via wet-etch microfabricated microneedles.* J. Drug Targeting 13 (2005) 415-21.

2 Henry S, McAllister DV, Allen M and Prausnitz MR. *Microfabricated Microneedles: A Novel Approach to Transdermal Drug Delivery.* J Pharm Sci 87 (1998) 922-925.

3 Kaushik S, Hord AH, Denson DD, McAllister DV, Smitra S, Allen MG and Prausnitz MR. *Lack of pain associated with Microfabricated Microneedles.* Anaesthesia and Analgesia 92 (2001) 502-504.

4 Chabri F, Bouris K, Jones T, Barrow D, Hann A, Allender C, Brain K and Birchall J. *Microfabricated silicon microneedles for nonviral cutaneous gene delivery.* Br J Dermatol 150 (2004) 869-877.

5 Coulman SA, Barrow D, Anstey A, Gateley C, Morrissey A, Willke N, Allender C, Brain K and Birchall JC. *Minimally invasive delivery of macromolecules and plasmid DNA via microneedles.* Current Drug Delivery 1 (2006) 65-75.

6 Porter RM, Lunny DP, Rugg EL, Morley SM, Ogden PH, McLean WHI, and Lane EB. *Keratin 15 has a unique role to keratin 14 in basal epithelial cells.* Lab Invest. 80 (2000), 1701-1710.

7 Porter, R. M., Reichelt, J., Lunny, D. P., Magin, T. M. and E. B. Lane. 1998. *The relationship between hyperproliferation and epidermal thickening in a mouse model for BCIE.* J. Invest. Dermatol. 110:951-957.

8 Tupker RA, Pinnagoda J, "Measurement of Transepidermal Water Loss by Semiopen Systems", *Handbook of Non-invasive Methods and the Skin*, ed Jorgen Serup et al. Taylor & Francis Group, Florida, 2006. pp.383-396.

APPENDIX IV

Participant rules post-application

Version 1.1 09/03/2007

RULES AND REQUIREMENTS FOR VOLUNTEER SUBJECT

AN ASSESSMENT OF PAIN, PERCEPTION AND WOUND HEALING FOLLOWING APPLICATION OF SILICON MICRONEEDLE ARRAYS

- Absolutely **NO** Alcohol 24 hours before or during the 24 period of the trial.
- Absolutely **NO** recreational drug use or use of prescription medicines other than regular medicines as disclosed in your medical history.
- Absolutely **NO** moisturising creams or makeup to be used on buttocks from 8pm the night before the trail **and** during the 24 period of the trial.
- Absolutely **NO** bathing for the duration of the trial.

****Please arrive exactly at the times stated on your timetable****

Location:

St Woolos Hospital
Dept. of Dermatology
2nd Floor Main Building

APPENDIX V

Example of timetable given to each participant

Timetable for Participant 1

Location: Dermatology Department, St Woolos Hospital, Newport

Updated Times (To complete on day)	Start Time	Saturday 10th March	Time Required
	10.35 AM	**ARRIVAL & START**	2hrs 30 mins
	1.05 PM	Break	3hrs 40 mins
	8:25 PM	**ARRIVAL VISIT 2**	40 mins
	9:05 PM	END of Day 1	
	Start Time	**Sunday 11th March**	
	12:20 PM	**ARRIVAL AND START**	45 mins
	1:05 PM	END	

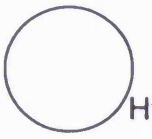
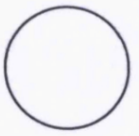
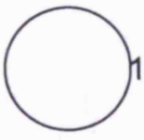
Thank you for taking part.

Please return in 10-14 days to have stitches removed.

APPENDIX VI

Example Template of application sites to guide physician

Application Sites for Participant X

2nd Left Buttock (TEWL)		1st Right Buttock (Perception)	
 Hypodermic	1st Application	3rd Application	180 
 280	3rd Application	1st Application	280 
 180	2nd Application	2nd Application	Hypodermic 

APPENDIX VII

Example of timetable given to each coordinator on trial day

Dr Kalavala's Timetable

Saturday 10th March

Time	Room 1 (Perception)		Room 2 (TEWL)	Time	EXTRA BIOPSY (AA)
08:15	ALL STAFF ARRIVAL & SET UP				BED 2
09:05	**280µm microneedle** length array applied **site C**, stained, VAS recorded **(6)**	09:05		09:05	
09:10		09:10		09:10	
09:15	**Hypodermic** needle applied **site B**, stained, VAS recorded **(6)**	09:15		09:15	
09:20		09:20		09:20	
09:25	**180µm microneedle** length array applied **site A**, stained, VAS recorded **(6)**	09:25	**180µm microneedle** applied **site B** **Hypodermic** applied **site A** **280µm microneedle** applied **site C** **(7)**	09:25	
09:55	**180µm microneedle** length array applied **site A**, stained, VAS recorded **(7)**	09:55		09:55	
10:00		10:00		10:00	
10:05	**280µm microneedle** length array applied **site B**, stained, VAS recorded **(7)**	10:05		10:05	
10:10		10:10		10:10	
10:15	**Hypodermic** needle applied **site C**, stained, VAS recorded **(7)**	10:15		10:15	
10:20		10:20		10:20	
10:25		10:25	**280µm microneedle** applied **site B** **Hypodermic** applied **site C** **180µm microneedle** applied **site A** **(6)**	10:25	
10:30		10:30		10:30	
10:50	**280µm microneedle** length array applied **site B**, stained, VAS recorded **(1)**	10:50		10:50	
10:55		10:55		10:55	
11:00	**Hypodermic** needle applied **site C**, stained, VAS recorded **(1)**	11:00		11:00	
11:05		11:05		11:05	
11:10	**180µm microneedle** length array applied **site A**, stained, VAS recorded **(1)**	11:10		11:10	
11:15		11:15		11:15	
11:30		11:30	**Hypodermic** applied **site C** **280µm microneedle** applied **site B** **180µm microneedle** applied **site A** **(12)**	11:30	
11:35		11:35		11:35	
11:50		11:50		11:50	**site B** Biopsy **(1)**

APPENDIX VIII

Case report form (CRF)

PERCEPTION AND WOUND HEALING FOLLOWING APPLICATION OF SILICON MICRONEEDLE ARRAYS

Participant Initials:

Participant Study Number:

Department of Dermatology
Royal Gwent Hospital
Cardiff Road
Newport
Gwent NP20 2UB

Visit 1 : Screen visit	Date: D D/ M M /2007

PARTICIPANT DETAILS
(Complete & Tick as appropriate)

Date of Birth dd.mm.yy • • **Age** (18 +)

Male / Female **Race** _____ **Skin Type** _____

ELIGIBILITY: The subject is: **Yes** **No**

Male or female, aged 18-65 years

Has no significant clinical or skin abnormalities
likely to interfere with the results

Has signed the consent form after the nature of the study
has been fully explained

The subject is not pregnant or lactating (females only)

Only if the answer to each question is yes, is the patient is eligible to take part in the study.

Signed: **Date:**
(Investigator)

Group Assignment: 1

Visit 1 : Screen visit	Date: D D/ M M /2007

Medical History

None

Description	Date Start	Date Stop
		Ongoing
		Ongoing
		Ongoing

Current Medication

None

Therapy & Indication	Date Start	Date Stop
		Ongoing
		Ongoing
		Ongoing

Gwent Healthcare
NHS Trust

Royal Gwent Hospital
Cardiff Road
Newport
South Wales
NP20 2UB
Tel: 01633 234234

Ymddiriedolaeth GIG
Gofal Iechyd Gwent

Ysbyty Brenhinol Gwent
Ffordd Caerdydd
Casnewydd
De Cymru
NP20 2UB
Ffôn: 01633 234234

VOLUNTEER SUBJECT CONSENT FORM

An Assessment Of Pain, Perception And Wound Healing Following Application Of Silicon Microneedle Arrays

Please initial each box to indicate that you have read and agree to each statement.

1. I confirm that I have read and understand the information sheet, version 2.0 (05.01.07) for this study, that I have had the opportunity to ask questions and that I have received satisfactory answers to the questions I have asked. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of any of my study notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I understand that anonymous data about me, as collected for this study, including information about my health may be used in publications about the study. ☐
5. I am happy for you to inform my GP that I shall be participating in this study. ☐
6. I consent to photographs being taken of me as a record for microchannel staining, for teaching healthcare professionals or for medical publications only. I understand that if the photographs are used for teaching purposes or medical publications, my identity will not be disclosed. ☐
7. I agree to take part in the above study. ☐

NAME OF SUBJECT	DATE	SIGNATURE
(Please print your name and date your own signature)		
NAME OF PERSON TAKING CONSENT	DATE	SIGNATURE
(Investigator/ subinvestigator)		

Gwent Healthcare
NHS Trust

Royal Gwent Hospital
Cardiff Road
Newport
South Wales
NP20 2UB
Tel: 01633 234234

Ymddiriedolaeth GIG
Gofal Iechyd Gwent

Ysbyty Brenhinol Gwent
Ffordd Caerdydd
Casnewydd
De Cymru
NP20 2UB
Ffôn: 01633 234234

VOLUNTEER SUBJECT CONSENT FORM – AUDIO RECORDING

An Assessment Of Pain, Perception And Wound Healing Following Application Of Silicon Microneedle Arrays

Please initial each box to indicate that you have read and agree to each statement.

1. I confirm that I have read and understand the information sheet, version 2.0 (05.01.07) for this study, that I have had the opportunity to ask questions and that I have received satisfactory answers to the questions I have asked. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that my participation will be audio recorded and transcribed later and this will be added to my study notes, which may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I consent to audio recordings being taken of me as a record for perception and these will be transcribed and held with my records and used for teaching healthcare professionals or for medical publications only. I understand that if the audio recordings or transcribed notes are used for teaching purposes or medical publications, my identity will not be disclosed. ☐
5. I agree to take part in the above study. ☐

NAME OF SUBJECT	DATE	SIGNATURE
(Please print your name and date your own signature)		
Name of Person taking consent (Investigator/ subinvestigator)	Date	Signature

Visit 2:

PAIN Perception

&

TEWL

Date:

D D/ M M /2007

Time:

h h : m m

1. Audio Recording

Q:

"Hi, hope you had a safe journey in today, can I just check your name please. Ok, here's what we are going to do: Please lie down on the coach and make yourself comfortable. We are going to apply the devices we are using in the study and we are going to record the SENSATION that you feel when the things are being applied. Ok?

[TEST TAPE RECORDER]

I need to check this is working first of all. Just tell me your name and tell me where you are from.

[PLAY BACK TAPE] [START RECORDING]

As you can hear, to get information on the tape we will need you to speak loudly and as clearly as possible.

First thing we are going to do is to apply a dummy device just you show you how we want to do this. So we are just going to push this device onto the skin and we want you to explain exactly what you are feeling and whether you feel any SENSATION at all. And it's very important that we do get some spoken words as this is the information we are actually using the study. Ok?

Dr Kalavala will now apply the device.

[APPLY PLACEBO DEVICE - take any notes below]

[DURING APPLICATION] **Please describe how that is feeling at the moment.**

[IMMEDIATELY AFTER] **Do you feel anything now?**

What we are going to do now is apply the first of our three devices and record your commentary and feelings whilst they are applied. So similarly to what we just did, please describe the SENSATIONs clearly and loudly for the recording.

[DURING APPLICATION] **Please describe how that is feeling at the moment.**

[IMMEDIATELY AFTER] **Do you feel anything now?"**

Comments: Please record key comments below.

2. FIRST APPLICATION TO SITE B

3. VAS

Present PAIN Intensity (PPI): Visual Analogue Scale (VAS) (To be taken immediately after needle application using VAS tool)

Q:

"To measure the PAIN intensity please move the slider to the position that best represents the PAIN felt during and after the application. The left end of the scale represents no PAIN and the right end of the scale represents the worst PAIN imaginable."

_____mm

NO PAIN _____ **WORST PAIN IMAGINEABLE**

4. SENSATION Rating Index

"You will now be shown some descriptive words. As each word is read out to you please state if it describes a SENSATION you felt during or after the application. Rating the feeling as either none, mild, moderate or severe. If you are unsure of the meaning please ask."

FIRST APPLICATION TO SITE B

SENSATION Rating Index (PRI):

The words below describe average **SENSATION**. Place a tick [✓] in the column that represents the degree to which you feel that type of **SENSATION**.

			None		Mild		Moderate		Severe
a	**Throbbing**	**0**		**1**		**2**		**3**	
	Shooting	0		1		2		3	
	Stabbing	**0**		**1**		**2**		**3**	
	Sharp	0		1		2		3	
	Cramping	**0**		**1**		**2**		**3**	
	Gnawing	0		1		2		3	
	Hot-Burning	**0**		**1**		**2**		**3**	
	Aching	0		1		2		3	
	Heavy	**0**		**1**		**2**		**3**	
	Tender	0		1		2		3	
	Splitting	**0**		**1**		**2**		**3**	
b	Tiring- Exhausting	0		1		2		3	
	Sickening	**0**		**1**		**2**		**3**	
	Fearful	0		1		2		3	
	Punishing-Cruel	**0**		**1**		**2**		**3**	

" Here are some other words to describe sensation. Place a tick [✓] in the column that represents the degree to which you feel that type of SENSATION.

			None		Mild		Moderate		Severe
	Pressing	0		1		2		3	
	Pricking	**0**		**1**		**2**		**3**	
	Cold	0		1		2		3	

5. Evaluative overall intensity of total PAIN experience.

Place a tick [✓] in the appropriate column.

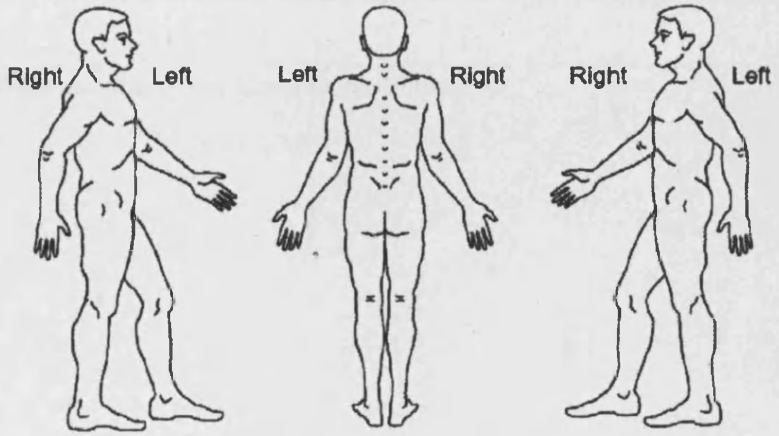
"Looking at the words, which one describes the overall intensity of the total PAIN you experienced during and after the application?"

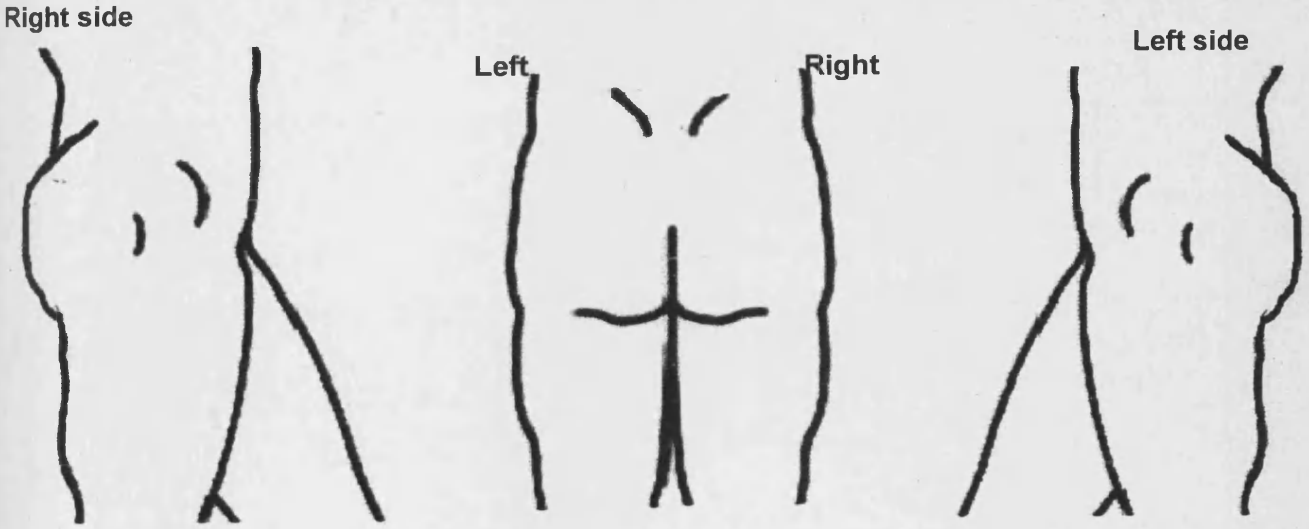
Evaluative Intensity Score		
0	**No pain**	
1	**Mild**	
2	**Discomforting**	
3	**Distressing**	
4	**Horrible**	
5	**Excruciating**	

6. Location of application.

"Please clearly mark on the diagram below where you feel the application occurred using a cross. And circle where, if at all, any PAIN is felt."

X = first application
O = location of PAIN





7. SECOND APPLICATION TO SITE C

"What we are going to do now is apply the second of our three devices. So as you just did, please describe the SENSATIONs clearly and loudly for the recording. Again do say if you do not feel anything.

Dr Kalavala will now apply the device.

[DURING APPLICATION] **Please describe how that is feeling at the moment.**

[IMMEDIATELY AFTER] **Do you feel anything now?"**

Comments: Please record key comments below.

8. VAS

Present PAIN Intensity (PPI): Visual Analogue Scale (VAS) (To be taken immediately after needle application using VAS tool)

Q:

"To measure the PAIN intensity please move the slider to the position that best represents the PAIN felt during and after this application."

_____mm

NO PAIN _____ **WORST PAIN IMAGINEABLE**

9. SENSATION Rating Index

"As each word is read out to you please state if it describes a SENSATION you felt during or after this application. Rating the feeling as either none, mild, moderate or severe."

SECOND APPLICATION TO SITE C

SENSATION Rating Index (PRI):

The words below describe average **SENSATION**. Place a tick [✓] in the column that represents the degree to which you feel that type of **SENSATION**.

			None		Mild		Moderate		Severe
a	**Throbbing**	**0**		**1**		**2**		**3**	
	Shooting	0		1		2		3	
	Stabbing	**0**		**1**		**2**		**3**	
	Sharp	0		1		2		3	
	Cramping	**0**		**1**		**2**		**3**	
	Gnawing	0		1		2		3	
	Hot-Burning	**0**		**1**		**2**		**3**	
	Aching	0		1		2		3	
	Heavy	**0**		**1**		**2**		**3**	
	Tender	0		1		2		3	
	Splitting	**0**		**1**		**2**		**3**	
b	Tiring- Exhausting	0		1		2		3	
	Sickening	**0**		**1**		**2**		**3**	
	Fearful	0		1		2		3	
	Punishing-Cruel	**0**		**1**		**2**		**3**	

" Here are some other words to describe sensation. Place a tick [✓] in the column that represents the degree to which you feel that type of SENSATION.

			None		Mild		Moderate		Severe
	Pressing	0		1		2		3	
	Pricking	**0**		**1**		**2**		**3**	
	Cold	0		1		2		3	

5. Evaluative overall intensity of total PAIN experience.

Place a tick [✓] in the appropriate column.

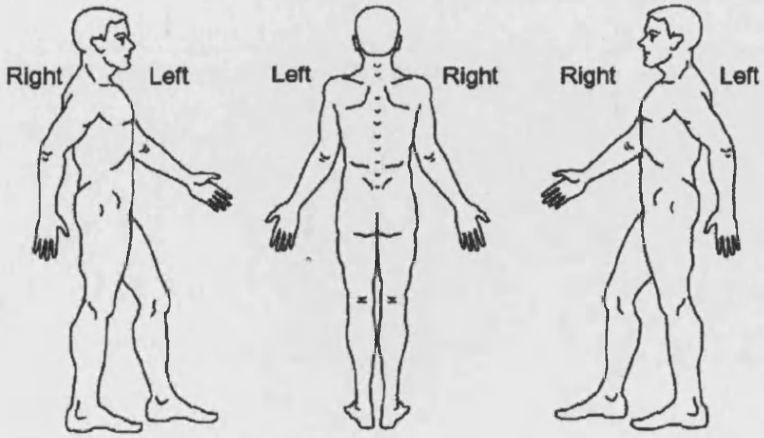
"Looking at the words, which one describes the overall intensity of the total PAIN you experienced during and after the application?"

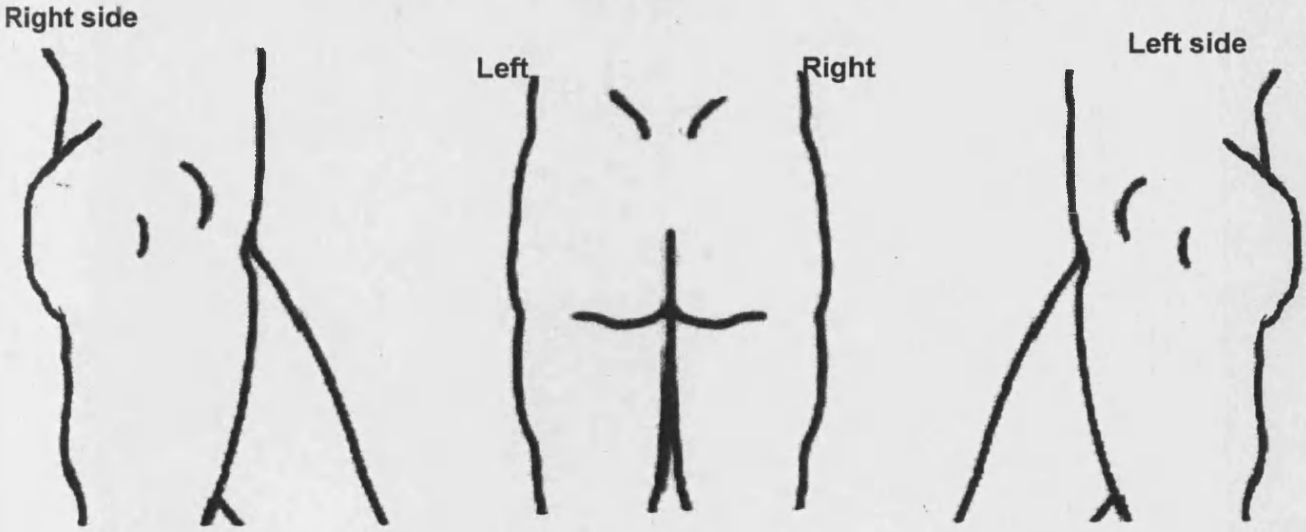
Evaluative Intensity Score		
0	**No PAIN**	
1	**Mild**	
2	**Discomforting**	
3	**Distressing**	
4	**Horrible**	
5	**Excruciating**	

11. Location of application.

"Please clearly mark on the diagram below where you feel the application occurred using a cross. And circle where, if at all, any PAIN is felt."

X = second application
O = location of PAIN





12. THIRD APPLICATION TO SITE A

"What we are going to do now is apply the final device. So as you just did, please describe the SENSATIONs clearly and loudly for the recording. Again do say if you do not feel anything."

Dr Kalavala will now apply the device.

[DURING APPLICATION] **Please describe how that is feeling at the moment.**

[IMMEDIATELY AFTER] **Do you feel anything now?"**

Comments: Please record key comments below.

13. VAS

Present PAIN Intensity (PPI): Visual Analogue Scale (VAS) (To be taken immediately after needle application using VAS tool)

Q:

"To measure the PAIN intensity please move the slider to the position that best represents the PAIN felt during and after this application."

_____mm

NO PAIN _____ **WORST PAIN IMAGINEABLE**

14. SENSATION Rating Index

"As each word is read out to you please state if it describes a SENSATION you felt during or after this application. Rating the feeling as either none, mild, moderate or severe."

THIRD APPLICATION TO SITE A

SENSATION Rating Index (PRI):

The words below describe average **SENSATION**. Place a tick [✓] in the column that represents the degree to which you feel that type of **SENSATION**.

			None		Mild		Moderate		Severe
a	**Throbbing**	**0**		**1**		**2**		**3**	
	Shooting	0		1		2		3	
	Stabbing	**0**		**1**		**2**		**3**	
	Sharp	0		1		2		3	
	Cramping	**0**		**1**		**2**		**3**	
	Gnawing	0		1		2		3	
	Hot-Burning	**0**		**1**		**2**		**3**	
	Aching	0		1		2		3	
	Heavy	**0**		**1**		**2**		**3**	
	Tender	0		1		2		3	
	Splitting	**0**		**1**		**2**		**3**	
b	Tiring- Exhausting	0		1		2		3	
	Sickening	**0**		**1**		**2**		**3**	
	Fearful	0		1		2		3	
	Punishing-Cruel	**0**		**1**		**2**		**3**	

" Here are some other words to describe sensation. Place a tick [✓] in the column that represents the degree to which you feel that type of SENSATION.

			None		Mild		Moderate		Severe
	Pressing	0		1		2		3	
	Pricking	**0**		**1**		**2**		**3**	
	Cold	0		1		2		3	

5. Evaluative overall intensity of total PAIN experience.

Place a tick [✓] in the appropriate column.

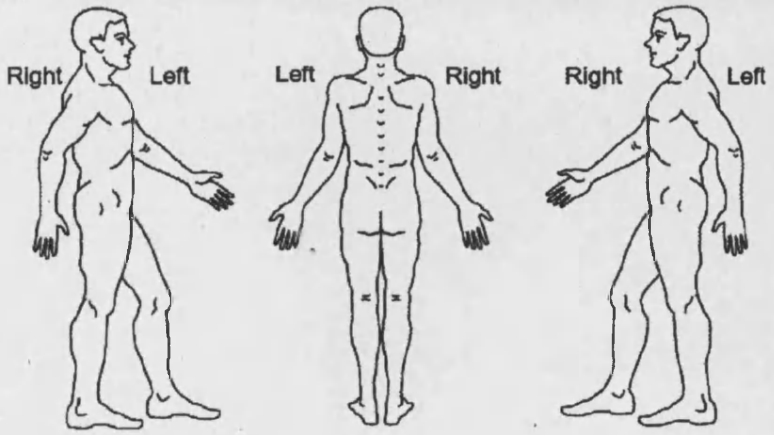
"Looking at the words, which one describes the overall intensity of the total PAIN you experienced during and after the application?"

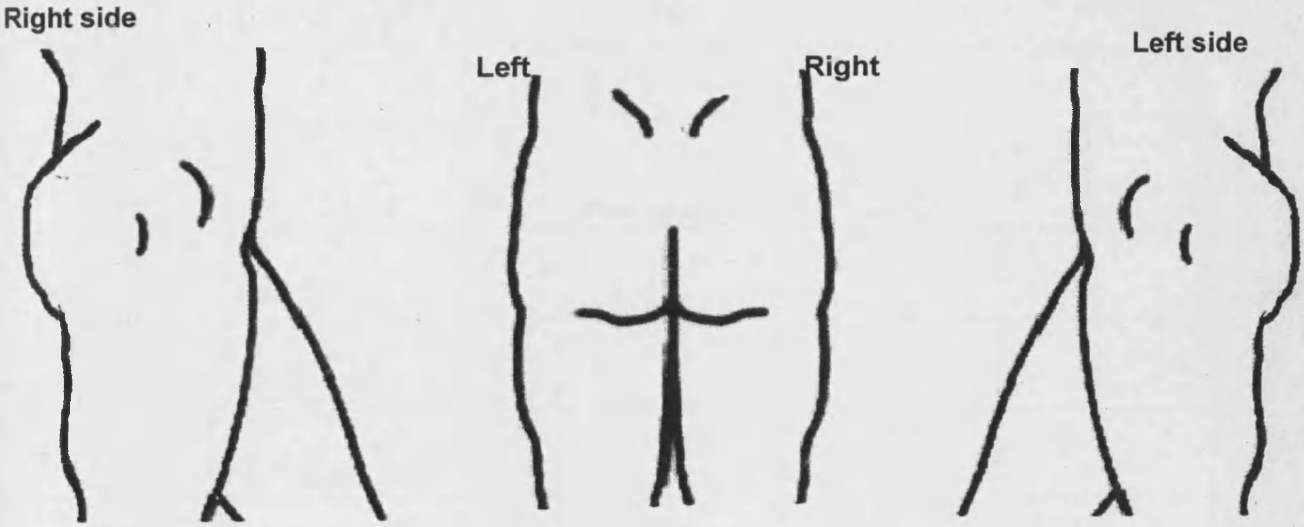
Evaluative Intensity Score		
0	**No PAIN**	
1	**Mild**	
2	**Discomforting**	
3	**Distressing**	
4	**Horrible**	
5	**Excruciating**	

6. Location of application.

"Please clearly mark on the diagram below where you feel this application occurred using a cross. And circle where, if at all, any PAIN is felt."

X = third application
O = location of PAIN





Comments: Please add any additional comments below.

Scoring: (For Internal Use Only)

		First Application	Second	Third
I-a	S-PRI (Sensory **PAIN** Rating Index)			
I-b	A-PRI (Affective **PAIN** Rating Index)			
I-a+b	T-PRI (Total **PAIN** Rating Index)			
II	PPI-VAS (Present **PAIN** Rating Intensity-Visual Analogue Scale)			
III	Evaluative overall intensity of total **PAIN** experience			

Clinical Record Form	Department of Dermatology, Royal Gwent Hospital, Newport	Participant Number:	1

Visit 2: Stain Photographs	Date: D D/ M M /2007	Time: h h : m m

Instruction: [STAIN IMMEDIATELY BEFORE BIOPSY]

1. **Cover application sites with Methylene Blue Stain.**
2. **Allow 10 minutes to settle.**
3. **Wipe off excess using Ethanol wipes.**
4. **Check with Dermatoscope and photograph.**
5. **Write the filename below once photograph is uploaded to PC.**

Site B:

Time: h h : m m

File name:

Site C:

Time: h h : m m

File name:

Site A:

Time: h h : m m

File name:

Visit 2: Baseline TEWL	Date: D D/ M M /2007	Time: h h : m m

1. **Turn participant onto other side to take TEWL from applications to their second buttock.**

2. **Participant to rest for 15 minutes.**

3. CONTROL SITE TEWL measurement:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

4. PRE-application TEWL measurement site A:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

5. POST-application TEWL measurement site A:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

6. PRE-application TEWL measurement site C:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

7. POST-application TEWL measurement site C:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

8. PRE-application TEWL measurement site B:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

9. POST-application TEWL measurement site B:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Clinical Record Form	Department of Dermatology, Royal Gwent Hospital, Newport	Participant Number:	1

Visit 3: TEWL @ 8hrs	Date: D D/ M M /2007	Time: h h : m m

1. TEWL measurement @ 8hrs

CONTROL SITE TEWL measurement:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Site A:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Site C:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Site B:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Visit 4: TEWL @ 24hrs	Date: D D/ M M /2007	Time: h h : m m

2. TEWL measurement @ 24hrs

CONTROL SITE TEWL measurement:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Clinical Record Form Department of Dermatology, Royal Gwent Hospital, Newport **Participant Number:** 1

Site A:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Site C:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Site B:

Time: h h : m m

Mean TEWL:

			.		g/hm^2

Standard Deviation

			.		

Adverse Event Form

None

Event 1 Diagnosis	Severity	Start Date	Stop Date
			Ongoing

Relationship to treatment

Action taken

Definitely unrelated Unlikely Possible Probable Definitely related

Event 2 Diagnosis	Severity	Start Date	Stop Date
			Ongoing

Relationship to treatment

Definitely unrelated Unlikely Possible Probable Definitely related

Action taken

Comments

Clinical Record Form Department of Dermatology, Royal Gwent Hospital, Newport **Participant Number:** 1

Visit 5: Suture Removal	Date: D D/ M M /2007	Time: h h : m m

Participant returned for removal of suture ☐

Payment Form Signed ☐

Any other comments:

End of Study

Date: : D D/ M M /2007

Study Completion

The subject completed the study

Discontinuation

Adverse event – complete adverse event form

Subject request

Major protocol violation

Other – please specify

As principle Investigator, I have reviewed this CRF and found it to be a complete and accurate record.

Signature Date: • •

APPENDIX IX

Example of complete verbatim transcription of audio-recorded data from the clinical trial

Transcribed commentary for participant 8

PLACEBO

P8: Yer, umm, I can feel these, umm, endo-pressure, like someone is pressing against me with perhaps a rough fingertip. And that's about it now.

FIRST APPLICATION:

P8: again it feels like, err, fingertip. Umm, a bit of pressure, nothing else.

P8: its not really pain, there is a sensation there but not really pain. It feels like err... you can tell its something sharp but its not painful as such over the overall area.

SECOND APPLICATION:

P8: ok, yer, that again feels similar to the first. Umm, pressing a bit more, err, it feels a little bit sharper, and you can tell there is something going into the skin but it's not a sharp pain, but you can feel something. Umm, similar to the first but a little bit more intense. Umm, but I wouldn't call it pain.

THIRD APPLICATION

P8: Yes that's a needle!

p8: ok, yep, it felt like a needle. Very different, can tell something pressing on the skin and going in, and then coming out as well. Umm, it feels like the injections you get from the nurse. Umm, again it is, there is an increase in pressure as it pierces the skin and then its not so bad. But you can definitely feel in terms of the other two umm, its far more intense, umm,

FINAL COMMENTS:

Dai: and the final thing is the box for comments you have on the 3 application. Perhaps the differences in between them.

P8: differences in between them... well the 3rd application you could tell was quite different to the other two, umm, and it did feel like pain a needle if I am honest. Umm, so I could feel it going in. with regards to comparing that to the other 2 applicators. Umm, yer you felt very different to, the application 2 felt more, umm, you could feel it more. If that's of any help

P8: yer you could tell that more pressure was being applied, umm, and you could feel. I suppose the only way of describing it is if you get like err, like some leaves and they've got little prickly things on the back and you are pressing on them. Like a nettle leaf but without the actual sting in it. So you can feel that its gone in, it feels a bit like that so the more you press on it the more prickly but again it doesn't feel painful. You can sense that are the pricks there... umm, that's more for the first one. But yer, 3 felt far more sort of penetrating than the other 2.

APPENDIX X

Summarised and tabulated data from all the collection methods for each participant

Data for Participant 1

Application order:

Device 1	Device 2	Device 3
280µm	Hypodermic	180µm

VAS:

Needle Device	VAS/ cm
180µm	0.95
280µm	0.14
Hypodermic	0.25

Sensation words:

	180µm		280µm		Hypodermic	
	Mild	Moderate	Mild	Moderate	Mild	Moderate
Stabbing	✓				✓	
Sharp			✓			✓
Aching			✓			
Heavy		✓		✓		
Tender	✓				✓	
Pressing		✓	✓			
Pricking					✓	

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain		✓	
1	Mild			✓
2	Discomforting	✓		
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
280µm	180µm	hypodermic

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"No can't really feel anything. There was a feeling of pressing wax in. I feel pressure, pressure and cold."	"There's pressure, slightly sharp feeling, umm, much like the first one (280), a little bit more uncomfortable and a greater feeling of pressure. In fact some dull pain this time. Umm, again it's quite mild, now pressure relief, release and now it's gone." [comment on VAS]: "Again not a huge amount of pain so (moves VAS meter), much like the second one (hypodermic) but err more than the first one (280)... but it's a different type of pain to the second one (hypodermic). The second one (hypodermic) was quite sharp type of pain, that was more of a dull pressure pain."	Fine, I can just feel pressure, erm stronger than last time (placebo), no particular discomfort, perhaps getting more so like a bruise now, like a dull pressure. And now its coming, leaving. Slight stinging now, but now it's gone." [comment on VAS]: "It would be minimal, no real pain."	"Sharp pain, more uncomfortable than last time (280). Feels err like a normal injection, so a sharp pain going in and leaving and now it's gone. Far less pressure than last time (280)."	"Erm, the first one (280) was the easiest to tolerate. Erm there is more a feeling of pressure, more like somebody's just pushing onto the skin without actually breaking the skin. Umm the second one (hypodermic) felt like a sharp needle application, so sort of you know a sub cut injection. Umm it was sharp, and as as uncomfortable as subcut injections normally are. Umm not crazily discomforting. The last (180) umm application felt heavier and more pressure. Same kind of feeling as the first one (280), umm with perhaps just err bit more of err err pressure pain in the middle, perhaps just a sharp in the middle. So like the first one (280) but it felt like perhaps its pushing a bit deeper."

Data for Participant 2

Application order:

Device 1	Device 2	Device 3
Hypodermic	280µm	180µm

VAS:

Needle Device	VAS/ cm
180µm	0.6
280µm	0
Hypodermic	0.54

Sensation words:

	180µm		280µm		Hypodermic
	Mild	Moderate	Mild	Moderate	Mild
Stabbing	✓				✓
Sharp	✓				✓
Heavy		✓	✓		
Pressing		✓		✓	✓
Pricking	✓				✓
Cold		✓			

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain		✓	
1	Mild			✓
2	Discomforting	✓		
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomforable (deduced from comments)		
Least	Middle	Most
280µm	180µm	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"Um, I can't really feel a lot, I feel just a bit of pressure on the skin."	"That one feels cold and pressing. Felt slightly sharp, that one does."	"It feels more like pressing, but there is no sharp pain at the moment. Can't feel anything at all now."... "I would say there isn't any pain."	"Felt er like a sharp scratch. I can't feel anything else." [comment on PRESSING]: "Yep, but I think that was before the application."	"The first one (hypodermic) was the normal needle, and the second one (280) was the least painful, or anything like that."

Data for Participant 3

Application order:

Device 1	Device 2	Device 3
Hypodermic	180µm	280µm

VAS:

Needle Device	VAS/ cm
180µm	0
280µm	0
Hypodermic	1.14

Sensation words:

	180µm		280µm		Hypodermic	
	Mild	Moderate	Mild	Moderate	Mild	Moderate
Throbbing					✓	
Shooting					✓	
Stabbing						✓
Sharp						✓
Hot-burning					✓	
Aching					✓	
Heavy	✓		✓			
Tender					✓	
Splitting					✓	
Punishing-cruel					✓	
Pressing		✓		✓	✓	
Pricking						✓
Cold					✓	

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain	✓	✓	
1	Mild			
2	Discomforting			✓
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
180µm & 280µm	-	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"I'm just feeling a slight pressure."	"Feel a slight pressure, continued pressure. There is no other sensation."	"Pressure."	"Just a slight prick. Maybe, a slight decrease. Sensation, obviously of the needle going through."	"I thought the first (hypodermic) was hypodermic needle. Erm when it entered the skin there was, there wasn't so much pressing, but there was more of err, there was a slight pain and it continued and umm that lasted until, until it was removed. It was quite cold as well. Umm in terms of the, the second (180) and third (280) applications were similar. Umm there was no pain at all, umm it was just pressing, but I wouldn't say it was a pain sensation. There was just a sensation of pressing down on the skin. So the last two (180, 280) were the most comfortable out of the three."

Data for Participant 4

Application order:

Device 1	Device 2	Device 3
280µm	Hypodermic	180µm

VAS:

Needle Device	VAS/ cm
180µm	0
280µm	0
Hypodermic	0.11

Sensation words:

	180µm		280µm		Hypodermic
	Mild	Moderate	Mild	Moderate	Mild
Stabbing					✓
Sharp			✓		✓
Heavy	✓				
Pressing	✓		✓		
Pricking	✓		✓		✓
Cold					✓

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain	✓	✓	
1	Mild			
2	Discomforting			✓
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
180µm	280µm	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"A very light prick, but no pain whatsoever."	"Umm, that was, it just felt like a big press, it just felt like a big press. And just new the end of it, I think as you were withdrawing it I felt it, a little prick but it was quite comfortable overall." [comment on VAS]: "I would say in terms of pain it would be probably nearer what the first one (280) was. Oh actually even less than the first one (280), so virtually no pain." [comment on HEAVY]: "A little heavy, a little heavy in terms of the press, I mean is that what heavy means? I would say 0 to 1." [comment on PRICKING]: "There was no pricking but I felt pricking at the end when he was taking it out. So I would say, you know, between 0 and 1... felt it while he was withdrawing the application."	"It's umm, very tiny prick, err, again not painful at all. Just barely felt it." [comment on SHARP]: "No, but I would probably classify it as none, its probably not mild, so in between." [comments on PRICKING & PRESSING]: "A bit, in the middle (of none and mild)."	"Ok, its quite, I felt that far more than the first one (280). It was a sharp prick this time, and a bit more painful than the first one." [comment on VAS]: "It's a bit more than the other one." [comments on STABBING & SHARP]: "Between 0 and 1 for stabbing and sharp."	"Overall no pain, if only injections were like that [laughs]."... "Out of those three I probably thought the second (hypodermic) was the most uncomfortable. Umm third one (180) perhaps the most comfortable. Err, err, I barely felt the first one (280). But I did feel a tiny pricking sensation (for the 280), so if I was to. Out of all the three I would probably prefer the third one (180) for an injection. Umm, apart from that they were all pretty minimal in terms of pain, or you know, umm, except second one (hypodermic) is probably highest in terms of pain, but they were all pretty minimal. I would prefer all three, I mean if I was given a choice its no problem."... "Err, I felt no the side effects apart from the odd press in the third one (180) and prickly sensations at times but that's it."

Data for Participant 5

Application order:

Device 1	Device 2	Device 3
180µm	Hypodermic	280µm

VAS:

Needle Device	VAS/ cm
180µm	2.3
280µm	1
Hypodermic	2.3

Sensation words:

	180µm		280µm		Hypodermic	
	Mild	Moderate	Mild	Moderate	Mild	Moderate
Shooting	✓				✓	
Stabbing	✓		✓		✓	
Sharp	✓		✓			✓
Tender	✓					
Fearful						✓
Pressing	✓		✓			✓
Pricking	✓		✓			✓

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain			
1	Mild	✓	✓	
2	Discomforting			✓
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
180µm & 280µm	-	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"Just a little pricking sensation, but its fine"..."No, not pain, but I could feel it going in, but not painful, no."	"Ok, I can feel something there, but I didn't really see it go in, I didn't really feel much, didn't hurt, it's a bit erm, yer I can feel it"..."prickling"..."I didn't really feel anything"... "I suppose it didn't, the thing is it didn't hurt when it entered, but when the doctor put it in and started shaking it hurt more."	"Err, its ok, more or less like the first one (180). Hurt much less than the second one (hypodermic). I can feel it, sharp, but it didn't hurt as much as the last one. Very similar to the first one (180)."	"Much sharper, ARGHH That is much sharper"... "Can feel it much more"	"The second one (hypodermic) was the most painful, I could feel it the most. Umm, the third one (280) I didn't feel as much, but maybe that's because I had the first 2 before. So maybe it didn't 'cause I've had the first 2 I didn't feel it, but the second one (hypodermic), oh, definitely the most painful. And I felt most were stabbing and it prickled it a bit"

Data for Participant 6

Application order:

Device 1	Device 2	Device 3
280µm	Hypodermic	180µm

VAS:

Needle Device	VAS/ cm
180µm	1.44
280µm	0
Hypodermic	2.17

Sensation words:

	180µm		280µm	Hypodermic
	Mild	Moderate	Mild	Mild
Throbbing	✓			
Shooting				✓
Stabbing				✓
Heavy		✓	✓	
Pressing		✓	✓	✓
Pricking				✓

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain		✓	
1	Mild	✓		
2	Discomforting			✓
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
280µm	180µm	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
[nothing audible]	"There's quite firm pressing down. Feel it going in. More discomfort than the first (280) and second one (hypodermic)."	"Feels as if there is something being pressed down. He's taken his hand off."	"Now its quite sharp, can feel something going in, inside. Yer it's really painful."	"Erm, you could tell quite easily the second one (hypodermic) was the needle. That was definitely more uncomfortable than the other two. You could actually feel it in terms of it going into you. Erm, third one (180) felt as if it was being pressed down harder, er, and the first one (280) was like someone holding onto your arm."

Data for Participant 7

Application order:

Device 1	Device 2	Device 3
180µm	280µm	Hypodermic

VAS:

Needle Device	VAS/ cm
180µm	0.21
280µm	0.14
Hypodermic	0.28

Sensation words:

	180µm		280µm		Hypodermic	
	Mild	Moderate	Mild	Moderate	Mild	Moderate
Sharp	✓		✓			✓
Heavy		✓	✓			
Pressing		✓		✓	✓	
Pricking	✓		✓		✓	

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain	✓	✓	
1	Mild			✓
2	Discomforting			
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
180µm & 280µm	-	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"It feels, loads of pressure, feels circular and I can feel an edge pressing in more on one side. Its getting lighter now, umm just feels a tickle now."	"Ok, I can feel heavy pressure pushing down, umm, slightly sharp towards the centre, but err, not excessively painful, now its pulling backwards and its gone." [comment on VAS]: "It was very little at all." [comment on HEAVY] "Moderate, that was the overriding sensation."	"Ok, pressure again, less sharp pain, ok there is more sharp pain now. Ok, can feel it pushing down, it slightly gripped my skin and then came out."... [during withdrawing of device]: "no." [comment on VAS]: "Very little again, probably about the same as last time (180)." [comment on HEAVY]: "A mild heavy, there wasn't quite such pressure as last time (180)." [comment on PRESSING]: "Umm, felt quite similar to last time (180), so I would say moderate pressing, that was the overriding feeling."	"Yes I can feel that, that's like a nipping feeling on the skin. Umm it's less now but it was worse to start with and it's gone." [comments on VAS]: "That was slightly sharper to start with."... [comments on HEAVY]: I didn't feel the heaviness that time."	"Umm, I think the first two (180, 280) felt fine because pressure doesn't feel as bad as this sharp. Well I suppose you would it as a pain, a sharp pain on the third one (hypodermic) like your skin's being nipped. But I think the third (hypodermic) was the worst. The first two (180, 280) were fine and not a lot of difference between the two."

Data for Participant 8

Application order:

Device 1	Device 2	Device 3
280µm	180µm	Hypodermic

VAS:

Needle Device	VAS/ cm
180µm	0.08
280µm	0.01
Hypodermic	0.74

Sensation words:

	180µm	280µm		Hypodermic	
	Mild	Mild	Moderate	Mild	Moderate
Stabbing				✓	
Sharp	✓	✓			✓
Tender				✓	
Pressing	✓		✓		✓
Pricking	✓	✓			✓

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain	✓	✓	
1	Mild			
2	Discomforting			✓
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
280µm	180µm	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"Yer, umm, I can feel this, umm, endo-pressure, like someone is pressing against me with perhaps a rough fingertip. And that's about it now."	"ok, yer, that again feels similar to the first (280). Umm, pressing a bit more, err, it feels a little bit sharper, and you can tell there is something going into the skin but it's not a sharp pain, but you can feel something. Umm, similar to the first (280) but a little bit more intense. Umm, but I wouldn't call it pain."	"Again it feels like, err, fingertip. Umm, a bit of pressure, nothing else."..."Its not really pain, there is a sensation there but not really pain. It feels like err... you can tell its something sharp but its not painful as such over the overall area."	"Yes, that's a needle!"... "Ok, yep, it felt like a needle. Very different, can tell something pressing on the skin and going in, and then coming out as well. Umm, it feels like the injections you get from the nurse. Umm, again it is, there is an increase in pressure as it pierces the skin and then its not so bad. But you can definitely feel in terms of the other two umm, it's far more intense, umm."	"Differences in between them. Well the third application (hypodermic) you could tell was quite different to the other two, umm, and it did feel like pain a needle if I am honest. Umm, so I could feel it going in. with regards to comparing that to the other two applicators. Umm, yer you felt very different to, the application two (180) felt more, umm, you could feel it more. If that's of any help"... "yer you could tell that more pressure was being applied, umm, and you could feel. I suppose the only way of describing it is if you get like err, like some leaves and they've got little prickly things on the back and you are pressing on them. Like a nettle leaf but without the actual sting in it. So you can feel that its gone in, it feels a bit like that so the more you press on it the more prickly but again it doesn't feel painful. You can sense that are the pricks there. Umm, that's more for the first one. But yer, three (hypodermic) felt far more sort of penetrating than the other two."

Data for Participant 9

Application order:

Device 1	Device 2	Device 3
180µm	280µm	Hypodermic

VAS:

Needle Device	VAS/ cm
180µm	1.08
280µm	0.88
Hypodermic	2.23

Sensation words:

	180µm	280µm	Hypodermic	
	Mild	Mild	Mild	Moderate
Shooting		✓		
Stabbing	✓			✓
Sharp		✓		✓
Cramping	✓			✓
Gnawing			✓	
Hot-burning				✓
Aching		✓		✓
Heavy		✓		✓
Tender	✓		✓	
Splitting		✓		✓
Sickening			✓	
Fearful	✓		✓	
Punishing-cruel	✓			
Pressing	✓	✓	✓	
Pricking				✓

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain			
1	Mild	✓	✓	
2	Discomforting			✓
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	**Middle**	**Most**
180µm & 280µm	-	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"Its just like something on my skin."	"I feel its er, er not clear, it's like something scratching on the area."... [comment on FEARFUL] "Because I expect it is going to cause me a pain, I don't know whether (I ancitipate it)."..."yer, but actually when the device was applied, but before that was feeling a bit fearful."	"No I think it feel quite similar to the previous one (180)."... [comment on associated pain]: "No, no, no."	"Sharp and pain."	<<No comments given by participants>>

Data for Participant 10

Application order:

Device 1	Device 2	Device 3
180µm	Hypodermic	280µm

VAS:

Needle Device	VAS/ cm
180µm	0.23
280µm	0.46
Hypodermic	0.92

Sensation words:

	180µm	280µm	Hypodermic
	Moderate	Moderate	Mild
Stabbing	✓	✓	
Sharp			✓
Pressing	✓	✓	
Pricking			✓

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain	✓	✓	
1	Mild			✓
2	Discomforting			
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
180µm	280µm	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"Umm literally just feel pressure."	"Alright, quite a bit of pressure, quite a lot of pressure really. Slight pain and a lot of pressure." [comment of VAS]: "Hardly any really." [comment on Evaluative Overall Pain]: "It was just mild pain, there wasn't pain as in sharp pain, but because there was pressure." ..."mild sensation"... "I think it was more of a sensation, it wasn't really a pain."	"Quite a lot of pressure. not pain, but its [pause] no pain. [comment on VAS]: "Same as the first one (180) really."	"feel a slight pressure and a slight pain." [comment on VAS]: "slightly more than the first one (180)"	"The second one (hypodermic) was painful, then slightly painful. The first (180) and the last one (280), they were similar. Couldn't feel any pain, could feel sensation and a lot, a lot of pressure. There was probably actually slightly more pressure on the last one (280)."

Data for Participant 11

Application order:

Device 1	Device 2	Device 3
Hypodermic	180µm	280µm

VAS:

Needle Device	VAS/ cm
180µm	0
280µm	0
Hypodermic	2.65

Sensation words:

	180µm		280µm	Hypodermic
	Mild	Moderate	Mild	Mild
Stabbing				✓
Sharp			✓	✓
Hot-burning				✓
Heavy	✓		✓	
Pressing		✓	✓	

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain	✓	✓	
1	Mild			
2	Discomforting			✓
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
180µm	280µm	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
<< No comment from participant audiable on recording>>	"Umm pressing, umm, umm pressure."	"Umm, sharp with a bit, with a lot of pressing."	"Yer, err feel kind a like a sting."	"First one (hypodermic) was really sharp. The second one (180) was more intense pressing. The third one (280) was like a mixture between both."

Data for Participant 12

Application order:

Device 1	Device 2	Device 3
280µm	180µm	Hypodermic

VAS:

Needle Device	VAS/ cm
180µm	0.21
280µm	0
Hypodermic	1.69

Sensation words:

	180µm	280µm	Hypodermic	
	Mild	Mild	Mild	Moderate
Shooting				✓
Sharp	✓		✓	
Tender			✓	
Pressing		✓	✓	
Pricking	✓		✓	

Overall Evaluative Pain Intensity:

		180µm	280µm	Hypodermic
0	No pain		✓	
1	Mild	✓		
2	Discomforting			✓
3	Distressing			
4	Horrible			
5	Excruciating			

Increasing order of Painful/ uncomfortable (deduced from comments)		
Least	Middle	Most
180µm & 280µm	-	hypodermic

Transcription of audio-recorded data:

Placebo	180µm	280µm	Hypodermic	*"Were there any additional comments you wanted to make in relation to the three devices or what just happened?"*
"I can feel a very light pressure, umm, on my right buttock. That's pretty much the only sensation really."	"Umm, I'm sorry, umm, ok, same sort of, same level of err pressure as the last time (280). Err, I wouldn't call it pain, but I did feel a slight, I did feel something err go into my skin but not pain. More sort of, don't really know the word to describe it really, sort of mild discomfort, but." [comment on SHARP]: "I would say mild sharp, and umm, 0.5 for this, because I did feel sharp but it wasn't sort of." [comment on Overall Evaluative Pain]: "Mild pain, again it was more than the first one (280) but you can spot the a mild pain. So I don't know."... [suggested between none and mild]: "Yer"	"yer, I can feel a heavier, heavier touch, feels as if there is something in the centre and sort of like a point in the centre of the circle. No, no pain at all. Just a slightly heavier touch."	"Ok I can feel, ok now I can actually feel err what I would describe as a pain, umm very mild, umm felt some needle going into the skin. Shucks, slight err shooting as the needle sort of punctured the skin, umm but quite quite, not a, not a major pain, but a pain all the same."... "Umm yer I knew it was there I could sort of, I could sense that a needle was going in that time."	"Err, my least favourite was the last one (hypodermic), umm which well obviously I didn't see but I am pretty confident that's the standard sort of hypodermic needle, umm. Yer that was my least favourite cause that felt, that was quite, if I had a choice out of the three I would definitely go for one of the first two (280, 180). Umm, err, I can't really remember which one out of the first two was the least actually, umm but I think they were pretty, you know they were quite close by. Umm, yer so the first two (280, 180) were better. Third one (hypodermic), yer, uncomfortable but not intolerable, but sort of out, out a preference I would go for either of the first two (280, 180)."

APPENDIX XI

Publication

Biomed Microdevices
DOI 10.1007/s10544-008-9208-1

Clinical administration of microneedles: skin puncture, pain and sensation

M. I. Haq · E. Smith · D. N. John · M. Kalavala · C. Edwards · A. Anstey · A. Morrissey · J. C. Birchall

Abstract Injections using hypodermic needles cause pain, discomfort, localised trauma and apprehension. Additionally, careful use and disposal of needles is required to avoid transmission of blood-borne pathogens. As an alternative, microneedles can facilitate drug delivery without significantly impacting on pain receptors or blood vessels that reside beneath the skin outer layers. In this study we aim to determine the pain and sensory response to the application of wet-etch silicon microneedles, when used in such a way as to reliably penetrate skin, and provide a preliminary indication of how skin responds to microneedle injury with time. Twelve subjects received single-blinded insertions of a 25-G hypodermic needle and two microneedle arrays (36 needles of 180 and 280 μm height). The optimal method for microneedle application was determined in a pilot study. Pain intensity was scored using a visual analogue scale (VAS) and sensory perception determined using an adapted McGill Pain Questionnaire Short Form. Skin penetration was determined by external staining and measurement of trans-epidermal water loss (TEWL). Mean VAS scores, verbal descriptions and questionnaire responses showed that the 180 and 280 μm microneedles caused significantly less pain and discomforting sensation in participants than the hypodermic needle. Methylene blue staining and TEWL analysis confirmed that microchannels were formed in the skin following microneedle application. Evidence of microchannel repair and resealing was apparent at 8–24 h post-application. In summary, this study shows that pyramidal wet-etch microneedles can penetrate human skin with minimal pain and sensory discomfort, creating transient pathways for potential drug, vaccine and DNA delivery.

Keywords Microneedle · Pain · Sensation · Visual analogue scale · Clinical trial

M. I. Haq · E. Smith · D. N. John · J. C. Birchall (✉)
Welsh School Of Pharmacy, Cardiff University,
Redwood Building, King Edward VII Avenue,
Cardiff CF10 3NB, UK
e-mail: birchalljc@cf.ac.uk

M. Kalavala · C. Edwards · A. Anstey
Gwent Healthcare NHS Trust, Royal Gwent Hospital,
Cardiff Road,
Newport NP20 2UB, UK

A. Morrissey
Biomedical Microsystems Team, Tyndall National Institute,
Prospect Row,
Cork, Ireland

1 Introduction

Over 15 billion injections are given worldwide each year, with at least 40 million curative or therapeutic injections being administered globally each day (WHO 1999, 2004). With only 5% of injections being used in prophylactic immunisations, the vast majority are prescribed for therapeutic medical interventions. Whilst injections using hypodermic needles are reliable and effective, they cause pain and discomfort and a considerable degree of apprehension in young and vulnerable patient groups. Indeed, patients suffering from needle-phobia, or "belonephobia", commonly avoid seeking medical and dental assistance due to their fear of injections (Kleinknecht 1994; Fredrikson et al. 1996; Marks 1988; Ost 1992; Milgrom et al. 1997; Nir et al. 2003). Needle use also carries the inherent risk of transmission of blood-borne pathogens from patient to patient where needles are re-used, or from patient to health care worker. In some countries the likelihood of cross infection with needles is further exaggerated where insuf-

ficient resources are available for consistent and effective disposal and where needle re-use may be unavoidable due to economic or supply constraints or necessity. In particular, the use of needles and syringes for delivering vaccines creates key challenges for immunisation programmes in developing countries including stability, transport and storage of the vaccine in liquid form, the requirement for trained clinicians to administer injections and sharps disposal. Other potential disadvantages of injections include hypersensitivity, lipohypertrophy, bruising and bleeding at the site of administration. It is also debatable whether localised injections are refined enough to deliver the medicament or antigen consistently to the most appropriate site to exert maximum effect. Clearly, conventional injection practices, whilst effective, are crude and invasive.

Transdermal drug delivery (TDD) is a proven patient choice, providing the potential for improved user convenience and compliance and controlled release of medicament for enhanced duration of action and reduced side effects (Hutin et al. 2003; Barry 2001). As the skin plays a definitive role as a protective barrier however, only a very limited number of drug candidates are able to penetrate through the skin and therefore TDD is currently not an option for most applications. To this end, various strategies for facilitating the transcutaneous delivery of a larger range of therapeutics have been developed. These include electroporation, ultrasound, thermal ablation, iontophoresis and skin bombardment (Barry 2001; Coulman et al. 2006a; Asbill et al. 2000; Denet et al. 2004; Dreher et al. 2005). Whilst demonstrating proof-of-concept, these methods all require complex and relatively expensive equipment and have yet to make a significant clinical impact.

The last 10 years has seen the development of minimally invasive needle devices, microneedles, that provide a new method for delivering medicaments and vaccines into and through skin (Coulman et al. 2006a; Henry et al. 1998; McAllister et al. 2000; Prausnitz 2001; Birchall et al. 2006). Microneedles are designed specifically to penetrate the outermost skin barrier layer, the stratum corneum (SC), creating transient pathways for transcutaneous delivery. It is purported that microneedles can facilitate drug delivery through SC interruption without necessarily stimulating the pain receptors or blood vessels that reside beneath the skin outer layers (Henry et al. 1998; McAllister et al. 2000; Kaushik et al. 2001). Kaushik et al. (2001) measured pain response to an array of 400 microneedles of approximately 150 μm length (Kaushik et al. 2001). The authors showed that pain response following microneedle application was statistically indistinguishable from application of a smooth surface and statistically inferior to pain response following insertion of a 26-gauge hypodermic needle. In this study pain was measured using a visual analogue scale, which is an efficient, reliable and validated tool for scoring subject pain, but provides limited information on sensation or overall assessment of the human perception of microneedle application. Importantly, this study did not attempt to simultaneously demonstrate both microneedle functionality and pain response as there was no *in vivo* assessment during the study to verify skin puncture due to microneedles.

Sivamani et al. (2005) also assessed pain when comparing *in vivo* human injections of 1 μl methyl nicotinate using 200 μm length hollow microneedle arrays to topical application. The data revealed increased blood flux post application of microneedles whilst comments from the volunteers describe the application of microneedles as a feeling of "pressure but no pain" (Sivamani et al. 2005). Shirkhanzadeh (2005) reported that microneedles coated with porous calcium phosphate would appear to be well tolerated in human subjects although pain scoring was not performed (Shirkhanzadeh 2005). Miyano et al. (2005) reported that detachable, biodegradable microneedles of 500 μm length manufactured from maltose do not cause any pain on skin insertion (Miyano et al. 2005). Further reports suggest that microneedles do not cause any significant pain when used for extraction of interstitial fluid or blood for glucose monitoring (Smart and Subramanian 2000; Wang et al. 2005).

Pain is subjective and has several dimensions and therefore, is hard to clinically measure or quantify. Any measurements of pain are dependent on how a person views and communicates the pain they are feeling and this is individual to them. A reliable device for assessing pain will be able to measure pain consistently without being affected by minor changes in environment, administration or circumstances, yet would identify if the experienced pain was to change. One such device is the McGill Pain Questionnaire (MPQ) which contains a visual analogue scale (VAS) to measure pain intensity, certain key descriptor words and an evaluative index using key words such as mild, discomforting and excruciating (Melzack 1975). The MPQ measures dimensions of pain quality by including a set of descriptor adjectives, an intensity scale and pain drawing. The MPQ is one of the most widely used of all pain measurement tools, it has been shown to be highly reliable, is able to measure multidimensional aspects of pain and provides both quantitative and qualitative data (Kahl 2005). It is also important however, not to use a measurement tool that is overly time consuming to administer, making it impractical to employ in a clinical setting. One of the disadvantages of the MPQ is that it takes at least ten minutes to administer, which in some studies is not viable. To overcome this, Melzack developed a short-form version (MPQ-SF), which uses key adjectives from the longer MPQ (Melzack 1987; Collins et al. 1997). As shown by Melzack and Katz (1994), the

MPQ-SF provides a reliable and valid method for assessing the qualitative nature of an individual's pain experience (Melzack and Katz 1994).

In this study we aim to utilise these tools to determine whether the wet-etch silicon microneedles we have previously used in our *ex vivo* studies (Pearton et al. 2008; Birchall et al. 2005; Coulman et al. 2006b) elicit pain on application to human volunteers. Uniquely, we will simultaneously confirm that the solid microneedles have been applied appropriately for clinical use, i.e. that they have been used in such a way as to reliably penetrate the stratum corneum. Our results also provide distinctive data for sensory perception on microneedle application and a primary indication of how skin responds to microneedle injury with time. We include details of a preliminary study that was performed to confirm that the data obtained in the clinical study relates to pain, sensation and injury arising from the microneedles themselves rather than the microneedle applicator.

2 Materials and methods

2.1 Materials

Methylene blue dye was from Fisher Scientific UK, Loughborough, UK. Dulbecco's modified Eagle's medium (DMEM; in 25 mmol l^{-1} HEPES) and fetal bovine serum were from Invitrogen Ltd, Paisley, UK.

The microneedle arrays used in this study were provided by The Tyndall National Institute, Cork, Ireland. These platinum-coated "wet-etch" manufactured silicon microneedles have been shown to be of appropriate dimensions to create microconduits, approximately 50 μm in diameter, extending through the stratum corneum (SC) and viable epidermis (Wilke et al. 2005). Wet etching using potassium hydroxide (KOH) provides a method of mass production with lower fabrication costs than dry etching (McAllister et al. 2000; Birchall et al. 2005; Wilke et al. 2005). The process starts with a standard silicon wafer which is coated with silicon nitride on a silicon oxide layer. Square shape patterns are transferred into the masking double layer by standard photolithography. After lithography, the patterned silicon wafer is etched using a 29% KOH solution at a temperature of 79°C. The needle formation is based on convex-corner undercut. The silicon wafers are subsequently coated with a thin (0.3 μm) layer of platinum. In this study the microneedle arrays comprised 36 pyramidal shaped microneedles of either 280 or 180 μm length with a base diameter of approximately 180 μm. The needle tips can be less than 1 μm wide. The microneedle arrays were characterised and checked for damage prior to use in any procedure using scanning electron microscopy (SEM; Fig. 1).

Aluminium applicator rods used in preliminary studies were supplied by Professor David Barrow at the Cardiff School of Engineering.

3 Methods

3.1 Preliminary study on microneedle application method

3.1.1 Design of microneedle applicators

Seven very simple and basic microneedle applicator designs were considered. The applicator designs were:

(a) Inverted 2 ml plastic syringe with the plunger surface heated and smoothed to remove any protrusions

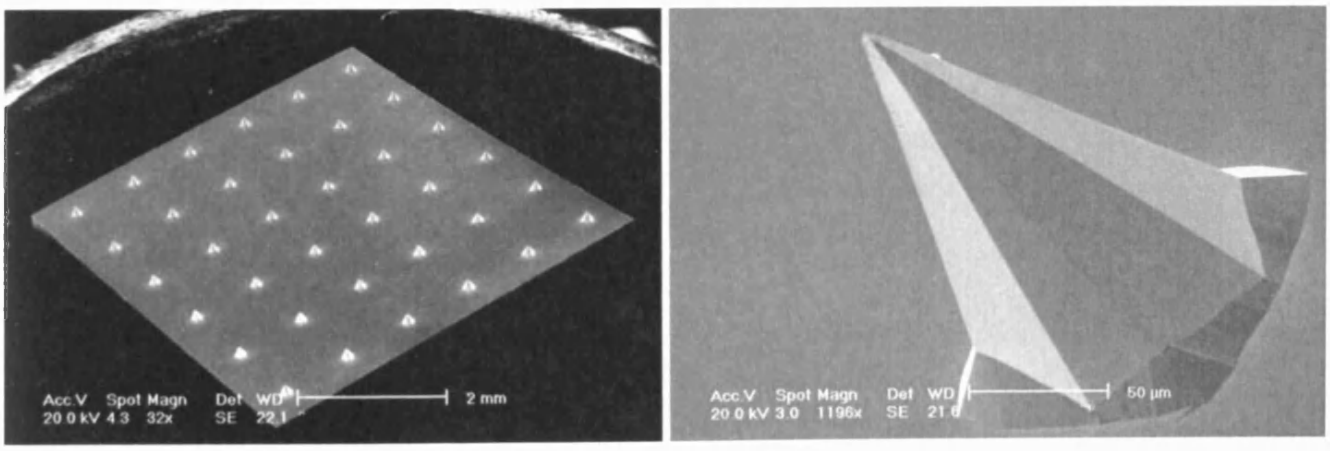


Fig. 1 Each of the 36 pyramidal 180 and 280 μm length microneedle arrays were characterized by SEM. Each array was inspected for damage (*left*) and each needle clearly observed for defects in its structure (*right*). The *left* image shows a complete 280 μm microneedle array, and the *right* image is a single magnified 180 μm microneedle

(b) Cylindrical aluminum rod with soft rubber (3×2 mm) cushion
(c) Cylindrical aluminum rod with an elastic band rubber (4×1 mm) cushion
(d) Cylindrical aluminum rod with hard rubber (2×1 mm) cushion
(e) Cylindrical aluminum rod with foam (5×4 mm) cushion
(f) Cylindrical aluminum rod with rubber (6×2 mm) cushion and foam (6×9 mm) mounting on the application face
(g) Cylindrical aluminum rod with foam (6×9 mm) mounting on the application face

These designs are shown in Fig. 2.

3.1.2 Ethical and consent approval

For the collection and use of excised human skin ethical approval was obtained from the South East Wales Local Research Ethics Committee. Skin samples were obtained from redundant skin in mastectomy or breast reduction specimens from women who had given their informed consented.

3.1.3 Study design for pain assessment

Participants (n=13) were recruited after obtaining written informed consent to have different applicators applied to the inside of their forearms. Initially, as a control, participants received a single application of unmodified cylindrical and square aluminium rods. Subsequently, the modified applicators were applied single-blind and randomised to either left or right forearm. The pressure of application was kept consistent for each applicator. After the application a simple pain questionnaire was administered. Recovery time of at least 1 h was provided between applications, in case previous applications had increased the sensitivity of the forearm. Verbal descriptions of the application from the volunteers were recorded during the application process and transcribed verbatim.

3.1.4 Ex vivo testing of applicators

Full-thickness human breast skin was obtained from mastectomy or breast reduction. Skin was collected from a variety of donors ranging from 45 to 65 years of age. The skin was frozen at −20°C and defrosted for 2 h before use. The two least painful applicators, as determined from

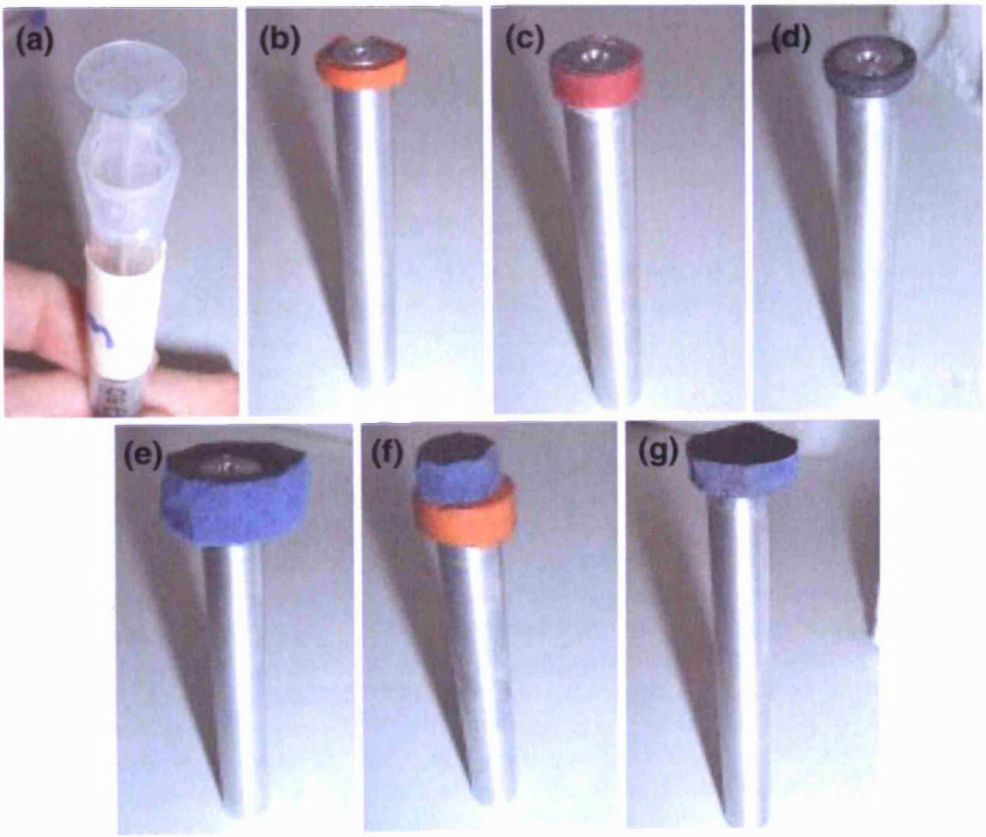


Fig. 2 Seven simple microneedle applicators designs. (**a**) Inverted 2 ml plastic syringe with the plunger surface heated and smoothed to remove any protrusions. (**b**) Cylindrical aluminum rod with soft rubber (3×2 mm) cushion. (**c**) Cylindrical aluminum rod with an elastic band rubber (4×1 mm) cushion. (**d**) Cylindrical aluminum rod with hard rubber (2×1 mm) cushion. (**e**) Cylindrical aluminum rod with foam (54 mm) cushion. (**f**) Cylindrical aluminum rod with rubber (6×2 mm) cushion and foam (6×9 mm) mounting on the application face. (**g**) Cylindrical aluminum rod with foam (6×9 mm) mounting on the application face

subject pain questionnaire, were tested *ex vivo* for skin puncturing efficiency. Araldite adhesive was applied to the top of the applicators and a microneedle array (in this case 49 microneedles of 250 μm length) bonded in a central position. Three different skin application techniques were tested:

1. Applying the applicator in a single rolling motion, whereby the applicator is placed at an angle of approximately 45° to the skin surface and rotated firmly forward through an angle of 90°, finishing at approximately 135°
2. Pushing the applicator vertically onto the skin, gently rotating the applicator for 10 s before removal
3. Punching the applicator vertically onto the skin with force, holding down for 10 s and then lifting off

Following application skin samples were treated topically with 10% methylene blue dye solution for 5 min before the excess dye was wiped from the skin surface. The samples were viewed under a light microscope.

3.2 Clinical study on microneedle pain, perception and skin puncturing

3.2.1 Ethical and consent approval

Ethical approval for this study was obtained from the South East Wales Local Research Ethics Committee. Written informed consent was obtained from healthy volunteers in adherence with the principles of the Declaration of Helsinki.

3.2.2 Study design

Participants (n=12) received single-blind applications of two types of microneedle array (36 pyramidal microneedles of either 180 or 280 μm height) and a 25-gauge subcutaneous hypodermic needle, injected at an angle appropriate for sub-cutaneous injection. Figure 3 shows the hypodermic needle in scale with one of the microneedle arrays. Each of these three applications were given to each buttock. The buttock was used as 6 mm skin biopsies were subsequently taken for further study. Applications of needle devices on one buttock were used to assess pain, perception and skin puncturing whereby the assessor was also blinded to the application device being administered. The second application was used to assess transepidermal water loss (TEWL) from the skin.

Following application to the human subjects each microneedle array was carefully examined by SEM for morphological changes. We observed no structural damage to any of the arrays or to any individual microneedles. The puncture sites on each patient were also carefully examined by dermatoscopy post-application and no microneedle or microneedle array artefacts were visible.

Fig. 3 The 25-G hypodermic needle used in this study placed next to 36 pyramidal microneedles on the 280 μm microneedle array (*Bar*= 1 cm)

3.2.3 VAS and sensation questionnaire

The pain intensity rating was taken immediately after application of each needle using an electronic sliding VAS whereby the participant moved a slider along a 10 cm slide where one end represented "No Pain=0 cm" and the other end the "Worst Pain Imaginable=10 cm. The slider was set to 0 cm at prior to each reading. The device does not disclose to the participant their rating, although a digital display viewed only by the assessor shows the rating for recording.

The perception questionnaire is based on the McGill Pain Questionnaire Short Form (MPQ-SF). It contains the four main assessment points of the MPQ-SF and three additional words (pressing, pricking and cold) taken from the long form MPQ, which unpublished research within our

Table 1 Summary of oral comments recorded during and immediately post-application of prototype applicator designs

Applicator	Summary of transcribed comments
A	Does not feel sharp on the skin. Pressure can be felt but no pain. Could offer advantage over both original applicators
B	Causes less sharpness than the square applicator but feels very similar to the round applicator. Therefore, although it offers advantage over the square applicator it is probably not significantly better than the round applicator
C	Similar to applicator B, causes less sharpness than the square applicator but feels very similar to the round applicator. Therefore, although it offers advantage over the square applicator it is probably not significantly better than the round applicator
D	Feels the same as the round applicator therefore would offer no advantage over it
E	Feels soft on the skin. Causes no pain or does not feel sharp. Could offer advantage over both original applicators
F	Cannot really feel the applicator on the skin. Causes no pain. Could offer advantage over both original applicators
G	Similar to applicator F, causes no pain and could offer advantage over both original applicators

group have shown to be of specific relevance in this study. The formatting and presentation of the MPQ-SF was designed to ensure reliability and validity of the data collected. To prevent leading of the participants the "Pain Rating Index" of descriptor words was relabelled the "Sensation Rating Index" as this provided a truer context of what was felt during device application.

3.2.4 Transepidermal water loss (TEWL)

TEWL was measured to determine the level of disruption to skin barrier function following application of the hypodermic needle and microneedle arrays. A Tewameter TM 210 (Courage and Khazaka Electronic GmbH, Köln, Germany) was used to measure TEWL at a control site as well as the three sites pre- and post-application of needle devices. The TEWL probe is a delicate measuring device, and was protected from shock, dirt, manual contact and liquids, using a cap when not in use. Before TEWL measurement each participant rested for 15 min to acclimatise to the ambient room temperature and relative humidity, which were maintained at 22°C and 45±5%, respectively. TEWL measurements were taken by carefully resting the TEWL probe horizontally on the application site, with the probe head vertical and perpendicular to the skin. The probe was held in place using a clamp stand to prevent any interferences arising from hand movement or heating of the probe by the assessor. Once the participant was comfortable, a reading was taken over 3 mins. The presented values represent the mean TEWL reading for the 20 s prior to stopping the measurement. If there were any uncharacteristic spikes during this period a more representative 20 s reading was used. TEWL readings were taken 1, 4, 8 and 24 h after the needle applications.

3.2.5 Dermatoscope and skin staining

Evidence of skin puncturing and microchannel healing and re-sealing was provided using an external stain. The 12 participants were randomised into four groups of three participants. At either 1 (group 1), 4 (group 2), 8 (group 3) or 24 (group 4) h after application of the needles 20 μl of methylene blue stain was applied to the needle treated skin surface. The stain was left in place for 10 min before excess stain was removed using ethanol wipes. The sites was then visually assessed through the dermatoscope and a photographic image recorded (Canon IXUS 500 digital camera; Canon, UK).

3.2.6 Statistical analysis of results

Non-parametric Wilcoxon signed rank test was performed using Prism GraphPad. Statistical significance was shown when $p<0.05$ and applied to both the VAS and TEWL data.

4 Results

4.1 Preliminary study on microneedle application method

The method of application of microneedles will be key to efficient and painless penetration through the skin barrier

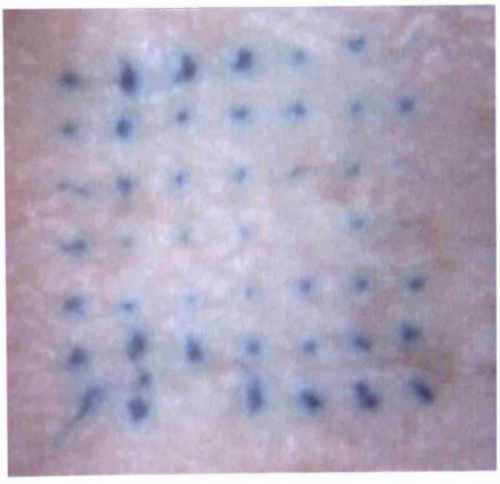


Fig. 4 Methylene blue staining of *ex vivo* human skin. A 49 microneedle array of 250 μm microneedle length was mounted onto the inverted syringe applicator and applied to human skin. The presence of blue staining confirms successful interruption of the stratum corneum barrier

Fig. 5 Microneedle arrays of 180 and 280 μm length mounted onto inverted syringe applicators

layer. A preliminary study assessed pain response and puncturing efficacy of different applicator designs prior to the clinical study. Seven potential basic applicator designs (Fig. 2) were investigated. A summary of oral comments post-application of the applicators are presented in Table 1.

As shown in Table 1, some of the applicator designs were less painful on administration than the applicators commonly used in laboratory studies, i.e. cylindrical and square-end metal rods. Figure 4 confirms that one of these applicator designs, i.e. the inverted syringe (A), was able to efficiently penetrate human skin when mounted with a microneedle array. Similar results were obtained with the foam applicator (E). Whilst Table 1 suggests that designs F and G are also suitable from a pain perspective, the *ex vivo* skin tests revealed that these applicators were not as efficient as penetrating human skin due to the cushioning effect of the rubber and/or foam mounting. *Ex vivo* testing also showed that the first method of application was shown to be optimal for applying the microneedles, i.e. applying the applicator in a single rolling motion.

Based on this simple pilot study the foam applicator and the syringe applicator were shown to be most appropriate for use in the clinical study. As the syringe applicators are easier and less costly to obtain and are supplied sterile, these applicators were selected for application of microneedles to human volunteers (Fig. 5).

4.2 Clinical study on microneedle pain, sensation and skin puncturing

The 12 subject clinical study firstly aimed to test the pain response against single-blinded application of microneedle arrays of two different microneedle heights (mounted on the reverse of a syringe barrel) and a hypodermic needle. The mean VAS pain scores following application of each 'needle' treatment show that the 180 and 280 μm microneedles caused significantly less pain (p=0.027 and p=0.0005 respectively) in participants than the hypodermic needle [Fig. 6(a)]. The 280 μm microneedles were perceived to be significantly less painful than the 180 μm microneedles (p=0.039). Individualised data in Fig. 6(b) further show that the hypodermic needle was always more painful than either microneedle device whilst 11 of the 12 participants found the 180 μm microneedles to be more painful than the 280 μm microneedles.

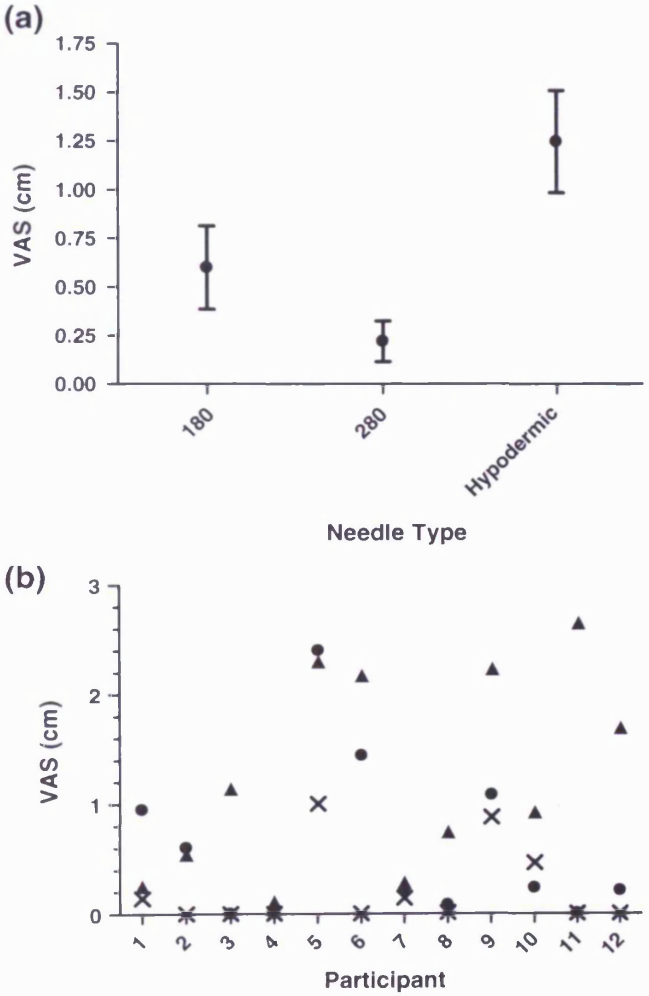


Fig. 6 (**a**) Overall mean±SD for VAS pain scores following application of a 180 μm microneedle array (180), a 280 μm microneedle array (280) and a 25-G hypodermic needle (Hypodermic). (**b**) Individual participant VAS scores following application of a 180 μm microneedle array (*circle*), a 280 μm microneedle array (*cross*) and a 25-G hypodermic needle (*triangle*)

Table 2 Ranking of reports of pain and discomfort following device applications

Ranking deduced from audio-recorded comments made by participants both during and after the application of each device

Participant (order of application)	Comparative pain/discomfort from participant oral descriptions following application of three needle devices		
	Least Pain/discomfort	Middle Pain/discomfort	Most Pain/discomfort
1 (280/hypo/180)	280	180	Hypo
2 (hypo/280/180)	280	180	Hypo
3 (hypo/180/280)	180/280	–	Hypo
4 (280/hypo/180)	180	280	Hypo
5 (180/hypo/280)	280	180	Hypo
6 (280/hypo/180)	280	180	Hypo
7 (180/280/hypo)	180/280	–	Hypo
8 (280/180/hypo)	280	180	Hypo
9 (180/280/hypo)	180/280	–	Hypo
10 (180/hypo/280)	180	280	Hypo
11 (hypo/180/280)	-	180/280	Hypo
12 (280/180/hypo)	180/280	–	Hypo

Oral comments confirmed that every participant found the hypodermic to be the most painful and uncomfortable of the three applications. Five of the 12 participants felt that treatment with the 180 μm microneedle array was slightly more discomforting than the application of the 280 μm microneedle array as clearly explained by participant 6: "Third one (180) felt as if it was being pressed down harder", "The first one (280) was like someone holding onto your arm". The hypodermic needle was immediately highlighted as the most painful and uncomfortable, as explained by participant 5: "definitely the most painful". Table 2 summarises the level of pain/discomfort felt by

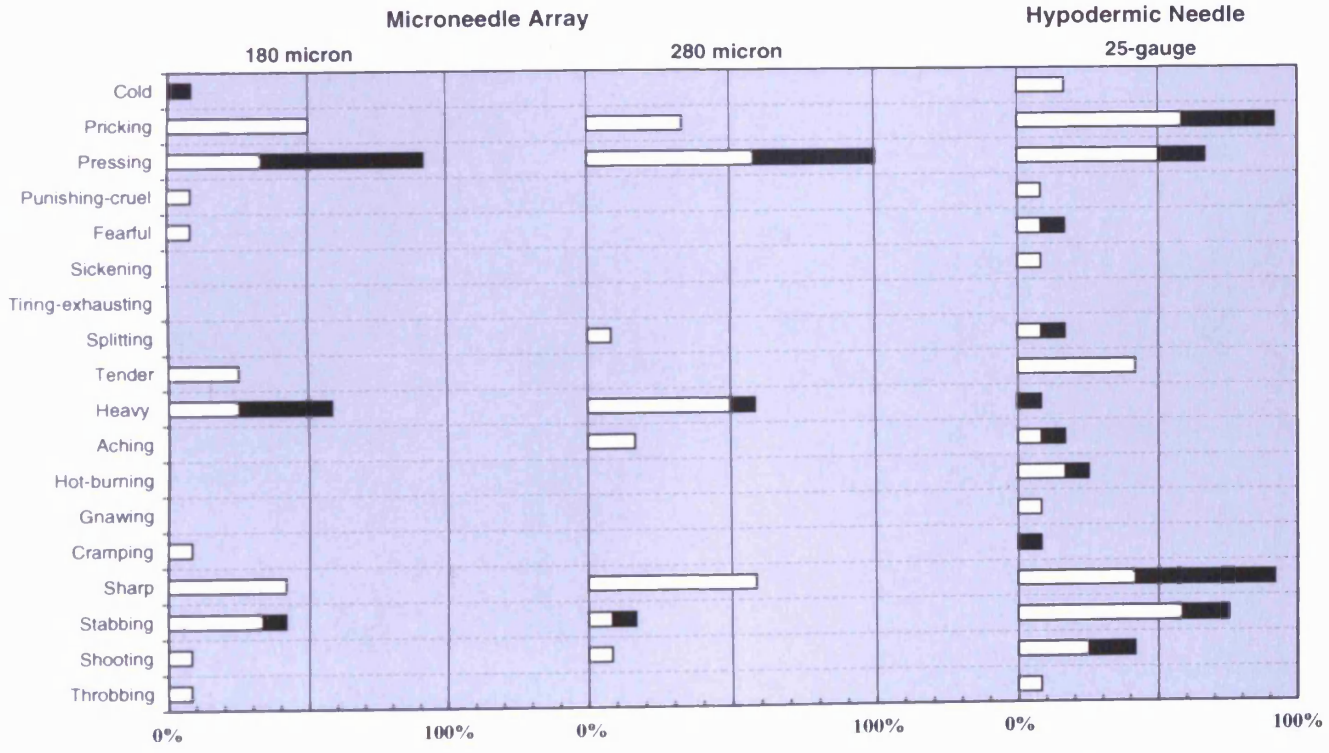


Fig. 7 Sensory evaluation following needle application. Each participant was asked to complete an adapted SF-MPQ to rate the sensations experienced during application of the 180 μm length and 280 μm length microneedle arrays and 25-G hypodermic needle. These data show the percentage of participants who rated each sensation occurring as mild (*empty square*) or moderate (*filled square*)

each participant following each needle application. The order of needle application, from the randomisation schedule, did not affect the participants' description of differences in pain and discomfort.

In addition to perceived pain this study further explored the sensation following microneedle and hypodermic needle treatment. Collated responses to the sensation questionnaire are shown in Fig. 7. Overall a greater number, variety and severity of sensations were experienced when participants were treated with the hypodermic needle when compared with either microneedle array. Participants felt greater 'sharp' and 'stabbing' sensations when the hypodermic needle was applied and more 'pressing' and 'heavy' sensations when the microneedles were applied.

Allied to pain and sensory response an important arm of this study was to demonstrate that the microneedle arrays had been applied in such a way as to penetrate the skin. This allows for meaningful comparison of pain response using an application procedure that is relevant, that is, using an application method and pressure that is shown to work in human skin. Monitoring the presence of skin punctures also provides a preliminary indication of skin healing following microneedle insult. The application sites of the two different microneedle array designs and the hypodermic needle used in pain and sensory measurements were subsequently stained using an external application of methylene blue dye. The stain was applied to the skin surface at 1 hr, 4 hrs, 8 hrs or 24 hrs after initial 'needle' application. Methylene blue staining clearly showed that microchannels are formed in the skin following application of the microneedles (Fig. 8). A larger puncture mark is observed when a 25-G hypodermic needle is applied and in this case, unlike microneedle treated skin, bleeding and erythema is also apparent. Whereas methylene blue staining plainly shows that the hypodermic injury is still amenable to staining at 24 h, evidence of microchannel repair and resealing following microneedle application is apparent at 8–24 h post-application. Generally the 280 μm length microneedle array was more effective at skin penetration than the 180 μm length microneedle array with a mean of

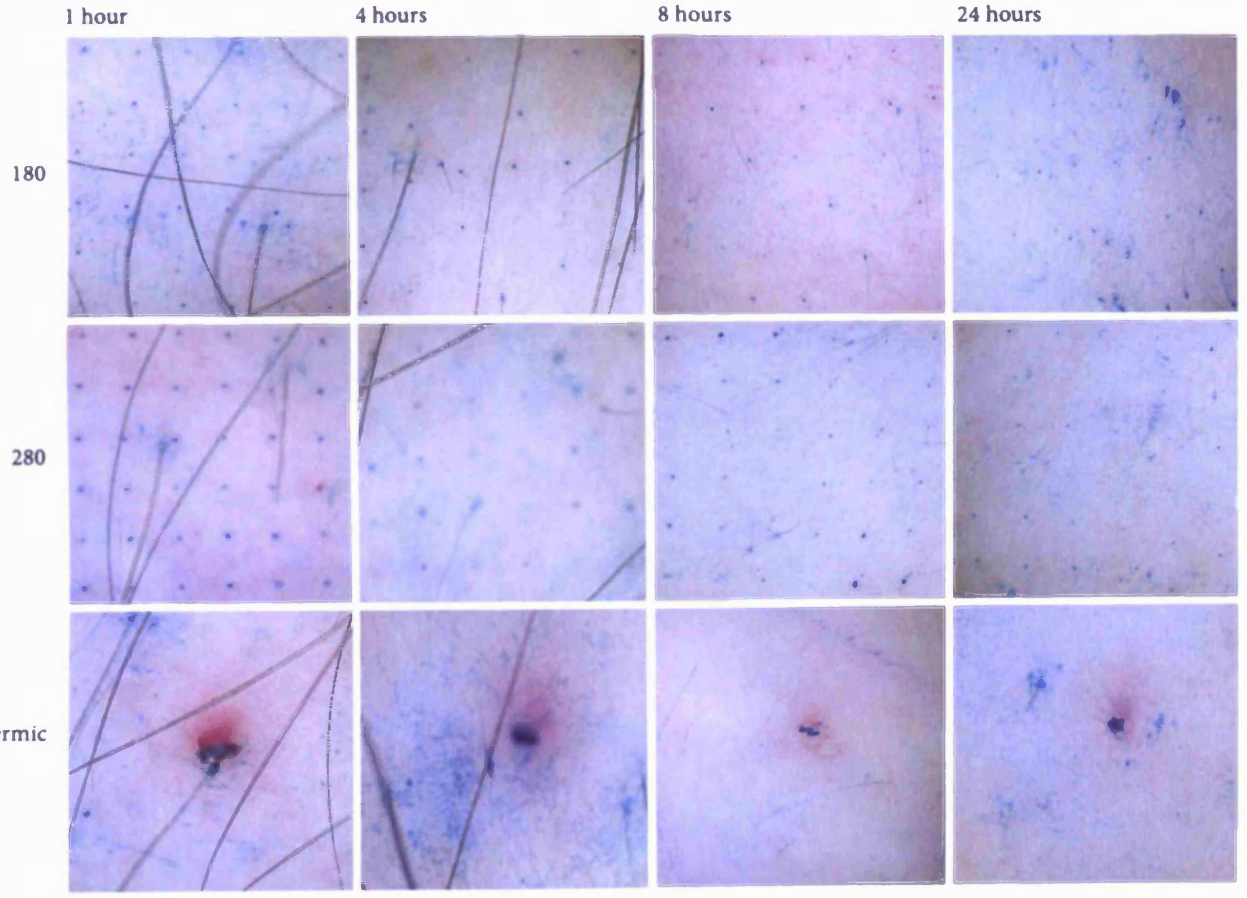


Fig. 8 Skin puncture marks for each needle device as observed with time following methylene blue staining. The blue dye was externally applied to the sites of puncture at 1, 4, 8 and 24 h after needle use. (180) 180 μm microneedle array, (280) 280 μm microneedle array, (Hypodermic) 25-G hypodermic needle

96% of the individual pyramidal needles puncturing the skin in participants (shown at 1 h staining) compared to a mean of 50% for the 180 μm length microneedle array.

Water loss from the skin is restricted by the SC layer. Transepidermal water loss (TEWL) measurement is a standardised method of determining changes in skin barrier properties, being frequently used in the cosmetics and dermatology industry (Treffel et al. 1994; Rosado et al. 2005; Aramaki et al. 2002; Schwindt et al. 1998). High TEWL values correspond to damaged skin whilst low TEWL correlate to healthy undamaged skin. The measurement of TEWL in this study was used to provide information concerning the compromised integrity of skin epidermis following application of the microneedle and hypodermic needle treatments.

Following skin puncture for pain, sensory and staining analysis, each participant received a second application of each of the 180 and 280 μm microneedle arrays and the 25-G hypodermic needle. TEWL was measured immediately after application and at three further intervals over a period of 24 h. In accordance with the external staining data, TEWL measurements further demonstrated perbation of the SC barrier following needle treatments with water loss increasing post application of each device (Fig. 9). Mean TEWL increased significantly ($P<0.05$) immediately post-application for all applications: from 5.1 (SD=3.8) to 8.8 (SD=5.4) g H_2O m^{-2} h^{-1} for the hypodermic needle; from 5.9 (SD=4.0) to 7.9 (SD=2.8) for 180 μm microneedles; and from 5.7 (SD=3.4) to 10.3 (SD=13.0) for the 280 μm microneedle array. In each case skin water loss recovered to baseline within 24 h with their being no significance ($P>0.05$) for any device when compared to control.

Figure 10 allows comparison of the percentage of puncture marks observed via methylene blue staining in microneedle treated skin with the TEWL readings at various timepoints. It is evident that as the number of distinct puncture marks reduces over the 24 h period the TEWL values normalise accordingly.

5 Discussion

In total seven designs of applicators were pre-tested, without any microneedles mounted, to ensure that the applicator itself would not bias the pain response data in the subsequent clinical study. Further, laboratory tests were performed to ensure that selected designs, once mounted with microneedle arrays, were able to puncture excised human skin. Our results showed that the simplest applicator design, i.e. the reverse end of a 2 ml plastic syringe, was the most appropriate for use in the clinical study from pain, accessibility, ease of use and skin penetration perspectives. *Ex vivo* testing also showed that the pyramidal micro-

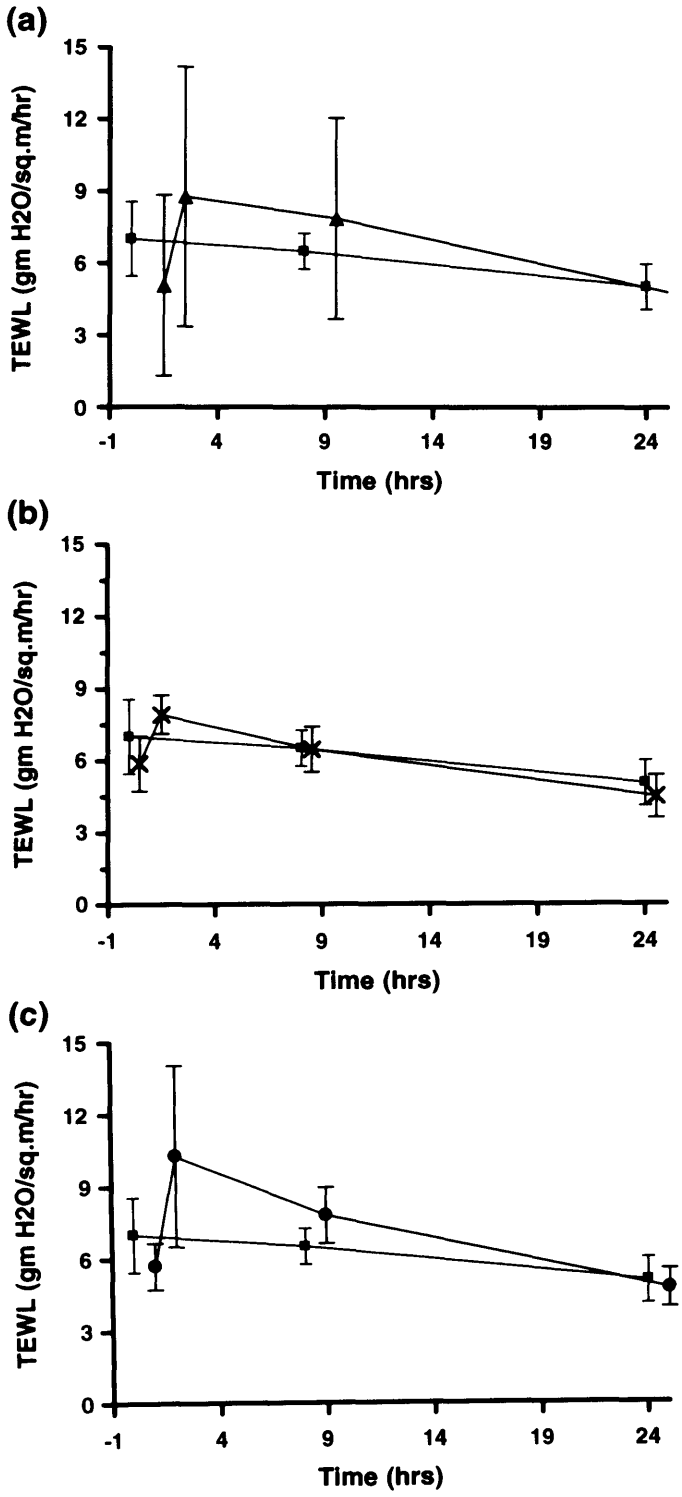


Fig. 9 Mean values ($n=12$) for trans-epidermal water loss (*TEWL*) at 1, 8 and 24 h post-application of (**a**) 25-G hypodermic needle (*triangle*), (**b**) 180 μm microneedle array (*cross*) and (**c**) 280 μm microneedle array (*circle*), each compared to control (*square*)

needles penetrated skin more effectively and reproducibly if applied in a single rolling motion rather than by downward vertical pressure. This indicates that these arrays pierce skin more efficiently when the force is distributed over one row at a time, rather than over the whole array. This is not surprising given the likely 'bed-of-nails' effect resulting from vertical application and the fact that similarly dimensioned microneedles mounted onto a cylindrical drum have been shown to effectively penetrate outer skin layers for cosmetic and medical applications (Dermaroller SARL, France).

A 12 subject human clinical study was used to explore whether clinically untested wet-etch silicon microneedles elicit pain when used in such a way as to reliably penetrate the stratum corneum, and compare microneedle pain response with that following insertion of a 25-G small hypodermic needle. VAS scores showed the hypodermic needle to be significantly more painful on insertion than either 180 or 280 μm microneedle arrays. This was further substantiated by descriptive comments and a sensation assessment from each participant. The fact that the participants scored and described the 180 μm microneedles to be more painful than the 280 μm microneedles may initially appear to be contradictory. This result is however perfectly understandable when you consider how the microneedles were used in this study. Uniquely, this study focuses on the experienced pain response to microneedle use when they are applied in such a way as to ensure sufficient penetration of the skin outer layer to facilitate drug delivery. The amount of application force required to provide reliable skin penetration is analogous with the force used to massage an aching muscle. Prior to applying the microneedle designs to human volunteers we have performed a large number of *ex vivo* studies using human skin, obtained from surgical procedures under ethical approval and informed patient consent. These studies have clearly shown that smaller microneedles (i.e. 180 μm in height) need to be more firmly applied to skin than larger microneedles (280 μm in height) to ensure comparable skin penetration. The clinician in our clinical study was therefore trained to apply the microneedle arrays to human skin, using more force with the 180 μm microneedle array. The oral commentaries from participants support the VAS data by highlighting key words and analogies given by the participants relating to pain response.

The sensory questionnaire further probed the perception of the participants to microneedle and hypodermic needle treatment. The microneedle applications were commonly perceived as being 'pressing' and 'heavy' with the hypodermic needle application perceived as more 'sharp' and 'pricking'. These data relate to surface area and ease of needle puncture. Microneedles spread over a larger surface area may require more force of penetration than an individual sharp hypodermic needle. Sensory responses suggest that further developments in microneedle array design, microneedle applicator morphology and clinical application technique that reduce the force required to ensure penetration of the SC would be beneficial. Importantly however, this study confirms that the pain and sensation felt from application of the wet-etch pyramidal microneedle devices were relatively similar, and in each case significantly lower than for the hypodermic needle. The verbal comments from the participants correlate with the pain questionnaire results with participants stating that application of the hypodermic was sharper and more pricking than either of the microneedle designs.

As previously mentioned, we thought it important to demonstrate that the pain and sensory response data would be relevant to clinical application of the microneedles. Therefore, the functionality of the microneedles, at least

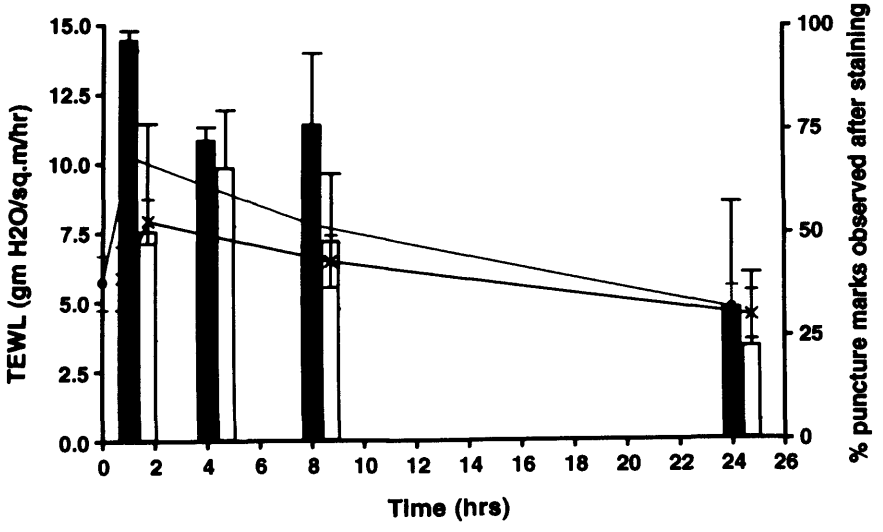


Fig. 10 TEWL measurements for 180 μm (*cross*) and 280 μm (*circle*) microneedle arrays compared against the percentage of puncture marks observed by external staining (out of 36) following application of the 180 μm (*white bars*) and 280 μm (*black bars*) microneedle arrays

from a skin penetration perspective if not a drug delivery viewpoint, was monitored. Following application of the microneedle arrays, skin puncture marks could be identified by staining with a topically applied solution of methylene blue dye. The intensity of puncture staining reduced with time after microneedle application indicating temporal sealing of the transient skin microchannels over the 24 h study period.

Trans-epidermal water loss (TEWL) measurements were used as an additional indicator of skin barrier disruption. It is acknowledged that TEWL is an extremely sensitive measurement of skin permeability and as the microneedle punctures are less than 100 μm at their widest and 280 μm at their theoretical deepest, it is likely that TEWL is affected by the participant's movements, diet and atmosphere over the 24 h study period, despite taking rigorous precautions at the time of measurement. Nevertheless, TEWL data correlated with the skin staining experiments in that an increase in TEWL is observed immediately after microneedle puncture and this increase diminishes to baseline over the 24 h study period. The TEWL values also reflect the relative efficiency of skin puncture with larger differences in TEWL observed when using 280 μm microneedles compared with 180 μm microneedles.

6 Conclusions

We have shown that pyramidal microneedles fabricated using low-cost wet-etching processes can penetrate human skin, providing transient microconduits for transcutaneous drug delivery, with minimal pain and discomfort. Our data suggest that the size of the microneedle must be optimised to ensure consistent skin penetration without excessive application force. The transient nature of skin disruption following application of microneedles was demonstrated with preliminary indications of microchannel healing and repair within the 24 h study period. More extensive studies investigating these skin repair responses at a cellular level are underway.

Acknowledgements The authors acknowledge the financial support of the British Skin Foundation and Mrs Stephanie Hill for preparing ethical documentation.

References

J. Aramaki, S. Kawana, I. Effendy et al., Differences of skin irritation between Japanese and European women Br. J. Dermatol **146**, 1052–1056 (2002)

C.S. Asbill, A.F. El-Kattan, B. Michniak, Enhancement of transdermal drug delivery: chemical and physical approaches Crit. Rev. Ther. Drug Carrier Syst **17**, 621–658 (2000)

B.W. Barry, Novel mechanisms and devices to enable successful transdermal drug delivery Eur. J. Pharm. Sci **14**, 101–14 (2001)

J. Birchall, S. Coulman, M. Pearton et al., Cutaneous DNA delivery and gene expression in ex vivo human skin explants via wet-etch micro-fabricated micro-needles J. Drug Target **13**, 415–421 (2005)

J. Birchall, S. Coulman, A. Anstey et al., Cutaneous gene expression of plasmid DNA in excised human skin following delivery via microchannels created by radio frequency ablation Int. J. Pharm **312**, 15–23 (2006)

S.L. Collins, R.A. Moore, H.J. McQuay, The visual analogue pain intensity scale: what is moderate pain in millimetres? Pain **72**, 95–97 (1997)

S. Coulman, C. Allender, J. Birchall, Microneedles and other physical methods for overcoming the stratum corneum barrier for cutaneous gene therapy Crit. Rev. Ther. Drug Carrier Syst **23**, 205–258 (2006a)

S.A. Coulman, D. Barrow, A. Anstey et al., Minimally invasive cutaneous delivery of macromolecules and plasmid DNA via microneedles Curr. Drug Deliv **3**, 65–75 (2006b)

A.R. Denet, R. Vanbever, V. Preat, Skin electroporation for transdermal and topical delivery Adv. Drug Deliv. Rev **56**, 659–674 (2004)

F. Dreher, B.S. Modjtahedi, S.P. Modjtahedi et al., Quantification of stratum corneum removal by adhesive tape stripping by total protein assay in 96-well microplates Skin Res. Technol **11**, 97–101 (2005)

M. Fredrikson, P. Annas, H. Fischer et al., Gender and age differences in the prevalence of specific fears and phobias Behav. Res. Ther **34**, 33–39 (1996)

S. Henry, D.V. McAllister, M.G. Allen et al., Microfabricated microneedles: a novel approach to transdermal drug delivery J. Pharm. Sci **87**, 922–925 (1998)

Y.J. Hutin, A.M. Hauri, G.L. Armstrong, Use of injections in healthcare settings worldwide, 2000: literature review and regional estimates BMJ **327**, 1075 (2003)

C.C.J. Kahl, Visual analogue scale, numeric pain rating scale and the McGill pain Questionnaire: an overview of psychometric properties Phys. Ther. Rev. **10**, 123–128 (2005)

S. Kaushik, A.H. Hord, D.D. Denson et al., Lack of pain associated with microfabricated microneedles Anesth. Analg **92**, 502–504 (2001)

R.A. Kleinknecht, Acquisition of blood, injury, and needle fears and phobias Behav. Res. Ther. **32**, 817–823 (1994)

I. Marks, Blood-injury phobia: a review Am. J. Psychiatry **145**, 1207–1213 (1988)

D.V. McAllister, M.G. Allen, M.R. Prausnitz, Microfabricated microneedles for gene and drug delivery Annu Rev. Biomed. Eng **2**, 289–313 (2000)

R. Melzack, The McGill Pain Questionnaire: major properties and scoring methods Pain **1**, 277–299 (1975)

R. Melzack, The short-form McGill Pain Questionnaire Pain **30**, 191–197 (1987)

R.K.J. Melzack, Katz J, in Pain measurement in persons in pain, ed. By PDMR Wall. Textbook of pain, 3rd edn. (Churchill Livingstone, New York, 1994), pp. 337–351

P. Milgrom, S.E. Coldwell, T. Getz et al., Four dimensions of fear of dental injections J. Am. Dent. Assoc **128**, 756–766 (1997)

T. Miyano, Y. Tobinaga, T. Kanno et al., Sugar micro needles as transdermic drug delivery system Biomed. Microdevices **7**, 185–188 (2005)

Y. Nir, A. Paz, E. Sabo et al., Fear of injections in young adults: prevalence and associations Am. J. Trop Med. Hyg **68**, 341–344 (2003)

L.G. Ost, Blood and injection phobia: background and cognitive, physiological, and behavioral variables J. Abnorm. Psychol **101**, 68–74 (1992)

M. Pearton, C. Allender, K. Brain et al., Gene delivery to the epidermal cells of human skin explants using microfabricated microneedles and hydrogel formulations Pharm Res **25**, 407–416 (2008)

M.R. Prausnitz, Overcoming skin's barrier: the search for effective and user-friendly drug delivery Diabetes Technol. Ther **3**, 233–236 (2001)

C. Rosado, P. Pinto, L.M. Rodrigues, Modeling TEWL-desorption curves: a new practical approach for the quantitative in vivo assessment of skin barrier Exp. Dermatol **14**, 386–390 (2005)

D.A. Schwindt, K.P. Wilhelm, H.I. Maibach, Water diffusion characteristics of human stratum corneum at different anatomical sites in vivo J. Invest. Dermatol **111**, 385–389 (1998)

M. Shirkhanzadeh, , Microneedles coated with porous calcium phosphate ceramics: effective vehicles for transdermal delivery of solid trehalose J. Mater Sci. Mater Med **16**, 37–45 (2005)

R.K. Sivamani, B. Stoeber, G.C. Wu et al., Clinical microneedle injection of methyl nicotinate: stratum corneum penetration Skin Res. Technol **11**, 152–156 (2005)

W.H. Smart, K. Subramanian, The use of silicon microfabrication technology in painless blood glucose monitoring Diabetes Technol. Ther **2**, 549–559 (2000)

P. Treffel, F. Panisset, B. Faivre et al., Hydration, transepidermal water loss, pH and skin surface parameters: correlations and variations between dominant and non-dominant forearms Br. J. Dermatol. **130**, 325–328 (1994)

P.M. Wang, M. Cornwell, M.R. Prausnitz, Minimally invasive extraction of dermal interstitial fluid for glucose monitoring using microneedles Diabetes Technol. Ther **7**, 131–141 (2005)

WHO. Safety of injections. In Secretariat of the Safe Injection Global Network, vol. 2007. Department of Essential Health Technologies, World Health Organization (2004)

WHO. Safety of injections: questions and answers. In *WHO fact sheet, no. 234*, vol. 2007. World Health Organization, Geneva (1999)

N. Wilke, C. Hibert, J. O'Brien et al., Silicon microneedle electrode array with temperature monitoring for electroporation Sens Actuators A Phys **123–124**, 319–325 (2005)