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Microneedles for intradermal and transdermal delivery

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Abstract

The formidable barrier properties of the uppermost layer of the skin, the *stratum corneum* impose significant limitations for successful systemic delivery of a broad range of therapeutic molecules, particularly macromolecules and genetic material. Microneedle delivery has been proposed as a strategy to breach the SC barrier function in order to facilitate effective transport of molecules across the skin. This strategy involves the use of micron sized needles fabricated from different materials and using different geometries to create transient aqueous conduits across the skin. Microneedles in isolation, or in combination with other enhancing strategies, have been shown to dramatically enhance the skin permeability of numerous therapeutic molecules including biopharmaceuticals either *in vitro*, *ex vivo* or *in vivo*. Progress in the areas of microneedle design, development and manufacture have proven promising in terms of the potential use of this emerging delivery method in clinical applications such as insulin delivery, transcutaneous immunisations and cutaneous gene delivery. This review article focuses on recent and potential future developments in microneedle technologies. This will include the detailing of progress made in microneedle design, an exploration of the challenges faced in this field and potential forward strategies to embrace the exploitation of microneedle methodologies, while considering the inherent safety aspects of such therapeutic tools.

Keywords

Transdermal drug delivery; microneedle; hydrogel-forming; safety; vaccination; drug monitoring; public perception

1. Introduction

There are a large volume of published studies describing the role of the skin as a promising site for systemic delivery of active pharmaceutical ingredients (APIs) (Banga, 2006; Gupta and Sharma, 2009). The transdermal delivery route also offers certain advantages including

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feasible controlled delivery or sustained release of drugs, avoidance of first pass-hepatic metabolism and a patient friendly approach to drug delivery. Despite this, to date, there are only approximately 35 transdermal products which have been commercially approved (Tanner and Mark, 2008). In particular, successful passive transdermal delivery is restricted to molecules sharing the following key properties of ideal skin permeant: low molecular weight of < 500 Da; the demonstration of adequate lipophilicity with log partition coefficient preferably in the range of 1 to 3.5; potent molecules with typical daily dose of < 10 mg and reasonable aqueous solubility of > 100 $\mu\text{g/ml}$ (Coulman *et al.*, 2006a; Williams, 2003). This stems from the formidable barrier properties of the skin's *stratum corneum* (SC), also known as the horny layer. This uppermost layer of the skin consisting of corneocytes embedded in lipid-enriched matrix with a thickness of approximately 10-15 μm , is a key factor in regulating drug flux through the tissue (Wiechers, 1998; Williams, 2003). Most APIs however, are rather hydrophilic, charged or of large molecular size, making them far from ideal skin permeants. Recent developments in transdermal drug delivery therefore have heightened the need for strategies to overcome the SC barrier function in order to facilitate rapid and effective permeation of a broader range of molecules, including macromolecular therapeutics and genetic materials. Such strategies to overcome the SC barrier properties namely *via* optimisation of the drug formulation or manipulation of the SC barrier function, can be achieved by one of two main approaches - either by chemical or physical methods. Conventional transdermal delivery strategies, well established for small molecules, are focused on optimisation of drug formulation. For macromolecules such as protein/peptide drugs, optimisation of the formulation can be performed by encapsulation of macromolecules within vesicular carrier systems such as liposomes, chemical modification for synthesising more lipophilic analogues, or incorporation of chemical penetration enhancers and proteolytic enzymes inhibitors. However, as this approach does not significantly disrupt the skin barrier, its application might be limited to only small peptides. Other transdermal enhancement technologies rely on manipulating the SC barrier properties by means of application of physical energy, or by physical abruption of the SC and, finally, by controlled removal of the SC so that permeation of drug molecules could be increased (Figure 1).

This review will focus on recent and emerging progress in microneedle (MN) technologies utilised to disrupt the barrier properties of the SC, thus enabling enhanced transdermal drug delivery. Emphasis will be given to the latest trends and advances in the areas of MN development and design. Challenges to successful MN advancement will be highlighted and attention will be paid to some of the important safety aspects which must be considered as MN technologies move towards commercial innovations.

2. Microneedle arrays

MN arrays consist of a plurality of micron-sized projections typically assembled on one side of a supporting base or patch. These microprojections generally range from lengths as short as 25 μm to those as long as 2,000 μm . The first concept for the use of MN as a drug delivery device was filed in 1971 in a United States Patent in which the inventors, Gerstel and Place, used the term 'puncturing projections' to describe this invention (Gerstel and Place, 1976). However, the first serious discussions and proof-of-concept analyses of MN

emerged in the late 1990s, when Henry *et al.* (1998) demonstrated the use of silicon MN to successfully facilitate the delivery of a model drug, calcein, across human skin. It is believed that a revolution in the microelectronics industry leading to the advent of microfabrication technology tools did, in part, enable the evolution of the manufacturing facilities necessary to produce such microconduits. Since then, a large and growing body of literature has investigated various microfabrication methodologies utilised to manufacture MN arrays from numerous materials. These materials have included silicon (Donnelly *et al.*, 2008; Khanna *et al.*, 2011; Wei-Zeet *et al.*, 2010), metals such as stainless steel, palladium, nickel and titanium (Chandrasekaran *et al.*, 2003; Gill and Prausnitz, 2007; Parker *et al.*, 2007), carbohydrates including galactose, maltose and polysaccharide (Donnelly *et al.*, 2009c; Lee *et al.*, 2008; Lee *et al.*, 2011; Li *et al.*, 2009), glass (Gupta *et al.*, 2009; Wang *et al.*, 2006), ceramics (Bystrova and Luttge, 2011) and various polymers (Donnelly *et al.*, 2011; Ito *et al.*, 2011; Noh *et al.*, 2010). In addition, MN arrays have been produced in various different geometries. These microstructure geometries can be in the form of needle-like (most common MN geometries which can be sharp-, tapered-, conical- or bevel-tipped), microblades, blunt-projections or shaped in an arrow-head.

MNs have been shown to effectively enhance the delivery of many therapeutic molecules across biological membranes including skin, mucosal tissue and sclera. Upon application of MN arrays, transient micropores, orders of magnitude larger than the molecular dimensions of the target molecule are created. In 2004, it was suggested that MN arrays could be used to permit the transport of, not only small molecular weight APIs but macromolecules and possibly supramolecular complexes and microparticles (Prausnitz, 2004). MNs could allow for the easy and patient-friendly administration of therapeutics to and across the skin at low cost with potential efficacy as a parenteral route of administration. Modulation of MN geometry and simple alteration of drug formulations can result in controlled drug deposition within targeted skin layers. MNs have been shown to penetrate the skin and cross the SC into the viable epidermis, avoiding contact with nerve fibres and blood vessels that reside primarily in the dermal layer. Therefore, the use of MNs would provide a pain-free, minimally invasive means of delivering both small and large molecular weight APIs with the prevention of bleeding at the application site (Bal *et al.*, 2008) (Figure 2). Over the last decade, extensive research has been carried out concerning MN design with the use of a wide range of techniques and fabrication methods. Importantly, enhancement of the delivery of drugs and biomolecules of a wide variety of physicochemical properties has been demonstrated in *in vitro*, *ex vivo* and *in vivo* experiments, using a broad variety of device designs. Figure 2 illustrates the classical mechanism of action of MN devices.

3. Delivery strategies using MN

Four typical MN designs which are commonly described in the literature are solid, coated, dissolving and hollow MNs (Figure 3 a-d). As the names suggest, these four types of MNs may be used in differing approaches for the delivery of therapeutics across the skin. In this review, we will also describe a recently reported MN category, namely hydrogel-forming MNs and explore some possible strategies for use with these novel MNs, the adoption of which will facilitate the effective delivery of therapeutics across the skin.

The first strategy of MN-mediated transdermal and dermal drug delivery is *via* the use of solid MN, also termed the “poke with patch” approach (Prausnitz, 2004). Upon removal of the MN, transient microchannels are created in the skin upon which a conventional drug formulation is subsequently applied. The movement of molecules through these microchannels occurs *via* passive diffusion. The drug formulation serves as an external drug reservoir and can be in the form of a transdermal patch, solution, cream, gel etc. The limitation of solid MNs however, is associated with the requirement of a two-step application process which may lead to practicality issues for the eventual end-users.

Coated MNs are prepared by coating a drug formulation onto the microstructures pre-application. Upon insertion of the coated MN arrays into the skin, drug will be deposited in the skin following the dissolution of the drug coating material. This drug delivery strategy is termed “coat and poke” (Prausnitz, 2004).

Dissolving MNs function by creating microporation in the skin, followed by the dissolution of the MNs upon contact with the skin interstitial fluid. The drug payload of the MN matrix is then released over time.

The use of hollow MNs allows the continuous delivery of a particular medication via the injection of a fluid formulation containing the medication of choice through the hollow needle bore-opening into the skin.

In a recently published study, Donnelly *et al.* (2012) have reported a novel strategy of MN-mediated drug delivery *via* the use of hydrogel-forming polymeric MN arrays. The methodologies underpinning the use of such innovative MN technology will be described further in Section 3.5.

3.1 Solid MNs

Solid MNs have been fabricated from silicon (Donnelly *et al.*, 2009b; Wei-Ze *et al.*, 2010), metals and polymers e.g. polycarbonate (Oh *et al.*, 2008). The first solid MNs were fabricated from silicon using microfabrication technology (Henry *et al.*, 1998). Other research groups also employed microfabrication methods to produce silicon-based solid MNs of varying shapes, heights and densities (Moon and Lee, 2005; Wilke *et al.*, 2005; Xie *et al.*, 2005). Although the manufacture of silicon MNs using microfabrication methods offers the potential for high throughput MN manufacturing, it is often expensive, highly specialized and includes complex multi-step processes (Banga, 2009; Jin *et al.*, 2009). Metals, such as stainless-steel and titanium have been used as structural materials for solid MN fabrication (Cormier *et al.*, 2004; Ding *et al.*, 2009; Verbaan *et al.*, 2008; Wermeling *et al.*, 2008) and various approaches, for example, photochemical etching (titanium) and laser cutting (stainless-steel), have been employed for fabricating solid metal MNs. Metals, such as stainless-steel (e.g. hypodermic needles) have been in medical use for decades. Due to the conventional exploitation of such materials in medical usages, MN manufacturing from the same materials should not raise new issues of safety, thus facilitating the regulatory pathway towards commercial acceptance and approval. Moreover, MN devices produced using materials with less established safety profiles (e.g. silicon) have already obtained FDA clearance for the intradermal delivery of vaccines and drugs (<http://www.nanopass.com/>). In

addition, various silicon MN-based systems are also now available for use in cosmetic applications. (<http://www.genuinedermaroller.co.uk>, http://www.nanomed-skincare.com/en/core_tech.asp, <http://www.dermaneedle.com/>).

3.1.1 Solid MN design considerations—A plethora of studies have been published which demonstrate the effectiveness of using solid MNs to increase the transport of molecules with varying physicochemical properties into and across the skin. These have included studies using insulin (McAllister *et al.*, 2003), calcein (McAllister *et al.*, 2003; Oh *et al.*, 2008), naltrexone (Wermeling *et al.*, 2008), photosensitizer meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate (TMP) (Donnelly *et al.*, 2009b) and bovine serum albumin (BSA) (McAllister *et al.*, 2003). The influence of different parameters, such as MN insertion force, tip sharpness and MN density on solid MN-mediated skin permeation of therapeutics has been reported in the literature. For example, Wei-Ze *et al.* (2010) evaluated the effect of different MN insertion forces (1, 3, 5, 7 and 8 N/array), MN tip sharpness (sharp and flat tip) and MN densities (8×8 , 10×10 , 12×12) on the performance of super-short silicon MNs (70–80 μm in height) used to enhance the transport of a model compound, galanthamine (GAL), across full-thickness rat skin (Wei-Ze *et al.*, 2010). It was demonstrated that the application of forces in the range between 1 and 5 N/array resulted in increased transdermal GAL transport. However, a further increase in the insertion force beyond this range did not lead to any further permeation enhancement. This indicated that insertion force has a significant effect on the transport of molecules, but only when complete MN penetration has not been achieved. GAL permeation was increased after skin pre-treatment with flat-tipped MNs in comparison to sharp-tipped MNs. In addition, the results revealed that increasing the number of MNs per unit area resulted in increased flux of GAL. However, there was no statistical difference in the cumulative amount of GAL permeated across skin treated with 10×10 and 12×12 MN array (Wei-Ze *et al.*, 2010).

The effect of different application scenarios on the efficacy of solid silicon MNs to enhance *in vitro* transdermal delivery of relatively small molecular (calcein) compounds as well as macromolecular (insulin, BSA and nanoparticles) compounds has been studied by McAllister *et al.* (2003). Two different scenarios were investigated. Firstly, MN arrays (400 needles, 150 μm in height) were inserted into human cadaver epidermis and left in place during the application of drug solutions. The results showed that the skin permeation of calcein, insulin and BSA were enhanced by orders of magnitude which were deemed by the authors to be significant findings as the skin is generally considered to be impermeable, particularly to macromolecules. In the second set of experiments, MN arrays were used to pierce the skin and then removed, after which drug formulations were applied. It was found that skin permeability to model compounds was increased by an additional order of magnitude in comparison to the first outlined scenario. Furthermore, pre-treatment of the skin with MNs resulted in the transport of detectable amounts of nanoparticles across the skin (McAllister *et al.*, 2003). This is in accordance with the study reported by Zhang *et al.* (2010) on synergistic enhancement of *in vitro* topical delivery of fluorescent poly (D,L-lactic-co-glycolic acid) (PLGA) nanoparticles in human skin pre-treated with solid silicon MNs. In this study, the deposition of the fluorescent nanoparticles within a 48-hour period was mainly in the epidermis layer (approximately 25%) rather than in the dermis (less than

2%). However both were significantly enhanced compared to the control groups without MN pre-treatment ($p < 0.01$) (Zhang *et al.*, 2010).

In another study using polymer-based polycarbonate solid MNs, the influence of various MN heights (200 and 500 μm), MN densities (45, 99 and 154 MN/cm^2) and MN application on the permeation of a model hydrophilic molecule, calcein (62.5 Da) across rat skin *in vitro* was investigated (Oh *et al.*, 2008). The following methods of MN/drug formulation and application were tested: the MN array was inserted into the skin for 30 min prior to the application of a calcein gel; the calcein gel was applied to the skin directly and the MN array was inserted into the same site for 30 min; or the calcein gel was applied into the skin simultaneously with the MN array. It was found that the highest skin permeation of calcein was achieved when the calcein gel was simultaneously applied with a MN array of 500 μm height. Furthermore, the skin permeation of calcein was dependent upon the design of the MN employed, with increasing amounts delivered as the height and the density of the MN arrays increased (Oh *et al.*, 2008). Therefore, based on the outlined studies, these various parameters are of integral importance when designing and fabricating solid MNs in order to maintain optimum penetration and performance characteristics.

3.1.2 Insulin delivery—The influence of different insulin concentrations (100 and 500 IU/ml), duration of MN insertion (10 sec, 10 min and 4 h), in addition to the influence of the number of MN array insertions (1 insertion and 5 insertions) on the transdermal delivery of insulin to diabetic rats using solid stainless steel MNs (105 needles, 1000 μm in height) was investigated by Martanto *et al.* (2004). When the duration of MN insertion was increased, this resulted in a lower reduction in blood glucose levels, when compared to shorter insertion times. This observation was attributed to the blockage of micro channels by MN arrays left *in situ* thus leading to the delivery of lower insulin concentrations, thus highlighting a limitation in the use of solid MN arrays when delivering therapeutics. Moreover, the insertion of a single MN array was found to be more effective in reducing blood glucose levels than multiple insertions of MN arrays. The authors hypothesised that this phenomenon was due to local damage of the skin which resulted in altered insulin clearance and its absorption by the capillary bed (Martanto *et al.*, 2004).

3.1.3 Combinatorial approaches—Solid MNs, used in combination with other enhancement strategies, such as iontophoresis and/or nanovesicles, have been shown to produce synergistic effects in the facilitation of the transdermal delivery of numerous molecules. One example of such was reported in 2012. In this study, the treatment of the skin with solid silicon MNs and iontophoresis was reported to enhance skin permeation of insulin, which had been incorporated into transdermal patches. The result of this was the maintenance of a sustained basal dose of insulin for the continuous reduction of blood glucose levels and also on-demand bolus dosing for mealtime coverage *via* the iontophoresis “switch on” effect (Qin *et al.*, 2012).

A combinatorial approach was again adopted by Chen and colleagues when skin was pre-treated with solid stainless steel MNs (296 needles; 800 μm in length) followed by the application of insulin-loaded nanovesicles *via* iontophoresis (Chen *et al.*, 2009a). Results reported following adoption of this approach showed comparable reductions in blood

glucose levels to those induced using conventional subcutaneous insulin injections, thus highlighting the potential of such a novel, combinatorial approach for the facilitation of insulin delivery.

Synergistic enhancement effects of MN pre-treatment using a solid MN device (484 needles; 150 μm in height) in combination with a docetaxel-containing liposome delivery system was shown to enhance transdermal flux across porcine skin *in vitro* (Qiu *et al.*, 2008). The authors suggested that this approach could be used to achieve higher and more stable transdermal delivery rates of drugs with high molecular weights and poor water solubility. Interestingly in this study, a significant reduction in the lag time of approximately 70% was observed following MN skin pre-treatment compared to that obtained from conventional liposomes used in isolation. This evidence suggests the potential of MN methodologies to not only enhance the overall rate and extent of the transdermal drug permeation, but also to allow faster pharmacological effects by eliminating the problem associated with the lag time of conventional transdermal delivery. This observation is of particular significance when considering the delivery of drugs such as lidocaine where rapid exertion of therapeutic effect is preferable in cases of emergency delivery.

3.1.4 Vaccine delivery—Successful transcutaneous intradermal vaccination, with or without adjuvant, has also been demonstrated using solid MNs. Ding *et al.* (2009) investigated immune responses in mice following MN-mediated transcutaneous immunization using a model antigen, diphtheria toxoid (DT). Stainless steel MN arrays (4×4 , 300 μm in height) were used to pierce the mouse skin and DT formulation with or without cholera toxoid as adjuvant was administered. The application of DT on untreated skin resulted in low serum IgG titres. However, MN pre-treatment led to significantly higher serum IgG titres, which were further increased in the presence of cholera toxin (Ding *et al.*, 2009). In a follow up study, the same research group investigated MN-assisted transport of DT formulations with N-trimethyl chitosan (TMC) as adjuvant into mice skin (Balet *et al.*, 2010). The formulations tested were: TMC nanoparticles loaded with DT, liquid mixtures of TMC and DT and DT solution alone. Application of DT solution to MN-treated skin was shown to elicit a stronger immune response in comparison to the administration of DT solution to skin which had not been MN treated. Administration of DT-loaded-TMC-nanoparticles did not result in further enhancement of immune response. However, when TMC/DT solutions were applied to MN-treated skin, an 8-fold increase in IgG titers was observed in comparison to the application of nanoparticles and DT solution alone (Bal *et al.*, 2010). In a more recent study, the combined effect of solid MN pre-treatment (4×4 MN array, 300 μm in height) with hepatitis B surface antigen encapsulated vesicle formulations for potential transcutaneous vaccination was evaluated in female BALB/c mice (Hirschberg *et al.*, 2012). The antigen-loaded vesicles alone failed to induce an immune response in the mice, however MN-treated skin resulted in a robust immune response and incorporation of adjuvant resulted in further improved immune response in mice (Hirschberg *et al.*, 2012). The results of these studies serve to enhance the profile of MN administration in the convenient, safe and efficacious delivery of vaccines.

3.1.5 Gene delivery—The potential application of solid MNs to enhance cutaneous gene delivery has also been investigated (Chabri et al., 2004; Coulman *et al.*, 2006b; Pearton *et al.*, 2008). In one study, Pearton *et al.* (2008) utilised an array of frustum-shaped silicon MNs (with 4×4 array, 260 μm in height). MN application was shown to produce micropores of approximate depths 150–200 μm and resulted in increased transepidermal water loss (TEWL) in comparison to untreated skin samples. Two hydrogel formulations loaded with plasmid DNA (pDNA): 1% w/v Carbopol®-940 and 23% w/w tri-block copolymer PLGA-PEG-PLGA were applied to the split-thickness human breast skin prior to MN array application. Release of functional pDNA from both hydrogel formulations was indicated by successful transfection of epidermal cells and subsequent expression of the gene product in the cutaneous tissue.

3.1.6 Clinical studies in humans—In addition to *in vitro* and *in vivo* studies conducted in animal models, some clinical studies in humans have also been carried out using solid MNs. The first study was carried out by Wermeling *et al.*, (2008) to demonstrate the MN-based transdermal delivery of an opioid blocker, naltrexone (NTX). This compound was deemed to be an ideal candidate for MN administration due to the fact that passive transdermal delivery of it is limited by its hydrophilic nature. Skin on the upper arm of healthy volunteers was pretreated with two solid stainless steel MN arrays (5×10 MNs, 620 μm in height) which was followed by NTX patch application and collection of blood samples over a 72 h sampling period. Results showed variable absorption of NTX (1.6 to 8.1 ng/ml) achieved within a wide timeframe (1.5 to 18 h) of patch application, followed by steady-state plasma concentrations of ~ 2.5 ng/ml which were maintained for at least 48 h. Application of NTX patches to untreated skin did not result in detectable drug plasma concentrations. This study served as a platform to demonstrate the feasibility of using MNs to enhance the transdermal delivery of hydrophilic molecules.

Taken together, these various studies suggest a potential role for solid MNs in promoting successful enhanced transdermal and intradermal delivery of various low and macromolecular therapeutic compounds, genetic materials and vaccines. Commercialisation of silicon-based solid MNs in the field of cosmetic applications should serve to promote the case for commercialisation of other solid MN devices.

3.2 Coated MNs

Coated MNs can be fabricated from silicon or metal and the drug in question is loaded onto the individual needles of the MN array in a dry state as a coating layer (Cormier *et al.*, 2004; Hooper *et al.*, 2007; Kim *et al.*, 2010, 2012b; Manhee Han *et al.*, 2009; Ng *et al.*, 2012; Tas *et al.*, 2012). Following insertion of the coated MNs into the skin, the drug is rapidly released into the tissue. Coated MNs offer the advantage of allowing a simplistic one-step application process in comparison to un-coated solid MNs which require a two-step approach (i.e. the “poke and patch approach”). Coated MNs have been considered as particularly attractive candidates for the rapid cutaneous delivery of macromolecules such as vaccines, proteins, peptides and DNA to the skin (Cormier *et al.*, 2004; Hooper *et al.*, 2007; Kim *et al.*, 2010, 2012b; Manhee Han *et al.*, 2009; Ng *et al.*, 2012; Tas *et al.*, 2012). However, one major concern and limiting factor in achieving this end is the restricted

amount of drug that can be coated onto the miniscule surface area of the MN structures. This therefore suggests that delivery *via* coated MNs is restricted only to potent molecules/drugs so as to ensure optimal drug dosage without compromising the mechanical strength of the needles which is required to achieve insertion into the skin. Furthermore, the optimisation of MN coating methods and formulation characteristics suffers from issues such as ensuring the consistency, uniformity, reproducibility and stability of the MN drug coating materials while minimising drug loss during the coating process. In addition to this, the premature and deleterious loss of drug coating from the MN surface prior to insertion into the skin, for example during handling, must be prevented (Gill and Prausnitz, 2007b).

3.2.1 Coating methodologies—Different methodologies have been developed and adapted to successfully coat all the individual MN shafts encompassing a MN array with particular drug compounds. For example, the micron-scale dip-coating process was proposed by Gill and Prausnitz (Gill and Prausnitz, 2007a; Gill and Prausnitz, 2007b) and was successfully applied for the deposition of a variety of molecules differing in physicochemical properties onto the surface of MNs. The two most important parameters affecting the dip-coating process are surface tension and viscosity of the formulation. The authors demonstrated that the reduction of surface tension by the addition of surfactants (e.g. Lutrol® F-68) and the increase in viscosity of the coating solution by the addition of viscosity enhancers (e.g. carboxymethylcellulose sodium salt, sucrose) resulted in uniform and thick drug coatings onto MNs (Gill and Prausnitz, 2007a; Gill and Prausnitz, 2007b). Coating solutions with lower surface tensions were determined to facilitate good wetting and decreased speed of film formation on the MN surface. In contrast, higher viscosity (and reduced contact angle of the coating solution) caused increased volume of liquid film adherence to the MNs and increased residence time of the film on the MN surface. The versatility of this coating technique was demonstrated by coating MNs with a hydrophobic molecule, curcumin, in addition to the model proteins, BSA and insulin, in either organic or aqueous-based coating solutions. The investigators then proposed a novel approach where drug could be deposited within holes created in the centre of the MNs. In the most complex design, a drug layering approach was adopted. The holes in the centre of the MN, termed pockets, were selectively filled with a drug formulation and a protective layer composed of biodegradable polymer poly(lactic-*co*-glycolic acid) (PLGA), was coated onto the surface of the MN shaft. Finally, a second drug layer was deposited onto the MN.

Chen *et al.* (2009b) proposed a novel coating technique utilizing gas jet to distribute coating solution evenly onto the surface of individual MN shafts. The microprojection patch was composed of densely packed ($\sim 20,000 \text{ cm}^2$) solid silicon MNs of heights of 30, 60 or 90 μm . The coating solution was applied to the patch and gas jet (6–8 m/s) at an incident angle of 20° was used to distribute and quickly dry the formulation onto the entire patch. The proposed method allowed for the uniform coating of a variety of compounds (OVA, rhodamine-labelled dextran, ethidium bromide) on short and densely packed microprojections (Chen *et al.*, 2009b).

3.2.2 Gene delivery—The use of coated MN for delivery of genetic materials for various clinical indications (e.g. genetic immunisations) has been reported by Pearton *et al.* (2012).

In this study, genetic material, in particular plasmid DNA (pDNA), was dry-coated onto in-plane stainless steel MNs 750 μm in length. The reliable loading capacity of pDNA onto the MNs, its stability and the performance of the MNs in skin penetration were reflected by successful gene expression in excised, but viable human cutaneous tissue. However, the authors highlighted the need to further investigate the dissolution characteristics of this coated pDNA prior to investigation in a live animal model (Pearton *et al.*, 2012). More recently, the same research group also demonstrated successful proof-of-concept gene silencing using steel MN coated with small interfering RNA (siRNA) (Chong *et al.*, 2013).

3.2.3 Vaccine delivery—Coated MN have been extensively studied to facilitate transcutaneous vaccination using MNs. An abundance of immune-presenting cells (APCs) makes the skin an extremely attractive site for antigen presentation. Antigens can be introduced into the skin *via* coated MNs to target Langerhans cells in the epidermis or dendritic cells in the dermis in order to induce a more pronounced immune response (Gill and Prausnitz, 2007b). The limited drug quantities that can be coated onto MNs does not in fact hinder their application in vaccine delivery as only minute quantities of antigen are necessary to elicit an immune response (Wermeling *et al.*, 2008). Stability concerns associated with conventional injectable vaccines such as the need for the cold chain preservation of vaccine potency during storage and transport is a critical issue. The storage of vaccines in a dry state coated onto MN arrays may circumvent this issue however as it allows the preservation of vaccine stability to a greater extent than storage in the form of an injectable (Gill and Prausnitz, 2007b). Most importantly, coated MN-mediated vaccinations manufactured from different fabrication materials (metals or polymer), with or without adjuvant and/or in combination with other enhancing technologies have been demonstrated to induce superior or comparable immunogenicity with attractive advantages of dose sparing compared to the conventional vaccination routes such as subcutaneous or intramuscular administration, as discussed in Table 1 (Chou *et al.*, 2012; Fernando *et al.*, 2012; Kim *et al.*, 2012b; Koutsonanos *et al.*, 2012; Weldon *et al.*, 2012). Most recent advances in the development of coated MNs for transcutaneous immunisation are also presented in Table 1.

3.3 Dissolving MNs

Dissolving MNs have been fabricated primarily from polymers mainly due to the biocompatibility, biodegradability and long established safety profiles of polymers in medical tools (Park *et al.*, 2005, 2007; Jin *et al.*, 2009). Examples of polymers used in the fabrication of dissolving MNs include polylactic acid (PLA) (Aoyagi *et al.*, 2008; Park *et al.*, 2005), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA) (Park *et al.*, 2005), polyvinylpyrrolidone (PVP), poly(vinylpyrrolidone-co-methacrylic acid) (PVP-MAA) (Sullivan *et al.*, 2008) and poly(methyl vinyl ether-maleic anhydride) (Donnelly *et al.*, 2010). Biopolymers such as sodium hyaluronate (Matsuo *et al.*, 2012a, 2012b), chondroitin sulphate (Ito *et al.*, 2011, Naito *et al.*, 2012) and carbohydrates e.g. sugars, carboxymethyl cellulose and amylopectin (Lee *et al.*, 2008; Park *et al.*, 2010) have also been employed. A variety of mould-based techniques were employed in the production of dissolving MNs including solvent casting (Mansoor *et al.*, 2012), modified thermal imprinting (Shibata *et al.*, 2011), hot embossing (Han *et al.*, 2007), laser machining (Aoyagi *et al.*, 2007; Donnelly *et*

al., 2011), micro-injection moulding (Wang *et al.*, 2008) and polymer investment moulding (Lippmann *et al.*, 2007).

3.3.1 Polymer choice - Considerations—In the dissolving MN system of delivery, the rates at which constituent polymers dissolve within the skin will influence the release kinetics of the incorporated drug. To this end, careful consideration when selecting the polymer of choice from which to fabricate the MN arrays plays an important role in dictating the dissolution characteristics of the drug in question. In addition, controlled drug delivery is achievable by simple adjustment of the polymeric composition of the MN array or by modification of the MN fabrication process. The use of water-soluble and biodegradable polymers or sugars eliminates the potential risk of leaving biohazardous sharp waste in the skin and in addition guarantees safe MN disposal by mechanical destruction or dissolution in a solvent (Park *et al.*, 2005; Prausnitz and Langer, 2008). The low cost of polymeric materials and their relatively easy fabrication in micro- moulding processes allows for the straight forward mass production of MN arrays formulated from these materials. Furthermore, the favourable biocompatibility and biodegradability profiles exhibited by polymeric materials such as those outlined above make them an attractive material for development in these drug delivery systems.

3.3.2 Dissolving MN fabrication - Limitations—Nevertheless, the development of dissolving MN systems in drug delivery methodologies has not been without obstacles. For example, drug loading can compromise the mechanical strength of MNs and the stability of the incorporated drug or macromolecule might be negatively affected by the fabrication processes used. One such example which illustrates the influence of the fabrication process on the stability of incorporated therapeutic molecules was reported by Donnelly and colleagues in 2009. Drug loaded galactose MNs (280 μm in height) were prepared in a micro-moulding process by melting galactose powder at 160°C and subsequent addition of model drugs: 5-aminolevulinic acid (ALA) and bovine serum albumin (BSA) (Donnelly *et al.*, 2009c). The authors highlighted in this study that MN fabrication resulted in substantial losses of the incorporated ALA and BSA (40% and 100%, respectively). The same authors also described a number of difficulties associated with the processing and storage of galactose MN arrays. The high viscosity of molten galactose and its tendency to solidify prevented the preparation of more than two MN arrays at a time, which excluded the possibility of easy scale up for mass production. In addition, storage of MN arrays at ambient conditions led to their deformation within 1 h and complete loss of MN shape in 6 h (Donnelly *et al.*, 2009c). Furthermore, the issue of degradation of the incorporated drug could also be expected when using MN fabrication processes which require elevated temperatures to facilitate polymer melting, such as maltose with a melting point of 110°C (Lee *et al.*, 2011). These concerns are obviously of particular importance when considering the encapsulation of any thermo-labile molecules. Alternative MN fabrication methods which do not involve exposure of drug molecules to such elevated temperatures have also been proposed. These include fabrication by room-temperature photo- polymerization of the liquid monomer vinyl pyrrolidone (Sullivan *et al.* 2008); the thread forming technique (Ito *et al.*, 2006); employment of aqueous blends of different types of polymers (Lee *et al.*, 2008) and low temperature vacuum-forming micromoulding method (Martin *et al.*, 2012).

3.3.3 The delivery of therapeutic and model compounds using dissolving MNs

—Dissolving MNs have been recently shown to enhance transdermal and dermal delivery of numerous substances including insulin (Ito *et al.*, 2012; Liu *et al.*, 2012), 5-aminolevulinic acid (Donnelly *et al.*, 2009c), sulforhodamine B (Lee *et al.*, 2008), low molecular weight heparin (Gomaa *et al.*, 2012), ovalbumin (Matsuo *et al.*, 2012b; Naito *et al.*, 2012), adenovirus vector (Matsuo *et al.*, 2012b) and a variety of vaccine antigens (Matsuo *et al.*, 2012a). Synergistic effects of dissolving MNs used in combination with other enhancing strategies have been reported recently by Garland *et al.* (2012) when the use of drug-loaded dissolving poly(methyl-vinyl-ether-co-maleic-acid) PMVE/MA MN arrays was coupled with iontophoresis. An approximate two-fold increment in the permeation of the model peptide (bovine insulin), in addition to a nearly three-fold increase in model protein permeation (fluorescein isothiocyanate - labelled bovine serum albumin, FITC-BSA) were observed across neonatal porcine skin after a 6 hour period. This observation was made following the simultaneous application of dissolving MN arrays consisting of 361 MNs/cm² 600 µm in height with iontophoresis, compared to MN alone. This implies that, when used in combination with iontophoresis, the delivery of therapeutic macromolecules is not only restricted to the drugs contained within the MN shafts alone, but therapeutic macromolecules contained within from the entire MN array matrix. Surprisingly however, the combination of both treatment modalities did not further enhance the *in vitro* permeation of model small hydrophilic solutes namely theophylline, methylene blue and fluorescein sodium across the porcine skin (Garland *et al.*, 2012).

Dissolving MNs have also been investigated for their potential application in transcutaneous immunisation. A recent study published within *Nature Medicine* (Sullivan *et al.*, 2010) on the use of dissolving polymeric MN patches for influenza vaccination received worldwide media attention, which highlights the level of interest that MN technology is currently receiving. The promise here is that the MNs rapidly dissolve in the skin interstitial fluid in the viable epidermis and/or dermis releasing their payload. This approach holds great promise, as the MN arrays would be unusable after removal from a patient's skin, meaning insertion into the skin on another person would not be possible. Therefore, this should greatly reduce any risk of infection transmission. Sullivan *et al.* (2010) fabricated MN arrays using a room photo-polymerised PVP, encapsulating inactivated influenza virus for a vaccination strategy in a mouse model *in vivo*. It was shown that a single vaccine dose with dissolving MNs induces protective immune responses superior to those obtained with intramuscular injection at the same dose, including increased lung viral clearance. Furthermore, MN vaccination generated a robust antibody and cellular immune response in mice that provided complete protection against lethal challenge (Sullivan *et al.*, 2010). The authors suggest that these results highlight the benefits of dissolving polymeric MN patches as a new technology for simpler and safer vaccination with improved immunogenicity that could facilitate increased vaccination coverage. More recent studies investigating the potential of dissolving MNs for transcutaneous vaccination demonstrated that dissolving MNs (MicroHyal), fabricated by sodium hyaluronate, were able to induce successful immune response in mice against tetanus toxoid, diphtheria toxoid, Se36 (malaria), influenza hemagglutinin, ovalbumin and adenovirus vector antigens (Matsuo *et al.*, 2012a, 2012b).

3.4 Hollow MNs

Hollow MNs have been fabricated in a wide range of heights and geometries mainly out of silicon and metal using MEMS techniques (Chandrasekaran et al., 2003; Griss and Stemme, 2003; Kolli and Banga, 2008; Roxhed et al., 2008a, 2008b; Stoeber and Liepmann, 2000). In addition, hollow glass (Wang et al., 2006), polymeric (Sammoura et al., 2007) and ceramic MNs (Ovsianikov et al., 2007) have also been manufactured.

Hollow MNs enable continuous delivery of molecules across the skin through the MN bore either by diffusion or pressure- or electrically-driven flow. In comparison to solid or coated MNs, which are capable of delivering small quantities of pharmaceuticals, this approach allows for infusion of larger amounts of drug substances (Roxhed *et al.*, 2008b). In addition, pressure-assisted injection *via* hollow MNs offers the potential to modulate drug delivery by altering the infusion rate. It was reported that an increase in the flow rate is proportional to the inner diameter of the MN and inversely related to MN length (Bodhale *et al.*, 2010). Martanto *et al.* (2006b) provided an extensive analysis of factors influencing flow rate of sulforhodamine B solution through a single hollow glass MN which was attached to a 250 μ l or 1 ml glass syringe (Martanto *et al.*, 2006b). Results showed that partial needle retraction, as well as the increase in pressure, caused a significant increase in flow rate. This result also proved that the infusion flow was greater in the presence of hyaluronidase as well as a beveled tip MN when comparing this to a blunt tip MN. Therefore, this study clearly demonstrated that by varying infusion parameters, different flow rates can be achieved and in turn, drug delivery could be controlled.

3.4.1 Limitations associated with hollow MN application—The successful use of hollow MNs can be hindered by potential clogging of the needle bore- opening with tissue during MN skin insertion (Gardeniers *et al.*, 2003). However, using innovative design to locate the bore-opening at the side of the MN tip, rather than at the extreme tip, this issue of clogging has been disabled (Griss and Stemme, 2003; Stoeber and Liepmann, 2000). Keeping the outlet off-centre not only prevents needle clogging but also increases the area of drug exposure to the tissue and retains tip sharpness. Another limitation associated with the hollow MNs is flow resistance due to dense dermal tissue compressed around MN tip during insertion (Martanto *et al.*, 2006a). Research has determined that partial needle retraction following insertion improved the fluid infusion due to relaxation of the compressed tissue and an increase in flow conductivity of skin beneath the MN tip (Wang *et al.*, 2006). Moreover, skin deformation during needle insertion could possibly be minimized by application of MN *via* drilling or vibrating motion (Wang *et al.*, 2006).

3.4.2 Cutaneous delivery of therapeutics by hollow MNs—To date, most of the studies regarding hollow MNs have been focused on fabrication aspects, whereas less attention has been given to their actual efficacy in delivering compounds across the skin. Insulin was the most extensively utilized molecule in that respect. Davis *et al.* (2005) reported successful transdermal insulin delivery *via* hollow MNs to diabetic rats. The authors inserted a MN array comprised of 16 metal needles (4×4) with a height of 500 μ m into rat skin. A glass chamber filled with insulin solution was adhered to the base of an array to serve as a drug reservoir. It was reported that the passive-diffusion-driven insulin delivery

resulted in the reduction of blood glucose levels over 4 h by 53% and remained constant in a 4 h post-delivery period (Davis *et al.*, 2005). Wang *et al.* (2006) investigated the efficacy of hollow glass MNs in enhancing the transport of insulin both *in vitro* and *in vivo* (Roxhed *et al.*, 2008a). FITC-insulin was successfully microinjected into hairless rat skin *in vitro*, as confirmed by bright-field and fluorescence microscopy. In this experiment, microinjection of insulin through the MNs inserted to a depth of 500-800 μm and infused for 30 min elicited a drop in blood glucose levels by 25% below the pretreatment values when approximately 5 μl of insulin solution was microinjected into diabetic rat skin. When the MNs were retracted back by $\sim 200 \mu\text{m}$, a greater volume of insulin solution was delivered ($\sim 30 \mu\text{l}$) and blood glucose levels were reduced by 70% below pretreatment values. The authors also demonstrated efficient microinjection of Caco-2 human intestinal epithelial cells through MNs into hairless rat skin *in vivo*. In addition, hollow MNs facilitated the delivery of microparticles into the skin of hairless rats *in vivo*. Nordquist and colleagues (2007) developed an integrated patch-like MN system where MNs were attached to a drug dispenser and evaluated the performance of the same *in vivo* in diabetic rats. The patch was composed of an array of 21 hollow silicon MNs and an electronically controlled drug dispenser where the drug was stored. This was then ejected through the hollow MNs when thermally expandable silicon material expanded into the liquid reservoir following supply of the voltage to the heater. Administration of insulin via the MN system to diabetic rats resulted in reduction of blood glucose levels at the end of 4 h monitoring period. The effect of electrically driven low and high insulin infusion rates (2 $\mu\text{l/h}$ and 4 $\mu\text{l/h}$) versus passive insulin infusion (0 $\mu\text{l/h}$ rate) on blood glucose levels have been demonstrated. Passive insulin infusion (0 $\mu\text{l/h}$ rate) and active infusion at a rate of 2 $\mu\text{l/h}$ and 4 $\mu\text{l/h}$ resulted in the decrease in blood glucose levels from the initial value of $19 \pm 1 \text{ mM}$ to $14 \pm 1 \text{ mM}$, $11 \pm 2 \text{ mM}$ and $9 \pm 1 \text{ mM}$, respectively. This finding suggested that greater reduction in blood glucose level can possibly be obtained by increasing the infusion rate of hollow MNs system. (Nordquist *et al.*, 2007).

The efficacy of hollow MNs has also been investigated in human subjects. Gupta *et al.* (2009) conducted a study to assess the effect of hollow MN on insulin delivery in Type 1 diabetic adults (1 male and 1 female) in comparison to that of a catheter infusion set (9 mm) (Gupta *et al.*, 2009). MN arrays were attached to 3 ml syringes which were further connected to a syringe pump that controlled the insulin delivery rate. MNs were inserted at a 90° angle into the abdominal skin to three different depths 1, 3.5 and 5 mm using a custom-made rotator device. Results showed that a MN insertion depth of 1 mm within the skin led to rapid insulin absorption and reduction in the glucose levels in fasting subjects, in comparison to either 9-mm catheter control or 3.5 and 5 mm insertion depths, thus proving the effectiveness of hollow MNs in minimally-invasive transdermal delivery of insulin. It was hypothesised that fast absorption of insulin administered at a depth of 1 mm was due to insertion of MNs in the close proximity of blood capillaries in the papillary region of the dermis.

Furthermore, Van Damme *et al.* (2009) conducted a single-blinded study to evaluate intradermal delivery of low-dose influenza vaccines (α -RIX®), using a novel MN device in 180 healthy men and women (Van Damme *et al.*, 2009). The MN device (MicronJet™)

consisted of an array of 4-silicon MNs (450 μm length) bonded to the tip of a plastic adapter which was mounted to a standard syringe. Subjects were randomly assigned to receive either a full-dose of vaccine administered intramuscularly (IM) by conventional needle and syringe or a low and a medium-dose administered intradermally by MicronJet™. It was concluded that the low-dose influenza vaccines delivered by MicronJet™ produced immunogenicity responses similar to that of full dose IM vaccinations, indicating the potential for vaccine dose sparing *via* the adoption of MN mediated intradermal administration of vaccines.

3.4.3 Combination of hollow MNs and other skin delivery methods—The combined effect of using hollow silicon MN with sonophoresis - termed SEMA (sonophoretic enhanced MNs array) to facilitate the delivery of hydrophilic and large molecular mass compounds, namely calcein and BSA, across pig skin has been investigated by Chen *et al.* (2010). Hollow MNs were used to breach the SC, hence facilitating delivery of drug deeper into the dermis layer, whilst ultrasounds improved the diffusion rates by cavitations effect in the epidermis and also in the hollow MNs drug reservoir. The key findings from this study were the incremental increase in the skin permeability to calcein of approximately 5 times by the hollow MNs alone, compared to passive diffusion of topically applied calcein formulation. The use of sonophoresis alone resulted in a 7-fold increase in skin permeability of calcein and a further 9-fold increase employing SEMA, proving the synergistic enhancement of calcein permeability by SEMA. Similar synergistic effects were also demonstrated with BSA in which the skin permeability of BSA was greatly increased to ~12 times using SEMA in comparison to ~7 times using MN alone and ~8.5 times using sonophoresis alone (Chen *et al.*, 2010).

3.5 Hydrogel-forming MNs

As previously mentioned (Section 3), unique hydrogel-forming MN systems have recently been described by Donnelly *et al.* (2012). Hydrogel MN arrays have been manufactured by using aqueous blends of polymeric materials (i.e. poly(methylvinylether/maleic acid) and poly(ethyleneglycol)) and a micromoulding process involving a novel laser-based methodology. As the manufacture process is performed under ambient conditions, such MNs can be employed to deliver thermolabile compounds such as peptide and proteins. Hydrogel MNs are integrated systems consisting of crosslinked blank needles projecting from a solid baseplate to which an adhesive drug reservoir is attached. Upon application of the MN array to the skin, the needles rapidly take up interstitial fluids from the tissue, thus inducing diffusion of the drug from the patch through the swollen microprojections (Figure 4).

Studies carried out in the Donnelly group have demonstrated the suitability of such MN technology as a means to enhance percutaneous delivery of a large variety of molecules such as small hydrophilic drugs (i.e. theophylline, caffeine, methylene blue and metronidazole) and high molecular weight compounds (i.e. insulin and bovine serum albumin) (Donnelly *et al.*, 2012). This system was shown to enable sustained transdermal transport of medicaments *in vitro* in dermatomed neonatal porcine skin where milligram doses of peptides and proteins per square centimetre were delivered over a period of 24 hours. The capability of such MN technology to enhance transdermal drug delivery was furthermore confirmed by *in vivo* animal experiments. In particular, application of insulin-loaded integrated hydrogel MNs

patch resulted in controlled reduction of blood glucose levels in diabetic rats (i.e. insulin blood glucose level was reduced to 90% of its original value when the MN was applied for 2 hours and it dropped further to 37% after 24 hours). Sustained release of FITC-BSA was also observed for protein-loaded integrated hydrogel MN patches (i.e. FITC-BSA plasma concentrations of 0.82 µg/ml and 8.86 µg/ml were registered after a MN application time of 2 and 12 hours respectively).

Complementary studies by the same research group (Garland *et al.*, 2011) indicated that transdermal drug delivery could be easily controlled by modulating the crosslink density of the hydrogel matrix. This implies that drug delivery can be tailored, on a case-by-case basis, to meet the requirements of different drugs with differing therapeutic windows, thus confirming the versatility of an integrated hydrogel-MN device.

Most interestingly, once applied to the skin, hydrogel MNs can be withdrawn intact from the tissue, leaving no polymeric residues behind. This represents a considerable advantage of the hydrogel MN technology in comparison to dissolvable MNs. In fact, although FDA-approved polymeric materials with long established safety profiles have been used to produce dissolvable microneedles (Park, 2006), these materials have never been administered intradermally. As a consequence, nothing is known about the fate of the polymeric materials which remain in the skin (and thus potentially within the entire body), following MN application. For example, nothing is known about the metabolic degradation which these compounds undergo before they are eventually eliminated from the body. In addition, the potential toxic effects which these polymeric residues may elicit following repeated MN applications and thus upon accumulation in the body are unknown. It is reasonable to assume that the absence of residual polymeric materials in the skin following hydrogel MN application may significantly accelerate the path to commercialisation path of this device, as the number of regulatory hurdles involved in this process would likely be reduced. This would also be aided by the fact that hydrogel MNs could tolerate the sterilisation process and thus the sterility of the devices could be assured if required.

Moreover, in contrast to that frequently observed when using hollow MNs (Gardeniers *et al.*, 2003), hydrogel MNs do not become blocked by compressed dermal tissue upon application (Donnelly *et al.*, 2012). As such, they offer the potential to enable better control of the delivery process and ultimately of the dose of medicament delivered. Hydrogel MNs would also overcome some of the limitations typically associated with coated MNs (Section 3.2), i.e. extremely reduced MN loading capacity, difficulty in achieving accurate drug coating and controlling extent and rate of drug release. Moreover, such MN devices would certainly be advantageous in comparison to uncoated solid MNs, as they would guarantee a simplified one-step application process.

Donnelly *et al.* (2012) also investigated the combined effect of iontophoresis and integrated hydrogel MN on drug permeation in both *in vitro* and *in vivo* studies. Enhancements in the rate and extent of transdermal delivery of all the compounds investigated (both low and high molecular weight compounds as mentioned above) were observed for MN combined with iontophoresis, in comparison to the MN alone. These experimental outcomes indicated that

electrically modulated delivery could also be achieved if specific therapeutic needs were required (e.g. bolus administration of insulin after a meal).

Interestingly, the use of the hydrogel MN may not be restricted to drug delivery only. The capability of the hydrogel MNs to imbibe interstitial fluids implies that such MNs could be used to extract molecules of interest from the skin for subsequent analysis. Given that drug concentrations in interstitial fluids often reflect those in plasma (Brunner and Derendorf, 2006), this system could prove of great use in bleeding free patient monitoring. This would overcome many of the issues associated with direct blood sampling and it is expected to be extremely advantageous for vulnerable patients such as neonates and the elderly.

4. Pain associated with MN application

Despite promising results from delivery studies and clinical trials, MN technology would be limited in its utility if application caused pain and distress in patients. Therefore, several studies have been conducted with the aim of establishing the scale of pain associated with MN insertion into skin, the temporary nature of the disruption of the skin's barrier function and the occurrence of other adverse skin reactions.

Kaushik *et al.* (2001) carried out the first MN safety evaluation study in human subjects (Kaushik *et al.*, 2001). A total of 12 male and female healthy volunteers, aged between 18 and 40 years, participated to the study. Silicon MN arrays comprised of 400 needles which were 150 μm long with a base diameter of 80 μm and tip radius of 1 μm where used in the study. Pain-scores from the subjects were recorded on a visual analogue scale (VAS) and it was found that MN application was painless and caused no skin damage or irritation.

Bal *et al.*, (2008) investigated safety and barrier disruption following application of MN arrays varying in length and shape of the tip (Bal *et al.*, 2008). A total of 18 healthy volunteers took part in the study. Parameters such as, barrier function of skin (measured by the TEWL), erythema and pain-score were measured. TEWL and erythema values after treatment with solid MN arrays of 400 μm height were significantly increased in comparison to solid MN arrays of 200 μm height. However, for all MN designs, the irritation was short-lasting (< 2 h) and application was perceived as painless.

Haq *et al.* (2009) investigated the pain and sensory responses in 12 human subjects (Haq *et al.*, 2009). The piercing of the skin with hypodermic needles was perceived by the subjects to be significantly more painful than MN insertion, whilst greater MN height resulting in higher pain score compared to shorter MN. This result was further supported by verbal comments from the participants who described hypodermic needle application as 'sharp' and 'stabbing' and perceived MN insertion as 'pressing' and 'heavy'.

In several other studies, focused mainly on the assessment of the efficacy of MN- assisted drug delivery to human subjects, the evaluation of pain and discomfort was also carried out. Wermeling *et al.* (2008) evaluated tolerability to not only MNs themselves but also MN arrays (5 $\times$ 10 MNs, 620 μm in height) in combination with drug formulations during investigation of MN-mediated systemic delivery of naltrexone (NTX) in human volunteers (Wermeling *et al.*, 2008). It was found that MN array insertion was 4 times less painful than

insertion of a hypodermic needle (the mean VAS score using 0 to 10 cm scale was 0.6 cm and 2.4 cm, respectively). In addition, after MN application, only transient erythema was observed which disappeared within a few hours. However, skin changes were more pronounced after MN insertion followed by application of NTX patch. In two out of six subjects, contact dermatitis occurred at the MN insertion site which was in contact with the NTX formulation and in another two subjects local irritation and mild erythema was observed. The increase in severity of adverse skin reactions was attributed to the properties of the NTX and the presence of benzyl alcohol in the NTX formulation, rather than the MN device itself.

In a study conducted by Sivamani *et al.* (2005), 1 µl of methyl nicotinate solution was injected to human volunteers by using 200 µm-long silicon MNs. MN application was perceived as a moderate pressure by human subjects and it was generally described as painless (Sivamani *et al.*, 2005). Similarly, Gupta *et al.* (2009) assessed pain when comparing insulin infusion *via* hollow MN with that administered *via* catheter. Both human subjects perceived MN-mediated insulin delivery as less painful than catheter infusion (Gupta *et al.*, 2009).

Van Damme *et al.*, (2009) reported that local reactions, such as erythema and swelling, were more frequent after intradermal injections of influenza vaccine (α-RIX®) using the novel MN device MicronJet™, in comparison to intramuscular injections (Van Damme *et al.*, 2009). However, the reactions were described as mild and of short duration. In addition, no significant difference was observed in the level of pain experienced by patients who received intradermal and intramuscular injections.

A more recent study by Gupta *et al.* (2011) highlighted that moderate pain sensation was generated following hollow MN insertion and the authors deduced that this may not only be associated with MN insertion, but also with the extrusion of liquid formulation from the MNs during injection. The authors concluded that regardless of MN length, the injection of up to 1 ml of drug solution was associated with minimal pain but as volumes increased above this limit, volunteers experienced increased pain due to the higher pressures associated with infusion of these volumes. (Gupta *et al.*, 2011)

5. Microbiological studies involving MNs application

The possibility for microorganism influx through MN-created skin conduits and the associated risk of skin infection have been investigated by several research groups (Donnelly *et al.*, 2009a, Wei-Ze *et al.*, 2010). Studies carried out in our group have demonstrated that specific microorganisms (*Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*) were capable of traversing the micron-sized pores induced in the SC by MNs (Donnelly *et al.* 2009a). Using either Silescol® membranes or excised porcine skin, representative Gram positive (*S. epidermidis*) and Gram negative (*P. aeruginosa*) bacteria and fungi (*C. albicans*) were shown to traverse the holes induced by MNs. In the experiments with Silescol® membranes, hypodermic needles with cross-sectional areas of 0.5 mm² were shown to induce significantly greater microbial penetration than MNs with combined cross-sectional areas of 1.5 mm². Therefore the results of this

study suggested that microbial infection risk associated with MN application is indeed very low and in fact less than that associated with conventional hypodermic needles. The safety of MN usage can of course be enhanced by the aseptic or sterile manufacture and fabrication of MNs from self-disabling materials (e.g. dissolving or biodegradable polymers) (Donnelly *et al.*, 2009a).

Wei-Ze *et al.* (2010) evaluated the potential for microorganism invasion through microconduits *in vivo* (Wei-Ze *et al.*, 2010). Rat skin was pretreated either with flat-tipped super-short MN array (10 × 10, 80 µm in height) or a macroneedle (1500 µm in height) and subsequently a culture solution of *Staphylococcus aureus* was applied. The development of infection was assessed by the measurement of white blood cells, leukomonocytes and neutrophil granulocyte levels within the blood. It was demonstrated that there was no significant difference in the populations of the three cell types between MN treated group and control group (untreated rats), indicating that the small size of the created microchannels did not allow for microorganism passage across the skin. In contrast, in rats treated with a macroneedle, the number of all three cell types was increased indicating development of an infection. In addition, the authors assessed changes in the skin pretreated with MN arrays by examining the skin expression of EIHA⁺(526 bp) segment, a sensitive marker of tissue injury. No expression of EIHA⁺(526 bp) segments in the skin was observed which was interpreted by the authors as evidence of a lack of MN-induced skin damage.

In a very recent and exciting development in this field, MNs fabricated using the acid anhydride copolymer, Gantrez® AN-169 BF, were shown to exhibit antimicrobial effects against several microorganisms (Boehm *et al.*, 2012). Agar diffusion assays were used to determine the antimicrobial properties of the MNs which were placed onto lawns of the microorganisms and subsequently incubated for 24 h. Of the microorganisms tested, *Bacillus subtilis*, *C. albicans*, *Enterococcus faecalis*, *Escherichia coli*, *P. aeruginosa* and *S. aureus*, large zones of growth inhibition were recorded for *E. coli*, *S. aureus*, *E. faecalis* and *B. subtilis*. This work serves to highlight the potential for use of this polymer and possibly polymer derivatives in future MN fabrication. Future work to assess the antimicrobial potential of this polymer against other skin pathogens, as well as *in vitro* assays with cadaveric skin and *in vivo* assays with animal models are warranted and planned by the authors of this work (Boehm *et al.*, 2012).

6. The perceptions of healthcare professionals and the public of MNs

The ultimate commercial success of MN-based delivery and monitoring devices will depend upon not only the ability of the devices to perform their intended function, but also their overall acceptability by both health care professionals (e.g. doctors, nurses and pharmacists) and patients. Accordingly, efforts to ascertain the views of these end-users will be essential moving forwards. The seminal study conducted by Birchall *et al.* (2011) in this regard was highly-informative. The majority of healthcare professionals and members of the public recruited into this focus-group-centred study were able to appreciate the potential advantage of using MNs. These advantages included reduced pain, tissue damage and risk of transmitting infections and needle stick injuries; the feasibility for self-administration and use in children, needlephobes and/or diabetics. However, some concerns regarding

effectiveness, a means to confirming successful drug delivery (such as a visual dose indicator that notifies the user that the correct dose has been delivered), delayed onset of action, costs of the delivery system, possible accidental use, misuse or abuse were also raised. Healthcare professionals were also concerned about inter-individual variation in skin thickness; problems associated with injecting small volumes and infection risk. Overall, the group reported that 100% of the public participants and 74% of the health care professional participants were optimistic about the future of MN technology. Further studies in the area of MN perceptions and acceptability will undoubtedly aid industry as it moves towards the marketing of MN devices.

7. Optical imaging methods for real time *in vivo* MN visualisation

In order for successful development and widespread commercialisation of MN technology, it is of paramount importance that the exact depth of MN penetration and the dimensions of the microchannels created within the skin, as well as the recovery of the skin's natural barrier function from patient to patient can be determined. The majority of studies to date have confirmed successful MN skin penetration by applying a coloured dye to the skin surface, or by measuring TEWL following MN removal. Although these techniques confirm whether the SC has been compromised, they provide no information with regards to the true depth of MN penetration. Recently, there has been a crucial development in optical imaging methods that could prove to be a major milestone for the progression of MN technology into a clinical reality. Researchers from three different groups have simultaneously highlighted the benefits of optical coherence tomography (OCT), enabling the non-invasive assessment of MN penetration depth, MN dissolution and pore closure kinetics, in real time and *in vivo*. Enfield *et al.* (2010) and Coulman *et al.* (2011) both utilised OCT to enable direct visualisation and quantification of the micropores created within the skin following MN insertion and subsequent removal, and followed the pore closure kinetics as the skin recovered *in vivo*. Whilst these two studies focused on the manual application of the MN array, it is envisaged that some form of quantifiable application method will be necessary for MN technology to be accepted by the regulatory authorities, healthcare professionals, as well as the general public. As such, Donnelly *et al.* (2010) evaluated the potential for OCT to enable an assessment of the effect that application force had upon MN penetration into skin *in vitro*. It was found that increasing the force used for MN application resulted in a significant increase in the depth of penetration achieved within neonatal porcine skin. Furthermore, it was shown that, at a constant application force, the density of a MN had no impact upon the depth of skin penetration achieved by a MN array. The authors believe that the use of OCT opens up the possibility to investigate the consistency of MN penetration, dissolution and also skin recovery on a patient-to-patient basis. Indeed, given the extensive *in vitro* characterisation of MN technology that has been completed to date, comprehensive studies to evaluate how MN design, skin penetration depth, and MN dissolution affect the performance of a MN device *in vivo* are warranted in anticipation of the widespread commercialisation of these devices..

8. MN products currently in clinical and commercial development

As previously mentioned (Section 3.1), several microneedle products are currently available on the market for cosmetic purposes. Dermalroller®, consisting of metal, 0.2-2.5 mm long MNs projecting from a cylindrical roller, was initially sold in Germany in 1999 and it has been commercialised worldwide (<http://www.dermalroller.com>). The device is designed for home use to improve skin texture or to be used in clinics to reduce the appearance of scars. Other examples of commercialised products are LiteClear® used in the treatment of acne and other skin conditions (http://www.nanomed-skincare.com/en/core_tech.asp; Kim *et al.*, 2012a) and MicroHyal® employed to reduce the appearance of wrinkles (Kim *et al.*, 2012a). The only MN-based system available on the market for therapeutic applications is Beckton-Dickinson's Soluvia®, a microinjection device, consisting of a 1.5 mm long, hypodermic needle attached to a syringe prefilled with influenza vaccine (<http://www.bd.com>). Soluvia® is currently commercialised worldwide as IDflu®, Intanza® and Fluzone Intradermal® for intradermal vaccination (http://en.sanofi.com/Images/14262_20081218_fluid_chmp_en.pdf; http://en.sanofi.com/Images/14032_20091223_FLU_HIGH_DOSE_en.pdf).

Moreover, there are currently a number of companies that are working towards commercialisation of their respective MN technologies including Zosano Pharma, Corium 3M and Nanopass Technology. Zosano Pharma are currently preparing to enter a Phase III pivotal trial using a solid drug coated MN patch system (MN height 190 µm) for the delivery of parathyroid hormone in the treatment of severe osteoporosis, with a high likelihood of positive outcome based upon Phase II results (<http://www.zosanopharma.com/index.php/20091106127/Research/Research-General/ZP-PTH.html>). Importantly, Zosano Pharma have incorporated an applicator device system as an essential component of this delivery system, which applies a consistent and pain free force, and allows for patient self-administration. Furthermore, focus groups (288 post-menopausal women with osteoporosis; aged 60-85 years) were conducted to evaluate patient perception of this technology, with positive outcomes noted. It was highlighted that 93% of patients liked the patch concept “extremely well”, whilst 90% rated it as easy to use, as exemplified by the fact that 82% of patients were capable of applying the patch correctly the first time without assistance (<http://www.zosanopharma.com/>). Indeed, it appears that incorporation of the applicator device led to enhanced patient acceptability and faith in the device as an efficient drug delivery system (Singh *et al.*, 2011). In terms of other MN devices, 3M's microstructured transdermal systems (MTS) encompassing either solid or hollow MNs have shown promising results in several preclinical studies for the delivery of proteins, peptides and vaccines (<http://solutions.3m.com>).

Finally, Micronjet® received FDA clearance in February 2010 (<http://www.nanopass.com>). This device consists of four hollow, less than 500 µm long, silicon microneedles protruding from a plastic device, which can be mounted onto any conventional syringe (Van Damme *et al.*, 2009). The Micronjet® has been used for intradermal delivery of commercially available drug formulations including influenza vaccines, lidocaine and insulin to human subjects and its application has been reported as effective, accurate and almost painless (<http://www.nanopass.com>; Van Damme *et al.*, 2009). The existence on the market of these

commercially available MN devices serves to encourage the MN field as it moves towards large-scale manufacture and marketing of even more varied and far-reaching MN innovations.

9. Conclusion

Since conception in the late 1990's, the MN field has continued to evolve and improve, with superior manufacturing materials, fabrication methods, and designs appearing within the scientific and patent literature. The introduction of biodegradable, polymeric MN devices may herald a new area in the development of MN technology, overcoming a number of disadvantages of previous MN designs. Firstly, solid MN devices may suffer from the fact that silicon is not a biomaterial with an established safety profile thus leading to concerns over the potential skin problems that could arise if breakage of silicon or metal MNs occurred. Secondly, the use of a solid, non-drug coated MN devices requires a two-step application process, which is undesirable, particularly for at home usage where a dosage form may not be positioned exactly over the area of skin where MN puncture was performed. Whilst coated MN devices may overcome this issue, accurate MN coating is a difficult process, which requires considerable research effort which must be optimised on a drug-to- drug basis. Furthermore, a coated MN device is only capable of delivering up to a maximum of 1mg of drug as a bolus dose only. Although hollow MNs offer the potential for continuous infusion, or as required dosing of a drug solution, central outlets may become blocked by compressed dermal tissue following MN insertion. The major advantages of polymeric MN systems include the possibility of loading a drug to be delivered into the MN matrix for release in skin by biodegradation or dissolution in skin interstitial fluid and, in many cases, their biocompatibility and biodegradability. Furthermore, the ability to produce MN devices from aqueous polymeric blends at ambient conditions, without the need for a heating step, could prove to be a notable advantage in preserving the stability of an incorporated drug, particularly in the case of protein/peptide and vaccine delivery.

Given the ever-increasing evidence available within the academic and patent literature that MNs of a wide variety of designs are capable of achieving successful intradermal and transdermal delivery of therapeutics, it is envisaged that the already concerted industrial effort into development of MN devices will now intensify. Furthermore, novel applications of MN technology are likely to come to the forefront. The ability of MN arrays to extract bodily fluids for determination of efficacy of medicines is particularly interesting. As technological advances continue, MN arrays may well become the pharmaceutical dosage forms and monitoring devices of the near future. However, there are a number of barriers that will firstly need to be addressed in order for microneedle technology to progress as discussed in this paper.

References

- Aoyagi S, Izumi H, Fukuda M. Biodegradable polymer needle with various tip angles and consideration on insertion mechanism of mosquito's proboscis. *Sensors and Actuators A: Physical*. 2008; 143(1):20–28.

- Aoyagi S, Izumi H, Isono Y, et al. Laser fabrication of high aspect ratio thin holes on biodegradable polymer and its application to a microneedle. *Sensors and Actuators A: Physical*. 2007; 139(1-2): 293–302.
- Bal SM, Ding Z, Kersten GF, et al. Microneedle-based transcutaneous immunisation in mice with N-trimethyl chitosan adjuvanted diphtheria toxoid formulations. *Pharmaceutical Research*. 2010; 27(9): 1837–1847. [PubMed: 20559701]
- Bal SM, Caussin J, Pavel S, et al. *In vivo* assessment of safety of microneedle arrays in human skin. *European Journal of Pharmaceutical Sciences*. 2008; 35(3):193–202. [PubMed: 18657610]
- Banga, AK. *Therapeutic Peptides and Proteins: Formulation, Processing and Delivery Systems*. CRC Press Taylor and Francis Group; Boca Raton: 2006.
- Banga AK. Microporation applications for enhancing drug delivery. *Expert Opinion on Drug Delivery*. 2009; 6(4):343–354. [PubMed: 19348604]
- Birchall JC, Clemo R, Anstey A, et al. Microneedles in clinical practice - an exploratory study into the opinions of healthcare professionals and the public. *Pharmaceutical Research*. 2011; 28(1):95–106. [PubMed: 20238152]
- Bodhale WD, Nisar A, Afzulpurkar N. Structural and microfluidic analysis of hollow side-open polymeric microneedles for transdermal drug delivery applications. *Microfluid Nanofluid*. 2010; 8:373–392.
- Boehm RD, Miller PR, Singh R, et al. Indirect rapid prototyping of antibacterial acid anhydride copolymer microneedles. *Biofabrication*. 2012; 4(1):011002. [PubMed: 22287512]
- Brunner M, Derendorf H. Clinical microdialysis: Current applications and potential use in drug development. *Trends in Analytical Chemistry*. 2006; 25(7):674–680.
- Bystrova S, Luttge R. Micromolding for ceramic microneedle arrays. *Microelectronic Engineering*. 2011; 88(8):1681–1684.
- Chabri F, Bouris K, Jones T, et al. Microfabricated silicon microneedles for nonviral cutaneous gene delivery. *British Journal of Dermatology*. 2004; 150(5):869–877. [PubMed: 15149498]
- Chandrasekaran S, Brazzle JD, Frazier AB. Surface micromachined metallic microneedles. *Journal of Microelectromechanical Systems*. 2003; 12(3):281–288.
- Chen H, Zhu H, Zheng J, et al. Iontophoresis-driven penetration of nanovesicles through microneedle-induced skin microchannels for enhancing transdermal delivery of insulin. *Journal of Controlled Release*. 2009a; 139(1):63–72. [PubMed: 19481577]
- Chen X, Prow TW, Crichton ML, et al. Dry-coated microprojection array patches for targeted delivery of immunotherapeutics to the skin. *Journal of Controlled Release*. 2009b; 139(3):212–220. [PubMed: 19577597]
- Chen B, Wei J, Iliescu C. Sonophoretic enhanced microneedles arrays (SEMA) - Improving the efficiency of transdermal drug delivery. *Sensors and Actuators B: Chemical*. 2010; 145(1):54–60.
- Choi HJ, Yoo D-G, Bondy BJ, et al. Stability of influenza vaccine coated onto microneedles. *Biomaterials*. 2012; 33(14):3756–3769. [PubMed: 22361098]
- Chong RHE, Gonzalez-Gonzalez E, Lara MF, et al. Gene silencing following siRNA delivery to skin *via* coated steel microneedles: *in vitro* and *in vivo* proof-of-concept. *Journal of Controlled Release*. 2013; 166(3):211–219. [PubMed: 23313112]
- Cormier M, Johnson B, Ameri M, et al. Transdermal delivery of desmopressin using a coated microneedle array patch system. *Journal of Controlled Release*. 2004; 97(3):503–511. [PubMed: 15212882]
- Coulman S, Allender C, Birchall J. Microneedles and other physical methods for overcoming the stratum corneum barrier for cutaneous gene therapy. *Critical Reviews in Therapeutic Drug Carrier Systems*. 2006a; 23(3):205–258. [PubMed: 17206925]
- Coulman SA, Barrow D, Anstey A, et al. Minimally invasive cutaneous delivery of macromolecules and plasmid DNA *via* microneedles. *Current Drug Delivery*. 2006b; 3(1):65–75. [PubMed: 16472095]
- Coulman SA, Birchall JC, Alex A, et al. *In vivo*, *in situ* imaging of microneedle insertion into the skin of human volunteers using optical coherence tomography. *Pharmaceutical Research*. 2011; 28(1): 66–81. [PubMed: 20464461]

- Crichton ML, Ansaldo A, Chen X, et al. The effect of strain rate on the precision of penetration of short densely packed microprojection array patches coated with vaccine. *Biomaterials*. 2010; 31(16):4562–4572. [PubMed: 20226519]
- Davis SP, Martanto W, Allen MG, et al. Hollow metal microneedles for insulin delivery to diabetic rats. *IEEE Trans. Biomed. Eng.* 2005; 52(5):909–915. [PubMed: 15887540]
- Davis SP, Landis BJ, Adams ZH, et al. Insertion of microneedles into skin: measurement and prediction of insertion force and needle fracture force. *Journal of Biomechanics*. 2004; 37(8): 1155–1163. [PubMed: 15212920]
- Ding Z, Verbaan FJ, Bivas-Benita M, et al. Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice. *Journal of Controlled Release*. 2009; 136(1):71–78. [PubMed: 19331846]
- Donnelly RF, Garland MJ, Morrow DI, et al. Optical coherence tomography is a valuable tool in the study of the effects of microneedle geometry on skin penetration characteristics and in-skin dissolution. *Journal of Controlled Release*. 2010; 147(3):333–341. [PubMed: 20727929]
- Donnelly RF, Majithiya R, Singh TR, et al. Design, Optimization and Characterisation of Polymeric Microneedle Arrays Prepared by a Novel Laser-Based Micromoulding Technique. *Pharmaceutical Research*. 2011; 28(1):41–57. [PubMed: 20490627]
- Donnelly RF, Morrow DI, McCarron PA, et al. Microneedle-mediated intradermal delivery of 5-aminolevulinic acid: potential for enhanced topical photodynamic therapy. *Journal of Controlled Release*. 2008; 129(3):154–162. [PubMed: 18556084]
- Donnelly RF, Singh TR, Tunney MM, et al. Microneedle arrays allow lower microbial penetration than hypodermic needles *in vitro*. *Pharmaceutical Research*. 2009a; 26(11):2513–2522. [PubMed: 19756972]
- Donnelly RF, Morrow DI, McCarron PA, et al. Microneedle arrays permit enhanced intradermal delivery of a preformed photosensitizer. *Photochemistry and Photobiology*. 2009b; 85(1):195–204. [PubMed: 18764907]
- Donnelly RF, Morrow DI, Singh TRR, et al. Processing difficulties and instability of carbohydrate microneedle arrays. *Drug Delivery and Industrial Pharmacy*. 2009c; 35(10):1242–1254.
- Donnelly RF, Singh TRR, Garland MJ, et al. Hydrogel-forming microneedle arrays for enhanced transdermal drug delivery. *Advanced Functional Materials*. 2012; 22(23):4879–4890. [PubMed: 23606824]
- Enfield J, O'Connell ML, Lawlor K, et al. *In vivo* dynamic characterization of microneedle skin penetration using optical coherence tomography. *J. Biomed. Opt.* 2010; 15(4):046001. [PubMed: 20799803]
- Fernando GJ, Chen X, Primiero CA, et al. Nanopatch targeted delivery of both antigen and adjuvant to skin synergistically drives enhanced antibody responses. *Journal of Controlled Release*. 2012; 159(2):215–221. [PubMed: 22306334]
- Gardeniers HJGE, Luttge R, Berenschot EJW, de Boer MJ, et al. Silicon micromachined hollow microneedles for transdermal liquid transport. *Journal of Microelectromechanical Systems*. 2002; 12(6):855–862.
- Garland MJ, Caffarel-Salvador E, Migalska K, et al. Dissolving polymeric microneedle arrays for electrically assisted transdermal drug delivery. *Journal of Controlled Release*. 2012; 159(1):52–59. [PubMed: 22265694]
- Garland MJ, Singh TR, Woolfson AD, et al. Electrically enhanced solute permeation across poly(ethylene glycol)-crosslinked poly(methyl vinyl ether-co-maleic acid) hydrogels: effect of hydrogel crosslink density and ionic conductivity. *Int. J. Pharm.* 2011; 406(1-2):91–8. [PubMed: 21236323]
- Gerstel, MS.; Place, VA. Drug Delivery Device. 1976. United States Patent, 3,964,482
- Gill HS, Prausnitz MR. Coating formulations for microneedles. *Pharmaceutical Research*. 2007a; 24(7):1369–1380. [PubMed: 17385011]
- Gill HS, Prausnitz MR. Coated microneedles for transdermal delivery. *Journal of Controlled Release*. 2007b; 117(2):227–237. [PubMed: 17169459]

- Gomaa YA, Garland MJ, McInnes F, et al. Laser-engineered dissolving microneedles for active transdermal delivery of nadroparin calcium. *European Journal of Pharmaceutics and Biopharmaceutics*. 2012; 82(2):299–307. [PubMed: 22836025]
- Griss P, Stemme G. Side-opened out-of-plane microneedles for microfluidic transdermal liquid transfer. *Journal of Microelectromechanical Systems*. 2003; 12(3):296–301.
- Gupta H, Sharma A. Recent trends in protein and peptide drug delivery systems. *Asian Journal of Pharmaceutics*. 2009; 3(2):69–75.
- Gupta J, Felner EI, Prausnitz MR. Minimally invasive insulin delivery in subjects with type 1 diabetes using hollow microneedles. *Diabetes Technology & Therapeutics*. 2009; 11(6):329–337. [PubMed: 19459760]
- Gupta J, Park SS, Bondy B, et al. Infusion pressure and pain during microneedle injection into skin of human subject. *Biomaterials*. 2011; 32(28):6823–6831. [PubMed: 21684001]
- Han M, Hyun D-H, Park H-H, et al. A novel fabrication process for out-of-plane microneedle sheets of biocompatible polymer. *Journal of Micromechanics and Microengineering*. 2007; 17(6):1184–1191.
- Han M, Kim DK, Kang SH, et al. Improvement in antigen-delivery using fabrication of a grooves-embedded microneedle array. *Sensors and Actuators B: Chemical*. 2009; 137(1):274–280.
- Haq MI, Smith E, John DN, et al. Clinical administration of microneedles: skin puncture, pain and sensation. *Biomedical Microdevices*. 2009; 11(1):35–47. [PubMed: 18663579]
- Henry S, McAllister DV, Allen MG, et al. Microfabricated Microneedles: A Novel Approach to Transdermal Drug Delivery. *Journal of Pharmaceutical Sciences*. 1998; 87(8):922–925. [PubMed: 9687334]
- Hirschberg H, van Kuijk S, Loch J, et al. A combined approach of vesicle formulations and microneedle arrays for transcutaneous immunization against hepatitis B virus. *European Journal of Pharmaceutical Sciences*. 2012; 46(1-2):1–7. [PubMed: 22330147]
- Hooper JW, Golden JW, Ferro AM, et al. Smallpox DNA vaccine delivered by novel skin electroporation device protects mice against intranasal poxvirus challenge. *Vaccine*. 2007; 25(10):1814–1823. [PubMed: 17240007]
- Ito Y, Hagiwara E, Saeki A, et al. Feasibility of microneedles for percutaneous absorption of insulin. *European Journal of Pharmaceutical Sciences*. 2006; 29(1):82–88. [PubMed: 16828268]
- Ito Y, Hirono M, Fukushima K, et al. Two-layered dissolving microneedles formulated with intermediate-acting insulin. *International Journal of Pharmaceutics*. 2012; 436(1-2):387–93. [PubMed: 22750407]
- Ito Y, Murano H, Hamasaki N, et al. Incidence of low bioavailability of leuprolide acetate after percutaneous administration to rats by dissolving microneedles. *International Journal of Pharmaceutics*. 2011; 407(1-2):126–31. [PubMed: 21277965]
- Ji J, Tay FEH, Miao J, et al. Microfabricated microneedles with porous tip for drug delivery. *Journal of Micromechanics and Microengineering*. 2006; 16(5):958–964.
- Jin CY, Han MH, Lee SS, et al. Usefulness verification of biocompatible microneedle patch for transdermal drug delivery. 2009 IEEE Sensors. 2009:1533–1537.
- Kaushik S, Hord AH, Denson DD, et al. Lack of pain associated with microfabricated microneedles. *Anesthesia & Analgesia*. 2001; 92(2):502–504. [PubMed: 11159258]
- Khanna P, Flam BR, Osborn B, et al. Skin penetration and fracture strength testing of silicon dioxide microneedles. *Sensors and Actuators A: Physical*. 2011; 170(1-2):180–186.
- Kim Y-C, Quan F-S, Compans RW, et al. Formulation and coating of microneedles with inactivated influenza virus to improve vaccine stability and immunogenicity. *Journal of Controlled Release*. 2010; 142(2):187–195. [PubMed: 19840825]
- Kim Y-C, Park J-H, Prausnitz MR. Microneedles for drug and vaccine delivery. *Advanced Drug Delivery Review*. 2012a; 64:1547–1568.
- Kim Y-C, Song J-M, Lipatov AS, et al. Increased immunogenicity of avian influenza DNA vaccine delivered to the skin using a microneedle patch. *European Journal of Pharmaceutics and Biopharmaceutics*. 2012b; 81(2):239–247. [PubMed: 22504442]
- Kolli CS, Banga AK. Characterization of solid maltose microneedles and their use for transdermal delivery. *Pharmaceutical Research*. 2008; 25(1):104–113. [PubMed: 17597381]

- Koutsouanos DG, Vassilieva EV, Stavropoulou A, et al. Delivery of subunit influenza vaccine to skin with microneedles improves immunogenicity and long-lived protection. *Scientific Reports*. 2012; 2:357. [PubMed: 22500210]
- Lee JW, Park JH, Prausnitz MR. Dissolving microneedles for transdermal drug delivery. *Biomaterials*. 2008; 29(13):2113–2124. [PubMed: 18261792]
- Lee K, Lee CY, Jung H. Dissolving microneedles for transdermal drug administration prepared by stepwise controlled drawing of maltose. *Biomaterials*. 2011; 32(11):3134–3140. [PubMed: 21292317]
- Li G, Badkar A, Nema S, et al. *In vitro* transdermal delivery of therapeutic antibodies using maltose microneedles. *International Journal of Pharmaceutics*. 2009; 368(1–2):109–15. [PubMed: 18996461]
- Lippmann JM, Geiger EJ, Pisano AP. Polymer investment molding: method for fabricating hollow, microscale parts. *Sensors and Actuators A: Physical*. 2007; 134(1):2–10.
- Liu S, Jin MN, Quan YS, et al. The development and characteristics of novel microneedle arrays fabricated from hyaluronic acid, and their application in the transdermal delivery of insulin. *Journal of Controlled Release*. 2012; 161(3):933–41. [PubMed: 22634072]
- Mansoor I, Häfeli UO, Stoeber B. Hollow out-of-plane polymer microneedles made by solvent casting for transdermal drug delivery. *Journal of Microelectromechanical Systems*. 2012; 21(1):44–52.
- Martanto W, Davis SP, Holiday NR, et al. Transdermal delivery of insulin using microneedles *in vivo*. *Pharmaceutical Research*. 2004; 21(6):947–52. [PubMed: 15212158]
- Martanto W, Moore JS, Couse T, et al. Mechanism of fluid infusion during microneedle insertion and retraction. *Journal of Controlled Release*. 2006a; 112(3):357–361. [PubMed: 16626836]
- Martanto W, Moore JS, Kashlan O, et al. Microinfusion using hollow microneedles. *Pharmaceutical Research*. 2006b; 23(1):104–113. [PubMed: 16308670]
- Martin CJ, Allender CJ, Brain KR, et al. Low temperature fabrication of biodegradable sugar glass microneedles for transdermal drug delivery applications. *Journal of Controlled Release*. 2012; 158(1):93–101. [PubMed: 22063007]
- Matsuo K, Hirobe S, Yokota Y, et al. Transcutaneous immunization using a dissolving microneedle array protects against tetanus, diphtheria, malaria, and influenza. *Journal of Controlled Release*. 2012a; 160(3):495–501. [PubMed: 22516091]
- Matsuo K, Yokota Y, Zhai Y, et al. A low-invasive and effective transcutaneous immunization system using a novel dissolving microneedle array for soluble and particulate antigens. *Journal of Controlled Release*. 2012b; 161(1):10–17. [PubMed: 22306332]
- McAllister DV, Wang PM, Davis SP, et al. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100(24):13755–13760. [PubMed: 14623977]
- Mikolajewska P, Donnelly RF, Garland MJ, et al. Microneedle pre-treatment of human skin improves 5-aminolevulinic acid (ALA)- and 5-aminolevulinic acid methyl ester (MAL)-induced PpIX production for topical photodynamic therapy without increase in pain or erythema. *Pharmaceutical Research*. 2010; 27(10):2213–2220. [PubMed: 20676735]
- Miksza JA, Dekker JP, Harvey NG, et al. Microneedle-based intradermal delivery of the anthrax recombinant protective antigen vaccine. *Infection and Immunity*. 2006; 74(12):6806–6810. [PubMed: 17030580]
- Moon SJ, Lee SS. A novel fabrication method of a microneedle array using inclined deep x-ray exposure. *Journal of Micromechanics and Microengineering*. 2005; 15(5):903–911.
- Naito S, Ito Y, Kiyohara T, et al. Antigen-loaded dissolving microneedle array as a novel tool for percutaneous vaccination. *Vaccine*. 2012; 30(6):1191–1197. [PubMed: 22172508]
- Ng H-I, Fernando GJP, Kendall MAF. Induction of potent CD8⁺ T cell responses through the delivery of subunit protein vaccines to skin antigen-presenting cells using densely packed microprojection arrays. *Journal of Controlled Release*. 2012; 162(3):477–84. [PubMed: 22841796]
- Noh YW, Kim TH, Baek JS, et al. *In vitro* characterization of the invasiveness of polymer microneedle against skin. *International Journal of Pharmaceutics*. 2010; 397(1–2):201–205. [PubMed: 20619328]

- Nordquist L, Roxhed N, Griss P, et al. Novel microneedle patches for active insulin delivery are efficient in maintaining glycaemic control: an initial comparison with subcutaneous administration. *Pharmaceutical Research*. 2007; 24(7):1381–1388. [PubMed: 17387600]
- Oh JH, Park HH, Do KY, et al. Influence of the delivery systems using a microneedle array on the permeation of a hydrophilic molecule, calcein. *European Journal of Pharmaceutics and Biopharmaceutics*. 2008; 69(3):1040–1045. [PubMed: 18411045]
- Ovsianikov A, Chichkov B, Mente P, et al. Two photon polymerization of polymer-ceramic hybrid materials for transdermal drug delivery. *Int. J. Appl. Ceramic Tech*. 2007; 4(1):22–29.
- Park J-H, Allen MG, Prausnitz MR. Biodegradable polymer microneedles: Fabrication, mechanics and transdermal drug delivery. *Journal of Controlled Release*. 2005; 104(1):51–66. [PubMed: 15866334]
- Park J-H, Allen MG, Prausnitz MR. Polymer microneedles for controlled- release drug delivery. *Pharmaceutical Research*. 2006; 23(5):1008–1019. [PubMed: 16715391]
- Park J-H, Choi S-O, Seo S, et al. A microneedle roller for transdermal drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics*. 2010; 76(2):282–9. [PubMed: 20624460]
- Park J-H, Yoon Y-K, Choi S-O, et al. Tapered conical polymer microneedles fabricated using an integrated lens technique for transdermal drug delivery. *IEEE Transactions on Biomedical Engineering*. 2007; 54(5):903–13. [PubMed: 17518288]
- Parker ER, Rao MP, Turner KL, et al. Bulk micromachined titanium microneedles. *Journal of Microelectromechanical Systems*. 2007; 16(2):289–295.
- Pearton M, Allender C, Brain K, et al. Gene delivery to the epidermal cells of human skin explants using microfabricated microneedles and hydrogel formulations. *Pharmaceutical Research*. 2008; 25(2):407–16. [PubMed: 17671832]
- Pearton M, Saller V, Coulman SA, et al. Microneedle delivery of plamid DNA to living human skin: formulation coating, skin insertion and gene expression. *Journal of Controlled Release*. 2012; 160:561–569. [PubMed: 22516089]
- Prausnitz MR. Microneedles for transdermal drug delivery. *Advanced Drug Delivery Reviews*. 2004; 56(5):581–587. [PubMed: 15019747]
- Prausnitz MR, Langer R. Transdermal drug delivery. *Nat. Biotechnol*. 2008; 26(11):1261–1268. [PubMed: 18997767]
- Qin G, Gao Y, Wu Y, et al. Simultaneous basal-bolus delivery of fast-acting insulin and its significance in diabetes management. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 2012; 8(2):221–7.
- Qiu Y, Gao Y, Hu K, et al. Enhancement of skin permeation of docetaxel: a novel approach combining microneedle and elastic liposomes. *Journal of Controlled Release*. 2008; 129(2):144–50. [PubMed: 18538885]
- Roxhed N, Samel B, Nordquist L, et al. Painless drug delivery through microneedle-based transdermal patches featuring active infusion. *IEEE Trans. Biomed. Eng*. 2008a; 55(3):1063–1071. [PubMed: 18334398]
- Roxhed N, Griss P, Stemme G. Membrane-sealed hollow microneedles and related administration schemes for transdermal drug delivery. *Biomedical Microdevices*. 2008b; 10(2):271–279. [PubMed: 17940907]
- Sammoura F, Kang JJ, Heo YM, et al. Polymeric microneedle fabrication using a microinjection molding technique. *Microsyst. Technol*. 2007; 13(56):517–522.
- Shibata T, Yukizono S, Kawashima T, et al. Modified imprinting process using hollow microneedle array for forming through holes in polymers. *Microelectronic Engineering*. 2011; 88(8):2121–2125.
- Singh TR, Dunne NJ, Cunningham E, et al. Review of patents on microneedle applicators. *Recent. Pat. Drug. Deliv. Formul*. 2011; 5(1):11–23. [PubMed: 21143128]
- Sivamani RK, Stoeber B, Liepmann D, et al. Microneedle penetration and injection past the stratum corneum in humans. *J. Dermatolog. Treat*. 2009; 20(3):156–9. [PubMed: 19016065]
- Sivamani RK, Stoeber B, Wu GC, et al. Clinical microneedle injection of methyl nicotinate: stratum corneum penetration. *Skin Research and Technology*. 2005; 11(2):152–156. [PubMed: 15807814]

- Stoeber B, Liepmann L. Two-dimensional arrays of out-of-plane needles. *Proc. ASME MEMS Dev. 2000 IMECE*. 2000; 2:355–359.
- Sullivan SP, Koutsonanos DG, Del Pilar Martin M, et al. Dissolving polymer microneedle patches for influenza vaccination. *Nature Medicine*. 2010; 16(8):915–920.
- Sullivan SP, Murthy N, Prausnitz MR. Minimally invasive protein delivery with rapidly dissolving polymer microneedles. *Advanced Materials*. 2008; 20(5):933–938. [PubMed: 23239904]
- Tanner T, Marks R. Delivering drugs by the transdermal route: review and comment. *Skin Res. Technol.* 2008; 14(3):249–260. [PubMed: 19159369]
- Tas C, Mansoor S, Kalluri H, et al. Delivery of salmon calcitonin using a microneedle patch. *International Journal of Pharmaceutics*. 2012; 423(2):257–63. [PubMed: 22172290]
- Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, et al. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults. *Vaccine*. 2009; 27(3):454–459. [PubMed: 19022318]
- Verbaan F, Bal S, Van den Berg D, et al. Assembled microneedle arrays enhance the transport of compounds varying over a large range of molecular weight across human dermatomed skin. *J. Control Release*. 2007; 117(2):238–245. [PubMed: 17196697]
- Verbaan FJ, Bal SM, van den Berg DJ. Improved piercing of microneedle arrays in dermatomed human skin by an impact insertion method. *J. Control Release*. 2008; 128(1):80–88. [PubMed: 18394741]
- Wang M-W, Fu G-L, Jeng J-H. Optimal molding parameter design of PLA micro lancet needles using Taguchi method. *IEEE/Service Operations and Logistic and Informatics (SOLI)*. 2008; 2008; 2:2731–2735.
- Wang PM, Cornwell M, Prausnitz MR. Minimally invasive extraction of dermal interstitial fluid for glucose monitoring using microneedles. *Diabetes. Technol. Ther.* 2005; 7(1):131–141. [PubMed: 15738711]
- Wang PM, Cornwell M, Hill J, et al. Precise microinjection into skin using hollow microneedles. *The Journal of Investigative Dermatology*. 2006; 126(5):1080–1087. [PubMed: 16484988]
- Wei-Ze L, Mei-Rong H, Jian-Ping Z, et al. Super-short solid silicon microneedles for transdermal drug delivery applications. *International Journal of Pharmaceutics*. 2010; 389(1-2):122–129. [PubMed: 20096759]
- Weldon WC, Zarnitsyn VG, Esser ES, et al. Effect of adjuvants on responses to skin immunization by microneedles coated with influenza subunit vaccine. *PloS ONE*. 2012; 7(7):e41501. doi:10.1371/journal.pone.0041501. [PubMed: 22848514]
- Wermeling DP, Banks SL, Hudson DA, et al. Microneedles permit transdermal delivery of a skin-impermeant medication to humans. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105(6):2058–2063. [PubMed: 18250310]
- Wiechers JW. The barrier function of the skin in relation to percutaneous absorption of drugs. *Pharm. Weekbl. Sci.* 1998; 11(6):185–198. [PubMed: 2694089]
- Wilke N, Mulcahy A, Ye S-R, et al. Process optimization and characterization of silicon microneedles fabricated by wet etch technology. *Microelectronics Journal*. 2005; 36(7):650–656.
- Williams, A. *Transdermal and topical drug delivery from theory to clinical practice*. Pharmaceutical Press; (Great Britain): 2003.
- Xie Y, Xu B, Gao Y. Controlled transdermal delivery of model drug compounds by MEMS microneedle array. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2005; 1(2):184–190.
- Yamamoto T, Yamamoto Y. Electrical properties of the epidermal stratum corneum. *Med. Biol. Eng.* 1976; 14(2):151–158. [PubMed: 940370]
- Zhang W, Gao J, Zhu Q, et al. Penetration and distribution of PLGA nanoparticles in the human skin treated with microneedles. *International Journal of Pharmaceutics*. 2010; 402(1-2):205–12. [PubMed: 20932886]

Websites

[Accessed on 19 March 2013] Dermaroller®. <http://www.genuinedermaroller.co.uk>

- [Accessed on 19 March 2013] Skin Permeation Enhancer (SPE™) Nanopin. http://www.nanomed-skincare.com/en/core_tech.asp
- [Accessed on 19 March 2013] Derma-Q and Mi-roll Micro Needle Roller. <http://www.dermaneedle.com/>
- [Accessed on 27 March 2013] Dermaroller®. <http://www.dermaroller.com>
- [Accessed on 27 March 2013] Beckton-Dickinson' Soluvia®. <http://www.bd.com>
- [Accessed on 27 March 2013] IDflu®, Intanza® and Fluzone Intradermal®. http://en.sanofi.com/Images/14262_20081218_fluid_chmp_en.pdfhttp://en.sanofi.com/Images/14032_20091223_FLU_HIGH_DOSE_en.pdf
- [Accessed on 27 March 2013] Zosano Pharma website. <http://www.zosanopharma.com/http://www.zosanopharma.com/index.php/20091106127/Research/Research-General/ZP-PTH.html>
- [Accessed on 27 March 2013] 3M Microneedle Technology (MTS) website. <http://solutions.3m.com>
- [Accessed on 27 March 2013] Micronjet®. <http://www.nanopass.com>

Figure 1. Various strategies for enhancing the delivery of macromolecules across the skin.

Figure 2. Schematic representation of the mechanism of action of a microneedle array device. The device perforates the stratum corneum (SC) providing direct access of drugs to the underlying viable epidermis, without reaching blood vessels and nerve fibres located in the dermis.

Figure 3. A schematic representation of four different MN application methods used to facilitate drug delivery transdermally.

(a) Solid MNs for increasing the permeability of a drug formulation by creating micro-holes across the skin. (b) Coated MNs for rapid dissolution of the coated drug into the skin. (c) Dissolvable MNs for rapid or controlled release of the drug incorporated within the microneedles. (d) Hollow MNs used to puncture the skin and enable release of a liquid drug following active infusion or diffusion of the formulation through the needle bores. This image was reproduced with the kind permission of Donnelly *et al.* (2012).

Figure 4. Novel hydrogel-forming MNs facilitate controlled transdermal drug delivery.

(a) An expanded view of the backing layer, drug-loaded adhesive patch and solid crosslinked hydrogel MN array which constitutes an integrated hydrogel MN patch. (b) Application of the integrated hydrogel MN patch to the skin surface. (c) Diffusion of water into the MN array leading to controlled swelling of the arrays and diffusion of drug molecules from the adhesive patch through the hydrogel conduit. (d) Intact hydrogel MN arrays following removal from the skin. This image was reproduced with kind permission from Donnelly *et al.* (2012).

Table 1
Most recent advances in development of coated MNs for potential transcutaneous immunisations

Fabrication material for coated MNs	Antigen/ vaccine type	MN geometries	Model animals used	Key findings	Ref.
Stainless steel	Influenza H1N1 subunit and adjuvant TLR ligands	MNs: 750 µm length, 200 µm width, assembled in rows of 5 MNs each	Female BALB/c mice	<ul style="list-style-type: none"> Investigate the influence of two types of TLR ligands adjuvant namely imiquimod and poly(I:C) on immune response triggered by coated MN with influenza subunit vaccine. 	Weldon <i>et al.</i> , (2012)
				<ul style="list-style-type: none"> Observed similar immune response between imiquimod/adjuvanted vaccine and vaccine alone delivered using coated MNs. 	
				<ul style="list-style-type: none"> However, poly(I:C) adjuvanted vaccine delivered using coated MN was found to further enhance the immune response compared to vaccine alone. 	
Stainless steel	Avian H5 influenza hemagglutinin DNA vaccine	MNs: 700 µm length, 160 µm base width, assembled in a row of 5 needles per device	Female BALB/c mice	<ul style="list-style-type: none"> More concentrated DNA coating solution and higher number of dip coating cycles will increase the dose of DNA coating. 	Kim <i>et al.</i> , (2012b)
				<ul style="list-style-type: none"> MN-mediated vaccination generates better immune response than the same dose of the DNA vaccine delivered by IM. 	
				<ul style="list-style-type: none"> Compromise immunogenicity if the MN coating solution containing carboxymethylcellulose and a surfactant. 	
Titanium	Influenza vaccine	MNs: 750 µm in length, tapered to a sharp tip.	Female BALB/c mice	<ul style="list-style-type: none"> Studied influence of crystallization and phase separation of the dried coating matrix on long term vaccine stability of MNs coated with whole inactivated influenza vaccine <i>in vitro</i>. 	Chou <i>et al.</i> , (2012)
				<ul style="list-style-type: none"> MN with crystallized or phase-separated coatings showed reduced vaccine immunogenicity <i>in vivo</i> and lost vaccine stability <i>in vitro</i>. 	
Nanopatch (NP) micro-projections (MP)	Commercial influenza vaccine (Fluvax) and adjuvant, saponinQuil -A	NP: 4 × 4 mm MP; 100 µm ultra high density	Female C57BL/6 mice	<ul style="list-style-type: none"> Investigate the synergistic effect of vaccine and adjuvant to improve the generated immune response. 	Fernando <i>et al.</i> , (2012)
				<ul style="list-style-type: none"> NP-based vaccine (+adjuvant) delivery with up to 900-fold reduction of antigen dose (dose sparing effect) demonstrated comparable immune response to normal IM dose in mice. 	
Stainless steel	Licensed influenza subunit vaccine	MNs: 700 µm height, tapering to a sharp tip, with five MNs per row	Female C57BL/6 mice	<ul style="list-style-type: none"> Single dose of MN treatment induced robust humoral and cellular immune response and improved long term immunity in mice compared to conventional injection 	Koutsouanos <i>et al.</i> , (2012)