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Microneedle-mediated vaccine delivery: Harnessing cutaneous immunobiology to improve efficacy

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Abstract

Introduction—We describe the use of microneedle arrays for delivery to targets within the skin itself. Breaching the skin's *stratum corneum* barrier raises the possibility of administration of vaccines, gene vectors, antibodies and even nanoparticles, all of which have at least their initial effect on populations of skin cells.

Areas Covered—Intradermal vaccine delivery, in particular, holds enormous potential for improved therapeutic outcomes for patients, particularly those in the developing world. Various vaccine-delivery strategies have been employed and here we discuss each one in turn. We also describe the importance of cutaneous immunobiology on the effect produced by microneedle-mediated intradermal vaccination.

Expert Opinion—Microneedle-mediated vaccine holds enormous potential for patient benefit. In order for microneedle vaccine strategies to fulfil their potential, however, the proportion of an immune response that is due to local action of delivered vaccines on skin antigen presenting cells and what is due to a systemic effect from vaccine reaching the systemic circulation must be determined. Moreover, industry will need to invest significantly in new equipment and instrumentation in order to mass produce microneedle vaccines consistently. Finally, microneedles will need to demonstrate consistent dose delivery across patient groups and match this to reliable immune responses before they will replace tried- and-tested needle-and-syringe based-approaches.

Keywords

Microneedle; intradermal; vaccine; antigen; cutaneous immunobiology

1. Vaccination

Vaccination is the most effective means of controlling infectious disease-related morbidity and mortality. The World Health Organization (WHO) estimates that vaccination prevents over 2.5 million child deaths each year worldwide. A vaccine is a biological preparation that improves immunity to a particular disease. The four traditional types of vaccines that have been used to date clinically are vaccines that contain either dead or live-attenuated microorganisms, inactivated toxic compounds (Toxoid), or protein subunits. A number of innovative vaccines are in development such as recombinant vector and DNA vaccines. These agents resemble a disease-causing microorganism and stimulate the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily challenge these microorganisms upon subsequent encounters.

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1.1 Disadvantages associated with conventional vaccination strategies

Appropriate vaccine administration is the key element to ensure successful vaccination. Typically, most vaccines are administered via the subcutaneous (SC) or intramuscular (IM) routes. Hypodermic injections are associated with pain and distress that might lead to poor patient compliance and require highly trained personnel for administration. They are associated with a risk of disease transmission due to the possibility of needle-stick injuries or reuse of contaminated needles. Insufficient vaccine supply or limitation of vaccine production may also prove problematic in instances when mass vaccination is necessary [1, 2].

At present, most vaccines are deposited into the subcutaneous fat or into the muscle beneath the skin. Relatively few vaccines are administered into the dermis [3], and even fewer are applied topically onto the skin, also known as transcutaneous [4][5] or epicutaneous route [5]. Each of these routes of application relies on the presence of dendritic cells (DCs) in the tissues that take up the antigen, process it and present it to T lymphocytes in the draining lymphoid organs. Whereas subcutaneous fat and muscle tissue contain relatively few DCs, the dermis and the epidermis are densely populated by different subsets of DCs. Consequently, antigen delivery by hypodermic injection will bypass the skin's immune cells leading to less efficient vaccination. For this reason, the skin represents an ideal site for vaccine delivery, as vaccination at this site will evoke strong immune responses at much lower doses of antigen than intramuscular vaccines [6]. The potential of skin immunization was observed in a clinical trial where epidermal Influenza vaccination induced Influenza-specific CD8 T cell response, while classical intramuscular route did not [7]. Dose-sparing approaches are critical to ensuring a sufficient supply of certain vaccines, especially in pandemic diseases [8].

1.2 Skin Structure and Function

The skin is the largest organ in the human body and is designed to carry out a wide range of functions [9, 10]. It has barrier properties to ensure that the underlying organs are protected from physical, chemical or microbial insults. The epidermis is composed of the viable epidermis and the *stratum corneum*. The viable epidermis consists of 4 histologically distinct layers; the *stratum germinativum*, *stratum spinosum*, *stratum granulosum* and the *stratum lucidum.* The thickness of the epidermis varies depending on location, ranging from 60 μm on the eyelids to 800 μm on the palms [11]. The layers of the epidermis are avascular and receive nutrients by diffusion of substances from the underlying dermal capillaries. The dermis (or corium) resides atop the subcutaneous fat layer and is approximately 3-5 mm thick [12]. The dermis is composed of a network of collagen and elastin fibres embedded in a mucopolysaccharide matrix. Collagen provides the skin with mechanical support [13], whilst the elastic properties of the skin are associated with elastin [14]. Physiological support to the dermis is provided by a network of blood vessels, lymphatics and nerve endings [15]. The cutaneous blood supply delivers oxygen and nutrients to the skin, and facilitates the removal of waste products. The subcutaneous fat layer, sub-cutis, subdermis or hypodermis, lies between the overlying dermis, and the underlying body constituents [13]. Its main functions are to impart physical support to the dermis and epidermis, act as a heat insulator (due to the high content of adipose tissue), and to provide nutritional support

[16]. The hypodermis also carries the main blood vessels and nerves to the skin, and may contain sensory organs. In terms of drug delivery, the hypodermis is thought to be of minor significance, as it resides beneath the vascular dermis [17].

1.3 The skin dendritic cell network

Research and development in the field of vaccination is an ever-evolving process, for both the discovery of new antigens for novel vaccine production, and developing improved administration strategies to ameliorate vaccine efficacy. The concept of delivery of vaccines through the skin has been gathering momentem in the past decade, largely due to the increasing recognition that a tight semi-contiguous network of immunregulatory cells that reside in the different skin layers is an ideal target for administration of antigenic agents. DCs, macrophages and neutrophilic granulocytes are the principal phagocytes in the skin, while numerous cells of the adaptive immune system, such are $CD8^+$ T cells and all different types of $CD4^+$ T cells can be found in normal skin.

DCs are the main recipients of intradermal vaccines in the skin, and are the key cells involved in generating robust antigen specific immune responses. DCs represent the most important family of professional antigen-presenting cells (APCs) specialized in capture and presentation of particulate and soluble substances [18]. The main role of DCs is to induce specific immunity against invading pathogens while maintaining tolerance to self-antigens [19, 20]. Several subsets of DCs that form a rich network of cells present in the different layers of the skin have been described in both mice and humans [21, 22].

Despite their heterogeneity, DCs share specific functional properties that distinguish them as a pivotal link between innate and adaptive immunity. As a part of an innate immune response, DCs can produce large amounts of IL-12, TNF-α and type-I interferons, and in such a way, attract and activate other innate lymphocytes, like NK cells, NKT cells and gamma-delta T cells. But more importantly DCs play a pivotal role in activation of adaptive immune responses. Following activation upon encounter of pathogens, under inflammatory settings, DCs undergo rapid maturation characterized by the upregulation of major histocompatibility complex [MHC] and costimulatory molecules and migrate to the draining lymph nodes. The antigen taken up by DCs is then processed and presented to T cells as peptides bound to the MHC class I or MHC class II molecules. In this way DCs of the skin can induce and stimulate $CD4^+$ T cells, but equally activate $CD8^+$ T cells. Importantly, the activation of the adaptive immune system induces immunologic memory, a prerequisite for successful vaccines/vaccination.

Conversely, in a tolerogenic setting, DCs can induce anergy in antigen-specific T cells or generate protective regulatory T cells upon arrival in the lymph node (LN) [23]. Thus, vaccines not only serve to induce robust immunity against bacterial and viral microbes, or toxins, but also to regulate immunological tolerance, as would be desired in autoimmune diseases. The specific role of the diverse DCs subsets in the skin is currently under investigation and there is an indication that skin DC subsets exhibit specific immune functions. Phenotypically, the cutaneous DC population in fact includes several distinct DC subsets, each with a specific phenotype, origin and function, and while significanrt progress

has been made in the last decade a complete understanding of these different DC subsets remains to be established.

The Langerhans cells [LCs] reside in the suprabasal layers of the epidermis and account for 3–5% of all nucleated cells in the murine epidermis [24]. They are arranged in a network occupying the interstices between neighboring keratinocytes, the epithelial cells forming the epidermis [25]. Murine LCs constitutively express the lectin receptor langerin, a type II C-type lectin receptor that binds mannose and related sugars [26, 27]. LCs have long been regarded as the exclusive APCs of the skin that detect pathogens which penetrate the skin barrier and, after undergoing a phase of maturation, convey this information via lymphatic vessels to T cells present in cutaneous LNs (CLNs) [28, 29]; however this remains to be unequivocally demonstrated.

In addition to LCs, the skin contains a second group of DCs known as dermal DCs (DDCs) that are distributed throughout the dermal connective tissue. Dermal DCs have been much less studied than LCs due to the lack of available specific markers which we are only now beginning to fully understand. DCs in the dermis include dermal resident DCs and migratory LCs on their way to the LNs [24].

Murine dermal resident DCs were thought to form a homogenous population easily distinguishable from migratory LCs based on their lack of langerin expression [27]. However, recent studies in mice showed that dermal langerin^{+} cells include both migratory LCs and a novel population of DDCs, known as langerin^{+} dermal DCs [30-32].

In summary, three main distinct subsets can thus be identified in steady-state murine skin: epidermal Langerhans cells, dermal langerin^{-} DCs and dermal langerin^{+} DCs. Further details about the expression of various markers by different murine skin DCs subsets are shown in Table 1.

Although these characteristics of different skin DC subsets have been elucidated, the functional diversity and specificity of skin DC subsets require investigation. However, some recent observations are of importance. Several laboratories have shown that murine Langerhans cells are especially capable of inducing cytotoxic T lymphocytes [33, 34] as opposed to dermal DCs. Studies in a mouse tumour model confirmed that Langerhans cells and dermal langerin^{+} DCs are essential for anti-tumour immunity *in vivo* [5]. Protection from an experimental tumour was lost when Langerhans cells and dermal langerin^{+} DCs were absent [35]. In addition, Geijtenbeek and coworkers have shown that LCs are important for protection from HIV infection [36].

On the other hand, recent studies in experimental cutaneous leishmaniasis have shown that LCs may have more of a regulatory role. Following the inoculation of the parasite *Leishmania major*, dermal DCs induce protective Th1 immunity after antigen presentation, whereas antigen-loaded LCs promote development of regulatory T cells, which prevents complete parasite eradication from the host [37]. In other immunologic-mediated inflammatory reactions, such as hapten-induced contact hypersensitivity reaction, LCs also seem to have regulatory function [38]; however this remains controversial.

Studies involving gene-gun technology to deliver DNA vaccines to the cutaneous DC subsets demonstrate that skin DCs are also important for humoral responses. They identified migratory langerin$^+$ DDCs as the subset that directly activated CD8$^+$ T cells in lymph nodes, while Langerin$^+$ DCs were also critical for IgG1 but not IgG2a antibody induction, suggesting differential polarization of CD4$^+$T helper cells by langerin$^+$ or langerin$^-$ DCs, respectively [39].

Similar to murine skin, multiple distinct DCs subsets can be distinguished by location, phenotype and function in homeostatic human skin. The outer epidermal layer of the human skin also contains LCs, that constitute approximately 2% of the total epidermal cell population in normal healthy skin. LCs are the only subset in human skin that expresses langerin. The search for the human equivalent of the murine dermal Langerin$^+$ DCs is still a topic of investigation and to date it is not clear whether the human dermal Langerin$^+$ DDCs exist. DDCs in humans can be further subdivided into a quantitatively minor population expressing CD14 but not CD1a and a major population characterized by strong CD1a but not CD14 expression [40]. The complexity of phenotypic characteristics of human skin DC subsets is described in more details in Table 2.

Possible functional differences between the different types of human skin DCs are essentially verified when LCs and CD14$^+$ DDCs were directly isolated from human skin. LCs were shown to be superior in cross-priming CD8$^+$ T cells, while CD14$^+$ DDCs were specialized to prime CD4$^+$ helper T cells that further turned B cells into antibody producing cells. CD14-CD1a$^+$ cells appear to be functionally in between LCs and the CD14$^+$ dermal DCs [41]. It has recently been confirmed that LCs are involved in the initiation of antiviral immunity, as they efficiently stimulate naive CD8$^+$ T cells to differentiate into effector cells that express IFN-γ,TNF-α, granzyme B, and high cytotoxic activity [42]. The human DCs of the skin, were reviewed by Ginhoux et al. [21] and Teunissen et al. [22].

2 Microneedles arrays for vaccination via the skin

The use of microneedle (MN) arrays is one attractive approach for ID vaccine delivery. MN arrays consist of a multiplicity of microprojections, ranging from 25 to 2000 μm in height, attached to a base support [43]. Application of MNs to the skin surface can mechanically perforate the stratum corneum barrier and create aqueous transport pathways of micron dimensions [44]. These micropores created by MNs readily permit transport of a wide range of micromolecules and macromolecules such as immunotherapeutic agents (e.g. vaccines and proteins) [45].

Academic and industrial research in development and application of MNs has been ongoing since their proposition by Henry et al., 1998 [46]. MNs are fabricated from various materials such as metals, glass, silicon, and FDA- approved polymers [47]. A review by Donnelly *et al.*, described in detail various methods of development and fabrication of a wide range of MNs [48]. There are four main modes of action of MNs, namely poke-and-patch, coated and poke, poke-and-release and poke-and-flow [49].

2.1 Poke and patch

The poke and patch approach uses solid MNs to puncture the skin, followed by application of antigen to the treated area to allow diffusion of antigen into the skin. Substantial work using this approach has been carried out by the Bouwstra Group, who in one report, investigated mouse immune responses after transcutaneous immunization (TCI) using two model antigens, diphtheria toxoid (DT) and influenza subunit vaccine [50]. Stainless steel MN arrays (16 × 300 μm MNs) were used to perforate the mouse skin followed by application of a DT formulation with or without cholera toxin (CT). The application of DT to MN-treated skin resulted in significantly higher serum IgG and toxin-neutralizing antibody titres than in unperforated skin. The presence of CT increased the immune response to similar levels as observed after subcutaneous injection of AlPO4-adsorbed DT (DT-alum). Unlike in the case of DT however, MN array pre-treatment elicited no effect on the immune response to influenza vaccine alone. This response was strongly improved by addition of CT, independent of MN treatment. The authors concluded that their study indicated that TCI of DT in the presence of CT after MN treatment results in similar protection to injection of DT-alum.

The effect of co-administration of various adjuvants with DT in the modulation of the immune response in TCI mice after application of MN arrays was studied [51]. Mice were treated with DT co-administrated with lipopolysaccharide (LPS), Quil A, CpG oligo deoxynucleotide (CpG) or CT as adjuvants. MN array pretreatment group resulted in high serum IgG levels that were significantly improved by co-administration of adjuvants. The IgG levels of the group treated with DT co-administered with CT were similar to the IgG level of the group treated with DT-alum subcutaneously. N-Trimethyl chitosan also proved beneficial in boosting the immune response to DT following MN pre-treatment when in solution with the antigen, although DT-loaded nanoparticles of N-Trimethyl chitosan did not improve immune response [52].

The impact of transdermal vaccination on the development of melanoma was reported by Bhowmik *et al.*, (2011), who delivered a novel microparticulate vaccine to the skin following puncture using of MN-based Dermaroller® [53]. Eight weeks following vaccination, mice were challenged with live melanoma cells. The results of this study showed that no measurable tumour growth was observed 35 days after tumour injection in mice that were treated using Dermaroller® MNs and SC injection of vaccine. A significant increase in IgG antibody levels was observed for both transdermal and subcutaneous vaccinated groups in comparison to control groups. However, the transdermally vaccinated group showed slightly increased IgG antibody levels compared to the SC group. The authors concluded that the developed formulation for melanoma cancer that can be administered using MNs technology opens up new avenues for prevention of melanoma cancer.

2.2 Coat and poke

The coat and poke approach involves the coating of solid MNs with an antigen of choice, that can be delivered in a one-step process, and is an attractive approach for mediating ID vaccine delivery as smaller amounts of antigen should be required to coat the MNs. Since

the antigen coating on the MNs is in a solid [54], long-term stability should be improved, ensuring optimal shelf life [55].

The Prausnitz group at Georgia Institute of Technology has carried out immunization studies using stainless steel monument-shaped arrays of 5 MNs dip-coated in vaccine. The MNs were fabricated by laser-cutting stainless steel sheets and were designed at a length of approximately 700 μm. Plasmid encoding hepatitis C virus, seasonal influenza: H1N1, H3N2, inactivated virus, influenza virus-like particles and recently BCG have been engaged successfully in MN mediated ID immunization. After optimization of the coating formulation that led to the inclusion of trehalose as an antigen stabiliser [56], MNs were coated and inserted into the skin of mice. It was found that using coated MNs for vaccination achieved a robust immune response and produced complete protection against lethal influenza virus challenge similar to conventional intramuscular injection. The study concluded that optimization of coating formulation and addition of a stabilizing agent protects antigen activity, ensuring effective vaccination. As a result, trehalose was added to the coating formulation in following studies.

Koutsonanos and colleagues reported that a single MN immunization with inactivated H3N2 influenza virus induced significantly higher hemagglutination inhibition (HI) titers in comparison with that observed by IM injection [2]. Solid metal MNs coated with inactivated influenza virus were found to be at least as effective as the conventional IM route in inducing similar levels of functional antibodies at low or high antigen concentrations, in clearing the virus from the lungs of infected mice, in conferring protection and in inducing short-lived as well as memory B immune responses. In IM immunization, the serum IgG responses were dose related, while MN administration produced similar responses at low or high antigen loadings, indicating a higher capacity of the skin to produce an immunologic response. The same system was used to evaluate the potential of BCG-coated MN vaccine patches [57]. The results of this study indicated that BCG vaccine-coated MNs can induce a strong antigen-specific cellular immune response in both the lung and spleen of guinea pigs that was comparable to that induced by using a 26-gauge needle. It was found that MN BCG vaccination induced similar frequencies of TNF-α secreting or both IFN-γ and TNF-α cytokine secreting bi-functional $CD4^+$ T cells to that induced by hypodermic injection. A strong IgG response was generated by both vaccination methods.

The group assessed the efficacy of modified recombinant trimeric soluble influenza virus hemagglutinin (sHA GCN4pII) coated MNs in inducing a protective immune response [58]. Results from the modified protein were compared with the results of unmodified protein (sHA). Mice that were vaccinated with MN coated sHA trimeric induced fully protective immune response against influenza virus challenge. Both sHA and sHA GCN4pII coated MNs induced improved clearance of replicating virus compared to the SC route. The MNs coated with sHA GCN4pII induced a stronger Th1 response in mice suggested by the ratio of IFN-γ^+ $CD4^+$ T cell to IL- 4^+. The study was concluded by proving that MNs coated with stabilized HA trimers promoted a protective immune response and showed the same level of protection as that induced by the subcutaneous route of vaccination.

Professor Mark Kendall's research has pioneered the development of Nanopatch™ technology. Nanopatch™ devices are fabricated from silicon using a process of deep reactive ion etching. The projections are solid silicon, sputter coated with a thin (~ 100 nm) layer of gold, and contain 3364 densely packed projections. These devices have been used group used Nanopatch™ technology to target ID vaccination against West Nile virus and chikungunya virus in mice. Nanopatch™ devices are fabricated from silicon using a process of deep reactive ion etching. The projections are solid silicon, sputter coated with a thin (~ 100 nm) layer of gold, and contain 3364 densely packed projections. The efficiency of Nanopatch™ immunization was demonstrated using an inactivated whole chikungunya virus vaccine and a DNA-delivered attenuated West Nile virus vaccine [59]. Nanopatch™ technology was also used to deliver the prophylactic cervical cancer vaccine, Gardasil®, and succeeded in delivering up to 300 ng of vaccine to the ears of mice. Moreover, the virus-neutralising response of mouse serum samples from mice vaccinated using nanopatch was not inferior to that of control mice that had been vaccinated by the IM route [60]. Similarly impressive results have also been reported when Nanopatch™ coating was with the influenza vaccine, Fluvax® [61].

The group of researchers at Zosano Pharma (formerly ALZA Corporation) assessed the performance of another device containing an array of microprojections, the Macroflux®, coated with the model antigen ovalbumin. microprojection array for intracutaneous delivery of model antigen, ovalbumin. The immunization studies showed that at all antigen doses, administration of OVA-coated Macroflux® resulted in immune response comparable to that observed after intradermal administration of the same doses of antigen. And that application of 1 μg and 5 μg of antigen via Macroflux® induced 10 and 50-fold increase in anti-ovalbumin levels in comparison to those produced by intramuscular or subcutaneous injections of equivalent doses [62]. Follow up mechanistic studies revealed that the immunologic response was unaffected by MN height (225 – 600 μm) and density (140 & 657/cm^2), but was dependent on the dose of antigen delivered [63].

2.3 Poke and flow

The poke and flow approach is based on diffusion of vaccine through conduits of solid MNs. The antigen can be delivered either by passive diffusion, pressure or electrical driven flow [43, 64]. The approach can, however, be limited by the same disadvantages associated with conventional vaccination technoiques, including requirement of a cold chain and possible need for trained personnel [65]. The narrow bore of MNs, and the dense elastic nature of the skin may also limit fluid flow; Wang and colleagues circumvented this problem by partially retracting the MN device prior to expulsion of fluid [66]. Frost (2007) suggested that the co-administration of hyaluronidase an enzyme that degrades hyaluronic acid in the extracellular matrix of the skin, might reduce skin resistance [67]. Martanto *et al* provided many explanations of the factors affecting the flow rate through hollow MNs. Recently hollow MNs have received attention due to their potential for use for TCI or ID vaccination [68].

Van Damme *et al*. (2009) investigated the safety and efficacy of a novel MN device for dose-sparing intradermal influenza vaccination in healthy adults [69]. The study was conducted in 180 healthy adults. The safety and immunogenicity of α-RIX® (GSK

Biologicals) influenza vaccines delivered using a hollow MN device (Micronjet®) was investigated. This novel device has been developed by Nanopass specifically for intradermal delivery. Micronjet® comprises an array of four MNs, each 450 μm in length. The needles are of silicon crystal bonded to a plastic adapter which can be mounted on any standard syringe. In a trial comprising 180 subjects, low-dose influenza vaccines delivered by MicronJet® elicited immune responses similar to those elicited by 15 μg HA per strain injected IM in human volunteers. One limitation however, was the prevalence of local reaction, although these were transient in nature. Similar developments have been made at BD Technologies. A 34G stainless steel MN with inner diameter of 76 μm, an outer diameter of 178 μm and an exposed length of 1 mm was used to deliver three distinct influenza vaccines. Results indicated that the dose required to elicit the full immunological response was at least 10 fold lower than with IM administration, and up to 100 fold more potent, depending on the nature of the antigen [70]. Further investigations by the same team of researchers revealed dose-sparing benefits of MN-delivered anthrax vaccinations in comparison with vaccinations delivered IM [71].

2.4 Dissolving/soluble microneedles

Dissolving MNs have been proposed as an innovative approach for vaccine delivery. They are fabricated from water soluble polymers or carbohydrate material that encapsulates drug within the needle matrix. The MNs will be completely dissolved upon insertion into the skin, releasing their payload. Dissolvable MNs show promise in vaccine delivery breakthrough for many reasons. Since the MNs will dissolve after insertion into the patient's skin, the possibility of cross infection is eliminated. Moreover, no sharp biohazardous waste is generated, and therefore no special disposal mechanism will be required. The solid state nature of the contained/encapsulated vaccine should also reduce the need for cold chain storage and transport. The MN patches could permit self-administration of vaccine during pandemics and simplify large-scale immunization programs in developing nations. As these self-disabling MNs lack many of the disadvantages of conventional vaccination techniques, and also some of those associated with the MN strategies mentioned to this point, poke and patch MNs are receiving increasing attention for their value in vaccination applications.

The first dissolvable MN patches for vaccine delivery purposes were introduced by the Prausnitz group. Sullivan et al (2010) fabricated and investigated dissolving MN patches for influenza vaccination using a simple patch-based system. MN patches of poly (vinyl pyrrolidone - MNs 650 μm in height) containing 3 μg lyophilised inactivated influenza virus vaccine generated robust antibody and cellular immune responses that provided complete protection against lethal influenza challenge [72]. Lung virus clearance and cellular recall responses were more impressive following MN vaccination than following IM.

The Kendall group described the micromoulding of dissolving MN arrays from master templates of one of their Nanopatch™ designs [73]. Replica MNs were formed from carboxymethylcellulose by multiple castings into poly (dimethylsiloxane) moulds. Dual-layer MNs containing the model antigen ovalbumin, along with the adjuvant Quil-A, elicited post-immunisation schedule antibody levels in mice that were comparable to an IM ovalbumin/Quil-A immunisation group at day 28 and superior to the IM group at day 102,

despite using a lower antigen dose in the MNs. Similar results were seen with influenza vaccine.

3 Microneedle-mediated delivery of antigen encapsulated nanoparticles as a vehicle for improved antigen targeting to skin DCs

In the past few years, particle-based vaccines have been proposed as additives to aid successful immunization. They have been used to protect antigen stability in vivo, and to deliver it in a controlled and sustained manner to the site of action [74]. Drug-loaded nanoparticles are colloidal systems, typically 10–1000 nm in diameter, with a therapeutic payload entrapped, adsorbed or chemically coupled to an orbicular matrix [75]. Nanoparticles are widely used for controlled delivery of small molecule drugs, oligonucleotides and protein antigens to a variety cell types, including dendritic cells [76]. Among the different parameters that need to be considered in design of particle-based vaccines, the particle size and their physicochemical properties are particularly important for skin vaccination. It has been demonstrated that polymeric nanoparticles <500 nm in diameter have high rate of intracellular uptake by variety of APC [77].

Several groups have demonstrated that nanoparticles have adjuvant effects comparable to those of CFA or ALUM, and as synthetic adjuvants can activate DCs to induce T cell immune responses against encapsulated antigens [78-80]. An important advance was the demonstration that nanoparticles as adjuvants promote activation of the NLRP3 inflammasome [81].

Nanoparticles have been extensively studied for oral and parenteral administration owing to their sustained drug release [82, 83]. This property of nanoparticles could also be utilized for topical antigen administration to target skin DCs with antigen over a prolonged period. Researchers have attempted to use nanoparticles for topical drug delivery, and they found that the drug permeation was enhanced by gradual drug release from the nanoparticles on the skin surface, but did not optimize way to deliver nanoparticles inside the skin [84-86]. This suggested that as a drug delivery vehicle, the nanoparticle could sustain drug release, but if it was applied as a drug reservoir to treat the skin disease, it must be delivered into the skin layers instead of remaining on the skin surface. Some other researchers tried to verify the penetration of nanoparticles across the skin, but found that few NPs were able to permeate into the skin passively through the hair follicles while most NPs were restricted by stratum corneum and unable to penetrate the skin [87, 88]. To investigate whether the microconduits on the epidermis produced by microneedles could act as channels for NPs to penetrate the skin, in vitro experiments have been designed and proved that nanoparticles could pass through the human epidermal membrane and get into skin layers [89, 90]. Moreover, Bal and colleagues showed that in intradermal antigen delivery to skin pretreated by metal MNs, antigen was more efficiently taken up by skin DCs when it was encapsulated into polymeric NPs, comparing with delivery in a soluble form [91]. These findings suggest that microneedles may be an effective vehicle for the intradermal delivery of antigen encapsulated nanoparticles *in vivo*.

4 Conclusion

Dendritic cells are key regulators of immune responses and play a critical role in the design of modern vaccines [18, 92]. The skin harbours a wide network of these cells and, for this reason, it is recognized as an attractive target for immunization. MN technology has the potential to favour the targeting of these specialised immunologic cells of the skin. The conventional methods that have been in use for more than 150 years are flawed or not optimal, as has been pointed out. MNs have the potential to replace these dated methods and, in so doing, improve the response to infectious diseases. In order to utilize the whole potential of MN-mediated intradermal immunization, better understanding of skin immunology, in particular cutaneous DCs is necessary. Therefore, it is essential that further study of these cells *in vivo* is undertaken, so that vaccines that directly take advantage of the specialized properties of DCs to control immunity can be designed. Additionally, the exciting developments that are being made in the field of MN research have already resulted in a number of products that have potential for use in targeting the specialised immunological tissue of the skin. Further developments, in particular in production of novel MN-based vaccine formulations, could lead to new vaccination strategies, with benefits to patients worldwide, with those in the developing world likely to be the principal beneficiaries.

5 Expert opinion

Microneedle-based systems for vaccine delivery have undoubted potential. Most studies appear to show dose-sparing with respect to conventional routes of administration. The ability to formulate vaccines in the dry state is a significant advantage in attempts to circumvent the cold chain, while the lack of medical training required for microneedle application should prove to be a real boon in developing countries. The absence of visible needles is likely to be useful in vaccination of the significant proportion of needle-phobic patients, as well as small children, due to the lack of pain upon administration.

Numerous types of microneedles have been used in *in vitro* and *in vivo* vaccine delivery studies carried out to date. Silicon and metal have been the most commonly-employed materials. However, the emerging science behind dissolving polymeric microneedles is what seems to hold most promise. Formulating microneedles from vaccine-loaded polymeric gels is straightforward and avoids complex and time-consuming coating processes, materials are cheap and can be processed at room temperature. Importantly, the microneedles are self-disabling, dissolving or biodegrading rapidly in skin to release their payload. This means that these microneedles can’t be reused following removal from a patient and also require no special disposal arrangements. Clinical vaccination studies in human volunteers are now required to demonstrate their safety and efficacy. However, it is not difficult to foresee the major impact vaccine-loaded polymeric microneedles could have on the health of human beings worldwide.

A number of factors must be considered before microneedle-based vaccination will become well-accepted in clinical practice, to the benefit of patients. Firstly, it is known that significant proportions of macromolecule doses are absorbed systemically when delivered

intradermally using microneedles [94]. It should be shown what proportion of an immune response is due to local action of delivered vaccines on skin antigen presenting cells and what is due to a systemic effect from vaccine reaching the systemic circulation. Secondly, industry will need to invest significantly in new equipment and instrumentation in order to mass produce microneedle vaccines consistently. Microneedles will also need to demonstrate consistent dose delivery across patient groups and match this to reliable immune responses before they will replace tried-and-tested needle-and-syringe based-approaches. Central to this will be design and utilisation of applicator devices which will not only guarantee consistent application forces with concomitant predictable skin insertion depths, but will also provide feedback to healthcare workers and/or patients that the microneedles have been inserted correctly. This will be necessary to provide the same level of assurance of vaccination currently seen with conventional administration devices.

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Article Highlights

- Vaccination is the most effective means of controlling infectious disease-related morbidity and mortality
- Most vaccines are deposited into the subcutaneous fat or into the muscle beneath the skin
- The skin possesses a rich supply of professional antigen-presenting cells
- Using microneedle-based vaccine delivery strategies to target skin immune cells may be dose-sparing and may avoid needle-stick injuries and injection-associated transmission of infection
- Nanoparticles delivery using microneedles may promote antigen stability during storage and enhance immune responses
- Industry needs to develop mass production strategies for microneedle-based vaccines and suitable applicator devices must be designed to guarantee vaccine administration

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Table 1

Phenotype of the murine cutaneous dendritic cell subsets

	Langerhans cells	Dermal Langerin$^+$ CD103$^+$	Dermal Langerin$^+$ CD103−	Dermal Langerin− CD11b$^+$	Dermal Langerin− CD11b−	Skin macrophages
CD45	+	+	+	+	+	+
CD11c	++	++	++	++	++	−/+
MHCII	+	+	+	+	+	−/+
Langerin	++	+	+	−	−	−
CD103	−	+	−	−	−	−
CD11b	+	−	−	+	−	++
EpCAM	+	−/+	−/+	−	−	−
F4/80	+	−	−	+	+	+

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Table 2

Phenotype of the human cutaneous dendritic cell subsets

	LCs	CD1a$^+$ DDCs	CD14$^+$ DDCs	Skin macrophages
CD45	+	+	+	+
CD11c	+	+	+	−
CD11b	−	+	+	+
Langerin	+	−	−	−
CD1a	+	+	−	−
CD14	−	−	+	+
E-cadherin	+	−	−	−