

EXPERT OPINION

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

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Introduction: Breaching the skin's *stratum corneum* barrier raises the possibility of the 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 holds enormous potential for improved therapeutic outcomes for patients, particularly those in the developing world. Various vaccine-delivery strategies have been employed, which are discussed in this review. The importance of cutaneous immunobiology on the effect produced by microneedle-mediated intradermal vaccination is also discussed.

Expert opinion: Microneedle-mediated vaccines hold enormous potential for patient benefit. However, in order for microneedle vaccine strategies to fulfill their potential, the proportion of an immune response that is due to the local action of delivered vaccines on skin antigen-presenting cells, and what is due to a systemic effect from vaccines 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: antigen, cutaneous immunobiology, intradermal, microneedle, vaccine

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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 more than 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.

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 (s.c.) or intramuscular

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 needlestick 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.

This box summarizes key points contained in the article.

(i.m.) 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 needlestick 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 s.c. 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 s.c. 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 i.m. 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 i.m. 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 four 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 s.c. fat layer and is approximately 3 – 5 mm thick [12]. The dermis is composed of a network of collagen and elastin fibers embedded in a mucopolysaccharide matrix. Collagen provides the skin with mechanical support [13], while 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 s.c. fat layer, subcutis, 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 DC 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 the development of improved administration strategies to ameliorate vaccine efficacy. The concept of delivery of vaccines through the skin has been gathering momentum in the past decade, largely due to the increasing recognition that a tight semi-contiguous network of immunoregulatory 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 as CD8⁺ T cells and all different types of CD4⁺ T cells can be found in normal skin.

DCs are the main recipients of intradermal (ID) 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

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	+	-	-	+	+	+

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, such as NK cells, NKT cells and gamma-delta T cells. But more importantly, DCs play a pivotal role in the 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 (LNs). 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 LN [23]. Thus, vaccines serve not only 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 DC 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 significant 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 [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. DDCs have been much less studied than LCs due to the lack of available specific markers that 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 homogeneous 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⁺ DDCs [30-32].

In summary, three main distinct subsets can thus be identified in steady-state murine skin: epidermal LCs, dermal langerin⁻ DCs and dermal langerin⁺ DCs. Further details about the expression of various markers by different murine skin DC 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 LCs are especially capable of inducing cytotoxic T lymphocytes [33,34] as opposed to DDCs. Studies in a mouse tumor model confirmed that LCs and dermal langerin⁺ DCs are essential for antitumor immunity *in vivo* [5]. Protection from an experimental tumor was lost when LCs 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*, DDCs 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.

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	+	-	-	-

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 LNs, 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 DC subsets can be distinguished by location, phenotype and function in homeostatic human skin. The outer epidermal layer of the human skin also contains LCs, which constitute approximately 2% of the total epidermal cell population in normal healthy skin. LCs are the only subset in human skin that express 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⁺ DDCs [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. Microneedle 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 micrometer 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 the 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 \times 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 titers than in unperforated skin. The presence of CT increased the immune response to similar levels as observed after s.c. injection of AlPO₄-adsorbed DT (DT-alum). Unlike in the case of DT, however, MN array pretreatment 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 coadministration 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 coadministered with lipopolysaccharide (LPS), Quil A, CpG oligodeoxynucleotide (CpG) or CT as adjuvants. MN array pretreatment group resulted in high serum IgG levels that were significantly improved by coadministration of adjuvants. The IgG levels of the group treated with DT coadministered 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 pretreatment when in solution with the antigen, although DT-loaded nanoparticles (NPs) 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 tumor growth was observed 35 days after tumor injection in mice that were treated using Dermaroller® MNs and s.c. injection of vaccine. A significant increase in IgG antibody levels was observed for both transdermal and s.c. vaccinated groups in comparison with control groups. However, the transdermally vaccinated group showed slightly increased IgG antibody levels compared with the s.c. group. The authors concluded that the developed formulation for melanoma cancer that can be administered using MN 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, which 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 five 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 stabilizer [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 i.m. injection. The study concluded that optimization of coating formulation and addition of a stabilizing agent protect 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 i.m. injection [2]. Solid metal MNs coated with inactivated influenza virus were found to be at least as effective as the conventional i.m. 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 i.m. 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 the spleen of guinea pigs that was comparable with 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 bifunctional 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 with 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 with the s.c. 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 s.c. 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 used Nanopatch technology to target ID vaccination against West Nile virus and chikungunya virus in mice. 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-neutralizing response of mouse serum samples from mice vaccinated using Nanopatch was not inferior to that of control mice that had been vaccinated by the i.m. 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 Corp.) 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 with that observed after ID administration of the same doses of antigen. And that application of 1 and 5 μg of antigen via Macroflux induced 10- and 50-fold increase in anti-ovalbumin levels in comparison with those produced by i.m. or s.c. 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 and 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 electrically driven flow [43,64]. The approach can, however, be limited by the same disadvantages associated with conventional vaccination techniques, 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 coadministration 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 ID influenza vaccination in healthy adults [69]. The study was conducted in 180 healthy adults. The safety and immunogenicity of $\alpha\text{-RIX}$ ® (GSK Biologicals) influenza vaccines delivered using a hollow MN device (Micronjet®) was investigated. This novel device has been developed by Nanopass specifically for ID 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 i.m. 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 i.m. 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 i.m. [71].

2.4 Dissolving/soluble MNs

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 lyophilized 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 i.m.

The Kendall group described the micromolding 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) molds. Dual-layer MNs containing the model antigen ovalbumin, along with the adjuvant Quil-A, elicited post-immunization schedule antibody levels in mice that were comparable with an i.m. ovalbumin/Quil-A immunization group at day 28 and superior to the i.m. group at day 102, despite using a lower antigen dose in the MNs. Similar results were seen with influenza vaccine.

3. MN-mediated delivery of antigen-encapsulated NPs 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 NPs are colloidal systems, typically 10 – 1000 nm in diameter, with a therapeutic payload entrapped, adsorbed or chemically coupled to an orbicular

matrix [75]. NPs are widely used for controlled delivery of small-molecule drugs, oligonucleotides and protein antigens to a variety of cell types, including DCs [76]. Among the different parameters that need to be considered in the design of particle-based vaccines, the particle size and their physico-chemical properties are particularly important for skin vaccination. It has been demonstrated that polymeric NPs < 500 nm in diameter have high rate of intracellular uptake by variety of APCs [77].

Several groups have demonstrated that NPs have adjuvant effects comparable with 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 NPs as adjuvants promote activation of the NLRP3 inflammasome [81].

NPs have been extensively studied for oral and parenteral administration owing to their sustained drug release [82,83]. This property of NPs could also be utilized for topical antigen administration to target skin DCs with antigen over a prolonged period. Researchers have attempted to use NPs for topical drug delivery, and they found that the drug permeation was enhanced by gradual drug release from the NPs on the skin surface, but did not optimize way to deliver NPs inside the skin [84-86]. This suggested that as a drug delivery vehicle, the NP 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 NPs 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 MNs could act as channels for NPs to penetrate the skin, *in vitro* experiments have been designed and proved that NPs could pass through the human epidermal membrane and get into skin layers [89,90]. Moreover, Bal and colleagues showed that in ID 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 MNs may be an effective vehicle for the ID delivery of antigen-encapsulated NPs *in vivo*.

4. Conclusion

DCs are key regulators of immune responses and play a critical role in the design of modern vaccines [18,92,93]. The skin harbors 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 favor the targeting of these specialized 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 ID 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 specialized immunological tissue of the skin. Further developments, in particular in the 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

MN-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 MN application should prove to be a real boon in developing countries. The absence of visible needles is likely to be useful in the 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 MNs 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 MNs is what seems to hold most promise. Formulating MNs 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 MNs are self-disabling, dissolving or biodegrading rapidly in skin to release their payload. This means that these MNs cannot 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 MNs could have on the health of human beings worldwide.

A number of factors must be considered before MN-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 MNs [94]. It should be shown what proportion of an immune response is due to local action of delivered vaccines on skin APCs 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 MN vaccines consistently. MNs 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 utilization of applicator devices, which will not only guarantee consistent application forces with concomitant predictable skin insertion depths, but also provide feedback to healthcare workers and/or patients that the MNs have been

inserted correctly. This will be necessary to provide the same level of assurance of vaccination currently seen with conventional administration devices.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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