Intradermal vaccine delivery: Will new delivery systems transform vaccine administration?

Paul Henri Lambert a,*, Philippe E. Laurent b

a CMU, Centre of Vaccinology, 1 rue Michel-Servet, CH1211 Geneva 4, Switzerland
b BD Medical-Pharmaceutical Systems, 11 rue Aristide Bergès, 38800 Le Pont de Claix, France

Abstract

There has been a recent resurgence of interest in intradermal vaccine delivery. The physiological advantages of intradermal vaccine delivery have been known for some time, but the difficulties associated with performing an intradermal injection have historically limited its use. New delivery systems currently in development facilitate convenient intradermal vaccination, unlocking the potential advantages of this delivery route, and potentially transforming vaccine delivery.

© 2008 Elsevier Ltd. All rights reserved.
1. History of intradermal vaccination

The discovery of the principles of vaccination is often described as one of the most important developments in public health. The practice of inoculating small amounts of material from sick patients, such as powdered smallpox scabs or pus, into the nose or skin of healthy individuals to prevent disease was widespread across parts of Africa, Asia and the Ottoman empire, before inoculation into the skin – variolation – was introduced to Europe in 1721. Inoculated patients would generally develop a milder form of the disease than that occurring naturally. However, the risk of death from smallpox remained. It was not until 1796 that the first vaccination was carried out as practiced today by Edward Jenner in the United Kingdom based on his observation that milkmaids who had contracted cowpox through contact with cowpox pustules were not getting smallpox. Initially Jenner’s findings were not well received and it took 44 years for variolation to be forbidden by an Act of Parliament and a further 13 years for vaccination against smallpox to be made compulsory in Britain in 1853 [1].

In 1967 the WHO launched a global programme to eradicate smallpox which, 150 years after Jenner’s discovery, was still affecting 10–15 million people each year. Eradication of the disease was finally confirmed by the World Health Assembly in 1980 [5]. A major contribution to this achievement was the development of the bifurcated needle by Benjamin A. Rubin. This needle was specifically designed to ensure the delivery of about 2 μl, but sufficient, quantity of this very potent vaccine into the dermis. It helped healthcare workers to correctly deliver vaccine to the most efficient site for immunization against smallpox. Vaccination was done by dipping the bifurcated needle into the vial of vaccine to pick up a minute drop of vaccine solution between the needle's two prongs, then by jabbing the skin – typically in the deltoid region – several times with a brisk movement perpendicularly to the skin surface [6].

The first renewed interest in intradermal immunization using a needle and syringe injection system in controlled clinical trials was reported by Tuft in 1930 [7]. This study reported an equivalent immune response and an improved adverse event profile with a smaller dose of typhoid vaccine when injected intradermally relative to subcutaneous injection [8]. Subsequently to these reports several studies aiming to evaluate the efficiency and utility of intradermal delivery route such as vaccine dose reduction were conducted using different commercially available vaccines including influenza [9–11], measles [12,13], cholera [14], rabies [15,16], hepatitis B [17–20], polio virus [21–24] aiming to evaluate the optimal route of immunization for preventive vaccination. In spite of the large number of published clinical trials comparing post-inmunization humoral immune responses, the evaluation of the benefit and utility of intradermal delivery suffers from the absence of a consistent clinical design and standardized investigational method permitting an efficient side-by-side comparison and meta-analysis. The vaccine antigen concentration/immune response curve has rarely been thoroughly evaluated to detect and characterize the minimal, maximal and optimal antigen concentrations in various population segments which correspond to the clinical indication of investigated vaccine. Nevertheless, vaccines can be generally categorized into three groups: (i) those for which intradermal delivery induces better responses than by intramuscular or subcutaneous injection; (ii) vaccines for which conflicting results have been observed in separate clinical trials; and (iii) vaccine remaining to be investigated such as combo vaccines, meningococcal. Potential benefits of the intradermal delivery route as measured by post-immunization immune response depend upon the type of vaccine. For example, it is well documented that the immune response after intradermal administration of one-tenth of an intramuscular dose is equivalent to the full dose given intramuscularly for rabies and hepatitis B vaccines, but not for trivalent influenza vaccine [25–29]. One confounding factors leading to mixed clinical study results with trivalent influenza vaccine is the priming by previous natural infection; primed adult subjects produce equivalent immune response with reduced dose of antigen delivered by intramuscular as well as intradermal delivery routes [29,30]. In contrast, intradermal influenza vaccination in elderly subjects (15 and 21 μg of haemaglutinin/strain/0.1 ml dose) induced a humoral immune response superior to the IM control against all three strains [31]. Clinical studies in subjects with chronic medical conditions such as kidney failure, with or without haemodialysis, suggest that intradermal delivery of hepatitis B vaccine induces a better immune response than intramuscular injection [27,28,32]. Meta-analysis of clinical trials evaluating rabies vaccine prepared on diploid cells indicated that the persistence of specific humoral antibodies is at least equivalent to that observed with intramuscular delivery; the same results are observed with hepatitis B vaccine [25,33]. The local skin reactivity usually observed at the injection site after intradermal vaccine inoculation reflects the physiological local inflammatory response due to immune response induction and is characterized by spontaneously reversible redness at the injection site for a maximum period of 2 days without local sequelae. Systemic adverse event profiles are equivalent whatever the delivery route.

2. Current situation and future needs of innovative vaccine delivery systems

An ideal vaccine is safe, cost-effective, and efficient after a single dose [34]. The way in which a vaccine is delivered can have considerable bearing on these factors through its influence on the efficiency of the procedure, the dose required, compliance, and safety. For vaccination to succeed holistically in contributing to public health, vaccine delivery systems must allow efficient delivery without compromising product stability during storage and transport and without negatively influencing patient perception. To be considered safe, new delivery systems should reduce the risk of injury and infection of healthcare workers, and prevent illicit re-use. A delivery system combining all these qualities would facilitate the vaccination of greater portion of the population.

Currently licensed vaccines are delivered via one of five main administration routes: intramuscular for the majority of vaccines including hepatitis A and B, rabies, influenza and diphtheria–tetanus–pertussis–based combination vaccines; subcutaneous for vaccines such as measles, mumps and rubella, and yellow fever; intradermal for BCG and rabies; intranasal for live attenuated influenza vaccine, and oral for poliomyelitis, cholera, rotavirus and typhoid fever. With the rare exception of jet injectors, intramuscular, subcutaneous, and intradermal routes are accessed using needles. These techniques, whilst having proven efficacy in terms of achieving the required immune response, have some drawbacks relating to safety and patient compliance [35,36]. The invasive nature of the parenteral injection procedure and the potential for inappropriate reuse of equipment exposes patients to the risk of transmission of blood borne pathogens. Additionally, the use and disposal of equipment is associated with the risk of needle stick injury. The introduction of safer devices engineered to prevent needle re-use and reduce the risk of needle stick infections is likely
to lessen these concerns. However, the perceived or real pain and trauma sometimes associated with needle-based vaccination can be barriers to vaccination uptake, particularly by needle-phobic individuals [37,38]. These drawbacks, and the development of new types of vaccines, are some of the reasons driving the pharmaceutical industry and public health organizations to search for new delivery methods that are safe, cost-effective and efficient.

While the majority of vaccines in clinical development are envisioned as needle and syringe products, a number of research groups and vaccine manufacturers are exploring the advantages of new parenteral delivery systems as well as of mucosal and transcutaneous delivery [39]. Mucosal delivery is currently only used for live attenuated vaccines against poliomyelitis, typhoid fever (oral), rotavirus and influenza (nasal) [40,41]. Mucosally administered vaccines have a number of benefits. They eliminate the risk of transmission of blood borne diseases and needle stick injury. They can potentially be given by personnel with little medical training, which provides significant practical and cost benefits, particularly in the context of large-scale immunisation programmes in the developing world [42]. This route can also, in theory, elicit both mucosal and humoral immunity, offering advantages against diseases contracted via mucosal surfaces [43]. However, there are also a number of drawbacks. The live attenuated viruses in oral poliomyelitis vaccine (OPV) can revert to virulence, causing vaccine-associated paralytic poliomyelitis (VAPP) in the vaccinated child or their close contacts, particularly in the immuno-depressed subjects [44]. This has resulted in a shift from the use of OPV to the use of injectable poliomyelitis vaccine containing inactivated virus, especially in countries that have eliminated naturally occurring polio [45]. Oral vaccines have to overcome problems associated with poor absorption or degradation within the digestive system that may require the concomitant administration of antacids [46]. Finally, to date no mucosal vaccine adjuvant is available with the required safety and efficacy [44]. Such safety issues were encountered with an intranasal adjuvant-containing influenza vaccine that was associated with the occurrence of facial palsy [47].

### 3. Skin physiology and immunology

#### 3.1. Skin anatomy

An increasing understanding of skin physiology means that this organ is now recognized as a potentially excellent site for vaccination. It is easily accessible and has both cellular and humoral immune system components. The skin is comprised of three primary layers from outside to inside: epidermis, dermis and hypodermis (Fig. 1). Vaccine delivery into these layers is known, respectively as transdermal, intradermal and subcutaneous vaccination.

The epidermis is the outermost layer of the skin and acts as a physical barrier, preventing chemicals and micro-organisms from entering the body and stopping excess body water loss. This layer is generally 50–200 μm thick, depending on the body region and has four sublayers: the outermost stratum corneum, below which is the stratum granulosum, the stratum of Malpighii or spinosum, and finally the stratum basale (or germinativium). Keratinocytes constitute approximately 90% of the epidermis; the remaining cells are melanocytes and Langerhans dendritic cells. While Langerhans cells account for only about 1% of cells, they cover nearly 20% of the surface area due to their horizontal orientation and long protrusions [48]. The epidermis does not have its own blood supply; cells in lower levels receive nutrients via diffusion from blood capillaries in the dermis. Cells form within the stratum basale and migrate through to the stratum corneum where they are sloughed off. During this process, which lasts approximately 30 days, cells become keratinised. It is the stratum corneum with its layer of keratinised cells that is so important in the skin’s role as a physical barrier. The stratum corneum is also the greatest barrier to effective transdermal vaccine delivery. To be effective, it is critical that the vaccine be delivered to the Langerhans cells. This implies that a transdermal delivery method must include a system to disrupt, either physically or chemically, the stratum corneum, allowing antigens to pass through this layer and onto the Langerhans cells for antigen presentation.

![Fig. 1. Skin anatomy. Skin thickness was measured by 20MHz ultrasound echography in usual body sites for vaccine delivery [55].](image-url)
The dermis lies beneath the epidermis and is comprised of collagen, elastin and reticular fibres. It is a tough, flexible and very elastic layer between 1.5 and 3 mm thick, arranged into two sub-layers: the papillary dermis and the reticular dermis. The papillary dermis is the upper and the thinner of the two and consists of loosely arranged connective tissue. The reticular dermis consists of a network of horizontally running collagen fibres, connective tissue, and a very dense network of capillary blood and lymphatic vessels in which dermal dendritic cells, monocytes, polymorphonuclear leukocytes and mast cells circulate. Lymphatic vessels drain the dermis to satellite lymph nodes. Fibroblasts are the most abundant type of cells in the dermis. Endothelial cells forming the wall of blood and lymphatic channels play a key role in the inflammatory and immune cells as well as fluid movements in dermis. Endothelial cell contribute to various physiological effects in the skin including vasodilation increased permeability, increased vasomotion, production of cytokines converting adherent leukocytes into mobile cells, angiogenesis and trafficking of antigen presenting cells, T and B effector cells [49].

The hypodermis, or subcutaneous tissue, is a layer of loose connective tissue and elastin located immediately beneath the dermis. The arteries and veins that drain the skin dermis issue from the vascular plexus located in subcutaneous tissue. When entering the skin dermal arteries form a dense network of capillary loops in the papillary dermis layer. Numerous lymphatic vessels draining the skin dermis pass through the hypodermis before reaching draining lymph nodes. The hypodermis is the main tissue for fat storage.

Anatomical variations of skin according to body site, gender, age and ethnic origin are important parameters to consider for dermal vaccination. For example, skin thickness – an essential parameter for intradermal vaccination – is known to vary significantly between different parts of the body [50–54]. In a recent study designed specifically to investigate skin thickness at the usual areas for intradermal vaccination (deltoid, suprascapular, upper abdomen and thigh) in groups of people of different age, sex and ethnic origin, skin was found to be on average 1.5 mm thick at the thigh and between 1.8 mm and 2.7 mm at the other body sites, with no major differences between the different population subgroups considered [55]. Indeed skin thickness was found to vary less between people of different body mass index, age, gender and ethnic origin than it did between different body sites on people with the same demographic characteristics [55]. The average thickness of the skin appears to remain relatively unchanged in the age range of 18–70 years [56]. Skin is thinner in women than in men by 0.06–0.2 mm, but minimal skin thickness in women is greater than 1.5 mm in all cases [55–58]. The absence of a significant effect of the ethnic origin on the skin thickness at deltoid, and suprascapular body sites has also been reported in studies in US [54] and Japan [59]. This consistency in skin thickness across people with different demographic profiles represents a major advantage over classic intramuscular vaccination as, to correctly perform an intramuscular vaccination, it is important to select the appropriate needle length based on considerations of the muscle mass of the injection site, the amount of subcutaneous fat, and the weight of the patient [52,60].

3.2. Skin and immune response

The skin generates both innate (antigen non-specific response without immunological memory) and adaptive immune responses (antigen specific response with immunological memory), Table 1. While the adaptive response is primordial in generating a response to vaccination and generally becomes more effective with each successive encounter with an antigen [63], innate immune mechanisms also play a key role as they are activated first in response to pathogen invasion or contact with foreign antigens. The key group of immune cells involved in the skin’s innate immune response is dendritic leukocytes: Langerhans cells in the epidermis and dermal dendritic cells in the dermis [63–68].

In 1868 Paul Langerhans, driven by the interest in the anatomy of skin nerves, identified a population of dendritically shaped cells in the suprabasal region of the epidermis after impregnating human skin with gold salt [65]. These cells are known as antigen-presenting cells, called Langerhans cells after their discoverer. Although substantial numbers of dendritic leukocytes reside and circulate in the skin, only some of them are Langerhans cells, the majority being phenotypically different from Langerhans cells and generically called dermal dendritic cells [64]. Both Langerhans cells and dermal dendritic cells are bone marrow-derived leukocytes highly specialized in antigen-presenting properties. These cells, in association with macrophages recruited from circulating blood and infiltrating dermis tissue, are the gatekeepers of the immune systems. Compelling evidence exists that Langerhans cells and dermal dendritic cells, as members of the family of antigen-presenting cells play a pivotal role in the induction of adaptive immune response against pathogens and any other antigens and haptenes which compromise the host homeostasis. The immunogenic potential of antigen-presenting cells from both epidermis and

Table 1

Innate and adaptive skin immune system

<table>
<thead>
<tr>
<th>Functional components of skin immune system</th>
<th>Cells of the skin immune system</th>
<th>Resident</th>
<th>Recruited</th>
<th>Recirculating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive oxygen</td>
<td>Keratinocytes</td>
<td></td>
<td></td>
<td>Natural killer cells</td>
</tr>
<tr>
<td>Ligands Toll receptors</td>
<td>Endothelial cells</td>
<td>Monocytes</td>
<td>Granulocytes</td>
<td>Dendritic cells</td>
</tr>
<tr>
<td>Heat shock proteins</td>
<td>Langerhans cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokines: IL-1, IL-6, TNFα</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chimiokines: CC, CXC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adhesion molecules</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropeptides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eicosanoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune tolerance: T regulator, IL-10, TGFβ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adapative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen recognition and presentation</td>
<td>Mast cells</td>
<td></td>
<td></td>
<td>Pro-macrophages</td>
</tr>
<tr>
<td>Cytokines: IL-1, IL-6, TNFα</td>
<td>Macrophages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2, IL-12, IL-18, INFα</td>
<td>T lymphocytes</td>
<td></td>
<td>T lymphocytes</td>
<td>T lymphocytes</td>
</tr>
<tr>
<td>Chimiokines: CC, CXC</td>
<td>Dendritic cells</td>
<td></td>
<td>B lymphocytes</td>
<td></td>
</tr>
<tr>
<td>Adhesion molecules</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T and B lymphocyte responses with high affinity effectors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
dermis tissues is regulated by cell surface receptors triggered by ligands secreted or presented by other somatic cells, or alternatively, by microbial products (danger or competence signals) [66]. Danger signals are represented for example by DNA rich in CpG repeats in bacteria, or other Toll-like receptor ligands [66,67]. Many of the receptor structures that sense such signals are essential components of the innate immune system. They are used to recognize molecular patterns demarking infectious nonself, as well as normal and abnormal self. The response to danger signals leads to tissue perturbation as evidenced by increased secretion of GM-CSF, TNF-α, IL-1 by keratinocytes and other skin cells. The antigen-presenting cells that pick up the antigen, process it, and re-express part of it as peptide/MHC complex on the surface are also profoundly affected by danger signals or danger signal-induced cytokine. The alterations of Langerhans cells and dermal dendritic cells include the increased expression of MHC antigens, co-stimulatory molecules, and cytokines such as IL-1β, IL-6, IL-12, as well as the enhanced emigration of these cells from the skin to the paracortical area of draining lymph nodes. At this site, the skin-derived dendritic cells provide the activation stimuli to naïve resting T cells surrounding them. This occurs in an antigen-specific fashion and thus results in the expansion of the respective clone(s) to mature into extremely potent immuno-stimulatory cells that controls the development of adaptive immunity [68]. Some evidence also exists that dermal dendritic cells that have not received such competence signals are not stimulatory, but actively down regulate or prevent potentially harmful immune responses by tolerizing T cells or by inducing T cells with suppressive properties (regulatory T cells) [69,70]. Several studies have indicated that protein or peptide delivery through the epidermis can lead to production of specific IgE due to a Th2-regulated response as well as immune tolerance status by regulatory T-cells [71–75]. As a consequence, in addition to the immuno-surveillance activity, the skin immune system secures the homeostasis of the skin integument by preventing the development of exaggerated, tissue destructive immune responses against per se innocuous moieties such as auto-antigens, allergens and haptens. Interestingly, a clinical study in healthy adults evaluating epidermal delivery of live-attenuated measles vaccine through disrupted stratum corneum relative to intramuscular route strongly suggests that resident antigen-presenting cells in epidermis were unable to boost the antibody response [76].

3.3. Skin immune response and sun exposure

It has been suggested that sun exposure may affect local or systemic immune responses through release of inflammatory mediators [80]. The question may be raised whether such effects may particularly influence responses to intradermal immunization. UV radiation below 290 nm is absorbed by the ozone layer in the stratosphere and does not reach the Earth’s surface. The UBV wave-lengths range from 280 to 315 nm and from 315 to 400 nm for UVA. Solar UV radiation is 95–98% UVA and 25% UBV. The most obvious clinical effects of the sun exposure are sunburn and tanning, but include more complex biological effects such as DNA photo damage, immunosuppression and vitamin D synthesis. These biological effects are radiation dose-dependent and the amount of UV radiation penetrating the epidermis and dermis is the critical factor. For instance, the stratum corneum of the epidermis is able to dissipate 90% of UBV radiation, and no more than 10% of UBV reaches the dermal-epidermal junction area. In addition, melanin present in high concentration in the epidermis acts as UV radiation filter. The biological effects of UVB on the skin immune response was actively investigated, the main changes being the depletion of Langerhans cells, the increased recruitment of macrophages in skin dermis and the release of pro-inflammatory cytokines such as TNF-α, IL-10, TGF-β, α-MSH and CGRP [77–80].

4. Clinical experience, techniques and devices for intradermal vaccination

Considerable clinical research has been conducted to compare the intradermal route with other routes of vaccine delivery (Table 2) and into new techniques for intradermal delivery to eliminate some of the problems associated with the methods currently available. This section will describe the available techniques, as well as those in clinical research or earlier development.

4.1. Current methods

4.1.1. Mantoux injection technique

The standard intradermal injection technique consists of stretching the surface of the skin and inserting the tip of a 27G, 3/8 in. short bevel needle attached to a plastic 1 ml disposable syringe. The needle is inserted bevel upwards, almost parallel to the skin surface and vaccine is injected slowly into the uppermost layer of the skin [81]. If placed properly, there is considerable resistance to injection and a raised papule immediately appears which can cause pain during injection. The correct placing of the needle-tip in the dermis is critical to avoid fluid injection difficulties due to inelastic skin or age-related anatomic changes [53,58,61,62,81]. This technique, introduced by Charles Mantoux over 95 years ago as a diagnostic skin test for tuberculosis disease [2] has not been pursued for the vast majority of vaccines due to its inherent difficulties. This technique is associated with a poor consistency of the injected volume, due in part to the difficulty of performing it correctly, but also to the unavoidable leakage of vaccine from the injection site, fluid wastage when filling disposable syringes and when purging the needle of air, and the large dead volume of the assembled disposable needle and syringe [82–84]. In many cases, intradermal vaccination according to Mantoux has proved to be comparably immunogenic to the comparator even at a reduced dose, due to the skin’s ability to generate a strong immune response [3,27,85–91]. This comparable efficacy at lower doses suggests that intradermal injection can have considerable benefits over other injection techniques when mass vaccination is necessary, as the reduced dose means improvement of vaccine availability and of health economic ratios if an injection system that is easier to practice becomes available.

4.1.2. Bifurcated needle

While working for Wyeth Laboratories in 1965, Benjamin Rubin developed his two-pronged needle for smallpox vaccination by skin scarification by grinding the eyelet of a sewing machine needle into a fork shape. This was the first example in modern medical history of a device specifically designed to deliver vaccine intradermally. The small space between the two tines was able to hold about 2 μl of vaccine solution but only part of this volume that was actually introduced into the skin's ability to generate a strong immune response [3,27,85–91]. This comparable efficacy at lower doses suggests that intradermal injection can have considerable benefits over other injection techniques when mass vaccination is necessary, as the reduced dose means improvement of vaccine availability and of health economic ratios if an injection system that is easier to practice becomes available.

4.1.3. Multipuncture

The percutaneous BCG delivery using single or multipuncture devices was introduced by Sol Roy Rosenthal in 1939, and developed worldwide by the Merieux Institute [92–94]. The multipuncture unit is a cylinder-like device with small needles, 1 mm length, which should be pressed firmly against the skin, within the
area where the **vaccine dose is spread on the skin surface.** In contrast with the intradermal technique, the percutaneous method does not allow a precise estimation of the dose injected in the epidermis or dermis skin layers. As a consequence, the number of live attenuated bacilli given per delivery is highly variable [95–97].

### 4.1.4. Needle-free jet injection

Needle-free jet injection uses a gas to force a liquid or powder vaccine through the skin, delivering it intradermally, intramuscularly or subcutaneously [98,99]. These devices can either be multidose or monodose. The latter are based either on entirely disposable devices or on a re-usable system with single dose cartridges [99]. This technology has been available since the 1940s and was used for several mass vaccination programmes [98–101]. However, when an outbreak of hepatitis B linked to the use of multidose jet injectors revealed the risk of contamination by the aspiration of small amounts of blood into the nozzle during the vaccination procedure, their use was discontinued by the WHO and health authorities around the world [101]. Recent research has focused on improved devices with single-use nozzles to remedy this problem [102] and on developing standardised single-dose cartridges that could potentially make monodose injectors financially viable for mass vaccination [99].

Studies have shown that vaccination via jet injection can elicit an immune response comparable to or better than other delivery methods. A comparison of jet injection with the standard needle-based techniques for five vaccines found that for four of them, jet injection had equivalent, if not improved efficacy [97,98,104]. Successful intramuscular or subcutaneous jet injection has been demonstrated with a wide range of vaccine technologies including DNA coated nanoparticles [105], naked DNA [106], inactivated virus, polysaccharide–protein conjugates, toxoids, and whole cell vaccines [103]. In one study with tetanus toxoid vaccine, jet injection was found to be less immunogenic than the standard needle and syringe technique in subjects younger than 40 years, although the techniques gave equivalent results in those aged over 40 [102]. In this study the jet injector delivered the vaccine into the deep subcutaneous layer.

In contrast to needle-based vaccination, jet injection results in a wide distribution of vaccine in the dermis, hypodermis and muscle, depending on jet injector settings and individual body characteristics. This distribution effect means that jet injection may face some problems when used to specifically deliver a precise dose of vaccine into the dermis, but is thought to lead to the increased immune responses observed [102,108]. It is also possible that the larger immune responses are a result of increased inflammation with jet injectors, leading to the recruitment of more immune cells to the injection site [107,108]. Indeed, while it has been suggested that jet injection results in less tissue damage than with needles as the injected liquid follows the path of least resistance [109] a number of studies refute this finding that jet injection (whether intradermal, intramuscular or subcutaneous) causes more adverse events including swelling, erythema, induration, haematoma and pain [98,103,110,111]. In a comparison of DNA vaccine delivered either by intramuscular injection or by intradermal jet injection, or a combination of the two, jet injection caused approximately twice as many adverse events. Despite this, the majority of subjects in this study stated that they preferred the jet injection to needle injection at the time of injection [106].

Jet injection with single use nozzles or single dose cartridges removes the risk of needle stick injuries after vaccine delivery and associated risk of blood borne transmission of diseases through contamination. In addition, the nature of jet injection is such that a large number of vaccines (in excess of 600 per hour) can potentially be treated in a short period of time [102]. This makes jet injectors an attractive technology for mass parenteral vaccination programmes. It has indeed been used in a number of mass campaigns for small-

### Table 2

**New technologies targeting vaccine delivery into the skin**

<table>
<thead>
<tr>
<th>Technology</th>
<th>Company</th>
<th>Vaccine (development phase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefilled microinjection system</td>
<td>BD Medical Pharmaceutical Systems/Sanofi Pasteur</td>
<td>Trivalent inactivated seasonal influenza (clinical phase 3)</td>
</tr>
<tr>
<td></td>
<td>BD Medical Pharmaceutical Systems/Oncovax</td>
<td>Cancer vaccine (clinical phase 2)</td>
</tr>
<tr>
<td>Non-prefilled microinjection needle</td>
<td>Nanopass, Micro-Pyramidal Needle</td>
<td>Flu (clinical phase 1)</td>
</tr>
<tr>
<td></td>
<td>Georgia Institute of Technology</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>Topical Patch with cholera toxin adjuvant</td>
<td>Iomai Corporation</td>
<td>Trivalent inactivated seasonal influenza (clinical phase 2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Travelers’ diarrhea (clinical phase 2)</td>
</tr>
<tr>
<td>Topical Patch</td>
<td>Vaxin, Inc.</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td></td>
<td>Ichor Medical Systems, TriGrid</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>Transdermal with electroporation</td>
<td>Cyto Pulse: Derma Vax, Easy Vax</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td></td>
<td>Inovio, MedPulsor DNA Delivery System</td>
<td>DNA dengue (clinical phase 1); HIV pre-clinical</td>
</tr>
<tr>
<td>Solid microneedle array</td>
<td>Genetronics Biomedical Corporation</td>
<td>DNA, proteins vaccine (pre-clinical)</td>
</tr>
<tr>
<td></td>
<td>Alza Corporation, Macroflux</td>
<td>Pre-clinical</td>
</tr>
<tr>
<td>Jet injector (Powder)</td>
<td>Biovalve, Micro-Trans</td>
<td>DNA vaccines (clinical phase 2)</td>
</tr>
<tr>
<td></td>
<td>Epidermal Powder Immunization</td>
<td>DNA HIV (clinical phase 1) herpes simples type 2 (clinical phase 1), DNA cancer vaccine (clinical phase 1)</td>
</tr>
<tr>
<td>Jet injector (liquid)</td>
<td>Bioject</td>
<td></td>
</tr>
<tr>
<td>Skin abrasion</td>
<td>BD Technology, Microenhancer Array</td>
<td></td>
</tr>
<tr>
<td>Skin permeation by low frequency (20 kHz)</td>
<td>Sonsics &amp; Materials, Newtown, CT</td>
<td></td>
</tr>
<tr>
<td>ultrasound associated with topical patch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanoparticle and microparticle formulation</td>
<td>Chiron</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Various</td>
<td></td>
</tr>
</tbody>
</table>

pox, yellow fever and IPV vaccination that included large numbers of recipients.

4.2. Methods in pre-clinical or clinical development

4.2.1. Microinjection system

A microinjection system has recently been developed as a new vaccine delivery platform employing a tiny hollow microneedle penetrating 1.5 mm into skin from the outer skin surface to deliver a volume in the range of 100–200 μL. This system is a pre-filled, ready to use syringe with a proprietary drug container and a staked microneedle, designed to protect the needle after injection, preventing risk of injury and impeding the re-use and misuse of the system. Due to its very small size and fine gauge, the microneedle is nearly imperceptible by the patient. The microneedle is inserted perpendicularly in the skin; this makes intradermal vaccination intuitive and easy to perform consistently and reliably, in contrast to conventional intradermal injection techniques as documented in clinical trials involving healthy volunteers and general practitioners [112]. Vaccine is injected into the dermis in all individuals, irrespective of age, weight or ethnic origin [55]. The deltoid, suprascapular or waist is the most appropriate sites for vaccination with this system [55]. The use of this system appears reliable since it ensures consistent dosing [112].

This type of delivery system is under clinical evaluation for seasonal influenza immunization. A pilot phase 2 study in adult volunteers with seasonal trivalent influenza vaccine indicated humoral immune responses comparable to those obtained by the standard IM route, but with a lower antigen dose [113]. The local adverse events were equivalent to the IM route except for more frequent oedema and redness at the site of injection, but with the absence of post immunization muscle pain [113]. In elderly volunteers administration of the trivalent inactivated vaccine against seasonal influenza using the BD microinjection system resulted in superior immune response for all three influenza strains compared with the IM delivery route [31].

Similar microinjection technology has been used to administer an anthrax vaccine to animals, resulting in complete protection against inhalational anthrax spore challenge [114,115]. Additionally, microneedle-based intradermal delivery of a Japanese encephalitis vaccine in monkeys resulted in the induction of protective levels of antibody titres that were higher than with conventional subcutaneous injection [116]. Pre-clinical dose-ranging studies with the microinjection platform also demonstrated that there was significant potential for greater vaccine efficiency for both anthrax and influenza applications. However, dose-sparing may not be universally applicable to all pathogen strains.

4.2.2. Transdermal delivery

Transdermal delivery involves the topical application of a vaccine or drug directly onto the skin surface using a patch or by applying a gel or solution. This has been successfully used for drugs such as nicotine and hormones, but does not yet allow the delivery of large molecules such as the proteins, polysaccharides, whole cells or DNA used in vaccines [117]. Although skin in its natural state is impermeable to large molecules, it has been shown that if skin permeability is disrupted, antigens can penetrate the stratum corneum to reach the Langerhans cells in the epidermis [69,73,119]. Techniques to temporarly disrupt skin permeability include, hydration, stripping with tape or wax strips, the use of electric currents, or ultrasound [119]. Alving and Glenn introduced the concept of using cholera toxin as a transcutaneous adjuvant by proving both in animal models and in humans that efficient immune responses can be induced against diphtheria and tetanus toxoid vaccine antigens [73,117–119,72,120,121]. Animal studies have shown that potent T cell and antibody responses can be induced using a variety of adjuvants applied directly to shaved and hydrated skin and that transdermal vaccination can induce both mucosal and systemic immunity. Another way of overcoming the stratum corneum is to enclose the molecule of interest in vesicles that are able to pass directly through this layer [118]. Transferosomes are one type of vesicle which can penetrate skin via the intercellular space in the stratum corneum; their movement is facilitated by their high deformability and the transdermal water gradient. The potential of this technology has been demonstrated using tetanus toxin in transferosomes to induce an immune response comparable to intramuscular injection of the same dose (equivalent to 10 LF) of alum-containing TT after secondary immunization [122].

The potential advantages of transdermal delivery for vaccination include the fact that such vaccines may not need to be administered by trained health professionals, as well as all the safety advantages of a needle-free administration. Transdermal vaccines could thus be ideally suited to mass vaccination campaigns, particularly in areas where access to healthcare personnel is an issue. This technology is currently in the early stages of development (up to phase 1) and patch manufacturing technology integrating vaccine antigen formulations and industrial scale-up remain to be overcome. Changes in skin permeability, caused for example by a skin disease, could render difficult to reliably assess the dose that has actually passed through the skin. Other techniques to increase the efficiency of transdermal delivery are being investigated. Electroportation – the application of high voltage electric pulses to the skin to temporarily increase the permeability of cell membranes – is one such technique. This is associated with changes in skin structure and could be painful to patients, although studies in animals suggest that it is well tolerated and associated with only mild skin irritation [72,123–126].

Electroporation has been shown to increase transfection of DNA vaccines when the vaccine itself is delivered by intradermal injection. Ionoporation or ionophoresis works in a similar manner but uses weaker transdermal voltages over a longer period to drive compounds across intact skin [109]. While such techniques have been in use in laboratories for over 20 years, their application to transcutaneous vaccination has not yet been proven or developed [72]. Sonoporation uses low frequency ultrasound to disrupt lipids in the stratum corneum [127,128]. This technique is relatively new, but studies in animals have shown that it can enhance the immune response to tetanus toxoid. The observed enhancement is thought to be due not only to increased skin permeability, but also to the activation of immune-competent cells within the skin by the ultrasound [128–131].

Photomechanical disruption uses a laser pulse to transiently increase permeability of the skin and it has been found to increase drug delivery through the skin without causing any pain or permanent changes to the stratum corneum [132]. Whilst these technologies have the potential to enable non-invasive vaccine delivery, their benefits may be limited by the need for specialist equipment and trained personnel.

4.2.3. Transdermal microneedle arrays

Hollow stainless steel microneedles have been successfully used to deliver Japanese encephalitis and anthrax vaccines in animal models, and trivalent influenza vaccine in humans [114–116]. Microneedle arrays made from glass, silicon or polymers have also been investigated, but limited experimental work has been published, and none with vaccines [133–135]. Solid microneedles as an alternative to hollow versions are also being investigated with three main delivery strategies. The first option consists of disrupting the stratum corneum with a microneedle array, then applying a transdermal vaccine patch on the disrupted zone [136–138]. The
reliability of stratum corneum disruption using solid microneedles has been investigated by trans-epidermal water loss and results suggest that the optimal needle length is within the range of 150–200 μm [139]. The second option is an antigen-coated microneedle array [140,141]. The use of this approach to deliver desmopressin [142] or a prototype antigen (ovalbumin) to hairless mice has suggested that this route is more efficient at eliciting an immune response as compared to other parenteral routes, this is particularly true because the administration with adjuvants may have to be occasionally considered to amplify immune responses and reduce the local adverse reactions that can occur at the site of injection. They are likely to reflect the long-term persistence of aluminium salts in skin macrophages. A second adjuvant effect that may be particularly beneficial for ID immunization is the activation and mobilization of skin dendritic cells that have captured vaccine antigens. This mechanism includes enhanced migration of appropriately pulsed APCs to local lymph nodes for initiation of T cell responses. Specific molecular targets for adjuvants aiming at the optimization of the activation and migration of antigen-pulsed skin DC are now becoming identified. For example, the repertoire of Toll-like receptors (TLR) expressed by human Langerhans cells (LC) is now better characterized. Highly enriched immature LC from human skin were shown to efficiently respond to TLR2 agonists peptidoglycan and lipoteichoic acid from Gram-positive bacteria, and to dsRNA which engages TLR3. In contrast, LC do not directly sense TLR7/8 ligands and LPS from Gram-negative bacteria, which signals through TLR4 [67]. Many other factors can influence the migration of epidermal LCs and dermal DCs. The most well-known are chemokines which regulate DC migration by virtue of their expression of chemokine receptors (CCR) and particularly CCR7, and inflammatory cytokines, e.g. Tumor Necrosis Factor (TNF)-α, interleukin (IL)-1β, and IL-18, that can also stimulate migration of DCs and their accumulation in draining lymph nodes. This knowledge should soon result in a novel generation of adjuvants specifically tailored for ID delivered vaccines. On a purely empirical basis some intradermal experimental vaccines that contained different adjuvants have been tested in clinical trials. A lipid containing adjuvant, MF59, was shown to be quite safe and efficient when given ID with an idiotype therapeutic vaccine for B cell lymphoma [149]. A Wilms tumor gene peptide vaccine was given ID with Montanide as adjuvant without major adverse effects [150]. A recent study in melanoma patients indicated that the intradermal administration of CpG-containing oligonucleotides (8 mg in 1.6 ml) at the excision site of the primary tumor resulted in very significant changes in the activation state and numbers of both plasmacytoid and myeloid dendritic cell populations and T-cell subsets in the melanoma sentinel lymph node. Some other promising adjuvants have only been tested in animal models, e.g. GM1 binding deficient LT exotoxin [151]. A critical issue for selecting new ID adjuvants will certainly be their safety profile at local and systemic levels.

### Table 3: Adjuvants and vaccine delivery routes

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Delivery route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium salts</td>
<td>IM, SC</td>
</tr>
<tr>
<td>Cholera toxin (phase 2)</td>
<td>Transdermal (topical application)</td>
</tr>
<tr>
<td>E. coli heat labile toxin</td>
<td>Mucosal (nasal)</td>
</tr>
<tr>
<td>ITK63 (phase 1–2)</td>
<td>Mucosal (nasal)</td>
</tr>
<tr>
<td>MF 59</td>
<td>Oral</td>
</tr>
<tr>
<td>Cytosine phosphate guanosine (CPG)</td>
<td>ID</td>
</tr>
</tbody>
</table>

4.3. **Intradermal delivery route and adjuvants**

Although many clinical studies evaluating intradermal delivery have suggested that this route is more efficient at eliciting an immune response as compared to other parenteral routes, this is not always the case and the use of adjuvants may have to be occasionally considered to amplify immune responses and reduce the vaccine dose [144] (Table 3).

Adjuvants may help ID-induced responses through two basic mechanisms. First adjuvants might be used to delay the clearance of locally injected antigen through a depot effect. This has been well demonstrated after IM administration of vaccines that contained aluminium salts. The adsorption of the vaccine antigens onto the alum-insoluble particles leads to the formation of a depot that is slowly released, prolonging the time of interaction with antigen presenting cells (APC). It also allows antigens to be presented in particulate form, which can be efficiently phagocytosed. However, adsorbed antigens appear to be released from alum particles within hours of the injection and other mechanisms may be involved [145]. Recent data indicate that alum can activate caspase-1 in a manner similar to urate crystals and induce the release of immunostimulatory cytokines including mature IL-1 and IL-18 [146].

Minor safety issues have been encountered with alum containing vaccines for ID delivery. Many studies have been done with intradermal hepatitis B vaccines at low doses. Specific local adverse effects that were occasionally encountered were induration, pigmentation abnormalities and nodules. Overall, the results of studies comparing ID versus IM vaccination with recombinant hepatitis B vaccines tend to indicate that similar antibody responses can be obtained by the ID route using lower doses of vaccine. However, there were some controversies and some studies show a faster decrease of antibody levels after ID immunization. Conversely, better responses can generally be obtained after repeated ID rather than IM immunization in patients with renal failure undergoing chronic haemodialysis. Intradermal administration of aluminium-salt adjuvanted hepatitis A vaccines was also shown to elicit antibody titres comparable to those obtained with the intramuscular route [147]. However, aluminium-adsorbed hepatitis A vaccines tend to cause some local adverse reactions [148]. Persisting skin nodules, which have the characteristics of foreign body reactions, can occur at the site of injection. They are likely to reflect the long-term persistence of aluminium salts in skin macrophages.

4.3.1. **Intradermal route and microparticulate delivery systems**

The potential of intradermal immunization associated with microparticulate vaccine delivery system opens a new avenue for improving immune responses. This technology consists of encapsulating of antigens in biodegradable polymers such as poly(lactide-co-ethylene glycol) (PEG), carboxymethyl-cellulose (CMC), polyesters, polyactic [PLA], poly(lactide-co-glycolides) [PLG] [152–155]. The observed enhancement in immune response upon intradermal administration is explained by the increased surface of interaction between antigens and antigen-presenting cells, a more efficient capture of antigens by phagocytes such as monocytes, and an improved distribution of injected particulates to draining lymph nodes [155].
4.3.2. What makes the intradermal route attractive? Are all types of vaccine compatible?

The intradermal route is attractive for two main reasons.

The first one is of a logistic nature. Vaccines that could be delivered using simplified intradermal delivery systems, such as those based on microneedles would be ideally suited for use in mass vaccination campaigns. The acceptance would be high in view of the painless character of such an injection procedure and vaccination could be done safely without extensive training.

The second reason is based on relative efficacy. Skin is optimally equipped in cells able to recognize and process foreign antigens. The high density of both LCs and dermal DCs confers unique properties to efficiently trigger innate immunity and initiate immune responses in the draining lymph nodes. In the absence of adjuvant, low doses of vaccine antigens are likely to be sufficient for vaccines that can be recognized by skin DCs through appropriate TLRs as seen with rabbies and adjuvant free formulations of hepatitis A. This may not be the case for all antigens and dose reduction strategies may require appropriate adjuvants and formulations specifically tailored for the ID route. Preference should be given to adjuvants that would optimally activate antigen-pulsed DCs without inducing neither extensive local inflammatory foci nor any long-lasting antigen depot. As seen with vaccinia and vaccinia-derived vectors, live attenuated viral vaccines that can infect skin DCs are also good candidates for ID immunization. The excellent T cell responses that have been seen after use of intradermal BCG in infancy demonstrate the potential of TLR2 activation at the level of skin DCs. Skin immunization may be optimal for induction of potent TH1 responses. The relative efficacy of the ID route for inducing CD8 responses has been suggested but this requires confirmation.

While it may not be possible to administer all vaccines through the intradermal route the availability of novel systems for simple and safe intradermal delivery is likely to increase the number of vaccines that can be administered to the skin. As such, it appears that the ID route should be considered when developing new vaccination strategies.

5. Conclusions

As recognition of the potential of the dermis to generate powerful immune responses has grown, pressure has increased to develop intradermal delivery methods to take full advantage of this ideal vaccination site, whilst overcoming the drawbacks of traditional intradermal injection methods. Indeed, although numerous clinical studies have confirmed the comparable or superior immunogenicity of this route compared with standard intramuscular or subcutaneous routes, the difficulty of correctly performing an intradermal injection with commercially available syringes has limited its use and has led to its association with dosing consistency issues. Intradermal delivery systems currently in development have the potential to reduce or remove the barriers to widespread adoption of intradermal vaccination. Systems designed to ensure that delivery into the dermis is both easy and consistent remove the need for highly trained medical staff and should improve dosing consistency and overall vaccine efficacy. The potential for this technology to reduce the required dose compared with intramuscular delivery could result in health economic benefits and increase the possibility of mass intradermal vaccination campaigns. The potential effects of different levels of sun exposure will have to be considered in clinical trials.

Non-invasive transdermal vaccination systems are also in development. However, the technology required to enable large molecules to cross the stratum corneum is not yet mature, and it has yet to be shown whether these systems can provide a viable answer to crucial vaccine supply chain and cost issues; which are particularly important to the success of vaccination campaigns in the developing world.

Improvements in invasive intradermal delivery currently offer the greatest opportunities to increase use of this route, thus improving the efficiency of and patient compliance to immunization programs.

References


