IMMUNIZATION WITHOUT NEEDLES

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Abstract | Most current immunization procedures make use of needles and syringes for vaccine administration. With the increase in the number of immunizations that children around the world routinely receive, health organizations are beginning to look for safer alternatives that reduce the risk of cross-contamination that arises from needle reuse. This article focuses on contemporary developments in needle-free methods of immunization, such as liquid-jet injectors, topical application to the skin, oral pills and nasal sprays.

LIVE ATTENUATED VIRUS
A weakened mutant of a wild-type virus that is antigenic but not infectious.

Needles and syringes are the most commonly used method for administering vaccines and protein therapeutics, such as insulin, to humans. The World Health Organization (WHO; see the Online links box) estimates that 12 billion injections are given annually, 5% of which are used for immunizations[1]. Despite their common use, needle-based immunizations have several limitations. Needle phobia is an important issue for both adults and children[2] and makes immunizations stressful[3]. In addition, accidental needle sticks are a serious problem in both developed and developing countries. The Centers for Disease Control and Prevention (CDC; see National Immunization Program in the Online links box), in the United States, estimates that more than 300,000 needle-stick injuries occur annually in US hospitals[4]. An estimated 5 accidental needle-stick injuries occur per 100 injections worldwide, posing a considerable risk to health-care providers[5].

An even greater shortcoming of injections is their improper and unsafe use. This mainly involves the reuse of needles and syringes, which is common in developing countries for reasons of cost[1]. (A detailed list of unsafe injection practices is given in REF. 1.) The WHO has estimated that as many as one-third of immunization injections are unsafe in four of its six geographical regions[5,6]. Each year, an overwhelming number of infections with HIV (80,000–160,000), hepatitis C virus (HCV; 2.3–4.7 million) and hepatitis B virus (HBV; 8–16 million) are thought to originate from the reuse of needles and syringes by health-care providers[7]. The WHO estimates that 32% of HBV infections, 40% of HCV infections and 5% of HIV infections in developing countries are attributable to unsafe injection practices[8]. Not surprisingly, the development of needle-free immunization methods has now been identified as an important goal in global health care[9].

Needle-free immunizations made their first notable appearance almost 50 years ago with the oral polio vaccine, which is still used in developing countries but has been discontinued in the United States since 2000 (BOX 1). This vaccine, which contains a LIVE ATTENUATED poliovirus, can infect the gastrointestinal tract and, subsequently, generate adequate immune protection in the host. Several other needle-free vaccines (oral typhoid fever, oral cholera, oral rotavirus and nasal influenza), which also contain live attenuated pathogens, are now available (TIMELINE). However, the administration of most vaccines without the use of needles has proved to be challenging, especially for non-living vaccines (that is, killed pathogens, and subunit, toxoid, peptide and DNA vaccines), which offer several advantages (BOX 1). Consequently, in developed countries, as well as developing countries, most childhood vaccines — including those against hepatitis B (a subunit vaccine), diphtheria–tetanus–pertussis (toxoid and inactivated bacteria), polio (killed virus), varicella (live attenuated virus), measles–mumps–rubella (live attenuated virus), tuberculosis (live attenuated bacteria) and yellow fever (live attenuated virus) — are administered using needles and syringes. In the past decade, however, there has been a strong step forward in addressing the technological challenges that are associated with immunization without needles[10,11].

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Current methods of needle-free immunization, either commercially available or under development, can be classified into two broad classes — cutaneous immunization and mucosal immunization — depending on the site of vaccine administration (FIG. 1). Cutaneous methods of immunization include the following: liquid-jet injection, which delivers a high-speed vaccine stream into intradermal, subcutaneous or intramuscular regions; ballistic methods (also known as epidermal powder immunization), which accelerate particulate vaccine material and deposit it in the skin; and topical application methods, which deliver the vaccine into or across the skin through passive diffusion or facilitated transdermal transport (FIG. 2). Mucosal immunization methods involve delivery of vaccines to a mucosal membrane, such as the ocular, oral, nasal, pulmonary, vaginal or rectal membrane (TABLE 1). This article provides an overview of the development of these methods, with an emphasis on the challenges that are associated with the delivery of non-living vaccines. Particular attention is paid to needle-free cutaneous immunization. Detailed discussion of mucosal immunization can be found elsewhere[11,12].

Liquid-jet injections

Jet injection is the oldest method of needle-free immunization. The origin of jet injections can be traced to the late 1800s, when a technique known as aquapuncture was reported in the medical literature[13]. This device was used to deliver jets of water and other liquids for applications other than immunization: for example, for the treatment of uncontrolled neuralgia. However, it was in the early 1950s when jet injections took their place as a needle-free method of delivering medications and vaccines[14].

A liquid-jet injector uses the kinetic energy of a high-velocity vaccine jet (typically more than 100 m per second) with a diameter that ranges from 76 μm to 360 μm, which is smaller than the outer diameter of a standard hypodermic needle (810 μm for a 21G needle). Liquid jets penetrate the skin and deliver the vaccine into the skin (that is, intradermally), the subcutaneous tissue or the muscle (intramuscularly) (FIG. 2A). Skin is a particularly attractive target for vaccine administration because it forms an integral part of the immune system[15,16]. The epidermis is enriched with LANGERHANS CELLS, which form a network that allows them to take up antigen efficiently and therefore to carry out immune surveillance[17]. The Langerhans-cell network is the next line of defence after the physical barrier of the skin has been breached. Langerhans cells initiate specific immune responses by processing and presenting antigen fragments to naive T cells in the lymph nodes[18]. This promotes the generation of both systemic (IgG and IgM) and mucosal (IgA) humoral immune responses[19,20]. Targeting the vaccine to the skin promotes its contact with Langerhans cells and reduces the required dose of vaccine[21,22], a factor that would become crucial at a time of vaccine shortage, such as the predicted H5N1 influenza-virus pandemic. Vaccines that are delivered by liquid-jet injectors typically spread throughout a larger tissue volume after injection than do vaccines that are administered with needles[23], which might allow them to establish better or faster contact with antigen-presenting cells before they are degraded.

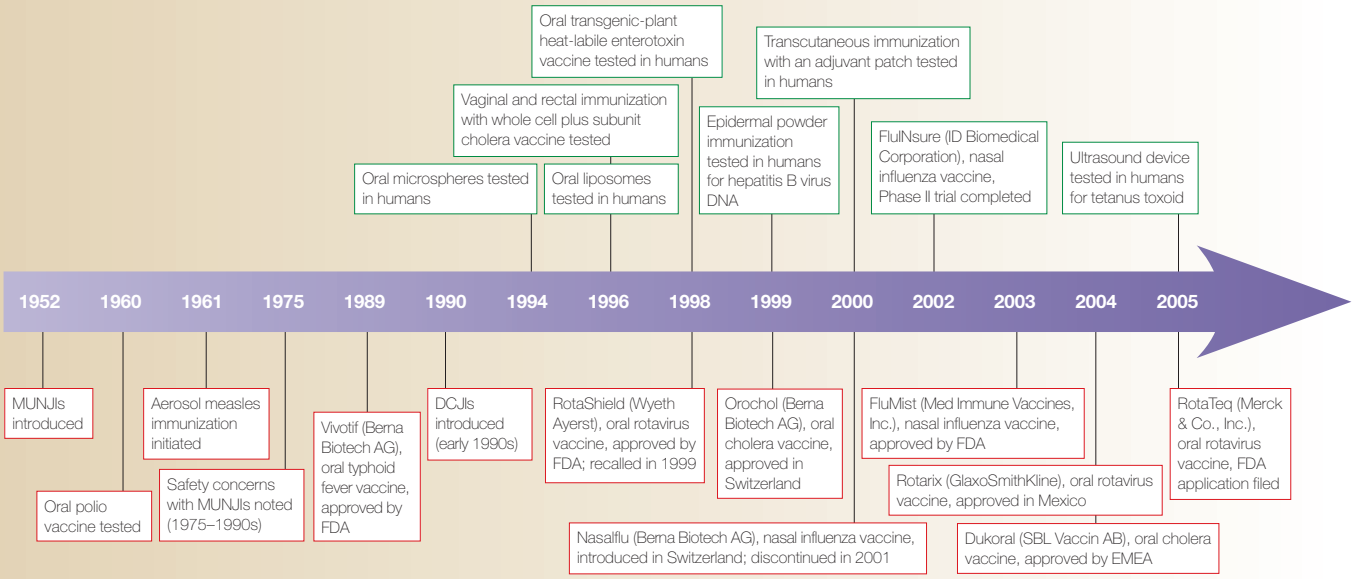
Box 1 | Live versus non-living vaccines

Live vaccines contain attenuated pathogens that have minimal virulence but high immunogenicity. These vaccines include natural viruses that are non-transmissible analogues of pathogens (for example, the smallpox vaccine), wild-type viruses that have been passaged *in vitro* to reduce their pathogenicity (for example, the measles–mumps–rubella vaccine) and cold-adapted viruses (for example, one type of influenza vaccine). Advantages of live vaccines include the generation of strong humoral, as well as cell-mediated, immune responses, the generation of immune responses of lengthy duration and the lack of requirement of an adjuvant. However, live vaccines, in theory, carry a risk of virulence, and occasionally, they have other vaccine-associated effects. For example, the use of oral polio vaccine was discontinued in the United States because of rare cases of vaccine-associated paralytic polio[144], and it was replaced by inactivated poliovirus vaccine. Another live vaccine, RotaShield (Wyeth Ayerst), was also recalled, owing to vaccine-related intussusception[145]. For a detailed review on vaccine safety, see REF. 146. Some live vaccines are also limited by practical constraints, such as difficulties in culturing (for example, hepatitis C virus[147]) and requirements for advanced storage and handling (for example, control of temperature) to maintain the pathogen.

Non-living vaccines encompass several types of vaccine: inactivated whole pathogens (for example, one type of influenza vaccine or the hepatitis A vaccine); subunit vaccines that include the epitope that is recognized by antibodies (for example, the hepatitis B vaccine); toxoids, which are deactivated pathogenic toxins (for example, the tetanus vaccine); synthetic peptides that mimic epitopes from the antigen; and DNA that encodes the antigen. Advantages of non-living vaccines include the absence of virulent pathogens, the ability to manufacture these vaccines to a high level of purity, and their stability under adverse conditions (for example, heat), which facilitates their use in field applications. Non-living vaccines, however, do not typically induce cellular immunity, which is partly because of their method of administration (by injection). They require frequent administration and larger doses than do live vaccines. Non-living vaccines require parenteral administration (except for one non-living cholera vaccine). Unlike live vaccines, which survive the low pH and the enzymatic environment of the stomach and can then infect the gastrointestinal tract and generate an immune response, non-living vaccines (especially subunit, synthetic peptide and DNA vaccines) are destroyed in the gastrointestinal tract. Strong adjuvants are typically required to counteract this vaccine loss and to make up for the lower immunogenicity of non-living vaccines.

LANGERHANS CELL
A type of dendritic cell (which are professional antigen-presenting cells) that is localized in the epidermal layer of the skin.

Timeline | **Important events in the development of needle-free methods of immunization**



Events below the arrow (red boxes) correspond to methods that have been used in commercial applications. Events above the arrow (green boxes) correspond to methods that are currently under development but that have been clinically tested. DCJI, disposable-cartridge jet injector; EMEA, European Medicines Agency; FDA, Food and Drug Administration (United States); MUNJI, multi-use-nozzle jet injector.

Liquid-jet injections were first popularized by multi-use-nozzle jet injectors (MUNJIs), which allow injection of several doses using the same nozzle and vaccine reservoir at a rate of up to 1,000 immunizations per hour. MUNJIs were successfully used for immunizing humans with live vaccines against measles and smallpox, as well as non-living vaccines against cholera, hepatitis B, influenza and polio[24]. Liquid-jet injectors offer several advantages in addition to avoiding the use of sharps. They have a long history of use, and they work with existing vaccine formulations that have been developed for needle-based administration. In one example, they resulted in higher SEROCONVERSION rates, but the reasons for this are not clear[25]. At the same time, liquid-jet injectors have several limitations. In some studies, they were associated with higher levels of pain than were needle-based injections[26], especially when using older MUNJI devices. Liquid-jet injectors have also been associated with more-frequent site reactions than have needles, such as soreness, redness and swelling of the injection site[25,27].

Perhaps the main safety issue that is associated with MUNJIs is the increased risk of subject-to-subject contamination. In the 1980s, the spread of HBV between subjects was linked to liquid-jet injections[28]. Splashes of small amounts of blood or interstitial fluid on the nozzle of the MUNJI were blamed for this spread of HBV. Systematic studies have shown that MUNJIs can transmit considerable volumes of blood (more than 10 pl) from one subject to another when the MUNJIs are used on multiple subjects. This volume of blood is presumed to be sufficient to transmit HBV infection[29]. The WHO and CDC recommend that MUNJIs should be used for mass immunization only when the gains from rapid immunization outweigh the risks of blood-borne disease: for example, during influenza pandemics or bioterrorism attacks[30]. To minimize the risk of contamination, protective devices that are disposable have been developed to cover the surface of the injector, and studies that have been carried out with these devices showed no risk of contamination[31]. Disposable-cartridge jet injectors (DCJIs; which are non-disposable injectors to which disposable nozzles are attached for each use) have also been developed to alleviate concerns about contamination. Single-use, pre-filled disposable devices are also under development to alleviate these concerns about contamination, and these devices present a new direction in liquid-jet injectors[32]. In addition to conventional vaccines, some DCJIs have also been effectively used to carry out DNA vaccination against dengue fever and influenza in animals[33–35].

Although MUNJIs are no longer used for routine immunizations, DCJIs are used for childhood immunizations at the physician's discretion. However, at present, the number of immunizations that is carried out with DCJIs is far less than the number that is carried out using needles, possibly because of the cost, the low level of awareness among health-care providers and patients, the potential pain, and the problems that were associated with earlier generations of liquid-jet injectors.

Numerous liquid-jet injectors are already on the market and are used for various vaccines, including those against influenza and hepatitis B. Newer, more convenient liquid-jet injectors are continually being developed, mainly by small companies. Although substantial technological advances have been made in

SEROCONVERSION
Development of a detectable concentration of pathogen-specific antibodies in the serum as a result of infection or immunization.

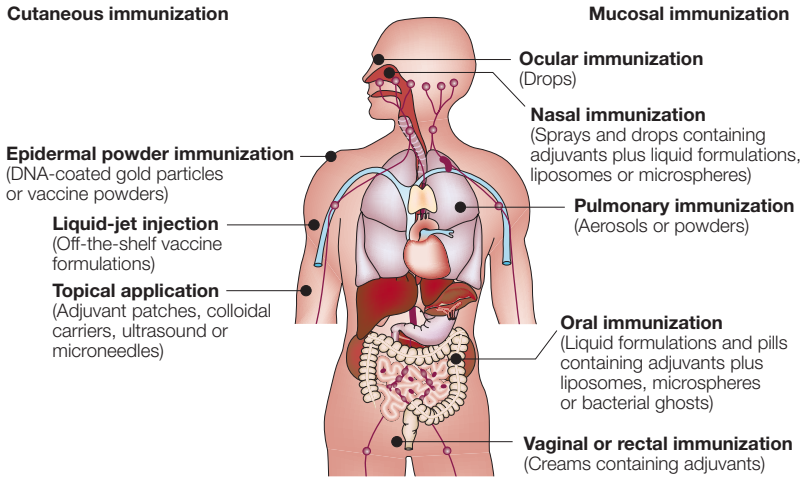


Figure 1 | **Schematic representation of various methods of needle-free immunization.** Four distinct modes of immunization are discussed in this article: liquid-jet injection, epidermal powder immunization and topical application (which are all forms of cutaneous immunization), and mucosal immunization, which is further classified into ocular, nasal, oral, pulmonary, and vaginal or rectal routes. Ocular immunization can be carried out using eye drops. Nasal immunization is carried out using sprays that comprise liquid formulations, liposomes or microspheres. Vaccines can be delivered orally in the form of liquid doses or pills, both of which can consist of various formulations: for example, microspheres. Vaccines can also be delivered to the vaginal or rectal mucosal membrane, using topical creams, or to the lungs, using aerosols or powders. Liquid-jet injection delivers vaccines to the skin, subcutaneous fat or muscles, depending on the parameters of the injection. Epidermal powder immunization delivers vaccine powders to the superficial layers of the skin. Transdermal patches also deliver vaccines to the superficial layers of the skin. Topical delivery of vaccines is facilitated by adjuvant patches, colloidal carriers, or physical methods, such as microneedles and ultrasound. For a general discussion of the issues that are associated with delivery of molecules (not just vaccines) through these routes, see the following references: ocular drug delivery[148,149], epidermal powder delivery[150], liquid-jet injection[23], transdermal drug delivery[51], nasal drug delivery[151,152], pulmonary drug delivery[153], oral drug delivery[86,154] and vaginal drug delivery[155].

the past decade, the fundamental science that underlies liquid-jet injections is not well understood. Studies on various basic aspects of liquid-jet injections, such as dynamics of jet fluid, mechanics of jet penetration and dynamics of jet dispersion, have only recently been reported in the literature[36,37]. It is hoped that this understanding, together with technological advances, will lead to better and cheaper devices.

Particle bombardment of the skin

Particle-based methods (also known as ballistic methods) accelerate powdered vaccines such that they penetrate the outer layer of the skin (that is, the stratum corneum) and are deposited in the epidermis or the superficial layers of the dermis, a method known as epidermal powder immunization (EPI)[38] (FIG. 2B). The ballistic technique was first developed in 1986, for the delivery of DNA-coated metal particles of ~1 μm in diameter into plants to genetically modify them, and it was known as the gene gun. In the early 1990s, the ballistic method was developed into devices for delivering both conventional and DNA vaccines to humans[39]. Unlike liquid-jet injectors, which routinely deliver the vaccine to the subcutaneous or intramuscular space, ballistic methods mainly deliver the vaccine to the superficial layers of the skin[39] and therefore naturally target Langerhans cells.

ADJUVANT
An agent that is mixed with an antigen and increases the immune response to that antigen following immunization.

Several vaccines have been delivered to animals using EPI. Influenza vaccine, when administered together with ADJUVANTS such as cholera toxin (CT) by EPI, elicited augmented serum and mucosal antibody responses in mice compared with those in unimmunized animals[40,41]. Similar results were reported for diphtheria toxoid (DT). Co-administration of adjuvants increased IgG titres after EPI-based immunization against diphtheria[42] and influenza[43]. EPI has also been used to deliver DNA vaccines to animals. Small (1–3 μm) DNA-coated gold or tungsten particles delivered by EPI directly penetrate epidermal keratinocytes or Langerhans cells, and they induce expression of encoded antigens[44]. Additional means to capitalize on the immunostimulatory properties of Langerhans cells — for example, co-administering DNA that encodes cytokines (such as interleukin-6) or inhibitors of apoptosis[45] — have also been adopted. Apoptosis inhibitors increase the survival of Langerhans cells after particle-induced mechanical trauma, and the expression of cytokines facilitates the migration of Langerhans cells, which is typically promoted by pro-inflammatory mediators.

There are fewer reports of EPI of humans. In one study, EPI efficiently delivered influenza vaccine to humans[46]. For all influenza-virus strains, titres of IgG were equivalent in groups in which EPI was used and in needle-immunized individuals. Clinical studies of immunization with DNA using ballistic methods have also yielded encouraging results[47–49]. EPI-based DNA immunization against infection with HBV induced high titres of protective antibody, as well as cell-mediated immune responses, in humans. Despite promising results in clinical studies, however, the commercial development of EPI for conventional vaccines seems to be stagnant. Instead, current development efforts in industry are focused entirely on DNA vaccines.

EPI offers several advantages as a mode of immunization. The use of powders simplifies handling and storage compared with liquid formulations. EPI also naturally targets Langerhans cells and allows their direct transfection. Initial safety studies of EPI seem to be satisfactory, although occasional bleeding was observed in some cases[46]. As is the case for liquid-jet injectors, fundamental studies that focus on the mechanics of particle penetration, which will be useful for understanding the mechanisms of EPI, have only recently been initiated[38,50]. These studies have documented the role in EPI of the properties of the particle (such as density and size), the operating conditions (that is, temperature, humidity and velocity) and the mechanical properties of the skin. Understanding gained from such studies might assist in the design of future EPI devices.

Topical application to the skin

The skin has been used for administering medication to treat local conditions (for example, inflammation) for thousands of years. Systemic drug delivery through the skin became prominent with the introduction of

COLLOIDAL CARRIER
A stable system of small particles of lipids, polymers or any other material that encapsulate a vaccine.

transdermal scopolamine patches for treating motion sickness, in 1979 (REF. 51). The use of the skin for the administration of vaccines has an even longer history. Immunization against smallpox was practiced in India more than 1,000 years ago by scratching dry scabs from smallpox lesions onto the skin of healthy individuals. The skin remains the site for immunization against smallpox in the modern era, using the bifurcated needle[52]. Although the skin has had a historical role in immunization, the use of topical vaccine application as a general mode of immunization has only recently (in the mid-1990s) received attention.

The simple topical application of a vaccine does not typically yield an adequate immune response, although rare cases can be found in the literature[53]. Topical delivery of vaccines into the skin is limited by the low permeability of the stratum corneum, the outer layer of skin, which is 15–20 μm in thickness and consists of cornified keratinocytes embedded in a lipid-rich matrix. The lipids of the stratum corneum are organized into an ordered bilayer structure and, consequently, form a strong barrier to molecular transport[54]. Increasing the permeability of the stratum corneum without irritating the underlying keratinocytes has been a considerable challenge in the field. Several innovative methodologies are being developed to facilitate antigen delivery into the skin. These include the use of topically applied adjuvants, COLLOIDAL CARRIERS to encapsulate vaccines, and physical methods to increase the permeability of the skin to vaccines (FIG. 2C).

Topical adjuvants. Topical application of adjuvants such as CT together with the vaccine on the skin generates a strong systemic and mucosal immune response[55–57]. This is the most studied of all topical-immunization methods. Topical application of CT provides the required activation signal for Langerhans cells to mature and become potent antigen-presenting cells that can prime the immune response to co-administered vaccines[58]. It is unclear how CT, a relatively large protein (86 kDa), diffuses across the stratum corneum, but hydration-induced permeabilization of the stratum corneum is one

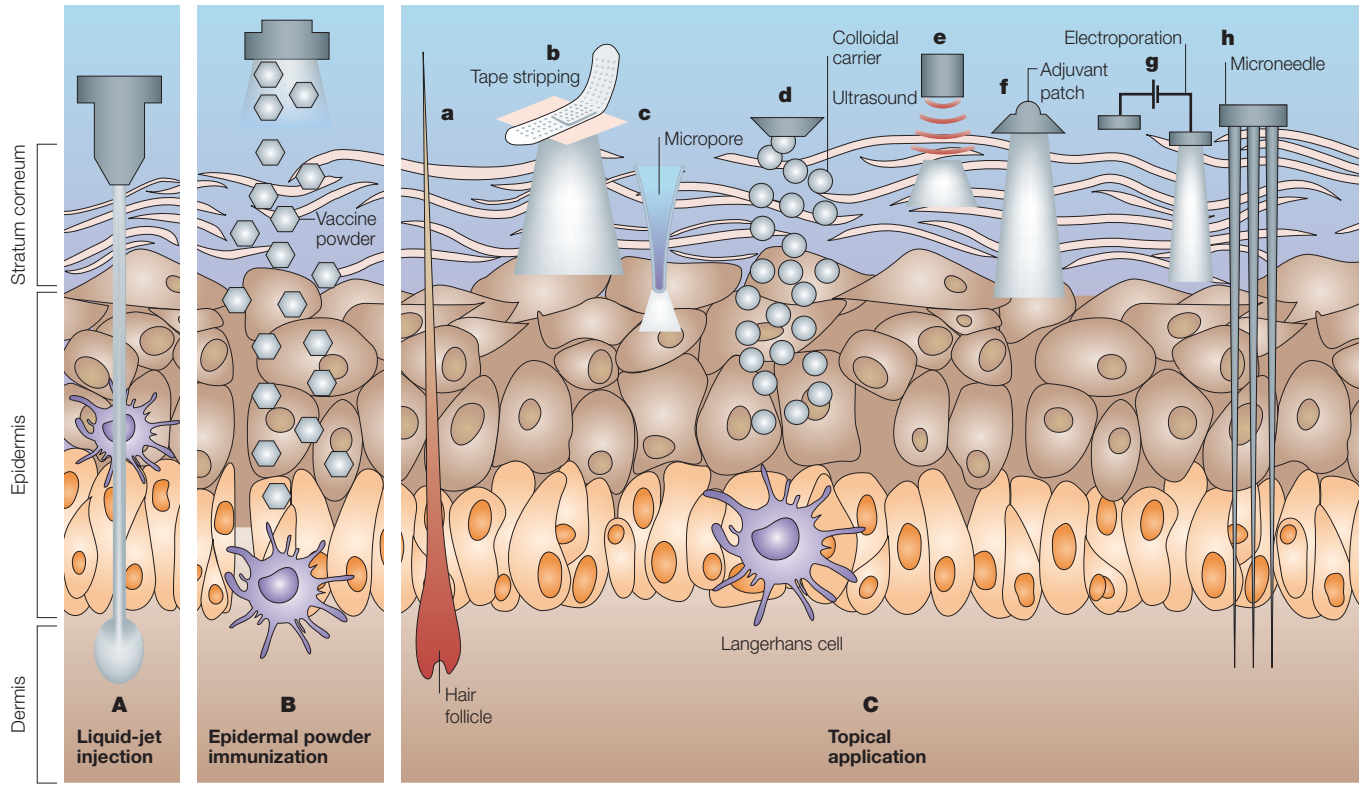


Figure 2 | **Immunization by cutaneous routes. A** | Liquid-jet injection delivers vaccine to muscular, subcutaneous or dermal regions, depending on the parameters of the injection. **B** | Epidermal powder immunization delivers vaccine powders to the superficial layers of the skin (that is, the epidermis and the superficial layers of the dermis), where they are recognized by Langerhans cells. **C** | Topical application of vaccines delivers vaccines to the epidermis, where they are recognized and processed by Langerhans cells. Immunization by topical vaccine application is facilitated by several methods. **Ca** | DNA immunization can be carried out through hair follicles. **Cb** | Tape stripping removes the stratum corneum and facilitates vaccine absorption. **Cc** | Thermal or radio-wave-mediated ablation of the stratum corneum creates micropores that increase vaccine delivery. **Cd** | Colloidal carriers such as microemulsions and transfersomes increase dermal absorption of topically applied vaccines. **Ce** | Low-frequency ultrasound is an adjuvant for topically applied vaccines, and it also increases vaccine delivery to the skin. **Cf** | Topically applied adjuvants, such as cholera toxin, can induce potent immune responses. **Cg** | Electroporation of the stratum corneum increases the delivery of DNA vaccines to the epidermis. **Ch** | Shallow microneedles that penetrate into the epidermis deliver vaccines effectively. For an overview of the issues that are associated with transdermal delivery of molecules (not just vaccines) by some of these methods, see REFS 56,72,156–158.

Table 1 | **Comparison of routes of needle-free delivery of non-living vaccines**

Method	Advantages	Limitations
Cutaneous immunization		
Liquid-jet injection	Long history of use, ability to work with existing formulations, and success with many forms of vaccine	Issues associated with cross-contamination when using MUNJIs, high cost of device, and occasional pain and bleeding
Epidermal powder immunization	Use of powders facilitates storage, strong data for DNA vaccines, and natural targeting of Langerhans cells	High cost of device, occasional bleeding, limited clinical data for non-DNA vaccines, and limited clinical history
Topical application	Ease of access, natural targeting of Langerhans cells, generation of mucosal and systemic immunity, and high patient compliance	Strong adjuvants or permeabilizing agents required, some permeabilization methods require expensive devices, and most delivery methods have limited clinical history
Mucosal immunization		
Oral	Ease of administration, high patient compliance, no complex devices necessary in most cases, primary site of infection of many pathogens, and long history of use for live attenuated pathogens	Gastrointestinal deactivation of vaccines, high doses required, variability of response, and mixed clinical data
Nasal	Easier access to mucosal membrane than for oral delivery, low cost, and one of the main sites of infection for airborne pathogens	Short contact time, enzymatic activity of nasal tissue, adjuvants required, safety concerns with earlier nasal vaccine, and limited applicability in patients with upper respiratory-tract infections
Pulmonary	Large surface area, one of the main sites of infection for airborne pathogens, and history of use for measles vaccine	Strong adjuvants required, high cost of some devices, and interference from upper respiratory-tract infections
Vaginal or rectal	High relevance for HIV and causative agents of other sexually transmitted diseases	Poor patient compliance for general applications, and strong adjuvants required

MUNJI, multi-use-nozzle jet injector.

possibility. In recent studies, however, disruption of the stratum corneum using emery paper (which is an abrasive paper) has been used before the application of vaccine and/or adjuvant to achieve immune responses[59,60]. Additional strategies, which also involve disruption of the stratum corneum, are discussed later.

Several other adjuvants that have fewer toxicity issues than CT, such as the B subunit of CT (CTB), also have adjuvant-like properties after topical administration[61]. The effectiveness of adjuvant-mediated cutaneous immunization has been shown in animals, using vaccines that include tetanus toxoid (TT)[19], DT[62] or *Bacillus anthracis* (the causative agent of anthrax)[59]. Clinical studies have also confirmed the generation of a strong IgG and IgA response in volunteers after topical application of a colonization factor from enterotoxigenic *Escherichia coli* together with adjuvants[63]. Earlier clinical studies that were carried out using *E. coli* heat-labile enterotoxin (LT) as an adjuvant showed that mucosal antibodies were generated, and these studies confirmed the role of Langerhans cells in immunization by topical vaccine application, as shown by the strong presence of these cells in the skin 24–48 hours after immunization[64].

Colloidal carriers. Encapsulation of vaccines in colloidal carriers facilitates the generation of an immune response after topical application. Few studies have reported on the use of colloidal carriers for the topical delivery of vaccines, all in animals. Topical application of TT encapsulated in lipid vesicles, after booster immunization, elicited a specific immune response (IgG) comparable to that produced by intramuscular injections of alum-adsorbed TT[65]. Another lipid-based system has been used to deliver DNA vaccines to animals[66]. Topical application of this DNA–lipid vaccine resulted in both antibody responses and cellular responses. Ethanol-in-fluorocarbon MICROEMULSION systems[67,68] and cationic nanoparticles coated with DNA vaccines have also been used for topical DNA immunization of animals[69]. The precise mechanisms by which colloidal carriers penetrate the stratum corneum remain a topic of research. Whether the results obtained from animal studies can be translated to humans also remains to be seen.

Physical methods. Physical techniques that use microneedles, tape stripping, ultrasound, microporation or electroporation have also been used to deliver vaccines across the skin. Most of these techniques, although well studied for general drug-delivery applications, have only recently emerged as potential immunization techniques. In the microporation technique, a vaporization process (which involves focused deposition of thermal energy into the skin through an electrically heated element) is used to remove small areas of the stratum corneum, thereby exposing the immunocompetent epidermis. In one study, in hairless mice, application of an adenoviral vector to microporated skin resulted in 10–100-fold greater cellular

MICROEMULSION
A stabilized emulsion (that is, a preparation of two immiscible liquids, in which one is dispersed in the other) in which the dispersed droplets are extremely small.

and humoral immune responses than did application to intact skin[70]. Microneedles (which are solid and hollow arrays of micrometre-scale silicon projections) have also been used on several occasions to carry out topical immunization with various vaccines[71–73]. Microprojection arrays have been used to deliver naked plasmid DNA, inducing stronger and less variable immune responses (as judged by serum IgG titres) than those induced by needle-based injections. They also reduced the number of immunizations that was required for full seroconversion[73]. In another study, microprojection-array patches were used to deliver a model antigen, ovalbumin, to generate a strong immune response[71]. Ovalbumin that was administered by microprojection array generated an immune response up to 50-fold greater than that observed after the same dose administered subcutaneously or intramuscularly using a needle.

A handful of studies have reported the use of tape stripping to facilitate transdermal vaccine absorption in animals[74,75]. Repeated peeling using tape (for example, Scotch tape) effectively removes the stratum corneum. Application of peptides that represent tumour-derived epitopes to tape-stripped mouse skin primed tumour-specific cytotoxic T cells in the lymph nodes and the spleen, protected mice against a subsequent challenge with the corresponding tumour cells and suppressed the growth of established tumours[76]. Skin abrasion using a razor and a toothbrush followed by application of adenoviral vectors has yielded promising results in humans[77].

Ultrasound, at low frequency (20 kHz), has also been shown to deliver a vaccine (consisting of TT) to mice in one study[78]. The immune response that was generated by ultrasonically delivered vaccine was about tenfold greater per unit dose of vaccine that entered the skin than occurred after subcutaneous injection. (About 1% of the topically applied dose entered the skin[78].) Compared with simple topical administration, pretreatment with ultrasound was shown to increase vaccine delivery, thereby allowing enough vaccine to enter the skin to activate the immune response. Ultrasound has been shown to increase skin permeability through disruption of the stratum corneum by acoustic cavitation (which involves the formation and collapse of gaseous cavities)[79]. Furthermore, application of ultrasound resulted in activation of Langerhans cells, the reasons for which are not clear. In another study, electroporation (which involves the application of high-voltage, short-duration electric pulses) has been used to increase the delivery of DNA vaccines across the skin of mice[80]. Electroporation has also been shown to induce an effective immune response after transdermal delivery of peptide vaccines[81]. Additional approaches, including the use of laser-assisted permeabilization of the stratum corneum and various designs of microneedles (other than those published in peer-reviewed literature), are also being pursued by industry. Most of the physical methods for topical immunization have only been tested on animals, specifically on mice or rats. It remains to be seen how many of these methods can be applied to human skin, which differs substantially in barrier properties from rodent skin. Some of the methods that are discussed in this section are purported to have been tested on humans for immunization purposes; however, these studies have not yet appeared in peer-reviewed literature.

Topical vaccine application (including the use of topical adjuvants, colloidal carriers and physical disruption) offers several advantages. Administration to the skin is generally easy to carry out and leads to high compliance of patients[82]. Topical vaccine application also naturally targets Langerhans cells. However, cost issues must be investigated in depth before these techniques can be adopted for wide-scale applications in humans. Methods that are based on physical techniques such as ultrasound, electroporation and microporation use expensive devices, which might pose constraints on their adoption by developing countries. The use of electric power might limit the widespread use of some of these methods, especially for field applications. Several companies, mostly small-scale businesses, are working to address these challenges.

Mucosal administration

Mucosal routes (especially oral and nasal routes) have been used for delivering medication for millennia, predating needles and syringes. Several centuries ago, nasal administration of dried scabs of smallpox lesions and oral administration of fleas from cows with cowpox were practiced in China as a means of immunization against smallpox. It was the Sabin oral polio vaccine, however, that brought mucosal immunization to prominence, in the early 1960s, and that had an important role in the programme for global eradication of polio. Since then, several mucosal vaccines have been marketed (TIMELINE). Because many pathogens — for example, HIV and influenza virus — enter the body through mucosal tissues, the development of vaccines that offer mucosal immunity has received considerable attention in the past 20 years[12,83].

Oral route. Oral delivery of vaccines is an attractive mode of immunization because of its acceptability and its ease of administration[84]. Orally delivered vaccines, especially particulates, are recognized by MICROFOLD (M) CELLS (which sample antigen) in the PEYER'S PATCHES of the intestine and by dendritic cells that reside there[12] (FIG. 3). At present, few vaccines (those against polio, typhoid fever and cholera) are administered orally, and most of these are based on live attenuated pathogens (TIMELINE). Oral delivery of non-living vaccines has proved to be extremely challenging, owing to poor stability of proteins, peptides and DNA in the acidic and enzyme-rich environment of the gastrointestinal tract[85]. Several strategies, including the use of biodegradable polymeric particles and LIPOSOMES, have been adopted to protect the antigens in the gastrointestinal tract[86,87]. In addition, strong adjuvants — for example, bacterial enterotoxins such as CT and LT — have also been successfully used for the oral immunization of animals[88]. However, toxicity of these enterotoxins limits their

MICROFOLD CELL
(M cell). A specialized type of epithelial cell that delivers antigens from the lumen directly to intraepithelial lymphocytes and to subepithelial lymphoid tissues, using transepithelial vesicular transport.

PEYER'S PATCH
A section of the intestinal epithelium that contains microfold cells. These regions form the mucosa-associated lymphoid tissue.

LIPOSOME
A lipid vesicle that encapsulates vaccines in a lipid-bilayer membrane and facilitates their delivery.

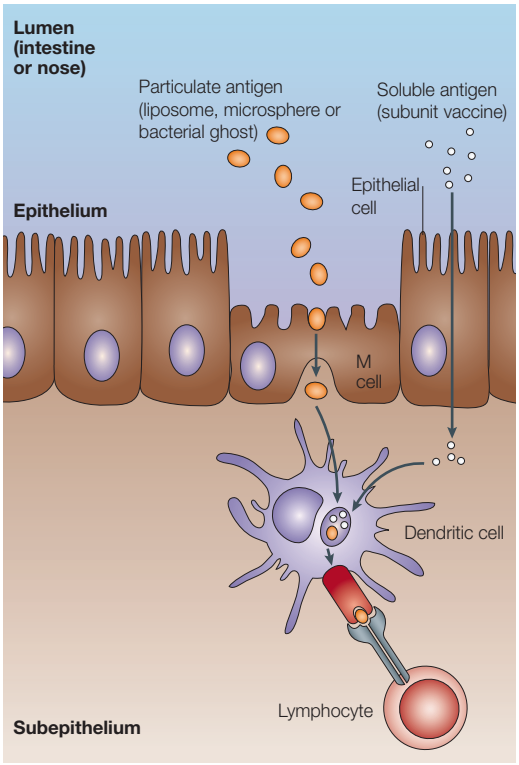


Figure 3 | **Immunization by mucosal routes.** Vaccines that are delivered by mucosal routes (that is, ocular, oral, nasal, pulmonary, vaginal or rectal routes) are recognized by microfold (M) cells and dendritic cells in the mucosa-associated lymphoid tissue. Particulate antigens (such as microspheres, liposomes and bacterial ghosts) are recognized by receptors at the surface of M cells and are presented to lymphocytes and macrophages. Soluble antigens, as well as small pathogens, can permeate the epithelium and be recognized by dendritic cells.

applications in humans[89]. To alleviate the toxicity issues, mutants and subunits of LT and CT have been used as adjuvants in many studies of oral immunization of animals[90]. A detailed review of mucosal adjuvants can be found in REFS 88,89.

Encapsulation of antigens in biodegradable polymer microspheres (especially poly(lactide-co-glycolide), PLG) has been successfully used for oral immunization of animals against HBV[91], TT[92] and other antigens[86,93]. Several other materials have also been used to encapsulate antigens, but a clear advantage of any particular material is not obvious[94–96]. In addition to protecting antigens from the hostile gastrointestinal environment, microspheres have been suggested to aid immunization through the sustained release of antigens, which might overcome the need for booster doses, which are typically required for vaccines that are administered by intramuscular injection[97]. Additional mechanisms, such as direct intracellular delivery of antigens through phagocytosis of particles, have also been proposed to explain the adjuvant activity of polymeric microspheres[98]. Oral delivery of DNA vaccines that have been encapsulated in PLG or chitosan microspheres has also received considerable attention[99–101]. Several strategies that involve the use of antibodies, IgA or lectins have also been proposed to target microspheres to M cells[102].

Liposomes offer an alternative means of protecting a vaccine[103–105]. Conventional liposomes are not particularly stable in the gastric environment, so polymerized liposomes have been developed as carriers for oral vaccines[106]. Modification of liposome composition using lipids from archaebacteria has been attempted to facilitate vaccine uptake by antigen-presenting cells[107,108]. Both nanoscale lipid particles that consist of lipids, adjuvants and antigens, which are known as immunostimulating complexes (ISCOMs)[107], and BACTERIAL GHOSTS[109], which are bacteria that lack their cytoplasmic contents, have also been successfully used as vaccine carriers in animals. Bacterial ghosts, the surface properties of which resemble those of live bacteria, are highly immunogenic and are therefore strong adjuvants.

Despite considerable effort, oral immunization with encapsulated antigens is still limited by several issues that are specific to this route. The effectiveness of oral immunization has been established in several animal studies (mostly in mice), but clinical experience in this field has been mixed[105,110–112]. Specifically, serum antibody titres after oral delivery of liposome-encapsulated TT or DT to humans were variable and were lower than those observed for animals[105]. In another clinical study, oral immunization against enterotoxigenic *E. coli* using a microsphere-encapsulated colonization-factor antigen rendered protection against subsequent challenge in only 30% of patients[110]. In a more recent study, oral delivery of PLG-encapsulated CS6 antigen from *E. coli* generated antigen-secreting cells and an IgA response, but the differences between responses that were generated by encapsulated and non-encapsulated antigen were not great[112]. Attempting to scale up the results of oral immunization from animals to humans is generally problematic. Typically, the doses that are required to elicit an immune response through the oral route are substantially higher (by up to 100-fold) than those that are required when using injection[113]. This raises the crucial issue of the cost of immunization. Furthermore, oral immunization with non-living vaccines requires the use of carriers and adjuvants, and the safety of exposing the sensitive gastrointestinal tract to these compounds, in addition to the safety of exposure to the vaccine itself, remains to be carefully studied. A completely different solution to this issue has been offered by the use of genetically engineered plants as immunizing agents. This approach has yielded encouraging results in animals[114] and humans[115,116], but the safety of transgenic-plant vaccines needs to be further evaluated.

Nasal route. Intranasal delivery of vaccines using a nasal spray delivered into the nostrils is an attractive mode of immunization. The nose, similar to the mouth, is a practical site for vaccine administration, and nasopharynx-associated lymphoid tissue efficiently induces antigen-specific immune responses in

BACTERIAL GHOST
A bacterium, the cytoplasmic contents of which have been removed.

both mucosal and systemic immune compartments[117,118]. A detailed review of nasal immunization can be found in REFS 118,119. FluMist (MedImmune Vaccines, Inc.), a live influenza-virus vaccine, has already been approved by the Food and Drug Administration (United States) for intranasal administration. The development of non-living nasal vaccines has proved to be challenging, but considerable progress has been made in the past decade[120]. One nasal vaccine, an inactivated influenza-virus product known as Nasalflu (Berna Biotech AG), was introduced in Switzerland in 2000. However, it was withdrawn from the market in 2001 because of the vaccine-associated incidence of Bell's palsy, which was thought to originate from the use of *E. coli* LT as an adjuvant[121]. Efforts to develop other nasal vaccines, however, have continued to make progress. In the past decade, several clinical studies have confirmed the generation of local and systemic immunity after nasal immunization of humans against diphtheria and tetanus[122], influenza[123] and infection with *Streptococcus mutans*[124]. Varying local reactions, ranging from good tolerance to stinging, were reported in response to intranasal administration of vaccine[122]. A far larger number of studies in mice, pigs and monkeys also confirm the effectiveness of nasal immunization with a variety of vaccines[119]. Nasal vaccines have been delivered in various physical forms, including aerosolized liquids[125], and liposome[126] and microsphere formulations[127], which can be administered together with various adjuvants[88,128,129]. Nasal immunization generally requires much lower antigen doses than does oral immunization, owing to lower enzymatic activity in the nasal cavity than in the gastrointestinal tract.

Vaginal or rectal route. Vaginal and rectal immunization through topical application of a cream has recently received attention for immunization against sexually transmitted diseases such as HIV/AIDS[130]. Vaginal immunization with a multicomponent peptide vaccine against HIV infection has been shown to induce local antibody responses in mice when administered together with strong adjuvants such as CTB[131,132]. DNA-vaccine strategies for preventing HIV infection using vaginal or rectal immunization have also been tested and have been shown to be effective at generating systemic and mucosal immune responses in mice[133]. Clinical experiments involving vaginal and rectal immunization against cholera, using a vaccine containing both whole cells and CTB, have yielded successful results[134]. In general, however, vaginal and rectal immunization with non-living vaccines has had limited success, owing to delivery and adjuvanticity issues.

Other routes. Additional mucosal routes, including pulmonary, ocular and sublingual administration of vaccines, have also been attempted in several cases. Aerosolized vaccines have been delivered through the pulmonary route, which aims to deliver vaccine at various levels of the bronchial tree, including the alveoli. Pulmonary delivery of vaccines, which targets bronchus-associated lymphoid tissue, has been effectively used for immunization of humans against measles, using a live attenuated virus[135]. Animal studies have also shown the effectiveness of non-living pulmonary vaccines, including inactivated influenza virus[136].

Ocular immunization has been attempted against infection with herpes simplex virus (HSV). This was motivated by the strong need to generate ocular mucosal immunity to HSV, which commonly infects the eye in addition to other sites. Heat-killed HSV, as well as HSV-based subunit vaccines, generated effective mucosal immunity to HSV in pre-clinical animal studies that involved direct administration to the eye in the form of drops[137,138]. The effectiveness of sublingual immunization has also been shown in several studies in animals[139,140]. However, ocular and sublingual routes have been less well studied as generalized methods for immunization than have other mucosal routes.

A large body of literature has confirmed the merits of all modes of mucosal immunization. Products for mucosal immunization, especially nasal immunization, are under development in several companies. Immunization through mucosal routes (oral, ocular, pulmonary, nasal, vaginal or rectal) generates IgA-producing cells at the site of infection[129]. However, it should be noted that mucosal administration of antigen is not essential for the generation of antigen-specific IgA-producing plasma cells. Topical application of vaccine has also been shown to generate these cells and to induce mucosal immunity in the host[19]. Among the methods of mucosal immunization, nasal immunization seems to offer an optimal balance of immunogenicity, dosing and accessibility, as well as patient acceptability. Other mucosal routes are limited as generic modes of immunization. The oral route is limited by the difficulty in accessing M cells in the gastrointestinal tract and by dosing issues. The vaginal and rectal routes are limited by acceptability and immunogenicity issues. The issues of dosing and immunogenicity could be addressed through future research focused on developing strategies for better encapsulation, adjuvanticity and targeting. Fundamental studies focused on the transport of antigens or antigen carriers from the point of administration to the mucosal membrane will also bring new insights to mucosal immunization. These studies should be complemented by research focused on gaining a better understanding of the immunology of mucosal membranes, in particular by identification of specific target cells and receptors for vaccines and by study of the crosstalk between different mucosal compartments[141].

Conclusions

The shortcomings of injections have led to active research and development of needle-free methods of immunization. The shift from needle-based to needle-free immunization is also catalysed, in part, by the realization that the skin and the mucosal membranes, which cannot be effectively accessed by conventional

needles, are ideal targets for vaccine delivery[12,142]. Until recently, needle-free methods of immunization were restricted to liquid-jet injection and oral delivery of live attenuated pathogens. Considerable advances have been made in the past decade, especially in transdermal and nasal immunization, but it should be noted that most of the technologies that are discussed here are still at an early stage and lack detailed evaluation in terms of safety, toxicity, reproducibility and economic feasibility.

Cost of immunization is an important factor in the acceptability of new methods. According to the WHO, the current cost of administering three doses of the diphtheria–tetanus–pertussis vaccine is US$4–70 per child, depending on the country. Use of needle-free immunization might push this cost even higher, owing to the increased cost of development. The potential higher cost of needle-free immunization needs to be viewed in light of its benefits. Care needs to be taken when carrying out cost–benefit analysis of needle-free methods, because several benefits of needle-free methods are difficult to quantify. It is hoped that needle-free methods will lower the economic burden that is associated with needle-borne infections[143] and will eventually prove to be economically feasible.

A comparison of the advantages and limitations of various methods of needle-free immunization (TABLE 1) makes it evident that there is no one method that is superior. Each method has advantages that are attractive for immunization. At the same time, all methods have limitations that need to be overcome. Each method is eventually likely to find its niche application, which will depend on the type of vaccine and the site of immunization, and on intellectual-property considerations. Opportunities in needle-free immunization have attracted an array of interdisciplinary researchers and businesses to the field of vaccine development. With the influx of new technologies and talent to this field, needle-free immunization is sure to become a reality.

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Competing interests statement

The author declares competing financial interests: see web version for details

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