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68 Technologies to Improve Immunization

Mark J. Papania, Darin Zehrung, and Courtney Jarrahian

NEW TECHNOLOGIES NEEDED TO REDUCE IMMUNIZATION LOGISTICS HURDLES

Immunization can be described as the process of delivering carefully packaged antigen to the appropriate destination in a vaccine recipient to produce a desired immune response. In this sense, immunization programs are package delivery systems: they manage the flow of antigens, formulated in vaccines and packaged in different presentations, between the point of origin at the vaccine manufacturer and the point of consumption, inside antigen-presenting cells (APCs) of the vaccinee. This simple concept of delivering antigen packages from point to point can help elucidate the complex logistical challenges inherent in the preservation, packaging, storage, transportation, and administration of vaccines.

Immunization is one of the most powerful tools for health, but many current vaccines are not affordable, accessible, and acceptable to everyone who needs them. Continual sharpening of this public health tool is needed to achieve the full potential of immunization for improving health. Some advances will come in the form of better vaccine antigens; however, significant potential also lies in improving the way vaccines are packaged and delivered. Reviewing immunization as a package delivery process and recognizing critical hurdles, bottlenecks, and barriers to vaccine flow is a first step toward making immunization programs more efficient and effective. Managing vaccine flow around those obstacles is the day-to-day work of immunization programs, which often requires heroic effort. This chapter briefly describes key restrictions to vaccine flow logistics in terms of complexity, cost, human resources requirements, distributability, and sources of errors in the immunization process. It reviews new technologies in various stages of development that have the potential to eliminate or reduce restrictions to vaccine flow. These new technologies have the potential to increase the capacity and efficiency of immunization programs and make immunization safer and more effective, affordable, accessible, and acceptable for everyone.

POINT-TO-POINT ANTIGEN PACKAGE DELIVERY

The four key points in the antigen package delivery system are the point of origin, storage and distribution point, the point of care (POC), and the final destination point in the recipient. The points of origin are the dozens of vaccine manufacturing plants where antigens are produced and, along with other components, formulated into vaccines, and where the vaccines are further packaged in multiple layers of containers for storage and distribution. The distribution and storage points form the network of thousands of sites from vaccine plants through national, regional, and local centers tasked with safely storing and transporting vaccines to the POCs. The POCs are the millions of hospitals, clinics, health posts, and homes where a competent vaccinator, an informed and willing vaccinee, and a safe and effective vaccine can be brought together for vaccine administration. The final destination points for antigen package delivery are the APCs inside the IM, subcutaneous (SC), cutaneous, or mucosal tissues of the billions of people who will benefit from immunization. Each of the four points along the package delivery system has distinct logistical challenges.

Points of Origin: Vaccine Manufacturing Plants

Purity and Stability

The antigen package delivery system originates in the vaccine manufacturing plant. The formulation, manufacture, and packaging of vaccines are well described in Chapter 5; this chapter highlights key factors relevant to immunization logistics. Vaccine manufacturing plants are highly capitalized, multimillion-dollar facilities that use sophisticated technology to mass-produce the billions of doses of vaccines used globally each year. Two critical vaccine flow logistics requirements start in the plant and span the entire immunization process up to vaccination of the individual: (a) maintenance of vaccine purity and (b) maintenance of vaccine potency by keeping it within the prescribed temperature range. These impose significant restrictions on vaccine flow logistics.

To maintain purity, the vaccine plant environment is highly regulated and monitored: people, equipment, and materials are introduced into the facilities in a precisely controlled manner. Expensive, high-speed filling equipment enables sterile packaging of bulk vaccine into specific-dose packages at low costs. Each step is carefully orchestrated, and even minor modifications to procedures or material may require approval from national regulatory authorities. Any change to the process implies regulatory consequences with significant associated economic costs (see Chapter 5).

For almost all currently licensed vaccines, the required storage conditions include constant maintenance at 2 °C to 8 °C (or −20 °C for several vaccines). This requirement, which is met by the system known as the cold chain, constricts the flow of vaccine to facilities and delivery mechanisms that have refrigeration or cold pack storage capabilities. Some vaccines, especially those that include aluminum adjuvants, are susceptible to damage from freezing temperatures. Others, especially live attenuated vaccines, are more susceptible to loss of potency at elevated temperatures. Through the addition of stabilizers and, in some cases, the additional step of lyophilization (freeze drying), vaccines maintain minimum potency under the required storage conditions, throughout the listed shelf life. When breaks in the cold chain are detected, valuable vaccines are discarded. When undetected, cold chain failures can result in administration of ineffective vaccine.

Presentation

Vaccine presentation affects flow logistics. In contrast to many pharmaceuticals, all currently licensed vaccines are administered as liquids—by needle and syringe injection, oral delivery, or nasal spray. Most vaccines are formulated, packaged, and shipped as liquids in single- or multidose vials or prefilled syringes. All others are first formulated as liquids, packaged in vials, and then lyophilized to enhance their stability. Although they are shipped in a dry format, lyophilized vaccines must be accompanied by a liquid diluent and reconstituted before on-site filling of the administration device and vaccination.

Liquid diluents increase the volume and weight of the vaccine, which may increase shipping costs and require more cold chain storage capacity. There is also the risk of spilling and leakage. Thus, the lyophilized format of vaccine with liquid diluent can slow down efficient vaccine flow.

There are three basic vaccine presentation schemes: prefilled delivery devices, liquid vaccine in vials or ampules, and lyophilized vaccine in vials. Prefilled delivery devices simplify the logistics at the POCs because they minimize on-site preparation. They also reduce the number of components to be shipped; eliminate the need for a separate supply chain for vaccine, diluent, and administration devices; and reduce filling overage required for vials and ampules. However, packaging vaccines in prefilled delivery devices is expensive, and the larger volumes of some devices increase the space needed in cold chain storage.

Liquid vaccines packaged and shipped in vials or ampules typically cost less per dose and occupy less cold chain space than prefilled vaccine presentations, but they require filling of the administration device at the POCs. This increases delivery complexity and creates an opportunity for errors, such as withdrawing the wrong dose amount from the vial or contamination of the vaccine. Vaccines in vials can be in single- or multidose format. Multidose vials typically cost less per dose and occupy less cold chain space than single-dose vials; however, vaccine wastage may be increased with multidose vials if a vial is opened when there are fewer people needing vaccine than the doses in the vial. Vaccinators also may be reluctant to open a 10-dose vial if only one or two people need vaccine, leading to missed opportunities to vaccinate those people. Repeated entry into a multidose vial and time lapse between vaccine withdrawals increase the risk of bacterial or fungal overgrowth, with subsequent injection of a contaminated vaccine. Single-dose vials avoid some of these problems but cost more per dose, occupy more cold chain space, and still require on-site filling of the delivery device.

Lyophilized vaccines can be packaged in single- or multidose vials, and they share the same problems as liquid vaccines in vials. The reconstitution step needed for lyophilized vaccines adds further complexity to delivery, creating the opportunity for errors such as use of a wrong amount of diluent or the wrong diluent. Other challenges with reconstitution include the potential for mismatched amounts of dry vaccine and diluent—which are often shipped and stored separately, with vaccine in the cold chain and diluent at ambient temperature—and a temperature difference between the diluent and the dried vaccine, which may render some vaccines ineffective upon reconstitution.

Inability to overcome the challenges presented by multidose liquid vial presentation and by lyophilized presentations can result in failure to immunize a vaccinee; cross-contamination of infectious pathogens among persons receiving vaccinations; and adverse reactions, including local abscesses, toxic shock syndrome, or even death.[1–9] Although rare, these errors can have grave consequences and may significantly undermine public confidence in vaccines. A recent example of a reconstitution error occurred in Syria in 2014, when an anesthetic agent was mistakenly used for a vaccine diluent, resulting in 15 infant deaths and many hospitalizations.[10]

Distribution and Storage Points

Complex networks of storage and shipping facilities manage the distribution of vaccines from dozens of manufacturers to millions of POCs. The challenges associated with timely ordering, purchase, inventory, and monitoring are staggering and result in ongoing attempts to improve immunization programs and develop software to assist with the logistics.

Vaccine distributability, a key logistics concept, is how easily a vaccine can be transported to POCs and administered to vaccine recipients. Cold chain requirements at every level of storage and distribution can significantly reduce vaccine distributability. In general, the complexity and difficulty associated with cold chain management increase with distance from the manufacturer. It is relatively easy to keep large quantities of vaccine refrigerated and monitored in cold rooms at vaccine plants or large national or regional storage facilities, and economies of scale reduce the per dose cost of storage. However, maintaining the refrigerated storage with around-the-clock monitoring and backup energy sources for the smaller quantities of vaccine present at each district and at the local level can be a daunting task.[11]

Package shipping must avoid delays that could allow vaccine temperatures to rise. Transporting vaccines at cold temperatures at the end of the cold chain, where transport is often by unrefrigerated vehicles, requires insulated boxes with cold packs, which significantly increase weight, expense, and difficulty.

Vaccination by needle and syringe injection requires highly skilled staff, which combined with limited staff supply can restrict distributability. Self-administered vaccines could significantly increase distributability, but only oral typhoid vaccine has been approved for self-administration.[12] Thermostable[13] self-administered vaccines distributed through regular mail systems and self-administered are a conceptual example of ideal distributability.

Points of Care: Vaccine Administration Settings

The POCs are the settings where three essential components meet: vaccine, vaccinator, and vaccinee. Ideally, a caring and competent vaccinator administers a safe and effective vaccine to an informed and willing vaccinee. While this chapter focuses on the logistics of getting vaccines safely to the POCs and technologies for vaccine administration, getting vaccinators and vaccinees to the POCs is equally important. As the complexity of the vaccination process increases, the skill level required of the vaccinator increases as well: safe injection requires highly trained staff, while oral vaccine delivery can be performed by minimally trained volunteers. Many pharmaceuticals and other treatments can be self-administered, but oral typhoid is currently the only vaccine approved for self-administration. Self-vaccination—enabling the vaccinee to be the vaccinator—could overcome the substantial logistical bottleneck resulting from skilled vaccinator shortages.

Getting potential vaccinees to the POCs can also be difficult, so these places need to be as close and convenient to everyone who needs vaccines as feasible. The vaccinee's home may be the most convenient POC, and many mass immunization campaigns in low-resource settings are conducted house-to-house to achieve maximum coverage. However, logistical challenges increase with rising numbers of POCs and with distance from distribution points. Vaccine hesitancy resulting from lack of confidence in vaccines or the health system, complacency about the risk of vaccine-preventable disease, or inconvenience of immunization can be another limiting factor in getting potential vaccinees to the POC. This is a multifaceted problem that must be addressed at many levels.[14] The pain associated with injection and needle phobia is one issue that may cause people not to seek vaccination. Many adults and children suffer from needle phobia.[15–17] Needle-free vaccine delivery technologies may increase the acceptability of vaccination.[12]

Vaccination, vaccine delivery, and vaccine administration are all terms that may refer to the act of transporting a vaccine across the skin of the vaccinee into the cutaneous, SC, or IM

tissue, or into an orifice to contact mucosal tissue. The vaccine delivery systems used for administration, such as injection by needle and syringe, jet injectors, or microarrays, may be considered *macrodelivery* systems. In contrast, vaccine *microdelivery* systems are the molecular antigen packaging technologies, such as viral vectors, microparticles, or virus-like particles (VLPs), that help transport the antigen to APCs once it has been administered to the vaccinee. Currently, most vaccines are administered by needle and syringe injection into the IM, SC, or intradermal (ID) tissues. Other vaccines are delivered mucosally by the oral or intranasal (IN) routes. Vaccine administration methods and devices are critical aspects of immunization logistics and many of the new immunization technologies described in this chapter focus on vaccine macrodelivery systems.

Current Vaccination Methods: Description and Logistical Hurdles

Vaccination by Injection. Hypodermic injection with a needle and syringe dates back to the mid-19th century and is such a predominant vaccine administration method that "shots" or "jabs" are often considered synonymous with vaccinations. Mass production of needles and syringes results in extremely low costs for these devices. Injection breaches the skin's stratum corneum, the protective layers of dead keratinized epithelial cells, to deposit vaccine in direct contact with the underlying dermal, SC, or muscle tissues. IM and SC delivery of vaccines by needle and syringe provides highly consistent dosing. For ID injection, because the dermal layer is so thin, precise targeted deposition is more difficult, so ID needle injection may provide less consistent delivery to the targeted tissues. Overall, needle and syringe vaccination produces excellent immune responses and is extremely safe when proper procedures are followed by trained vaccinators.

However, significant logistical limitations are inherent in needle and syringe injection. The high level of skill required for safe injections limits the availability of vaccinators. Reuse of contaminated syringes and needles is common in some developing countries and can lead to transmission of bloodborne diseases such as hepatitis and HIV.[18–22] Inexpensive autodisabling syringes and needles can prevent reuse and mitigate this problem. However, while autodisabling devices are used in many developing countries, risk of needlestick injuries to healthcare workers remains a concern and increases healthcare costs.[23,24] Safety syringes which include needlestick protection features, described later in this chapter, can reduce the risk of needlestick injuries. Finally, the cost and complexity of safe disposal of sharps in the medical waste stream represent major logistical challenges.

To overcome many of the difficulties of needle and syringe injection, multiuse nozzle jet injectors (MUNJIs) were commonly used in the past for IM and SC injection. However, repeated use of the jet injection nozzles without cleaning between patients was shown to have a risk of transmission of bloodborne pathogens, and use of these devices was discontinued.[25] Disposable syringe jet injectors (DSJIs) eliminated this contamination problem and are discussed in detail later in this chapter.

Mucosal Vaccination. In addition to vaccination by injection, current macrodelivery methods include mucosal vaccination via oral ingestion or nasal spray delivery. Both methods share the advantages of being needle free and present the possibility for simple administration methods. Mucosal vaccination mimics the route of entry, through portals to mucosal tissues, of many infectious agents and typically provides higher levels of mucosal immunity. The major challenge of mucosal vaccination is that delivery of antigen to the target tissues can be much less consistent than IM or SC vaccination by injection. Mucosal vaccination deposits vaccine on internal surfaces of the body. Although the vaccine is inside the body, it still must evade a variety of defense mechanisms to penetrate the mucosal surface and contact target tissues. Mucus flow, gastric acid, mucosal antibodies, and other antimicrobial substances continually destroy or remove substances on mucosal surfaces. As a result, mucosal vaccination generally requires higher doses of antigen, macrodelivery technologies to assure tissue contact, and more specialized antigen microdelivery packaging to reach APCs consistently.

Destination Points: Inside the Vaccinee's Antigen-Presenting Cells

Vaccination delivers the antigen package into the person receiving the vaccine, but delivery is not complete until APCs internalize the antigen. Immunization involves mimicry: vaccines must never be pathogenic, but must cause the APCs to respond to antigens as pathogens. One of the first principles of this mimicry is that the antigen package should be "pathogen-sized." Free antigen in solution is typically ignored by immune cells; however, APCs readily take up microparticle packages that are in the size range of pathogens, such as viruses and bacteria, by phagocytosis and endocytosis. These microparticle antigen packages are likely to be assessed as threatening and to initiate an immune response. Molecular antigen packages may include adjuvants, which are nonantigen components designed to trigger or modify an immune response. Some adjuvants act as package "warning labels" to alert activation of the innate immune system. Other adjuvants create a depot effect, which causes the antigen package to be opened gradually, prolonging the presence of antigen at the delivery site. Another molecular antigen packaging strategy is inclusion of specific cellular address labels. Typically, vaccine macrodelivery systems place the molecular antigen package in or near the tissues where APCs reside or traverse. For some vaccines, using molecules that match receptors on APCs such as dendritic cells or M cells increases the likelihood of delivery specifically to these cells. Many molecular packaging strategies for antigen delivery that are in use or in development are described in detail in Chapters 64 and Chapter 65, and examples are described briefly later in this chapter. Once the APCs have received the antigen package, the next step is processing of antigen and presentation to lymphocytes to initiate the immune response, which is well described in Chapter 2.

Logistical Hurdles in Special Settings

Emergency Settings

Preparation for emergencies should be included in every national immunization program plan. National programs in developed and developing countries have had varying degrees of success achieving high routine vaccine coverage rates, and strengthening routine systems is a key step for emergency immunization preparedness. Special situations and high-risk populations increase immunization logistical challenges and create the need for rapid delivery of vaccines to vastly increased numbers of people. War and other armed conflicts interrupt routine vaccination services and displace large populations away from available services, often concentrating people in refugee settings that significantly increase the risk of disease and the need for vaccines. Natural disasters such as earthquakes, tsunamis, or hurricanes disrupt local infrastructure, including transportation, electricity needed to maintain the

cold chain, and vaccine supply. Emergency situations expand the demand for skilled vaccinators and often the supply is diminished as vaccinators are personally affected by the emergency or pulled to other emergency duties. Reducing logistical barriers to routine vaccine delivery is critical to assuring the barriers are not insurmountable in an emergency situation.

Low- and Middle-Income Countries

All low- and middle-income countries (LMICs) have national immunization programs, and most provide vaccines recommended by the World Health Organization (WHO) for the following diseases: diphtheria, hepatitis B, *Haemophilus influenzae* type b (Hib), measles, pertussis, poliovirus, tetanus, and tuberculosis.[26] For routine immunization, vaccines can be administered at health posts or clinics (fixed post), or in communities through mobile outreach on a daily, weekly, or monthly schedule. Many countries also provide vaccines against pneumococcal disease, rotavirus, and other pathogens.[26] Supplemental immunization activities include mass campaigns that can be fixed post (often including extra posts), mobile outreach, or house-to-house campaigns. Three critical immunization delivery hurdles in LMICs are the cold chain requirement, the need for skilled vaccinators, and sharps safety.

The 2°C to 8°C cold chain storage requirement is especially challenging in countries with high ambient temperatures or with unreliable access to electricity.[27,28] Keeping vaccines tethered to refrigeration limits the capacity to distribute them to everyone in need because transporting them to remote locations requires cold packaging equipment many times larger and heavier than the vaccine itself. In a cold chain study in Vietnam, 46% of community health centers assessed did not have refrigeration.[11] This limits vaccination to days when vaccine can be transported from district facilities in cold boxes to the health centers for immediate use.

In many countries, cold chain equipment is antiquated and storage capacity is stretched to the limit, restricting the implementation of new vaccinations and eliminating surge capacity for mass-vaccination storage in case of an epidemic. For many immunization programs, purchase and maintenance of cold chain equipment consumes a significant portion of the immunization budget. One study estimated that cold chain equipment and overhead costs would account for 23% of the $25.4 billion needed for non-vaccine immunization program costs for low- and lower-middle-income countries from 2011 to 2020.[29]

Some vaccines may have thermostability that allows them to be managed outside the cold chain for limited periods of time. For example, a major reason for the successful MenAfriVac campaigns in Africa was the relaxation of the cold chain requirements in a number of countries for the last miles of delivery.[30,31] In these countries, a controlled temperature chain was implemented, allowing the vaccine to be shipped and stored at ambient temperatures for up to 4 days. In addition to increasing access to difficult-to-reach areas, a modeling study estimated that use of the controlled temperature chain could potentially reduce cold chain and associated logistics costs.[29] Additional studies based on field implementation are required. Research on the thermostability of other vaccines may enable their use under a controlled temperature chain to simplify logistics for the last difficult miles of delivery, improve vaccine access in remote areas, and potentially reduce costs.

The second hurdle for immunization programs in many LMICs—and often the rate-limiting factor—is the shortage of skilled vaccinators. There is a global shortage of all healthcare workers,[32] and safe and effective vaccination requires highly skilled workers for many vaccines, particularly for needle and syringe injection. Needle-free vaccines that could be administered by minimally trained staff or volunteers, or that could be self-administered, would significantly increase vaccine delivery capacity.

The third important logistics factor for immunizations in LMICs is sharps safety. Needlestick injuries are common; however, unlike in high-income countries, needlestick protection devices and prophylaxis to prevent bloodborne disease transmission following these injuries are often not available in low-resource settings because of cost. Safe disposal of used needles requires an expensive biowaste disposal infrastructure that is not always present. In some countries, used needles are harvested from the common waste stream and repackaged to the unsuspecting for reuse, presenting a high risk for transmission of bloodborne pathogens.

In the long run, thermostable needle-free vaccines could significantly reduce these logistical hurdles to vaccine delivery in developing countries and extend the benefits of immunization to all people. In the short term, intermediate technologies that are in development can improve cold chain shipping and storage and provide needlestick injury protection.

Bioterror and Pandemic Settings

The United States—used here as an example of a developed country—has a relatively strong immunization program infrastructure, which is reflected in high vaccination coverage rates (except for certain communities where vaccine hesitancy by parents has led to historically low coverage rates). In the United States, POCs include doctors' offices, health clinics, and, within the last several years, pharmacies. However, in an emergency, new vaccination POCs may be established—such as sports arenas, schools, convention centers, and other nontraditional locations—to allow mass vaccination to reach large populations rapidly.[33–35] At-risk individuals are a key consideration and include children, pregnant women, people with disabilities, and the elderly, among other groups.

In the event of a pandemic or bioterror event, local, regional, and even national healthcare resources and infrastructure may be overwhelmed. From the immunization logistics perspective, key factors will include ensuring vaccine supply and distribution, establishing accessible POCs for large numbers of people, and providing sufficiently skilled vaccinators to meet the vaccine demand. The U.S. Strategic National Stockpile maintains supplies of key vaccines. Cold chain logistics may become an issue in emergency settings if there is collateral damage to the power infrastructure, as in a natural disaster such as a hurricane, or if massive increases in vaccine volumes overwhelm local cold chain capacity.

In contrast to many developing countries, the United States has little recent experience with mass vaccination campaigns. In a pandemic setting or a bioterror event involving an infectious agent, gathering masses of people in central locations for vaccination may represent an increased risk for transmission of the disease; thus, more discrete methods of vaccine distribution may be preferable.

Summary: Desirable Features of Practically Ideal Vaccine Delivery Systems

The major immunization logistical challenges to vaccine flow include the following: distribution of multicomponent products, purity and sterility requirements, cold chain requirements, availability of highly skilled vaccinators, on-site filling and reconstitution, needle safety issues, and transfer of antigen into APCs.

Technologies described in this chapter address some or many of these challenges. Overall, a "practically ideal vaccine

delivery system" would be a thermostable vaccine in a prefilled unit dose for needle-free delivery with minimal waste. The vaccine would be optimized for shipping, even by mail in some cases, and would be self-administered or administered by minimally trained personnel. The molecular antigen packaging would ensure safe delivery of the antigen into the APC and induction of the immune response with minimal adverse reactions.

NEW TECHNOLOGIES FOR VACCINE STABILIZATION, PACKAGING, STORAGE, SHIPPING, AND ON-SITE PREPARATION: POINTS OF ORIGIN AND DISTRIBUTION POINTS

Maintaining the integrity, potency, and safety of vaccines from their point of origin at the vaccine manufacturer, through the point of preparation and use, requires attention to the vaccine's formulation, packaging (primary, secondary, and tertiary containers), and reconstitution.

Formulation

The development of vaccine formulations includes the chemical and physical characterization of the antigen, potency assays for lot release and to demonstrate stability, preclinical and clinical evaluation of the optimal administration route to include the potential use of adjuvants, and formulation stability development.[36] Current live-attenuated vaccines are not formulated with an adjuvant. Nonreplicating vaccines, including inactivated viruses and bacteria, VLPs, carbohydrate antigens, and purified or recombinant subunit protein antigens, are typically presented as liquid solutions or suspensions and usually contain adjuvants to induce the desired immune response.

A vaccine's formulation consists of the antigen as well as the other supporting ingredients, called excipients. Excipients include preservatives to prevent contamination and adjuvants to enhance potency. A carefully developed formulation can also increase the thermostability of the vaccine and avoid damage to the antigen due to freezing or high temperatures. Live-attenuated vaccines are often lyophilized and freeze stable but tend to be more heat sensitive for long-term storage (months to years) in the cold chain and immediately after reconstitution.[37] Nonreplicating vaccines can be more stable in high temperatures compared with live-attenuated vaccines, but can also be damaged by freezing, particularly if they include aluminum adjuvants.[37]

Preservatives

Thimerosal, an organic mercury preservative (approximately 50% by weight in the form of ethyl mercury), is used in some inactivated vaccines for multidose vial formats to prevent microbial growth in opened and partially used vials. It is also used during some vaccine manufacturing processes as an inactivation agent. Thimerosal is intended to kill or prevent the growth of a broad spectrum of pathogens (bacteria, fungi). The safety of thimerosal has been evaluated by the Global Advisory Committee on Vaccine Safety as well as other national-level expert groups and regulatory bodies, such as the European Agency for the Evaluation of Medicinal Products, the American Academy of Pediatrics, and the U.S. Food and Drug Administration (FDA). These evaluations concluded that, given the short biological half-life of ethyl mercury—which is excreted via the gut and does not accumulate in the body—evidence of long-term toxic effects has not been demonstrated.[38] In 1999, however, the U.S. Public Health Service urged vaccine manufacturers to reduce or eliminate thimerosal from vaccines as a precautionary measure. Currently in the United States, routine vaccines are thimerosal-free or have levels less than or equal to 1 µg of mercury per dose.[39] Two other preservatives in WHO prequalified vaccines are 2-phenoxyethanol, used with inactivated poliovirus vaccine (IPV), and phenol, used for inactivated typhoid vaccine.

Many industrialized countries, such as the United States, have switched to single-dose vials for use in routine immunization, and thimerosal is not used because the vial is accessed only once. However, in multidose vial formats for seasonal influenza vaccines and vaccines for epidemic or pandemic response, preservatives such as thimerosal continue to be used. Furthermore, many vaccines used in LMICs still contain thimerosal, including diphtheria, tetanus, and pertussis, hepatitis B, Hib, influenza, and meningococcal vaccines. LMIC use of single-dose vial presentations is limited by vaccine manufacturer production capacity, the increased cold chain volume, and cost, which many countries cannot absorb.

Thermostability

Extended exposure to elevated and freezing temperatures—those temperatures outside of the recommended range (normally 2°C to 8°C)—can damage vaccines. Heat can denature or otherwise alter the protein tertiary structure; this may reduce viability of live-attenuated vaccines or, in the case of polysaccharide conjugate vaccines, result in increased rates of hydrolysis of the polysaccharide from the protein in the vaccine formulation. Formation of ice crystals can result in freeze damage to the antigen, and freezing of vaccines containing aluminum adjuvants can reduce potency from agglomeration of the adjuvant.[40] Thermostability (resistance to high ambient temperatures or freezing) could reduce potency loss and have positive impacts on vaccine efficacy.[27,41] It could also allow removal of vaccines from the cold chain, reduce costs, and lead to increased coverage by allowing flexibility in time to reach remote populations. Some thermostable products could be dry cakes similar to lyophilized vaccine and would require reconstitution prior to injection. Other dry formulations could be incorporated into unit-dose dry-format delivery systems such as microarray patches, or dry powder aerosols for respiratory administration (see "Cutaneous Vaccination" and "Mucosal Vaccination" later). These formats would have the combined benefits of being needle free and thus simple to administer or self-administer. They would not require refrigeration or reconstitution.

Protection From High Ambient Temperatures. Enhanced thermostability of liquid formulation vaccines can be achieved through selection of buffering agents and by the use of excipients (vaccine formulation ingredients other than the antigen) that can further stabilize the formulation. Examples include nonreducing sugars, nonionic surfactants, and polymers or protein stabilizers. Excipient stabilization can enhance protection from shifts in pH, decrease antigen loss as a result of surface adsorption and aggregation, and prevent or reduce protein-to-protein interactions.[41]

Freeze Protection. Propylene glycol, polyethylene glycol, and glycerol have been used to protect aluminum-adjuvanted vaccines from freezing. Various concentrations of propylene glycol have prevented vaccine freezing or loss of potency and have prevented destructive particle aggregation if physical freezing occurred.[41]

Dry-Format Delivery. Alternative processes to lyophilization for vaccine drying (removing water molecules from the vaccine

suspension) include spray drying, spray-freeze drying (SFD), vacuum-foam drying, and supercritical fluid drying. These processes have been evaluated for increasing vaccine thermostability.[42] Research with aluminum-hydroxide–adjuvanted hepatitis B surface antigen (HBsAg) or human papillomavirus (HPV) vaccines and aluminum-phosphate–adjuvanted diphtheria and tetanus toxoids has been conducted using alternative freeze-drying processes. These studies have typically found aluminum coagulation and difficulty in reconstitution for lyophilized adjuvanted vaccines, although additional excipients and a thin film freeze-drying process may provide freeze protection.[43,44] The SFD method produced a homogenous suspension, indicating the feasibility of this approach for aluminum-adjuvanted vaccines.[45] Evaluation of SFD of HBsAg without aluminum in combination with inulin or dextran/trehalose stabilizers demonstrated enhanced thermostability (up to 60°C). However, preclinical immunogenicity studies of this formulation demonstrated immunoglobulin (Ig) G immune responses that were lower than responses to aluminum-adjuvanted HBsAg.[46] SFD has also been assessed for meningitis A and measles vaccines.[47,48]

In addition, alternative delivery or packaging methods, such as dry powder inhalation, microarray patches, biodegradable implants, or integrated reconstitution technologies (discussed later in this chapter), are currently being developed or used for biologics or pharmaceuticals and could be adapted for vaccines. Primary excipients include nonreducing sugars such as trehalose or sucrose because of the high glass-transition temperatures they exhibit. Glass (amorphous solid) is formed instead of crystals when these excipients are dried, which contributes to vaccine stability.[41]

Packaging

Vaccine packaging is the collection of components that surround the vaccine and protect its integrity (potency/stability/shelf life), from production through the supply chain to the POCs. Vaccine packaging is typically divided into three categories: primary, secondary, and tertiary.[49,50] Primary packaging protects against light, oxygen, and moisture vapor ingress, and it must not allow pH shifts that could affect vaccine stability or antigen binding to the material of the container that could reduce the available dose. The packaging also provides information and identification of its contents.[51] Labeling to identify the product must be integral to the packaging, or affixed to it. Primary containers are nested together in secondary packaging, and larger containers such as boxes or cartons provide tertiary packaging.

Primary Packaging

Vial and Ampoule (Vaccine and Diluent). Primary packaging, such as ampoules, vials, prefilled syringes, and prefilled oral dispensers, comes into direct contact with the vaccine product or diluent and may affect the vaccine formulation itself. The WHO has issued guidelines covering vaccine formulation, presentation, labeling, and packaging to ensure that vaccines submitted for prequalification have been optimized to address LMIC needs. These guidelines include mandatory, critical, unique and innovative, and preferred characteristics. Mandatory and critical characteristics must be met to achieve WHO prequalification. In the case of critical characteristics, some deviation from defined values may be allowed when taking into account public health needs. Unique and innovative characteristics may be vaccine specific and are reviewed as such. Preferred characteristics are not required, but they represent the buyer preference (procurement agencies and national immunization programs).[52]

Vaccines must be WHO prequalified for purchasing by UN agencies. The WHO requirements on vaccine quality, safety, and efficacy are included in the prequalification process, as well as compliance requirements for manufacturing and specifications for packaging and presentation. Prequalification provides assurance that vaccines used by national immunization programs are safe, effective, and meet quality standards.[53]

The Vaccine Presentation and Packaging Advisory Group (VPPAG) is a WHO and United Nations Children's Fund (UNICEF)-led forum for both the public sector and industry to discuss and provide advice on vaccine presentation and packaging. VPPAG has developed a generic preferred product profile for vaccines for LMIC use, which recommends "ready-to-use" presentations that do not require mixing (i.e., reconstitution) and formats that reduce the number of user steps (and potential errors). Vaccine formulations with improved heat and freeze stability also are recommended, to provide for higher temperature storage (target threshold 40°C) and potential use beyond the cold chain. Prefilled syringes or injection systems should reduce the volume required in the cold chain and should incorporate an autodisable feature that prevents reuse. These designs are designated as compact prefilled autodisable injection devices (cPADs). The generic preferred product profile also includes vaccine vial dimensional recommendations that conform to ISO 8362 and are the most efficient size for the cold chain. Vial labeling should include a vaccine vial monitor (per UNICEF and WHO recommendations), which consists of a temperature-sensitive material and serves as a visual indicator of cumulative heat exposure. Labeling should also include standard product, date, and lot information, among other requirements.

Unit Dose Versus Multidose Formats. Multidose presentations are common in LMICs, a result of both lower cost and reduced per-dose volume compared with single-dose presentations. Industrialized or high-income countries are less vaccine-price sensitive and have been switching to single-dose presentations for adult and childhood vaccines. The shift to single dose was accelerated by public concerns regarding thimerosal,[54–58] healthcare provider preference for single-dose presentations including prefilled syringes, and increased awareness of injection safety.[58] In the United States, the awareness of safety issues came in response to the Needlestick Safety and Prevention Act and subsequent revision of the Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens standard. This led to requirements for workplace reporting and maintenance of a log of needlestick injuries as well as broader implementation of engineered safety features to reduce or prevent the risk of needlestick injury.[59]

The use of single-dose and small multidose presentations also has increased in LMICs, in part because of the higher costs of vaccine wastage for newer vaccines.[60,61] The use of preservatives in multidose vials is critically important for vaccines that are used in more than one immunization session because repeated access of the vial through the septum, as well as storage between sessions, presents potential contamination risk from pathogen ingress. The WHO's multidose vial policy permits open vials of vaccine with preservative that have been handled under specific conditions to be used for up to 28 days after the first dose is withdrawn.[5] These guidelines are consistent with U.S. recommendations, which also require that an open vial be discarded in 28 days except if there are different manufacturer requirements in the product labeling, which may specify an alternative number of days.[63]

These policies apply only to liquid vaccines that contain preservative; lyophilized live attenuated vaccines—such as measles-containing vaccines, bacillus Calmette-Guérin (BCG), and yellow fever vaccines—generally do not contain preservatives and must be discarded at a maximum of 6 hours after

reconstitution, per the multidose vial policy. Because of vaccine wastage concerns, some healthcare workers are hesitant to reconstitute a multidose vial if there are insufficient numbers of people available to be vaccinated.

Preservative-Free Multidose Primary Packaging. MEDInstill, Inc., a U.S.-based firm, has developed a primary packaging pharmaceutical filling and dispensing technology called Intact that may reduce or eliminate the need for preservatives in multidose vaccine vials. The technology is designed to reduce the risk of contamination during both filling and dispensing from a primary container. To maintain sterility during the filling process, a closed-system valve is used for the filling needle. The prevention of contamination allows for sterile filling in a nonaseptic facility.[64]

In addition, the Intact design has been incorporated into the dispensing port for MEDInstill primary containers. The valve allows multiple withdrawals from the container, maintaining sterility of the contents even in the presence of external contaminants. Multidose vials (Fig. 68.1A) and pouch designs are undergoing evaluation for pharmaceuticals and vaccines.[65]

Prefilled Syringes. Prefilled syringes represent a fully integrated vaccine presentation. Multiple studies comparing prefilled syringes with standard vial presentations have demonstrated increased efficiency and improved vaccination throughput with this packaging format. For example, a study of U.S. nurses preparing and delivering influenza vaccine reported that the time necessary for providing an injection was 12.4 seconds for a prefilled syringe compared with 49.7 seconds for a multidose vial.[66] The increased efficiencies possible with prefilled syringes for both parenteral and nonparenteral administration (oral/nasal) could greatly enhance pandemic/epidemic outbreak response capacity.

Glass Prefilled Syringes. Glass prefilled syringes are manufactured from type 1 borosilicate glass, which has high chemical resistance, low alkali content, and barrier properties appropriate for long-term storage of vaccines and other pharmaceuticals.[67] A leading example is the Becton, Dickinson and Company (BD) Hypak SCF (sterile, clean, ready to fill) glass prefilled syringe used widely in the United States and Europe (see Fig. 68.1B). This design comes in different models to include fixed-needle, Luer slip, and Luer-Lok varieties. Other manufacturers include Gerresheimer, Schott, Nuova Ompi, Nipro, and Catalent. Glass has been used for decades for a variety of primary containers (prefilled syringes, vials, cartridges). Problems associated with its use include the possibility of cracks or breakage.

Plastic Prefilled Syringes. Various plastic materials have been used as alternatives to glass, for both prefilled syringes and other primary container technologies. Plastic syringes are injection molded, allowing for tighter dimensional tolerances and ability to generate alternative geometries. Plastic prefilled syringes are lighter in weight and more resistant to breakage than glass during production, fill/finish, shipping, and programmatic use. Polypropylene (PP) is a polymer used for standard- and large-volume prefilled syringes (up to 50 mL) and vials.[68] Cyclic olefin copolymer and cyclic olefin polymer (COP) are highly transparent polymers that have been used increasingly for prefilled syringes.[69,70] Compared with PP, cyclic olefin copolymer and cyclic olefin polymer have lower water vapor and oxygen permeability, allowing long-term storage of vaccines[71] and have been demonstrated to be biocompatible, resistant to heat, and compatible with various terminal sterilization processes.[68]

The selection of glass or plastic is determined by the formulation or stability requirements of the pharmaceutical, needs of the patient/user, and other requirements. Plastic prefilled syringes are typically more expensive than glass.[72] Examples include the BD Sterifill SCF, SCHOTT TopPac, Gerresheimer ClearJect, Baxter Clearshot, and West Daikyo Crystal Zenith Ready-to-Use silicone-free syringes. Cyclic olefin copolymer and cyclic olefin polymer have also been used for vials; for example, Aseptic Technologies, Inc.'s, AT-Closed Vial composed of cyclic olefin copolymer (see Fig. 68.1C) has been validated for use with GlaxoSmithKline's Synflorix pneumococcal vaccine and was approved by the European Medicines Agency.[73]

Compact Prefilled Autodisable Devices. A cPAD is a prefilled single-dose injection system comparable to a prefilled syringe, but with an autodisable feature that prevents reuse. Like prefilled syringes, cPADs provide accurate doses, fast injection preparation, and quick delivery time (ready to use). In addition, because they are typically smaller than standard syringes, they bring the logistical benefits of decreased volume and weight.[74]

The BD Uniject injection system (see Fig. 68.1D) is a cPAD technology with a small reservoir prefilled with vaccine or other pharmaceutical. It has four main components: reservoir, port, needle assembly, and needle shield. The reservoir is a three-layer laminate film with linear low-density polyethylene in contact with the contained fluid. The port and valve are in contact with the fluid in the reservoir and are also constructed of low-density polyethylene. The needle assembly connected to the port consists of a polystyrene hub and a steel needle. The PP needle shield is removed from the needle for administration. Typically, a foil-laminate secondary pouch maintains stability of the material in the Uniject units. Crucell has developed a novel hybrid secondary packaging for the Uniject system that can store up to 20 filled devices.[75] A needleless version of the Uniject platform, designated "Uniject DP," also has been developed by BD for oral delivery and is commercially available.

To deliver the prefilled dose, after removal of the needle shield and insertion of the needle, the plastic reservoir of the Uniject system is squeezed between the thumb and fingers. The Uniject system is available in four dose-volume sizes: 0.25, 0.5, 1.0, and 2.0 mL. It is available with various needle gauges and lengths, ranging from 18 to 26 gauge and needle lengths of ⅜ to 1.5 inches (for SC or IM injection). The container is provided sterile in "ready-to-fill" reels of 1500. The sterile reel is loaded onto a custom filling machine where the containers are filled and then heat sealed. They are kept on the reel throughout the process and either accumulated in reel form or transferred out of the sterile area for final packaging.[76]

Hepatitis B and tetanus toxoid vaccines made by PT Bio Farma (Indonesia) have been WHO-prequalified in Uniject formats, and Crucell developed a new presentation of Quinvaxem fully liquid pentavalent vaccine in Uniject, which was prequalified but is not being commercialized.[77] A time-and-motion study in Kenya comparing the average time for health workers to prepare and inject 20 doses of pentavalent vaccine in five different presentations found that the prefilled Uniject system was faster to deliver than fill-onsite presentations (single-dose or multidose vials, liquid or lyophilized).[78]

Novel Primary Container Technologies. Blow-fill-seal (BFS) ampoules are plastic primary containers manufactured from polyethylene or PP and used for a variety of pharmaceuticals (see Fig. 68.1E). The containers are extruded, blown, filled, and sealed in an automated, continuous process. Although these ampoules have not yet been approved for vaccines, they have been evaluated with MedImmune's live attenuated influenza vaccine (LAIV)[79] and are being evaluated by rotavirus vaccine manufacturers.[80] For parenteral delivery, a Luer interface can be incorporated into the neck of the ampoule, allowing for connection of a Luer-tip needle. A needle and syringe can also simply withdraw a dose from a standard BFS ampoule

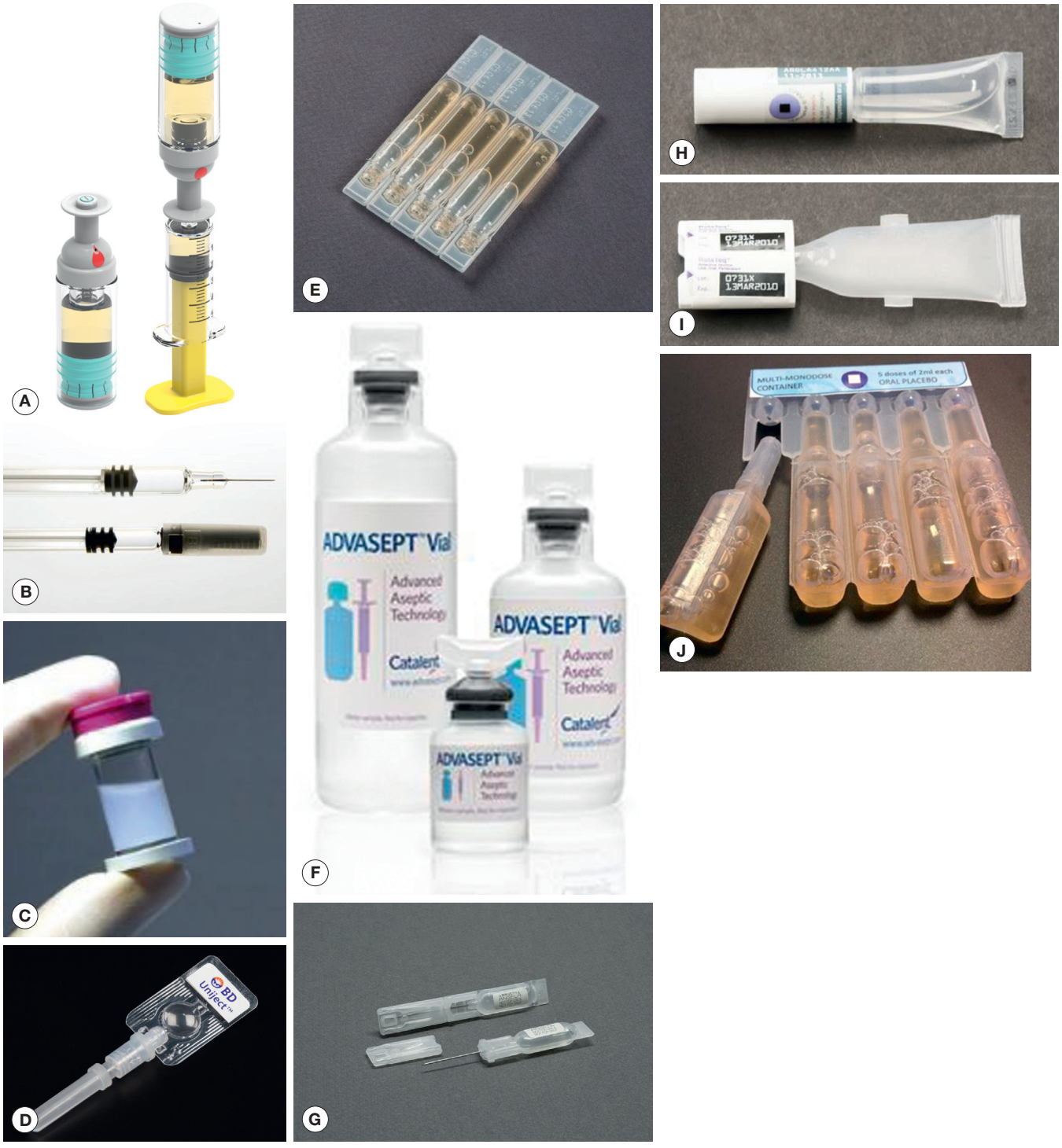

Figure 68.1. Vaccine primary containers. A, MEDInstill vial represents a preservative-free technology that incorporates a novel valve to prevent contamination. **B,** BD Hypack SCF is a widely used prefill glass syringe used for a variety of pharmaceutical applications. **C,** The Aseptic Technologies AT-Closed Vial uses cyclic olefin copolymer, an alternative material to glass. **D,** Uniject is the only WHO-prequalified compact prefilled autodisable (cPAD) technology. **E,** Rommelag produces blow-fill-seal (BFS) technology for small- and large-volume pharmaceuticals. **F,** Catalent ADVASEPT Vial technology uses the advanced aseptic manufacturing process of BFS to incorporate a septum through an insertion to form fill and seal the primary container. **G,** Brevetti Angela has also developed an integrated needle design for use in parenteral delivery, the SECURE-JECT- SQUEEZABLE. **H,** GlaxoSmithKline uses a polymer tube manufactured by Rexam for use with its Rotarix vaccine. **I,** Merck RotaTeq comes in a plastic injection molded tube composed of polyethylene. **J,** PATH in cooperation with Rommelag has developed a multi-mono-dose design with a single vaccine vial monitor to maximize cold chain volume. *(**A,** Courtesy of MEDInstill [Debashis Sahoo]. **B, D, E,** and **H–J,** Courtesy PATH. **C,** Courtesy Aseptic Technologies, Inc. **F,** Courtesy Catalent [Bill Hartzell]. **G,** Courtesy Brevetti Angela [Rajeev Kabbur, Daniel Martinez].)*

or vial with integrated septum such as Catalent's ADVASEPT Vial Technology (see Fig. 68.1F). Rommelag, Weiler Engineering, and Brevetti Angela are leading examples of BFS machine manufacturers. Brevetti Angela and Rommelag have BFS systems with integrated needles incorporated during the forming process (see Fig. 68.1G).

Plastic tubes that are injection molded or extruded have been developed primarily for oral administration. These containers are manufactured, terminally sterilized, and shipped to the pharmaceutical manufacturer for filling and heat sealing. Examples are the Rexam dispenser tube, used for GlaxoSmithKline's Rotarix vaccine, and the Lameplast tube, used for Merck's RotaTeq vaccine (see Figs. 68.1H and I). Under funding from the Bill & Melinda Gates Foundation, PATH, in collaboration with Rommelag, has developed a multi-mono-dose BFS container design targeted for rotavirus vaccine delivery. The design has individual containers conjoined by a shared tab. When one container is separated from the tab, it is rendered open and must be used to deliver the vaccine. This attribute provides key advantages compared with single-dose presentations, including cold chain volume reduction and potential cost savings (see Fig. 68.1J).

Safety Syringes. The Needlestick Safety and Prevention Act, signed into law in 2000, led to the standard use of syringes with engineered safety features for vaccine and drug delivery in the United States. One study found that beginning in 2001, this resulted in a drop of more than one-third in needlestick injuries—with approximate annual reductions of 100,000—and a cost savings of $69 million to $415 million.[81] However, a Cochrane review concluded there was no clear evidence of benefit with use of these devices, despite their increased costs.[82]

To achieve WHO prequalification, vaccines in prefilled syringes are required to have an autodisable or reuse-prevention feature.[52] Autodisable syringes have attached needles and a method to prevent reuse of needle and syringe after delivery of a fixed dose. A reuse-prevention feature syringe allows variable dosing, necessary for volume diluent transfer, for vaccine reconstitution. The WHO recommends autodisable syringes or other syringes with reuse-prevention features for vaccine administration.[84]

WHO also recommends syringes with a sharps injury protection (SIP) feature for immunizations.[85] A sharps injury protection feature covers, shields, or retracts the needle into the syringe barrel to prevent needlestick after use or during disposal. Multiple self-shielding needle technologies have been introduced to reduce the risk of needlestick injuries.[86,87] Currently, the costs of such syringes limit their use in LMICs.

The Unifill safety syringe (Unilife Medical Solutions) has a reuse-prevention feature that retracts the needle after delivery. Other developers, including Tip-Top, Safety Syringes, and Credence MedSystems, have prefilled syringe-compatible sharps injury protection designs that can be incorporated into the syringe after filling.[86,88] Merck's Gardasil HPV vaccine is delivered in a single-dose prefilled syringe with UltraSafe needle guards.[89] Clip-on prefilled syringe safety mechanisms also include the BD Preventis, the West Clip'n'Ject, and the Specialized Health Products International LuproLoc.

Secondary and Tertiary Packaging

Secondary packaging, such as cartons, trays, or foil pouches, hold one or more primary containers, such as vials or prefilled syringes of vaccine. These packages generally represent the volume for calculation of cold chain storage requirements.[52] Secondary packaging is important for vaccine stability; for example, a foil pouch protects a polymer-based primary container such as the Uniject system from water vapor loss, oxygen ingress, and light.

Tertiary packaging includes cartons or cases that enclose multiple secondary packaging units. Insulated shipping boxes with internal walls of polystyrene, isocyanurate panels, or polyurethane foam also maintain cold chain temperatures and prevent shifting during shipment. Pallets are used for international shipment of these containers. In the GS1 General Specifications, these are designated as export packaging or shipping containers, whereas the WHO puts them into the category of tertiary packaging.[90,91]

VPPAG secondary package recommendations include minimizing weight, volume, and the need for in-country repackaging for distribution in the cold chain. Specific vial arrays (rectangular) by number of vials for packing are also recommended. Tertiary packaging recommendations also include minimizing dimensions as well as limiting the need for repackaging, with vials in multiples of 100 for easier inventory control. Weight and width dimension recommendations are also included, with reference to ISO pallet sizes. Labeling recommendations also include the use of bar codes that conform to GS1 standards. Materials used for packaging (primary, secondary, tertiary) should minimize environmental impact.[92]

Environmentally Friendly Secondary Cartons and Tertiary Shipping Containers. Numerous companies have developed insulation products to replace polystyrene. Green Cell Foam technology is a biodegradable, starch-based polymer that can break down within four weeks, dissolves in water, and can be incinerated or burned cleanly (Fig. 68.2A). It maintains temperatures for 24 to 48 hours.[93,94] Ecovative is developing compostable plastics derived from agricultural byproducts and mushroom mycelium.[95] Wool packaging uses this renewable and compostable material for thermal insulation.[96] The Coldpack AirLiner is an inflatable insulation liner that can be shipped flat when empty for space saving and deflated for landfill disposal.[97] Placon plastics (polyethylene terephthalate) for secondary packaging are composed of at least 35% postconsumer content.[98] Softbox has developed a new thermal insulation foam with 100% recyclable materials that has increased thermal efficiency compared with Styrofoam. Softbox maintains the cold chain for more than half the world's top 50 pharmaceutical companies.[99]

The Credo container from Pelican Biothermal has been used for influenza vaccines and other temperature-sensitive drugs (see Fig. 68.2B).[100] Repeat flu vaccine shipments and alternative logistics/distribution models of use have been successfully demonstrated with this technology.[103] Container reuse could provide reduced environmental impact and disposal burden at the program level.[103] The Sonoco ThermoSafe Greenbox is another reusable container technology, composed of 100% biodegradable, bio-based materials.[105] A plant-based phase-change material allows cold chain temperature maintenance of up to 6 days.[96] The phase-change material can be reconditioned and used repeatedly, replacing the need for other refrigerants. Approximately 20,000 repeat uses have demonstrated the thermal management properties of this material.

On-Site Reconstitution and Filling

While shipping vaccines in lyophilized form has advantages such as improved thermostability, reconstitution can introduce errors noted earlier, such as using the wrong diluent. For reconstitution, diluent is withdrawn from its container, typically with a needle and syringe, and injected into the primary vaccine container for mixing. Then the reconstituted vaccine is withdrawn from the vaccine container for administration. Vaccines currently available in lyophilized form include yellow fever,

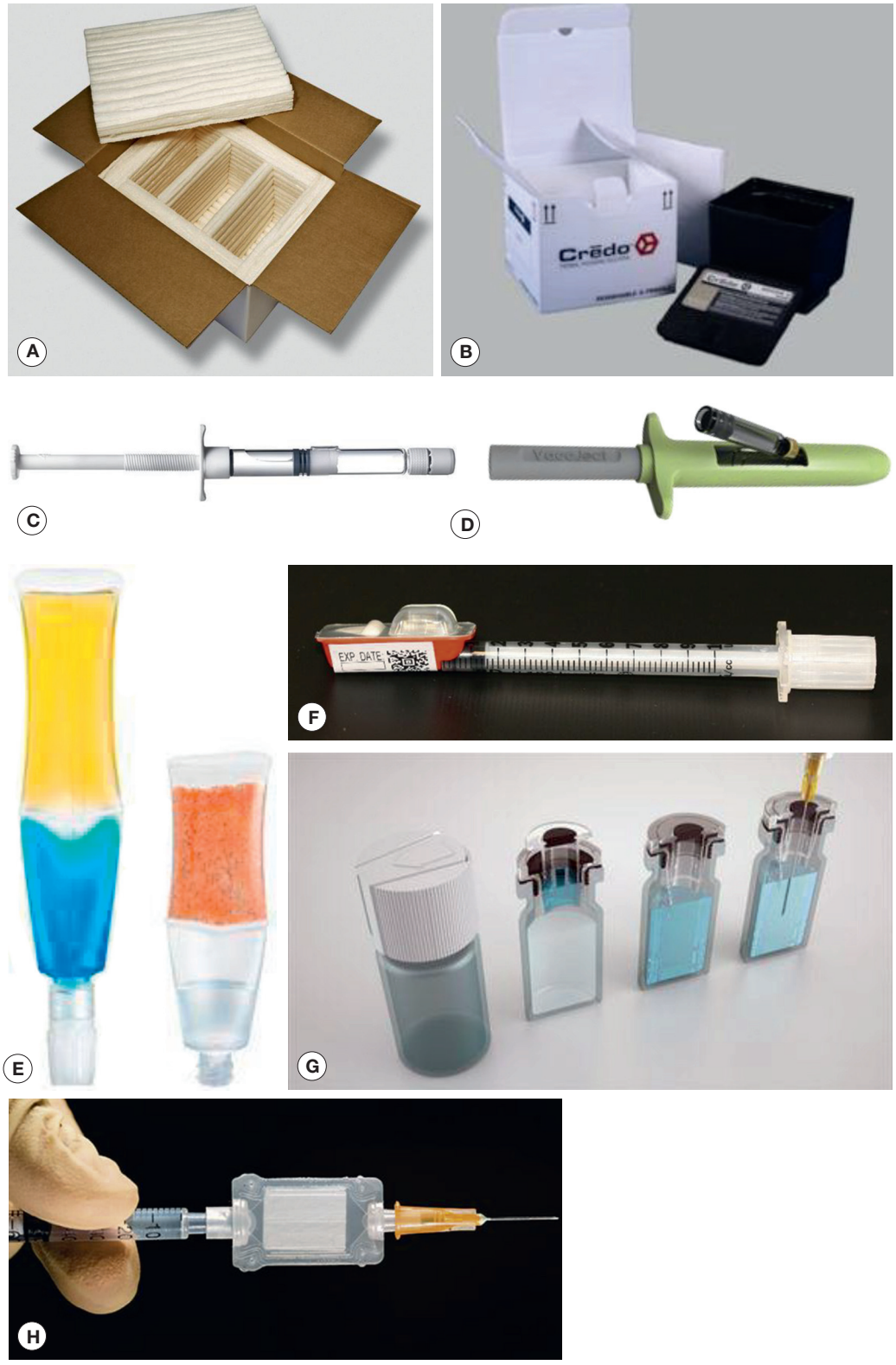

Figure 68.2. Vaccine packaging and reconstitution technologies. A, Landaal Green Cell Foam is a biodegradable polymer that can be easily disposed of by dissolving or incinerating. **B,** Credo Container from Pelican BioThermal can be repeatedly reused and has been used for influenza vaccines and other temperature-sensitive drugs. **C,** The Vetter Lyo-Ject (syringe) is a dual-chamber technology for simplified reconstitution. **D,** Duoject VaccJet is a cartridge-based technology that uses dual-sided needles for parenteral injection. **E,** Neopac's Fleximed Easymix uses a frangible seal separating the wet and dry compartments. Pressurizing (squeezing) one end breaks the seal allowing for reconstitution. **F,** AktiVax's ARCH design uses blisters with frangible seals to separate the dry vaccine from diluent prior to mixing. **G,** Eulysis' SVS is a vial-based design with a compartment that separates the dry powder/lyophilized pharmaceutical from the diluent, which is pierced by a piston cap allowing for mixing. **H,** HydRIS from NOVA Laboratories, is distal hub/attachment for prefilled diluent syringes that is filled with the lyophilized pharmaceutical in a sugar glass based membrane. Reconstitution occurs upon expelling the diluent from the syringe into the compartment during the process of delivery. (***A,*** *Courtesy Landaal Packaging Systems.* ***B,*** *Courtesy Pelican BioThermal.* ***C,*** *Courtesy Vetter.* ***D,*** *Courtesy Duoject.* ***E,*** *Courtesy Neopac [Ralf Künzi].* ***F,*** *Courtesy PATH.* ***G,*** *Courtesy Eulysis.* ***H,*** *Courtesy Nova Technologies.)*

measles, rubella, varicella, measles-containing combination vaccines (measles-rubella, measles-mumps-rubella [MMR], and measles-mumps-rubella-varicella), BCG, Hib (standalone and in some combination vaccines), rabies, rotavirus, Japanese encephalitis, meningococcus, and LAIVs. Vaccines in development, including those for cholera, dengue, enterotoxigenic *Escherichia coli*, influenza, tuberculosis, and rotavirus, will likely be available in a format requiring reconstitution.[37]

Preparation and delivery of reconstituted vaccines requires more vaccinator time than preparation and delivery of liquid vaccines. A time-motion study conducted in Kenya, comparing the amount of time required for vaccinators to prepare and deliver mock injections with various single-dose and 10-dose vaccine presentations, found that more time was required to administer lyophilized vaccines than liquid vaccines. The difference was greatest for single-dose, lyophilized presentations, since reconstitution is done once for each vial.[78]

Novel reconstitution technologies might be useful for current or future lyophilized vaccines. A variety of reconstitution technologies have been developed to reduce the chance of user error and simplify delivery. These technologies can be grouped into several categories: (1) fully integrated reconstitution technologies, which include primary packaging, diluent transfer, reconstitution, and delivery features; (2) partially integrated reconstitution technologies that include everything but the delivery device; (3) diluent transfer devices that facilitate reconstitution of existing lyophilized vaccine vials; and (4) hybrid reconstitution technologies that incorporate one or more features. Some of these technologies are currently in use for high-cost pharmaceuticals, particularly those intended for self-administration by patients at home. For vaccines, except diluent transfer devices such as vial adapters, reconstitution technologies are in varying stages of development, and no vaccines are available currently in these formats.

Fully Integrated Reconstitution Technologies

This type of technology incorporates packaging, reconstitution, and delivery and represents the most complex of the reconstitution technologies. Some technologies incorporate valved stoppers that are actuated with plungers in a glass syringe or a cartridge. Others use polymer tubes, pouches, or blister packs with frangible seals that rupture when pressure is applied.

Syringe- or Cartridge-Based Technologies. In this reconstitution technology, a prefilled syringe or cartridge contains both the dry and liquid components separated by an elastomeric internal stopper. Reconstitution occurs when mechanical pressure is applied to either overcome a valve in the plunger or push diluent through a bypass channel in the syringe. Parenteral delivery is possible with these technologies. These technologies may be more suitable for vaccine-drying methods other than lyophilization, which may represent a barrier to consideration because alternative drying methods are not currently in use for vaccines.

Examples of syringes include the Vetter Lyo-Ject Syringe (see Fig. 68.2C) and the LyoGo Dual Chamber Pre-Filled Syringe technologies. The commercially available Vetter Lyo-Ject Syringe incorporates a standard stopper with a bypass channel that is accessed upon advancement of the plunger, allowing diluent to flow through the channel into the dry powder chamber.[106] Vetter has also developed and marketed the Vetter V-LK Reconstitution Cartridge, which has a similar mechanism of action.

The LyoGo Dual Chamber Pre-Filled Syringe, currently in development,[107] has a chamber-separating stopper with a novel valve. Advancement of the plunger creates a pressure differential that opens the valve, allowing the diluent to enter the dry powder chamber. LyoGo also is developing a dual-chamber cartridge version of their novel valve technology. Another manufacturer, Unilife, has developed the dual-chamber syringe EZMix Genesis, which incorporates its retractable needle feature.[108]

Cartridge-based reconstitution technologies require incorporation into a separate novel delivery device. Duoject's VacJect technology is designed for single-use delivery using a retractable needle that allows cartridge access (see Fig. 68.2D). A reconstitution-capable version of the VacJect has been developed with a feature to allow for diluent transfer from one cartridge to a cartridge containing the lyophilized vaccine or drug.

Frangible Seal–Based Technologies (Tubes, Pouches, Blister Packs). An alternative to syringes and cartridges for reconstitution is the use of tubes, pouches, or blister packs with a frangible seal separating the diluent from the lyophilized or dry powder vaccine. Frangibility refers to property of breaking up into fragments when deformed or otherwise placed under sufficient stress. The frangible seal is ruptured upon application of pressure, allowing the diluent to mix with the pharmaceutical. Materials used for the seal must be compatible with heat sealing and can include PP, polyesters, or coextruded laminates.[98] Water vapor and oxygen transfer rates related to these different materials must be considered because they will affect product stability. An aluminum dual-chamber foil pouch was used for oral cholera vaccine (Orochol from Crucell [formerly Berna Biotech]); however, this product is no longer available. Frangible seal pouches have been used in non-pharmaceutical point-of-use component mixing.[109]

An example of a commercially available tube with a frangible seal is the Neopac Fleximed Easymix, a two-compartment, coextruded, polymer-based tube with the seal separating two compartments containing the dry and liquid or two liquid components (see Fig. 68.2E). Reconstitution occurs when the user applies pressure to rupture the seal between compartments. Nozzles for oral delivery are available, or a Luer interface can allow filling into a syringe or attachment of a Luer-fit needle. The pharmaceutical must be in dry format and dispensed into the tube and sealed. Use of this technology for vaccines may require shifting to a drying technique other than lyophilization (e.g., SFD).

The AktiVax ARCH (aseptic reconstitution cartridge hybrid) is a polymer prefilled syringe incorporating a flexible package on a solid backing (see Fig. 68.2F).[109a] The ARCH package can accommodate one or more constituents of the pharmaceutical, stored in adjacent high-barrier compartments separated by a frangible seal, which can be broken by depressing with a thumb for reconstitution just prior to injection. The mixed pharmaceutical is drawn into the attached syringe for injection. The ARCH syringe can incorporate a staked needle or Luer interface. As for the other technologies discussed above, use of flexible packaging may require updated vaccine lyophilization or other drying techniques. An integrated needle-based design using ARCH features—the Immunoject—also has been developed by AktiVax. In this design, the two-compartment blister is attached to a needle and a foldable plastic backing. The plastic backing shields the needle before use and allows compression of the blister compartments to rupture the frangible seal for reconstitution. Once reconstituted, the integrated needle allows parenteral administration, and refolding the plastic backing disables and covers the needle.[109b]

Partially Integrated Reconstitution Technologies

These technologies are characterized by an integrated primary container with a reconstitution feature such as a dual chamber, but they are without an integrated delivery feature for parenteral

delivery. The Eulysis Single Vial System (SVS) is composed of two compartments: the lyophilized or dry powder pharmaceutical is in a thin plastic cup resting on a glass vial that contains the diluent (see Fig. 68.2G). Actuation of a piston-like cap punctures the base of the plastic cup, causing the contents to fall into the diluent and permitting reconstitution; a needle and syringe must be used to withdraw a dose from the vial. A tamper-evident cap protects the piston cap from activation during shipment. This technology is in early-stage development and may potentially have wide-ranging applications for all freeze-dried injectable products. The Pfizer Injectable Act-O-Vial System demonstrates a similar concept. A rubber septum separates diluent and dry compartments of a nonstandard glass vial. Pushing down on the plastic cap dislodges the septum, forcing it into the lower dry compartment where reconstitution occurs. The Act-O-Vial is used with Pfizer's glucocorticoids Solu-Cortef and Solu-Medrol.[110,111]

Diluent Transfer Technologies

Diluent transfer technologies facilitate mixing of dry and liquid components of a pharmaceutical from two separate primary containers. These technologies eliminate the reconstitution needle to improve safety and reduce user error. An example, the Duoject E-Z-Link, incorporates a Luer lock fitting with a socket-style plastic spike vial adapter (socket sizes 13 mm and 20 mm) that can be used only once. A prefilled diluent syringe can be used to dispense into a standard-sized vial with lyophilized or dry powder pharmaceutical and draw from the contents once reconstituted. The E-Z-Link contains a protective disk to prevent contamination and shield the plastic spike.[112]

The Mix2Vial from West Pharmaceutical Services, Inc., allows connection of two vials with diluent and lyophilized pharmaceutical via two vial-adapter sockets with plastic spikes. The Mix2Vial requires the dry powder vial to be manufactured under vacuum to facilitate the transfer from the diluent vial. After reconstitution, a needle and syringe are used for dosing and delivery.[113] The West Needle-free Transfer Device has a Luer fitting to allow the attachment of a Luer lock syringe, which can be used to transfer the diluent from its vial into the lyophilized pharmaceutical vial and then back into the syringe. The syringe is then detached and a needle attached for parenteral delivery.[114] The West MixJect consists of a capped needle, a 13-mm vial adapter, and a Luer lock fitting for a prefilled syringe attachment. Diluent from the syringe is transferred into a vial of lyophilized pharmaceutical through the vial adapter, reconstituted, and drawn back into the syringe. The vial is then detached and the device is ready for drug administration.[115]

Hybrid Reconstitution Technologies

Some technologies present a fully or partially integrated hybrid approach to primary packaging, diluent transfer, reconstitution, and delivery. The Integrity Bio LyoTip distal hub incorporates a spiral-shaped channel to facilitate mixing of diluent and lyophilized pharmaceutical. The hub requires a Luer lock needle and syringe for delivery. The NOVA Laboratories Hydration Rehydration Injection System (HydRIS) is similar to the LyoTip: it is an attachment filled with lyophilized pharmaceutical for a prefilled diluent syringe (see Fig. 68.2H). A unique feature of the HydRIS is use of a stabilization technology based on sugar glass (an amorphous solid, clear crystallized form of the sugar): the pharmaceutical is dried onto a membrane, which is then integrated into the distal hub. The sugar-based membrane dissolves when it contacts the diluent, allowing the instantaneous mixing of diluent and lyophilized pharmaceutical.

Duoject has designed several reconstitution formats. One example is the PEN-PREP EVO device used to transfer reconstituted pharmaceuticals from a vial into multidose cartridges for use with traditional pen injectors. An integrated vial adapter incorporates a protective disk to shield the user from sharps injuries and to prevent contamination prior to use.[116] The Duoject Inter-Vial and the Duoject Smart-Rod XR combine a prefilled diluent syringe and a vial adapter. The vial adapter on the Inter-Vial detaches from the device once the drug is reconstituted and drawn back into the syringe. Detaching the vial adapter exposes a standard Luer lock for needle attachment. The vial adapter on the Smart-Rod is located on the plunger and cannot be removed for reuse. The Duoject Inter-Vial Plus and Duoject Smart-Rod Plus are nearly identical to the Inter-Vial and Smart-Rod XR except for the location of the diluent and lyophilized contents. The syringe components are prefilled with a lyophilized drug and attached to a diluent-filled vial.

Reconstitution Aids

Bundling or copackaging lyophilized vaccine and diluent in the same secondary packaging is common for many pharmaceutical applications as well as for select vaccine applications such as rabies vaccine. For the Rabipur rabies vaccine that is produced by CSL (formerly Chiron), the secondary package contains the vaccine vial, the diluent ampoule, and a syringe.[117]

Both vaccines and diluents can be supplied in vials or ampoule primary container presentations, and diluent can be provided in a prefilled syringe or other container such as a BFS ampoule. GlaxoSmithKline's Tritanrix HB+Hib vaccine presentation included a one-dose vial containing diphtheria, tetanus, pertussis (DTP)-hepatitis B (HepB) (liquid) and a one-dose vial of lyophilized Hib. A plastic clip bound both vials together to aid in easier reconstitution in that the "diluent" vial (the DTP-HepB vial) was immediately adjacent to the lyophilized vaccine vial.[118] The plastic clip binding either vial–vial or vial–ampoule combinations could reduce adverse events associated with incorrect diluent use.

Summary

The traditional glass vial and needle and syringe continue to be used with standard vaccine formulations and pharmaceutical filling techniques; however, new technologies are available that represent paradigm shifts in manufacturing and healthcare worker use. The use of polymer materials has enabled design and development of new primary packaging technologies, and alternative materials are informing new research in secondary and tertiary containers. Given the challenges of vaccine shipment and storage in the cold chain, new technologies that can reduce volume or the need for the cold chain would be of great benefit to high-income countries as well as LMICs.

NEW TECHNOLOGIES FOR VACCINE ADMINISTRATION: VACCINE MACRODELIVERY SYSTEMS AT THE POINTS OF CARE

New vaccine administration technologies are classified here into three major categories: SC and IM injection, cutaneous vaccine delivery, and mucosal vaccine delivery.

Subcutaneous and Intramuscular Injection

Most vaccines are delivered via SC or IM injection with needles and syringes. As described previously, in high-income–country

markets, immunizations are increasingly provided in prefilled syringes or single-dose presentations, while in lower-income countries serviced by UNICEF, vaccines are commonly delivered from multidose vials using autodisable needles and syringes, although use of single-dose vials and prefilled devices is increasing. An alternative to needle and syringe administration for SC and IM delivery is the jet injector, which can deliver vaccines in their current formulations. Another method in an early stage of development is solid dissolving needles, for which vaccines are reformulated as biodegradable implants.

Anatomy

The SC tissue (also known as the hypodermis) lies below the dermis of the skin and consists mainly of lobules of adipocytes as well as connective tissue (see Fig. 68.3A). Blood and lymphatic vessels transiting this layer serve the dermal layer of the skin. The thickness of the SC tissue layer varies widely between individuals of different age and body mass and at different body sites. For SC injection of vaccines, a $\frac{5}{8}$-inch needle (23 to 25 gauge) is recommended. The skin should be pinched up to avoid injecting intramuscularly and the needle inserted at a 45-degree angle. In infants younger than 12 months of age, SC injections are typically given in the thigh, while for adults and children older than 12 months, SC injections are given in the back of the upper arm, over the triceps.

Beneath the SC layer are skeletal muscles. Individual muscle cells are arranged into bundles called fascicles. These fascicles in turn are grouped in bundles within a layer of connective tissue to form muscles. For infants and toddlers up to 2 years of age, the vastus lateralis muscle in the anterolateral thigh is the preferred IM injection site, and the deltoid muscle in the upper arm is used for older children and adults. Recommended needle lengths vary by age, injection site, and body mass, because of the variation in thickness of the SC layer.

Immunological Rationale

The SC and IM routes of administration are typically used for vaccines because they are relatively convenient to access using a needle and syringe, enable consistent delivery, and provide adequately reproducible immunogenicity among individuals. However, compared with internal and external body surfaces that are more frequently in contact with the environment, SC and IM tissues have relatively fewer immune cells. Following injection of a vaccine, the antigen or adjuvant generates a local innate immune response, attracting APCs which ingest the vaccine package. These infiltrating dendritic cells (see Fig. 68.3B) and monocytes migrate to local draining lymph nodes and induce B- and T-cell immune responses. In general, SC and IM injection of vaccines tends to be less effective at producing mucosal immunity than mucosal vaccination.[119]

Jet Injection

Jet injectors force liquid under high pressure through a tiny orifice, producing a focused stream that penetrates the skin to deliver medication into targeted tissues without needles. Most modern jet injectors are powered by the release of energy stored in a compressed metal spring, and a few use compressed gas such as CO_2 or N_2. The velocity of the jet stream exceeds 100 meters per second. The depth achieved depends primarily on the power imparted to the liquid and then on variables such as orifice diameter, distance from the nozzle to the skin, and angle of injection.[120–125] This section focuses on jet injectors for SC and IM delivery of vaccines; devices capable of ID delivery are discussed "Cutaneous Vaccination" later.

Over the past half-century, jet injectors have been used to administer hundreds of millions, if not billions, of vaccine doses for mass campaigns in humans against smallpox,[126–132] measles,[133–135] polio,[136,137] meningitis,[138–140] influenza,[141,142] yellow fever,[143,144] cholera, and other diseases.[128,129,145–149] During the swine influenza mass campaign of 1976–77 in the United States, a substantial proportion of the approximately 43 million doses administered were by jet injection (Centers for Disease Control and Prevention [CDC], unpublished data).[150,151] Jet injectors have also been used for a wide variety of therapeutic drugs.[152–171] In recent years, the devices have been used to administer antigens to humans and animal models for a variety of investigational vaccines, including dengue,[172–175] herpes simplex type 2,[176] HIV/AIDS,[177–179] Japanese encephalitis,[180] malaria,[181] and melanoma.[182] A wide variety of investigational recombinant nucleic acid vaccines are being delivered in preclinical and clinical trials using various jet injectors.[183–198]

Because of safety issues with the earlier generation of MUNJIs, a new generation of DSJIs has been developed, many with features designed to be suitable for global immunization programs.

Multiuse Nozzle Jet Injectors. Jet injector technology was invented in France in the 1860s[199,200] and reintroduced in the 1940s as the Hypospray[157] for patient self-injection with insulin. In the 1950s, the U.S. military developed a high-speed system called the Ped-O-Jet[201] (Fig. 68.4A), and the units once referred to as jet guns were widely used for mass-vaccination programs.[127,202–206]

A large body of clinical literature shows the immunogenicity of vaccine delivered by jet injectors to be usually equal to, and sometimes better than, that induced by conventional needle and syringe for a wide variety of vaccines.[207,208] The pain associated with jet injection depends on the medication or vaccine involved. Insulin, other nonirritating drugs, and vaccines without adjuvants are usually reported to result in reduced or equivalent pain compared with needles,[121,124,133,141,154,171,209,210] but not always.[211] Vaccines with aluminum adjuvants or other irritating components tend to result in higher frequencies of mild, transient, local reactions (e.g., soreness, edema, erythema) when jet injected, probably because small amounts remain in the track left through the skin.[212–224] Bleeding and, less often, ecchymosis are reported to occur at the jet injection site more frequently than with needle injections.* Rarely, the jet stream causes a laceration if the injector moves during injection.[132,195,200,204] Safety features on some modern DSJIs reduce this risk.

Beginning in the 1960s, concerns arose for potential iatrogenic transmission of bloodborne pathogens by MUNJIs, which use the same nozzle to inject consecutive patients without intervening sterilization.[229,230] Bench and animal studies indicated cross-contamination could occur because blood or virus remained in nozzle orifices despite recommended alcohol swabbing between injections.[231–233] Fact superseded theory when a Med-E-Jet caused an outbreak of several dozen cases of hepatitis B among patients in a California clinic.[234] Subsequent clinical,[235] field,[236,237] bench,[238] animal,[239,240] and epidemiologic[241,242] studies added more evidence that MUNJIs could transmit pathogens between patients. This led to warnings and discontinuation of their use by public health authorities[24] and to market withdrawal of the Ped-O-Jet and discontinuation of its U.S. military use in 1997.[243,244]

*References 121, 124, 136, 137, 141, 154, 158, 167, 170, 202, 204, 209, 213, 225–228.

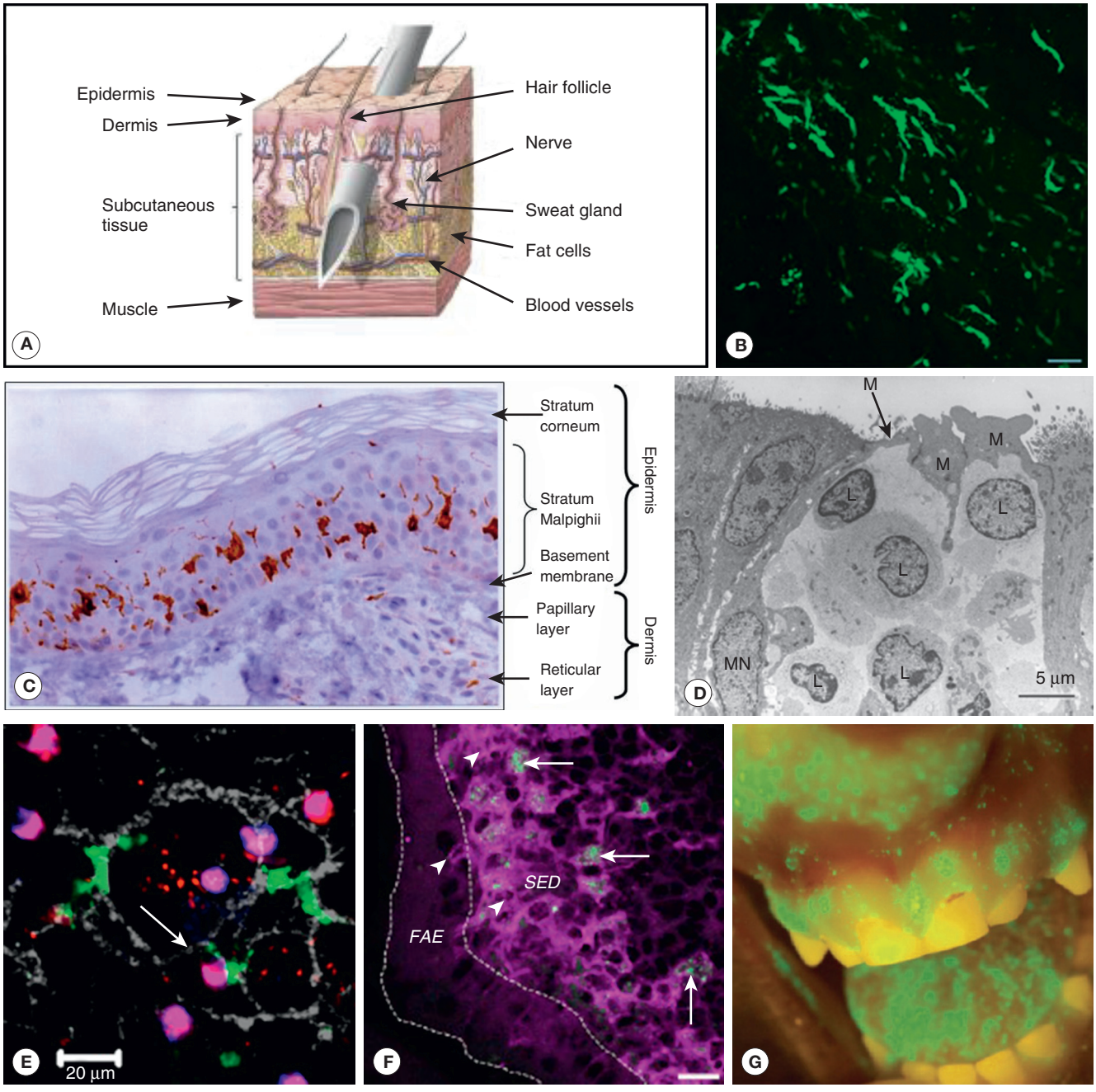

Figure 68.3. Examples of vaccine antigen delivery target cells and tissues. A, Diagram of subcutaneous injection showing epidermal, dermal, subcutaneous, and muscle tissue layers. **B,** Confocal microscopy of macaque muscle tissue 5 days following IM injection of live attenuated measles vaccine strain encoding enhanced green fluorescent protein (EGFP) demonstrates virus concentration in in dendritic cells by green fluorescence. Scale bar, 50 µm.[714] **C,** Activated Langerhans cells (dark stain) in the epidermal Malpighian layer 48 hours after immunization by application of cutaneous patch containing heat-labile enterotoxin of *Escherichia coli.* Full depth of dermis not shown. **D,** Transmission electron micrograph of nasal-associated lymphoid tissue from excised human adenoids, showing lack of apical cilia at the endothelial lumen *(top)* of an M cell *(M)*, the M cell nucleus *(MN)*, and the lymphocytes *(L)* enfolded in the cell's invaginated pocket, which remains contiguous with the extracellular space. M cells sample particulates from the lumen, presenting them to lymphocytes, macrophages, and dendritic cells, which congregate in the pockets. **E,** Two-photon intravital imaging of mice lungs: alveolar macrophages stained blue with rhodamine B-dextran appear purple when laden with *Bacillus anthracis* spores stained red with Alexa Fluor 633. Alveolar outline shown in gray. The white arrow on the lower image highlights the contact between infected macrophage and a green fluorescent protein (GFP) expressing dendritic cell (DC). Alveolar macrophages capture spores within minutes, DCs transport spores to the lymph nodes. Bar, 20 µm. **F,** Confocal laser scanning microscopy of murine Peyer patch. After administration by gavage, glucan particles *(green; arrows)* traverse follicle-associated epithelium (FAE) M cells and accumulate within $CD11c^+$ DCs *(magenta; arrowheads)* located within the subepithelial dome (SED). Scale bar, 50 mm. **G,** Macroscopic EGFP fluorescence in macaque mouth 9 days following infection with wild-type measles virus encoding EGFP shows extensive measles virus infection in tongue, buccal mucosa, gingiva, and tonsils (not shown). (***A,*** *From Clayton BD, Stock YN.* Basic Pharmacology for Nurses, *ed 13. St. Louis: Mosby; 2004.* ***B,*** *From Rennick LJ, de Vries RD, Carsillo TJ, et al. Live-attenuated measles virus vaccine targets dendritic cells and macrophages in muscle of nonhuman primates.* J Virol. *2015;89[4]:2192–2200.* ***C,*** *From Glenn GM, Taylor DN, Li X, et al. Transcutaneous immunization: a human vaccine delivery strategy using a patch.* Nat Med. *2000;6:1403–1406.* ***D,*** *From Fujimura Y. Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans.* Virchows Arch. *2000;436:560–566, 2000.* ***E,*** *From Fiole D, Deman P, Trescos Y, et al. Two-photon intravital imaging of lungs during anthrax infection reveals long-lasting macrophage-dendritic cell contacts.* Infect Immun. *2014;82[2]:864–872.* ***F,*** *From De Jesus M, Ostroff GR, Levitz SM, et al. A population of Langerin-positive dendritic cells in murine Peyer's patches involved in sampling beta-glucan microparticles.* PLoS One. *2014;9[3]:e91002.* ***G,*** *From Ludlow M, de Vries RD, Lemon K, et al. Infection of lymphoid tissues in the macaque upper respiratory tract contributes to the emergence of transmissible measles virus.* J Gen Virol. *2013;94[Pt 9]:1933–1944.)*

Figure 68.4. Selected devices for subcutaneous and intramuscular vaccine delivery. A, Ped-O-Jet (Keystone Industries), the most widely used multiuse nozzle jet injector (MUNJI) worldwide, before withdrawal from public health use by the 1990s for cross-contamination risk. Its metal spring is compressed by hydraulic fluid pumped by a foot pedal in its carrying case (**A,** *inset*) or by electric pump (not shown). Depth of delivery determined by removable nozzle used, either a subcutaneous (SC)/IM nozzle (shown here) or an intradermal (ID) nozzle (see Fig. 68.3E). **B,** Bioject Biojector 2000 DSJI, powered by gas. It has Food and Drug Administration (FDA) clearance for SC, IM, and ID delivery. **C,** PharmaJet Stratis DSJI, for 0.5-mL dose delivery. Delivery IM or SC set by vaccinator technique (fat layer pinched up for SC). Spring is reset using separate carrying case. Syringe is filled by pulling back and breaking off its blue shaft and thumb tab from conventional single-dose and multidose vials using needle-free vial adaptor (not shown). On insertion into device, any excess liquid is returned to vial to minimize wastage of overfill. Cleared for U.S. marketing by the FDA in 2011 and received World Health Organization (WHO) prequalification in 2013.[715,716] **D,** Biojet ZetaJet metal-spring-powered DSJI, features built-in crank for manual recocking of metal spring (Bioject Medical Technologies). Uses different autodisabling cartridges for SC, IM, and ID injections (SC and IM delivery was licensed by U.S. FDA in 2009; ID use is investigational). **E,** Investigational LectraJet HS (high-speed) motorized DSJI (D'Antonio Consultants International) features built-in motor and rechargeable battery for rapidly compressing metal spring between injections at rates exceeding 600 per hour, with battery capacity of >3000 injections per charge. Capable of rapid loading and unloading of single-use syringes from (**E,** *inset*) a sterile-packaged, 30-unit magazine for mass vaccination. **F,** Med-Jet H4 DSJI (Medical International Technology), gas-powered with entirely disposable single use fluid path. Licensed in Canada in 2014. A manually powered DSJI, the Dart (not shown), is in development and uses the same disposable cartridges. **G,** Investigational Solid Dose Injector (SDI) from Glide Pharma is powered by a compressed metal spring, which is released as the disposable drug cassette (white component extending beyond blue hub) is pressed fully against the skin. It inserts a (**G,** *inset*) pointed, hardened, approximately 1-mm-diameter drug formulation (shown compared with conventional matchstick tip) into subcutaneous tissues, where it dissolves. (***A*** *and* ***inset,*** *Courtesy James Gathany, Greg Knobloch [CDC Photographic Services].* ***B*** *and* ***D,*** *Courtesy Bioject Medical Technologies.* ***C,*** *Courtesy PharmaJet, Inc.* ***E,*** *Courtesy D'Antonio Consultants International, Inc.* ***F,*** *Courtesy Medical International Technologies [MIT Canada] Inc. [Karim Menassa].* ***G,*** *Courtesy Glide Pharma.)*

In the mid-2000s, a MUNJI was reengineered with disposable caps to try to prevent contaminating blood or tissue fluid from splashing back onto the reusable nozzle. However, after injections with saline of volunteers who carried hepatitis B virus, 8% of subsequent injections into vials—representing the next vaccinees in a clinic or mass campaign—were found to contain hepatitis B antigen.[245] High-speed microcinematography also revealed extensive splash back from the skin during injection with MUNJIs.

This body of evidence supports the conclusion that the design of MUNJIs is inherently unsafe for use in immunization settings, and any reuse of fluid pathways or unsterile components that are in direct or indirect contact with consecutive patients should be abandoned.[25] Even if contamination could be shown to be extremely rare, it is unlikely that policy makers could be convinced to set any level of acceptable risk.[246] Although public health authorities recommend against MUNJI use for vaccination,[25,247] MUNJIs continue to be used in the United States and other countries in clinical specialties such as dentistry, urology, and podiatry where cleaning and sterilization (such as autoclaving) of the entire device fluid path between patients is possible. Also, the Med-Jet line of MUNJIs has received licensure in multiple countries including Canada, China, and Russia for human applications including physiatrics, dermatology, and mesotherapy indications.[248] MUNJIs allowed a single health worker to vaccinate 600 or more patients per hour.[132] The withdrawal of the device posed challenges for conducting mass-immunization campaigns for disease control programs and in response to pandemic or bioterrorism threat.

Disposable-Syringe Jet Injectors. To overcome concerns about MUNJIs and their withdrawal, a new generation of safer DSJIs has been developed since the early 1990s.[249–252] Each sterile syringe (or cartridge) has its own orifice and nozzle and is discarded between patients. Although some are used for self-administration of insulin, other hormones, and drugs such as sumatriptan for migraines,[253] others are targeted for vaccine administration.

One early system, developed by predecessor companies of Sanofi Pasteur, was the manufacturer-prefilled Imule syringe for use in the Mini-Imojet DSJI.[254,255] Although demonstrated in the clinic and field to be immunogenic and safe for several vaccines,[256] the system was eventually abandoned upon corporate merger. The pioneering DSJI for the vaccine market was the Biojector 2000 (see Fig. 68.4B), introduced in the United States in the 1990s. During the first decade of the 2000s, it delivered approximately 1 million IM and SC vaccine doses per year at private, public, and U.S. Navy and Coast Guard immunization clinics in the United States, and it has been used in many vaccine studies, including a number of investigational recombinant nucleic acid vaccines.*

To meet developing-world needs, several DSJIs have been developed that are economical, autodisabling to prevent reuse, and suitable for mass campaigns and routine immunization. PharmaJet entered the market in 2009 with licensure of its eponymous device for IM and SC injections. A recent study with this now-discontinued DSJI found that delivery of MMR vaccine did not meet noninferiority criteria in comparison with needle and syringe delivery.[267] PharmaJet subsequently received market clearance for the Stratis device (see Fig. 68.4C), which was the first DSJI to receive WHO prequalification, in 2013. Various PharmaJet models have been studied for veterinary[186–188,268] and human[164,165,255,257,258,269–271] applications. In a bench study testing injection of MMR vaccine through pig skin into a vial with three PharmaJet injectors, there was no loss of measles or mumps vaccine virus infectivity but some loss of rubella infectivity with two of the devices.[271a] Other examples of DSJIs developed for vaccine delivery include the Bioject ZetaJet (see Fig. 68.4D),[272] the DCI Lectrajet (see Fig. 68.4E),[273,274] and the MIT Med-Jet H-4 (see Fig. 68.4F) and Dart.[248]

Use of DSJIs in the United States was curtailed by a 2011 FDA communication advising that vaccines should be delivered with a needle and syringe unless specifically labeled for jet injector delivery. Following consultation with the FDA, PharmaJet and bioCSL conducted a clinical trial demonstrating noninferiority of delivering bioCSL's Afluria influenza vaccine with a DSJI compared with a needle and syringe.[271] Based on these data, in 2014 Afluria was licensed by the FDA for delivery with the PharmaJet Stratis device. This "vaccine relabeling" has enabled DSJIs to reenter the U.S. market; however, the FDA-approved language limits delivery to a specific manufacturer's DSJI device.

Advantages of DSJIs are the elimination of needlestick injuries associated with possible transmission of bloodborne pathogens to health workers as well as reduction in the volume of sharps waste disposal, which is not required for used DSJI syringes. They also have the potential to increase acceptability and immunization coverage rates. Reduced crying has been observed when vaccines are delivered to infants with jet injection compared with needle and syringe injections.[258,267] However, like syringes with needles, DSJI syringes require onsite filling following vaccine reconstitution (if required). Prefilled DSJI syringes in development by PharmaJet and other manufacturers may enable greater efficiency and speed of administration, similar to that of the old generation of MUNJIs. Use of DSJIs likely will entail higher costs because of the higher cost of manufacturing a DSJI syringe and the cost of the durable injector, but modeling indicates the potential for overall cost savings compared with needles and syringes when the indirect costs of iatrogenic disease resulting from the latter are included.[257,275] As with all vaccination systems that require a durable administration device, distributability of the vaccine is restricted by the need to have a device available at every POC when vaccine is delivered. The cost of a jet injector can be negligible if amortized over many doses in high-vaccination–volume settings. However, in POCs with low-vaccination volumes, the cost of these devices may be prohibitive.

Solid Dissolving Needles

Dissolving needles are a novel biodegradable implant technology consisting of solid doses of vaccine that are administered subcutaneously. A disposable cartridge containing the implant is loaded into a reusable handheld applicator that uses gas or spring pressure to insert the implant into the skin. Once the implant is in the tissue, it begins to dissolve and the vaccine is released.

The Bioneedle Technologies Group (The Netherlands) has developed Bioneedles, small implants of biodegradable polymer (extruded starch) filled with concentrated liquid vaccine, which are freeze-dried. After insertion into the SC tissue, the implants have been shown to break down within a few hours and dissipate from the delivery site within 3 days. Administration of a placebo Bioneedle implant in a clinical trial found adequate safety and acceptability of the technology and no histological findings at the implantation site. Administration of tetanus toxoid, hepatitis B, influenza, and IPVs via Bioneedles has been studied in animal models.[276–279] In Wistar rats, the same dose of IPV given by SC or IM liquid injection or by Bioneedle implantation was found to produce similar antibody titers after two immunizations.

*References 154, 160, 161, 175–179, 182–185, 188–191, 198, 211, 214, 217, 254, 256a, 258–266.

Glide Pharma (United Kingdom) has also developed a solid-dose injector. It uses a spring-loaded device to quickly push into SC tissue a sharp, biodegradable implant the size of a grain of rice (see Fig. 68.4G). The solid-dose injector has been tested with influenza, diphtheria, and *H. influenzae* vaccines and is in development with an anthrax vaccine.[282]

Potential benefits of this technology class include dry-format unit-dose administration; increased thermostability of the solid vaccine formulation in comparison to a liquid formulation; reduced cold chain footprint because of the small dose and package size; avoidance of needles and syringes with reduced risk of needlestick injury and reuse; and potential for reduced number of booster doses if slow release is successfully demonstrated to provide improved immunogenicity. Challenges to development include demonstrating the feasibility of the formulation and manufacture of solid vaccine implants, acceptability, and the complexity and costliness of the administration device.

Cutaneous Vaccination

Cutaneous administration of vaccines includes delivery to either the dermis or epidermal layers of the skin. The skin was one of the first tissues into which variola (smallpox) virus and, later, cross-protecting cowpox virus were introduced to prevent smallpox. The cutaneous route for variolation involved breaking the skin with a sharp instrument, and it was used in India and China at least as early as the 16th century. Variolation was supplanted by safer cutaneous application of material from cowpox lesions (vaccinia virus), the 18th-century method first given the term *vaccination* and first published by Edward Jenner. The cutaneous route remains the standard for smallpox vaccine (see Chapter 33) as well as for administering BCG to prevent tuberculosis (see Chapter 54). Vaccines for poliovirus[284] and yellow fever[285] were also delivered by this route in the past. The cutaneous route has demonstrated and hypothetical advantages over other delivery methods, including the potential to enable dose-sparing.[286–307] Cutaneous delivery is often associated with an increase in mild local reactions, because of the visibility of the administration site, but is less likely to result in unanticipated serious adverse reactions than other routes. Mixed results for the immunogenicity of the ID route have been reported for a variety of live attenuated, inactivated, and subunit vaccines, including those from dose-sparing studies for currently available vaccine formulations as well as for investigational vaccines, particularly DNA vaccines.

In a classical ID injection, a bolus of liquid is deposited into the dermis to raise a visible bleb, as in the traditional Mantoux method. A variety of novel delivery methods and technologies have been developed to deliver vaccines into the skin with the goals of (a) improving the ease, acceptability, and safety of vaccination, and (b) taking advantage of the potential for skin tissue to enhance immunogenicity or enable dose-sparing.

Anatomy

The outermost layer of the skin is the epidermis (see Fig. 68.3A), a stratified squamous epithelium that is usually about 0.1-mm thick but can be from 0.8 to 1.4 mm on the palms and soles. The stratum Malpighi layer is the primary component of the epidermis, and its dividing and growing keratinocytes serve both a structural function—limiting the passage of water and other molecules—and an immunologic role. Keratinocytes germinate just above the basement membrane, which demarcates the boundary between epidermis and deeper dermis. These cells then grow, flatten, mature, and senesce in increasingly superficial strata until they reach the surface and are sloughed. The main product of this cell is keratohyalin, a dense lipid that helps form a waterproof barrier. The lateral edges of adjacent keratinocytes are tightly linked by desmosomes, which maintain the strength of the epidermis and also contribute to its resistance to the passage of foreign matter or molecules.[308,309]

The topmost horny layer of the epidermis is the stratum corneum, composed of staggered courses of dead keratinocytes—also known as corneocytes—in a lipid bilayer matrix. This stack of 10 to 20 cells, 10 to 20 µm thick, is the principal obstacle to the introduction of vaccine antigen for cutaneous vaccination. Below the epidermis and basement membrane lies the dermis, approximately 1.5 to 3.0 mm thick, in which fibroblasts, fine collagen, elastic fibers, and most skin organelles—including small blood vessels, lymphatic vessels, nerves, hair follicles, and sweat and sebaceous glands—are found.

Skin thicknesses have been mapped in children to identify the histologic suitability of sites for cutaneous vaccination. Equally important is selecting skin sites that are easily accessed so as to minimize disrobing and loss of privacy. In smallpox eradication, the volar surface of the forearm was commonly used because it was quickly accessible.[126]

Immunological Rationale

The specific mechanisms that produce an immune response when vaccine antigen is introduced into the skin are not entirely clear. With stimulation, keratinocytes can produce proinflammatory cytokines (e.g., interleukin-1) and can function as APCs by displaying major histocompatibility complex class II antigens (human leukocyte antigen–DR) as well as intercellular adhesion molecule-1. Epidermal Langerhans cells—a type of APC—are believed to play a key role in cutaneous immunization (see Fig. 68.3C), although dermal dendritic cells and other well-known immune system components such as $CD8^+$ and $CD4^+$ T lymphocytes, mast cells, and macrophages also circulate or reside in the epidermis or dermis.[298,299,302,310–314]

The immature Langerhans cells reside like sentinels among the keratinocytes in the epidermis, composing about a quarter of the skin surface area,[315] where they efficiently capture foreign antigens by phagocytosis or endocytosis. Like dendritic cells in other tissues (see Chapter 2), on activation (see Fig. 68.3C) these professional APCs process the antigen as they migrate to draining lymph nodes. There, now mature, they express high levels of class II major histocompatibility complex molecules and present the antigen brought from the skin to T-helper lymphocytes, a critical step for the subsequent immune responses orchestrated by the latter cells.

Macrodelivery of vaccines precisely into the extremely thin epidermal tissue or the dermis layer creates a technological challenge that has resulted in a wide array of cutaneous delivery methods and devices. Researchers investigating cutaneous delivery of existing vaccines and those in development have used the Mantoux method of injection by needle and syringe (see "Mantoux Method" later) as well as a variety of novel devices such as ID jet injectors, mini-needles, microneedles, electroporation devices, and microarray patches.

Traditional Methods

During the more than 200 years of cutaneous vaccination against smallpox (see Chapter 54), a variety of sharp

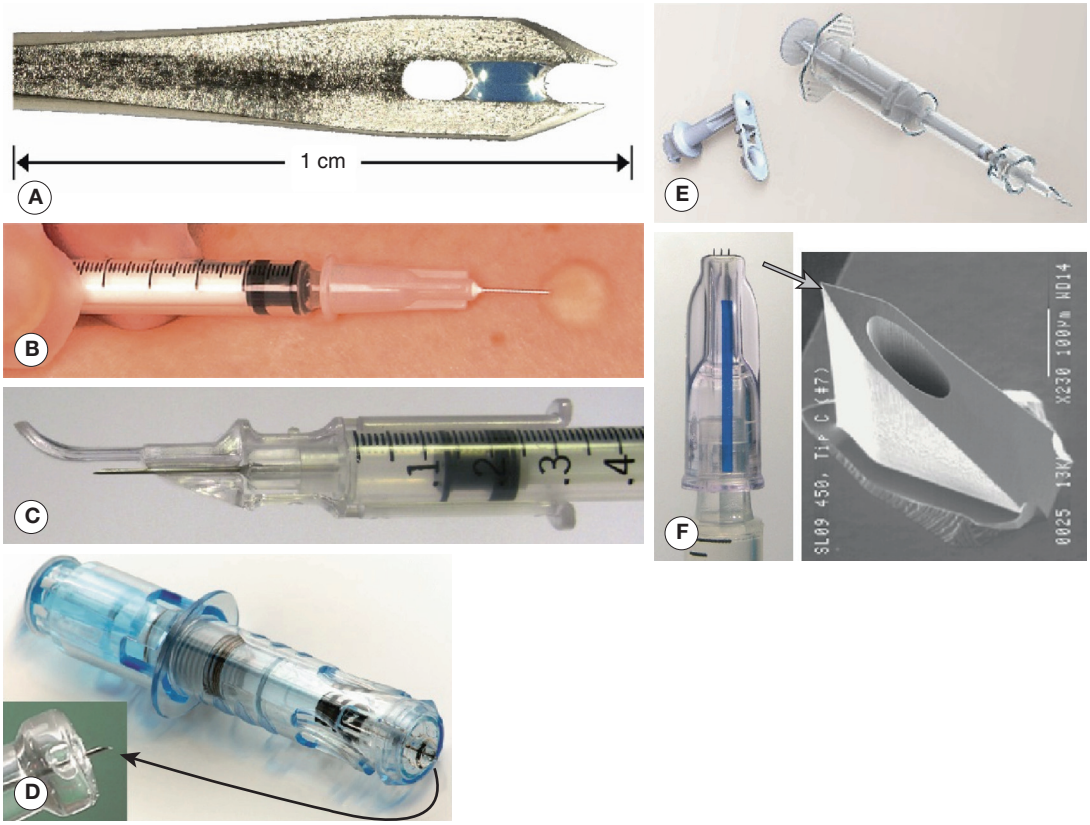

Figure 68.5. Selected needle-based technologies for cutaneous vaccine delivery. A, Pronged end of bifurcated needle (full length is 5 to 7 cm), which is the current preferred device for smallpox vaccination, holds between its tines approximately 2.5 μL of fluid by capillary action. **B,** Traditional Mantoux method for creating intradermal (ID) wheal using 26-gauge hypodermic needle and conventional 1-mL syringe. **C,** ID adapter for conventional needle and syringe for quick and consistent Mantoux ID injection (West Pharmaceutica Services), the length and depth of the protruding needle underneath the "Ski-tip" guide manipulates the skin to optimize needle placement regardless of bevel orientation.[338,339] **D,** Prefilled version of Soluvia mini-needle ID syringe (BD Micro-Delivery System; Becton, Dickinson and Co.). Used for ID delivery of Sanofi Pasteur brands of inactivated influenza vaccine (Intanza, IDflu,[717] Fluzone Intradermal[718]).[719] **D** *inset,* The 30-gauge staked mini-needle projects 1.5 mm beyond its hub to limit the depth of injection upon perpendicular insertion into the skin. Marketing of vaccine-device combination product approved in European Union in 2009 and in United States in 2011. **E,** ID syringe in development (Star Syringe) with an autodisable feature and an integrated plastic spike for filling from a vial, and a mini-needle adapter for providing an ID injection. **F,** MicronJet hollow microneedle device with Luer fitting onto conventional syringe for ID delivery (NanoPass Technologies Ltd). Cleared for marketing in the European Union and the United States. The blue line on the hub indicates to the user that the bevel and lumen of the microneedle needle are on the opposite side. **F** *inset*, Microphotograph shows the pyramidal shape and lumen of an individual microneedle. *(**A,** Courtesy James Gathany [CDC Photographic Services]. **B,** Courtesy of James Gathany and Greg Knobloch [CDC Photographic Services]; **C** and **F,** courtesy of Bruce G. Weniger; **D,** courtesy of Sanofi Pasteur; **E,** courtesy of Star Syringe Ltd [Paul Mallins]; **F,** from Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, et al. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults.* Vaccine. *2009;27[3]:454–459.)*

instruments have been used to penetrate the epidermis (and avoid penetrating unnecessarily deeper into the dermis) for inoculation of cowpox or vaccinia virus. These included scarification with a needle, scalpel, lancet, or knife and rubbing vaccine into the resulting lesion; twirling a rotary lancet in a drop of vaccine on the skin; and the multiple-pressure method of pressing a straight surgical needle sideways into the skin through a drop of vaccine. Multitine devices consisting of multiple short needles in a circular or square array dipped in solution and pressed into the skin have also been used.[316,317]

The BCG vaccine for the prevention of disease from *Mycobacterium tuberculosis* was originally administered orally in the 1920s (see Chapter 60). Safety concerns prompted a shift to cutaneous administration by ID needle injection using the Mantoux technique in 1927. BCG has also been delivered cutaneously by multiple puncture devices,[318–321] scarification, jet injectors,[322] bifurcated needles (Fig. 68.5A),[323] and multitine devices,[316,324] but the Mantoux method remains the standard delivery method (see Fig. 68.5B).

Bifurcated Needle. In the 1960s, Benjamin Rubin invented the bifurcated needle (see Fig. 68.5A), for which Wyeth waived the royalties so that the WHO could produce it for smallpox eradication. The device holds approximately 2.5 μL of fluid by capillary action between its tines and is applied perpendicularly into the skin. This uses one-fifth of the typical dose volume needed by earlier multiple-pressure methods, but it

requires a higher virus concentration. Its simplicity, portability, and economy greatly facilitated the latter half of smallpox eradication, particularly in Asia and East Africa.

Mantoux Method. The needle technique for classical ID injection, as used for BCG, was developed in the early 20th century by Felix Mendel and separately by Charles Mantoux for the administration of tuberculin (now replaced by purified protein derivative) used for diagnosis of tuberculosis infection. Now referred to as the Mantoux method (see Fig. 68.5B), this procedure has become the common route for ID injection of both BCG and rabies vaccines. Although the latter was typically delivered intramuscularly, research found that reduced doses of rabies vaccines delivered via ID are similarly effective for both postexposure and preexposure prophylaxis regimens.[325–333] Because of the high cost of cell-culture rabies vaccines, the WHO has recommended ID delivery for resource-constrained settings since 1991, and this route is widely used in the developing world, particularly in Asia.[333a,334–336]

In the Mantoux technique, a short-bevel, fine-gauge needle, usually 27 gauge, is inserted, bevel up, at a 5- to 15-degree angle into slightly stretched skin, often the volar surface of the forearm. The tip is advanced approximately 3 mm until the entire bevel is covered. Upon injection of fluid, proper location of the bevel in the dermis creates a bleb, or a wheal, as the basement membrane and epidermis above are stretched by the fluid (see Fig. 68.5B). Leakage onto the skin indicates insufficient penetration to cover the bevel. Failure to produce a bleb indicates an improperly deep location of the fluid in the SC tissue. Drawbacks to the Mantoux method are the training, skill, and extra time needed to accomplish it correctly.

Needle-Based Technologies

Intradermal Adapters. A novel ID adapter (see Fig. 68.5C), designed to improve ease and consistency of the traditional Mantoux technique (see Fig. 68.5B), fits over a conventional syringe with a fixed needle and guides the needle to its appropriate position in the skin to produce the desired bleb. The device is marketed by West Pharmaceutical Services[337] and has FDA clearance. In clinical studies, the ID adapter has been found to reliably produce blebs, regardless of the orientation of the needle bevel during injection.[338,339] Similar to a fill-on-site mini-needle device, the ID adapter has the potential to be a relatively low-cost option to increase the ease and reliability of ID injections. Because the angle of injection mimics the Mantoux technique, it may also serve as a training tool for users inexperienced with Mantoux. One challenge is that the device design is syringe specific and must be paired with a particular brand of syringe.

Mini-Needles. As for cutaneous vaccination in general, a diverse terminology is applied to microscopic projections for perforating the superficial skin to deliver the drug or vaccine.[340–343] This chapter uses the term *mini-needles* for hollow projections of 1 mm or longer for liquid delivery. The term *microneedles* refers to hollow projections shorter than 1000 µm. Microarray patches are arrays of solid projections (without lumens) shorter than 1000 µm. These patches deliver vaccine in a dry format and are not classified as needles.

To circumvent the amount of skill and time needed for successful Mantoux injection, BD developed a prefilled glass syringe with a staked 30-gauge mini-needle that projects only 1.5 mm beyond its depth-limiting hub for intuitive perpendicular insertion into the skin (see Fig. 68.5D). Termed the Soluvia Micro-Delivery System, it was licensed exclusively by Sanofi Pasteur for certain vaccine applications. Sanofi Pasteur undertook a series of clinical trials with trivalent inactivated influenza vaccine,[345–349] which led to marketing approval in Europe in 2009 for ID delivery of its Intanza and IDflu products. These contained either 9 µg of viral hemagglutinin per strain per 0.1 mL for adults through age 59 years,[350] or a full (non–dosage-sparing) 15 µg for those age 60 years and older.[345–347] Sanofi Pasteur's U.S. brand of ID influenza vaccine (Fluzone Intradermal), similarly containing 9 µg per strain, was found to induce geometric mean titers of hemagglutination-inhibiting antibody that were noninferior to those of control patients receiving conventional Fluzone by the IM route with 15 µg per strain. In 2011, the FDA licensed the vaccine and its unique prefilled delivery system with an indication that it be used only for patients 18 to 64 years of age. Several other countries (e.g., Canada, Australia, and New Zealand) have also licensed a Sanofi influenza vaccine in the Soluvia mini-needle delivery system.

A fill-on-site version of the Soluvia mini-needle syringe was used in a rabies vaccine clinical trial and demonstrated protective seroconversion comparable to a full dose by the IM route. In preclinical animal experiments, delivery with this device produced good immune responses to anthrax recombinant protective antigen,[351–353] conventional hemagglutinin and plasmid DNA antigens for influenza, and live recombinant yellow fever vector for Japanese encephalitis vaccines.[354]

Novosanis has developed another prefilled mini-needle device, the VAX-ID, which was tested for delivery of saline in humans.[355] Star Syringe has developed a fill-on-site mini-needle ID syringe (see Fig. 68.5E). The Star ID device is designed to be low cost and suitable for use with existing vial formats, is filled through an integrated plastic spike, and has an autodisable feature. A clinical study assessing the injection performance with this device is planned.

Mini-needle devices can facilitate ID delivery and enable the dose-sparing potential of some vaccines while retaining the familiar interface and use method of common IM needle and syringe injections. ID injections with the Soluvia device frequently do not produce the characteristic wheals in the skin that are indicative of correct Mantoux injection technique,[356,357] so some education for healthcare workers may be needed. Prefilled presentations such as the Soluvia and VAX-ID offer additional benefits in ease of use and dose accuracy, but they are more expensive and require much more cold chain storage space than vial presentations. The recent development of low-cost fill-on-site devices such as the Star ID syringe, if demonstrated to perform clinically, could be more appropriate for the requirements of LMIC immunization programs.

Hollow Microneedles. Hollow microneedles, which deliver liquid vaccines intradermally, share attributes with the needle-based technologies for ID delivery described previously (see "Mini-Needles" and "Intradermal Adapters" earlier). Hollow microneedles are designed to inject therapeutic liquids through their tiny lumens and are used with either a syringe or a small wearable pump.[292,303,305,342,358] Flow rates of microneedles have been measured up to a remarkable 1 mL/min per cannula. Common lengths of 200 to 1000 µm are short enough, in theory, to be painless because they would not reach nerve endings in the dermis.[294,358–360]

The MicronJet 600 device developed by NanoPass is unique in its availability as a licensed, sterile, disposable microneedle device. It consists of three hollow microneedles 600 µm in height in a beveled pyramidal shape. These are mounted on an adapter with a Luer interface for fitting onto a conventional syringe for liquid vaccine or drug (see Fig. 68.5F). In 2010, it was cleared by the FDA for injection of any drug approved for ID delivery. It also holds a CE mark for marketing in Europe.

Adult volunteers vaccinated intradermally with a similar MicronJet version (four microneedles 450 µm in height)

received reduced single doses of 3 or 6 µg per strain, or full 15-µg doses by IM route, of licensed alpha-Rix (Fluarix, GlaxoSmithKline) 2007–08 seasonal influenza vaccine. By day 21, all three study arms developed comparable increases in antibody titers.[361] Local reactions were more frequent with MicronJet than by the IM route, but they were mild and transient. Similar dosage-sparing trials for 2009–10 monovalent H1N1 influenza vaccine and 2010–11 trivalent vaccine confirmed comparable or superior immune responses for the ID route versus IM. The MicronJet has also been studied for reduced-dose delivery of IPV[362,363] and varicella-zoster vaccine.[364]

Another hollow microneedle system is 3M's hollow microstructured transdermal system (hMTS).[289,369] Its patient-contact surface contains 18 microneedles of 500 to 900 µm in height, whose lumina of 10 to 40 µm in diameter delivers liquid volumes ranging from 0.3 to 1.5 mL. A spring-powered device contains liquid drug prefilled into a glass dose chamber. Upon triggering, a spike pierces the stopper of the chamber through which the dose passes and is forced slowly, over a period of 5 to 40 minutes, through the microneedles into the skin of the upper arm or thigh. Adhesive keeps the system in place until delivery is complete. Delivery of equine tetanus antitoxin to swine as a model for delivery of monoclonal antibodies resulted in pharmacokinetic profiles of tetanus antitoxin similar to dosages via SC injection.[369] Other industry and academic groups have pursued development of hollow microneedles, including Debiotech and the Georgia Institute of Technology.[249,370]

Hollow microneedles have advantages similar to those of other needle-based ID devices for delivery of liquid vaccines: they can increase reliability and ease of ID injection, potentially enabling vaccine dose-sparing. A clinical study found that delivery of influenza vaccine with the NanoPass MicronJet hollow microneedle device enhanced immunogenicity compared with the same dose of vaccine delivered ID by the Mantoux method, which suggests that the reliability and shallower depth of delivery of some hollow microneedle devices may enable superior immune responses.[371] However, separate needles or vial adapters are required to fill the syringes for hollow microneedle devices from a conventional vial, adding to the steps required for injection. Prefilled hollow microneedle technologies in development would simplify usage but are likely to increase costs and cold chain requirements.

Electroporation. Electroporation uses very short electrical pulses to produce temporary pores of nanometer-range diameters in the intercellular lipid matrix of the stratum corneum, which remain open and permeable for hours.[294,372–376] In vitro and in vivo preclinical studies of this technique demonstrated skin entry of larger molecules, such as heparin (12 kDa), peptides, proteins (such as luteinizing hormone–releasing hormone), and nucleic acids,[260,377–380] with potentially extensive use for investigational DNA vaccines in animals and humans.[194,381,382]

Inovio's CELLECTRA series of electroporation devices[383] and Ichor's TriGrid delivery system[385] are capable of ID and IM delivery. A hollow needle injects the drug conventionally, while parallel solid needles surrounding the injected dose create the current to generate pores in the target muscle tissue.[376,386,387] These devices have been tested preclinically and clinically for delivery of several DNA vaccines, including those for HPV,[388] HIV,[389,390] malaria,[391] and tuberculosis, with results demonstrating improved immunogenicity of the DNA antigen when electroporation is applied. Noninvasive surface electroporation devices have also been developed by Inovio and may have better acceptability.[392]

The Easy Vax and related Derma Vax epidermal electroporation systems combine the application of antigen- or drug-coated mini-needles 2 mm long, followed by electroporation. Smallpox antigen in plasmid DNA was dried onto the tips of arrays and inserted into the skin of mice, and when followed by six electric pulses, it induced protection from smallpox challenge.[393] A prostate cancer DNA vaccine was similarly administered.[394]

Electroporation has promise as an enabling technology for DNA vaccines, which have lower efficacy by traditional delivery methods. Acceptability is a challenge, particularly for IM electroporation devices, although ID devices have been found to be more tolerable.[395] The cost and complexity of the devices could preclude use in most low-resource settings.

Needle-Free Technologies

Jet Injection. Since the 1960s, multiuse nozzle jet injectors (see "Subcutaneous and Intramuscular Injection: Jet Injection" earlier) have allowed ID delivery of smallpox, BCG, and other vaccines via specialized nozzles or standoffs to achieve the shallower penetration required compared with IM or SC injection.[396,397–399] With discontinuation of the use of MUNJIs, DSJIs have been used for some ID injections.[128,129,249,400–402] For some of these devices, such as the Biojector 2000 and the Bioject ZetaJet, spacers can be added to a nozzle intended for SC delivery, creating a gap between orifice and skin, which weakens the jet and provides space for a bleb that leaves the dose in the skin. Other designs, such as the MIT Med-Jet line of gas-powered jet injectors and the MIT Dart spring-powered DSJI, can achieve ID delivery with variable pressure settings that allow the user to reduce the force of the injection stream. The PharmaJet Tropis (Fig. 68.6A) and the Bioject ID Pen (see Fig. 68.6B) are two new spring-powered DSJIs designed solely for ID delivery of 0.05 and 0.1-mL volumes.

The Ped-O-Jet (and, to a much lesser extent, other MUNJIs) administered tens of millions of smallpox vaccine doses for the first half of the WHO Smallpox Eradication Program in South America and West Africa from the late 1960s to the early 1970s, until invention of the simpler and swifter bifurcated needle.[126,132,399] MUNJIs also delivered yellow fever[128,143,404,405] and BCG vaccines[405a,406–410] by the ID route, as well as various tuberculosis skin testing antigens.[401,411–417] However, variations in subsequent tuberculosis skin testing reaction sizes when delivered by MUNJIs[322,418] led the WHO to discourage jet injector use for BCG and tuberculosis skin testing.[419,420]

To achieve polio eradication goals, injectable IPV is being introduced worldwide to supplement use of oral poliovirus vaccine (OPV). However, IPV is more expensive to produce and more difficult to deliver in campaign settings than OPV, promoting a search for cost-saving strategies that also avoid the introduction of needles into the polio eradication program. Recent clinical research has compared full doses of IPV given intramuscularly (0.5 mL) with ID delivery of 0.1 mL by either needle-free jet injectors or the Mantoux technique. In studies evaluating a primary series of immunizations in infants, some have found equivalent seroconversion rates, while others have shown reduced responses with ID jet injection.[258,259,421] In all the trials conducted in infants, ID delivery of reduced doses of IPV has resulted in lower titers of neutralizing antibodies, whether reported as geometric mean titers or median titers, and whether or not the study was performed in poliovirus-naïve infants or in infants receiving supplemental doses of IPV.[258,259,420,422] It has been suggested, however, that any detectable neutralizing antibody titer should be sufficient to prevent paralytic disease.[258] Clinical trials using a single dose of IPV for boosting immunity in previously immunized children and adults generally have found that ID delivery of a 20% (0.1 mL)

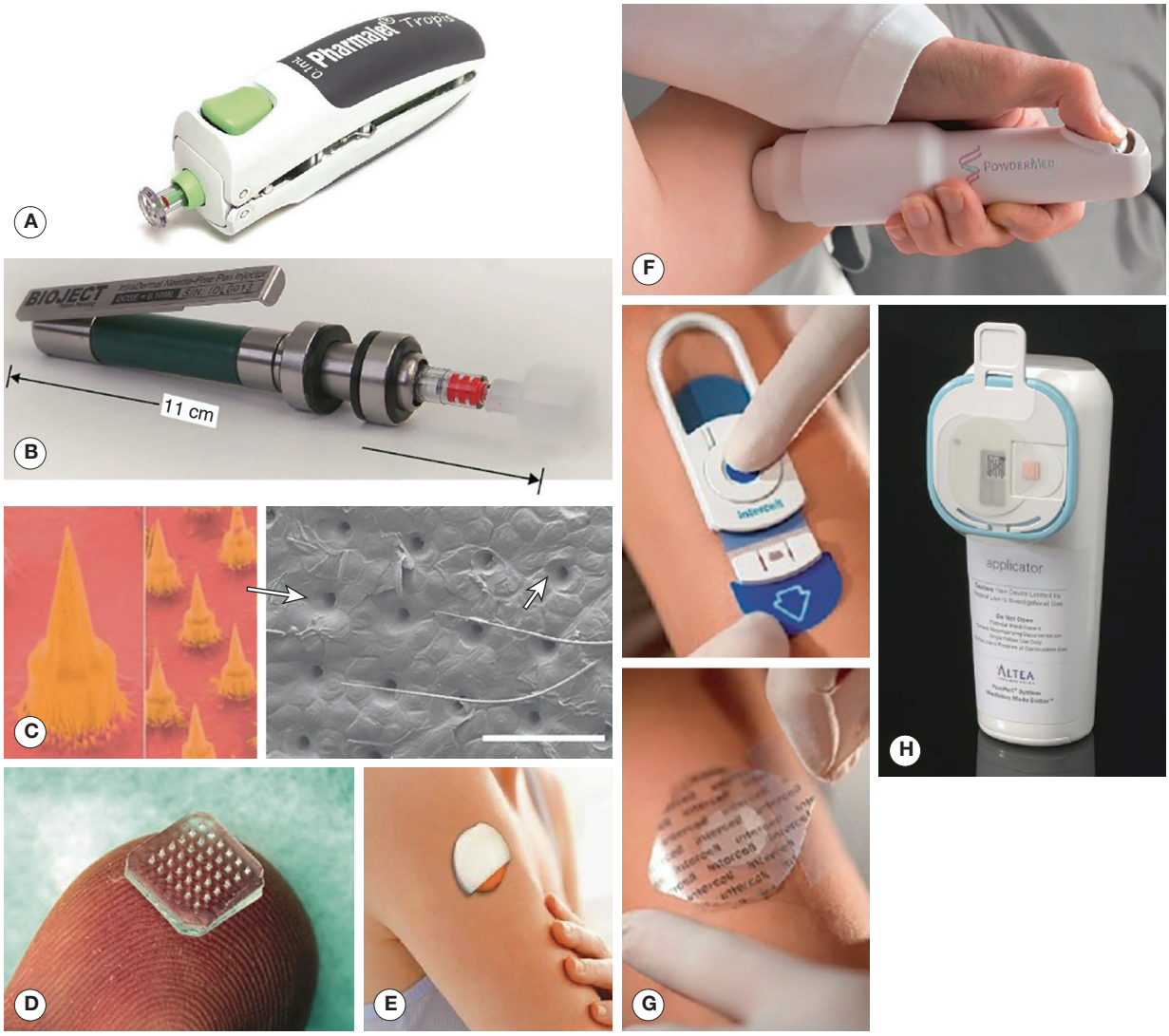

Figure 68.6. Selected needle-free technologies for cutaneous vaccine delivery. A, Investigational Tropis needle-free intradermal (ID) jet injector (PharmaJet). **B,** Investigational Bioject ID Pen needle-free jet injector for ID delivery of 0.1 mL (0.05-mL–dose model not shown) (Bioject Medical Technologies). Powered by metal spring reset with built-in lever. Disposable polypropylene spacer on disposable syringe creates the desired air gap to weaken the jet stream for ID delivery. **C,** *Left* and *middle*, Investigational Nanopatch microneedle array of silicon, after application to mouse skin. Microprojections are 30 μm wide at base and from 65 to 110 μm in height, and sputter-coated with 100 nm of gold. The red coating of antigen/adjuvant elutes to reveal the original gold coating.[721] **D,** Investigational dissolving microarray patch (Georgia Institute of Technology).[722] **E,** Corium dissolving microarray patch. Adhesive applicator provides correct pressure to apply patch and remains on the skin for several minutes while the patch projections dissolve. **F,** Investigational Particle-Mediated Epidermal Delivery (PMED) device (PowderMed) propels (usually gold) microparticles coated with (usually DNA) antigen into skin using a stream of supersonic helium gas. **G,** Investigational Skin Preparation System (SPS) for *transcutaneous immunization* (Intercell AG, originally developed by Iomai Corporation). Blue push-button requires the correct amount of abrasion pressure on the stratum corneum by a sandpaper-like strip pulled with the blue tab. **H,** Investigational PassPort thermoporation device and patch applied to patient arm. Heat induced by the device in metallic filaments embedded in the patch creates micropores in the stratum corneum for subsequent entry of drug within the patch (Altea Therapeutics). *(**A,** Courtesy of PharmaJet [Chris Cappello]. **B,** Courtesy Bioject Medical Technologies. **C,** Courtesy University of Queensland/Vaxxas [Mark Kendall]. **D,** Courtesy Jeong Woo Lee, Georgia Institute of Technology. **E,** Courtesy Corium International, Inc. [Bobby Singh]. **F,** Courtesy PowderMed. **G,** Courtesy Intercell AG [Andi Bruckner]. **H,** Courtesy Altea Therapeutics.)*

dose is immunogenic but often does not meet noninferiority criteria compared with full-dose IM delivery.[266,269,270]

A recent clinical trial of BCG vaccine given via ID found that DSJI delivery and Mantoux method delivery were similar in safety and immunogenicity.[423] Other vaccines that have been studied for ID delivery by DSJIs include those for HIV,[179,198] influenza, dengue,[424] and HPV.[425] Jet injectors for ID delivery offer the advantage of speed, simplicity, and patient acceptability as well as the ability to use off-the-shelf vaccines without reformulation. Challenges include developing devices that reliably deliver small ID doses to the correct depth,[266,269] obtaining clinical data to license vaccines for ID delivery by DSJIs, and the higher cost of DSJI devices compared with an ID needles and syringes.

Microarray Patches. Microarray patches (also referred to as microneedle patches) deliver solid formulations of vaccines into the skin and have a different set of potential benefits and development challenges from the liquid-delivery technologies for cutaneous vaccination that have been described. Microarray patches meet many of the criteria for a practically ideal vaccine delivery system because they are needle free in a prefilled unit-dose dry format, with potential for increased thermostability. They also can be shipped by common shipping methods such as mail and can potentially be self-administered or administered by minimally trained personnel. Microarray patches can be further subdivided into solid-coated and dissolving types.

Solid-Coated Microarray Patches. A common strategy pursued by a number of commercial and academic teams to carry antigens across the stratum corneum is to coat the vaccine onto solid microscopic projections, which are held for variable periods of time in the dermis or epidermal layer (depending on height) while the antigen (or other drug) elutes and diffuses.[290,293–295,297,301–305,342,343,426] Some microarray patch technologies have been designed for application by finger pressure, while other developers have found that a mechanical applicator is needed for consistency of skin penetration. To date, therapeutic drug applications have reached a more advanced stage of development than those for vaccines; nevertheless, a substantial amount of preclinical research has been conducted with vaccines, and several early-stage vaccine clinical trials are in progress.

One example of drug-coated microarray patches is the investigational Zosano Pharma ZP Patch platform (formerly known as Macroflux). Its titanium projections vary from 225 to 600 µm in height and are packed into an area of 1 to 2 cm^2 at densities from 140 to 650 tines/cm^2. They are inserted by a spring-mounted applicator and held in place by an adhesive patch. The most advanced applications for these microarray patches are delivery of parathyroid hormone to treat osteoporosis,[427] already studied clinically, and erythropoietin to treat anemia. Regarding vaccine applications,[428] Zosano Pharma's ZP-Flu influenza vaccine patch, applied for 5 or 10 minutes onto the skin, trended toward increased titers and seroprotection compared with an IM control injection.[430] Other preclinical studies of the system demonstrated delivery of ovalbumin,[428,431] oligonucleotides,[432] and the peptide hormone desmopressin.[433] The company reports animal work with tetanus, diphtheria, Lyme disease, and hepatitis B (DNA) vaccine antigens.

Another coated microarray patch platform is the solid microstructured transdermal system from 3M.[289,434] Its drug-coated pyramidal projections vary from 250 to 750 µm in height, in arrays of 300 to 1500 microarray patches mounted on an adhesive patch at a density of 1300 per square centimeter.[435–438] Application to the skin is by a manual finger-thumb Press&Patch device[439–441,480] or by a spring-powered applicator, shown elsewhere.[249] Coatings of the microarray patches are said to hold up to 0.5 mg of active pharmaceutical ingredient. In a rabbit model, coatings of tetanus toxoid and aluminum adjuvant in various ratios induced antibody using just a fraction of the standard IM dosage.[443] In swine, a model virus-like protein (HBsAg) demonstrated dosage sparing via solid microstructured transdermal system compared with antigen delivered by IM control route.[442] A more recent public registration described a safety trial without antigen.[444]

The Georgia Institute of Technology, a pioneering center for microarray technology, has worked with Emory University to conduct numerous studies of coated microarray patches[342,444a] in animal models for cutaneous vaccine delivery. In a series of murine studies using solid metal microarray patches coated with inactivated influenza viruses, cutaneous vaccination induced robust immune responses—often better than equivalent dosages in controls injected by the SC route—as well as protection against lethal viral challenge.[445–454]

When coated with BCG, the same microarray platform was highly immunogenic in guinea pigs, with robust cell-mediated responses in lungs and spleen, comparable to those with Mantoux injection.[455] Similarly, plasmid DNA antigen for hepatitis C, coated on needles 500 µm in length, primed specific cytotoxic T lymphocytes in vaccinated mice more readily than conventional needle.[456] Inactivated rotavirus vaccine—developed to avoid the inhibitory effect of maternal antibodies on live oral vaccines[457]—was coated onto this microarray platform and found immunogenic in an animal model.[458]

Another center for microarray research, the University of Queensland and the nearby company Vaxxas in Australia,[459,460] developed a novel nitrogen gas jet-drying method for coating antigen onto silicon that overcomes the challenges of dip-coating closely spaced projections,[444a,459,461] but still allows antigen to elute within 2 to 3 minutes upon skin entry. This Nanopatch technology has among the smallest projections (less than 300 µm in length) with more than 20,000 projections per square centimeter on silicon chips (see Fig. 68.6C). It has achieved $\frac{1}{30}$ to $\frac{1}{100}$ dosage-sparing compared with the IM route in a mouse model for influenza.[462,463] Other antigens studied with good results in murine models with this platform include HPV, chikungunya, DNA plasmid vaccines (herpes simplex virus 2, West Nile virus), and viral vector DNA vaccine (malaria).[463–470] Research on and development of coated microarray patches for vaccination are also underway by many other groups.[340,381,471]

Experimental placement of the solid microstructured transdermal system microarray patches device on human volunteers found it to be "well tolerated" and "nonintimidating and not painful." The Georgia Institute of Technology microarray patch (without coating) has been used in clinical studies to assess usability and completeness of skin penetration when applied by self-administration, and study participants indicated they would be more willing to be vaccinated with influenza vaccine if microarray patches were available.[12] In focus group discussions, both public and private healthcare providers were also positive toward microarray patches as a change from conventional needle and syringe delivery.[472] Microarray patches can also allow use of dry formulations, which improve vaccine thermostability and reduce the need for cold chain storage. For example, in formulations prepared at Georgia Institute of Technology, a key ingredient of the carboxycellulose matrix of the dried coating was trehalose, one of several sugars, including sucrose, that have been found useful in protecting protein antigens from damage by drying and freezing, thereby improving vaccine thermostability.[473,474] Microarray patches have the potential to enable dose sparing of some vaccines, and some patches may also provide an adjuvant-like effect themselves by increasing local cell death.[475]

Although they have the potential to be a transformative technology for vaccine delivery, microarray patches are in an early stage of development for use with vaccines, and challenges include generating clinical evidence and scaling up manufacturing processes. The required wear times for microarray patches are not yet fixed and differ among designs. In preclinical studies, wear times for some microarray patches for vaccines have been greater than 5 minutes but can also be around 2 minutes. Ideally, delivery times would be sufficiently rapid to allow for quick application, removal, and proper disposal by a healthcare worker. If longer wear times are required for successful delivery, early removal is a risk, leading to possible insufficient antigen delivery as well as disposal of vaccine antigen outside the location of the supervised immunization session. If the vaccine recipient remains in the clinic

or otherwise-supervised environment of delivery, increased vaccination time and thus reduced throughput might have a negative impact on overall program efficiencies. Coated microarray patches might be considered both sharps and infectious waste material after they have been used and in contact with bodily fluids, so the risk of needlestick injury remains a consideration. The need for an applicator (whether separate or integrated with the patch) to reliably apply the microarray patches is another key question, as are cost and logistical implications for immunization programs. A visual or auditory indicator may also be important to reassure users and recipients that the vaccine has been delivered correctly.[472,476]

Dissolving Microarray Patches. An elegant strategy to decrease risk from intentional reuse of, or inadvertent contact with, used microarray patches is for the sharps to dissolve in the skin with hydration, thus releasing the antigen.[249,305,477–481] The most common matrix for dissolvable microarray patches hard enough to penetrate skin is carboxymethylcellulose, "generally recognized as safe" for parenteral delivery by the FDA, among other compounds.[477,478] Georgia Institute of Technology and Emory University have encapsulated inactivated influenza vaccine virus into biocompatible polymer, which dissolves within minutes after its application to skin (see Fig. 68.6D). Robust antibody and cellular immune responses provided complete protection from lethal challenge in mice, and a clinical trial is in progress.[481a,482] Several sugars, such as trehalose, sucrose, and maltose, have been found to be key ingredients in stabilizing and maintaining the potency of antigen during the process of forming dissolvable microarray patches, and studies have shown up to 4 months of stability at 40°C for measles vaccine in a microarray patch.[473,483–485] Formal thermostability studies will be needed to assess whether such formulations would resist heat degradation and allow transport and storage outside the cold chain.

Corium International has developed the MicroCor dissolvable microarray patch, with an integrated applicator that adheres to the skin (see Fig. 68.6E). TheraJect has developed the DrugMAT and VaxMAT dissolvable microarray patches, which have been investigated preclinically for delivery of several pharmaceuticals, including influenza vaccine. TheraJect's patches are designed for manual application without an applicator, but an applicator is also in development if needed for reliable delivery. In Japan, CosMED markets a cosmetic MicroHyala microarray patch containing hyaluronate, which dissolves in 60 to 90 minutes, and a Phase I clinical trial using this technology to deliver influenza vaccine has been conducted.[486] Many others also pursue dissolvable microarray patches.[486a,487,488]

Advantages and challenges for dissolving microarray patches are similar to those for solid-coated patches (see "Solid-Coated Microarray Patches" earlier). An important distinction is that dissolving patches leave no sharps waste.

Powder Injection. The transfection of cells by kinetic methods to deposit DNA-coated gold particles into them was pioneered in the 1980s. The Helios or PDS 1000/He gene guns170 and the Accell injector[518] have become standard bench tools for biolistic delivery of nucleic acid plasmids into a wide variety of plants and animals to transfect them to express the coded genes.[304,489,490] Delivery of DNA into the skin overcomes the usual polarized T-helper cell type 1 response when DNA is delivered into muscle.[293,491,492] These devices are unavailable for human vaccination (patent rights are held by PowderMed). The proprietary terms "epidermal powder immunization" and "particle-mediated epidermal delivery" (PMED) refer to the use of helium gas to blow powdered proteins, polysaccharides, or inactivated pathogens (epidermal powder immunization) or DNA-coated particles (PMED) into the epidermis at supersonic speeds. This unique method of vaccination was developed in the early 1990s by Oxford BioSciences, which over the years was renamed PowderJect, acquired by Chiron,[493] spun off as PowderMed, and finally acquired by Pfizer[494] in 2006. Delivery is by either reusable (XR series) or single-use disposable (ND series) devices (see Fig. 68.6F), with the ND series targeted for commercialization.

Conventional protein antigens for delivery by epidermal powder immunization are spray-dried into powders of suitable density and size (20 to 70 μm),[495,496] but the economics of manufacturing such formulations may be an obstacle.[293] For DNA vaccines delivered by PMED, plasmids coding for desired antigens are coated onto gold beads (1 to 3 μm in diameter) and, when deposited into epidermal APCs, they are eluted and transcribed.[497] A number of preclinical studies in various animal models have been conducted.[495,498–500]

Human trials of DNA vaccines containing up to one order of magnitude less antigen than the amount used for IM routes have induced humoral and cellular immune responses for hepatitis B in subjects both naïve and previously vaccinated with conventional vaccine.[501,502] PMED vaccination has also been studied for DNA priming in trials of malaria vaccine,[503,504] has produced seroprotective immune responses by DNA vaccine for seasonal strains of influenza,[505] and has reduced influenza symptoms and viral shedding after human challenge.[506]

In the hepatitis B and influenza trials cited earlier, there were no severe local reactions, but erythema, swelling, and flaking or crust formation occurred in nearly all subjects, albeit resolving by day 28. Skin discoloration, however, persisted through day 56 in 29 (97%) of 30 subjects,[510] through day 180 in 21 (25%) of 84 injection sites,[511] and beyond 12 months in 5 (25%) of 20 patients with long-term follow-up.[510] No anti–double-stranded DNA antibodies were detected. The deposition of the gold particles was studied in pigs, in which most were deposited in the stratum corneum and epidermis and were eventually sloughed by exfoliation by 28 days.[512] At days 56 and 141 after administration, a few particles remained in the basal epidermal layer and in macrophages in the dermis and regional lymph nodes.

Preclinical studies of epidermal powder immunization or PMED in murine, porcine, and primate models have shown immunogenicity or protection for either powdered or DNA plasmid antigens for various other pathogens, including Eurasian encephalitic viruses,[514] hantaviruses,[515] HIV,[516,517] influenza H5N1,[499] malaria,[518] severe acute respiratory syndrome (SARS) coronavirus,[519] smallpox,[393] and Venezuelan equine encephalitis.[520]

Abrasion/Transcutaneous Delivery. Various commercial patch delivery systems developed since 1981 have demonstrated the ability of certain therapeutic agents (e.g., scopolamine, nitroglycerin, clonidine, estradiol, fentanyl, nicotine, testosterone) to diffuse passively into bare, untreated skin without the use of active technologies or enhancers. However, such passive diffusion usually works only for small molecules with certain physical characteristics. Thus, there are but a few animal models of immunization onto bare, untreated skin.[521–523]

Newer methods to facilitate antigen delivery to the epidermis involve painlessly stripping or abrading the skin to increase the permeability barrier of the stratum corneum. A variety of simple tools have been used to remove the stratum corneum. Common cellophane adhesive tape may be applied to the skin and pulled away, carrying away dead keratinocytes with each repetition. Such tape-stripping has been shown to enhance cytotoxic T-cell and cytokine immune responses on subsequent application of various antigens and adjuvants

to the skin in mice.[524–530] Similarly, rubbing gauze, emery paper, or pumice on the skin removes cells by their abrasive effects, and this has been found to enhance immune responses in humans. Application of cyanoacrylate glue followed by stripping the skin to apply antigen to the exposed hair follicles has been described,[531] but its practicality has been questioned.[299]

Among methods that strip the skin, perhaps the most advanced is one that combines this step with the use of a remarkably potent adjuvant, the heat-labile enterotoxin (LT) of *Escherichia coli*. This effort was originally championed by Gregory M. Glenn, first at the Walter Reed Army Institute of Research, then at Iomai Corporation, and later at Intercell. The vaccinator or the patient holds against the skin a device, the Skin Preparation System (see Fig. 68.6G). With the push of a button and the pull of a tab, a controlled pressure is applied to a sandpaper strip, which gently abrades and removes approximately 25% of the stratum corneum.[532] Then, a patch containing LT as antigen alone, or LT as an adjuvant for another antigen, is applied to the skin; the process is called transcutaneous immunization.[534–537] LT alone is intended to induce immunity against enterotoxigenic *E. coli* (ETEC), the cause of traveler's diarrhea, or against *Vibrio cholerae*, with[538] or without[539,540] ETEC colonization factor.

An initial randomized, blinded field trial among travelers to Guatemala and Mexico found 75% efficacy for the patch with LT alone in protecting from moderate to severe diarrhea.[541] In 2010, Intercell reported mixed results from two follow-up field studies. In a pivotal Phase III trial for traveler's diarrhea (N = 2036), again in Guatemala and Mexico, the trial's primary target end point of greater than 60% efficacy against moderate to severe ETEC diarrhea was not met, finding only approximately 35% protection. Nor was there an effect on the frequency of all causes of diarrhea. However, there was a 60% reduction in the incidence of LT-positive diarrhea of all degrees of severity, along with a significant reduction in duration and severity of all diarrhea causes. The patch also induced measurable immune responses and was well tolerated.[542]

In a smaller Phase II trial in India (N = 723), the LT patch also did not reach its targeted end point, perhaps because of a low attack rate (approximately 1%) for LT-positive ETEC.[543] As a result of these two trials, Intercell discontinued work on the LT patch for traveler's diarrhea but still pursues its use with the Skin Preparation System device for other applications.

Applying the Intercell LT patch near the site of injection of parenteral influenza vaccine (an application referred to as a vaccine enhancement patch) was found to improve hemagglutination inhibition titers in the serum and mucosa of both young and aged mice[544,545] and increase the hemagglutination inhibition titer or show an improving trend for adult human volunteers older than 60 years of age.[546] In preclinical studies of other applications, use of LT or a structurally similar cholera toxin as cutaneous adjuvants resulted in improved immune responses or challenge protection in animal models for tetanus,[547] anthrax,[548,549] malaria, *Helicobacter pylori*, and Shiga toxin–producing strains of enterohemorrhagic *E. coli*.[550,551]

Other methods take advantage of low-cost fabrication techniques adapted from the microelectronics industry to convert silicon, metal, or other material into arrays of micrometer- to millimeter-sized microrasps designed to abrade the stratum corneum.[294,297,301–303,325,552] One example is the microenhancer array (also known as Onvax), an investigational technology that scrapes the skin before or after topical application of the antigen or therapeutic agent.[553] The microenhancer array consists of a square or round chip containing about a 1-cm^2 area of silicon or plastic microprojections mounted on a finger-held applicator.[249,342,353] Preclinical studies of the microenhancer array device have been conducted using hepatitis B, anthrax, and Japanese encephalitis vaccines.[288,354] A human trial, however, in which rabies vaccine was applied before or after four "rubs" of the device over four separate deltoid skin sites did not detect any immune response after three dosings.[325]

Technologies for abrading the skin to enhance transdermal delivery have the potential to be low cost, acceptable to users, compatible with vaccines, and suitable for use in a variety of vaccination settings, although device design must ensure ease of correct use. Reproducibility of dosing and effectiveness of vaccination has been a challenge for this technology, particularly the disappointing results of the Intercell ETEC vaccine. As a result, the field of cutaneous vaccination has focused mainly on technologies that inject or deliver the vaccine into the skin, rather than relying on passive diffusion.

Microporation. Microporation, also termed thermoporation, uses heat to vaporize tiny openings in the stratum corneum.[294,295,377,554,555] In the PassPort system, developed by Altea and now owned by Nitto Denko,[289,556] this heat is generated by a disposable array of metallic filaments held momentarily against the skin by a device the size of a computer mouse (see Fig. 68.6H). At activation, electric pulses are induced to heat the filaments. An adhesive patch containing vaccine or therapeutic agent is then applied over the micropores just created. In a hairless mouse model, this technique elicited 10- to 100-fold greater cellular and humoral responses to an adenovirus vaccine than intact skin, as well as 100% protection to surrogate tumor challenge (27% for intact skin).[377] In the same model, adenovirus-vectored melanoma antigen applied to the micropores roughly doubled the average onset time of tumors after challenge, and it protected one of six mice compared with none of eight vaccinated controls with intact skin. Microporated recombinant influenza H5 hemagglutinin protected BALB/c mice from challenge with a lethal H5N1 strain.[557] Skin micropores also permitted the passage of insulin in pharmacokinetic human trials with historical controls, and in the other direction allowed interstitial fluid to be extracted for potential glucose monitoring.[558]

Another device similarly generates micropores with heat induced by radiofrequency waves (ViaDerm). A different technique uses short, 100-microsecond pulses of superheated steam in microliter amounts to remove the stratum corneum.[559] Pantec Biosolutions has developed a laser microporation platform that could also be used for cutaneous vaccine delivery.

Microporation systems provide a reliable means of forming pores in the stratum corneum to enhance transdermal delivery, although the dose and reproducibility of vaccine delivered across the skin following this procedure remain to be verified. Devices can be designed for rapid use and acceptable levels of local reactivity, but for any microporation system, the cost and distribution of the reusable device could be a challenge in low-resource settings, particularly due to electrical power requirements.

Mucosal Vaccination

Most human pathogens initiate infection through mucosal portals of entry—the respiratory, gastrointestinal, and genitourinary tracts. Mucosal immunity can prevent these infections. In contrast, systemic immunity clears infection only after successful invasion, by limiting replication and destroying the pathogens. Ideally, both mucosal and systemic immunity should be raised against targeted pathogens. Strong mucosal immunity may enhance the benefits of immunization for some diseases. For example, by preventing the initial infection, mucosal immunity can reduce the risk of transmission to

others in addition to preventing clinical disease. Prevention of infection at the mucosal surface may be especially important for diseases for which effective systemic immunity has been difficult to achieve, such as tuberculosis and AIDS.

Anatomy

As described above, the external surfaces of the human body are protected by the keratinized stratified squamous epithelium, which composes the outermost layer of the skin. In contrast, the internal surfaces of the mucosal tracts are protected by a variety of epithelial types commensurate with the different functions of the gastrointestinal, respiratory, and genitourinary tracts. Nonkeratinized stratified squamous epithelium predominates in the oral cavity, oropharynx, vagina, and anal canal. Ciliated and nonciliated pseudostratified epithelia line the nasal airway, nasopharynx, and bronchi. Various specialized simple (single layer) epithelia are found at other mucosal sites. For example, the enterocytes of the gastrointestinal tract have microvilli at their apical surfaces to facilitate nutrient absorption, and the pulmonary squamous alveolar cells are extremely thin to permit diffusion of oxygen and other gases between the alveoli and the vascular systems.

Lacking the skin's protective layer of keratin, mucosal epithelial surfaces are protected from pathogen invasion by several alternative mechanisms. First, a complex dynamic mucus coat restricts access to most mucosal epithelial surfaces. Next, proteolytic enzymes are abundant on many mucosal surfaces and the highly acidic gastric environment degrades most pathogens before entry into the small intestine. Mucosal systems also dynamically move potential threats to limit the duration of exposure. For example, the ciliated epithelia of the upper and lower respiratory tract continually drive the mucus layer and its contents to the oropharynx to be swallowed and degraded by gastric acid. Peristalsis and other gastrointestinal mechanisms result in the eventual expulsion of gastrointestinal contents. Finally, the importance of mucosal microbiota to the prevention of infection and promotion of healthy immune responses is becoming increasingly realized.[560–562]

In addition to the physical defenses described above, mucosal immunity provides additional defensive layers to prevent infection. Innate mucosal immune factors include endogenous antimicrobial peptides such as defensins and cathelicidins. Proteins such as lysozyme and lactoferrin also reside in many mucosal surfaces and inhibit pathogen incursions. Each of these pathogen invasion defenses is a barrier that mucosal vaccine delivery systems must overcome to deliver the antigen package to host cells and elicit a consistent protective immune response.

Anatomical features of the upper respiratory tract affect IN delivery of medications. Particles are first filtered by the hairs in the nostrils, whose surface is covered with keratinized stratified squamous epithelia. Next, the particles must traverse the external nasal valves, slit-like passages that limit airflow from the nostrils into the internal nasal airways. Djupesland and colleagues estimated that only 25% of large, high-speed droplets (average: 43 μm) of a traditional nasal spray reach beyond the external nasal valve.[563] Almost all large, high-speed particles that transit the nasal valve deposit in the internal nasal airway, which has an average of approximately 150 cm^2 (0.015 m^2) of surface area covered with columnar epithelia, most of which is ciliated. In the internal nasal airway, particles deposit on the nasal mucosa and then join the flow of mucus that is swept by ciliated epithelia toward the pharynx, where it is swallowed. Immune surveillance of antigens in the flow of mucus begins as they are taken up into epithelial cells, intraepithelial dendritic cells, surface macrophages, and M cells (see Fig. 68.3D).

For delivery into the lower respiratory tract, small particles inhaled via nose or mouth share a common pathway through the pharynx, larynx, and trachea. The bifurcation of the trachea into the right and left bronchi starts a series of bifurcations, providing further surfaces to trap airborne particles. Only very small, slow-moving particles succeed in navigating the tortuous passages to deposit in the lower airways. The smallest particles (≤3 μm) may reach the alveoli, where they can be rapidly absorbed into systemic circulation. The complex branching of the lung passages also results in an astonishing alveolar surface area, exceeding 100 m^2 in a human adult male. The lower airways in humans do not typically have organized lymphoid tissues, but they do have abundant intraepithelial dendritic cells and alveolar macrophages that process antigens (see Fig. 68.3E).[564]

Immunological Rationale

While IM, SC, and ID vaccine delivery can induce both mucosal and systemic immunity, administration of vaccines to mucosal tissues tends to induce the strongest mucosal response. Several cell types are active in the mucosal antigen surveillance system. Although epithelial cells may play a role in the immune response, critical targets include the professional APCs, such as dendritic cells and macrophages, which are present in all mucosal tissues (see Figs. 68.3E and F), and in the case of alveolar macrophages, on the mucosal surface (see Fig. 68.3E). Phagocytosis of foreign microparticles is an important component of innate mucosal immunity and of mucosal antigen surveillance. Because mucosal surfaces are exposed to a myriad of nonpathogenic macromolecules, there are mechanisms for downregulating the immune response to antigenic exposure. Thus the potential for inducing immunologic tolerance must be considered when developing mucosal immunization strategies.

Dendritic cells are active throughout mucosal tissues and are present in high concentrations in the mucosal-associated lymphoid tissue, which include the tonsils, adenoids, and Peyer patches (see Fig. 68.3F). Mucosal-associated lymphoid tissue are also rich in microfold (M) cells, specialized epithelial cells with apical surfaces that take up macromolecules, viruses, and bacteria by endocytosis. On their basal surfaces, M cells have invaginated pockets that communicate with the extracellular space and host collections of lymphocytes and dendritic cells (see Fig. 68.3D). M cells transfer the endocytosed materials to these APCs for processing.[565–568]

APCs from the mucosa track to regional lymph nodes, where B-cell activation occurs. These B cells preferentially switch to IgA plasmablasts that "home" back to the exposed mucosal tissue to provide antigen-specific IgA protection. T cells also home back to mucosal sites and play a major role in mucosal immunologic memory responses. Lymphocytes exposed to antigen from one mucosal site, such as the respiratory tract, will migrate to provide protection at remote mucosal sites, such as the vagina. This integrated network of immune cells and tissues is known as the common mucosal immune system.[119,569] In some studies, IN vaccination has resulted in higher vaginal IgA titers than has vaginal vaccination.[570]

Almost every mucosal surface available for administering vaccines has been studied with a variety of antigens in animal models, including oral, respiratory, rectal, vaginal, and ocular tissues. In general, the respiratory and gastrointestinal tracts have more organized lymphoid tissue sites and more capacity for induction of immune responses.[571]

Several human vaccines are licensed and have been used successfully for delivery to the enteric tract by oral ingestion. Preclinical studies have investigated the potential utility of administering oral vaccines that target the oral mucosa rather

than the enteric tract. IN LAIVs are the only respiratory-delivered vaccines in use. However, multiple clinical trials have assessed vaccine delivery to the respiratory tract.

Challenges for Mucosal Delivery of Vaccines

Many of the technologies in development for mucosal vaccination target the oral or respiratory route and these will be the major focus of this section. Although vaginal and rectal vaccines may be effective, they could have limited acceptability for social, cultural, and practical reasons.

The first challenge in mucosal immunization is determining the appropriate target tissue. The optimal target tissues are not fully understood and may vary for different vaccines. Oral and respiratory vaccination strategies each have two discrete target areas. Vaccination via the oral route may target the oral mucosa or the intestines. Respiratory vaccination may target the upper respiratory tract (nasal passages, pharynx and larynx) or the lower tract (trachea, bronchi, bronchioles and lungs). For both nasal and oral mucosal delivery, the pharyngeal tonsils are likely target tissues. Some respiratory vaccines may require deposition in the lower airways for uptake by alveolar macrophages and dendritic cells. Lower airway deposition can be achieved by oral or nasal inhalation. Scientific methods for evaluating and comparing different target tissues are not yet well developed.

A second challenge is selecting animal models and extrapolating results to human mucosal vaccine delivery. Interspecies differences in immunologic tissue limit interpretation of animal target-tissue research results for humans. Moreover, the size and anatomy of common research animals differ greatly from those in humans. For example, in small animals such as rodents, nose drops may deposit to the entire respiratory tract, which would not be the case in humans. Balmelli and colleagues estimated that 30% of 20 µL of vaccine given to mice as IN drops deposited into the lungs.[572] Many viruses and bacteria that infect humans do not grow well in animal models. For example, species-specific differences in the distribution of sialic acid receptors on cell surfaces is a crucial factor in tissue and host specificity of influenza A viruses, which limits the number of animal models suitable for influenza research. Such species-specific differences can make it difficult to use animals to study live attenuated vaccines or vaccine vectors, and difficult to challenge animals to assess protection. This impedes the development of safe and effective mucosal vaccines for humans.

A third hurdle for mucosal immunization is assuring delivery of an accurate dose to the target tissue. The mass or volume of the antigen delivered depends on many factors. For intestinal delivery, gastric acids may destroy much of the antigen dose. For respiratory delivery, variability in performance by delivery device, the technique of the vaccinator, and differences in the anatomy and physiology of persons being vaccinated affect the dose delivered.[573] Fortunately, for many vaccines there is a wide margin between the dosage necessary to induce protection and the dosage at which the risk of adverse events increases.

A fourth challenge is the lack of accepted correlates of protection for mucosal immunity. For many diseases, laboratory assays have well-established criteria for systemic immunity—such as antibody titers above certain cutoffs—that have served for many years to predict protection from disease. In the absence of accepted serologic or cellular correlates of protection induced by mucosal vaccines, clinical trials must use specific disease-prevention end points, which can make the studies required for licensure much larger and more expensive.

Several immunization safety concerns represent further challenges for mucosal vaccines. Live virus or bacterial vaccines might pose an increased risk to immunocompromised persons if administered mucosally. Live-attenuated vaccines that revert to more virulent genotypes can adversely affect the vaccinee and be spread to contacts in the community. For nasal vaccines, another risk is that vaccine antigen (live or inactivated), adjuvant, or excipients might affect nearby cranial nerves[574] or travel along the olfactory nerve through the cribriform plate into the brain, with resulting adverse nervous system effects. Vaccines targeting the lower airways may induce or exacerbate bronchospasm or pulmonary inflammation, which can be life threatening. Another risk is cross-contamination: respiratory pathogens from one patient may contaminate the respiratory immunization device and be spread to subsequent patients.[575] Also, vaccine aerosols may spread beyond the intended recipient and affect other persons in the vicinity.

With so many challenges, new delivery technologies to achieve mucosal immunization are required if this route is to become practical and acceptable. In this young field, published research on delivery systems in animals or humans is limited. In most reported animal studies, the delivery system is not mentioned at all. For most systems designed for human respiratory systems, testing is very difficult or impossible in an animal model.

Finally, perhaps the most significant challenges to implementation of novel vaccine delivery systems in routine immunization practice are the regulatory requirements needed to ensure that the systems are safe and effective. The required studies and clinical trials can be extremely expensive. Vaccine manufacturers typically are reluctant to assume such cost and risk to relicense an existing product already delivering profits unless the potential benefits and market advantages will be significant. The best opportunity to bring alternative delivery into routine practice may be to use new delivery systems—from the start—for new vaccines early in development and licensure processes.

Oral Vaccination

Oral Ingestion (Intestinal Delivery). Several human vaccines are licensed and have been used successfully for delivery by ingestion, including those for polio, cholera, rotavirus, typhoid, and adenovirus (see Chapters 10, 14, 48, 49, 52, and 61). OPV, a prototypical mucosal vaccine, demonstrates the key advantages and challenges of mucosal vaccination. The ease of delivery of OPV drops enables minimally trained volunteers to distribute it in house-to-house campaigns that have been essential for the current successes of the global polio eradication effort. OPV is a live attenuated vaccine, as are most successful mucosal vaccines to date. Vaccination results in increased mucosal immunity, particularly in the intestine, as evidenced by increased secretory IgA and decreased shedding of vaccine poliovirus on subsequent challenge compared with IPV given by injection (see Chapter 48). However, even minor shedding of vaccine virus can result in transmission of the vaccine virus to others. This is especially concerning because the vaccine virus has the potential for reversion to virulence, which can result in vaccine-associated paralytic poliomyelitis in vaccinees and their contacts, and even in sustained outbreaks of circulating vaccine-derived polioviruses. Another concern regarding mucosal vaccine delivery demonstrated by OPV is the inconsistency of the immune response across different populations. OPV produces immunogenic responses less consistently in children in developing countries than in children in industrialized countries. This may be caused by differences in intestinal microbiota (see Chapter 48).

Because intestinal delivery by oral ingestion is simple, especially for liquids such as OPV drops, there is little

technology development required for macrodelivery of oral vaccines. Most of the new technologies for intestinal vaccination focus on microdelivery systems, which are reviewed in a separate section. One of the biggest hurdles to consistent delivery of vaccine antigen to APCs in the intestinal tract is ensuring that the antigen survives degradation from gastric acids. Like wild poliovirus, OPV is stable in acid environments for several hours. Other enteric vaccines, such as cholera and rotavirus vaccine, are delivered with a buffer solution to neutralize gastric acid. Typhoid fever vaccine is delivered as a lyophilized powder protected in an enteric coated capsule. However, capsules are not appropriate for infant vaccination.

Oral Mucosal Vaccination (Sublingual or Buccal Delivery). Preclinical studies have been conducted to explore the potential of administering vaccines via the sublingual (SL) or buccal mucosa. These routes have long been used for low-molecular-weight drugs such as nitroglycerin SL tablets. Several SL immunotherapy vaccines are marketed in Europe to suppress allergic hypersensitivity.[576] The major advantages of oral mucosal immunization are the ease of administration, including the potential for self-administration, and the probability of inducing robust mucosal immunity. In contrast to intestinal delivery, orally absorbed antigens are not subjected to degradation by gastric acid.

The oral cavity is lined with stratified epithelium. Like the skin, the oral epithelium is replete with Langerhans cells, the principal target APCs for oral vaccination. Some oral surfaces, such as the hard palate, have a keratinized upper layer, while the epithelia of the buccal and SL mucosa are nonkeratinized.[576] The lack of keratin increases permeability and potential for transportation of antigen to the mucosal APCs without the disruption or penetration of the stratum corneum required for cutaneous vaccination. As an example, Fig. 68.3G demonstrates wild-type measles virus replication in the oral mucosa. However, salivary and mucus flow rapidly moves substances on oral surfaces toward the pharynx to be swallowed and digested, and many salivary components initiate the processes of digestion and degradation. The oral cavity does not have organized lymphoid tissue such as the intestinal Peyer patches; however, movement of antigen across the pharyngeal tonsils may play a role in induction of an immune response.[577] Unlike nasal delivery, oral delivery does not have the potential of exposing the cribriform plate to vaccine. Shim and colleagues reported that SL vaccination with a recombinant replication-deficient adenovirus expressing SARS-associated coronavirus antigen induced protective immunity against SARS-associated coronavirus comparable to that seen with IN vaccination. Adenoviral DNA was detected in the olfactory bulbs of the mice vaccinated intranasally, but not in those mice vaccinated sublingually.[578]

In addition to immunotherapy vaccines, two novel SL vaccines are commercially available in Spain for the prevention of recurrent infections. One of these, Uromune, is a mixture of inactivated whole bacteria of selected strains that are common causes of urinary tract infections (UTIs). The vaccine is self-administered as a daily SL spray, maintained under the tongue for a period of 1 to 2 minutes, and then swallowed. In a clinical study of 319 patients with recurrent UTIs, subjects who received the vaccination daily for 3 months had significantly reduced occurrence of UTIs compared with subjects who received the standard therapy of sulfamethoxazole/trimethoprim for a period of 6 months. The reduced incidence of UTIs persisted for 12 months after completion of vaccination.[579]

Immunotherapy for allergy patients and use of polyvalent inactivated bacterial preparations to suppress recurrent infections are the major focus of published clinical trials for oral mucosal vaccination.[580–583] There are few clinical data on traditional prophylactic vaccines delivered via the SL or buccal route, although studies are registered on ClinicalTrials.gov for influenza, cholera, and HPV.[584,585] Preclinical studies of SL vaccines have demonstrated immunogenicity in mice using viral vectors such as adenovirus-based vaccines[586–588] and bacterial vectors such as *Bacillus subtilis*.[589,590] For example, SL immunization of mice with recombinant adenovirus-based vaccine expressing influenza virus hemagglutinin induced significant levels of sustained hemagglutinin-specific mucosal IgA and IgG, and provided complete protection from challenge with a lethal dose of homologous virus.[590a]

Despite the potential benefits of oral mucosal immunization, significant challenges have delayed progress toward commercially available prophylactic vaccines. First, antigens presented to the oral mucosa may result in immune tolerance, as evidenced by the success of the SL immunotherapy products designed to induce tolerance. Producing the desired protective immune response may require manipulation of the antigen formulation and dose, as well as addition of adjuvants. Another problem is that the rapid flow of saliva limits the residence time of antigen in the mouth and the opportunity for contact with APCs. Several formats have been designed or adapted from oral drug delivery systems to increase oral residence time.[576] For preclinical testing of vaccines, oral delivery is complicated by the extremely small oral cavities in small animal models: delivery of liquid vaccines to the mouth in mice is limited to 5 to 15 μL and alternate delivery formats are extremely difficult to use. Also, unlike humans, most rodents have keratinized epithelia in the buccal mucosa, which limits comparability of antigen uptake.[576]

To overcome the inherent mucosal tolerance for antigens, a variety of mucosal adjuvants have been studied.[576,591–594] For example, mice sublingually immunized with influenza vaccine (hemagglutinin split vaccine) with a lipopolysaccharide adjuvant demonstrated both hemagglutinin-specific IgG (systemic) and IgA (mucosal) antibody responses, which led to a significant increase in survival rate against lethal influenza virus challenge compared with SC vaccination.[591] Two mucosal adjuvants—α-galactosylceramide, a potent stimulator of natural killer T cells, and cytosine phosphate guanine–oligodeoxynucleotide a Toll-like receptor-9 agonist—effectively increased gp140-specific serum IgG and vaginal IgA levels following SL vaccination in mice. Combining both significantly improved these responses, and serum and vaginal washes collected 60 days after immunization had significant neutralization activity against simian/human immunodeficiency virus.[595]

To enhance residence time on the oral mucosa, a thermoresponsive gel—which changes from aqueous solution to viscous gel upon contact with the mucosa at body temperature—was combined with a double mutant of a bacterial heat-labile toxin. SL immunization of mice with a trivalent IPV with the gel delivery system produced both mucosal and serum antibodies, including IgA.[596]

Interest in SL vaccination has accelerated significantly: a 2015 PubMed search of "sublingual vaccine" returned more than 175 published articles since 2010 compared with 115 prior to 2010.

Progress of candidate oral mucosal vaccines through clinical trials to commercialization will require significant additional research to increase our understanding of the pharmacokinetics and pharmacodynamics, as well as immunological mechanisms, of this route of delivery. Because of the inherent mucosal tolerance of antigens and limited oral residence time,[576] development of optimized delivery formulations and predictive assays for mucosal immunity are particularly challenging.

Respiratory Vaccination

Background. The earliest known route of vaccination was IN insufflation of powdered scab material from smallpox patients, reportedly practiced in China as early as the 10th century AD. However, IN LAIVs are the only respiratory vaccines in modern use (see Chapter 54). In contrast, the respiratory route is used to deliver a wide and expanding variety of pharmaceutical products.[597,598] Respiratory drug administration typically targets one of two major areas: the upper airway or the lower airway. Most common products, such as nasal decongestants and inhaled asthma medications, are intended for local therapy in the target area. Newer products, such as inhaled insulin, target deposition in alveoli to achieve systemic delivery. The first inhalable insulin (Exubera) was a commercial failure, partly because of a cumbersome delivery device.[599,600] However, another product for insulin inhalation, Afrezza, was approved by the FDA in 2014.[600a] Afrezza uses a small, simple dry powder inhaler, the Mannkind Dreamboat.[600b] Efficient and effective delivery to respiratory target tissues is determined largely by the aerodynamic size and speed of the vaccine particles, which are functions of the device engineering and the product formulation.

Devices for upper respiratory tract drug delivery include droppers, nasal sprayers, nasal nebulizers, and dry powder inhalers. Very few are designed for or have been tested with vaccines. Most drug devices deliver repetitive doses to a single patient. In contrast, vaccination delivers single doses to multiple patients, which raises the issue of device cross-contamination. Although unit-dose disposable devices or disposable components could solve this problem, they must be inexpensive to be cost-effective. Many drug-delivery devices are designed for self-administration; however, this requires a level of patient cooperation difficult to achieve in young children and impossible in infants.

With mucosal vaccination in general, the optimal target tissues are not yet understood for most potential respiratory vaccines, and they vary for different antigens. Liquid droppers for IN delivery are perhaps the simplest devices, and inexpensive prefilled single-use droppers are commonly used for administering drugs to the upper respiratory tract. However, drops administered intranasally tend to drip out of the nose or roll back to the pharynx where they are swallowed, resulting in less residence time in the nasal passages and nasopharynx and less contact with mucosal surfaces. Recumbent patient positioning may increase nasal residence time but may not be practical at all POCs. Prefilled and fill-on-site nasal spray devices generate smaller particles, typically in the 50- to 100-μm range, and nasal nebulizers generate even smaller (20 to 30 μm) particles. While these devices may increase mucosal surface contact and residence time, there are no definitive studies to their effect on immunogenicity.

Deposition of liquid vaccines in the lower airways requires a nebulizer device that generates the very small droplets required to pass the vocal cords and reach the trachea and bronchi (<10 μm) or the alveoli (<5 μm). Nebulizers are durable devices that require an energy source to generate aerosols. The device transits aerosol to the patient through a mask, oral prong, or nasal prongs. Single-use disposable components, including patient interfaces, are needed to prevent cross-contamination.

Dry powder aerosols can also be used to deposit vaccine in the upper or lower respiratory tract. Liquid delivery has the advantage of being the standard format: all currently licensed aerosol vaccines are in liquid presentation. Generating sprays and aerosols from existing liquid vaccine formulations could accelerate respiratory vaccination research. In contrast, a number of obstacles must be overcome to produce vaccines as dry particles of sizes suitable for upper or lower respiratory tract deposition.[601–603] First, formulating powders requires significant and extensive changes in manufacturing methods. Second, many potential dry-formulation ingredients are extremely hygroscopic and become sticky when exposed to humidity. Third, once the powders are deposited in the respiratory tract, they must be sufficiently hygroscopic to dissolve and release the vaccine for uptake. However, if these challenges can be met, dry aerosols have several advantages over liquid aerosols. Doses can be filled into inexpensive single-use presentations and delivered without on-site aqueous reconstitution. Secondary packaging that seals the dose container in an impermeable overwrap, such as metal foil, could maintain low humidity to prolong potency and increase shelf life. Minimal energy is required for powder dispersion compared with liquid droplet generation, so electromechanical devices are not necessary.

Upper Airway Vaccine Deposition

Nasal Sprays. The only vaccine currently licensed and in use in the United States for respiratory delivery is the cold-adapted LAIV FluMist, delivered with the AccuSpray IN syringe. LAIV development, testing, and licensure are reviewed in detail in Chapter 32. LAIV demonstrates several potential benefits of respiratory delivery. It produces both mucosal and systemic immunity, and it generally has been found to provide higher protective efficacy than injected inactivated vaccine in young children.[604–612] It also provides heterotypic immunity against nonvaccine strains.[604] IN delivery of LAIV may reduce the risk of influenza transmission because it reduces respiratory shedding among immunized children challenged later with a vaccine virus.[604] Finally, modest coverage with LAIV among schoolchildren reduced influenza-related illness rates in unvaccinated adults in a community.[612] However, despite the previous positive results for LAIV, vaccine effectiveness data for the 2015–16 influenza season found that FluMist did not provide protective benefit for children, and the Centers for Disease Control and Prevention (CDC)'s Advisory Committee on Immunization Practices has recommended that FluMist not be used in the United States.[613]

The AccuSpray device is a sterile, single-patient–use, disposable, prefilled glass syringe fixed with a nonremovable plastic nozzle (Figs. 68.7A and B). Its total dose is 0.2 mL of which 0.1 mL is sprayed consecutively into each nostril. An attachment on the plunger stops the first dose to allow the user to switch nostrils. FluMist vaccination delivered by AccuSpray is highly effective in most populations (see Chapter 54). Key advantages of AccuSpray delivery are that it is simple to use, low cost, capable of disposed without needing to use a sharps container, and has a low possibility of refill and reuse. The large particle sizes generated by the sprayer minimize deposition to the lower airways, reducing the risk of adverse pulmonary events. A limitation of the system is that the particle size emitted depends on the speed at which the vaccinator depresses the plunger. The median diameters of the particles can range from 200 μm or greater at plunger speeds of up to 33 mm per second to 50 μm or less at speeds of 80 mm per second and greater.[614] Although this wide variability might in theory affect the efficiency of vaccine deposition, LAIV by AccuSpray typically produces a high rate of protective immunity at the current dosage.

An IN LAIV developed in Russia has been in use in that country for more than 50 years (see Chapter 54). The vaccine is lyophilized and must be reconstituted and filled into the syringe at the POC. Each dose comes with a Lindal Group atomizer tip that fits the syringe, after removal of the reconstitution needle, for delivery into the nostril. No dose divider is provided and the 0.25-mL dose for each nostril must be drawn

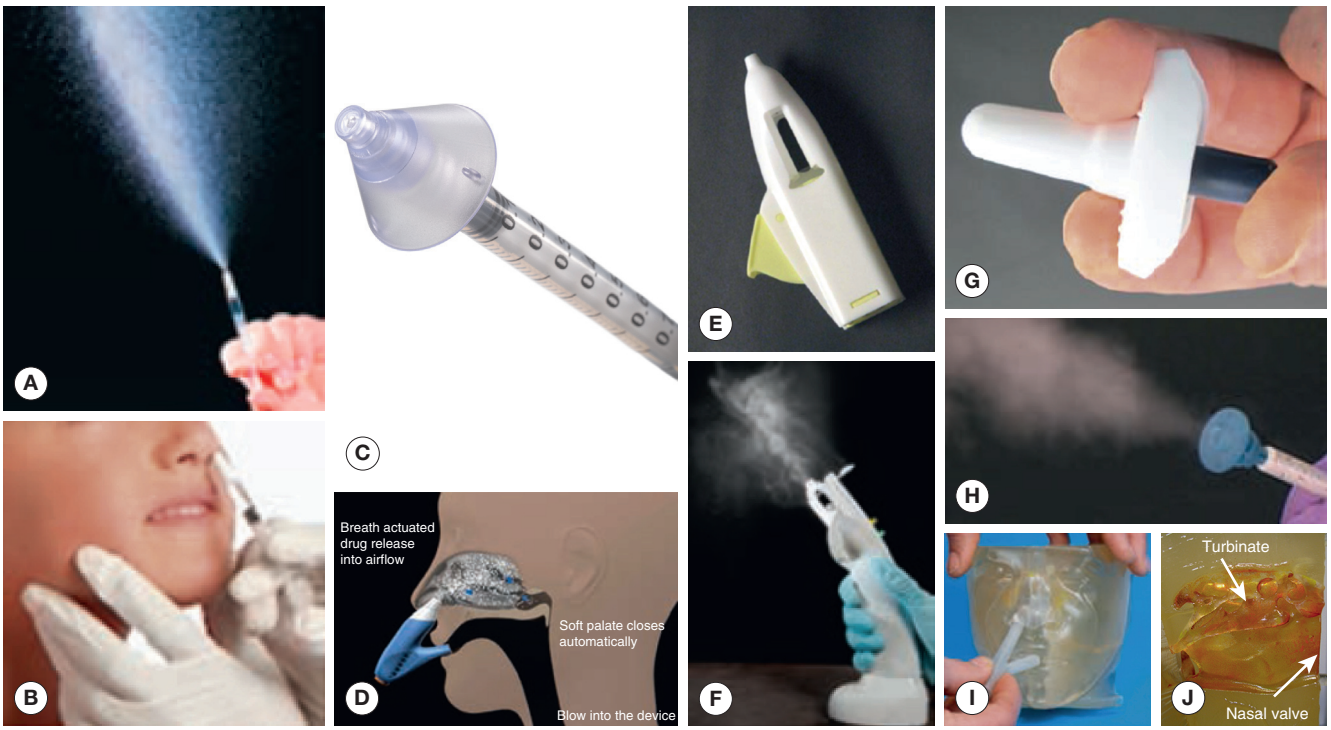

Figure 68.7. Selected devices for upper respiratory tract vaccine delivery. A and **B,** AccuSpray nasal spray syringe (Becton, Dickinson and Co.) **A,** AccuSpray produces an aerosol plume of particles reported from 50 to 200 μm in diameter, depending on plunger speed. **B,** AccuSpray used for intranasal delivery of FluMist influenza vaccine (MedImmune, Inc.). Prefilled liquid vaccine is stored refrigerated for single patient use. The total volume is 0.2 mL. A dose separator interrupts delivery at 0.1 mL and, when removed, allows the remaining 0.1 mL to be administered into the opposite nostril. **C,** Teleflex VaxINinator nasal spray device currently used to deliver Serum Institute of India Ltd's Nasovac-S influenza vaccine. The conical spray tip is attached to the syringe by a Luer fitting. **D,** Sagittal computer-assisted image illustrating intranasal delivery by the OptiNose delivery device (OptiNose, Inc.). **E,** Dolphin side-activated nasal spray disposable unit-spray device designed to deliver 200 μL of product in two 100 μL sprays. **F,** Investigational AeroVax prototype (Creare, Inc.) uses battery-powered piezoelectric energy to drive an aerosol from a disposable drug cartridge via a microperforated mesh plate through a disposable patient interface, such as nasal prong in patient nostril, oral prong, or mask (not shown). Droplet diameters can be tailored from <5 μm to 10 to 25 μm for upper or lower airway delivery, respectively. **G,** BesPak, UniDose DP a dry powder prefilled unit-dose device which can deliver up to 10 μg of medication. Requires a predefined amount of pressure to be applied to the plunger before the device will operate, thus ensuring proper dispersion. **H,** Investigational Solovent (Becton, Dickinson and Co.) dry powder inhaler prototype, adaptable for intranasal or pulmonary formats. The Solovent is a small cylindrical plastic capsule open on both ends. One end has a Luer fitting for attachment to any standard syringe to provide air flow, while the other end is the patient interface, either a nasal prong or a snap-fit adapter to mate with a mask or reservoir. The capsule contains the powder dose between two burstable plastic membranes, which rupture when airflow is provided by the syringe. Plume of powder upon release from Solovent in open air for visualization purposes. **I,** Investigational nasal dry powder inhaler developed by the CDC and Creare, Inc. A prefilled cup (shown between right thumb and forefinger) containing the powdered vaccine is opened by its attachment to the device. The breath of the patient blowing into the device tube carries the dry powder into the nose. Dispersion during patient exhalation limits pulmonary deposition from the posterior nasal space. The plastic face is a phantom model of the airway of a 5-year-old child (CFDRC, Inc.). **J,** Medial section of the right internal nasal airway of a plastic, phantom model shown and described in **I**. The external nares and face (not shown) are to the right, proximal to the nasal valve. The pharynx (not shown) is to the left, distal to the nasopharyngeal opening. The red-pigmented powder indicates the deposition pattern from the investigational nasal dry powder inhaler shown in **I**. *(**A**–**B,** Courtesy Nuphar Rozen-Alder [Becton, Dickinson and Co.]. **C,** Courtesy Teleflex Medical Europe Limited. Unauthorized use prohibited. **D,** Courtesy Gisle Djupesland. **E,** Courtesy Aptar Pharma and from Riddle MS, Kaminski RW, Williams C, et al. Safety and immunogenicity of an intranasal Shigella flexneri 2a Invaplex 50 vaccine.* Vaccine. *2011;29[40]:7009–7019. **F,** Courtesy James Gathany [CDC Photographic Services]. **G,** Courtesy BesPak [Ian Anderson] and from Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness.* N Engl J Med. *2011;365[23]:2178–2187. **H,** Courtesy Becton, Dickinson and Co. [Vincent Sullivan]. **I,** Courtesy Creare, Inc. [Darin Knaus]. **J,** Courtesy Creare, Inc. The research was supported by the U.S. Department of Health and Human Services/CDD through SBIR Contract 200-2009-32519. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. We gratefully acknowledge Dr. Vincent Harrand of CFD Research Corporation for supplying the nasal phantom models used for this work.)*

up separately by reattaching the fill needle. Potential reuse of the device with cross-contamination is a risk.

BioDiem has commercialization rights for the Russian LAIV outside of Russia and has licensed the technology to the Serum Institute of India Ltd. The Serum Institute of India Nasovac-S vaccine is currently delivered with the Teleflex VaxINator, a single-use device that consists of an atomization spray tip compatible with any standard Luer lock fill-on-site syringe (see Fig. 68.7C). A dose divider attached to the syringe plunger separates the 0.5-mL dose for delivery of 0.25 mL per nostril. One advantage of the VaxINator is that the conical tip fits any nostril size and limits the depth of

insertion, providing the vaccinator with physical guidance to device placement. The device is potentially reusable. Teleflex offers an optional autodisable syringe, which automatically disables both the syringe and the VaxINator after the drug is delivered.

An inactivated virosomal-subunit influenza vaccine (Nasalflu, Berna Biotech) was licensed in Switzerland in 2000 for administration as a nasal spray. Nasalflu contained *E. coli* heat-labile toxin as a mucosal adjuvant. The vaccine was withdrawn from the market after an increased incidence of temporary paralysis of the seventh cranial nerve (Bell palsy), which was considered to be related to the heat-labile toxin adjuvant.[574,615]

Multiple clinical trials have been conducted with other LAIVs in addition to the licensed vaccines.[616,617] Clinical trials of other types of influenza vaccines—including inactivated, virosomal, live replication-deficient, and proteasome vaccines—also have demonstrated promising immune responses.[618–621] Most studies used standard nasal spray or did not describe the IN delivery method. One study used a novel breath-actuated nasal spray device, OptiMist, which delivers liquid or dry powder aerosols only during oral exhalation. Because this method raises the soft palate to close the connection between nose and throat, pulmonary deposition is avoided and delivery to the posterior nasal segments is increased (see Fig. 68.7D).[563] Inactivated influenza vaccine self-administered using the OptiMist resulted in significant increases in virus-specific IgA in nasal secretions, as well as protective levels of virus-specific serum antibodies, after two doses in more than 80% of subjects.[622]

Other IN vaccines for which clinical trials demonstrate encouraging immune responses include live attenuated respiratory syncytial virus, parainfluenza, *Bordetella pertussis* vaccines,[623–626] a Norwalk virus VLP vaccine, and hepatitis B vaccine. For example, live attenuated respiratory syncytial virus vaccines administered as IN drops to seronegative infants 5 to 24 months of age produced a functional immune response in 59% of subjects.[626] Riddle and colleagues reported that a *Shigella flexneri* subunit vaccine was safe and well tolerated, producing robust levels of antigen-specific intestinal IgA when administered IN with a Dolphin spray device (see Fig. 68.7E).[627]

Nasal Drops. Multiple clinical trials and almost all preclinical studies of IN vaccines have been conducted with nasal drops. Unfortunately, the dropper device and delivery method is often not described. Several studies compared LAIV delivery by nasal spray with liquid dropper delivery.[628] In a multicenter study of children 18 to 71 months of age, King and colleagues detected no statistically significant differences in reactogenicity or immunogenicity between subjects who received LAIV by drops or spray at four different doses.[628] In another study, four weekly doses of inactivated influenza vaccine were given as drops, 250 µL per nostril, with the vaccinee in a supine position on a tilted couch with the head lowered for 1 minute in order to retain the vaccine within the nasopharyngeal area. IN vaccine drops produced hemagglutination-inhibiting antibody titers ≥40, considered protective in more than 80% of subjects.[618]

Nasal Nebulizer. Several nebulizers have the capacity to deliver IN aerosols. Live attenuated measles vaccine administered via nasal prong with the AeroVax nebulizer (see Fig. 68.7F; Creare, Inc.) was shown to be safe and immunogenic in macaques.[629] AeroVax delivery of LAIV to ferrets elicited high levels of serum-neutralizing antibodies and protected them from homologous virus challenge at conventional (median tissue culture infective dose [$TCID_{50}$]: 10^7) and significantly reduced ($TCID_{50}$: 10^3) dosages, and it provided a significant level of subtype-specific cross-protection.[630]

Intranasal Dry Powders. Multiple clinical trials of an IN powder vaccine including chitosan as a mucoadhesive have demonstrated robust immune responses.[631,632] Two doses of a combination dry powder *Neisseria meningitidis*–diphtheria IN vaccine administered by a 5-mL Combitips Plus syringe produced protective antibody titers for both pathogens comparable to those generated by conventional injection. The Combitips is a sterile PP syringe with a solid displacement plunger that expels powder without residual airspace and has a very narrow opening that generates a fine aerosol. The tip of the syringe was placed as far into the nostril as possible without causing discomfort, with the subject in a semireclined position. One-third of IN vaccinees reported mild side effects compared with two-thirds of injection vaccinees who reported mild injection pain.[631] In another clinical study, IN powder diphtheria vaccine formulated with chitosan significantly augmented systemic T-cell responses compared with the same vaccine without chitosan.[632] An IN norovirus VLP vaccine that included chitosan and monophosphoryl lipid A as adjuvants significantly reduced the frequency of Norwalk virus gastroenteritis in human subjects following viral challenge with a homologous strain. The vaccine was delivered intranasally using the Bespak Unidose DP (dry powder) device[633,634] (see Fig. 68.7G), a single-use unit-dose design that allows for IN delivery of up to 100 µg of medication.

IN dry powder vaccines have been formulated and tested in preclinical studies for influenza, anthrax, diphtheria, meningococcus, measles, hepatitis B, plague, and Norwalk virus. The BD Solovent device has been used in several preclinical studies (see Fig. 68.7H). Live attenuated measles vaccine delivered intranasally to macaques with Solovent provided an immune response equivalent to SC injection, including protection from virus challenge.[635] Anthrax vaccine by the IN route with Solovent provided complete protection against inhalational challenge with roughly 90 times the median lethal dose in rabbits, while providing better stability than liquid formulations.[353,635a,636] Solovent IN delivery of a whole inactivated influenza virus product in rats elicited high titers of nasal antiinfluenza IgA, as well as serum antibody titers equivalent to those obtained with injected vaccine.[637] No loss of potency was found when it was stored at 25°C and 25% relative humidity for up to 12 weeks and at 40°C and 75% relative humidity for 2 weeks.[637]

An investigational nasal powder delivery device developed by Creare, Inc., operates by patient exhalation through the mouth, blowing the powder into the nose while simultaneously generating air flow that limits entry to the lower respiratory tract. In three-dimensional plastic models developed from computerized tomography airway anatomy of children ages 2, 5, 7, and 12 years of age, the device consistently delivered a significant fraction (>66%) of a fluorescein powder dose to the target IN airways (see Figs. 68.7I and J).

To increase nasal residence time, a dry powder formulation, GelVac, was developed with an inert in situ gelling polysaccharide. IN vaccination with GelVac Norwalk-virus VLPs induced systemic and mucosal antibody titers in rats and guinea pigs equal to or greater than those achieved by liquid VLPs plus adjuvant.[638] The GelVac powder vaccine was administered using a laboratory-constructed delivery device using a 5-mL syringe to provide airflow through a modified pipette tip, which served as a nasal prong. Although many dry powder and liquid-spray nasal devices are in use for drug delivery in humans, testing them in vaccine studies in animals is difficult or impossible because of the nostril size.

Lower Airway Vaccine Deposition. The vast alveolar surface area and abundant APCs make the lungs an attractive target for vaccine delivery. However, inflammatory adverse reactions in this vital organ could have grave consequences, so a high degree of caution is required. Significant development in

pulmonary drug delivery has been achieved, which might be adapted for vaccine delivery.

Pulmonary Liquid Aerosols. Measles vaccine is the most widely studied vaccine targeting pulmonary delivery. Early clinical studies of drops or sprays delivered to the conjunctiva, or the oral or nasal mucosa, produced inconsistent immune responses,[639–648] prompting researchers to consider aerosol pulmonary delivery. Albert Sabin was an early pioneer and advocate of this delivery method.[649–652] A significant body of clinical research in aerosol measles has been published and summarized in review articles and metaanalyses.[653–656] The primary finding is that delivery of nebulizer-generated small-particle liquid aerosols via pulmonary inhalation to children 10 months of age or older typically produced immune responses in very high proportions of subjects, usually equivalent to or greater than responses to injected vaccines.[630–632,640–642,656–671] For example, Dilraj and colleagues found that 96%, 94%, and 86% of schoolchildren who received the aerosol measles vaccine had antibody titers of greater than 300 IU/mL at 1, 2, and 6 years after vaccination, respectively, compared with 91%, 87%, and 73% among those receiving the injected vaccine.[659,669,672] In contrast, generally lower immune responses were found for the aerosol route compared with parenteral injection, among children younger than 10 months.[649–652,673–675] For example, Wong-Chew and coworkers found vaccination of 12- and 9-month-old infants by injection induced immunity in 100%, but by aerosol route in only 86% and 23%, respectively.[673,674] One hypothesis was that the very low respiratory volume of infants results in an inhaled dose of aerosol vaccine that is too small in that period. A follow-up study by Wong-Chew and colleagues demonstrated that increasing exposure time to aerosol measles vaccine elicits immune responses that are comparable to those seen by the SC route in 9-month-old infants.[676]

With regard to vaccine safety, the same reviews and metaanalyses[653–655] noted that no severe adverse events were reported after aerosol measles vaccination in any of the studies. Rates of minor adverse events, when reported, were typically less than or the same as vaccination by injection.[659,666,668,673,674] Experience in mass campaigns was similar, with de Castro and colleagues reporting no serious adverse events among more than 3.7 million children in Mexico vaccinated by aerosol.

The jet nebulizer system known as the Classic Mexican Device (see Figs. 68.8A and B) was used in many measles aerosol clinical studies and during mass campaigns in Mexico.[659,669,672,674,677] The system consists of a general-use (non–medical-grade) compressor that delivers air to a jet nebulizer, which holds the vaccine in crushed ice to maintain potency during the vaccination session. The vaccine aerosol is delivered through reusable plastic tubing to a single-use disposable paper cone (modified from a drinking cup), which is held close to the patient's face for 30 seconds. Typically, the aerosolized dose volume is roughly 0.15 mL, and the mass median aerosol diameter of droplets is 4.3 μm.[678] In a study that assessed the distribution of viable vaccine virus across the range of droplet sizes emitted from a Classic Mexican Device, Coates and coworkers estimated that 30% of infective viral particles were contained in droplets with diameters of 5 μm or less, and 23% were in droplets of greater than 10 μm. As with all vaccine delivery to the lower respiratory tract, it has not been possible to identify which target tissues are essential for production of an immune response. Although the Classic Mexican Device has demonstrated a level of safety and immunogenicity, it is heavy, cumbersome, and noisy, and it requires outlet (mains) electricity and crushed ice. It is thus not practical for routine vaccination.

Because of the encouraging results of early measles aerosol vaccine trials, in 2002 the WHO, in partnership with the CDC and the American Red Cross, initiated the Measles Aerosol Project. Its goal was licensure of at least one live attenuated measles vaccine and its associated aerosol delivery system in the developing world. The project documented immunogenicity and safety (i.e., the lack of local or systemic toxicity) in animal studies.[629] Three existing therapeutic nebulizers were used for Phase I clinical trials: the AeroEclipse, the CompAIR, and the Aeroneb.[679,680] Measles vaccine delivery by the three devices, delivered to 145 subjects in India, was reported to be safe, well tolerated, and immunogenic.[681] A modified version of the battery-operated Aeroneb vibrating mesh nebulizer (Aerogen) device (see Fig. 68.8C) was selected for use in the Phase II/III pivotal trial initiated in 2009 by the Measles Aerosol Project. The study was a randomized, open-label, active-control, noninferiority trial of the measles vaccine in unvaccinated healthy infants from 9 to 11.9 months of age. For aerosol delivery, vaccine was reconstituted in 2-mL diluent and administered as a single 0.2-mL dose, nebulized for 30 seconds through a single-use nonvented face mask. The nebulizer generated aerosol with a median diameter of 5.1 μm. In the full-analysis set, 673 (85.4%) of 788 children in the aerosol group and 754 (94.7%) of 796 children in the SC group were seropositive at day 91, a difference of 9.3 percentage points (95% confidence interval, 12.3 to 6.4), which failed to meet the predetermined 5% noninferiority margin. No serious adverse events were attributable to measles vaccination and adverse-event profiles were similar in the two groups.[682]

In other recent clinical studies, rubella and mumps vaccines have been used in combination with measles vaccine for aerosol delivery. Bennett and colleagues found that aerosol vaccination of preschool children with a combination MMR vaccine produced antibody responses to all antigens equivalent to those produced by injection.[658] Sepúlveda and coworkers found that aerosolized measles-rubella combination vaccine in school-age children produced levels of measles and rubella antibodies equivalent or higher than levels seen after SC injection.[666] Fewer adverse events were reported in the aerosol group. Diaz-Ortega and colleagues found that MMR vaccination by aerosol in college students produced immune responses similar to those produced by injection, with seropositivity retained in all vaccinees 1 year after vaccination.[670,671]

Few clinical trials of pulmonary aerosol delivery of other vaccines have been reported. In a small Phase I double-blind trial, aerosol delivery was compared with ID delivery for a modified vaccinia Ankara–vectored vaccine delivering the *Mycobacterium tuberculosis* Ag85A antigen. The aerosol was administered as a 1-mL liquid dose using an Omron MicroAIR U22 vibrating mesh nebulizer. Both administration routes were well tolerated with few adverse events and were immunogenic with Ag85A-specific systemic responses similar across groups. Ag85A-specific CD4 T cells were detected in bronchoalveolar lavage cells from both groups, and responses were higher in the aerosol group than in the ID group.[683]

Pulmonary Dry Powders. Measles vaccine has been a pathfinding application for respiratory dry powder vaccination. Early formulations were finely milled and retained adequate potency, but immune responses were poor when delivered to the respiratory tract of macaques.[601,603] Aktiv-Dry was awarded a Grand Challenges in Global Health grant in 2005 for a measles vaccine dry powder (MVDP) project. The project goals were to refine a dry powder measles vaccine formulation, establish production capacity at the Serum Institute of India, and complete preclinical and Phase I clinical testing[684] A novel spray-drying system was used to manufacture inhalable MVDP with minimal potency losses in the drying process.

Figure 68.8. Selected devices for lower respiratory tract vaccine delivery. A–B, Investigational Classic Mexican Device for aerosol vaccine delivery, illustrated by component diagram **(A)** and use in clinical trials **(B)**. A nonmedical electric compressor (not shown) delivers roughly 9 L of air per minute at a pressure of 30 to 40 psi (207 to 276 kPa) to a jet nebulizer that is kept in crushed ice to maintain vaccine potency. The vaccine aerosol (roughly 0.15 cm^3 of particles averaging 4.3 μm in diameter) is delivered through a disposable paper cone held close to the patient's face for 30 seconds. **C,** Aerogen Aeroneb GO battery powered vibrating mesh nebulizer used as the basis for the Measles Aerosol Project clinical trials device. Cap (between thumb and finger) is removed to fill upper chamber. Microperforated vibrating mesh disk at the base of the chamber aerosolizes dose into lower chamber. Patient inhales aerosol through the mouthpiece or attached mask (not shown). Cord connects to battery control pack. **D,** Investigational Solovent dry powder inhaler prototype pulmonary delivery format (Becton, Dickinson and Co.). Air from the empty syringe ruptures the membranes of the vaccine container, releasing into the cardstock spacer a plume of dry powder. Patient inhales from the cardstock spacer directly or via a mask. The vaccine container (capsule), spacer, and mask are single-use disposables. **E** and **F,** Investigational PuffHaler dry powder inhaler (Aktivdry, LLC). **E,** Air from the squeeze bulb lofts vaccine powder from the disperser into the reservoir once the pressure threshold of the burst valve is exceeded. **F,** Patient inhales from the single-use-only reservoir of PuffHaler after detachment from the device, either directly (as shown) or via a disposable mask (not shown). **G,** Investigational Twincer single-use, disposable, dry powder inhaler for pulmonary delivery (University of Groningen). The drug formulation is stored in the snap-together plates of the device in an aluminum blister for maximal moisture protection. The powder becomes available for inhalation upon pulling a foil cover that protrudes from the rear of the inhaler (not shown). *(**A–B,** Courtesy José Luis Valdespino [Instituto Nacional de Salud Pública, Mexico] and from Valdespino-Gómez JL, de Lourdes Garcia-Garcia M, Fernandez-de-Castro J, et al. Measles aerosol vaccination.* Curr Top Microbiol Immunol. *2006;304:165–193; **C,** Courtesy Philips Respironics and from Laube BL. The expanding role of aerosols in systemic drug delivery, gene therapy, and vaccination.* Respir Care. *2005;50[9]:1161–1176. **D–F,** Courtesy James Gathany [CDC Photographic Services] and [**D** and **F**] from MVDP author group, Cape S, Chaudhari A, et al. Safety and immunogenicity of dry powder measles vaccine administered by inhalation: a randomized controlled Phase I clinical trial.* Vaccine. *2014;32[50]:6791–6797. **G,** Courtesy University of Groningen [A.H. de Boer] and from de Boer AH, Hagedoorn P, Westerman EM, et al. Design and in vitro performance testing of multiple air classifier technology in a new disposable inhaler concept [Twincer] for high powder doses.* Eur J Pharm Sci. *2006;28[3]:171–178.)*

Pulmonary delivery of the vaccine powder with the Solovent device (see Fig. 68.8D) and the Aktiv-Dry developed PuffHaler device (see Figs. 68.8E and F) demonstrated robust immune responses in macaques, including serum antibody, T-cell responses, and protection from viral challenge. Both devices delivered aerosol into reservoirs for mask delivery.[635] Toxicology studies found no test-article–related effects or delayed onset of toxicity after inhalation by Sprague Dawley rats and no effects in mortality, clinical observations, respiratory function, clinical pathology, or histopathology in rhesus macaques.[685]

A Phase I MVDP safety trial of the vaccine was conducted in adults in India, with subjects randomly assigned in 1 : 1:1 ratio to receive MVDP via PuffHaler device, Solovent device, or the licensed SC measles vaccine. No adverse events were reported. MVDP produced serologic responses similar to SC vaccination.[686]

Other vaccines in development for pulmonary powder delivery include influenza, tuberculosis, and hepatitis B.[687] For example, a pulmonary powder formulation produced by SFD, with influenza subunit viral antigen and inulin stabilizer, induced humoral (IgG), cell-mediated (interleukin-4, interferon-γ), and mucosal (IgA, IgG) immune responses in BALB/c mice.[688] When tested separately with a novel dry powder inhaler, the single-use disposable Twincer (see Fig. 68.8G), inulin-based dry powder subunit influenza vaccine was dispersed with an aerodynamic particle size distribution suitable for pulmonary administration.[689]

VACCINE MICRODELIVERY SYSTEMS: ANTIGEN DELIVERY TO DESTINATION-POINT ANTIGEN-PRESENTING CELLS

Once vaccine has been delivered to the appropriate target tissue, sufficient quantities of the antigen must gain access to APCs to activate the immune system. The microdelivery vehicles or vectors that can be used for this purpose include live attenuated viruses (including those acting as vectors for exogenous antigen), live attenuated bacteria (including vectors), commensal bacterial vectors, virosomes, VLPs, liposomes, lipopeptides, immune stimulating complexes, microparticles, nanoparticles, and dendritic cells.[690–696] These vaccine microdelivery systems are described in detail in Chapter 64. Here we include an abbreviated description of these systems to complete the discussion of immunization as an antigen package delivery system.

Replicating Systems

Live Viruses and Bacteria

Viruses and bacteria are prototypical antigen delivery vehicles for several reasons: (a) the package size is optimal for cellular uptake; (b) viral surfaces are coated with molecules that attach to cellular receptors, a process that "rings the package delivery bell" (i.e., cells recognize them as potential pathogens, activating innate immunity and increasing cellular uptake); (c) once inside, viruses commandeer cellular systems for replication, multiplying exponentially the vaccine antigen that they encode; and (d) bacteria self-replicate, in tissues or inside cells, multiplying the vaccine antigen available to APCs. Live attenuated strains of pathogenic viruses and bacteria are among the most common type of vaccines and have been used for every delivery route. The major risks of attenuated pathogens as vaccines are potential pathogenic effects, which may occur in immunocompromised persons, following viral reversion to a virulent form, or with exposure of an unintended tissue, such as neurotoxicity following exposure via the olfactory route.[697]

Recombinant viruses and bacteria act as vaccine vectors by incorporation of genes that express a heterologous antigen. Some recombinant vectors also express adjuvants. The replicating systems have advantages similar to those of conventional live attenuated virus vaccines. They deliver the genetic code for the vaccine antigens and adjuvants to host cells for replication, and the cells manufacture the vaccine components to activate the immune system. Viruses and bacteria used as vaccine vectors should, ideally, have very low pathogenic potential, even in immunocompromised people, and they should have the capacity to incorporate the necessary foreign genes for desired antigens, promoters, and adjuvants. As vectors, bacteria have an advantage over viruses because of their higher capacity for insertion of heterologous genes expressing antigens, adjuvants, or plasmids (for DNA vaccination). One caveat for the use of vectored vaccines is that preexisting immunity to the vector may reduce vaccine effectiveness.[698]

DNA Vaccines

DNA vaccination involves the delivery of eponymous plasmids directly into host cells to express the desired antigens and adjuvants. Naked DNA lacks cellular entry mechanisms, so the critical hurdle is getting the DNA into the cells without degradation. Live attenuated bacteria, especially *Salmonella* and *Shigella*, have been vectored to produce DNA for IN vaccination.[699–704] Virosomes, liposomes, and microparticles have also been used to deliver DNA vaccines.[705–708]

Nonreplicating Vaccine Delivery Systems

Synthetic constructs, including liposomes, VLPs, virosomes, immune stimulating complexes, microparticles, and nanoparticles, are nonreplicating delivery systems that mimic live viruses in the way they appear to the immune system to enhance antigen delivery. They may also carry adjuvant. The particles are about the size of viruses, allowing similar uptake by APCs. Many include a lipid component to increase cell membrane permeability and may contain unrelated viral or bacterial proteins to activate the immune system.

One of the most novel methods of delivering antigen into a recipient's APCs is to extract the APCs, by peripheral blood draw for example, culture them with the antigen, and then administer the autologous antigen-laden APCs via intravenous, IM, or SC injection. Autologous dendritic cell-based vaccines are under development as curative cancer vaccines.[695,696]

Adjuvants

Nonreplicating antigens are typically poorly immunogenic and may require adjuvants to stimulate an appropriate immune response. One mechanism of adjuvant action is to provide "warning labels," implying the antigen package is a potential pathogen, to alert cells and induce innate immunity. Adjuvants studied for this purpose include pathogen components such as bacterial toxins and their derivatives, other bacterial components, and bacterial DNA motifs. Other adjuvants include the cytokines and chemokines that cells produce to induce innate immunity. Another adjuvant mechanism is to increase the residence time of the antigen in the target tissue, prolonging the opportunity for the antigen package to be taken up by APCs. Aluminum adjuvants provide this depot effect for many inactivated vaccines delivered intramuscularly or subcutaneously. For mucosal delivery, nanoemulsions and natural polymers such as chitosan have mucoadhesive properties that delay mucosal antigen clearance.[621,708a,708b,709–712]

CONCLUSION

New technologies for delivering antigen packages have the potential to overcome logistical hurdles that impede delivery of vaccines to all populations in need. Practically ideal vaccines would be safe, effective, and inexpensive. They would have these characteristics: (1) packaged as single-unit doses to minimize wastage and missed opportunities to vaccinate; (2) thermostable at ambient temperatures to make vaccines easy to store and ship, even via routine delivery systems such as postal services; (3) available in prefilled dose format to obviate on-site reconstitution or filling; (4) simple to administer or self-administer to reduce dependence on highly skilled healthcare workers; and (5) needle-free to reduce the risks of sharps injuries, needle reuse, and the burden of sharps waste disposal.

Combining new technologies for stabilizing and packaging vaccines with new vaccine administration technologies, new molecular antigen delivery systems, and adjuvants could yield practically ideal vaccines in the next few decades. However, the promising technologies described in this chapter face daunting obstacles to (1) bridge the gap between successful proof of principle and the expensive and complicated series of clinical trials, related studies, and regulatory steps to ensure that the novel systems are safe and effective, and (2) achieve licensure and general availability.

The initial costs of developing, licensing, and supplying practically ideal vaccines are very high. To encourage financing, a clear demand—a willingness to pay—must be expressed in advance by key stakeholders, including public health and immunization program policy makers, vaccine purchasers, and independent philanthropic entities. To create this demand, stakeholders must be aware of the potential of new technologies and convinced that the cost of developing practically ideal vaccines is outweighed by the benefit of reducing the long-term costs of current inefficiencies in the immunization system. In February 2014, the WHO held a meeting on Next-Generation Vaccine Delivery Technologies to conduct a rapid review of existing and future technologies, provide a vision for the future of vaccine technologies, and determine the strategic next steps to guide and enable the development, introduction, and uptake of new technologies with potential positive public health impact. Integrated products that would eliminate the need for reconstitution, provide improved thermostability, and offer needle-free delivery were all highlighted as priority technology development areas.[713]

DISCLOSURE

Mark Papania is a coinventor with corresponding financial interests in the AeroVax (CDC, Creare, Inc.) and dry powder inhaler (CDC, Creare, Inc.) devices illustrated in Figs. 68.7F and I.

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References for this chapter are available at ExpertConsult.com.

REFERENCES

1. World Health Organization (WHO). Vaccine Safety Basics e-learning course. Module 3: Adverse events following immunization: Immunization error-related reaction. Available at: <http://vaccine-safety-training.org/immunization-error-related-reaction.html>.
2. World Health Organization (WHO). Global Vaccine Safety: Information for health-care workers—managing adverse events. Available at: <http://www.who.int/vaccine_safety/initiative/detection/managing_AEFIs/en/index4.html>.
3. Logez S, Soyolgerel G, Fields R, et al. Rapid assessment of injection practices in Mongolia. *Am J Infect Control*. 2004;32(1):31-37.
4. World Health Organization (WHO). *Immunization Safety Surveillance: Guidelines for Immunization Programme Managers on Surveillance of Adverse Events Following Immunization*. Geneva, Switzerland: World Health Organization; 2013.
5. World Health Organization (WHO), Department of Vaccines and Biologicals. *The Use of Opened Multi-Dose Vials of Vaccine in Subsequent Immunization Sessions. WHO Policy Statement: Document WHO/V&B/00.09*. Geneva, Switzerland: World Health Organization; 2000. Available at: <https://www.scribd.com/document/201645841/WHO-Policy-Statement-The-Use-of-Opened-Multi-dose-Vials-of-Vaccine-in-Subsequent-Immunization-Sessions#>.
6. Phadke MA, Joshi BN, Warerkar UV, et al. Toxic shock syndrome: an unforeseen complication following measles vaccination. *Indian Pediatr*. 1991;28(6):663-665.
7. Sood DK, Kumar S, Singh S, et al. Measles vaccination in India and controversies regarding adverse reactions. *Vaccine*. 1995;13(8):785-786.
8. John TJ. Death of children after measles vaccination. *Indian Pediatr*. 2008;45(6):477-478.
9. Vaccine supply and quality. Surveillance of adverse events following immunization. *Wkly Epidemiol Rec*. 1996;71(32):237-242.
10. World Health Organization (WHO). Statement regarding interim findings of WHO assessment of deaths of children in Idleb Governorate, Syria. Published September 27, 2014. Available at: <http://www.who.int/mediacentre/news/statements/2014/interim-findings-idleb-syria/en>.
11. Mvundura M, Kien VD, Nga NT, et al. How much does it cost to get a dose of vaccine to the service delivery location? Empirical evidence from Vietnam's Expanded Program on Immunization. *Vaccine*. 2014;32(7):834-838.
12. Norman JJ, Arya JM, McClain MA, et al. Microneedle patches: usability and acceptability for self-vaccination against influenza. *Vaccine*. 2014;32(16):1856-1862.
13. Vivotif Typhoid Vaccine Live Oral Ty21a. Vivotif Package Insert USA–Updated September 2013–Increase of Upper Specification Limit. Available at: <http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM142807.pdf>.
14. Report of the SAGE Working Group on Vaccine Hesitancy. 2014. Available at: <http://www.who.int/immunization/sage/meetings/2014/october/1_Report_WORKING_GROUP_vaccine_hesitancy_final.pdf>.
15. Lieu TA, Black SB, Ray GT, et al. The hidden costs of infant vaccination. *Vaccine*. 2000;19(1):33-41.
16. Jacobson RM, Swan A, Adegbenro A, et al. Making vaccines more acceptable—methods to prevent and minimize pain and other common adverse events associated with vaccines. *Vaccine*. 2001;19(17-19):2418-2427.
17. Nir Y, Paz A, Sabo E, et al. Fear of injections in young adults: prevalence and associations. *Am J Trop Med Hyg*. 2003;68(3):341-344.
18. Simonsen L, Kane A, Lloyd J, et al. Unsafe injections in the developing world and transmission of bloodborne pathogens: a review. *Bull World Health Organ*. 1999;77(10):789-800.
19. Popp W, Rasslan O, Unahalekhaka A, et al. What is the use? An international look at reuse of single-use medical devices. *Int J Hyg Environ Health*. 2010;213(4):302-307.
20. Hagan H, Des Jarlais DC. HIV and HCV infection among injecting drug users. *Mt Sinai J Med*. 2000;67(5-6):423-428.
21. Drucker E, Alcabes PG, Marx PA. The injection century: massive unsterile injections and the emergence of human pathogens. *Lancet*. 2001;358(9297):1989-1992.
22. Hauri AM, Armstrong GL, Hutin YJ. The global burden of disease attributable to contaminated injections given in health care settings. *Int J STD AIDS*. 2004;15(1):7-16.
23. Prüss-Üstün A, Rapiti E, Hutin Y. *Sharps Injuries: Global Burden of Disease from Sharps Injuries to Health-Care Workers. Environmental Burden of Disease Series, No. 3*. Geneva, Switzerland: World Health Organization; 2003. Available at: <http://www.who.int/quantifying_ehimpacts/publications/9241562463/en>.
24. Panlilio AL, Orelien JG, Srivastava PU, et al. Estimate of the annual number of percutaneous injuries among hospital-based healthcare workers in the United States, 1997-1998. *Infect Control Hosp Epidemiol*. 2004;25(7):556-562.
25. National Center for Immunization and Respiratory Diseases. General recommendations on immunization-recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep*. 2011;60(2):1-64.
26. World Health Organization (WHO). *State of the World's Vaccines and Immunization*. 3rd ed. Geneva, Switzerland: World Health Organization; 2009. Available at: <http://apps.who.int/iris/bitstream/10665/44169/1/9789241563864_eng.pdf>.
27. Karp CL, Lans D, Esparza J, et al. Evaluating the value proposition for improving vaccine thermostability to increase vaccine impact in low and middle-income countries. *Vaccine*. 2015;33(30):3471-3479.
28. World Health Organization (WHO). WHO/UNICEF Guidance Note, October 10, 2011. Criteria to determine if a given vaccination is a routine or supplemental dose. Available at: <http://www.who.int/immunization/programmes_systems/policies_strategies/guidance_note_on_vaccination_doses_oct_10_2011.pdf?ua=1>.
29. Lydon P, Gandhi G, Vandelaer J, et al. Health system cost of delivering routine vaccination in low- and lower-middle income countries: what is needed over the next decade? *Bull World Health Organ*. 2014;92(5):382-384.
30. Zipursky S, Djingarey MH, Lodjo JC, et al. Benefits of using vaccines out of the cold chain: delivering meningitis A vaccine in a controlled temperature chain during the mass immunization campaign in Benin. *Vaccine*. 2014;32(13):1431-1435.
31. Lydon P, Zipursky S, Tevi-Benissan C, et al. Economic benefits of keeping vaccines at ambient temperature during mass vaccination: the case of meningitis A vaccine in Chad. *Bull World Health Organ*. 2014;92(2):86-92.
32. World Health Organization (WHO). *The World Health Report 2006–Working Together for Health*. Geneva, Switzerland: World Health Organization; 2006. Available at: <http://www.who.int/whr/2006/en/>.
33. U.S. Food and Drug Administration (FDA). Pandemic and All-Hazards Preparedness Reauthorization Act of 2013 (PAHPRA). Available at: <http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/ucm359581.htm>.
34. The White House. FACT SHEET: The Global Health Security Agenda. July 28, 2015. Available at: <https://www.whitehouse.gov/the-press-office/2015/07/28/fact-sheet-global-health-security-agenda>.
35. U.S. Food and Drug Administration (FDA). Medical Countermeasures Initiative Strategic Plan 2012–2106. Available at: <http://www.fda.gov/downloads/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/UCM286151.pdf>.
36. Kumru OS, Joshi SB, Smith DE, et al. Vaccine instability in the cold chain: mechanisms, analysis and formulation strategies. *Biologicals*. 2014;42(5):237-259.
37. PATH. *Summary of Vaccine Stability Data*. Seattle, WA: PATH; 2012. Available at: <http://www.path.org/publications/detail.php?i=1696>.
38. World Health Organization (WHO). Global Advisory Committee on Vaccine Safety. Global Vaccine Safety: Statement on thiomersal. July, 2006. Available at: <http://www.who.int/vaccine_safety/committee/topics/thiomersal/statement_jul2006/en/>.
39. U.S. Food and Drug Administration (FDA). Preservatives in Vaccines. Available at: <http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/VaccineSafety/UCM096228#pres>.
40. Chen D, Tyagi A, Carpenter J, et al. Characterization of the freeze sensitivity of a hepatitis B vaccine. *Hum Vaccin*. 2009;5(1):26-32.

41. Chen D, Kristensen D. Opportunities and challenges of developing thermostable vaccines. *Expert Rev Vaccines*. 2009;8(5):547-557.
42. McAdams D, Chen D, Kristensen D. Spray drying and vaccine stabilization. *Expert Rev Vaccines*. 2012;11(10):1211-1219.
43. Li X, Thakkar SG, Ruwona TB, et al. A method of lyophilizing vaccines containing aluminum salts into a dry powder without causing particle aggregation or decreasing the immunogenicity following reconstitution. *J Control Release*. 2015;204:38-50.
44. Hassett KJ, Meinerz NM, Semmelmann F, et al. Development of a highly thermostable, adjuvanted human papillomavirus vaccine. *Eur J Pharm Biopharm*. 2015;94:220-228.
45. Maa YF, Zhao L, Payne LG, et al. Stabilization of alum-adjuvanted vaccine dry powder formulations: Mechanism and application. *J Pharm Sci*. 2003;92(2):319-332.
46. Tonnis W, Amorij JP, Vreeman MA, et al. Improved storage stability and immunogenicity of hepatitis B vaccine after spray-freeze drying in presence of sugars. *Eur J Pharm Sci*. 2014;55:36-45.
47. Ohtake S, Martin RA, Yee L, et al. Heat-stable measles vaccine produced by spray drying. *Vaccine*. 2010;28(5):1275-1284.
48. Chen D, Kapre S, Goel A, et al. Thermostable formulations of a hepatitis B vaccine and a meningitis a polysaccharide conjugate vaccine produced by a spray-drying method. *Vaccine*. 2010;28(31):5093-5099.
49. World Health Organization (WHO). *Guidelines on the International Packaging and Shipping of Vaccines. WHO/IVB/05.23*. Geneva, Switzerland: World Health Organization; 2005. Available at: <http://www.unicef.org/supply/files/Annex_C__Guidelines_on_the_International_Packaging_and_Shipping_of_Vaccines.pdf>.
50. Centers for Disease Control and Prevention (CDC). Immunization Information Systems (IIS). NDC Crosswalk tables: Vaccine National Drug Code (NDC). Available at: <http://www2a.cdc.gov/vaccines/iis/iisstandards/ndc_crosswalk.asp>.
51. Zadbuke N, Shahi S, Gulecha B, et al. Recent trends and future of pharmaceutical packaging technology. *J Pharm Bioallied Sci*. 2013;5(2):98-110.
52. World Health Organization (WHO). Assessing the programmatic suitability of vaccine candidates for WHO prequalification. *Revision*. 2014. Available at: <http://apps.who.int/iris/bitstream/10665/148168/1/WHO_IVB_14.10_eng.pdf>.
53. World Health Organization (WHO). WHO Expert Committee on Biological Standardization. *World Health Organ Tech Rep Ser*. 2014;987:1.
54. Rios M, Harrison B. Big shot: developments in prefilled syringes. *Pharm Technol*. 2007;31(3). Available at: <http://www.pharmtech.com/big-shot-developments-prefilled-syringes>.
55. Ng K, Malone R, Xiong C, Yi X. An integrated prefilled syringe platform approach for vaccine development. *Biopharm Int*. 2013;23(6):22-29.
56. Drug Development & Delivery (). Special Feature—Prefilled Syringes & Parenteral Contract Manufacturing—Product Differentiation Is Critical. 2014. Available at: <http://www.drug-dev.com/Main/Back-Issues/SPECIAL-FEATURE-Prefilled-Syringes-Parenteral-Cont-695.aspx#sthash.9qaMiT16.dpuf>.
57. Joshi HN. Quality by design (QbD) of sterile dosage form packaging. American Pharmaceutical Review. August 22, 2012. Available at: <http://www.americanpharmaceuticalreview.com/Featured-Articles/119645-Quality-by-Design-QbD-of-Sterile-Dosage-Form-Packaging/>.
58. Schmidt K. Prefilled syringes quality by design: Designed, sealed and delivered. Catalent. 2012. Available at: <http://www.catalent.com/index.php/thinking/market-insights/Prefilled-Syringes-Quality-By-Design-Designed-Sealed-and-Delivered>.
59. OSHA, Department of Labor: Occupational Safety and Health Administration. Occupational exposure to bloodborne pathogens: Final rule. 66:5317–5325. January 18, 2001. 29 CFR Part 1910. Available at: <https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=FEDERAL_REGISTER&p_id=16265>.
60. Drug Development & Delivery. The new industry paradigm for prefilled success. March 12, 2013. Available at: <http://www.drug-dev.com/Main/Back-Issues/The-New-Industry-Paradigm-for-Prefilled-Success-528.aspx>.
61. Kiang P. Injectables—future materials for pre-filled syringe components. *Am Pharm Rev*. 2011;14(5):54.
62. Deleted in review.
63. U.S. Pharmacopeial Convention (USP). *Guidebook to Pharmaceutical Compounding—Sterile Preparations*. Ann Arbor, MI: United States Pharmacopeial Convention; 2008. Available at: <http://www.usp.org/usp-nf/official-text/revision-bulletins/general-chapter-pharmaceutical-compounding-sterile-preparations>.
64. Py D, Turner A. Genesis of the closed vial technology. Advanced Aseptic Processing Technology. In: Agalloco J, Akers J, eds. *Advanced Aseptic Processing Technology*. Boca Raton, FL: CRC Press; 2010:172-180.
65. Greb E. In Search of a Sterile Fill in Pharmaceutical Technology. *Pharm Technol*. 2010;34(3). Available at: <http://medinstill.com/pdf/ePrint_PharmaTech_X03.pdf>.
66. Pereira C, Bishai D. Vaccine presentation in the USA: economics of prefilled syringes versus multi-dose vials for influenza vaccination. *Expert Rev Vaccines*. 2010;9(11):1343-1349.
67. ISO 11040-4:2015. Prefilled syringes—Part 4: Glass barrels for injectables. Available at: <http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=58079>.
68. Eakins M. Plastic pre-fillable syringes and vials: progress towards a wider acceptance. *Am Pharm Rev*. 2010;13:12-16.
69. Cidra Precision Services. Cyclic olefin copolymer (COC)/cyclic olefin polymer (COP). Available at: <http://www.cidraprecisionservices.com/life-sciences-materials-polymer-materials-coc-cop.html>.
70. Plas-Tech Engineering home page. Available at: <http://www.plastechengineering.com/>.
71. Polyplastics. TOPAS Thermoplastic Olefin Polymer of Amorphous Structure (COC). Available at: <http://www.polyplastics.com/en/product/lines/topas/general_e.pdf>.
72. Dowlat HA. Prefilled Syringes and Related Biologic Drug/Devices: Market Trends and Regulatory Acceptability. Pharmaceutical Outsourcing. 2013. Available at: <http://www.pharmoutsourcing.com/Featured-Articles/150836-Prefilled-Syringes-and-Related-Biologic-Drug-Devices-Market-Trends-and-Regulatory-Acceptability/>.
73. Aseptic Technologies. A first product approved in the Crystal Closed Vial for all European countries. 2011. NEWS 17. Available at: <http://www.aseptictech.com/ASEPTIC/Edit.aspx?Ctrl=NewsletterDetails&LetterId=517&mid=12984&Page=e-news>.
74. Vaccine Presentation and Packaging Advisory Group (VPPAG). Generic Preferred Product Profile for Vaccines, Version 2.1: Recommendations. Seattle, WA: VPPAG; 2009. Available at: <http://www.who.int/immunization/policy/committees/VPPAG_Generic_PPP_and_Workplan.pdf>.
75. World Health Organization (WHO), UNICEF. Information Bulletin: DTwP-HepB-Hib vaccine available in a compact, pre-filled auto-disable injection technology (cPAD). 2015. Available at: <http://www.who.int/immunization/programmes_systems/service_delivery/InfoBulletin_Uniject_March2015_FINAL_ENG.pdf?ua=1>.
76. BD Medical-Pharmaceutical Systems, Technical Manual. Uniject Prefillable Injection System. Edition June 4, 2009.
77. World Health Organization (WHO). Quinvaxem prequalification reference. Immunization Standards: Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-*Haemophilus influenzae* type b (single dose compact prefilled autodisable device). Available at: <http://www.who.int/immunization_standards/vaccine_quality/pq_283_dtphepbhib_1dose_uniject_Crucell_Korea/en/>.
78. PATH. *Pentavalent Vaccine in the Uniject Injection System—A Time and Motion Study*. Seattle, WA: PATH; 2014.
79. Sheldon EA, Jeanfreau R, Sliman JA, et al. Immunogenicity of a quadrivalent Ann Arbor strain live attenuated influenza vaccine delivered using a blow-fill-seal device in adults: a randomized, active-controlled study. *Influenza Other Respir Viruses*. 2013;7(6):1142-1150.
80. Leslie P. Benefits with BFS container vs a preformed LDPE container. Blow-fill-seal International Operators Association Meeting. Seattle, WA: GlaxoSmithKline. October 1, 2015.
81. Phillips EK, Conaway M, Parker G, et al. Issues in understanding the impact of the Needlestick Safety and Prevention Act

on hospital sharps injuries. *Infect Control*. 2013;34(09):935-939.
82. Lavoie MC, Verbeek JH, Pahwa M. Devices for preventing percutaneous exposure injuries caused by needles in healthcare personnel. *Cochrane Database Syst Rev*. 2014;(3):CD009740.
83. Deleted in review.
84. World Health Organization (WHO). *"First, Do No Harm": Introducing Auto-disable Syringes and Ensuring Injection Safety in Immunization Systems of Developing Countries*. Geneva, Switzerland: WHO; 2002. Available at: <http://apps.who.int/iris/bitstream/10665/67612/1/WHO_V-B_02.26_eng.pdf>.
85. World Health Organization (WHO). *WHO Guideline on the Use of Safety-Engineered Syringes for Intramuscular, Intradermal and Subcutaneous Injections in Health-Care Settings*. Geneva, Switzerland: World Health Organization; 2015.
86. Liversidge B. Safety-engineered needle technologies from tip-top gaining recognition amongst syringe manufacturers. In: *Pre-filled Syringes: With the Patient & Safety at Its Core, the Market Re-Aligns*. OnDrugDelivery; 2011:4-7. Available at: <http://www.ondrugdelivery.com/publications/Prefilled%20Syringes%20Sep%202011/Prefilled%20Syringes%20September%202011%20Low%20Res.pdf>.
87. Lee JM, Botteman MF, Xanthakos N, et al. Needlestick injuries in the United States. Epidemiologic, economic, and quality of life issues. *AAOHN J*. 2005;53(3):117-133.
88. Credence MedSystems. The Credence Companion Safety Syringe System. Available at: <http://www.credencemed.com/>.
89. U.S. Food and Drug Administration (FDA). June 8, 2006 Approval Letter—Human Papillomavirus Quadrivalent (Types 6, 11, 16, 18) Vaccine, Recombinant. Available at: <http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm111283.htm>.
90. World Health Organization (WHO). Performance, Quality, and Safety (PQS) performance specification: Vaccine Cold Box. 2008. Available at: <http://www.who.int/immunization_standards/vaccine_quality/pqs_e004_cb01_2_pps.pdf>.
91. Centers for Disease Control and Prevention (CDC). *Guidelines for Smallpox Vaccine Packaging & Shipping*. Atlanta, GA: CDC; 2003. Available at: <http://emergency.cdc.gov/agent/smallpox/vaccination/pdf/packing-shipping.pdf>.
92. Kristensen D, Chen D. Strategies to advance vaccine technologies for resource-poor settings. *Vaccine*. 2013;31:B157-B162.
93. Landaal Packaging Systems. Biodegradable Foam Products. Available at: <http://www.landaal.com/environmentally-focused-packaging/biodegradable-foam-products/>.
94. Greener Package. Biodegradable foam protects Sandoz's shipments, 2010. Available at: <http://www.greenerpackage.com/renewable_resources/biodegradable_foam_protects_sandozs_shipments>.
95. Ecovative corporate website. Available at: <http://www.ecovativedesign.com>.
96. Fischer E. Diminishing vaccine wastage: next-gen temperature-controlled packaging page. Pharmaceutical Technology. April 2, 2012. Available at: <http://www.pharmaceutical-technology.com/features/featurediminishing-vaccine-wastage-the-next-gen-of-temperature-controlled-packaging/>.
97. Coldpack Airliner website. Available at: <http://www.coldpack.com/usa/about.html>.
98. Dupont. Surlyn, ionomer resin page. Available at: <http://www.dupont.com/products-and-services/plastics-polymers-resins/ethylene-copolymers/brands/surlyn-ionomer-resin.html>.
99. Softbox Systems. Available at: <https://www.softboxsystems.com/>.
100. Pelican BioThermal. Available at: <http://pelicanbiothermal.com/>.
101. Deleted in review.
102. Deleted in review.
103. Fisher BioServices Adopts Credo Container for Flu Vaccines. PMP News Magazine; 2009. Available at: <http://www.pmpnews.com/article/fisher-bioservices-adopts-credo-container-flu-vaccines-0>.
104. Deleted in review.
105. Thermosafe. Greenbox. Available at: <https://www.thermosafe.com/greenbox>.
106. Vetter Packaging Solutions build in safety, quality, and opportunity. Available at: <http://www.vetter-pharma.com/en/services-solutions-en/vetter-packaging-solutions/specialized-packaging-technologies/vetter-dual-chamber-systems>.
107. LyoGo. LyoGo Dual Chamber Pre-Filled Syringe page. Available at: <http://www.lyogo.com/products/l-pfs/>.
108. Unilife. EZMix page. Available at: <http://www.unilife.com/product-platforms/reconstitution-and-mixing/ezmix>.
109. Fisher Container. Pouches page. Available at: <http://www.fishercontainer.com/pouches.html>.
109a. AktiVax. Core Technology ARCH: the vial of the future. Available at: <http://planasolutions.brinkster.net/wordpress/index.php/products/>.
109b. Aktivax Immunoject. Available at: <http://planasolutions.brinkster.net/wordpress/index.php/products/immunoject/>.
110. *Solu-Cortef 100 mg Act-O-Vial*. Houston, TX: The University of Texas Anderson Cancer Center; 2007.
111. *PrSOLU-MEDROL (methylprednisolone sodium succinate) Product Monograph*. Kirkland, Quebec: Pfizer Canada Inc; 2014.
112. Duoject. Z-Link—A reconstitution device. Available at: <http://www.duoject.com/en/product-details/2/>.
113. Reconstitution Devices—Mix2Vial page. Available at: <http://www.westpharma.com/en/products/Documents/SAS%20SS%20Mix2Vial%207250%20%20LR.pdf>.
114. Reconstitution Devices—Needle-Free Transfer Device. Available at: <http://www.westpharma.com/SiteCollectionDocuments/Needle-free%20Transfer%20Device%20LR.pdf>.
115. Reconstitution Devices—MixJect. Available at: <http://www.westpharma.com/en/products/Documents/SAS%20SS%20MixJect%207250%20%20LR.pdf>.
116. Systems D. *Duoject in Perspective: Where We are Now, and Where We are Going*. East Sussex, UK: Frederick Furness Publishing Ltd; 2013.
117. Novartis Vaccines. RABIPUR Package leaflet. Available at: <http://www.who.int/immunization_standards/vaccine_quality/Rabipur_Product_Insert.pdf?ua=1>.
118. World Health Organization (WHO). WHO Immunization Standards: Diphtheria-Tetanus-Pertussis (whole cell)-Hepatitis B-Haemophilus influenzae type b (1 dose vial + vial). Available at: <http://www.who.int/immunization_standards/vaccine_quality/PQ_56_penta_GSK_1_dose/en/>.
119. Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. *Nat Rev Immunol*. 2006;6(2):148-158.
120. Mitragotri S. Current status and future prospects of needle-free liquid jet injectors. *Nat Rev Drug Discov*. 2006;5(7):543-548.
121. Hingson RA, Hughes JG. Clinical studies with jet injection; a new method of drug administration. *Curr Res Anesth Analg*. 1947;26(6):221-230.
122. Schramm J, Mitragotri S. Transdermal drug delivery by jet injectors: energetics of jet formation and penetration. *Pharm Res*. 2002;19(11):1673-1679.
123. Figge FH, Barnett DJ. Anatomic evaluation of a jet injection instrument designed to minimize pain and inconvenience of parenteral therapy. *Am Pract Dig Treat*. 1948;3(4):197-206.
124. Hughes JG, Jordan RG, Hill FS. Jet injection in pediatric practice. *Pediatrics*. 1949;3(6):801-812.
125. Schramm-Baxter J, Mitragotri S. Needle-free jet injections: dependence of jet penetration and dispersion in the skin on jet power. *J Control Release*. 2004;97(3):527-535.
126. Fenner F, Henderson DA, Arita I, et al. *Smallpox and Its Eradication*. Geneva, Switzerland: World Health Organization; 1988.
127. Barclay EM, Hingson RA, Abram LE, et al. Mass vaccination against smallpox in Liberia. *Bull Acad Med Clevel*. 1962;47(suppl 8):16-23.
128. Meyer HM Jr, Hostetler DD Jr, Bernheim BC, et al. Response of Volta children to jet inoculation of combined live measles, smallpox and yellow fever vaccines. *Bull World Health Organ*. 1964;30(6):783-794.
129. Kalabus F, Sansarricq H, Lambin P, et al. Standardization and mass application of combined live measles-smallpox vaccine in Upper Volta. *Am J Epidemiol*. 1967;86(1):93-111.
130. Millar JD, Foege WH. Status of eradication of smallpox (and control of measles) in West and Central Africa. *J Infect Dis*. 1969;120(6):725-732.

131. Millar JD, Morris L, Macedo Filho A, et al. The introduction of jet injection mass vaccination into the national smallpox eradication program of Brazil. *Trop Geogr Med*. 1971;23(1):89-101.
132. Ruben FL, Smith EA, Foster SO, et al. Simultaneous administration of smallpox, measles, yellow fever, and diphtheria-pertussis-tetanus antigens to Nigerian children. *Bull World Health Organ*. 1973;48(2):175-181.
133. Meyer HM Jr. Mass vaccination against measles in Upper Volta. *Arch Gesamte Virusforsch*. 1965;16:243-245.
134. Hendrickse RG, Montefiore D, Peradze T, et al. Measles vaccination. Report of a large scale trial of further attenuated measles vaccine in Nigeria. *J Trop Med Hyg*. 1966;69(5):112-116.
135. de Quadros CA, Hersh BS, Nogueira AC, et al. Measles eradication: experience in the Americas. *Bull World Health Organ*. 1998;76(suppl 2):47-52.
136. Hingson RA, Davis HS, Rosen M. Clinical experience with one and a half million jet injections in parenteral therapy and in preventive medicine. *Mil Med*. 1963;128:525-528.
137. Hingson RA, Davis HS, Bloomfield RA, et al. Mass inoculation of the Salk polio vaccine with the multiple dose jet injector. *GP*. 1957;15(5):94-96.
138. Mohammed I, Obineche EN, Onyemelukwe GC, et al. Control of epidemic meningococcal meningitis by mass vaccination. I. Further epidemiological evaluation of groups A and C vaccines in northern Nigeria. *J Infect*. 1984;9(2):190-196.
139. Spiegel A, Greindl Y, Lippeveld T, et al. Effect of 2 vaccination strategies on developments during the epidemic of meningococcal A meningitis in N'Djamena (Chad) in 1988. *Bull World Health Organ*. 1993;71(3-4):311-315. [in French].
140. Spiegel A, Moren A, Varaine F, et al. Epidemiological and control aspects of meningococcal meningitis epidemics in Africa]. *Sante*. 1994;4(3):231-236. [in French].
141. Anderson EA, Lindberg RB, Hunter DH. Report of large-scale field trial of jet injection in immunization for influenza. *J Am Med Assoc*. 1958;167(5):549-552.
142. Ivannikov Iu G, Efimenko IB, Marinich IG, et al. Evaluation of mass influenza prevention effectiveness using an inactivated chromatographic vaccine in Leningrad]. *Zh Mikrobiol Epidemiol Immunobiol*. 1980;11:18-27. [in Russian].
143. Meyer HM Jr. Field experience with combined live measles, smallpox and yellow fever vaccines. *Arch Gesamte Virusforsch*. 1965;16:365-366.
144. Artus JC. Mass vaccination with the Rockefeller 17 D strain vaccine in Senegal. Use of "Ped-o-Jet". *Med Trop (Mars)*. 1966;26(5):527-536. [in French].
145. Foege WH, Eddins DL. Mass vaccination programs in developing countries. *Prog Med Virol*. 1973;15:205-243.
146. Barrett C, Molner J. Automated multiple immunization against diphtheria, tetanus and poliomyelitis. *J Sch Health*. 1962;32:48-50.
147. Veronesi R, Gomes LF, Soares MA, et al. Importance of the jet injector (injection without a needle) in mass immunization projects in Brazil: results with anti-tetanus and anti-smallpox vaccines. *Rev Hosp Clin Fac Med Sao Paulo*. 1966;21(2):92-95. [in Portuguese].
148. Rey M, Triau R, Diop Mar I, et al. *Single shot tetanus immunization and its application to mass campaign. In: 3rd International Conference on Tetanus; August 17-22, 1970; São Paulo, Brazil, Scientific Publication No. 253*. Washington, DC: Pan American Health Organization; 1972:94-101.
149. Rey M, Diop Mar I, Gbezo P, et al. Mass antitetanus vaccination in Africa]. *Nouv Presse Med*. 1973;2(8):514. [in French].
150. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, et al. Guillain-Barre syndrome following vaccination in the National Influenza Immunization Program, United States, 1976–1977. *Am J Epidemiol*. 1979;110(2):105-123.
151. Ehrengut W, Allerdist H, Erdmann G. Clinical reactions to an adsorbed killed trivalent influenza vaccine (including A/New Jersey 8/76 antigen) with different immunization methods. *Dev Biol Stand*. 1977;39:283-287.
152. Munshi AK, Hegde A, Bashir N. Clinical evaluation of the efficacy of anesthesia and patient preference using the needleless jet syringe in pediatric dental practice. *J Clin Pediatr Dent*. 2001;25(2):131-136.
153. Jimenez N, Bradford H, Seidel KD, et al. A comparison of a needle-free injection system for local anesthesia versus EMLA for intravenous catheter insertion in the pediatric patient. *Anesth Analg*. 2006;102(2):411-414.
154. Greenberg RS, Maxwell LG, Zahurak M, et al. Preanesthetic medication of children with midazolam using the Biojector jet injector. *Anesthesiology*. 1995;83(2):264-269.
155. Zsigmond EK, Kovacs V, Fekete G. A new route, jet-injection for anesthetic induction in children: I. Midazolam dose-range finding studies. *Int J Clin Pharmacol Ther*. 1995;33(10):580-584.
156. Hingson R, Easley E. Hypospray administration of penicillin in the treatment of gonorrhea. *J Vener Dis Inf*. 1948;29(3):61-63.
157. Hirsh HL, Welch H, Milloff B, Katz S. Administration of penicillin and streptomycin by means of the hypospray apparatus; absorption, toxicity, and stability. *J Lab Clin Med*. 1948;33(7):805-810.
158. Black J, Nagle CJ, Strachan CJ. Prophylactic low-dose heparin by jet injection. *Br Med J*. 1978;2(6130):95.
159. Baer CL, Bennett WM, Folwick DA, et al. Effectiveness of a jet injection system in administering morphine and heparin to healthy adults. *Am J Crit Care*. 1996;5(1):42-48.
160. Harris M, Joy R, Larsen G, et al. Enfuvirtide plasma levels and injection site reactions using a needle-free gas-powered injection system (Biojector). *AIDS*. 2006;20(5):719-723.
161. Gottlieb M, Thommes JA, WAND Study Team. Short communication safety, tolerability and pharmacokinetics of enfuvirtide administered by a needle-free injection system compared with subcutaneous injection. *Antivir Ther*. 2008;13(5):723-727.
162. Clarke AK, Woodland J. Comparison of two steroid preparations used to treat tennis elbow, using the hypospray. *Rheumatol Rehabil*. 1975;14(1):47-49.
163. Martins JK, Roedl EA. Medijector—a new method of corticosteroid-anesthetic delivery. *J Occup Med*. 1979;21(12):821-824.
164. Nathan CF, Kaplan G, Levis WR, et al. Local and systemic effects of intradermal recombinant interferon-gamma in patients with lepromatous leprosy. *N Engl J Med*. 1986;315(1):6-15.
165. Brodell RT, Bredle DL. The treatment of palmar and plantar warts using natural alpha interferon and a needleless injector. *Dermatol Surg*. 1995;21(3):213-218.
166. Perkin FS, Todd GM, Brown TM, Abbott HL. Jet injection of insulin in treatment of diabetes mellitus. *Proc Am Diabetes Assoc*. 1950;10:185-199.
167. Bremseth DL, Pass F. Delivery of insulin by jet injection: recent observations. *Diabetes Technol Ther*. 2001;3(2):225-232.
168. Resman Z, Metelko Z, Skrabalo Z. The application of insulin using the jet injector DG-77. *Acta Diabetol Lat*. 1985;22(2):119-125.
169. Welty TK, Josimovich JB, Gerende JH, et al. Reduction of variability in the anovulatory period following medroxyprogesterone acetate injection by using jet injectors. *Fertil Steril*. 1970;21(9):673-682.
170. Bareille P, MacSwiney M, Albanese A, et al. Growth hormone treatment without a needle using the Preci-Jet 50 transjector. *Arch Dis Child*. 1997;76(1):65-67.
171. Dorr HG, Zabransky S, Keller E, et al. Are needle-free injections a useful alternative for growth hormone therapy in children? Safety and pharmacokinetics of growth hormone delivered by a new needle-free injection device compared to a fine gauge needle. *J Pediatr Endocrinol Metab*. 2003;16(3):383-392.
172. Osorio JE, Huang CY, Kinney RM, et al. Development of DENVax: a chimeric dengue-2 PDK-53-based tetravalent vaccine for protection against dengue fever. *Vaccine*. 2011;29(42):7251-7260.
173. Velez ID. Safety and immunogenicity study to assess DENVax, a live attenuated tetravalent vaccine for prevention of dengue fever. Available at: <https://clinicaltrials.gov/ct2/show/NCT01224639>.
174. George S. Tetravalent chimeric dengue vaccine trial. Available at: <https://clinicaltrials.gov/ct2/show/NCT01110551>.
175. Beckett CG, Tjaden J, Burgess T, et al. Evaluation of a prototype dengue-1 DNA vaccine in a Phase 1 clinical trial. *Vaccine*. 2011;29(5):960-968.
176. Cattamanchi A, Posavad CM, Wald A, et al. Phase I study of a herpes simplex virus type 2 (HSV-2) DNA vaccine administered to healthy, HSV-2-seronegative adults by a needle-free injection system. *Clin Vaccine Immunol*. 2008;15(11):1638-1643.
177. Bakari M, Aboud S, Nilsson C, et al. Broad and potent immune responses to a low dose intradermal HIV-1 DNA boosted with HIV-1 recombinant MVA among healthy adults in Tanzania. *Vaccine*. 2011;29(46):8417-8428.

178. Jaoko W, Karita E, Kayitenkore K, et al. Safety and immunogenicity study of multiclade HIV-1 adenoviral vector vaccine alone or as boost following a multiclade HIV-1 DNA vaccine in Africa. *PLoS ONE*. 2010;5(9):e12873.
179. Brave A, Gudmundsdotter L, Sandström E, et al. Biodistribution, persistence and lack of integration of a multigene HIV vaccine delivered by needle-free intradermal injection and electroporation. *Vaccine*. 2010;28(51):8203-8209.
180. Imoto J, Ishikawa T, Yamanaka A, et al. Needle-free jet injection of small doses of Japanese encephalitis DNA and inactivated vaccine mixture induces neutralizing antibodies in miniature pigs and protects against fetal death and mummification in pregnant sows. *Vaccine*. 2010;28(46):7373-7380.
181. Epstein JE, Tewari K, Lyke KE, et al. Live attenuated malaria vaccine designed to protect through hepatic CD8+ T cell immunity. *Science*. 2011;334(6055):475-480.
182. Ginsberg BA, Gallardo HF, Rasalan TS, et al. Immunologic response to xenogeneic gp100 DNA in melanoma patients: comparison of particle-mediated epidermal delivery with intramuscular injection. *Clin Cancer Res*. 2010;16(15):4057-4065.
183. Epstein JE, Gorak EJ, Charoenvit Y, et al. Safety, tolerability, and lack of antibody responses after administration of a PfCSP DNA malaria vaccine via needle or needle-free jet injection, and comparison of intramuscular and combination intramuscular/intradermal routes. *Hum Gene Ther*. 2002;13(13):1551-1560.
184. Wang R, Epstein J, Baraceros FM, et al. Induction of CD4(+) T cell-dependent CD8(+) type 1 responses in humans by a malaria DNA vaccine. *Proc Natl Acad Sci USA*. 2001;98(19):10817-10822.
185. Rao SS, Gomez P, Mascola JR, et al. Comparative evaluation of three different intramuscular delivery methods for DNA immunization in a nonhuman primate animal model. *Vaccine*. 2006;24(3):367-373.
186. Carter EW, Kerr DE. Optimization of DNA-based vaccination in cows using green fluorescent protein and protein A as a prelude to immunization against staphylococcal mastitis. *J Dairy Sci*. 2003;86(4):1177-1186.
187. Imoto J, Konishi E. Needle-free jet injection of a mixture of Japanese encephalitis DNA and protein vaccines: a strategy to effectively enhance immunogenicity of the DNA vaccine in a murine model. *Viral Immunol*. 2005;18(1):205-212.
188. Mumper RJ, Cui Z. Genetic immunization by jet injection of targeted pDNA-coated nanoparticles. *Methods*. 2003;31(3):255-262.
189. Marshall JL, Hoyer RJ, Toomey MA, et al. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol*. 2000;18(23):3964-3973.
190. Evans LS, Johnson M, et al. Microsphere encapsulation or Biojector delivery enhances adjuvanted DNA vaccines in rhesus macaques. 19th Annual Symposium on Nonhuman Primate Models for AIDS. 2001: Monterey, CA.
191. Timmerman JM, Singh G, Hermanson G, et al. Immunogenicity of a plasmid DNA vaccine encoding chimeric idiotype in patients with B-cell lymphoma. *Cancer Res*. 2002;62(20):5845-5852.
192. Lundholm P, Leandersson AC, Christensson B, et al. DNA mucosal HIV vaccine in humans. *Virus Res*. 2002;82(1-2):141-145.
193. Konishi E, Terazawa A, Fujii A. Evidence for antigen production in muscles by dengue and Japanese encephalitis DNA vaccines and a relation to their immunogenicity in mice. *Vaccine*. 2003;21(25-26):3713-3720.
194. Borggren M, Nielsen J, Bragstad K, et al. Vector optimization and needle-free intradermal application of a broadly protective polyvalent influenza A DNA vaccine for pigs and humans. *Hum Vaccin Immunother*. 2015;11(8):1983-1990.
195. Kwilas S, Kishimori JM, Joselyn M, et al. A hantavirus pulmonary syndrome (HPS) DNA vaccine delivered using a spring-powered jet injector elicits a potent neutralizing antibody response in rabbits and nonhuman primates. *Curr Gene Ther*. 2014;14(3):200-210.
196. Ault A, Zajac AM, Kong WP, et al. Immunogenicity and clinical protection against equine influenza by DNA vaccination of ponies. *Vaccine*. 2012;30(26):3965-3974.
197. Gorres JP, Lager KM, Kong WP, et al. DNA vaccination elicits protective immune responses against pandemic and classic swine influenza viruses in pigs. *Clin Vaccine Immunol*. 2011;18(11):1987-1995.
198. Brave A, Ljungberg K, Boberg A, et al. Multigene/multisubtype HIV-1 vaccine induces potent cellular and humoral immune responses by needle-free intradermal delivery. *Mol Ther*. 2005;12(6):1197-1205.
199. Hingson RA, Figge FH. A survey of the development of jet injection in parenteral therapy. *Curr Res Anesth Analg*. 1952;31(6):361-366.
200. Béclard F. Présentation de l'injecteur de Galante, Séance du 18 décembre 1866, Présidence de M. Bouchardat. *Bulletin de l'Académie Impériale de Médecine*. 1866;32:321-327.
201. Hoke CH Jr, Egan JE, Sjogren MH, et al. Administration of hepatitis A vaccine to a military population by needle and jet injector and with hepatitis B vaccine. *J Infect Dis*. 1995;171(suppl 1):S53-S60.
202. Warren J, Ziherl FA, Kish AW, et al. Large-scale administration of vaccines by means of an automatic jet injection syringe. *J Am Med Assoc*. 1955;157(8):633-637.
203. Benenson AS. Mass immunization by jet injection. in Proceedings of the International Symposium of Immunology, 28 September-1 October 1959 (International Committee for Microbiological Standardization, Section of the International Association of Microbiological Societies). Opatija, Yugoslavia.
204. Hingson RA, Davis HS, Rosen M. Historical development of jet injection and envisioned uses in mass immunization and mass therapy based upon 2 decades experience. *Mil Med*. 1963;128(6):516.
205. Neufeld PD, Katz L. Comparative evaluation of three jet injectors for mass immunization. *Can J Public Health*. 1977;68(6):513-516.
206. Eli Lilly and Company. *Influenza Virus Vaccine Polyvalent (Types A and B) [vaccine product insert; 03516, 80:12, PA 1787 AMP]*. Indianapolis, IN: Eli Lilly and Company; 1962:102.
207. Vorob'ev AA, Nekrasov IL, Bandakov LF. Bezygol'nyi sposob vvedeniia biologicheskikh preparatov v organizm [Needle-free method for the introduction of biological preparations into organisms]. *Meditsina*. 1972;1-102.
208. Reis EC, Jacobson RM, Tarbell S, Weniger BG. Taking the sting out of shots: control of vaccination-associated pain and adverse reactions. *Pediatr Ann*. 1998;27(6):375-386.
209. Kutscher AH, Hyman GA, Zegarelli EV, et al. A comparative evaluation of the jet injection technique (Hypospray) and the hypodermic needle for the parenteral administration of drugs: a controlled study. *Am J Med Sci*. 1962;54:418-420.
210. Sarno MJ, Blase E, Galindo N, et al. Clinical immunogenicity of measles, mumps and rubella vaccine delivered by the Injex jet injector: comparison with standard syringe injection. *Pediatr Infect Dis J*. 2000;19(9):839-842.
211. Jackson LA, Austin G, Chen RT, et al. Safety and immunogenicity of varying dosages of trivalent inactivated influenza vaccine administered by needle-free jet injectors. *Vaccine*. 2001;19(32):4703-4709.
212. Stanfield JP, Bracken PM, Waddell KM, et al. Diphtheria-tetanus-pertussis immunization by intradermal jet injection. *Br Med J*. 1972;2(5807):197-199.
213. Parent du Châtelet I, Lang J, Schlumberger M, et al. Clinical immunogenicity and tolerance studies of liquid vaccines delivered by jet-injector and a new single-use cartridge (Imule): comparison with standard syringe injection. Imule Investigators Group. *Vaccine*. 1997;15(4):449-458.
214. Williams J, Fox-Leyva L, Christensen C, et al. Hepatitis A vaccine administration: comparison between jet-injector and needle injection. *Vaccine*. 2000;18(18):1939-1943.
215. Whittle HC, Lamb WH, Ryder RW. Trials of intradermal hepatitis B vaccines in Gambian children. *Ann Trop Paediatr*. 1987;7(1):6-9.
216. Lemon SM, Scott RM, Bancroft WH. Subcutaneous administration of inactivated hepatitis B vaccine by automatic jet injection. *J Med Virol*. 1983;12(2):129-136.
217. Matheï C, Van Damme P, Meheus A. Hepatitis B vaccine administration: comparison between jet-gun and syringe and needle. *Vaccine*. 1997;15(4):402-404.
218. Schlumberger M, Châtelet IP, Lafarge H, et al. Coût de l'injection d'anatoxine tétanique par injecteur sans aiguille (Imule tm) lors d'une vaccination collective au Sénégal: comparaison avec l'injection par seringues et aiguilles restérilisables. *Sante*. 1999;9(5):319-326.

219. Rey M, Triau R. Essais de primo-vaccination antitétanique en un temps avec une anatoxine concentrée inoculée par injecteurs sans aiguille (note préliminaire). *Bull Soc Med Afr Noire Lang Fr*. 1967;12(2):230-239.
220. Agafonov VI, Beliakov VD, Ishkil'din MI, et al. Immunological effectiveness of immunization against smallpox and tularemia by the jet injection method]. *Voen Med Zh*. 1973;4:48-51. [in Russian].
221. Wegmann A, Heiz R, Baumann T. Booster vaccination with a diphtheria-tetanus vaccine for Dermo-Jet with low diphtheria toxoid content]. *Schweiz Med Wochenschr*. 1976;106(4):112-114. [in German].
222. Edwards EA, Johnson DP, Pierce WE, et al. Reactions and serologic responses to monovalent acetone-inactivated typhoid vaccine and heat-killed TAB when given by jet injection. *Bull World Health Organ*. 1974;51(5):501-505.
223. Agafonov VI, Bulatova TI, Gamleshko KhP, et al. Effectiveness of comprehensive immunization with typhoid fever vaccine and polyanatoxin in combination with plague and small pox antigens]. *Voen Med Zh*. 1978;10:51-54. [in Russian].
224. Agafonov VI, Babkin EI, Bulatova TI, et al. Jet method of immunizing with associated sorbed vaccines. *Voen Med Zh*. 1974;12:44-48. [in Russian].
225. Spiegel A, Lemardeley P, Germanetto P, et al. Mini-Imojet et vaccination anti-grippale dans les armées françaises: tolérance immédiate et faisabilité. In: Parent du Châtelet I, Schlumberger M, da Silva A, et al., Association pour l'Aide à la Médecine Préventive, eds. *Actes: 4ème Séminaire International sur les Vaccinations en Afrique-'Bâtir des Partenariats Durables pour les Vaccinations en Afrique', Yamoussoukro, Côte d'Ivoire, 13–16 March 1994*. Lyon: Fondation Mérieux Collection; 1994:344-345.
226. Lipson MJ, Carver DH, Eleff MG, et al. Antibody response to poliomyelitis vaccine administered by jet injection. *Am J Public Health Nations Health*. 1958;48(5):599-603.
227. Schneider U, Birnbacher R, Schober E. Painfulness of needle and jet injection in children with diabetes mellitus. *Eur J Pediatr*. 1994;153:409-410.
228. Kremer MG. Jet vaccination [letter]. *Br Med J*. 1970;4:303.
229. Horn H, Opiz B, Schau G. Investigations into the risk of infection by the use of jet injectors. *Health Soc Serv J*. 1975;85:2396-2397.
230. Rosenthal SR. Transference of blood by various inoculation devices. *Am Rev Respir Dis*. 1967;96(4):815-819.
231. Petersen NJ, Bond WW, Carson LA. *Special Investigations Section, Hepatitis Laboratories Division. Informal quarterly report of October-December 1977 [memorandum]*. Phoenix, AZ: Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control; 1977:1-3.
232. Grabowsky M, Hadler SC, Chen RT, et al. *Risk of Transmission of Hepatitis B Virus or Human Immunodeficiency Virus from Jet Injectors and from Needles and Syringes*. Atlanta, GA: Centers for Disease Control and Prevention; 1994.
233. Brink PR, van Loon AM, Trommelen JC, et al. Virus transmission by subcutaneous jet injection. *J Med Microbiol*. 1985;20(3):393-397.
234. Canter J, Mackey K, Good LS, et al. An outbreak of hepatitis B associated with jet injections in a weight reduction clinic. *Arch Intern Med*. 1990;150(9):1923-1927.
235. Zachoval R, Deinhardt F, Gürtler L, et al. Risk of virus transmission by jet injection. *Lancet*. 1988;1(8578):189.
236. de Souza Brito G, Chen RT, Stefano IC, et al. The risk of transmission of HIV and other blood-born diseases via jet injectors during immunization mass campaigns in Brazil. In: *10th International Conference on AIDS*. Vol. 10. Japan: Yokohama; 1994:301. Abstract PC0132.
237. Department of Defense. Issues of administration: jet injector use. In: Poland GA, ed. *Vaccines in the Military: A Department of Defense-wide Review of Vaccine Policy and Practice. A Report for the Armed Forces Epidemiological Board, August 1999*. Falls Church, VA: Infectious Diseases Control Subcommittee of the Armed Forces Epidemiological Board; 1999:60. Available at: <http://web.archive.org/web/20060615065932/http:/www.ha.osd.mil/afeb/reports/vaccines.pdf>.
238. Weintraub AM, Ponce de Leon MP. Potential for cross-contamination from use of a needleless injector. *Am J Infect Control*. 1998;26(4):442-445.
239. Lukin EP, Evstigneev VI, Makhlaĭ AA, et al. Needle-fee injections and "syringe"-related infections. *Voen Med Zh*. 1997;318(3):48-52.
240. Hoffman PN, Abuknesha RA, Andrews NJ, et al. A model to assess the infection potential of jet injectors used in mass immunisation. *Vaccine*. 2001;19(28-29):4020-4027.
241. Wenger JD, Spika JS, Smithwick RW, et al. Outbreak of *Mycobacterium chelonae* infection associated with use of jet injectors. *JAMA*. 1990;264(3):373-376.
242. Souto FJ, Santo GA, Philippi JC, et al. Prevalência e fatores associados a marcadores do vírus da hepatite B em população rural do Brasil central. *Rev Panam Salud Publica*. 2001;10(6):388-393.
243. Centers for Disease Control and Prevention. Needle-free injection technology. Archived May 20, 2007. Available at: <http://web.archive.org/web/20070520070259/www.cdc.gov/nip/dev/jetinject.htm>.
244. Department of Defense. MMQC-97-1169 Automatic jet hypodermic injection units/withdrawal. (DPSC 970147). Fort Detrick, MD: Quad Service MMQC USAMMA/AFMLO/NMLC. December 3, 1997. <https://asknod.files.wordpress.com/2015/11/1997-ped-o-jet-letter-to-dod.pdf>.
245. Kelly K, Loskutov A, Zehrung D, et al. Preventing contamination between injections with multiple-use nozzle needle-free injectors: a safety trial. *Vaccine*. 2008;26(10):1344-1352.
246. U.S. Department of Health and Human Services. *Guidance for Industry and FDA Staff: Technical Considerations for Pen, Jet, and Related Injectors Intended for Use with Drugs and Biological Products. Silver Springs*. Maryland: U.S. Department of Health and Human Services; 2013. Available at: <http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM147095.pdf>.
247. World Health Organization (WHO) Expanded Programme on Immunization. Safety of injections in immunization programmes: WHO recommended policy. In: *Logistics for Health Information Series (WHO)*. Geneva, Switzerland: World Health Organization; 1994.
248. PR Newswire. MIT Canada launches MED-JET products in Russia after receiving regulatory approval. April 7, 2011. Available at: <http://www.prnewswire.com/news-releases/mit-canada-launches-med-jet-products-in-russia-after-receiving-regulatory-approval-119401189.html>.
249. Weniger BG, Papania MJ. Alternative vaccine delivery methods. In: Plotkin SA, Orenstein WA, Offit PA, eds. *Vaccines*. 5th ed. Philadelphia, PA: Elsevier; 2008.
250. Ziegler A. Needle-free injection—science fiction or comeback of an almost forgotten drug delivery system? *Med Monatsschr Pharm*. 2007;30(8):297-303. [in German].
251. Panchal A, Shah V, Upadhyay U. Insulin drug delivery systems: a review. *Int J Res Pharm Sci*. 2011;2(4):484-492.
252. Hogan NC, Taberner AJ, Jones LA, Hunter IW. Needle-free delivery of macromolecules through the skin using controllable jet injectors. *Expert Opin Drug Deliv*. 2015;12(10):1637-1648.
253. Brandes JL, Cady RK, Freitag FG. Needle-free subcutaneous sumatriptan (Sumavel DosePro): bioequivalence and ease of use. *Headache*. 2009;49(10):1435-1444.
254. Giudice EL, Campbell JD. Needle-free vaccine delivery. *Adv Drug Deliv Rev*. 2006;58(1):68-89.
255. Galy M, Genet A, Saliou P. Un progrès dans le domaine de l'injection sans aiguille: le système Imule. *STP Pharma Prat*. 1992;4:261-266.
256. Fisch A, Cadilhac P, Vidor E, et al. Immunogenicity and safety of a new inactivated hepatitis A vaccine: a clinical trial with comparison of administration route. *Vaccine*. 1996;14(12):1132-1136.
256a. O'Grady KA, et al. FluMum: a prospective cohort study of mother-infant pairs assessing the effectiveness of maternal influenza vaccination in prevention of influenza in early infancy. *BMJ Open*. 2014;4(6):e005676.
257. Griffiths UK, Santos AC, Nundy N, et al. Incremental costs of introducing jet injection technology for delivery of routine childhood vaccinations: comparative analysis from Brazil, India, and South Africa. *Vaccine*. 2011;29(5):969-975.
258. Mohammed AJ, Al Awaidy S, Bawikar S, et al. Fractional doses of inactivated poliovirus vaccine in Oman. *N Engl J Med*. 2010;362(25):2351-2359.
259. Resik S, Tejeda A, Lago PM, et al. Randomized controlled clinical trial of fractional doses of inactivated poliovirus vaccine

administered intradermally by needle-free device in Cuba. *J Infect Dis*. 2010;201(9):1344-1352.
260. Babiuk S, Baca-Estrada ME, Foldvari M, et al. Needle-free topical electroporation improves gene expression from plasmids administered in porcine skin. *Mol Ther*. 2003;8(6):992-998.
261. Cartier R, Ren SV, Walther W, et al. In vivo gene transfer by low-volume jet injection. *Anal Biochem*. 2000;282(2):262-265.
262. Jackson J, Dworkin R, Tsai T, et al. Comparison of antibody response and patient tolerance of yellow fever vaccine administered by the Biojector needle-free injection system versus conventional needle/syringe injection. In: *Third International Conference on Travel Medicine*. Vol. 264. Paris: 1993:209.
263. Bioject, Inc. *Guide to selection and use of Biojector syringes*. Portland, OR: Bioject, Inc; 1997. Document 171-0134-00 Rev C 5/97.
264. Perlman D, Copeland K, McAllister L. *A rabies vaccine trial utilizing two versions of a needle-free jet injection device versus standard needle and syringe: safety and efficacy*. Washington, DC, USA (Terrapinn): World Vaccine Congress; 2012. [poster presentation].
265. Harris M, Larsen G, Valyi M, Walker E, Montaner JS. Transient neuropathy after needle-free injection outside of recommended sites. *AIDS*. 2006;20(5):784-785.
266. Resik S, Tejeda A, Mach O, et al. Immune responses after fractional doses of inactivated poliovirus vaccine using newly developed intradermal jet injectors: a randomized controlled trial in Cuba. *Vaccine*. 2015;33(2):307-313.
267. de Menezes Martins R, Curran B, Maia Mde L, et al. Immunogenicity and safety of measles-mumps-rubella vaccine delivered by disposable-syringe jet injector in healthy Brazilian infants: a randomized non-inferiority study. *Contemp Clin Trials*. 2015;41:1-8.
268. Ambuel Y, Young G, Brewoo JN, et al. A rapid immunization strategy with a live-attenuated tetravalent dengue vaccine elicits protective neutralizing antibody responses in non-human primates. *Front Immunol*. 2014;5:263.
269. Estivariz CF, Jafari H, Sutter RW, et al. Immunogenicity of supplemental doses of poliovirus vaccine for children aged 6-9 months in Moradabad, India: a community-based, randomised controlled trial. *Lancet Infect Dis*. 2012;12(2):128-135.
270. Soonawala D, Verdijk P, Wijmenga-Monsuur AJ, et al. Intradermal fractional booster dose of inactivated poliomyelitis vaccine with a jet injector in healthy adults. *Vaccine*. 2013;31(36):3688-3694.
271. McAllister L, Anderson J, Werth K, et al. Needle-free jet injection for administration of influenza vaccine: a randomised non-inferiority trial. *Lancet*. 2014;384(9944):674-681.
271a. Coughlin MM, Collins M, Saxon G, et al. Effect of jet injection on infectivity of measles, mumps, and rubella vaccine in a bench model. *Vaccine*. 2015;33(36):4540-4547.
272. Stout RR, Gutierrez MJ, Freeland PJ, et al. Needle-free injections using a spring-powered device for subcutaneous, intramuscular and intradermal injections. *Drug Deliv Technol*. 2007;7:40-43.
273. LectraJet. East Syracuse, NY: D'Antonio Consultants International, Inc. Available at: <http://www.dantonioconsultants.com>.
274. Simon JK, Carter M, Pasetti MF, et al. Safety, tolerability, and immunogenicity of inactivated trivalent seasonal influenza vaccine administered with a needle-free disposable-syringe jet injector. *Vaccine*. 2011;29(51):9544-9550.
275. Ekwueme DU, Weniger BG, Chen RT. Model-based estimates of risks of disease transmission and economic costs of seven injection devices in sub-Saharan Africa. *Bull World Health Organ*. 2002;80(11):859-870.
276. Kraan H, Ploemen I, van de Wijdeven G, et al. Alternative delivery of a thermostable inactivated polio vaccine. *Vaccine*. 2015;33(17):2030-2037.
277. Soema PC, Willems GJ, van Twillert K, et al. Solid bioneedle-delivered influenza vaccines are highly thermostable and induce both humoral and cellular immune responses. *PLoS ONE*. 2014;9(3):e92806.
278. Hirschberg HJ, van de Wijdeven GG, Kraan H, et al. Bioneedles as alternative delivery system for hepatitis B vaccine. *J Control Release*. 2010;147(2):211-217.
279. Hirschberg HJ, van de Wijdeven GG, Kelder AB, et al. Bioneedles as vaccine carriers. *Vaccine*. 2008;26(19):2389-2397.
280. Deleted in review.
281. Deleted in review.
282. Potter C, Bennet S. A solid alternative to needle and syringe technology. *Drug Deliv Technol*. 2009;9:24-30.
283. Deleted in review.
284. Salk J. Recent studies on immunization against poliomyelitis. *Pediatrics*. 1953;12(5):471-482.
285. Roukens AH, Gelinck LB, Visser LG. Intradermal vaccination to protect against yellow fever and influenza. *Curr Top Microbiol Immunol*. 2012;351:159-179.
286. Hickling JK, Jones KR, Friede M, et al. Intradermal delivery of vaccines: potential benefits and current challenges. *Bull World Health Organ*. 2011;89(3):221-226.
287. Hickling JK, Jones R. *Intradermal delivery of vaccines: a review of the literature and the potential for development for use in low- and middle-income countries*. Seattle, WA: PATH; 2009:1-94. Available at: <http://www.path.org/publications/detail.php?i=1746>.
288. Picot V. Intradermal immunization: an alternative route for vaccine administration. Articles as per sessions meeting report. *Vaccine*. 2008;26(suppl 9):S1-S5.
289. Dubin CH. Transdermal delivery: making a comeback! *Drug Deliv Technol*. 2010;10:24-28.
290. Kis EE, Winter G, Myschik J. Devices for intradermal vaccination. *Vaccine*. 2012;30(3):523-538.
291. Levine MM. Can needle-free administration of vaccines become the norm in global immunization? *Nat Med*. 2003;9(1):99-103.
292. Cross SE, Roberts MS. Physical enhancement of transdermal drug application: is delivery technology keeping up with pharmaceutical development? *Curr Drug Deliv*. 2004;1(1):81-92.
293. O'Hagan DT, Rappuoli R. Novel approaches to vaccine delivery. *Pharm Res*. 2004;21(9):1519-1530.
294. Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. *Nat Rev Drug Discov*. 2004;3(2):115-124.
295. Prausnitz MR, Langer R. Transdermal drug delivery. *Nat Biotechnol*. 2008;26(11):1261-1268.
296. Mitragotri S. Immunization without needles. *Nat Rev Immunol*. 2005;5(12):905-916.
297. Brown MB, Traynor MJ, Martin GP, et al. Transdermal drug delivery systems: skin perturbation devices. *Methods Mol Biol*. 2008;437:119-139.
298. Lambert PH, Laurent PE. Intradermal vaccine delivery: will new delivery systems transform vaccine administration? *Vaccine*. 2008;26(26):3197-3208.
299. Nicolas JF, Guy B. Intradermal, epidermal and transcutaneous vaccination: from immunology to clinical practice. *Expert Rev Vaccines*. 2008;7(8):1201-1214.
300. Levine MM, Campbell JD. Mucosal immunization and needle-free injection devices. In: *New Generation Vaccines*. New York, NY: Marcel Dekker; 2004:393-399.
301. Prausnitz MR, Mikszta JA, Cormier M, et al. Microneedle-based vaccines. *Curr Top Microbiol Immunol*. 2009;333:369-393. Fig. 4 right.
302. Bal SM, Ding Z, van Riet E, et al. Advances in transcutaneous vaccine delivery: do all ways lead to Rome? *J Control Release*. 2010;148(3):266-282.
303. Mikszta JA, Laurent PE. Cutaneous delivery of prophylactic and therapeutic vaccines: historical perspective and future outlook. *Expert Rev Vaccines*. 2008;7(9):1329-1339.
304. Kendall MA. Needle-free vaccine injection. *Handb Exp Pharmacol*. 2010;197:193-219.
305. Kim YC, Jarrahian C, Zehrung D, et al. Delivery systems for intradermal vaccination. *Curr Top Microbiol Immunol*. 2012;351:77-112.
306. Combadiere B, Mahe B. Particle-based vaccines for transcutaneous vaccination. *Comp Immunol Microbiol Infect Dis*. 2008;31(2-3):293-315.
307. Zehrung D, Jarrahian C, Wales A. Intradermal delivery for vaccine dose sparing: overview of current issues. *Vaccine*. 2013;31(34):3392-3395.
308. Cheng X, Koch PJ. In vivo function of desmosomes. *J Dermatol*. 2004;31(3):171-187.
309. Huber O. Structure and function of desmosomal proteins and their role in development and disease. *Cell Mol Life Sci*. 2003;60(9):1872-1890.
310. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature*. 1998;392(6673):245-252.

311. Romani N, Holzmann S, Tripp CH, et al. Langerhans cells-dendritic cells of the epidermis. *APMIS*. 2003;111(7-8):725-740.
312. Sallusto F. Origin and migratory properties of dendritic cells in the skin. *Curr Opin Allergy Clin Immunol*. 2001;1(5):441-448.
313. Kindt TJ, Osborne BA, Kuby J. *Immunology*. 6th ed. New York, NY: WH Freeman; 2006:603.
314. Teunissen MB, Haniffa M, Collin MP. Insight into the immunobiology of human skin and functional specialization of skin dendritic cell subsets to innovate intradermal vaccination design. *Curr Top Microbiol Immunol*. 2012;351:25-76.
315. Yu RC, Abrams DC, Alaibac M, Chu AC. Morphological and quantitative analyses of normal epidermal Langerhans cells using confocal scanning laser microscopy. *Br J Dermatol*. 1994;131(6):843-848.
316. Merieux C, Merieux A, Triau R. Tuberculination, BCG or smallpox vaccination using a ring with points. *Arch Mal Prof*. 1966;27(4):444-449. [in French].
317. Kravitz H. A simplified technique for vaccination against smallpox. *Pediatrics*. 1961;27:219-226.
318. Birkhaug K. An experimental and clinical investigation of a percutaneous (Rosenthal) method of BCG vaccination. *Nord Med*. 1941;10:1224-1231.
319. Briggs I, Smith C. BCG vaccination by the multiple puncture method in Northern Rhodesia. *Tubercle*. 1957;38(2):107-112.
320. Griffith ABCG. vaccination by multiple puncture. *Lancet*. 1959;273(7084):1170-1172.
321. Gheorghiu M. The present and future role of BCG vaccine in tuberculosis control. *Biologicals*. 1990;18(2):135-141.
322. ten Dam HG, Fillastre C, Conge G, et al. The use of jet-injectors in BCG vaccination. *Bull World Health Organ*. 1970;43(5):707-720.
323. Darmanger AM, Nekzad SM, Kuis M, et al. BCG vaccination by bifurcated needle in a pilot vaccination programme. *Bull World Health Organ*. 1977;55(1):49-61.
324. Kamigawara N. [Retrospective assessment of the effectiveness of BCG vaccination by multiple puncture method (Kuchiki's needle) in childhood tuberculosis] [Article in Japanese]. *Kekkaku*. 1989;64(4):305-311.
325. Laurent PE, Bourhy H, Fantino M, et al. Safety and efficacy of novel dermal and epidermal microneedle delivery systems for rabies vaccination in healthy adults. *Vaccine*. 2010;28(36):5850-5856.
326. Nicholson KG, Prestage H, Cole PJ, et al. Multisite intradermal antirabies vaccination. Immune responses in man and protection of rabbits against death from street virus by postexposure administration of human diploid-cell-strain rabies vaccine. *Lancet*. 1981;2(8252):915-918.
327. Bernard KW, Roberts MA, Sumner J, et al. Human diploid cell rabies vaccine. Effectiveness of immunization with small intradermal or subcutaneous doses. *JAMA*. 1982;247(8):1138-1142.
328. Harverson G, Wasi C. Use of post-exposure intradermal rabies vaccination in a rural mission hospital. *Lancet*. 1984;2(8398):313-315.
329. Warrell MJ, Nicholson KG, Warrell DA, et al. Economical multiple-site intradermal immunisation with human diploid-cell-strain vaccine is effective for post-exposure rabies prophylaxis. *Lancet*. 1985;1(8437):1059-1062.
330. Phanuphak P, Khawplod P, Sirivichayakul S, et al. Humoral and cell-mediated immune responses to various economical regimens of purified Vero cell rabies vaccine. *Asian Pac J Allergy Immunol*. 1987;5(1):33-37.
331. Chutivongse S, Wilde H, Supich C, et al. Postexposure prophylaxis for rabies with antiserum and intradermal vaccination. *Lancet*. 1990;335(8694):896-898.
332. Briggs DJ, Banzhoff A, Nicolay U, et al. Antibody response of patients after postexposure rabies vaccination with small intradermal doses of purified chick embryo cell vaccine or purified Vero cell rabies vaccine. *Bull World Health Organ*. 2000;78(5):693-698.
333. Madhusudana SN, Sanjay TV, Mahendra BJ, et al. Comparison of safety and immunogenicity of purified chick embryo cell rabies vaccine (PCECV) and purified vero cell rabies vaccine (PVRV) using the Thai Red Cross intradermal regimen at a dose of 0.1 ML. *Hum Vaccin*. 2006;2(5):200-204.
333a. Permpalung N, Wongrakpanich S, Korpaisarn S, et al. Trend of human rabies prophylaxis in developing countries: toward optimal rabies immunization. *Vaccine*. 2013;31(38):4079-4083.
334. Rabies vaccines. WHO position paper. *Wkly Epidemiol Rec*. 2007;82(49-50):425-435.
335. Wilde H, Khawplod P, Khamoltham T, et al. Rabies control in South and Southeast Asia. *Vaccine*. 2005;23(17-18):2284-2289.
336. Warrell MJ. Intradermal rabies vaccination: the evolution and future of pre- and post-exposure prophylaxis. *Curr Top Microbiol Immunol*. 2012;351:139-157.
337. West Pharmaceutical Services. Intradermal delivery: an easy and reliable solution for intradermal injection. Available at: <http://www.westpharma.com/products/intradermal-delivery>.
338. Jarrahian C, Zehrung D, Saxon E, et al. Clinical performance and safety of the ID adapter, a prototype intradermal delivery technology for vaccines, drugs, and diagnostic tests. *Procedia Vaccinol*. 2012;6:125-133.
339. Tsals I, Jarrahian C, Snyder FE, et al. Clinical performance and safety of adapters for intradermal delivery with conventional and autodisable syringes. *Vaccine*. 2015;33(37):4705-4711.
340. Sachdeva V, Banga AK. Microneedles and their applications. *Recent Pat Drug Deliv Formul*. 2011;5(2):95-132.
341. Garland MJ, Migalska K, Mahmood TM, et al. Microneedle arrays as medical devices for enhanced transdermal drug delivery. *Expert Rev Med Devices*. 2011;8(4):459-482.
342. Prausnitz MR, Mikszta JA, Raeder-Devens J. Microneedles. In: Smith EW, Maibach HI, eds. *Percutaneous Penetration Enhancers*. 2nd ed. Boca Raton, FL: CRC Press; 2006:239-255.
343. Prausnitz MR. Microneedles for transdermal drug delivery. *Adv Drug Deliv Rev*. 2004;56(5):581-587.
344. Deleted in review.
345. Holland D, Booy R, De Looze F, et al. Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial. *J Infect Dis*. 2008;198(5):650-658.
346. Arnou R, Icardi G, De Decker M, et al. Intradermal influenza vaccine for older adults: a randomized controlled multicenter phase III study. *Vaccine*. 2009;27(52):7304-7312.
347. Van Damme P, Arnou R, Kafeja F, et al. Evaluation of non-inferiority of intradermal versus adjuvanted seasonal influenza vaccine using two serological techniques: a randomised comparative study. *BMC Infect Dis*. 2010;10:134.
348. Leroux-Roels I, Vets E, Freeze R, et al. Seasonal influenza vaccine delivered by intradermal microinjection: a randomised controlled safety and immunogenicity trial in adults. *Vaccine*. 2008;26(51):6614-6619.
349. Arnou R, Eavis P, Pardo JR, et al. Immunogenicity, large scale safety and lot consistency of an intradermal influenza vaccine in adults aged 18-60 years: randomized, controlled, phase III trial. *Hum Vaccin*. 2010;6(4):346-354.
350. Beran J, Ambrozaitis A, Laiskonis A, et al. Intradermal influenza vaccination of healthy adults using a new microinjection system: a 3-year randomised controlled safety and immunogenicity trial. *BMC Med*. 2009;7:13.
351. Morefield GL, Tammariello RF, Purcell BK, et al. An alternative approach to combination vaccines: intradermal administration of isolated components for control of anthrax, botulism, plague and staphylococcal toxic shock. *J Immune Based Ther Vaccines*. 2008;6:5.
352. Mikszta JA, Dekker JP, Harvey NG, et al. Microneedle-based intradermal delivery of the anthrax recombinant protective antigen vaccine. *Infect Immun*. 2006;74(12):6806-6810.
353. Mikszta JA, Sullivan VJ, Dean C, et al. Protective immunization against inhalational anthrax: a comparison of minimally invasive delivery platforms. *J Infect Dis*. 2005;191(2):278-288.
354. Dean CH, Alarcon JB, Waterston AM, et al. Cutaneous delivery of a live, attenuated chimeric flavivirus vaccine against Japanese encephalitis (ChimeriVax-JE) in non-human primates. *Hum Vaccin*. 2005;1(3):106-111.
355. Van Mulder TJ, Verwulgen S, Beyers KC, et al. Assessment of acceptability and usability of new delivery prototype device for intradermal vaccination in healthy subjects. *Hum Vaccin Immunother*. 2014;10(12):3746-3753.
356. Laurent PE, et al. Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system. *Vaccine*. 2007;25(52):8833-8842.

357. Hung IF, et al. Dose sparing intradermal trivalent influenza (2010/2011) vaccination overcomes reduced immunogenicity of the 2009 H1N1 strain. *Vaccine*. 2012;(45):6427-6435.
358. McAllister DV, Wang PM, Davis SP, et al. Microfabricated needles for transdermal delivery of macromolecules and nanoparticles: fabrication methods and transport studies. *Proc Natl Acad Sci USA*. 2003;100(24):13755-13760.
359. Haq MI, Smith E, John DN, et al. Clinical administration of microneedles: skin puncture, pain and sensation. *Biomed Microdevices*. 2009;11(1):35-47.
360. Kaushik S, Hord AH, Denson DD, et al. Lack of pain associated with microfabricated microneedles. *Anesth Analg*. 2001;92(2):502-504.
361. Van Damme P, Oosterhuis-Kafeja F, Van der Wielen M, et al. Safety and efficacy of a novel microneedle device for dose sparing intradermal influenza vaccination in healthy adults. *Vaccine*. 2009;27(3):454-459. Figs. 1 (adapter) and 2A (MicroPyramid).
362. Troy SB, Kouiavskaia D, Siik J, et al. Comparison of the immunogenicity of various booster doses of inactivated polio vaccine delivered intradermally versus intramuscularly to HIV-infected adults. *J Infect Dis*. 2015;211(12):1969-1976.
363. Anand A, Zaman K, Estívariz CF, et al. Early priming with inactivated poliovirus vaccine (IPV) and intradermal fractional dose IPV administered by a microneedle device: a randomized controlled trial. *Vaccine*. 2015;33(48):6816-6822.
364. Beals CR, Railkar RA, Schaeffer AK, et al. Immune response and reactogenicity of intradermal administration versus subcutaneous administration of varicella-zoster virus vaccine: an exploratory, randomised, partly blinded trial. *Lancet Infect Dis*. 2016;16(8):915-922.
365. Deleted in review.
366. Deleted in review.
367. Deleted in review.
368. Deleted in review.
369. Burton SA, Ng CY, Simmers R, et al. Rapid intradermal delivery of liquid formulations using a hollow microstructured array. *Pharm Res*. 2011;28(1):31-40.
370. Nanoject microneedle platform. Lausanne, Switzerland: Debiotech S.A. Available at: <http://www.debiotech.com/newsite/page/index.php?page=product_01&id=1&id_prod=47>.
371. Levin Y, Kochba E, Kenney R. Clinical evaluation of a novel microneedle device for intradermal delivery of an influenza vaccine: are all delivery methods the same? *Vaccine*. 2014;32(34):4249-4252.
372. Prausnitz MR, Bose VG, Langer R, Weaver JC. Electroporation of mammalian skin: a mechanism to enhance transdermal drug delivery. *Proc Natl Acad Sci USA*. 1993;90(22):10504-10508.
373. Prausnitz MR. A practical assessment of transdermal drug delivery by skin electroporation. *Adv Drug Deliv Rev*. 1999;35(1):61-76.
374. Lombry C, Dujardin N, Preat V. Transdermal delivery of macromolecules using skin electroporation. *Pharm Res*. 2000;17(1):32-37.
375. Sintov AC, Krymberk I, Daniel D, et al. Radiofrequency-driven skin microchanneling as a new way for electrically assisted transdermal delivery of hydrophilic drugs. *J Control Release*. 2003;89(2):311-320.
376. Prud'homme GJ, Glinka Y, Khan AS, et al. Electroporation-enhanced nonviral gene transfer for the prevention or treatment of immunological, endocrine and neoplastic diseases. *Curr Gene Ther*. 2006;6(2):243-273.
377. Bramson J, Dayball K, Evelegh C, et al. Enabling topical immunization via microporation: a novel method for pain-free and needle-free delivery of adenovirus-based vaccines. *Gene Ther*. 2003;10(3):251-260.
378. Zhang L, Nolan E, Kreitschitz S, et al. Enhanced delivery of naked DNA to the skin by non-invasive in vivo electroporation. *Biochim Biophys Acta*. 2002;1572(1):1-9.
379. Andre F, Mir LM. DNA electrotransfer: its principles and an updated review of its therapeutic applications. *Gene Ther*. 2004;11(suppl 1):S33-S42.
380. Banga AK. *Electrically Assisted Transdermal and Topical Drug Delivery*. London, UK: Taylor & Francis Group; 1998.
381. Kraynyak KA, Bodles-Brakhop A, Bagarazzi M. Tapping the potential of DNA delivery with electroporation for cancer immunotherapy. *Curr Top Microbiol Immunol*. 2015;[Epub ahead of print].
382. Mann JF, McKay PF, Fiserova A, et al. Enhanced immunogenicity of an HIV-1 DNA vaccine delivered with electroporation via combined intramuscular and intradermal routes. *J Virol*. 2014;88(12):6959-6969.
383. Inovio. Interdermal delivery: intradermal electroporation delivery. Cellectra 3000. Available at: <http://www.inovio.com/technology/electroporation-delivery/electroporation-devices/intradermal-delivery/>.
384. Deleted in review.
385. Luxembourg A, Hannaman D, Ellefsen B, et al. Enhancement of immune responses to an HBV DNA vaccine by electroporation. *Vaccine*. 2006;24(21):4490-4493.
386. Otten GR, Schaefer M, Doe B, et al. Potent immunogenicity of an HIV-1 gag-pol fusion DNA vaccine delivered by in vivo electroporation. *Vaccine*. 2006;24(21):4503-4509.
387. Li Z, Zhang H, Fan X, et al. DNA electroporation prime and protein boost strategy enhances humoral immunity of tuberculosis DNA vaccines in mice and non-human primates. *Vaccine*. 2006;24(21):4565-4568.
388. Trimble CL, Morrow MP, Kraynyak KA, et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. *Lancet*. 2015;386(10008):2078-2088.
389. Muthumani K, Wise MC, Broderick KE, et al. HIV-1 Env DNA vaccine plus protein boost delivered by EP expands B- and T-cell responses and neutralizing phenotype in vivo. *PLoS ONE*. 2013;8(12):e84234.
390. Kopycinski J, Cheeseman H, Ashraf A, et al. A DNA-based candidate HIV vaccine delivered via in vivo electroporation induces CD4 responses toward the alpha4beta7-binding V2 loop of HIV gp120 in healthy volunteers. *Clin Vaccine Immunol*. 2012;19(9):1557-1559.
391. Ferraro B, Talbott KT, Balakrishnan A, et al. Inducing humoral and cellular responses to multiple sporozoite and liver-stage malaria antigens using exogenous plasmid DNA. *Infect Immun*. 2013;81(10):3709-3720.
392. McCoy JR, Mendoza JM, Spik KW, et al. A multi-head intradermal electroporation device allows for tailored and increased dose DNA vaccine delivery to the skin. *Hum Vaccin Immunother*. 2015;11(3):746-754.
393. Hooper JW, Thompson E, Wilhelmsen C, et al. Smallpox DNA vaccine protects nonhuman primates against lethal monkeypox. *J Virol*. 2004;78(9):4433-4443.
394. Roos AK, Eriksson F, Walters DC, et al. Optimization of skin electroporation in mice to increase tolerability of DNA vaccine delivery to patients. *Mol Ther*. 2009;17(9):1637-1642.
395. Diehl MC, Lee JC, Daniels SE, et al. Tolerability of intramuscular and intradermal delivery by CELLECTRA® adaptive constant current electroporation device in healthy volunteers. *Hum Vaccin Immunother*. 2013;9(10):2246-2252.
396. Foege WH. *House on Fire: The Fight to Eradicate Smallpox*. Berkeley: University of California Press; 2011.
397. Ismach A. Intradermal nozzle for jet injection devices (US Patent 3,140,713), assigned to Secretary of the Army on behalf of United States of America. Washington, DC: US Patent Office; issued July 14, 1964.
398. Millar JD, Roberto RR, Wulff H, et al. Smallpox vaccination by intradermal jet injection. I. Introduction, background and results of pilot studies. *Bull World Health Organ*. 1969;41(6):749-760.
399. Neff JM, Millar JD, Roberto RR, et al. Smallpox vaccination by intradermal jet injection. 3. Evaluation in a well-vaccinated population. *Bull World Health Organ*. 1969;41(6):771-778.
400. Weibel RE, Stokes J Jr, Buynak EB, et al. Clinical-laboratory experiences with combined dried live measles-smallpox vaccine. *Pediatrics*. 1966;37(6):913-920.
401. Dull HB, Herring LL, Calafiore D, et al. Jet injector tuberculin skin testing: methodology and results. *Am Rev Respir Dis*. 1968;97:38-45.
402. Meyer HM, Bernheim BC, Rogers NG. Titration of live measles and smallpox vaccines by jet inoculation of susceptible children. *Proc Soc Exp Biol Med*. 1965;118:53-57.

403. Deleted in review.
404. Chambon L, Tommasi UB, Barme M, et al. Vaccination associée BCG-fièvre jaune avec un injecteur du type Ped-O-Jet, vol. I. Rapport Final de la Xème Conférence Technique de l'OCCGE. Bobo-Dioulasso, Upper Volta: Organisation de Coopération et de Coordination pour la Lutte Contre les Grandes Endémies; 20–24 April, 1970. p. 282–288.
405. Gateff C, Robin Y, Labusquiere R, et al. Comparison of 2 yellow fever vaccines administered by pressure injector without needle. *Med Trop (Mars)*. 1972;32(2):193. [in French].
405a. Chambon L, Barme M, Tommasi UB, et al. The use of an injector without needle for intradermal BCG vaccination. *Med Trop (Mars)*. 1970;30(6):809-828. [in French].
406. A comparison of intradermal BCG vaccination by jet injection and by syringe and needle. *Tubercle*. 1971;52(3):155-165.
407. Carnus H. Ped-O-Jet and the viability of BCG. *Med Trop (Mars)*. 1973;33:20-23. [in French].
408. Carnus H. Influence of Ped-O-Jet on the viability of lyophilized BCG intradermal vaccine: laboratory study. *Bull World Health Organ*. 1974;51(1):101-102. [in French].
409. Parker V. Jet gun or syringe? A trial of alternative methods of BCG vaccination. *Public Health*. 1984;98(6):315-320.
410. Paul SS, Raveendra Nath K, Chhabra AK, et al. Comparison of BCG inoculation by conventional intradermal and jet methods. *Indian Pediatr*. 1978;15(4):341-347.
411. Cockburn TA, Witt MT, Ludlow CE, et al. A comparison of jet injection with the Mantoux test in mass skin testing with tuberculin. *Am Rev Respir Dis*. 1965;92(6):982-985.
412. Hendrix C Jr, Nichols C, Hursh L. A new method of administering the tuberculin skin test. *Am J Public Health Nations Health*. 1966;56(5):818-820.
413. De Partearroyo R, Ruiz Benítez G. Considerations on the diagnostic tuberculin test. Comparative study of the Mantoux test and the Dermo-jet syringe. *Rev Clin Esp*. 1966;100(2):119-125. [in Spanish].
414. Bettag OL, Hall C. Mantoux tuberculin testing; standard method vs. jet injection. *Dis Chest*. 1967;51(5):530-536.
415. Morse D, Hall C, Kaluzny A, et al. Comparative tuberculin testing. Intradermal gun versus intradermal needle. *Am Rev Respir Dis*. 1967;96(1):107-110.
416. Luby JP, Kaiser RL, Herring LL, et al. Jet injector tuberculin skin testing: a comparative evaluation: quantitative aspects. *Am Rev Respir Dis*. 1968;97:46-53.
417. Marsallon, Magnin, Jego, et al. Intradermo-réaction tuberculinique et vaccination B.C.G. intradermique par injecteur à jet sous pression. *Rev Corps Sante Armees Terre Mer Air*. 1972;13:57-61.
418. Wijsmuller G, Snider DE Jr. Skin testing: a comparison of the jet injector with the Mantoux method. *Am Rev Respir Dis*. 1975;112(6):789-798.
419. ten Dam HG. Jet-injectors in BCG vaccination. *Clin Pediatr (Phila)*. 1971;10(1):4-5.
420. World Health Organization (WHO). The Immunological Basis for Immunization Series: Module 5. *Tuberculosis*. 2011. Available at: <http://apps.who.int/iris/bitstream/10665/44733/1/9789241502412_eng.pdf>.
421. Cadorna-Carlos J, Vidor E, Bonnet MC. Randomized controlled study of fractional doses of inactivated poliovirus vaccine administered intradermally with a needle in the Philippines. *Int J Infect Dis*. 2012;16(2):e110-e116.
422. Resik S, Tejeda A, Sutter RW, et al. Priming after a fractional dose of inactivated poliovirus vaccine. *N Engl J Med*. 2013;368(5):416-424.
423. Geldenhuys HD, Mearns H, Foster J, et al. A randomized clinical trial in adults and newborns in South Africa to compare the safety and immunogenicity of bacille Calmette-Guerin (BCG) vaccine administration via a disposable-syringe jet injector to conventional technique with needle and syringe. *Vaccine*. 2015;33(37):4719-4726.
424. Osorio JE, Velez ID, Thomson C, et al. Safety and immunogenicity of a recombinant live attenuated tetravalent dengue vaccine (DENVax) in flavivirus-naive healthy adults in Colombia: a randomised, placebo-controlled, phase 1 study. *Lancet Infect Dis*. 2014;14(9):830-838.
425. Nelson EA, Lam HS, Choi KC, et al. A pilot randomized study to assess immunogenicity, reactogenicity, safety and tolerability of two human papillomavirus vaccines administered intramuscularly and intradermally to females aged 18-26 years. *Vaccine*. 2013;31(34):3452-3460.
426. Kendall M. Engineering of needle-free physical methods to target epidermal cells for DNA vaccination. *Vaccine*. 2006;24(21):4651-4656.
427. Daddona PE, Matriano JA, Mandema J, et al. Parathyroid hormone (1-34)-coated microneedle patch system: clinical pharmacokinetics and pharmacodynamics for treatment of osteoporosis. *Pharm Res*. 2011;28(1):159-165.
428. Matriano JA, Cormier M, Johnson J, et al. Macroflux microprojection array patch technology: a new and efficient approach for intracutaneous immunization. *Pharm Res*. 2002;19(1):63-70.
429. Pearson FE, et al. Dry-coated live viral vector vaccines delivered by nanopatch microprojections retain long-term thermostability and induce transgene-specific T cell responses in mice. *PLoS One*. 2013;8(7):e67888.
430. Zosano Pharma website. Available at: <http://www.zosanopharma.com/technology/>.
431. Widera G, Johnson J, Kim L, et al. Effect of delivery parameters on immunization to ovalbumin following intracutaneous administration by a coated microneedle array patch system. *Vaccine*. 2006;24(10):1653-1664.
432. Lin W, Cormier M, Samiee A, et al. Transdermal delivery of antisense oligonucleotides with microprojection patch (Macroflux) technology. *Pharm Res*. 2001;18(12):1789-1793.
433. Cormier M, Johnson B, Ameri M, et al. Transdermal delivery of desmopressin using a coated microneedle array patch system. *J Control Release*. 2004;97(3):503-511.
434. Hansen K, Haldin B. A Solid Microstructured Transdermal System (sMTS) for systemic delivery of salts & proteins. *Drug Deliv Technol*. 2008;8:38-42.
435. 3M Corporation. The 3M Microstructured Transdermal System (MTS): White Paper. Spring 2009. Available at: <http://solutions.3m.com/3MContentRetrievalAPI/BlobServlet?lmd=1262009493000&locale=en_WW&assetType=MMM_Image&assetId=1258559455435&blobAttribute=ImageFile>.
436. Peterson TA, Wick SM, Ko C. Design, development, manufacturing, and testing of transdermal drug delivery systems. In: Ghosh T, Pfister W, Yum SI, eds. *Transdermal and Topical Drug Delivery Systems*. Buffalo Grove, IL: Interpharm; 1997:268.
437. Gordon RD, Peterson TA. 4 Myths about transdermal drug delivery. *Drug Deliv Technol*. 2003;3:1-4.
438. Johnson PR, Li J, Emery MR. Method development for quantification of tetanus toxoid and aluminum on microneedle arrays. In: *WCBP 2005-9th Symposium on the Interface of Regulatory and Analytical Sciences for Biotechnology Health Products*. 2005. Washington, DC. 10–13 January. Abstract P-05-T.
439. Hansen KJ, Duan D, Determan A, et al. Transdermal delivery of vaccines and proteins using a hand-applied, polymeric microstructured array (sMTS-hand and Press&Patch) (poster 439). In: Portland, OR: 37th Annual Meeting and Exposition of the Controlled Release Society. 10–14 July 2010. Available at: <http://solutions.3m.com/wps/portal/3M/en_US/CNDDSD/Home/technology-solutions/transdermal-technologies/publications/>.
440. Moeckly C, Hansen K, Gysbers J, et al. Formation of microchannels in skin with a hand-applied, plastic microneedle array. Annual Meeting of the American Association of Pharmaceutical Scientists, Washington, DC; 23–27 October 2011 (presentation R6196). Abstract available at: <http://solutions.3m.com/3MContentRetrievalAPI/BlobServlet?locale=en_WW&lmd=1319832968000&assetId=1319208150455&assetType=MMM_Image&blobAttribute=ImageFile>.
441. Brandwein D, Gysbers J, Johnson P, et al. Transcutaneous immunization of two vaccine antigens using 3M Drug Delivery System's solid Microstructured Transdermal System (sMTS) technology, and synergy with resiquimod, a toll-like receptor (TLR) 7/8 agonist, in hairless guinea pigs (poster). In: *First International Conference on Microneedles*. Atlanta, GA: Georgia Institute of Technology; 2010.
442. Sullivan SP, Murthy N, Prausnitz MR. Minimally invasive protein delivery with rapidly dissolving polymer microneedles. *Adv Mater*. 2008;20(5):933-938.
443. Wolter J, Brandwein D, Choi H, et al. Antigen-adjuvant dose response in a rabbit model using 3M's Microstructured

Transdermal System. In: *33rd Annual Meeting of the Controlled Release Society*. Miami, FL: 2005.
444. Alam M (principal investigator). Tolerability study of the application of a 3M microstructure transdermal system. ClinicalTrials.gov identifier NCT01257763. Available at: <http://clinicaltrials.gov/ct2/show/NCT01257763>.
444a. Gill HS, Prausnitz MR. Coated microneedles for transdermal delivery. *J Control Release*. 2007;117(2):227-237.
445. Koutsonanos DG, del Pilar Martin M, Zarnitsyn VG, et al. Transdermal influenza immunization with vaccine-coated microneedle arrays. *PLoS ONE*. 2009;4(3):e4773.
446. Weldon WC, Martin MP, Zarnitsyn V, et al. Microneedle vaccination with stabilized recombinant influenza virus hemagglutinin induces improved protective immunity. *Clin Vaccine Immunol*. 2011;18(4):647-654.
447. Koutsonanos DG, del Pilar Martin M, Zarnitsyn VG, et al. Serological memory and long-term protection to novel H1N1 influenza virus after skin vaccination. *J Infect Dis*. 2011;204(4):582-591.
448. Kim YC, Quan FS, Yoo DG, et al. Improved influenza vaccination in the skin using vaccine coated microneedles. *Vaccine*. 2009;27(49):6932-6938.
449. Quan FS, Kim YC, Compans RW, et al. Dose sparing enabled by skin immunization with influenza virus-like particle vaccine using microneedles. *J Control Release*. 2010;147(3):326-332.
450. Kim YC, Quan FS, Compans RW, et al. Formulation of microneedles coated with influenza virus-like particle vaccine. *AAPS PharmSciTech*. 2010;11(3):1193-1201.
451. Kim YC, Quan FS, Yoo DG, et al. Enhanced memory responses to seasonal H1N1 influenza vaccination of the skin with the use of vaccine-coated microneedles. *J Infect Dis*. 2010;201(2):190-198.
452. Song JM, Kim YC, Barlow PG, et al. Improved protection against avian influenza H5N1 virus by a single vaccination with virus-like particles in skin using microneedles. *Antiviral Res*. 2010;88(2):244-247.
453. Kim YC, Quan FS, Song JM, et al. Influenza immunization with trehalose-stabilized virus-like particle vaccine using microneedles. *Procedia Vaccinol*. 2010;2(1):15-19.
454. Kim YC, Quan FS, Compans RW, et al. Stability kinetics of influenza vaccine coated onto microneedles during drying and storage. *Pharm Res*. 2011;28(1):135-144.
455. Hiraishi Y, Nandakumar S, Choi SO, et al. Bacillus Calmette-Guerin vaccination using a microneedle patch. *Vaccine*. 2011;29(14):2626-2636.
456. Gill HS, Söderholm J, Prausnitz MR, et al. Cutaneous vaccination using microneedles coated with hepatitis C DNA vaccine. *Gene Ther*. 2010;17(6):811-814.
457. Wang Y, Azevedo M, Saif LJ, et al. Inactivated rotavirus vaccine induces protective immunity in gnotobiotic piglets. *Vaccine*. 2010;28(33):5432-5436.
458. Moon S, Wang Y, Edens C, et al. Dose sparing and enhanced immunogenicity of inactivated rotavirus vaccine administered by skin vaccination using a microneedle patch. *Vaccine*. 2013;31(34):3396-3402.
459. Crichton ML, Ansaldo A, Chen X, et al. The effect of strain rate on the precision of penetration of short densely-packed microprojection array patches coated with vaccine. *Biomaterials*. 2010;31(16):4562-4572.
460. Nanopatch microneedle platform. Sydney, Australia: Vaxxas Pty, Ltd. Available at: <http://www.vaxxas.com/>.
461. Park K. Dry coating of immunotherapeutics to densely packed and short microprojection arrays. *J Control Release*. 2009;139:171.
462. Fernando GJ, Chen X, Prow TW, et al. Potent immunity to low doses of influenza vaccine by probabilistic guided micro-targeted skin delivery in a mouse model. *PLoS ONE*. 2010;5(4):e10266.
463. Chen X, Fernando GJ, Crichton ML, et al. Improving the reach of vaccines to low-resource regions, with a needle-free vaccine delivery device and long-term thermostabilization. *J Control Release*. 2011;152(3):349-355.
464. Kendall MAF. Vaccinating against influenza in children without needles: a new initiative. *J Pediatr Infect Dis*. 2012;7(2):83-88.
465. Fernando GJ, Chen X, Primiero CA, et al. Nanopatch targeted delivery of both antigen and adjuvant to skin synergistically drives enhanced antibody responses. *J Control Release*. 2012;159(2):215-221.
466. Chen X, Fernando GJ, Raphael AP, et al. Rapid kinetics to peak serum antibodies is achieved following influenza vaccination by dry-coated densely packed microprojections to skin. *J Control Release*. 2012;158(1):78-84.
467. Chen X, Corbett HJ, Yukiko SR, et al. Site-selectively coated, densely-packed microprojection array patches for targeted delivery of vaccines to skin. *Adv Funct Mater*. 2011;21(3):464-473.
468. Chen X, Kask AS, Crichton ML, et al. Improved DNA vaccination by skin-targeted delivery using dry-coated densely-packed microprojection arrays. *J Control Release*. 2010;148(3):327-333.
469. Corbett HJ, Fernando GJ, Chen X, et al. Skin vaccination against cervical cancer associated human papillomavirus with a novel micro-projection array in a mouse model. *PLoS ONE*. 2010;5(10):e13460.
470. Prow TW, Chen X, Prow NA, et al. Nanopatch-targeted skin vaccination against West Nile Virus and Chikungunya virus in mice. *Small*. 2010;6(16):1776-1784.
471. Pearton M, Kang SM, Song JM, et al. Influenza virus-like particles coated onto microneedles can elicit stimulatory effects on Langerhans cells in human skin. *Vaccine*. 2010;28(37):6104-6113.
472. Birchall JC, Clemo R, Anstey A, et al. Microneedles in clinical practice—an exploratory study into the opinions of healthcare professionals and the public. *Pharm Res*. 2011;28(1):95-106.
473. Shire SJ. Formulation and manufacturability of biologics. *Curr Opin Biotechnol*. 2009;20(6):708-714.
474. Choi HJ, Bondy BJ, Yoo DG, et al. Stability of whole inactivated influenza virus vaccine during coating onto metal microneedles. *J Control Release*. 2013;166(2):159-171.
475. Depelsenaire AC, Meliga SC, McNeilly CL, et al. Colocalization of cell death with antigen deposition in skin enhances vaccine immunogenicity. *J Invest Dermatol*. 2014;134(9):2361-2370.
476. Donnelly RF, Moffatt K, Alkilani AZ, et al. Hydrogel-forming microneedle arrays can be effectively inserted in skin by self-application: a pilot study centred on pharmacist intervention and a patient information leaflet. *Pharm Res*. 2014;31(8):1989-1999.
477. Migalska K, Morrow DI, Garland MJ, et al. Laser-engineered dissolving microneedle arrays for transdermal macromolecular drug delivery. *Pharm Res*. 2011;28(8):1919-1930.
478. Chu LY, Prausnitz MR. Separable arrowhead microneedles. *J Control Release*. 2011;149(3):242-249.
479. Sullivan SP, Koutsonanos DG, Del Pilar Martin M, et al. Dissolving polymer microneedle patches for influenza vaccination. *Nat Med*. 2010;16(8):915-920.
480. Sullivan SP, Murthy N, Prausnitz MR. Minimally invasive protein delivery with rapidly dissolving polymer microneedles. *Adv Mater*. 2008;20(5):933-938.
481. Chu LY, Choi SO, Prausnitz MR. Fabrication of dissolving polymer microneedles for controlled drug encapsulation and delivery: Bubble and pedestal microneedle designs. *J Pharm Sci*. 2010;99(10):4228-4238.
481a. Vassilieva EV, Kalluri H, McAllister D, et al. Improved immunogenicity of individual influenza vaccine components delivered with a novel dissolving microneedle patch stable at room temperature. *Drug Deliv Transl Res*. 2015;5(4):360-371.
482. Prausnitz M. Inactivated Influenza Vaccine Delivered by Microneedle Patch or by Hypodermic Needle. Available at: <https://clinicaltrials.gov/ct2/show/NCT02438423>.
483. Li G, Badkar A, Nema S, et al. In vitro transdermal delivery of therapeutic antibodies using maltose microneedles. *Int J Pharm*. 2009;368(1-2):109-115.
484. Miyano T, Tobinaga Y, Kanno T, et al. Sugar micro needles as transdermic drug delivery system. *Biomed Microdevices*. 2005;7(3):185-188.
485. Edens C, Collins ML, Goodson JL, et al. A microneedle patch containing measles vaccine is immunogenic in non-human primates. *Vaccine*. 2015;33(37):4712-4718.
486. Hirobe S, Azukizawa H, Hanafusa T, et al. Clinical study and stability assessment of a novel transcutaneous influenza vaccination using a dissolving microneedle patch. *Biomaterials*. 2015;57:50-58.
486a. Qiu Y, Guo L, Zhang S, et al. DNA-based vaccination against hepatitis B virus using dissolving microneedle arrays adjuvanted by cationic liposomes and CpG ODN. *Drug Deliv*. 2016;23(7):2391-2398.

487. Raphael AP, Prow TW, Crichton ML, et al. Targeted, needle-free vaccinations in skin using multilayered, densely-packed dissolving microprojection arrays. *Small*. 2010;6(16):1785-1793.
488. McCrudden MT, Torrisi BM, Al-Zahrani S, et al. Laser-engineered dissolving microneedle arrays for protein delivery: potential for enhanced intradermal vaccination. *J Pharm Pharmacol*. 2015;67(3):409-425.
489. Wang S, Joshi S, Lu S. Delivery of DNA to skin by particle bombardment. *Methods Mol Biol*. 2004;245:185-196.
490. Williman J, Lockhart E, Slobbe L, et al. The use of Th1 cytokines, IL-12 and IL-23, to modulate the immune response raised to a DNA vaccine delivered by gene gun. *Vaccine*. 2006;24(21):4471-4474.
491. McCluskie MJ, Brazolot Millan CL, Gramzinski RA, et al. Route and method of delivery of DNA vaccine influence immune responses in mice and non-human primates. *Mol Med*. 1999;5(5):287-300.
492. Weiss R, Scheiblhofer S, Freund J, et al. Gene gun bombardment with gold particles displays a particular Th2-promoting signal that over-rules the Th1-inducing effect of immunostimulatory CpG motifs in DNA vaccines. *Vaccine*. 2002;20(25-26):3148-3154.
493. Novartis Vaccines & Diagnostics. (Acquired former Chiron Corporation, Emeryville, CA), Cambridge, MA. Available at: <http://www.novartisvaccines.com>.
494. Pfizer, Inc., New York, NY. Available at: <http://www.pfizer.com>.
495. Chen D, Weis KF, Chu Q, et al. Epidermal powder immunization induces both cytotoxic T-lymphocyte and antibody responses to protein antigens of influenza and hepatitis B viruses. *J Virol*. 2001;75(23):11630-11640.
496. Chen D, Endres R, Maa YF, et al. Epidermal powder immunization of mice and monkeys with an influenza vaccine. *Vaccine*. 2003;21(21-22):2830-2836.
497. Dean HJ, Fuller D, Osorio JE. Powder and particle-mediated approaches for delivery of DNA and protein vaccines into the epidermis. *Comp Immunol Microbiol Infect Dis*. 2003;26(5-6):373-388.
498. Chen D, Maa YF, Haynes JR. Needle-free epidermal powder immunization. *Expert Rev Vaccines*. 2002;1(3):265-276.
499. Sharpe M, Lynch D, Topham S, et al. Protection of mice from H5N1 influenza challenge by prophylactic DNA vaccination using particle mediated epidermal delivery. *Vaccine*. 2007;25(34):6392-6398.
500. Loudon PT, Yager EJ, Lynch DT, et al. GM-CSF increases mucosal and systemic immunogenicity of an H1N1 influenza DNA vaccine administered into the epidermis of non-human primates. *PLoS ONE*. 2010;5(6):e11021.
501. Tacket CO, Roy MJ, Widera G, et al. Phase 1 safety and immune response studies of a DNA vaccine encoding hepatitis B surface antigen delivered by a gene delivery device. *Vaccine*. 1999;17(22):2826-2829.
502. Roy MJ, Wu MS, Barr LJ, et al. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. *Vaccine*. 2000;19(7-8):764-778.
503. McConkey SJ, Reece WH, Moorthy VS, et al. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. *Nat Med*. 2003;9(6):729-735.
504. Moorthy VS, McConkey S, Roberts M, et al. Safety of DNA and modified vaccinia virus Ankara vaccines against liver-stage P. falciparum malaria in non-immune volunteers. *Vaccine*. 2003;21(17-18):1995-2002.
505. Dean HJ, Chen D. Epidermal powder immunization against influenza. *Vaccine*. 2004;23(5):681-686.
506. Jones S, Evans K, McElwaine-Johnn H, et al. DNA vaccination protects against an influenza challenge in a double-blind randomised placebo-controlled phase 1b clinical trial. *Vaccine*. 2009;27(18):2506-2512.
507. Deleted in review.
508. Deleted in review.
509. Deleted in review.
510. Roberts LK, Barr LJ, Fuller DH, et al. Clinical safety and efficacy of a powdered Hepatitis B nucleic acid vaccine delivered to the epidermis by a commercial prototype device. *Vaccine*. 2005;23(40):4867-4878.
511. Drape RJ, Macklin MD, Barr LJ, et al. Epidermal DNA vaccine for influenza is immunogenic in humans. *Vaccine*. 2006;24(21):4475-4481.
512. Pilling AM, Harman RM, Jones SA, et al. The assessment of local tolerance, acute toxicity, and DNA biodistribution following particle-mediated delivery of a DNA vaccine to minipigs. *Toxicol Pathol*. 2002;30(3):298-305.
513. Deleted in review.
514. Schmaljohn C, Vanderzanden L, Bray M, et al. Naked DNA vaccines expressing the prM and E genes of Russian spring summer encephalitis virus and Central European encephalitis virus protect mice from homologous and heterologous challenge. *J Virol*. 1997;71(12):9563-9569.
515. Hooper JW, Custer DM, Thompson E, et al. DNA vaccination with the Hantaan virus M gene protects hamsters against three of four HFRS hantaviruses and elicits a high-titer neutralizing antibody response in Rhesus monkeys. *J Virol*. 2001;75(18):8469-8477.
516. Chen D, Zuleger C, Chu Q, et al. Epidermal powder immunization with a recombinant HIV gp120 targets Langerhans cells and induces enhanced immune responses. *AIDS Res Hum Retroviruses*. 2002;18(10):715-722.
517. Fuller DH, Shipley T, Allen TM, et al. Immunogenicity of hybrid DNA vaccines expressing hepatitis B core particles carrying human and simian immunodeficiency virus epitopes in mice and rhesus macaques. *Virology*. 2007;364(2):245-255.
518. Sakai T, Hisaeda H, Nakano Y, et al. Gene gun-based co-immunization of merozoite surface protein-1 cDNA with IL-12 expression plasmid confers protection against lethal Plasmodium yoelii in A/J mice. *Vaccine*. 2003;21(13-14):1432-1444.
519. Kim TW, Lee JH, Hung CF, et al. Generation and characterization of DNA vaccines targeting the nucleocapsid protein of severe acute respiratory syndrome coronavirus. *J Virol*. 2004;78(9):4638-4645.
520. Dupuy LC, Richards MJ, Reed DS, et al. Immunogenicity and protective efficacy of a DNA vaccine against Venezuelan equine encephalitis virus aerosol challenge in nonhuman primates. *Vaccine*. 2010;28(46):7345-7350.
521. Fan H, Lin Q, Morrissey GR, et al. Immunization via hair follicles by topical application of naked DNA to normal skin. *Nat Biotechnol*. 1999;17(9):870-872.
522. Bolgiano B, Mawas F, Yost SE, et al. Effect of physico-chemical modification on the immunogenicity of Haemophilus influenzae type b oligosaccharide-CRM(197) conjugate vaccines. *Vaccine*. 2001;19(23-24):3189-3200.
523. Partidos CD, Beignon AS, Mawas F, et al. Immunity under the skin: potential application for topical delivery of vaccines. *Vaccine*. 2003;21(7-8):776-780.
524. Lu B, Federoff HJ, Wang Y, et al. Topical application of viral vectors for epidermal gene transfer. *J Invest Dermatol*. 1997;108(5):803-808.
525. Seo N, Tokura Y, Nishijima T, et al. Percutaneous peptide immunization via corneum barrier-disrupted murine skin for experimental tumor immunoprophylaxis. *Proc Natl Acad Sci USA*. 2000;97(1):371-376.
526. Takigawa M, Tokura Y, Hashizume H, et al. Percutaneous peptide immunization via corneum barrier-disrupted murine skin for experimental tumor immunoprophylaxis. *Ann N Y Acad Sci*. 2001;941:139-146.
527. Kahlon R, Hu Y, Orteu CH, et al. Optimization of epicutaneous immunization for the induction of CTL. *Vaccine*. 2003;21(21-22):2890-2899.
528. Godefroy S, Peyre M, Garcia N, et al. Effect of skin barrier disruption on immune responses to topically applied cross-reacting material, CRM(197), of diphtheria toxin. *Infect Immun*. 2005;73(8):4803-4809.
529. Choi MJ, Kim JH, Maibach HI. Topical DNA vaccination with DNA/lipid based complex. *Curr Drug Deliv*. 2006;3(1):37-45.
530. Skountzou I, Quan FS, Jacob J, et al. Transcutaneous immunization with inactivated influenza virus induces protective immune responses. *Vaccine*. 2006;24(35-36):6110-6119.
531. Vogt A, Mahe B, Costagliola D, et al. Transcutaneous anti-influenza vaccination promotes both CD4 and CD8 T cell immune responses in humans. *J Immunol*. 2008;180(3):1482-1489.

532. Frerichs DM, Ellingsworth LR, Frech SA, et al. Controlled, single-step, stratum corneum disruption as a pretreatment for immunization via a patch. *Vaccine*. 2008;26(22):2782-2787.
533. Deleted in review.
534. Glenn GM, Villar CP, Flyer DC, et al. Safety and immunogenicity of an enterotoxigenic *Escherichia coli* vaccine patch containing heat-labile toxin: use of skin pretreatment to disrupt the stratum corneum. *Infect Immun*. 2007;75(5):2163-2170.
535. Glenn GM, Rao M, Matyas GR, et al. Skin immunization made possible by cholera toxin. *Nature*. 1998;391(6670):851.
536. Glenn GM, Kenney RT, Hammond SA, et al. Transcutaneous immunization and immunostimulant strategies. *Immunol Allergy Clin North Am*. 2003;23(4):787-813.
537. Kenney RT, Glenn GM. Transcutaneous immunization using the heat-labile enterotoxin *E. coli* as an adjuvant. In: Schijns VE, O'Hagan DT, eds. *Immunopotentiators in Modern Vaccines*. Amsterdam, Holland: Elsevier Academic Press; 2006:253-273.
538. Güereña-Burgueño F, Hall ER, Taylor DN, et al. Safety and immunogenicity of a prototype enterotoxigenic *Escherichia coli* vaccine administered transcutaneously. *Infect Immun*. 2002;70(4):1874-1880.
539. Glenn GM, Taylor DN, Li X, et al. Transcutaneous immunization: a human vaccine delivery strategy using a patch. *Nat Med*. 2000;6(12):1403-1406.
540. McKenzie R, Bourgeois AL, Frech SA, et al. Transcutaneous immunization with the heat-labile toxin (LT) of enterotoxigenic *Escherichia coli* (ETEC): protective efficacy in a double-blind, placebo-controlled challenge study. *Vaccine*. 2007;25(18):3684-3691.
541. Frech SA, Dupont HL, Bourgeois AL, et al. Use of a patch containing heat-labile toxin from *Escherichia coli* against travellers' diarrhoea: a phase II, randomised, double-blind, placebo-controlled field trial. *Lancet*. 2008;371(9629):2019-2025.
542. Behrens RH, Cramer JP, Jelinek T, et al. Efficacy and safety of a patch vaccine containing heat-labile toxin from *Escherichia coli* against travellers' diarrhoea: a phase 3, randomised, double-blind, placebo-controlled field trial in travellers from Europe to Mexico and Guatemala. *Lancet Infect Dis*. 2014;14(3):197-204.
543. Steffen R, Cramer JP, Burchard G, et al. Efficacy of a travelers' diarrhea vaccine system in travelers to India. *J Travel Med*. 2013;20(6):374-379.
544. Guebre-Xabier M, Hammond SA, Epperson DE, et al. Immunostimulant patch containing heat-labile enterotoxin from *Escherichia coli* enhances immune responses to injected influenza virus vaccine through activation of skin dendritic cells. *J Virol*. 2003;77(9):5218-5225.
545. Guebre-Xabier M, Hammond SA, Ellingsworth LR, et al. Immunostimulant patch enhances immune responses to influenza virus vaccine in aged mice. *J Virol*. 2004;78(14):7610-7618.
546. Frech SA, Kenney RT, Spyr CA, et al. Improved immune responses to influenza vaccination in the elderly using an immunostimulant patch. *Vaccine*. 2005;23(7):946-950.
547. Hammond SA, Walwender D, Alving CR, et al. Transcutaneous immunization: T cell responses and boosting of existing immunity. *Vaccine*. 2001;19(17-19):2701-2707.
548. Kenney RT, Yu J, Guebre-Xabier M, et al. Induction of protective immunity against lethal anthrax challenge with a patch. *J Infect Dis*. 2004;190(4):774-782.
549. Matyas GR, Friedlander AM, Glenn GM, et al. Needle-free skin patch vaccination method for anthrax. *Infect Immun*. 2004;72(2):1181-1183.
550. Yu J, Cassels F, Scharton-Kersten T, et al. Transcutaneous immunization using colonization factor and heat-labile enterotoxin induces correlates of protective immunity for enterotoxigenic *Escherichia coli*. *Infect Immun*. 2002;70(3):1056-1068.
551. Zhu C, Yu J, Yang Z, et al. Protection against Shiga toxin-producing *Escherichia coli* infection by transcutaneous immunization with Shiga toxin subunit B. *Clin Vaccine Immunol*. 2008;15(2):359-366.
552. Tao SL, Desai TA. Microfabricated drug delivery systems: from particles to pores. *Adv Drug Deliv Rev*. 2003;55(3):315-328.
553. Mikszta JA, Alarcon JB, Brittingham JM, et al. Improved genetic immunization via micromechanical disruption of skin-barrier function and targeted epidermal delivery. *Nat Med*. 2002;8(4):415-419.
554. Banga AK. Microporation applications for enhancing drug delivery. *Expert Opin Drug Deliv*. 2009;6(4):343-354.
555. Hadgraft J. Skin, the final frontier. *Int J Pharm*. 2001;224(1-2):1-18.
556. Altea Therapeutics. Nitto Denko to expand transdermal patch product lineup. Nitto Denko acquires all assets of Altea Therapeutics [press release]. April 25, 2012. Available at: <http://www.nitto.com/us/en/press/2012/0425.jsp>.
557. Garg S, Hoelscher M, Belser JA, et al. Needle-free skin patch delivery of a vaccine for a potentially pandemic influenza virus provides protection against lethal challenge in mice. *Clin Vaccine Immunol*. 2007;14(7):926-928.
558. Smith A, Yang D, Delcher H, et al. Fluorescein kinetics in interstitial fluid harvested from diabetic skin during fluorescein angiography: implications for glucose monitoring. *Diabetes Technol Ther*. 1999;1(1):21-27.
559. Lee JW, Gadiraju P, Park JH, et al. Microsecond thermal ablation of skin for transdermal drug delivery. *J Control Release*. 2011;154(1):58-68.
560. Minton K. Microbiota: a "natural" vaccine adjuvant. *Nat Rev Immunol*. 2014;14(10):650-651.
561. Valdez Y, Brown EM, Finlay BB. Influence of the microbiota on vaccine effectiveness. *Trends Immunol*. 2014;35(11):526-537.
562. Huda MN, Lewis Z, Kalanetra KM, et al. Stool microbiota and vaccine responses of infants. *Pediatrics*. 2014;134(2):e362-e372.
563. Djupesland PG, Skretting A, Winderen M, et al. Breath actuated device improves delivery to target sites beyond the nasal valve. *Laryngoscope*. 2006;116(3):466-472.
564. Bienenstock J. Mucosal and other mechanisms of resistance in the respiratory tract: an overview. In: Mestecky J, Lamm ME, McGhee JR, et al., eds. *Mucosal Immunology*. 3rd ed. Burlington, MA: Elsevier; 2005:1401-1402.
565. Kelsall BL, Leon F, Smythies LE, et al. Antigen handling and presentation by mucosal dendritic cells and macrophages. In: Mestecky J, Lamm ME, McGhee JR, et al., eds. *Mucosal Immunology*. 3rd ed. Burlington, MA: Elsevier; 2005:451-486.
566. Vijay-Kumar M, Gewirtz AT. Role of epithelium in antigen presentation. In: Mestecky J, Lamm ME, McGhee JR, et al., eds. *Mucosal Immunology*. 3rd ed. Burlington, MA: Elsevier; 2005:423-434.
567. Fujimura Y. Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans. *Virchows Arch*. 2000;436(6):560-566.
568. Kraal G. Nasal associated lymphoid tissue. In: Mestecky J, Lamm ME, McGhee JR, et al., eds. *Mucosal Immunology*. 3rd ed. Burlington, MA: Elsevier; 2005:415-434.
569. Mestecky J. The common mucosal immune system and current strategies for induction of immune responses in external secretions. *J Clin Immunol*. 1987;7(4):265-276.
570. Mann JF, McKay PF, Arokiasamy S, et al. Mucosal application of gp140 encoding DNA polyplexes to different tissues results in altered immunological outcomes in mice. *PLoS ONE*. 2013;8(6):e67412.
571. Stober W, McGhee JR. Inductive and effector tissues and cells of the mucosal immune system. In: Mestecky J, Lamm ME, McGhee JR, et al., eds. *Mucosal Immunology*. 3rd ed. Burlington, MA: Elsevier; 2005:371-374.
572. Balmelli C, Demotz S, Acha-Orbea H, et al. Trachea, lung, and tracheobronchial lymph nodes are the major sites where antigen-presenting cells are detected after nasal vaccination of mice with human papillomavirus type 16 virus-like particles. *J Virol*. 2002;76(24):12596-12602.
573. Laube BL. The expanding role of aerosols in systemic drug delivery, gene therapy, and vaccination. *Respir Care*. 2005;50(9):1161-1176.
574. Mutsch M, Zhou W, Rhodes P, et al. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. *N Engl J Med*. 2004;350(9):896-903.
575. Tablan OC, Anderson LJ, Besser R, et al. Centers for Disease Control and Prevention. Guidelines for preventing health-care-associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. *MMWR Recomm Rep*. 2004;53(RR-3):1-36.
576. Kraan H, Vrieling H, Czerkinsky C, et al. Buccal and sublingual vaccine delivery. *J Control Release*. 2014;190:580-592.
577. Hellings P, Jorissen M, Ceuppens JL. The Waldeyer's ring. *Acta Otorhinolaryngol Belg*. 2000;54(3):237-241.

578. Shim BS, Stadler K, Nguyen HH, et al. Sublingual immunization with recombinant adenovirus encoding SARS-CoV spike protein induces systemic and mucosal immunity without redirection of the virus to the brain. *Virol J*. 2012;9:215.
579. Lorenzo-Gomez MF, Padilla-Fernández B, García-Criado FJ, et al. Evaluation of a therapeutic vaccine for the prevention of recurrent urinary tract infections versus prophylactic treatment with antibiotics. *Int Urogynecol J*. 2013;24(1):127-134.
580. Alecsandru D, Valor L, Sánchez-Ramón S, et al. Sublingual therapeutic immunization with a polyvalent bacterial preparation in patients with recurrent respiratory infections: immunomodulatory effect on antigen-specific memory CD4+ T cells and impact on clinical outcome. *Clin Exp Immunol*. 2011;164(1):100-107.
581. Cogo R, Ramponi A, Scivoletto G, Rippoli R. Prophylaxis for acute exacerbations of chronic bronchitis using an antibacterial sublingual vaccine obtained through mechanical lysis: a clinical and pharmacoeconomic study. *Acta Biomed*. 2003;74(2):81-87.
582. Lorenzo-Gómez MF, Padilla-Fernández B, García-Cenador MB, et al. Comparison of sublingual therapeutic vaccine with antibiotics for the prophylaxis of recurrent urinary tract infections. *Front Cell Infect Microbiol*. 2015;5:50.
583. Di Rienzo V, Cadario G, Grieco T, et al. Sublingual immunotherapy in mite-sensitized children with atopic dermatitis: a randomized, open, parallel-group study. *Ann Allergy Asthma Immunol*. 2014;113(6):671-673, e1.
584. Lewis D (principal investigator). Measuring Responses to Sublingual Antigens. Clinical trials.gov identifier NCT00949572. <https://clinicaltrials.gov/ct2/show/NCT00949572>.
585. Hofman P (principal investigator). Cholera Toxin B Subunit (CTB) Administered by Mucosal Way in Healthy Adult Volunteer. ClinicalTrials.gov identifier NCT00820144. <https://clinicaltrials.gov/ct2/show/NCT00820144?term=NCT00820144&rank=1>.
586. Singh S, Nehete PN, Yang G, et al. Enhancement of mucosal immunogenicity of viral vectored vaccines by the NKT cell agonist alpha-galactosylceramide as adjuvant. *Vaccines (Basel)*. 2014;2(4):686-706.
587. Choi JH, Jonsson-Schmunk K, Qiu X, et al. A single dose respiratory recombinant adenovirus-based vaccine provides long-term protection for non-human primates from lethal Ebola infection. *Mol Pharm*. 2015;12(8):2712-2731.
588. Fu YH, Jiao YY, He JS, et al. Sublingual administration of a helper-dependent adenoviral vector expressing the codon-optimized soluble fusion glycoprotein of human respiratory syncytial virus elicits protective immunity in mice. *Antiviral Res*. 2014;105:72-79.
589. Rosales-Mendoza S, Angulo C. Bacillus subtilis comes of age as a vaccine production host and delivery vehicle. *Expert Rev Vaccines*. 2015;14(8):1135-1148.
590. Tavares Batista M, Souza RD, Paccez JD, et al. Gut adhesive *Bacillus subtilis* spores as a platform for mucosal delivery of antigens. *Infect Immun*. 2014;82(4):1414-1423.
590a. Kim SH, Kim JY, Choi Y, et al. Mucosal vaccination with recombinant adenovirus encoding nucleoprotein provides potent protection against influenza virus infection. *PLoS ONE*. 2013;8(9):e75460.
591. Fukasaka M, Asari D, Kiyotoh E, et al. A lipopolysaccharide from Pantoea agglomerans is a promising adjuvant for sublingual vaccines to induce systemic and mucosal immune responses in mice via TLR4 pathway. *PLoS ONE*. 2015;10(5):e0126849.
592. Lee HJ, Cho H, Kim MG, et al. Sublingual immunization of trivalent human papillomavirus DNA vaccine in baculovirus nanovector for protection against vaginal challenge. *PLoS ONE*. 2015;10(3):e0119408.
593. Gonzalez Aznar E, Romeu B, Lastre M, et al. Mucosal and systemic immune responses induced by a single time vaccination strategy in mice. *Can J Microbiol*. 2015;61(8):531-538.
594. Crosby CM, Nehete P, Sastry KJ, et al. Amplified and persistent immune responses generated by single-cycle replicating adenovirus vaccines. *J Virol*. 2015;89(1):669-675.
595. Singh S, Yang G, Byrareddy SN, et al. Natural killer T cell and TLR9 agonists as mucosal adjuvants for sublingual vaccination with clade C HIV-1 envelope protein. *Vaccine*. 2014;32(51):6934-6940.
596. White JA, Blum JS, Hosken NA, et al. Serum and mucosal antibody responses to inactivated polio vaccine after sublingual immunization using a thermoresponsive gel delivery system. *Hum Vaccin Immunother*. 2014;10(12):3611-3621.
597. Bitter C, Suter-Zimmermann K, Surber C. Nasal drug delivery in humans. *Curr Probl Dermatol*. 2011;40:20-35.
598. Henning A, Hein S, Schneider M, et al. Pulmonary drug delivery: medicines for inhalation. *Handb Exp Pharmacol*. 2010;197:171-192.
599. Dunn C, Curran MP. Inhaled human insulin (Exubera): a review of its use in adult patients with diabetes mellitus. *Drugs*. 2006;66(7):1013-1032.
600. Johnson A. Insulin flop costs Pfizer 2.8 billion. *The Wall Street Journal*. 2007;A1. Available at: <http://online.wsj.com/article/SB119269071993163273.html>.
600a. Goldberg T, Wong E. Afrezza (insulin human) inhalation powder: a new inhaled insulin for the management of type-1 or type-2 diabetes mellitus. *P T*. 2015;40(11):735-741.
600b. Mannkind Corporation. Inhalation Devices. Available at: <http://www.mannkindcorp.com/research-development/tech-platforms/oral-inhalers/>.
601. LiCalsi C, Maniaci MJ, Christensen T, et al. A powder formulation of measles vaccine for aerosol delivery. *Vaccine*. 2001;19(17-19):2629-2636.
602. Smith JD, Bot S, Dellamary L, et al. Evaluation of novel aerosol formulations designed for mucosal vaccination against influenza virus. *Vaccine*. 2003;21:2805-2812.
603. de Swart RL, LiCalsi C, Quirk AV, et al. Measles vaccination of macaques by dry powder inhalation. *Vaccine*. 2007;25(7):1183-1190.
604. Belshe RB, Gruber WC, Mendelman PM, et al. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. *J Infect Dis*. 2000;181(3):1133-1137.
605. Nichol KL, Mendelman PM, Mallon KP, et al. Effectiveness of live, attenuated intranasal influenza virus vaccine in healthy, working adults: a randomized controlled trial. *JAMA*. 1999;282(2):137-144.
606. Ashkenazi S, Vertruyen A, Arístegui J, et al. Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections. *Pediatr Infect Dis J*. 2006;25(10):870-879.
607. Fleming DM, Crovari P, Wahn U, et al. Comparison of the efficacy and safety of live attenuated cold-adapted influenza vaccine, trivalent, with trivalent inactivated influenza virus vaccine in children and adolescents with asthma. *Pediatr Infect Dis J*. 2006;25(10):860-869.
608. Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med*. 2007;356(7):685-696.
609. Vesikari T, Fleming DM, Aristegui JF, et al. Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. *Pediatrics*. 2006;118(6):2298-2312.
610. Tam JS, Capeding MR, Lum LC, et al. Efficacy and safety of a live attenuated, cold-adapted influenza vaccine, trivalent against culture-confirmed influenza in young children in Asia. *Pediatr Infect Dis J*. 2007;26(7):619-628.
611. Belshe RB, Gruber WC, Mendelman PM, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *J Pediatr*. 2000;136(2):168-175.
612. Piedra PA, Gaglani MJ, Kozinetz CA, et al. Herd immunity in adults against influenza-related illnesses with use of the trivalent-live attenuated influenza vaccine (CAIV-T) in children. *Vaccine*. 2005;23(13):1540-1548.
613. Centers for Disease Control and Prevention (CDC). *ACIP votes down use of LAIV for 2016-2017 flu season [media statement]*. Atlanta, GA: CDC; 2016. Available at: <http://www.cdc.gov/media/releases/2016/s0622-laiv-flu.html>.
614. Sullivan V, Bhuta-Wills A, Hajiloo S. AccuSpray on demand. *Fluent News Spring*. 2003;2003:14. Available at: <http://www.yumpu.com/en/document/view/6503014/fluent/15>.

615. Couch RB. Nasal vaccination, *Escherichia coli* enterotoxin, and Bell's palsy. *N Engl J Med*. 2004;350(9):860-861.
616. Rudenko L, Desheva J, Korovkin S, et al. Safety and immunogenicity of live attenuated influenza reassortant H5 vaccine (phase I-II clinical trials). *Influenza Other Respir Viruses*. 2008;2(6):203-209.
617. Rudenko L, Kiseleva I, Naykhin AN, et al. Assessment of human immune responses to H7 avian influenza virus of pandemic potential: results from a placebo-controlled, randomized double-blind phase I study of live attenuated H7N3 influenza vaccine. *PLoS ONE*. 2014;9(2):e87962.
618. Samdal HH, Bakke H, Oftung F, et al. A non-living nasal influenza vaccine can induce major humoral and cellular immune responses in humans without the need for adjuvants. *Hum Vaccin*. 2005;1(2):85-90.
619. Treanor J, Nolan C, O'Brien D, et al. Intranasal administration of a proteosome-influenza vaccine is well-tolerated and induces serum and nasal secretion influenza antibodies in healthy human subjects. *Vaccine*. 2006;24(3):254-262.
620. Morokutti A, Muster T, Ferko B. Intranasal vaccination with a replication-deficient influenza virus induces heterosubtypic neutralising mucosal IgA antibodies in humans. *Vaccine*. 2014;32(17):1897-1900.
621. Read RC, Naylor SC, Potter CW, et al. Effective nasal influenza vaccine delivery using chitosan. *Vaccine*. 2005;23(35):4367-4374.
622. Bakke H, Samdal HH, Holst J, et al. Oral spray immunization may be an alternative to intranasal vaccine delivery to induce systemic antibodies but not nasal mucosal or cellular immunity. *Scand J Immunol*. 2006;63(3):223-231.
623. Jahnmatz M, Amu S, Ljungman M, et al. B-cell responses after intranasal vaccination with the novel attenuated Bordetella pertussis vaccine strain BPZE1 in a randomized phase I clinical trial. *Vaccine*. 2014;32(27):3350-3356.
624. Thorstensson R, Trollfors B, Al-Tawil N, et al. A phase I clinical study of a live attenuated Bordetella pertussis vaccine—BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers. *PLoS ONE*. 2014;9(1):e83449.
625. Bernstein DI, Malkin E, Abughali N, et al. Phase 1 study of the safety and immunogenicity of a live, attenuated respiratory syncytial virus and parainfluenza virus type 3 vaccine in seronegative children. *Pediatr Infect Dis J*. 2012;31(2):109-114.
626. Malkin E, Yogev R, Abughali N, et al. Safety and immunogenicity of a live attenuated RSV vaccine in healthy RSV-seronegative children 5 to 24 months of age. *PLoS ONE*. 2013;8(10):e77104.
627. Riddle MS, Kaminski RW, Williams C, et al. Safety and immunogenicity of an intranasal *Shigella flexneri* 2a Invaplex 50 vaccine. *Vaccine*. 2011;29(40):7009-7019.
628. King JC Jr, Lagos R, Bernstein DI, et al. Safety and immunogenicity of low and high doses of trivalent live cold-adapted influenza vaccine administered intranasally as drops or spray to healthy children. *J Infect Dis*. 1998;177(5):1394-1397.
629. de Swart RL, Kuiken T, Fernandez-de Castro J, et al. Aerosol measles vaccination in macaques: preclinical studies of immune responses and safety. *Vaccine*. 2006;24(40-41):6424-6436.
630. Smith JH, Papania M, Knaus D, et al. Nebulized live-attenuated influenza vaccine provides protection in ferrets at a reduced dose. *Vaccine*. 2012;30(19):3026-3033.
631. Huo Z, Sinha R, McNeela EA, et al. Induction of protective serum meningococcal bactericidal and diphtheria-neutralizing antibodies and mucosal immunoglobulin A in volunteers by nasal insufflations of the Neisseria meningitidis serogroup C polysaccharide-CRM197 conjugate vaccine mixed with chitosan. *Infect Immun*. 2005;73(12):8256-8265.
632. McNeela EA, Jabbal-Gill I, Illum L, et al. Intranasal immunization with genetically detoxified diphtheria toxin induces T cell responses in humans: enhancement of Th2 responses and toxin-neutralizing antibodies by formulation with chitosan. *Vaccine*. 2004;22(8):909-914.
633. Brosius E, Egozy E. Game controller simulating a musical instrument. Harmonix Music Systems, Editor. (US Patent no. 7320643 B1). Washington, DC: US Patent and Trademark Office.
634. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness. *N Engl J Med*. 2011;365(23):2178-2187.
635. Lin WH, Griffin DE, Rota PA, et al. Successful respiratory immunization with dry powder live-attenuated measles virus vaccine in rhesus macaques. *Proc Natl Acad Sci USA*. 2011;108(7):2987-2992.
635a. Huang J, Mikszta JA, Ferriter MS, et al. Intranasal administration of dry powder anthrax vaccine provides protection against lethal aerosol spore challenge. *Hum Vaccin*. 2007;3(3):90-93.
636. Jiang G, Joshi SB, Peek LJ, et al. Anthrax vaccine powder formulations for nasal mucosal delivery. *J Pharm Sci*. 2006;95(1):80-96.
637. Huang J, Garmise RJ, Crowder TM, et al. A novel dry powder influenza vaccine and intranasal delivery technology: induction of systemic and mucosal immune responses in rats. *Vaccine*. 2004;23(6):794-801.
638. Velasquez LS, Shira S, Berta AN, et al. Intranasal delivery of Norwalk virus-like particles formulated in an in situ gelling, dry powder vaccine. *Vaccine*. 2011;29(32):5221-5231.
639. Kok PW, Kenya PR, Ensering H. Measles immunization with further attenuated heat-stable measles vaccine using five different methods of administration. *Trans R Soc Trop Med Hyg*. 1983;77(2):171-176.
640. McCrumb F, Bulkeley J, Hornick R, et al. Clinical trials with living attenuated measles virus vaccines. *Am J Public Health*. 1962;52:11-15.
641. McCrumb FR, Kress S, Saunders E, et al. Studies with live attenuated measles virus vaccine: I. clinical and immunologic responses in institutionalized children. *Am J Dis Child*. 1961;101(6):689-700.
642. Black F, Sheridan SR. Studies on an attenuated measles-virus vaccine—administration of vaccine by several routes. *N Engl J Med*. 1960;263:165-169.
643. Cernescu C, Cajal N. Antimeasles vaccination by natural routes—experimental background and practical consequences. *Virologie*. 1984;35(4):259-271.
644. Whittle HC, Rowland MG. Failure of measles vaccine sprayed into the oropharynx of infants. *Lancet*. 1983;1(8332):1045.
645. Terskikh II, Danilov AI, Shchelchkov GI, et al. Theoretical basis and effectiveness of immunization with aerosols of liquid measles vaccines. *Vestn Akad Med Nauk SSSR*. 1971;26(2):84-90. [in Russian].
646. Kress S, et al. Studies with live attenuated measles virus vaccine. *Am J Dis Child*. 1961;101:57-63.
647. Simasathien S, Migasena S, Bellini W, et al. Measles vaccination of Thai infants by intranasal and subcutaneous routes: possible interference from respiratory infections. *Vaccine*. 1997;15(3):329-334.
648. Beck M, Smerdel S, Dedić I, et al. Immune response to Edmonston-Zagreb measles virus strain in monovalent and combined MMR vaccine. *Dev Biol Stand*. 1986;65:95-100.
649. Sabin AB, Albrecht P, Takeda AK, et al. High effectiveness of aerosolized chick embryo fibroblast measles vaccine in seven-month-old and older infants. *J Infect Dis*. 1985;152(6):1231-1237.
650. Sabin AB, Fernández de Castro J, Flores Arechiga A, et al. Clinical trials of inhaled aerosol of human diploid and chick embryo measles vaccine. *Lancet*. 1982;2(8298):604.
651. Sabin AB, Flores Arechiga A, Fernández de Castro J, et al. Successful immunization of infants with and without maternal antibody by aerosolized measles vaccine. II. Vaccine comparisons and evidence for multiple antibody response. *JAMA*. 1984;251(18):2363-2371.
652. Sabin AB, Flores Arechiga A, Fernández de Castro J, et al. Successful immunization of children with and without maternal antibody by aerosolized measles vaccine. I. Different results with undiluted human diploid cell and chick embryo fibroblast vaccines. *JAMA*. 1983;249(19):2651-2662.
653. Cutts FT, Clements CJ, Bennett JV. Alternative routes of measles immunization: a review. *Biologicals*. 1997;25(3):323-338.
654. Low N, Kraemer S, Schneider M, et al. Immunogenicity and safety of aerosolized measles vaccine: systematic review and meta-analysis. *Vaccine*. 2008;26(3):383-398.
655. Hiremath GS, Omer SB. A meta-analysis of studies comparing the respiratory route with the subcutaneous route of measles vaccine administration. *Hum Vaccin*. 2005;1(1):30-36.
656. Valdespino-Gómez JL, de Lourdes Garcia-Garcia M, Fernandez-de-Castro J, et al. Measles aerosol vaccination. *Curr Top Microbiol Immunol*. 2006;304:165-193.

657. Fernandez de Castro J, Kumate J. Vaccination against measles. The situation in Mexico and America. Advances in the method of aerosol immunization. *Bol Med Hosp Infant Mex*. 1990;47(7):449-461. [in Spanish].
658. Bennett JV, Fernandez de Castro J, Poblete RM, et al. A new, rapid, and promising approach to aerosol immunization: inflatable bags and valved masks. *Vaccine*. 2009;27(34):4571-4575.
659. Dilraj A, Cutts FT, Bennett JV, et al. Persistence of measles antibody two years after revaccination by aerosol or subcutaneous routes. *Pediatr Infect Dis J*. 2000;19(12):1211-1213.
660. Khanum S, Uddin N, Garelick H, et al. Comparison of Edmonston-Zagreb and Schwarz strains of measles vaccine given by aerosol or subcutaneous injection. *Lancet*. 1987;1(8525):150-153.
661. Ekunwe EO. Immunization by inhalation of aerosolized measles vaccine. *Ann Trop Paediatr*. 1990;10(2):145-149.
662. Okuno Y, Ueda S, Hosai H, et al. Studies on the combined use of killed and live measles vaccines. II. Advantages of the inhalation method. *Biken J*. 1965;8(2):81-85.
663. Ueda S, Hosai H, Minekawa Y, et al. Studies on the combined use of killed and live measles vaccines. 3. Conditions for the "take" of live vaccine. *Biken J*. 1966;9(2):97-101.
664. Fernández de Castro J, Valdespino Gómez JL, Díaz Ortega JL, et al. Diploid cell measles vaccine. *JAMA*. 1986;256(6):714.
665. Torigoe S, Biritwum RB, Isomura S, et al. Measles in Ghana: a trial of an alternative means of administration of measles vaccine. *J Trop Pediatr*. 1986;32(6):304-359.
666. Sepúlveda-Amor J, Valdespino-Gómez JL, García-García Mde L, et al. A randomized trial demonstrating successful boosting responses following simultaneous aerosols of measles and rubella (MR) vaccines in school age children. *Vaccine*. 2002;20(21-22):2790-2795.
667. Bennett JV, Fernandez de Castro J, Valdespino-Gomez JL, et al. Aerosolized measles and measles-rubella vaccines induce better measles antibody booster responses than injected vaccines: randomized trials in Mexican schoolchildren. *Bull World Health Organ*. 2002;80(10):806-812.
668. Bellanti JA, Zeligs BJ, Mendez-Inocencio J, et al. Immunologic studies of specific mucosal and systemic immune responses in Mexican school children after booster aerosol or subcutaneous immunization with measles vaccine. *Vaccine*. 2004;22(9-10):1214-1220.
669. Dilraj A, Sukhoo R, Cutts FT, Bennett JV. Aerosol and subcutaneous measles vaccine: measles antibody responses 6 years after re-vaccination. *Vaccine*. 2007;25(21):4170-4174.
670. Díaz-Ortega JL, Bennett JV, Castañeda D, Martinez D, de Castro JF. Antibody persistence in young adults 1 year after MMR immunization by aerosol or by subcutaneous route. *Vaccine*. 2010;28(44):7228-7232.
671. Diaz-Ortega JL, Bennett JV, Castaneda D, et al. Successful seroresponses to measles and rubella following aerosolized Triviraten vaccine, but poor response to aerosolized mumps (Rubini) component: comparisons with injected MMR. *Vaccine*. 2010;28(3):692-698.
672. Dilraj A, Cutts FT, de Castro JF, et al. Response to different measles vaccine strains given by aerosol and subcutaneous routes to schoolchildren: a randomised trial. *Lancet*. 2000;355(9206):798-803.
673. Wong-Chew RM, Islas-Romero R, García-García Mde L, et al. Induction of cellular and humoral immunity after aerosol or subcutaneous administration of Edmonston-Zagreb measles vaccine as a primary dose to 12-month-old children. *J Infect Dis*. 2004;189(2):254-257.
674. Wong-Chew RM, Islas-Romero R, García-García Mde L, et al. Immunogenicity of aerosol measles vaccine given as the primary measles immunization to nine-month-old Mexican children. *Vaccine*. 2006;24(5):683-690.
675. Whittle HC, Rowland MG, Mann GF, et al. Immunisation of 4-6 month old Gambian infants with Edmonston-Zagreb measles vaccine. *Lancet*. 1984;2(8407):834-837.
676. Wong-Chew RM, García-León ML, Espinosa-Torres Torrija B, et al. Increasing the time of exposure to aerosol measles vaccine elicits an immune response equivalent to that seen in 9-month-old Mexican children given the same dose subcutaneously. *J Infect Dis*. 2011;204(3):426-432.
677. Fernández-de Castro J, Kumate-Rodríguez J, Sepúlveda J, et al. La vacunación antisarampionosa en Mexico por el metodo de aerosol [Measles vaccination by the aerosol method in Mexico]. *Salud Publica Mex*. 1997;39(1):53-60.
678. Leung K, Louca E, Gray M, et al. Use of the next generation pharmaceutical impactor for particle size distribution measurements of live viral aerosol vaccines. *J Aerosol Med*. 2005;18(4):414-426.
679. Paessler S, Weaver SC. Vaccines for Venezuelan equine encephalitis. *Vaccine*. 2009;27(suppl 4):D80-D85.
680. Cohen BJ, Parry RP, Andrews N, et al. Laboratory methods for assessing vaccine potency retained in aerosol outputs from nebulizers: application to World Health Organization measles aerosol project. *Vaccine*. 2008;26(27-28):3534-3539.
681. Henao-Restrepo AM, Greco M, Laurie X, et al. WHO Product Development Group for Measles Aerosol Project. Measles aerosol vaccine project. *Procedia Vaccinol*. 2010;2:147-150.
682. Low N, Bavdekar A, Jeyaseelan L, et al. A randomized, controlled trial of an aerosolized vaccine against measles. *N Engl J Med*. 2015;372(16):1519-1529.
683. Satti I, Meyer J, Harris SA, et al. Safety and immunogenicity of a candidate tuberculosis vaccine MVA85A delivered by aerosol in BCG-vaccinated healthy adults: a phase 1, double-blind, randomised controlled trial. *Lancet Infect Dis*. 2014;14(10):939-946.
684. Sievers RE (principal investigator). Grand challenges in global health: needle-free delivery of stable respirable powder vaccine. Aktiv-Dry LLC. Available at: <http://gcgh.grandchallenges.org/grant/needle-free-delivery-stable-respirable-powder-vaccine>.
685. Godin CS, Krause E, Griffin D, et al. Safety and immunogenicity of measles vaccine dry powder in rhesus monkeys. Presented at American College of Toxicology meeting 2010, November 7–10, Baltimore, MD. Corresponding author: Robert E. Sievers, Bob.Sievers@colorado.edu.
686. MVDP author group, Cape S, Chaudhari A, et al. Safety and immunogenicity of dry powder measles vaccine administered by inhalation: a randomized controlled phase I clinical trial. *Vaccine*. 2014;32(50):6791-6797.
687. Lu D, Hickey AJ. Pulmonary vaccine delivery. *Expert Rev Vaccines*. 2007;6(2):213-226.
688. Amorij JP, Saluja V, Petersen AH, et al. Pulmonary delivery of an inulin-stabilized influenza subunit vaccine prepared by spray-freeze drying induces systemic, mucosal humoral as well as cell-mediated immune responses in BALB/c mice. *Vaccine*. 2007;25(52):8707-8717.
689. de Boer AH, Hagedoorn P, Westerman EM, et al. Design and in vitro performance testing of multiple air classifier technology in a new disposable inhaler concept (Twincer) for high powder doses. *Eur J Pharm Sci*. 2006;28(3):171-178.
690. Michalek SM, O'Hagen DT, Childers NK, et al. Antigen delivery systems, I: non-living microparticles, liposomes, and immune stimulating complexes (ISCOMS). In: Mestecky J, Bienenstock J, Lamm ME, et al., eds. *Mucosal Immunology*. Burlington, MA: Elsevier; 2005:987-1008.
691. Mestecky J, Michalek SM, Moldoveanu Z, Russell MW. Routes of immunization and antigen delivery systems for optimal mucosal immune responses in humans. *Behring Inst Mitt*. 1997;98:33-43.
692. Curtiss R. Antigen delivery systems, II: development of live recombinant attenuated bacterial antigen and DNA vaccine delivery vector vaccines. In: Mestecky J, Lamm ME, McGhee JR, et al., eds. *Mucosal Immunology*. 3rd ed. Burlington, MA: Elsevier; 2005:1009-1038.
693. Kersten G, Hirschberg H. Antigen delivery systems. *Expert Rev Vaccines*. 2004;3(4):453-462.
694. Rosenthal KL. Recombinant live viral vectors as vaccines for mucosal immunity. In: Mestecky J, Lamm ME, McGhee JR, et al., eds. *Mucosal Immunology*. 3rd ed. Burlington, MA: Elsevier; 2005:1039-1051.
695. Mody N, Dubey S, Sharma R, et al. Dendritic cell-based vaccine research against cancer. *Expert Rev Clin Immunol*. 2015;11(2):213-232.
696. Pizzurro GA, Barrio MM. Dendritic cell-based vaccine efficacy: aiming for hot spots. *Front Immunol*. 2015;6:91.
697. Tree JA, Williams A, Clark S, et al. Intranasal bacille Calmette-Guerin (BCG) vaccine dosage needs balancing between protection and lung pathology. *Clin Exp Immunol*. 2004;138(3):405-409.

698. Roberts M, Bacon A, Li J, et al. Prior immunity to homologous and heterologous Salmonella serotypes suppresses local and systemic anti-fragment C antibody responses and protection from tetanus toxin in mice immunized with *Salmonella* strains expressing fragment C. *Infect Immun*. 1999;67(8):3810-3815.
699. Anderson RJ, Pasetti MF, Sztein MB, et al. DeltaguaBA attenuated *Shigella flexneri* 2a strain CVD 1204 as a *Shigella* vaccine and as a live mucosal delivery system for fragment C of tetanus toxin. *Vaccine*. 2000;18(21):2193-2202.
700. Vecino WH, Morin PM, Agha R, et al. Mucosal DNA vaccination with highly attenuated *Shigella* is superior to attenuated *Salmonella* and comparable to intramuscular DNA vaccination for T cells against HIV. *Immunol Lett*. 2002;82(3):197-204.
701. Fennelly GJ, Khan SA, Abadi MA, et al. Mucosal DNA vaccine immunization against measles with a highly attenuated *Shigella flexneri* vector. *J Immunol*. 1999;162(3):1603-1610.
702. Shata MT, Hone DM. Vaccination with a Shigella DNA vaccine vector induces antigen-specific CD8(+) T cells and antiviral protective immunity. *J Virol*. 2001;75(20):9665-9670.
703. Xu F, Hong M, Ulmer JB. Immunogenicity of an HIV-1 gag DNA vaccine carried by attenuated Shigella. *Vaccine*. 2003;21(7-8):644-648.
704. Pasetti MF, Barry EM, Losonsky G, et al. Attenuated *Salmonella enterica* serovar Typhi and *Shigella flexneri* 2a strains mucosally deliver DNA vaccines encoding measles virus hemagglutinin, inducing specific immune responses and protection in cotton rats. *J Virol*. 2003;77(9):5209-5217.
705. Hamajima K, Kojima Y, Matsui K, et al. Chitin micro-particles (CMP): a useful adjuvant for inducing viral specific immunity when delivered intranasally with an HIV-DNA vaccine. *Viral Immunol*. 2003;16(4):541-547.
706. Singh M, Vajdy M, Gardner J, et al. Mucosal immunization with HIV-1 gag DNA on cationic microparticles prolongs gene expression and enhances local and systemic immunity. *Vaccine*. 2001;20(3-4):594-602.
707. Cusi MG, Zurbriggen R, Valassina M, et al. Intranasal immunization with mumps virus DNA vaccine delivered by influenza virosomes elicits mucosal and systemic immunity. *Virology*. 2000;277(1):111-118.
708. Yang X, Yuan X, Cai D, et al. Low molecular weight chitosan in DNA vaccine delivery via mucosa. *Int J Pharm*. 2009;375(1-2):123-132.
708a. Eriksson K, Holmgren J. Recent advances in mucosal vaccines and adjuvants. *Curr Opin Immunol*. 2002;14:666-672.
708b. Yuki Y, Kiyono H. New generation of mucosal adjuvants for the induction of protective immunity. *Rev Med Virol*. 2003;13(5):293-310.
709. Moyle PM, McGeary RP, Blanchfield JT, et al. Mucosal immunisation: adjuvants and delivery systems. *Curr Drug Deliv*. 2004;1(4):385-396.
710. Cox E, Verdonck F, Vanrompay D, et al. Adjuvants modulating mucosal immune responses or directing systemic responses towards the mucosa. *Vet Res*. 2006;37(3):511-539.
711. Holmgren J, Czerkinsky C, Eriksson K, et al. Mucosal immunisation and adjuvants: a brief overview of recent advances and challenges. *Vaccine*. 2003;21(suppl 2):S89-S95.
712. Hamouda T, Sutcliffe JA, Ciotti S, et al. Intranasal immunization of ferrets with commercial trivalent influenza vaccines formulated in a nanoemulsion-based adjuvant. *Clin Vaccine Immunol*. 2011;18(7):1167-1175.
713. World Health Organization (WHO). Next-Generation Vaccine Delivery Technology Meeting, February 18–19, 2014. Meeting report. Geneva, Switzerland: WHO. Available at: <http://www.who.int/immunization/highlights/Vacc_Deliv_Tech_Pack_Agenda_Feb2014.pdf>.
714. Rennick LJ, de Vries RD, Carsillo TJ, et al. Live-attenuated measles virus vaccine targets dendritic cells and macrophages in muscle of nonhuman primates. *J Virol*. 2015;89(4):2197. Fig. 3b.
715. Potter C, Bennet S. A solid alternative to needle and syringe technology. *Drug Deliv Technol*. 2009;9:24-30.
716. Potter C. Needle-free injection. *Pharm Technol Eur*. 2011;23: 30-32.
717. European Medicines Agency. Assessment report for Intanza. Intanza: common name: influenza vaccine (split virion, inactivated). Procedure no. EMEA/H/C/000957 2009. p. 1–63. Available at: <http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000957/WC500033850.pdf>.
718. Sanofi Pasteur. Prescribing information: Fluzone, Fluzone High-Dose, Fluzone Intradermal (Influenza Virus Vaccine), suspension for injection, 2011–2012 formula. Swiftwater, PA; 2011. Available at: <http://www.vaccineshoppe.com/image.cfm?doc_id=12427&image_type=product_pdf>.
719. Kis EE, Winter G, Myschik J. Devices for intradermal vaccination. *Vaccine*. 2012;30(3):526. Fig. 3.
720. Deleted in review.
721. Prow TW, Chen X, Prow NA, et al. Nanopatch-targeted skin vaccination against West Nile virus and Chikungunya virus in mice. *Small*. 2010;6(16):1776-1784.
722. Jacoby E, Jarrahian C, Hull HF, et al. Opportunities and challenges in delivering influenza vaccine by microneedle patch. *Vaccine*. 2015;33(37):4699-4704.