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REVIEW ARTICLE

Advances Towards Painless Vaccination and Newer Modes of Vaccine Delivery

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Abstract Vaccines have been successful in reducing the mortality and morbidity, but most of them are delivered by intramuscular or intravenous route. They are associated with pain to the baby and bring lot of anxiety for the parents. There has been a marked increase in the number of injections required in first two years of life for completing the vaccination schedule. Hence, there is a need to have a painless vaccine delivery system. Numerous new routes of vaccination like, oral, nasal and transdermal routes are being tried. Oral polio and intranasal influenza have already been a success. Other newer approaches like edible vaccines, nasal sprays, dry powder preparations, jet injectors, microneedles and nanopatches are promising in delivering painless vaccines. Many of them are under clinical trials. These vaccine delivery systems will not only be painless but also cost effective, safe and easy to administer in mass population. They may be devoid of the need of cold chain. Painless delivery system will ensure better compliance to vaccination schedule.

Keywords Painless vaccination · Needle-free vaccination · Newer vaccine delivery

Introduction

Vaccines are a cost effective way of decreasing mortality and morbidity due to various childhood infectious diseases. The Expanded Programme on Immunization (EPI) was adopted by WHO in 1977 against diphtheria, polio, tuberculosis, pertussis, measles, and tetanus with prime aim of immunization to all children [1]. It was started in India in 1978 with BCG, DPT (3 doses) and typhoid vaccine. In 1979, OPV and in 1985, measles vaccine was added to the list subsequent to omission of typhoid vaccine [2]. Later Hepatitis B and *Haemophilus influenzae* type b (Hib) were included in the same. So far, vaccination has successfully eradicated small pox, polio and maternal and neonatal tetanus along with decreasing the burden of many other diseases.

Majority of the vaccines available are injectable preparations. A child receives as many as 18–24 shots of vaccination till he/she reaches the age of two according to CDC vaccine schedule [3]. Besides the prototype oral Sabin polio vaccine, rotavirus vaccine, cholera vaccine, typhoid vaccine and *Shigella flexneri* 2a vaccine are available for oral administration. Nasal spray for influenza vaccine is also available but CDC advisory committee abandoned its use during 2016–2017 flu season [4].

Life saving benefits aside, the very thought of vaccination comes with pain and anxiety associated with that needle prick. It is difficult for the parents to handover their child to the nurse knowing that the child does not understand why he is being hurt. It is a helpless feeling as the parents know that the discomfort and the side-effects far outweigh the morbidity and mortality due to the disease. The most common vaccine-related concern the parents have is that injection will be painful to the child. Study by Kennedy et al. demonstrated that 44.2% of parents are concerned about pain to the child [5]. Studies suggest nearly 24% of parents and 63% of children have needle fear and it is the primary reason for immunization non-compliance amongst 7% of parents and 8% children [6]. So being a pediatrician we must ensure vaccine delivery in a painless manner.

There are guidelines to assist clinicians in managing vaccination-related fear, pain and anxiety among children. To reduce pain at the time of injection, breastfeeding, administration

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of a sweet-tasting solution (sucrose), offering to rub or stroke the skin near the injection site with moderate intensity before and during vaccination, parent and clinician-led distraction, and use of topical anesthetics (lidocaine–prilocaine 5% cream or patch, amethocaine 4% gel and liposomal lidocaine 4% cream) all have been tried [7]. Topical application of lidocaine-prilocaine cream is proven to be effective and does not interfere with immunogenicity of the vaccine. Parents have shown good acceptance towards it even though it is expensive and take 60 min to work [8, 9]. Distraction is defined as the use of strategies to take an individual's attention away from the procedure [7]. Its efficacy is variable. Music seems to reduce distress and pain during vaccinations in adolescents but not in younger children [9]. However, video distraction relieves anxiety before and after vaccinations but not the pain. Distraction by blowing a party blower and toys reduces vaccination pain in children. Party blower also aids in breathing techniques which also decreases the pain. Breastfeeding relieves the pain by sweet taste, distraction, suckling, and physical contact and the 24% oral sucrose solution too decreases the pain by releasing endogenous opioids and distraction [8]. Tactile stimulation in the form of pressure at the site reduces the pain or not, is controversial [7, 9, 10].

Immunization procedure matters a lot. Parents are advised to hold the infant comfortably and not to place them supine. Injecting the vaccine without aspiration and giving the most painful vaccine (MMR-II and Prevnar) last when administering multiple vaccines at the same visit reduces the pain [7, 8]. Finally, in the era of multiple injections, parents want that multiple injections be given simultaneously, rather than sequentially [10]. This has insufficient level of evidence in reducing the pain.

The said interventions may though decrease the pain but the fear of needles and anxiety associated with procedure of vaccination needs new ways of delivering the vaccines. The need of the hour is to find out ways of vaccine delivery which do not cause any pain at all. Various vaccine delivery methods already exist that are painless *vis a vis* oral and nasal vaccination.

The newer non-invasive routes of administering vaccines are the following

1. Oral
2. Nasal (aerosols and dry powder inhalations)
3. Transdermal (microneedles and nanopatch)

Table 1 enlists various non-injectable vaccines available for use and trial.

Oral Vaccines

There is a considerable amount of exposure of infectious agents from our gut mucosa. Needle vaccination may not be just sufficient to provide protection from the gut infection routes. Initially the oral vaccines were shunned, considering their digestion into small fragments by the gastric enzymes rendering them ineffective. However, now oral vaccines are being made using lipids and fats which are not broken down in the stomach and traverse till the intestine where they are absorbed.

The well-known oral polio vaccine (OPV) has been used for mass vaccination campaigns due to its ease of administration. Trivalent OPV consists of a mixture of live attenuated poliovirus strains of each of the three serotypes. OPV produces both, local and humoral immune response. Antibodies in the blood protect the individual against polio paralysis by preventing the spread of poliovirus to the nervous system. The local immune response in the lining ('mucous membrane') of the intestine inhibits the multiplication of subsequent infections of 'wild' (naturally occurring) virus and also stop person-to-person transmission of wild poliovirus [11]. As it has been nearly 5 y that India is free of polio, now bivalent OPV is used.

Similar oral vaccine is available for typhoid with 50% to 80% efficacy and it confers protection for at least 5 to 7 y in 62% to 78% of recipients [12]. The oral live-attenuated vaccine (manufactured from the Ty21a strain of Salmonella serotype typhi) is known for primary vaccination. It consists of an enteric-coated capsule taken on alternate days (day 0, 2, 4, and 6), for a total of four capsules [13]. Presently this vaccine is not available for use in India.

Oral cholera vaccines (OCVs): single-dose live oral cholera vaccine and two other oral inactivated, or non-live cholera vaccines have proven to be effective in epidemics and outbreaks [14]. Live oral cholera vaccine strain CVD 103-HgR, which is an attenuated, live vaccine, administered as a single dose has an efficacy of 62% to 100% [12]. The establishment of the global OCV stockpile in 2013 has been a major advance in cholera preparedness. New killed and live-attenuated vaccines are being actively explored as candidate vaccines for endemic settings and/or as a traveller's vaccine. While two doses of the currently available OCVs are recommended by manufacturers, a single dose would be easier to implement. Qadri et al. proved a single-dose of the current killed oral cholera vaccines, that have been prequalified by the World Health Organization, to be efficacious in epidemics [15].

Rotavirus is responsible for fever, nausea, vomiting and watery diarrhea in young infants. Oral vaccines for rotavirus are in use since 2006 and are effective. Both the brands of available vaccines, are administered orally and are only different in the number of doses. With five valent vaccine, three doses are required (2 mo, 4 mo, and 6 mo) and monovalent human strain vaccine requires two doses (2 mo and 4 mo).

Edible vaccine is an unusual and new concept. Scientists suggest that plants and plant viruses can be genetically engineered to produce vaccines against diseases. These are produced by introducing selected genes encoding bacterial

Table 1 List of non-injectable vaccines available for use or under trial

Route	Available	Under trial
Oral	Polio vaccine Rotavirus vaccine Typhoid vaccine Cholera vaccine	
Edible vaccines		Cholera, Norwalk virus, Hepatitis B, Malaria, Measles, Human Papilloma Virus (HPV-11), Human Immunodeficiency Virus (HIV), Respiratory Synctial virus and *Mycobacterium tuberculosis*
Sublingual vaccines		*Helicobacter pylori*, HPV16, HPV18, and HPV58 pseudoviruses, Influenza Virus, Respiratory Syncytial virus (RSV) and Severe Acute Respiratory Syndrome (SARS) virus
Melt in mouth strips		Rotavirus
Nasal vaccines	Influenza spray Ebola vaccine spray	Pneumococcal infection, Dengue, Hepatitis B, Whole Influenza virus, Vaccinia viruses, Anthrax and HIV
Pulmonary vaccines		Hepatitis B virus, Measles, Tuberculosis, Yersinia pestis, Measles, MMR (Measles, Mumps, Rubella) vaccine, Bacillus Calmette-Guérin (BCG) vaccine, Influenza, *Streptococcus pneumoniae*, HPV and *Mycoplasma hyopneumoniae*
Microneedles		Diphtheria, Hepatitis B, recombinant anthrax vaccine, Live-attenuated Japanese encephalitis vaccine, Rabies vaccine, Influenza vaccine, BCG vaccine, Measles, Rotavirus vaccine, Vaccine for travellers' diarrhea
Nanopatch		West Nile virus, Chikungunya, Influenza

and viral antigens in plants which are used to form immunogenic proteins [16]. Edible plant vaccines are like conventional subunit vaccines *i.e.*, immunogenic preparations containing antigenic proteins rather than pathogens [17]. This process is known as "transformation" and the altered plants are called "transgenic plants". Thus, they are highly safe and cannot cause disease. It will be easy to administer them and they will have a low production cost. It eliminates need of fermentation and purification systems, sterile delivery and being heat stable does not require cold chain maintenance. It confers both mucosal (IgA) and systemic (IgG) immunity. Over past 5 y significant progress has been made in expressing vaccine antigens in edible leaves (especially lettuce) and processing them to achieve antigen stability and efficacy after prolonged storage at ambient temperatures [18]. They can be grown using local production facilities and are thus, cutting expensive manufacturing cost. And as there is no need of needles, it prevents infection.

Bioencapsulation of antigens in plant cells protects them from the digestive system; the fusion of antigens to transmucosal carriers enhances efficiency of their delivery to the immune system and facilitates successful development of plant vaccines as oral boosters [18]. Antigen expression in plants has been successfully shown for LT-B (ETEC) in tobacco and potato; rabies virus-G protein in tomato; HBsAg in tobacco and potato; Norwalk virus in tobacco and potato; CT-B (Vibrio cholerae) in potato [19]. Clinical trials are undergoing for malaria, measles, human papilloma virus (HPV-11), Human Immunodeficiency Virus, Respiratory Synctial Virus and *Mycobacterium tuberculosis* [19]. Human clinical trials using transgenic potatoes having cholera toxin have shown successful seroconversion in volunteers.

The *sublingual route i.e., via* the mucosal surfaces under the tongue and the buccal route have been used for many years to deliver drugs (cardiovascular drugs, steroids, barbiturates, benzodiazepines, opioid analgesics) and small molecules to the bloodstream. The potential of sublingual and buccal vaccine delivery is largely unexplored. They are superior to oral vaccination method where there is a risk of degradation by gastric enzymes. Similarly in skin vaccination method, impermeable thick keratinized stratum corneum acts as a physiological barrier and chemical disruption and/or microneedle penetration is required which is not so in sublingual and buccal vaccine delivery. Intranasal immunization also induces mucosal immunity but retrograde transport of antigen and/or adjuvant from vaccine formulations to the brain and other neural tissues, causes serious side-effects. This is in contrast to the sublingual route of delivery wherein no antigenic migration to the central nervous system occurs. Many laboratories have documented the efficacy of sublingual immunization in inducing adequate immune response in experimental animal systems using a variety of antigens, including soluble proteins, inert particulate antigens (killed viruses, virus-like particles, bacterial extracts) as well as live-attenuated viruses [20].

Researchers have successfully demonstrated protection against *H. pylori* [21], HPV16, HPV18, and HPV58 pseudoviruses [22] by sublingual vaccination in animals. The sublingual mucosa is a promising vaccine delivery route for other respiratory pathogens including influenza virus, Respiratory Syncytial virus (RSV) and Severe Acute Respiratory Syndrome (SARS) virus [23]. However in a clinical trial, where HPV vaccine was applied sublingually to humans, antibody titre were 1000-fold lower than in the intramuscular group. So researchers concluded that alternative delivery systems and adjuvants would be required to enhance and evaluate immune responses following sublingual immunization in humans [23]. It is an attractive option for vaccine delivery because it is efficient, accessible, and relatively clean.

"*Melt in mouth strips*" is an another innovation in vaccine delivery. Researchers at McMaster University are inspired from the chemistry of a consumer product: breath-freshening strips that melt on tongue [24]. Pullulan, a polysaccharide derived from a common fungus is the key ingredient of these strips. It normally rests in a solid state but dissolves easily in water. By casting enzymes and other substrates within this material; they can be preserved in a form that will remain inert until it interacts with water. Undergraduate biomedical engineering students at John Hopkins University have developed such strips laced with vaccine against rotavirus [25].

Nasal and Aerosol Vaccines

We respire every moment and with every breath we are exposed to several viral and bacterial pathogens that transmit through air-borne particles. The large surface area of respiratory system can provide adequate interaction between the immune system and the antigen. The nasal mucosa and lungs can be considered as an important route for vaccination. In addition, the extensive vascularization and thin epithelium in the alveolar lung tissue [26] facilitates efficient systemic delivery of antigens, thereby ensuring both local and systemic antibodies. The delivery of vaccines *via* these routes is recently emerging as an attractive alternative to injection. It is more potent and a practical way of inducing effective immunity against infectious diseases. It elicits rapid immune response, both locally and systemically. It is gaining advances in the future for being a self-administrative and non-invasive technique, thus causing little discomfort to the patients. Another advantage is that both liquid and dry powder formulations can be given, thus saving the cost spend in transportation *via* cold chain.

The various devices available for nasal route of vaccination are single dose nasal spray, bi-dose nasal spray, multi-dose pump with tip-seal technology (prevents contamination of bottle content), unit-dose nasal powder delivery system, bi-dose nasal powder delivery system [27] and jet nebulizers.

The live, attenuated influenza vaccine (called LAIV) may be given to healthy, non-pregnant people (2 to 49 y of age) as a nasal spray. It is made from attenuated flu virus so it does not cause flu [28]. There are many flu viruses, and they keep on changing every year. Initially it was believed to provide some protection even when the vaccine does not match the current season virus. However, recently, CDC's Advisory Committee on Immunization Practices (ACIP) declared that it should **not** be used during the 2016–2017 flu season as studies conducted showed just 3% protective benefit [4].

Needle-free nasal immunization with recombinant HBsAg using nanoemulsions (NEs) has also proved to be a safe and effective hepatitis B vaccine, and has provided an alternative booster administration for the parenteral hepatitis B vaccines [29]. NEs (< 400 nm) are emulsions formulated with surfactants, distilled water, refined soybean oil and ethanol as a solvent. Initially they were used as broad-spectrum antimicrobial agents. NEs proved effective as mucosal adjuvants for whole influenza virus, vaccinia viruses, recombinant anthrax protective antigen and HIV gp120 [29].

Suzuki et al. have developed an efficient nasal vaccine delivery system against pneumococcal infection. They fused C-terminal fragment of *Clostridium perfringens* enterotoxin (C-CPE) with pneumococcal surface protein A (PspA). Nasal immunization with PspA-C-CPE induces PspA-specific IgG in the serum and bronchoalveolar lavage fluid (BALF) as well as IgA in the nasal wash and BALF, which proved sufficient to protect against pneumococcal infection [30].

Nantachit et al., delivered dengue immunogen (domain III of dengue serotype-3 E protein -EDIII-D3) loaded into trimethyl chitosan nanoparticles (EDIII-D3 TMC NPs) intranasally. This stimulated a strong local innate antiviral response which helped in systemic adaptive immunity [31].

Studies have also been conducted for Ebola virus, which was recently a dreadful outbreak in western Africa. Researchers at The University of Texas at Austin have developed nasal vaccine which provided long-term protection for non-human primates against the deadly Ebola virus. Results from a small pre-clinical study represent the only proof to date that a single dose of a non-injectable vaccine platform for Ebola is long-lasting [32].

Aerosol vaccine delivery involves creating small particles, usually generated by a nebulizer, that reach the lungs [12]. Two types of pulmonary delivery devices are available and useful for vaccination: Dry Powder Inhalers (DPI) and jet nebulizers. Pulmonary vaccine formulations for Hepatitis B virus [33], Measles [34], Tuberculosis [35] and *Yersinia pestis* are in preclinical research phase. Measles vaccine, MMR (Measles, Mumps, Rubella) vaccine, BCG vaccine, Influenza, *Streptococcus pneumoniae* and Human Papilloma virus [36] are in clinical research phase. Clinical trials in

Mexico and South Africa have demonstrated significantly higher rate of measles seroconversion following measles vaccination and combined rubella and measles vaccination in children after aerosol delivery than after subcutaneous delivery [37, 38]. Animal experiments have also demonstrated efficacy of *Mycoplasma hyopneumoniae* aerosol vaccine [39]. Most of the successful clinical trials have been done using jet nebulizers but the need for a pressurized (clean) air system and stability concerns of aqueous vaccine formulations limits their applicability in mass vaccination programs. As production of powder formulations for pulmonary administration is a one-step process, it reduces the risks of contaminations and batch-to-batch differences, as well as production costs. Thus, a simple, cheap, compact, disposable, and effective DPI is the most optimal device for pulmonary vaccination for target population.

Transdermal Route

Skin has an outermost layer called the stratum corneum, below which lies the viable epidermis that comprises 2% of Langerhans cells. These are extremely effective antigen-presenting cells, and generate an immune response. Vaccination in this cutaneous environment rich in specialized antigen-presenting cells using microneedles and nanopatches has practical and immunological advantages over conventional needle delivery.

Microneedles are micro structured projections which range from solid to hollow. There are (i) solid microneedles for skin pretreatment to increase skin permeability, (ii) microneedles coated with drug that dissolves off in the skin, (iii) polymer microneedles that encapsulate drug and fully dissolve in the skin and (iv) hollow microneedles for drug infusion into the skin [40]. Volunteers of clinical studies reported no pain and minimal sensation of these microneedle arrays, likely due to not enough length of these microneedles to stimulate nerves present in the deeper tissues [41].

Microneedles have been employed successfully to vaccinate with diphtheria toxoid adjuvanted with cholera toxin and to increase delivery of a DNA vaccine against hepatitis B, recombinant anthrax vaccine, and live-attenuated Japanese encephalitis vaccine in animal models as well as rabies vaccine in human subjects [40]. Influenza vaccination with coated microneedles has shown complete protection against lethal viral infection after vaccination using H1N1 and H3N2 seasonal strains in mice [40]. Substantially improved immunity resulted following administration of Bacillus Calmette-Guérin (BCG) vaccine in guinea pigs using similar coated-microneedle devices [40]. The World Health officials are aiming to eliminate measles and such a microneedle patch can be the game changer [12]. Study by Levin et al. and Behrens et al. confirmed the immunogenicity and safety of intradermal delivery of virosomal influenza vaccine and vaccine containing heat-labile toxin from *Escherichia coli* against travellers' diarrhea respectively in humans [42, 43].

The '*Nanopatch*' (NP) comprises arrays of densely packed projections with a defined geometry and distribution designed to physically target vaccines directly to thousands of epidermal and dermal antigen presenting cells [44]. These miniaturized arrays are smaller than standard needles used for vaccination and are also much smaller than current microneedle arrays. The NP immunization has been seen to be efficient using commercial available influenza vaccine antigen [45], inactivated whole chikungunya virus vaccine and DNA-delivered attenuated West Nile virus vaccine [44].

Others

Another mode of vaccine delivery known for more than 50 y is *via* jet injectors. Jet injectors are needle-free devices that deliver a prescribed drug, vaccine, or compound intradermally, subcutaneously, or intramuscularly *via* high pressure produced by either a carbon-dioxide-filled or nitrogen-filled cartridge or a spring [12]. Antigens delivered by jet injectors are dispersed more widely in the tissue because of high pressure of the fluid stream allowing for a larger contact volume between the vaccine antigen and immune cells. Several studies have shown that it elicits higher antibody titers and seroconversion rates than traditional needle and syringe [12]. Earlier, multiuse-nozzle jet injectors were used that delivered vaccine through the same fluid stream and nozzle to multiple patients. It was commonly used for vaccinating military personnel, and for other mass immunization campaigns. However, when the year 1985 witnessed an outbreak of hepatitis B infection due to contamination of the jet injector by body fluids, health authorities, including the Department of Defense and the World Health Organization, discontinued the use of multiuse-nozzle jet injectors.

Now-a-days disposable-cartridge jet injectors, where fluid stream is delivered within a disposable vaccine cartridge and nozzle, with a new cartridge and nozzle for each patient and no splash back of blood are under development. Biovalve's Mini-Ject, Bioject and Powderject are few developing technologies for vaccination. Trials are underway to deliver Inactivated polio vaccine, Measles-Mumps-Rubella vaccine, Yellow fever vaccine, DTP-Hib-hep B vaccine, BCG vaccine and rabies vaccine by jet injection technology [12].

On August 15, 2014, the U.S. Food and Drug Administration (FDA) approved use of one jet injector device (the PharmaJetStratis) for delivery of one particular flu vaccine in individuals 18 through 64 y of age [46]. However post-vaccination, patients have reported tenderness, swelling, pain, redness, itching and bruising at the site of vaccination.

Epidermal powder immunization (EPI) is similar to liquid jet injection, but here dried-powder particles of vaccine, rather

than liquid, are injected into the skin at supersonic speed. Another similar concept is particle-mediated epidermal delivery (PMED) in which DNA vaccine coated on gold microparticles are shot into the skin. Clinical studies of PMED immunization are promising but immunogenic responses were low compared to conventional vaccination methods [47].

As a whole, jet injection offers multiple benefits. They are less painful, thus improve compliance, reduce risks of needlestick injuries and cross-contamination, eliminate the need for "sharps" disposal, and ensure reliable, reproducible, and accurate delivery of medication with minimal training.

Conclusions

The future of immunization depends on the how successfully we are able develop methods for vaccination that are simpler to administer, do not need cold chain for their maintenance, provide long-lasting immune response with minimal side-effects and, most importantly in a child-friendly way. Needle-free and painless vaccination will ensure improved safety for the vaccinator, vaccinee, and community; improved compliance with immunization schedules; reduced anxiety and pain related to injection; easier and speedier vaccine delivery with reduced cost of production, storage and transportation. This will mean less healthcare training needed to give vaccines, especially in mass vaccinations on national or sub-national immunization days (campaigns), natural pandemics, and bioterrorism emergencies.

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Compliance with Ethical Standards

Conflict of Interest None.

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