Expert Opinion

Needle-free vaccine delivery

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The need for minimally invasive delivery methods is urgent. As the number of registered vaccines increases, so does the number of injections. The use of sharps can be unsafe and needle immunisation is less suitable for mass immunisations during emergencies such as pandemics or bioterrorist attacks. The approach of combining vaccines has limitations due to high development costs, risk of pharmaceutical or immunological interference and economic risks. Advancements in the development of alternatives to injection with syringes and needles are discussed in this paper, and include: mucosal vaccination, injection without needles and vaccine delivery via the skin.

Keywords: adjuvants, dermal vaccination, jet injection, mucosal vaccination, needle free, vaccine

Expert Opin. Drug Deliv. (2007) 4(5):459-474

1. Introduction

This review provides an overview of the rationale behind the many attempts to develop alternatives to the classical needle and syringe vaccinations. The need for alternatives is based on problems that exist with regard to present vaccine formulations. These include injection safety, patient fear of injections and drawbacks associated with the present alternative: combining vaccines for parenteral application. Using a number of case studies, this paper demonstrates that the development of these and other alternatives, although difficult, expensive and prone to failures, can be very successful. The availablity of affordable, safe and efficacious alternatives is crucial in order to maintain confidence in vaccines and vaccination programmes by users of vaccines. Incidents with measles [1] and oral polio vaccine (OPV) [2] have shown that this confidence is easily shattered.

In this review, the advances in needle-free vaccine delivery are also discussed. In the recent past, several excellent reviews on needle-free vaccination in general [3-6], fluid jet injectors [7,8], elastic vesicles [9,10], topical DNA immunisation [11], needle-free influenza vaccination [12], dermal vaccination and adjuvant patches [13], dermal vaccination [14], microneedles [15], mucoadhesive microspheres [16], mucosal delivery [17], mucosal immunity [18], nasal drug delivery [19], oral delivery [20], pulmonary vaccination [21], mucosal vaccines [22], mucosal adjuvants [23] have been published. The present review is, therefore, mainly focussed on literature published in 2006 and the first half of 2007.

2. Why do we need needle-free vaccines?

Of the more than 5 billion human vaccine doses that are given each year, 3 billion are delivered by injections. Needle-free vaccination consists mostly of OPV and must be seen in the light of the polio eradication programme by the WHO. Injected vaccines are very successful but have a number of drawbacks that warrant the development of alternative delivery systems.

2.1 Safety

The reuse of needles and syringes, as well as needle stick injuries, cause many infections in patients as well as medical personnel. Only a few years ago, global

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estimates were 10.4 – 20.9 million infections per year due to unsafe injections [201]. This concerns all injections given. Between 5 and 15% of all injections given are vaccines. Most infections transmitted by needles are hepatitis B (80%), followed by hepatitis C. A much smaller group is infected with HIV (< 1%). The reuse of needles is mainly a problem in developing countries. For this reason, needle-free alternatives should be cheap and/or should have additional advantages, such as increased thermostability and shelf life. These alternatives are not yet in the market, but safer needle-based alternatives are already available. Syringes such as PATH's SoloShot™ (now trademark of Becton, Dickinson and Company; 1992) and Univec (Univec; 1995) are examples of autodisable syringes used on a large scale. PATH also developed the single-use Uniject™ (now trademark of Becton, Dickinson and Company). Although a huge improvement, these solutions do not circumvent needlestick injuries. The transmission risk from an infected person to a healthcare worker following a needlestick injury is estimated at 0.3% for HIV, to 3 – 10% for hepatitis B. Of 35 million healthcare workers, ~ 2 million are infected by needlestick injuries [24].

2.2 Number of injections

If given the choice between a vaccination by needle or via a needle-free route, the vast majority of people choose the latter. In one clinical study comparing an intranasal virosomal influenza vaccine with a classical syringe and needle formulation, participants could choose between the two formulations. Ninety-seven percent chose the nasal vaccine [25]. When they were asked to explain their choice, 14% answered that they were afraid of injections. This is in accordance with other studies, where ~ 10% of the people reported needle phobia [26]. However, it is certain that the number of vaccines (e.g., those in national paediatric vaccination programmes) will expand in the coming decades. The Dutch Health Council has recently published a report on the future of the Dutch national vaccination programme [202]. Two of the conclusions were that all vaccines presently in use should stay in the programme, and that 15 (out of 23 assessed) candidate vaccines had a high enough disease burden to justify inclusion. Presently, most Dutch children receive 13 injections against 10 diseases, most of them in their first 14 months of life; two injections are received per session. Participation in immunisation programmes is voluntary, and this policy, and the fact that vaccines are given free of charge, results in a vaccine coverage of > 95%. There is concern that vaccine coverage will decrease when more than two injections per session are given. The fear of needles and pain are important factors contributing to decisions to avoid vaccination. It is possible to reduce perceived pain by distraction and other psychological means [27], but this becomes increasingly difficult when the number of injections per session increases.

2.3 Mass vaccinations

Classical vaccines are not very suitable for mass vaccinations during emergencies. These circumstances occur when there is an outbreak of a disease that is usually contained by vaccination (e.g., polio), in the case of emerging diseases (pandemic influenza, severe acute respiratory syndrome) and during attacks with infectious agents. In these cases, important parameters are speed (the number of vaccinations per unit of time), ease of application (no trained personnel needed) and stability (fewer logistical problems). Vaccines given by needle and syringe do not meet these criteria.

3. The drawbacks of combination vaccines

The present solution for the problems mentioned above is to combine vaccines. The applicability of combination vaccines has its limitations.

3.1 High development costs

Combination vaccines are expensive to develop, as combining two existing components into a combination is almost as expensive as developing the individual components. Apart from the necessity to redevelop or at least to revalidate release tests, the new combination must be reformulated, the stability and toxicity testing repeated, and at least some of the clinical studies performed for the individual components have to be redone.

3.2 Pharmaceutical interference

The stability profiles of antigens in a combination vaccine differ, for example as a function of pH. This may result in a reduced shelf life of the combination vaccine or the need for additional formulation work to select stabilising excipients. Bulk concentrations may also be limiting. Eventually, all components must be formulated in preferably 0.5 ml, but at the most 1.0 ml. The more components in the combination, the more concentrated the bulk materials must be. Sometimes concentration limits are reached because the production process cannot be optimised further or because the antigen aggregates to undesired levels or too quickly at high concentrations. This may require optimisation of the formulation of the 'monovalent' bulk materials.

3.3 Impurities

The impurity profile (proteins, nucleic acids, endotoxins) in the combination vaccine may also reach unwanted levels. Specifications, apart from clear-cut regulations, are often based on the impurities in the separate components or existing vaccine (i.e., vaccine developers should give at least some preclinical proof that the new combination is at least as safe as the old vaccines). Exceeding impurity limits will increase the risk of failure during clinical trials. Therefore, attempts should be made to match the impurity profile of the old, non-combined vaccines. This may result in substantially adapted production processes and increased costs

(more unit operations, lower yields), if possible at all. In addition, sometimes the refined antigens turn out to be less immunogenic because the removed impurities have some adjuvant effect.

3.4 Immunological interference

Optimal immunisation schedules may differ between antigens in a combination vaccine. Some antigens, polysaccharides for instance, are not very immunogenic in very young children, whereas others, such as vaccines against whooping cough, must be given as early as possible, as most victims are very young children.

Another problem that can occur is inhibition of the response after mixing with another antigen, although the reason for this phenomenon is often not known, it has been observed regularly [28,29]. The absence of immune interference in preclinical studies is not reliable and, therefore, expensive clinical studies are needed. An example of immunological interference with serious consequences is the Hexavac® (Aventis Pasteur MSD) vaccine, consisting of six vaccines: diphtheria, acellular pertussis, tetanus, inactivated polio, *Haemophilus influenzae* and hepatitis B. The existing pentavalent vaccine was extended by adding the hepatitis B component. Nine clinical studies were done and the product was approved in Europe in 2000. In 2005, registration was suspended because there were concerns due to lower and varying immunogenicity of the hepatitis B component. Hepatitis B and *Haemophilus influenzae* type b after immunisation with the hexavalent vaccine were lower compared with the pentavalent vaccine plus hepatitis B on its own [29]. For hepatitis B this may be a problem, as it is mainly a sexually transmitted disease, and decades of protection are needed when the immunisation takes place at a very young age.

3.5 Economic risks

The production of complex combination vaccines poses economic risks. If one component in the final product fails, the whole combination fails and has to be discarded.

4. Alternatives

The previous section illustrates the need for alternatives for classically injected vaccines. These alternatives can be divided into three groups that will be discussed below. **Tables 1** and **2** summarise the characteristics of the different approaches and refers to recent literature.

4.1 Mucosal vaccination

Mucosal vaccination has several advantages above the systemic route. It can lead to simultaneous local and systemic immune responses, it may lead to sterilising immunity (i.e., infection as well as disease are prevented) and the natural point of entry is thought to result in an optimal response (although in a qualitative sense only). In principle, all mucosal tissues can act as a site for immunisation. This refers to the existence of the common mucosal immune system. Immunisation at one site results in local secretory responses on the other mucosal sites and often in systemic responses. The strength of the response at distant mucosal sites is dependent on the site of application. The reason is that within the mucosal immune system, a degree of compartmentalisation is present [18]. For example, rectal immunisation will not lead to substantial immune responses in the nasopharyngeal area. On the other hand, intranasal immunisation usually induces strong responses in the urogenital tract.

Oral and nasal routes are the most frequently studied. Some other routes are less practical because mucosal tissues are difficult to reach, for instance the mucosal tissue in the urogenital tract and the lungs.

4.1.1 Oral vaccination

The problem with oral vaccination is its inefficiency both with respect to the magnitude of the response and the duration. The mucosal tissue of the gut is continuously exposed to large amounts of microbial and food antigens, and in that respect the gut is rather immune tolerant. Other problems relate to strong dilution of the antigen and the type of immunity that is needed for protection. Targeting strategies to mucosal lymphoid cells can improve the potency, but 'targeting' works at a short distance and merely enhances binding that would otherwise still occur. Other delivery problems relate to the low pH in the stomach and the presence of proteolytic enzymes. This can be solved to some extent by using enteric-coated capsules.

Although antigens expressed in plant cells are often used in the context of oral immunisation (e.g., [30]), the present paper considers this technique as a production platform for antigens. Quality control issues such as potency and dosing, prevent the use of non-processed plant material. The use of plant-produced purified antigens is not limited to the oral route. Therefore, this approach is not discussed.

Relatively simple solutions such as the use of inactivated whole cell vaccines may have an improved chance of success. These vaccines are relatively cheap to produce and, therefore, higher doses can be given. This can lead to strong responses [31-33]. Long-lasting immune responses after oral vaccination are still difficult to achieve with inactivated vaccines [34]. Inactivated cholera vaccines confer up to 50% protection [35,36], which is very substantial but less than desired. The development of powerful but non-toxic mucosal adjuvants may lead to improvement [37] but, until now, successful oral vaccines have been live attenuated vaccines with the ability to replicate in the gut. Presentation platforms based on attenuated Salmonella or commensal microorganisms are under development (see **Table 1**). Experiences with live oral vaccines show that they are efficacious, but that there are risks of rare but serious adverse effects. Another characteristic, which can be both an advantage and a concern, is shedding of the attenuated pathogen by the person vaccinated. This can contribute to herd immunity due to indirect immunisation of

Table 1. Mucosal vaccine delivery.

Route and characteristics	Technology/delivery platform	Development stage*
Oral	Live attenuated	Market (e.g., oral polio vaccine, rota [43], cholera [85,18], typhoid fever [86]). Clinical: improved typhoid [87]
- ease of administration - 'natural' route (mucosal and systemic response) - cheap (live vaccines require lower dose) - inefficient (with inactivated vaccines)		
	Live vectors: *Salmonella typhi* [86]	Early clinical [88]
	Live vector: *Salmonella typhimurium* [40]	Early clinical [89]
	Live vector: *Escherichia coli*	Early clinical [90]
	Live vector: *Listeria monocytogenes*	Research [91]
	Live vectors: *Lactobacillus casei* (oral better than intranasal)	Research [92]
	Inactivated *Lactococcus lactis* (GEM technology); also intranasal; oral better than intranasal	Research [93]
	plants or plant cells [28]	Clinical [94,95]
	Adjuvants: ADP ribosylating exotoxins (also other mucosal routes i.e., nasal)	Research LTR72, LTK63 (HLT analogues): [96,97] Clinical [98]
	Adjuvants: lipopeptides (MALP-2)	Research [49]
	Adjuvant: saponins	Research [99]
	Oral route to induce IgE with antitumour properties	Research [100]
Nasal	Live attenuated [58,57]	Market (Flumist) Research: live *B pertussis* [101]
- easy - 'natural' route, - can induce pulmonary protection - rapid clearance		
	Chitosans (also oral)	Research [102-104,45]
	Live vectors: *Salmonella typhi*	Research [105]
	Adjuvant: *Bacillus anthracis* edema toxin	Research [106]
	Live vectors: *Lactococcus lactis*	Research [107]
	Adjuvants: lipid emulsions (L3, N3)	Research [108]
	Adjuvants: Shigella invasin complex (Invaplex)	Early clinical (announced in [109])
	Adjuvants: bacterial flagellin (TLR 5 ligand)	Research [110]
	Adjuvant: surfacten (pulmonary surfactant preparation)	Research [111]
	Adjuvant: bacterial second messenger cdiGMP	Research [112]
	CT-conjugate (intranasal better than intravaginal)	Research [113,114]
	double stranded RNA	Research [115]
	Adjuvants: ADP ribosylating exotoxin (LTK63) ± 'biovector'	Early clinical [52]
	LT/flu virosomes	Market, but withdrawn
	Proteosomes (Neisseria sp.) [116]	Early clinical [117]
Rectal	Virus-like particles [118]	Early clinical [119,120]
- circumvents most part of digestive tract - inefficient (with inactivated vaccines)	CT [119]	
	Microparticles [121]	
Vaginal		
- easy, - 'natural' route - perhaps only suitable route for local STD vaccination [120] - only half of the population - responsiveness dependent on hormonal status [122]	Multivalent inactivated bacteria	Early clinical [120,123]
	CT conjugate	Early clinical [124] Research [125] (mechanistic study demonstrate induction of CD4 and CD8 T-cell responses; conjugation is essential)

*Stages: research, early clinical, late clinical, market.

CT: cholera toxin; GEM: Gram-positive enhancer matrix; HSV: Herpes simplex virus; LT: Heat labile toxin.

Table 1. Mucosal vaccine delivery (continued).

Route and characteristics	Technology/delivery platform	Development stage*
Pulmonal:	Nebulised measles vaccine	Late clinical [126,127]
- possibly efficient	Powder	Research [128]
- high shear forces in fluid jet nebulisers		
- dosing difficult		
Sublingual		Research [129]
Easy		
Ocular (lacrimal glands)		Research [130]
Easy		
Against infectious agents targeting the eye (e.g., HSV-1)		
Inefficient?		
Small dose		

*Stages: research, early clinical, late clinical, market.

CT: cholera toxin; GEM: Gram-positive enhancer matrix; HSV: Herpes simplex virus; LT: Heat labile toxin.

contacts of the vaccinee, although the importance of this effect is limited, at least for OPV [38]. On the other hand, shedding raises safety issues because the risks associated with the shedding of reverted mutants [39] or the spread of recombinant microorganisms in the environment [40,41]. The most successful oral vaccine is without doubt OPV. Developed in 1956 by Albert Sabin, the vaccine is largely responsible for a huge decrease in poliomyelitis cases. In 2000, ~ 40% of the vaccine doses given worldwide was OPV. However, OPV alone will not be enough to successfully complete the present eradication initiative by the WHO because OPV causes rare cases of vaccine-associated paralytic poliomyelitis and excretion of virulent virus by vaccinees.

An interesting case study regarding oral vaccines are rotavirus vaccines. Rotavirus causes diarrhoea. In infants it causes many deaths in developing countries and many hospitalisations in industrialised countries. The first registered vaccine, Rotashield® (Biovirx), was approved in the US in 1998. It consisted of four live attenuated strains. The vaccine was withdrawn after 9 months. During the period of use, 1.5 million doses were given and it became apparent that there were an unexpected number of cases of intussusception, compared with non-immunised children. Intussusception is the prolapse of a part of the gut into itself, causing obstruction. Sometimes surgery is needed to cure the painful condition. The initial clinical trials did not indicate increased risk of intussusception, but post-marketing research showed a clear correlation between vaccination and intussusception [42]. Today, two other live oral rotavirus vaccines are on the market: the pentavalent Rotateq® (Merck) and the monovalent Rotarix® (GlaxoSmithKline). Because of the risk of rare but serious intussusception, the clinical studies preceding registration of both vaccines were very large, including 60,000 – 70,000 children [43,44]. These studies showed no higher risk for intussusception compared with the placebo-administered groups.

The initial rotavirus vaccine problem sparked a debate on whether it was ethical to withdraw an affordable vaccine that could save many lives in developing countries at the cost of a relatively small number of vaccine-induced deaths. With the existence of safer alternatives, it is clear that a less safe vaccine cannot be used in any country. However, expensive clinical development results in expensive vaccines. The result is that the new vaccines are not affordable for the people that need them most.

4.1.2 Nasal vaccination

There is a substantial body of literature describing good results after nasal vaccination. Understanding of the immunology of the nasopharyngeal mucosal tissue is improving, including T-cell responses [45], induction of memory [46] and cytokine patterns [47]. The use of adjuvants such as macrophage-activating lipopeptide [48,49,45] and adamantylamide dipeptide [50] is probably mandatory to achieve sufficiently high immune responses with inactivated vaccines. A clinical study with an experimental adjuvant-free hepatitis B vaccine has shown high seroconversion rates, but this was achieved with high (100 µg) antigen doses and frequent (five) immunisations [51]. Even with potent adjuvants, the response to inactivated vaccines often does not match parenteral immunisation [52] when comparable antigen doses are used. The importance of prolonged contact times by adding mucoadhesives has been debated [53]. It was suggested that increasing the contact time with mucoadhesive excipients is less relevant than boosting with sufficient time intervals, at least to induce robust systemic responses [54], although the cationic polymer chitosan and its derivatives, which are thought to increase contact time with negatively charged cell surfaces, have clear mucosal immune-stimulating properties [19]. The exact mechanism of action of chitosans is unclear and may be a combination of mucoadhesive properties,

Table 2. Needle-free injection techniques and dermal vaccination.

Route and characteristics	Technology/delivery platform	Development stage*
NEEDLE-FREE INJECTION		
Fluids	Disposable cartridge jet injectors, spring loaded or with gas capsule [8]	Disposable cartridge jet injectors: market.
Long history Relatively easy Less (re)fomulation Local adverse effects bit higher than syringe & needle	Pulsed microjet [61]	Multi-use nozzle jet injectors withdrawn from market
Solids	Powder [63], coated gold particles [62]	Clinical [63]
Increased stability/no cold chain, Smaller volume (less adverse effects?) Extensive reformulation needed	Biodegradable mini-implants Glide Pharma, Bioneedle Group	Research
DERMAL		
Noninvasive patch	Adjuvant: LT [71]	Late clinical
Easy, Relatively cheap Inefficient (for other antigens than LT and CT) Standardisation dosing	Delivery system: elastic vesicles [79]	Research
	Delivery system: transporter peptides [76]	Research
Microstructures	Steel cut-out in plane for pretreatment or coated needles [82]: Macroflux®‡ skin patch (titanium) [67,131]	Research
Efficient Expensive Formulation problems Standardisation dose Technically demanding	Steel 30G needle tips for pretreatment [132]	Research
	Coated needles: steel coated with porous $CaPO_4$/trehalose [133]	Research
	Hollow (single needle) [68]	Research
	Onvax™§ silicon array [81]	Early clinical (announced, not published?)
	Hollow (needle array): problems with *in vivo* delivery [83]	Research
Ultrasound/sonoporation	Sonicator [134]	Research
No adverse effects (?) Less suitable for mass vaccinations		
Microporation	Electrode arrays generating superficial heat (pretreatment technique) [135]	Research
Electroporation	Electrodes plus pulse generator (short high voltage pulses) [136-138]	Research
Relatively fast Limited to < 10 kDa antigens, Not necessarily needle free Adverse effects (muscle contractions)	DNA-coated microneedles (EasyVax™¶) [139]	Research

*Stages: Research, early clinical, late clinical, market.

‡Alza Corp.

§Becton, Dickinson and Company.

¶Vaxin, Inc.

CT: cholera toxin; LT: Heat labile toxin.

penetration enhancement, increased cell interaction and immune-modulating effects.

When an immune response is induced after intranasal administration it may result in pulmonary immunity [55]. Also, T-cell responses in the lung, as demonstrated with lipopeptides, have been identified [45]. This would reduce the need to develop pulmonary immunisation routes, which are technically much more difficult.

Valorisation of this knowledge to marketed products is another matter. Illustrative examples are the nasal influenza vaccines Nasalflu® (Berna) and Flumist® (Medimmune, Inc.). The former vaccine, an inactivated nasal influenza vaccine,

adjuvated with heat labile toxin of *Escherichia coli* (LT), was introduced in Switzerland in 2000. In the 2000 – 2001 influenza season, 46 cases of Bell's palsy, a one-sided paralysis of the face, were reported in the vaccinated population. This temporary condition was probably due to uptake and transport of LT by facial nerves. The use of the vaccine was suspended in early 2001. A case-control study confirmed that vaccination increased the risk of Bell's palsy [56].

The live trivalent influenza vaccine Flumist was approved in the US in 2003 and has some desirable characteristics: efficacy at least as good as parenteral vaccines, few adverse effects and ease of administration. Nevertheless, the initial vaccine was not optimal. The vaccine needed storage at -15ºC, was not registered for the most important age groups, namely young children and the elderly, but for people of 5 – 49 years of age, and it was also expensive (initially $46 per dose). This resulted in modest use. However, the next-generation product, which is stable at 4 – 8ºC, has been available since January 2007. This new vaccine is suitable for young children and has demonstrated superior efficacy compared with inactivated parenteral vaccine in this age group [57]. The vaccine is also suitable for asthmatic children [58].

4.2 Needle-free injection

4.2.1 *Fluid jet injection*

Needle-free injection has a long history. As early as 1866 a jet injector was described in France (see [203] for a history of jet injectors). Its purpose was to inject spring water. In the first half of the 20th century, the procedure was reinvented and used for mass vaccination purposes for 20 – 30 years. These multi-use nozzle jet injectors were developed for US army recruits. Up to 1000 vaccinations per hour could be given. Their use was abolished when it became clear that cross contamination from one vaccinee to another could occur. Today, safe disposable cartridge jet injectors are available. The main advantages are the absence of sharps, the high-throughput, and improved immunogenicity. Clinical studies show consistently that the number of responders and the mean antibody response are comparable or, often, better, compared with needle injection [59,60]. This may be caused by better tissue distribution of the vaccine. Instead of a bolus, the fluid is dispersed more homogeneously. Local adverse effects are either comparable or higher after jet injection, although still mild. Taken together, jet injection may be advantageous in cases of (emergency) mass vaccinations, and in the veterinary field where high-throughput is crucial. A possible solution against local adverse effects is better control of injection depth by improved design of classical jet injectors or the use of pulsed microjets [61]. A piezoelectric pulse generator drives a piston, delivering 2 – 15 nl fluid per stroke through a micronozzle. At a frequency of 1 Hz, ~ 1 µl/min can be delivered to the skin. Due to the small volume per pump cycle, the injection depth is only 200 – 400 µm (i.e., true dermal delivery is easier to achieve). This may reduce or prevent pain, bleeding and other local adverse effects sometimes seen after 'conventional' jet injection. Delivery of larger volumes may be achieved by the use of nozzle arrays and increased piston frequency. These improved designs may also be suitable for standard vaccination.

4.2.2 *Needle-free injection of solids*

The needle-free injection of solids is also possible; best known is powder injection [62-64]. This approach shows promise with respect to DNA vaccination. Protein-containing powders are suitable but require extensive (re)formulation work [65]. Uniform dosing is difficult, as relatively small differences in particle size results in large differences in kinetic energy and, as a result, in penetration depth. Even in the case of monodisperse particles, it is difficult to control particle deposition. Improvements in the design of the injection device may lead to a more uniform penetration [66].

Solids can also be injected as monolithic formulations, circumventing the problem of particle size differences. The biodegradable implant contains the antigen and is injected by air pressure or a released spring. The implant dissolves and the vaccine is released. Although in its infancy, this approach holds promise because of several advantages such as the absence of sharps, the expected absence of adverse effects, including pain, due to the high velocity of the implant, the small volume of the implant and the high thermostability. Tetanus toxoid has been formulated in implants called bioneedles (**Figure 1**) and appears to be stable at 60ºC for 1 week (Hirschberg *et al.*, unpublished data). Loss of the cold chain and limited space requirements are valuable assets in both developing countries, as well as during emergency mass vaccinations.

4.3 Dermal application

The skin is one of the most attractive locations for immunisation. The epidermis – the upper 300 µm of the skin – is well populated with Langerhans cells, which are the prime antigen-presenting cells in the skin. These cells process antigen or microorganisms that managed to pass the stratum corneum, the upper 15 µm of the epidermis. The stratum corneum consists of corneocytes composed mainly of keratin. The corneocytes are embedded in a lipidic 'mortar' of ceramides, fatty acids and cholesterol. When intact, the stratum corneum is impermeable for microorganisms, macromolecules and even for many small molecules. Therefore, the main objective in dermal vaccination is to get past the stratum corneum. An advantage of the epidermis is that it does not contain nerves. Puncturing or otherwise damaging the epidermis is painless. Pain-sensing nerves end in the dermis, located under the epidermis.

Dermal vaccination is efficient compared with other immunisation routes (i.e., once the antigen has passed the stratum corneum). Intradermal injection of a vaccine generally leads to higher immune responses than subcutaneous or intramuscular immunisation [67]. Work by Mikszta and co-workers suggests that the kinetics of the response after dermal application is different [68]. The dermal application of

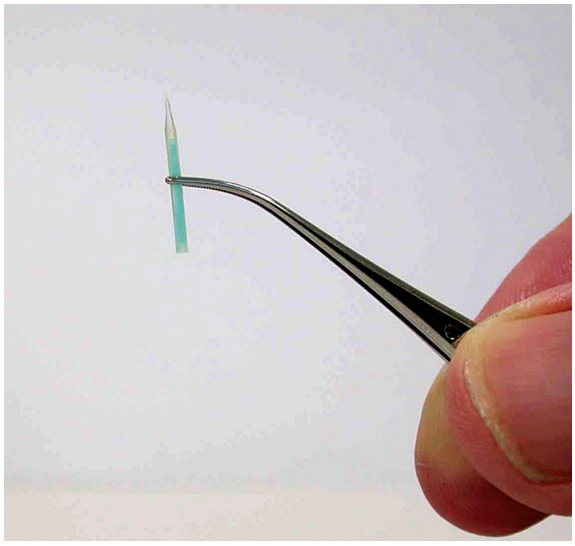

Figure 1. A biodegradable minineedle for subcutaneous or intramuscular delivery. The implant contains freeze-dried vaccine and is applied by air pressure. Picture by courtesy of G van de Wijdeven, Bioneedles Group.

anthrax protective antigen resulted in more potent early antibody responses compared with intramuscular injection, especially when low antigen doses were given. This may be beneficial in situations of emergency vaccinations. The differences became less pronounced longer after vaccination. Dermal vaccination by classical injection cannot be applied routinely because true dermal injection is difficult to perform and more painful than subcutaneous or intramuscular injection, although this may be related to the skills and experience of the vaccinating personnel. Auewarakul *et al.* found similar pain sensations in an intradermal group and a group receiving intramuscular influenza vaccine injection [69].

Many approaches are being investigated to deliver antigens to the skin (**Table 2**), and some of them are discussed below in more detail. Ongoing efforts are directed to increasing the efficiency of delivery.

4.3.1 *Non-invasive dermal delivery*

Some proteins, notably ADP-ribosylating exotoxins such as LT and cholera toxin (B subunit), are able to induce potent immune responses against themselves and are strong mucosal adjuvants. Glenn and co-workers demonstrated that the application of a patch containing LT to the skin leads to potent immune responses in humans [70]. Phase II clinical studies with LT patches against enterotoxigenic *E. coli*-induced travellers diarrhoea are promising [71]. The mechanism of action of the adjuvant activity is far from clear. Are these proteins better able to pass the stratum corneum or do they act as powerful adjuvants? The latter may be the case. The presence of ribosyltransferase activity, is important and the topical application of LT leads to the maturation of Langerhans cells [72]. Although only minute amounts of proteins are delivered into the epidermis, this is sufficient to induce immune responses. The adjuvant potential of ADP-ribosylating exotoxins via topical administration has been developed further by the Iomai Corporation [73]. LT it is available as purified recombinant *E. coli* [74] or plant-expressed material [75]. The dose of an injected vaccine can be lowered by combining the injection with a skin patch containing the adjuvant. This dose-sparing approach has the advantage that no reformulation of the existing vaccine is needed. The adjuvant patch and the antigen injection need to target the same draining lymph node [73]. The delivery of antigens other than LT and cholera toxin via a patch is still difficult. In the case of LT, pretreatment of the skin with an abrasive pad improves the immunogenicity of skin-applied antigen to levels higher than after a challenge with enterotoxigenic *E. coli*, as a clinical study has shown [71].

Non-invasive methods to increase the efficiency of delivery include the use of elastic vesicles and other penetration enhancers, iontophoresis and ultrasound.

Penetration enhancers are mostly amphiphilic molecules such as surfactants and fatty acids. The mechanism of action of some types of elastic vesicles (see below), consisting of surfactants, may be the adsorption-enhancing capabilities. Other adsorption enhancers are less suitable in combination with large molecules (typically > 500 Da). Certain peptides containing so-called protein transduction domains that facilitate transport across cell membranes also facilitate transdermal transport. This requires association between the transporter peptide and the cargo. A recently discovered peptide may possibly provide new opportunities [76]. A cyclic 11-mer peptide has been shown to facilitate the transport of insulin to the circulation without the need of association. The mechanism is yet unclear. Transport via hair follicles seems to play a role, making its applicability in humans perhaps less interesting.

Elastic vesicles are liposome-like structures consisting of surfactants alone or in combination with phospholipids with a low transition temperature. Due to the high bilayer fluidity and/or presence of destabilising micelle-forming surfactants, they are ultradeformable. Applied to the skin, they are able to penetrate the stratum corneum, possibly via channel-like imperfections in the stratum corneum [77]. Antigens formulated in elastic vesicles can induce potent immune responses in experimental animals [78,79]. The mechanism of action, apart from the abovementioned adsorption enhancement is thought to be movement from the skin surface into the epidermis via a transepidermal osmotic gradient [9,80]. Deformable, liquid-state vesicles will diffuse into the skin, especially when the vesicles are applied in a non-occlusive manner. Occlusive application on the other hand does not lead to penetration of intact vesicles, but lipid plaques are formed in the stratum corneum [77]. This may be enough for the immunisation purposes. Physical association of the antigen and vesicle will make the process more efficient,

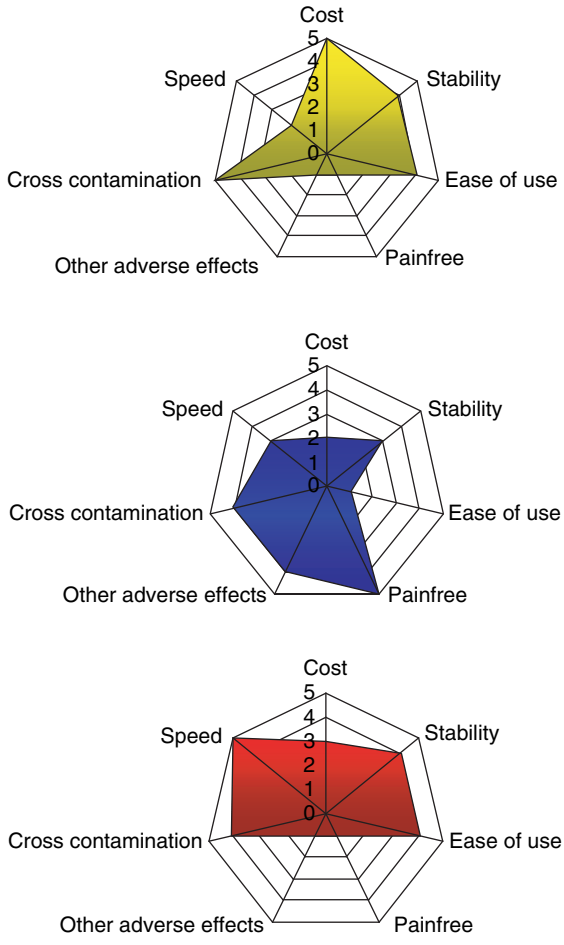

Figure 2. The product characteristics of needle-free vaccine delivery systems for developing countries (top), industrialised countries (middle) and emergency vaccinations (bottom). Scale: 0 less important, 5 important.

although mixing the antigen and vesicle (which may result in unnoticed association) also can result in potent immune responses [79]. Association of antigen to the delivery vehicle may affect the elastic properties of the vesicles, reducing transport ability, although Mishra *et al.* achieved extraordinary results with hepatitis B surface antigen associated with optimised elastic vesicles. Immune responses in mice were comparable to parenterally given equal doses of alum-adsorbed antigen. This indicates very efficient transport into the dermis, which is in accordance with *in vitro* transport efficiency of > 60% [79]. It is unknown what happens with antigen association after application, when pH and ionic strength may be different compared with the formulated material.

Other non-invasive skin vaccination techniques such as electroporation and the use of ultrasound are, at least in combination with macromolecues, still immature techniques. A disadvantage in the case of vaccines is the hardware for the energy supply. External supplies should work quickly (a few seconds per vaccination) to make them acceptable for vaccination purposes.

4.3.2 *Minimally invasive skin delivery*

Piercing or abrasion of the stratum corneum can facilitate entrance of antigens to the epidermis by several orders of magnitude. If the damage is restricted to the stratum corneum, no pain will be perceived. The feasibility of the concept, using microstructures, has been proven by several research groups (**Table 2**). Three approaches are being followed: abrasive blunt microstructures, solid microneedles and hollow microneedles. These approaches will be discussed below.

Apart from statum corneum disruption as pretreatment, followed by application of antigen [71], abrasive, blunt microstructures have been described that were coated with DNA [81]. The device is wiped over the skin, resulting in genetic immunisation.

Solid microneedles that pierce the stratum corneum are used prior to vaccine application. The vaccine enters the skin passively via diffusion. In other cases, the needles are coated with the vaccine. Relatively simple dip coating procedures onto stainless steel microneedles have been described [82]. A variety of materials, from proteins to microparticles, could be coated in a reproducible manner and released into cadaver skin in a quantitative manner. Matriano *et al.* performed an interesting study in guinea-pigs comparing intramuscular, subcutaneous, intradermal injection and intradermal delivery with coated microneedles [67]. The microneedle system was, together with intradermal injection, superior to subcutaneous and intramuscular immunisation. How massive microneedles perform in humans remains to be seen.

Solid non-coated microneedles used for pretreatment of the skin, are not very efficient with respect to transport capacity: most of the antigen applied to pierced skin will not enter it. Hollow needles with an active injection system would substantially reduce the amount of vaccine that needs to be applied to the skin. Although these systems are under development and prototypes have been described [83], this is a technically demanding task. Piercing must be reproducible and even. If some needles in the array are blocked, the fluid will leak at the back. If a needle does not pierce, most of the fluid will leave the device via this needle because there is low back pressure, unless each needle has a separate reservoir. Due to the small tip opening, high pressure is also needed to inject. This increases the chance of leakage. After injection, backflush must be minimal. In this respect, the approach by Becton Dickinson to develop a dermal injection device consisting of only one needle is sensible [68,84].

5. Conclusions

The number of registered vaccines that are applied via other routes than classical injections is still very limited. Until about a decade ago, vaccine manufacturers solved the problems associated with needle and syringe application via relatively

straightforward approaches such as the development of more and more complex combination vaccines and the design of single use or autodisable syringes. With the availablity of an ever-increasing number of vaccines and the need for easy, painless, fast and safe administration techniques, many alternatives are under development, and impressive progress has been made in many areas of needle-free vaccine delivery. The results of clinical studies indicate that nasal, oral, dermal and needle-free injection can be safe and result in strong immune responses. It is not possible to predict those approaches or products that will make it to the market. Both successful and failed attempts to develop oral rotavirus vaccines and nasal influenza vaccines have demonstrated that success and failure are separated by a thin line.

6. Expert opinion

Today, most human vaccines are given by subcutaneous or intramuscular injection. Of all the vaccines against infectious diseases, estimated to be 25 – 30, there are four oral vaccines (OPV, typhus, rotavirus vaccine and cholera), one dermal (smallpox) and one nasal formulation (influenza). All except smallpox and rotavirus vaccines are also available as injectable formulations. Despite the fact that the first-line of immune defence is at the surface of the body (the skin) and at mucosal tissues, we apply vaccines at places where the immune system is very 'dilute': in muscles and under the skin. This is done with needle injections, a technique that is not without risk or discomfort. The reason for the use of this method is because the skin and mucosal sites have disadvantages for antigen delivery. The mucosal immune system encounters many antigens that are non-self, but still harmless (e.g., food antigens, other non-infectious antigens and non-pathogenic microorganisms). As a result, the level of immunological tolerance is high. The immune system in the skin is less tolerant but the stratum corneum forms a physical barrier that is difficult to penetrate. Despite these difficulties, we think that it is inevitable that the number of marketed needle-free vaccines will increase. However, for this to happen, numerous challenges have to be overcome, as summarised below.

6.1 Scientific advancement

Progress in immunology has been huge in the last decades. Our knowledge of the innate immune system, T-cell regulation, immunological memory and mechanistic aspects of adjuvant action has grown rapidly. Most of this work concerns the systemic immune system (i.e., immune cells, cytokines and antibodies present in lymphoid tissues, blood and bone marrow). Our knowledge with regard to immunological events in the skin and to a lesser extent in the mucosal immune system is still quite limited. The immune system of the skin seems to have hybrid properties: it may serve both the systemic and secretory immune compartments. Indications for this are the ability to induce secretory immune responses after skin vaccination and the successful application of mucosal adjuvants. Our limited knowledge is caused by the difficult access to these parts of the immune system and the absence of suitable animal models, especially to perform skin immunisation studies. These are difficult to perform because the skin of small laboratory animals is much thinner compared with human skin. Immunisation devices such as microneedle arrays need optimisation depending on the species for which they are intended, because needles that penetrate human skin may deposit antigen too deep for true intradermal delivery in, for instance, mice.

Another field where research efforts are needed in order to accelerate the development of needle-free vaccines is mucosal and skin adjuvant research. ADP-ribosylated exotoxins are potent adjuvants, not only via mucosal entry, but also via the dermal route. Relatively little is known about the mechanism of action when these adjuvants are applied to the skin, but also the effects in different mucosal sites (e.g., nasal, oral, urogenital). Rational adjuvant design – something that has been happening for parenteral vaccines for some years now – is still in its infancy. The same holds true for assessment of the safety of adjuvants given via new routes.

6.2 Technological advancement

This refers mostly to devices and methods to immunise via the skin. The availability of pain-free devices for the delivery of relatively large amounts of antigen in the skin is mandatory for successful skin immunisation. With regard to oral immunisation – and to a lesser extent nasal immunisation – targeting devices, absorption-promoting and antigen-stabilising methods are needed unless safe and efficacious live vectors are developed. These formulations and devices need to be relatively cheap.

6.3 Costs

Vaccine prices vary greatly, but are cheap compared with other biopharmaceuticals. Basic pediatric vaccines such as oral polio vaccine, diphtheria–tetanus–whole cell pertussis vaccine and measles vaccine cost $0.05 – 0.30 per dose. These are multidose vials for use in low-priced markets. At the other end of the spectrum are proprietary paediatrics, such as bacterial conjugates, Human papilloma virus and rotavirus vaccines. These vaccines cost $35 – 60 per dose. Production costs of vaccines are orders of magnitude lower: $0.05 – 4.0 per dose. Approximately 60% of the production costs are fixed costs, leaving $0.03 – 2.40 for production, testing and fill and finish. So if a needle-free formulation is introduced, the maximal costs of that formulation or device will depend on the type of vaccine: for existing vaccines the price should not exceed existing formulation costs, which are $0.01 – 0.60 per dose. Devices for new vaccines with high market prices may cost more because the formulation costs hardly add to the total development costs.

6.4 Markets and applications

The ideal vaccine should be cheap, stable at elevated temperatures, easy and reliable to apply, painless and free of other adverse effects, exclude cross contamination, and enable fast vaccination (i.e., the number of vaccines per unit

of time). As this is realistically speaking not possible, the requirements for needle-free devices should be selected on their intended use (**Figure 2**). The main differentiating factors are low costs, absence of pain and logistical factors such as ease of application and stability.

6.5 Registration strategy

Reformulation of an existing vaccine into a new device is considered as a new product, which means that at least part of the clinical studies have to be repeated. Efficacy studies are in those cases mostly not possible. Bridging studies with existing vaccines, demonstrating non-inferiority, can sometimes be applied. A prerequisite is that some correlate of protection is available as well as excellent *in vitro* antigen characterisation methods to demonstrate comparability.

Entirely new vaccines have to go through the entire clinical phase. Vaccine developers are reluctant to enter this costly stage. This again stresses the importance of the presence of correlates of protection such as relevant animal models or serological parameters in the blood of vaccinees. The former allows finetuning of formulations in the preclinical stage, the latter allows smaller efficacy studies because the end point is not (prevention of) disease but a relevant immune response, such as the induction of neutralising antibodies or a T-cell response.

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