Vaccine Delivery Systems: Roles, Challenges and Recent Advances

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Abstract

The overwhelming majority of vaccine antigens are biological macromolecules, such as proteins and polysaccharides, typically with a molecular weight greater than 10,000. As such, they need to be delivered to the body in the correct conformation in order to elicit the desired immune response and to effectively target the immune cells. Currently, most vaccines are administered parenterally via the intradermal, subcutaneous or intramuscular route, the choice largely dependent on whether

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the antigen is in the adsorbed or nonadsorbed state. However, these routes have major drawbacks, including pain associated with the use of needles, the potential for needle contamination, practicalities of needle disposal and the need for a primary healthcare worker. There is now a particular focus on the development of mucosal vaccines, designed for direct application to mucosal surfaces such as those present in the mouth, nose, vagina and rectum.

Often, simple antigen solutions are immunologically ineffective when delivered by these mucosal routes, owing to difficulties associated with mucosal retention and uptake. A diverse range of formulation strategies, including microspheres, liposomes, nanoparticles and virus-like particles, are now being actively investigated. In addition to the design and selection of the antigen candidate, the choice and preparation of the antigen delivery system is crucial to achieve the end goal of vaccination. In this chapter, an overview of the role and considerations for the design of vaccine delivery systems is presented, with particular focus on the challenges and recent advances in the field of colloidal and nano-sized delivery systems.

46.1 Introduction

In the current age of molecularly defined vaccines, the necessary focus on ensuring product safety has inevitably impinged upon clinical efficacy [1]. Most antigen candidates are biological macromolecules and therefore highly susceptible to inactivation, loss of conformation and poor absorption by the peroral route. As a result, most vaccines are administered parenterally by needle injection. These problems are further compounded by the fact that antigens are extremely prone to physical, chemical and conformational degradation resulting in vaccine inefficacy [2]. Current vaccination requires the need for trained medical personnel for the administration of parenteral vaccines and for the disposal of used needles and syringes [3]. It is likely that induction of strong, antigen-specific mucosal immune responses will play a critical role in developing vaccines for diseases such as HIV/AIDS. Achieving this goal may necessitate antigen dosing at mucosal sites, with particular measures to overcome the challenges associated with local degradation [4, 5] and retention (e.g. in the nose and vagina). To this end, there is substantial scope globally for the development of accessible and affordable vaccine delivery strategies that maintain or enhance the delivery and efficacy of a given vaccine.

46.2 Roles of Vaccine Delivery Systems

A wide range of advanced drug delivery systems have been developed to overcome the various problems and obstacles associated with more conventional drug delivery methods. Table 46.1 provides an overview of the main formulation approaches adopted for the efficient delivery of drug molecules.

Table 46.1 Roles of drug delivery systems

Role of delivery system	Examples
Protection against chemical and physical degradation, improved shelf life and elimination of cold chain	Freeze-dried delivery systems/spray-dried vaccines [6]
Protection against (chemical/enzymatic) degradation at mucosal sites	Microparticles/nanoparticles [7]
Improved efficacy by immune system modulation	Liposomes [8, 9], nanoparticles [10, 11]
Improved efficiency through sustained release	Poly(lactide-co-glycolide) microparticles [12]
Obviation of the need for trained medical personnel	Microneedles [13]
Remove the need for special disposal of needles/syringes	Dissolving microneedles [14, 15]
Improved mucosal retention	Vaginal gels [16], nasal gels [17], vaginal rods [18, 19]

46.3 Important Considerations and Challenges for the Design of Vaccine Delivery Systems

46.3.1 The Conformation of an Antigen Needs to Be Protected

The preparation process of vaccine delivery systems or constituent excipients of vaccine delivery formulations may cause physical or chemical changes to the vaccine that result in loss of or altered activity [2]. The final activity of the dosage form must be adequately assessed by various immunological and in vivo techniques. The ultimate aim should be stabilisation to such an extent that the need for cold-chain transport is eliminated.

46.3.2 The Vaccine Delivery Rate Should Be Adequately Evaluated

Many delivery systems are designed to deliver the vaccine over a prolonged period of time. However, it is known that prolonged or repeated administration of some vaccine candidates can lead to the development of tolerance [20, 21]. This aspect should be carefully evaluated.

46.3.3 The Adjuvant Component Is Often as Important as the Antigen Itself

Adjuvants are useful, and sometimes essential, in potentiating an immune response. Thus, adjuvant formulation, dose and release rate should be given equal consideration and may need to be correlated to that of the accompanying antigens.

46.3.4 Dosage Form Design Must Aim to Eliminate the Need for Trained Personnel for Administration of the Vaccine

Ideally a vaccine intended for quick and effective mass immunisation should be able to be administered without the need for trained personnel. This would facilitate global and equitable access to the vaccine.

46.4 Concepts in Designing Delivery Systems for Vaccines

Clinical experience favours use of subunit vaccines as a safer alternative to traditional organism-based vaccines, despite their often impaired immunogenicity. Use of vaccine delivery systems may overcome this compromise.

46.4.1 Particulate Antigens Are More Potent Compared to Soluble Antigens

One advantage of particulate vaccines over soluble antigens arises from their facilitated uptake by antigen-presenting cells (APCs). Antigens associated with particles mimic the particulate nature of pathogens. For example, particulate vaccines are typically a few hundred nanometres to a few microns in size, dimensions comparable to those of common pathogens against which the immune system has evolved to react, and promoting efficient uptake by APCs. Internalisation of particulate vaccines into phagosomes through the mechanism of phagocytosis has important consequences since phagosomes are known to be competent organelles for antigen cross-presentation [22]. This makes particulate vaccines attractive for inducing cellular immune responses and in contrast to soluble antigens which are preferentially presented by the MHC class-II pathway and only poorly cross-presented. Other attractive features of particulate antigens include (i) the possibility to deliver relatively large quantities of particle-associated antigen inside the APCs, (ii) a prolonged intracellular [23] or extracellular [24] release leading to prolonged antigen presentation compared with soluble antigen, and (iii) concomitant delivery of antigen and immunostimulatory components to the same APC [25].

Table 46.2 Delivery systems with inherent adjuvanticity that have been tested in humans

Delivery systems	Composition (mechanism of action)	Disease (antigen)
Liposomes	One or several bilayers of phospholipids	Influenza (monovalent split)
Liposomes + MTP-PE	Liposomes that incorporate the synthetic lipid MTP-PE	HIV (gp120)
Virosomes	Liposomes that incorporate in their membrane viral fusion proteins.	HAV, influenza, DT, TT
ISCOMS	Micellar assemblies made of saponin Quil-A, cholesterol and phospholipids	Influenza (trivalent split), HPV16 (E6/E7), Helicobacter pylori
PLGA microparticles	Particles made of homo- and copolymers or lactic and glycolic acids.	TT, HIV
MF59®	Squalene/water emulsion stabilised with Span85 and Polysorbate 80	Influenza (trivalent split), HBV, HSV-2 (gB + gD), HIV-1(gp120), CMV(gB)
SBAS-2	Squalene/water emulsion that incorporate MPL® and QS21®	Malaria (RTS, S), HIV-1 (gp 120)
SBAS-4	Alum gel with MPL®	HBV (HBsAg), HSV
Incomplete Freund adjuvant	Water/Drakeol emulsion stabilised with mannide monooleate	HIV-1, Melanoma (gp100)
Montanide ISA720	Emulsion with a metabolizable oil	Malaria (MSP1, MSP2)
Detox®	Squalene/water emulsion that incorporate MPL® and CWS	Malaria (R32NS18), Melanoma cell lysates

Abbreviations: *CMV* cytomegalovirus, *CWS* cell wall skeleton from Mycobacterium phlei, *DT* diphtheria toxoid, *HAV* hepatitis A virus, *HBV* hepatitis B virus, *HPV* human papilloma virus, *HSV* herpes simplex virus, *MPL* monophosphoryl lipid, *MTP-PE* muramyl tripeptide dipalmitoyl phosphatidyl ethanolamine, *PLGA* poly-(D,L)-lactide-co-glycolic acid, *TT* tetanus toxoid

46.4.2 Particulate Delivery Systems Have Inherent Adjuvant Action

There are inherent safety concerns associated with traditional vaccines based upon killed, live-attenuated microorganisms or attenuated toxins. For some pathogens (i.e. tuberculosis, HIV), these types of vaccines are not available [26]. Nowadays, there is increasing interest in vaccines based on proteins, peptides or antigen-expressing DNA or RNA. Safer than the use of whole micro-organisms, they are, however, poorly immunogenic and require the use of adjuvants [27]. Examples of particulate delivery systems with inherent adjuvanticity that have been tested in humans are summarised in Table 46.2. Adjuvants can enhance specific immune response against the co-administered antigen by two major mechanisms [27]. (i) The particulate delivery system increases the uptake of antigen by APC by either they are directly engulfed by APC or they form a depot of antigen that prolongs exposure thus increasing the chance of the antigen to be uptaken by APC. (ii) The particulate delivery system acts as an immunopotentiator (e.g. cytokines) type of adjuvant directly activating innate immune cells.

46.5 Designing Antigen Delivery Systems

Pharmaceutical vaccine formulations that are presently being tested in various experimental and clinical models are generally particulate in nature and obtained by aggregation/cross-linking of antigen [28] or by adsorption or precipitation of antigen on aluminium salts. Alternatively, the antigen can be chemically attached to a pre-formed particular carrier [29] or chemically or physically distributed in or on particles in a more (in liposomes and virus-like particles) or less (in polymeric microspheres) organised way [29, 30]. Typically, any type of particle facilitates the recognition of the vaccine by professional APC as well as the uptake of the vaccine into these cells. Prior to APC uptake, the formulation itself may influence the properties of the recruited

Table 46.3 Various factors affecting uptake of polymeric particles

Particle size	Animal species used for evaluation
Particle hydrophobicity	Age of the animal
Dose of particle (antigen dose)	Fed state of the animal
Administration vehicle	Mucosal layer characteristics
Polymer composition	Use of targeting agent on the particles
Effect of additives	Method for the quantitation for the extent of uptake
Particle surface charge	

phagocytic cells. The design of the vaccine delivery system may also influence other critical factors, as discussed below.

46.5.1 Transport of Antigen Across a Biological Barrier

Vaccine delivery systems should improve antigen passage through relevant biological barriers, such as the intestinal and nasal mucosa. Based on observations over the past few decades, it has been noted that smaller particle sizes generally enhance the ability of particles to transport antigens across the intestinal barrier [31]. The ideal size for a mucosal vaccine carrier would be within the 50–500 nm range, although other factors that affect particle uptake are summarised in Table 46.3 [7].

46.5.2 Biodistribution of the Antigen Delivery System and Their APC-Targeting Potential

The size of the polymeric particles influences their distribution after subcutaneous, intradermal or intramuscular administration. When administered by the intramuscular or subcutaneous route, particles with size 20–100 nm can penetrate the extracellular matrix and enter directly into the lymphatic vessels. Once in the lymph, the particles travel to the lymphatic nodes where they are captured by the dense population of immune cells, mostly by dendritic cells, and generate effective immune responses [32]. In addition to the critical role of the particle size, it has been observed that the uptake of nanoparticles by macrophages and dendritic cells can be strongly enhanced if the particles have a cationic surface [33].

46.5.3 Antigen Stability in the Delivery System

Vaccine delivery systems may offer the advantage of antigen protection from harsh physiological conditions. However, formulation development conditions are of critical importance since the use of organic solvents, extreme temperatures or high energy inputs can also degrade or aggregate the antigen. Also, the materials used in the fabrication of the delivery systems or their degradation products can also enhance protein deterioration [34].

46.5.4 Concomitant Delivery of Antigen and Co-stimulatory Molecules

If the vaccine by itself is not capable of stimulating the expression of molecules necessary for T-cell activation, the combined delivery of antigen and co-stimulation factors within the same pharmaceutical formulation may improve the priming of lymphocytes. This concept has been demonstrated with recombinant viral vectors and liposomes, which provided co-stimulatory molecules such as those of the B7 family [35].

46.5.5 Immunomodulating Activities of Polymers

Some polymers can be used as effective protein carriers, and the development of vaccine delivery systems based on liposomes, microspheres, nanoparticles or water-soluble synthetic polymers has received considerable attention, as they can be tailored to meet the specific physical,

chemical and immunogenic requirements of a particular antigen [36]. Antigen delivery systems which assist the acidification of endosomes also promote the MHC class-II presentation of the antigen they are carrying. This might be one mechanism by which nano- and microparticles of poly(lactide-co-glycolide) function. On the other hand, pharmaceutical formulations, which contain basic polymers or excipients (e.g. chitosan, ethylene imine, collagen, anionic lipids and surfactants), may prevent antigen presentation by the MHC class-II pathway. It has been shown that chitosan promotes Th1 cytokine responses and MHC class-I antigen presentation [37].

46.5.6 Antigen Dose and Structure Affect the Type of Immunity Induced

The amount and amino acid sequence of the antigen that initiates the response also influence the differentiation of CD4 T cells into distinct effector subsets, with high and low density of peptide on the surface of APCs stimulating Th1 or Th2 cell responses, respectively [38]. Hence, when the stability of the antigen is compromised, as may occur in poly(lactide-co-glycolide) microspheres [39], this might also have consequences for the Th1/Th2 skewing of the immune response, since the relative accessibility of different epitopes may have changed. Moreover, peptides that interact strongly with the T-cell receptor tend to stimulate Th1-like responses, whereas peptides that bind weakly tend to stimulate Th2-like responses [38].

46.6 Advanced Vaccine Delivery Systems

46.6.1 Liposomes

Liposomes consist of one or more phospholipid bilayers enclosing an aqueous phase. Antigens can be encapsulated within the aqueous compartment, linked to the liposomal surface or embedded in the lipid bilayer, all of which can protect the antigen from the surroundings. Variations in size, composition and physicochemical characteristics can render liposomes a versatile platform for antigen delivery. Immunostimulatory properties of liposomes are supposed to arise from (i) their capacity to associate and release antigens over a prolonged time and (ii) their preferential internalisation by APCs. Thus, this higher immunoavailability of the antigen might promote the maturation and antigen presentation by APCs [40]. In liquid form, liposomes have limited stability and tend to aggregate and fuse together, an issue that can be solved to some extent through lyophilization. Among the innumerable types of liposomal vaccine formulations studied over the past few decades, cationic liposomes appear to be particularly immunogenic. For example, liposomes made of dimethyldioctadecylammonium and the immune-modulating glycolipid trehalose dibehenate efficiently promoted the cell-mediated and the humoral immune responses and are currently being evaluated in a Phase I study for the management of tuberculosis [41].

46.6.2 Polymeric Microparticles/Nanoparticles

Microparticles were first used as delivery systems for entrapped antigens in the early 1990s [42]. Owing to a long history in medical applications, the biodegradable poly(D,L-lactide) and poly(D,L-lactic-co-glycolic acid) are probably the most studied materials for parenteral and mucosal antigen delivery [43]. Long-lasting immunity can be induced by the parenteral administration of microparticles made with different ratios of polymers of different molecular weights that hydrolyse over a wide range of times. Long-lasting immunity can also be induced by using particles of mixed sizes, especially larger particles that avoid uptake by macrophages and hence break down at a slower rate [44]. In this manner, one injection of a vaccine can result in long-lasting immunity obviating the need to boost. Besides the encouraging immunological performances of poly(lactide-co-glycolide) (PLGA) based particles, the ensuring stability of

encapsulated protein antigens has been found to be an important issue. Indeed, some protein antigens tend to aggregate or degrade upon entrapment into PLGA or during release from the matrix. The exposure of antigen to organic solvent and acidic microenvironment generated during polymer hydrolysis may lead to antigen inactivation. These problems have been partially solved by optimised manufacturing methods or addition of stabilising agents such as $Mg(OH)_2$, other proteins, surfactants or sugars [45, 46].

46.6.3 Virosomes

Virosomes are liposomes containing functional viral membrane proteins and have a particle size that resembles that of the viruses. As such, virosomes represent reconstituted empty influenza virus envelopes where the viral proteins confer immunostimulatory properties [47]. Thus, when they are formulated to carry heterologous antigens, virosomes can be considered as delivery systems with intrinsic adjuvant activity. To date, virosomes have been approved in Europe for systemic vaccination against hepatitis A and influenza, and they have also been formulated for intranasal immunisation [48].

46.6.4 Immunostimulatory Complexes (ISCOMS)

ISCOMS are characterised by a cagelike structure that incorporates the antigen. They consist of lipids and Quil-A, the active component of the saponin derived from the plant *Quillaja saponaria* that has adjuvant activity [49]. Hydrophobic antigens can be embedded or anchored directly into the lipidic colloidal domains, whereas hydrophilic antigens require modification for efficient entrapment [50]. An interesting feature of ISCOMs is their good stability under varying conditions. ISCOMs have been evaluated in clinical trials in humans, and as well as being well tolerated, they induced strong humoral and cytotoxic T lymphocyte (CTL) responses even at very low antigen doses [51]. Despite their potential and good performance in clinical trials, ISCOM-based vaccines have only approved for veterinary use.

46.6.5 Emulsions

The investigation of emulsions as vaccine adjuvants started with Freund's complete adjuvant (a water-in-oil emulsion of a mineral oil, paraffin and killed mycobacteria) [52]. Although capable of generating high antibody titres, this emulsion led to strong adverse reactions which hampered its clinical use. A less toxic version, the Freund's incomplete adjuvant which lacks the mycobacterial component, is still applied in veterinary medicine but has been prohibited for use in human vaccines because of severe adverse events. In the 1990s, a squalene oil-in-water emulsion (MF59) was developed and generated good antibody titres, demonstrating good tolerability and general safety [53]. MF59 is currently approved in Europe for influenza vaccines.

46.6.6 Mucoadhesive Polymer/Gel

Some mucoadhesives have adjuvant properties when injected (e.g. sodium alginate), and others (e.g. sodium carboxymethyl cellulose) have been selected for their aqueous viscosity-enhancing properties when used as depot agents in experimental formulations for parenteral vaccines. Recently, mucoadhesive gels have received considerable attention for vaginal vaccine delivery. From a formulation perspective, inducing effective antigen-specific immune responses by cervicovaginal instillation of buffer solution containing solubilized antigen is far from ideal owing to the potential for leakage at the administration site, rapid enzymatic degradation of the antigen, the influence of the menstrual cycle and inadequate exposure of antigen to the mucosal associated lymphoid tissue. In order to improve the efficacy of vaginal vaccine delivery, various mucoadhesive delivery systems, including hydroxyethyl cellulose-based rheologically structured gel vehicles [16] and lyophilised solid dosage formulations [18, 54], have been investigated.

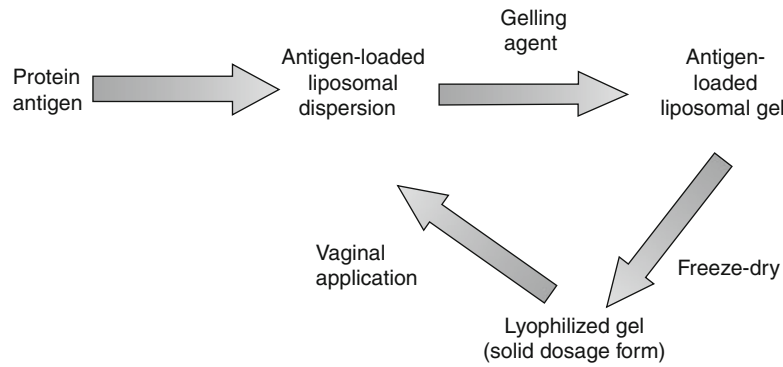

Fig. 46.1 Scheme for the development of lyophilised dosage form for vaginal vaccine administration

Recently, our group reported the development of liposomal gel formulations, and novel lyophilised variants, comprising HIV-1 envelope glycoprotein, CN54gp140, encapsulated within neutral, positively charged or negatively charged liposomes [19]. Scheme for the development of lyophilised dosage form for vaginal vaccine administration is shown in Fig. 46.1. The CN54gp140 liposomes were evaluated for mean vesicle diameter, polydispersity, morphology, zeta potential and antigen encapsulation efficiency before being incorporated into hydroxyethyl cellulose (HEC) aqueous gel and subsequently lyophilised to produce a rod-shaped solid dosage form for practical vaginal application. The lyophilised liposome–HEC rods were evaluated for moisture content and redispersibility in simulated vaginal fluid. Since these rods are designed to revert to gel form following intravaginal application, mucoadhesive, mechanical (compressibility and hardness) and rheological properties of the reformed gels were evaluated. The liposomes exhibited good encapsulation efficiency and the gels demonstrated suitable mucoadhesive strength. The freeze-dried liposome–HEC formulations represent a novel formulation strategy that could offer potential as stable and practical dosage form.

Conclusions

Safety concerns associated with traditional vaccine strategies have placed constraints on emerging vaccines that can render them less effective. Integral to overcoming this are vaccine delivery systems, acting not just as simple carriers of vaccines, but as adjuvants and targeting agents offering additional benefits such as needle-free delivery and stabilisation that facilitate wider access. There is huge scope for the development of vaccine delivery systems with uncharted potential to positively impact future global immunisation strategies.

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