Int. J. Adv. Pharm. Biotech., 2018; 4(1): 31-47
doi.org/10.38111/ijapb.20180401004

IJAPB

International Journal of Advances in Pharmacy and Biotechnology
Vol.4, Issue-1, 2018, 31-47
ISSN: 2454-8375
Review Article
Open Access

A POTENT VACCINE DELIVERY SYSTEM: THE PROMISE AND THE CHALLENGE

N. Kanaka Durga Devi*, Vennela Chakka, Reshma Borra, Divya Presingu

KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada

*Corresponding author e-mail: nelluriss@rediffmail.com

Received: 20 February 2018 Revised: 06 March 2018 Accepted: 12 March 2018

ABSTRACT:
A vaccine typically contains an agent that resembles a live attenuated or killed pathogen, its toxins or surface proteins. It stimulates the body's immunity to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can Successfully defend the body from later encounters. Vaccines are now being delivered through carriers, adjuvants to enhance the recipient's immunological response to a supplied antigen, while keeping the injected foreign material to a minimum. this eliminates the need for booster doses. Adjuvants can act as a depot for the antigen thus leading to their sustained release and targeting. This paper reviews liposomes, microspheres, nanoparticles, dendrimers, micellar systems, ISCOMs, plant-derived viruses and needle-free technologies used to administer the vaccines.

Key words: *Adjuvants, liposomes, ISCOMS, plant derived vaccines, micro needles, needle free delivery systems.*

1. INTRODUCTION

Vaccines take advantage of our body's innate ability to eliminate almost any disease-causing pathogen. Our body has an immunological memory that "remembers" how to protect itself from the microbes it has encountered before (Fig.1) [1]. Collectively, the parts of our body that repel microbial invasion are called the immune system.

Traditional vaccines contain either parts of microbes or whole microbes that have been killed or attenuated so that they don't cause disease. When our immune system confronts these harmless versions of the germs, it quickly clears them from our body at the same time acquires an immunological memory that helps in defending against future infections.

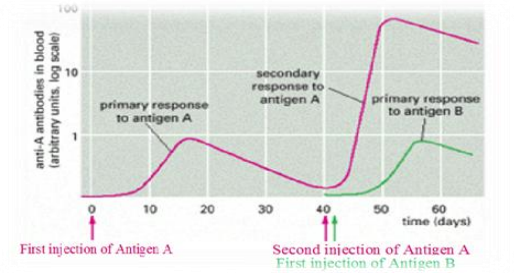

Fig. 1: Working of vaccines

Now-a-days toxoid vaccines, subunit vaccines, conjugate vaccines, DNA vaccines, recombinant vector vaccines are also being used to treat HIV, rabies, measles, influenza, hepatitis, herpes etc. Subunit vaccines include

only the antigen that best stimulates the immune system. Vaccines also use epitopes; the very specific parts of the antigen that antibodies or T cells recognize and bind to. Subunit vaccines can be made in one of two ways:

- Growing the microbe in laboratory and then breaking it apart to gather the important antigens
- Manufacturing the antigen molecules from the microbe using recombinant DNA technology (recombinant subunit vaccines).

Vaccines generally require the addition of an adjuvant which boosts the potency and longevity of specific immune responses to antigens. This reduces the number of immunizations required and improves the efficacy of vaccines in immune compressed individuals, newborns or the elderly. Adjuvants are generally divided in to two classes:

- *Vaccine delivery systems* (e.g., emulsions, microparticles, immune-stimulating complexes ISCOMs, liposomes).
- *Immunostimulatory adjuvants:* Conserved molecular patterns of pathogens stimulate immunity as they are identified by pattern recognition receptors like "Toll" receptors located mainly on B-cells, dendritic cells of mammals (e.g., unmethylated CpG containing DNA).

Clinically, the list of approved adjuvants is very limited. For decades, aluminum hydroxide or phosphates (alum) are the only approved adjuvants in the USA [1].

2. VACCINE DELIVERY SYSTEMS

Solid particulates: Solid particulate systems such as microspheres and lipospheres are being exploited for vaccine delivery based on the fact that the intestine is an imperfect barrier to small particulates. Antigens entrapped in such particulates when taken up by M-cells can generate immunity. In general, the particulate carriers as vaccine adjuvants have a number of functions, which include:

- Particulate carriers can serve as an effective antigen delivery system and, thus, facilitate the uptake of antigens by antigen-presenting cells (APCs) such as dendritic cells (DCs) or macrophages [2,3].
- They may serve as a depot for controlled release of antigen which enhances the quality of immune responses.
- They may possess the ability to modulate the type of immune responses.
- They have the ability to protect the integrity of antigens against degradation [4]. This is particularly important in oral vaccine formulations where antigens must be protected from the harsh acidic conditions of the stomach and enzymatic degradation in the GI tract.
- They can potentially cross-present antigen, and antigen cross-presentation is especially important to generate $CD8^+$ T-cell responses against viral infections [5, 6].

Particle size is an important consideration while formulating microparticulate systems; as it influences their uptake and release and hence immune responses. Small (<10 μm) microspheres due to their large surface to mass ratio leads to faster release and increased antigen processing.

Table 1: Representative list of studies summarizing the effects of sizes of particulate adjuvants and the resultant immune responses.

Materials	Particle size (nm)	Route of administration	Immune responses measured	Comments	Ref.
Polystyrene	40–49/93–123	i.d.	IFN-γ, IL-4, IgG1	Production of IgG1 was observed across all size ranges. IFN-γ was significantly higher with particles of 40–49 nm than 93–123-nm, but particles of 93–123 nm gave a higher IL-4 response.	[8]
PVA-grafted PLG PLG	100/500/1500 450–600/1000–3000/6000–32,000	p.o., i.p., s.c.	IgG, IgA $CD8^+$	Antibody titers were higher with particles of 100-nm size when compared with those of 500 nm. Particles of 1500 nm did not induce Ab titers. Particles of sizes less than 450–600 nm induced the strongest immune response	[7] [9]
Chitosan PLA	700–3000 7500–50,000	i.n., i.p.	IgA IgG	Particles of 400 and 1000 nm induced significantly higher IgA responses than particles of 3000 nm. Particles of sizes 7500–15,000 nm gave higher Ab titers than particles of sizes 50,000 nm.	[10] [11]
PLGA	1000/5000	p.o.	IgG	Particles of 1000 nm were observed to give better immune response than particles of 5000 nm in less than 5 weeks of immunization.	[12]

id.: Intradermal; in.: Intranasal; ip.: Intraperitoneal;); p.o.: Oral;
OVA: PLA: Poly(lactic acid); PLG: Poly(D,L-lactide-co-glycolide); PLGA: Poly(lactic-co-glycolic acid

2.1 Nano-microparticles as immune adjuvants

Examples of materials used to prepare nano-microparticles as vaccine-delivery systems include polymers [13], copolymers [14] and lipids [15]. The choice of material in particle preparation depends on factors such as biocompatibility, degradation rate, hydrophilicity or lipophilicity, and polarity. Polymers used are polylactic acid (PLA), polyortho esters and the copolymer polylactic-co-glycolic acid (PLGA), bioeliminable polyethylene glycol[16], and polyphosphazene[17]. Natural polymers such as albumin, gelatin, collagen, chitosan and alginate are also used. The attractiveness of some of these polymers is that they are biodegradable or biocompatible polymers with the US FDA's approval for human use in sutures or in drug-delivery systems [18]. Solid lipid nanoparticles prepared with materials such as emulsifying wax or lecithin-glyceryl monostearate have also been explored [19, 20].

2.2 Liposomal delivery systems

Liposomes-DNA complex is usually termed a lipoplex (Fig.2) [28]. Favorable, stable and small lipoplex particles were produced with the development of the novel liposomal formulation, liposomes/protamine/DNA (LPD).

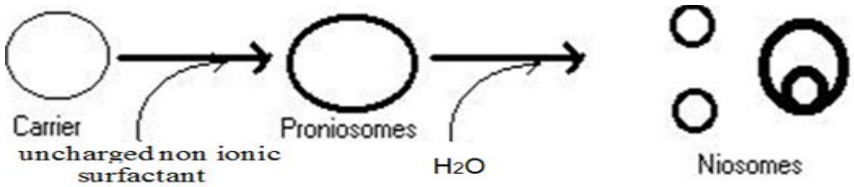

Fig. 2: Niosomes

However, one of the most important drawbacks of these systems is the lack of targeting and nonspecific interaction with cells. If the liposomal nanoparticles (LNs) possess certain properties, they tend to accumulate at sites of disease, such as tumors, where the endothelial layer is 'leaky' and allows extravasation of particles with small diameters. These properties include a diameter centered on 100 nm, a high drug-to-lipid ratio, excellent retention of the encapsulated drug, and a long circulation lifetime (> 6 h). Niosomes are a novel drug delivery system. Niosomes are microscopic lamellar structures, which are formed by the admixture of non-ionic surfactant of alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media.[1]

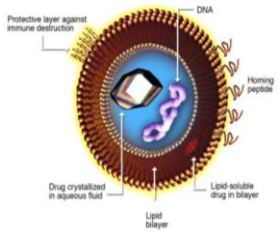

Fig. 3: Formation of niosomes

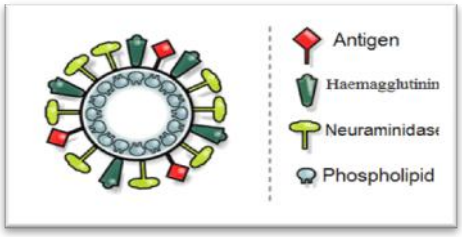

Fig. 4: Virosome: Influenza virus envelope with functional viral envelope of glycoprotein.

2.3 Virosomes

Virosomes are an innovative, broadly applicable adjuvant and carrier system. They are one of only three adjuvant systems approved by regulatory authorities. Virosomes are spherical, unilamellar vesicles with a mean diameter of 150 nm. Essentially, virosomes represent reconstituted empty influenza virus envelopes, devoid of the nucleocapsid including the genetic material of the source virus. In contrast to liposomes, virosomes contain a functional viral envelope of glycoproteins: influenza virus has hemagglutinin (HA) and neuraminidase (NA) intercalated in the phospholipid bilayer membrane (Fig. 4)[30]. Antigens can be incorporated into virosomes, adsorbed to the virosome surface, or integrated into the lipid membrane, either via hydrophobic domains or lipid moieties cross-linked to the antigen.

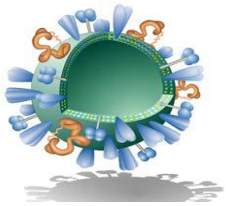

Fig. 5a: PeviPROTM

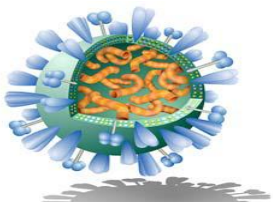

Fig. 5b: PeviTERTM

The nature of the elicited immune response to virosome formulations is dependent on whether the epitopes of the antigen are located on the surface of the virosome (PeviPROTM) (Fig. 5a) [30,31] or inside the virosome (PeviTERTM) (Fig. 5b). PeviPROTM elicits a humoral immune response.

2.4 Nanoparticles as vaccine adjuvants

Polymeric nanoparticles because of their size are preferentially taken up by the mucosa

associated lymphoid tissue. They are extensively reviewed for nasal and oral delivery of vaccines. Limited doses of antigen are sufficient to induce effective immunization. Hence, the use of nanoparticles for oral delivery of antigens is suitable because of their ability to release proteins and to protect them from enzymatic degradation in the GIT.

Polymeric nanoparticles formulated from biodegradable polymers are being widely explored as carriers for controlled delivery of different agents including proteins, peptides, plasmid DNA (pDNA), and low molecular weight compounds. The commonly used biodegradable polymers are aliphatic polyesters such as polylactic acid (PLA), polyglycolic acid (PGA), poly(e-caprolactone) (PCL), polyhydroxybutyrate (PHB) and their copolymers. In particular, poly(lactide-co-glycolide) (PLGA) has been the most extensively investigated polymer for developing nano- and microparticles encapsulating therapeutic drugs in controlled release applications due to their inherent advantages. Nanoparticles range from 10 to 500 nm, while microparticles are larger; around 1–100 μm in diameter.

The encapsulation of antigenic proteins or peptides into PLGA nanoparticle carrier system can be carried out principally through three methods: the water-in-oil-in-water (w/o/w) emulsion technique (Fig. 6), the phase separation method, and spray drying. The w/o/w double emulsion process is popularly used to load proteins into nanoparticles. In this process, an antigen is first dissolved in an aqueous solution, which is then emulsified in an organic solvent to make a primary water-in-oil emulsion. This initial emulsion is further mixed in an emulsifier-containing aqueous solution to make a w/o/w double emulsion. The ensuing removal of the solvent leaves nano- and microparticles in the aqueous continuous phase, making it possible to collect them by filtration or centrifugation.

Antigen-loaded polymeric nanoparticles represent an exciting approach to the enhancement of antigen-specific humoral and cellular immune responses via selective targeting of the antigen to antigen-presenting cells (APCs). Dendritic cells (DCs) are considered to be initiators and modulators of immune responses and are capable of processing antigens through both major histocompatibility complex (MHC) class I and II pathways. Immature DCs encounter pathogens (e.g., virus or bacteria), antigens, or particulate materials at the injection site and, after phagocytosis, the foreign bodies taken up into the DCs present antigens on MHC class II molecules or even on MHC class I molecules by cross-priming. Therefore, the antigen delivery to DCs is of key importance in the development of effective vaccines.

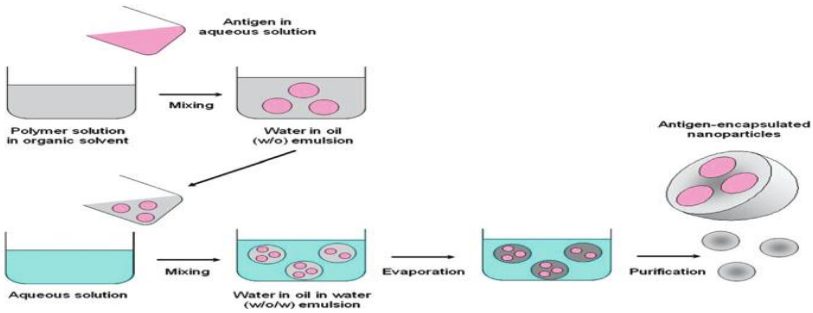

Fig. 6: Water-in-oil-in-water (w/o/w) emulsion technique

2.5 ISCOMS-Immunostimulatory Complexes

Immune stimulating complexes (ISCOMs) are spherical open cage-like structures (typically 40 nm in diameter) that are spontaneously formed when mixing together cholesterol, phospholipids and Quillaia saponins under a specific stoichiometry. ISCOMs combine certain aspects of virus particles such as their size and orientation of surface proteins, with the powerful immunostimulatory activity of saponins. Unlike other vaccine adjuvants, ISCOMs have shown to promote a broad immune response by simultaneously promoting high levels of antibody and strong T cell responses, including enhanced cytokine secretion and activation of cytotoxic T lymphocyte responses in a variety of experimental animal models and have now progressed to phase I and II human trials. ISCOM-based veterinary vaccine against equine influenza is commercially available.

2.6 DNA Vaccines

DNA-based immunization has been promoted as a new approach to prime specific humoral and cellular immune responses to protein antigens [21]. In mouse models, DNA vaccines have been successfully directed against a wide variety of tumors. DNA vaccines consist of bacterial plasmids into which specific sequences are incorporated. Gene expression is promoted by the cytomegalovirus promoter and its adjacent intron A sequence and elements like a transcription termination signal and a prokaryotic antibiotic resistance gene. Nowadays, two basic strategies have been applied for increasing DNA vaccine potency including

a) physical delivery to achieve higher levels of antigen production and

b) formulation with microparticles to target antigen-presenting cells (APCs). Both approaches are effective in animal models, but have yet to be evaluated fully in human clinical trials.

Generally, the methods of delivering a DNA plasmid are divided into:

I. Physical approaches including:

1. Tattooing
2. Gene gun
3. Ultrasound

4. Electroporation

5. Laser

II. Viral and non-viral delivery systems (Non-physical delivery methods) including:

1. Biological gene delivery systems (viral vectors)

2. Non-biological gene delivery systems (non-viral vectors) such as:

2.1. Cationic lipids/liposomes

2.2. Polysaccharides and cationic polymers

2.3. Micro-/Nano-particles

2.4. Cationic peptides/Cell-penetrating peptides (CPP)

Generally, DNA may be administered by different methods such as intradermal (i.d.), intramuscular (i.m.), intranasal (i.n.) and subcutaneous (s.c.). In many cases, cutaneous administration has been associated with immunological benefits, such as the induction of greater immune responses compared with those elicited by other routes of delivery.

Tattooing:

Tattooing has been recently described as a physical delivery technology for DNA injection to skin cells, which is similar to the effective smallpox-vaccination technique, it seems to decrease the time required for the induction of potent immune responses and protective immunity. Gene expression after DNA tattooing has been shown to be higher than that after intradermal injection and gene gun delivery. Gene expression after tattooing showed a peak after six hours that disappeared over the next four days. Furthermore, the effect of two adjuvants, cardiotoxin and plasmid DNA carrying the mouse granulocyte macrophage colony-stimulating factor (GM-CSF) has been evaluated on the efficacy of a DNA vaccine delivered either by tattoo or in needle injection [23]. In this study, a codon modified gene encoding the L1 major capsid protein of the human papillomavirus type 16 (HPV16) was used as a model antigen [15]. The results indicated that molecular adjuvants substantially enhance the efficiency of the HPV16 L1 DNA vaccine when administered intramuscularly. However, the tattoo delivery of DNA is a cost-effective method that may be used in laboratory conditions when more rapid and more robust immune responses are required [23].

Gene gun

The particle-mediated or gene gun technology (Fig.7) [24] has been developed as a non-viral method for gene transfer into various mammalian tissues. The gene gun is a biolistic device that enables delivered DNA to directly transfect keratinocytes and epidermal Langerhans cells. Recently, gene gun mediated transgene delivery system has been used for skin vaccination against melanoma using tumor-associated antigen (TAA) human gpl00 and reporter gene assays as experimental systems [24]. In a study in

mouse, the immunological and antitumor responses were evaluated by administration of the plasmid DNA encoding extracellular domain of human EGFR (epidermal growth factor receptor) through three different methods: needle intramuscular administration, gene gun administration using gold coated DNA and gene gun administration using non-coating DNA.

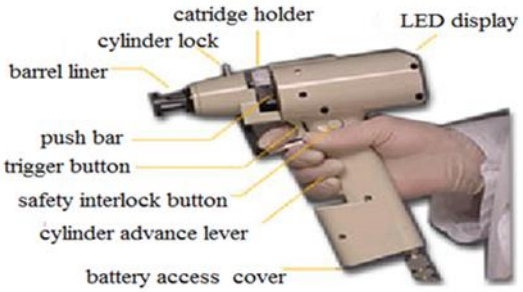

Fig. 7: Gene gun

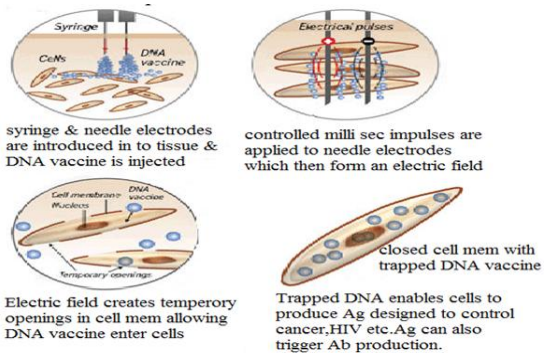

Fig. 8: Delivery of vaccines through electroporation

Electroporation

This technique involves application of electrical pulses to the skin thereby creating transient pores in the skin promoting the entry of DNA into the cell (Fig.8). ChronVac-C is a therapeutic DNA vaccine given to patients already infected with the virus in order to clear the infection by boosting immune response. It showed acceptable safety when delivered by electroporation in phase I/II clinical study at Karolinska University Hospital.

Ultrasound

Ultrasound (US) can be used to transiently disrupt cell membranes to enable the incorporation of DNA into cells. In addition, the combination of therapeutic US and microbubble echo contrast agents could enhance gene transfection efficiency. This system has been applied to deliver proteins into cells [25], but not yet to deliver antigens into DCs for cancer immunotherapy.

Laser

In vitro studies have shown that laser beam can deliver a certain amount of energy (e.g., up to 20 mega electron volts for the first time) onto a target cell, modifying permeability of the cell membrane by a local thermal effect. For therapeutic applications, a further increase in the amount of energy (e.g., up to 250 mega electron volts) is necessary. Recently, this novel technology has been described to be an effective method of enhancing the transfection efficiency of injected plasmids intradermally and inducing antigen-specific CD4+ and CD8+ T cell

immune response as well as humoral immunity. This novel technology was only used to show a high potential for therapeutic HPV DNA vaccine development in a limited number of studies.

Viral and non-viral methods of DNA vaccine delivery

Viral vectors such as retrovirus, adeno virus, herpes simplex virus, vaccinia virus are efficient in DNA transfer due to their nanoscale dimensions, well characterized surface properties allowing the incorporation of immunogenic components (e.g., virosomes). However drawbacks such as the limited DNA carrying capacity, toxicity, immunogenicity, the possibility of insertional mutations in host DNA and high cost warrants their use. Non-viral carriers including microspheres, nanospheres, liposomes as discussed in the above sections find potential application as carriers for DNA vaccines.

Needle Free Delivery

Needle-free vaccine delivery is gaining popularity these days due to the following reasons.

- Patient's concern about pain associated with the injections; disposal issues and potential for cross contamination due to blood borne diseases is eliminated.
- Differentiate their products from the existing products as the pharmaceutical industry faces massive losses in revenues from the expired patents and to withstand pressure from generic companies.
- Search for alternative ways to deliver growing list of new biopharmaceutical and molecular entities like vaccines, DNA, peptides and proteins that cannot be delivered orally.
- Urge to evolve into specialty pharmaceutical companies developing their own branded pharmaceutical products.
- Bioject's needle-free injection technology works by forcing liquid medication at high speed through a tiny orifice that is held against the skin. Bioject's technology is unique because it delivers injections to a number of injection depths and supports a wide range of injection volumes. For instance, the Biojector 2000 (Fig.9a) can deliver intramuscular or subcutaneous injections up to 1 mL in volume.
- Iject®(Fig.9b) and Iject® R(Fig.9c) are in investigational use only and are in development; not yet cleared by the FDA. The Iject® is a small, lightweight, gas-powered injection system being developed for home or professional use. This system has two versions, one is a pre-filled, single-use disposable injector, and the other is a reusable injector that accepts pre-filled medication cartridges.

Fig. 9a: Biojector 2000

Fig. 9b: Iject®

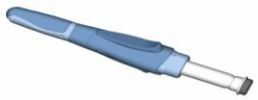

Fig. 9c: Iject® R

Fig. 9d: biojectzetajet

Fig. 9e: Jupiter jet

The Bioject®ZetaJet™ (Fig.9d), Bioject's latest advance in needle-free delivery systems, consists of two components, the portable injector and an auto-disabling disposable syringe. The ZetaJet™ is self-powered by a spring and is ideal for use in mass immunization programs world-wide. The syringe assembly has a unique "auto-disable" feature that prevents re-use of the syringe. TheBioject®ZetaJet™ has FDA clearance for delivering subcutaneous or intramuscular injections of liquid medication, including vaccines and other injected medications.

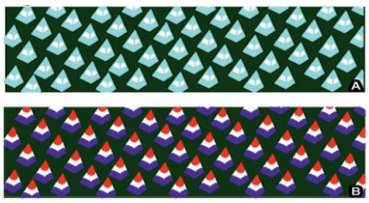

Fig. 10: Diagrammatic representation of scanning electron microscopy images of (A) unloaded and (B) plasmid DNA-coated

Microneedles (Fig. 10) are promising microfabricated devices for minimally invasive drug delivery applications. Needles are also designed to be extremely sharp, with submicron tip radii. Microneedles offer an attractive way for advanced drug delivery systems by mechanically penetrating the skin and injecting drug just under the stratum corneum where it is rapidly absorbed by the capillary bed into the bloodstream. In order to deliver drug or skin cosmetic components to all the layers or to a certain skin layer, the micro-needles are preferably fabricated to have an upper end diameter of 5-40 μm and an effective length of 1000-2000 μm.

Currently, the smallest needles that are commercially available for injections are 30 gauge for conventional syringes and 31 gauge for pen injectors, which are utilized mainly for insulin delivery. The 30 and 31 gauge needles have outer diameters of 305 and 254

μm, respectively. Drug delivery with micro-needles aims to deliver a drug through the skin rather than biological circulatory systems such as blood vessels or lymphatic vessels. The method for fabricating biodegradable solid microneedles comprises following main steps:

1. Coating the surface of a substrate with a viscous material for forming biodegradable solid microneedles.
2. Bringing the surface of a frame having pillar patterns formed thereon, into contact with the surface of the coated viscous material
3. Drawing the coated viscous material using the frame, while solidifying the viscous material
4. Cutting the drawn material at a given position thereof, thus obtaining biodegradable solid micro-needles. Various materials, such as hydrogel, maltose, drugs for the treatment for skin diseases, cosmetic components, water-soluble materials and polymeric proteins, may be used to form the biodegradable solid micro-needles.

Applications of micro-needles

- Solid micro-needles could be used with drug patches to increase diffusion rates; increase permeability by poking holes in skin, rub drug over area, or coat needles with agent to be delivered.
- Hollow needles could be used with drug patches and timed pumps to deliver drugs at specific times.
- Also, these micro-needles could be used to remove fluid from the body for analysis - such as blood glucose measurements - and to then supply micro liter volumes of insulin or other drug as required.
- These are capable of very accurate dosing, complex release patterns, promote local delivery and biological drug stability enhancement by storing in a micro volume that can be precisely controlled.

Matriano *et al.* examined the use of Microneedles coated with a dry-film of antigen to deliver ovalbumin as a model protein antigen by inserting them into the skin of hairless guinea pigs *in vivo* using a high-velocity injector.

2.7 Mucosal Delivery of Vaccines

Mucosal vaccination offers protection against microorganisms which gain access to body via mucosal membranes. Patient compliance, ease of administration, reduction in possibility of needle-borne injections, stimulation of both systemic and mucosal immunity are some of the advantages. Delivery systems like PLG microspheres, PLGA microparticles carrying immunogenic agents etc are taken up by Peyers patches. Particles of <5 μm further move into lymph

nodes and spleen-stimulating-specific IgG, IgM responses.

Nasal mucosal delivery

Nasal mucosa is the first contact site for antigens being inhaled, systemic and local immunity can be stimulated by activation of T-cells, B-cells, and dendritic cells present in nasal associated lymphoid tissue located beneath nasal epithelium in the form of IgG and secretory IgA. Intranasal vaccines include those against influenza A and B virus, proteosoma-influenza, adenovirus-vectored influenza, group B meningococcal native, attenuated respiratory syncytial virus and parainfluenza 3 virus.

Edible vaccines

Creating edible vaccines involves introduction of selected desired genes into plants and then inducing these altered plants to manufacture the encoded proteins. This process is known as "transformation," and the altered plants are called "transgenic plants." Like conventional subunit vaccines, edible vaccines are composed of antigenic proteins and are devoid of pathogenic genes. Thus, they have no way of establishing infection, assuring its safety, especially in immuno-compromised patients.

Conventional subunit vaccines are expensive and technology-intensive, need purification, require refrigeration and produce poor mucosal response. In contrast, edible vaccines would enhance compliance, especially in children and because of oral administration, would eliminate the need for trained medical personnel. Fear of contamination with animal viruses - like the mad cow disease, which is a threat in vaccines manufactured from cultured mammalian cells - is eliminated, because plant viruses do not infect humans.

2.8 Production of edible vaccines:

Edible vaccines are produced by integrating gene cloning, tissue culture and plant transformation techniques. The first step in the process of creating an edible vaccine is the selection of a suitable immunogen. The gene encoding the immunogen is cloned into an expression vector that contains plant regulatory sequences capable of driving gene expression and indicating the gene's terminus. This vector is then used in plant transformation.

3. CONCLUSION

Vaccine drug delivery systems are gaining popularity these days due to the benefits they offer. They are now being proven to be patient friendly as they avoid the need to administer booster doses and provide a long term therapy in small doses. Their use is further encouraged by administering them via needle-free technologies. Edible vaccines on the other hand open an attractive avenue for the oral delivery of vaccines. Currently, many modifications to the current delivery systems and novel carrier systems have been developed to optimize the immune efficiency.

Furthermore, the route of immunization can influence the outcome of the immune response through altering the interaction between the vaccine and different APCs at the site of injection. Hence, the routes of administration and formulation of DNA clearly affect the therapeutic response by altering immune pathway. Among the commonly used methods of DNA vaccination, the highest efficacy was achieved after in vivo electroporation and gene gun delivery. However, it is critical to further analyze the results of ongoing clinical trials, specifically, in the aspect of their success or failure of certain delivery methodology.

REFERENCES

1. Schmidt CS, Morrow WJW, Sheikh NA. Smart adjuvants. *Expert Review of Vaccines*, 2007; 6: 391–400.
2. Walter E, Dreher D, Kok M, et al. Hydrophilic poly(D,L-lactide-co-glycolide) microspheres for the delivery of DNA to human-derived macrophages and dendritic cells. *Journal of Controlled Release*, 2001; 76(1–2):149–168.
3. Reddy ST, Rehor A, Schmoekel HG, Hubbell JA, Swartz MA. *In vivo* targeting of dendritic cells in lymph nodes with poly (propylene sulfide) nanoparticles. *Journal of Controlled Release*, 2006; 112(1):26–34.
4. Slutter B, Soema PC, Ding Z, Verheul R, Hennink W, Jiskoot W. Conjugation of ovalbumin to trimethyl chitosan improves immunogenicity of the antigen. *Journal of Controlled Release*, 2010; 143(2):207–214.
5. Jain S, Yap WT, Irvine DJ. Synthesis of protein-loaded hydrogel particles in an aqueous two-phase system for coincident antigen and CpG oligonucleotide delivery to antigen presenting cells. *Biomacro-molecules*, 2005; 6(5):2590–2600.
6. Shen Z, Reznikoff G, Dranoff G, Rock K. Cloned dendritic cells can present exogenous antigens on both MHC class I and class II molecules. *Journal of Immunology*, 1997; 158(6):2723–2730.
7. Jung T, Kamm W, Breitenbach A, Hungerer K-D, Hundt E, Kissel T. Tetanus toxoid loaded nanoparticles from sulfobutylated poly(vinyl alcohol)-graft-poly(lactide-co-glycolide): evaluation of antibody response after oral and nasal application in mice. *Pharmaceutical Research*, 2001; 18(3):352–360.

8. Mottram PL, Leong D, Crimeen-Irwin B, et al. Type 1 and 2 immunity following vaccination is influenced by nanoparticle size: formulation of a model vaccine for respiratory syncytial virus. *Molecular Pharmaeutics*, 2006; 4(1):73–84.
9. Nixon DF, Hioe C, Chen P-D, et al. Synthetic peptides entrapped in microparticles can elicit cytotoxic T cell activity. *Vaccine*, 1996; 14(16):1523–1530.
10. Nagamoto T, Hattori Y, Takayama K, Maitani Y. Novel chitosan particles and chitosan-coated emulsions inducing immune response via intranasal vaccine delivery. *Pharmaceutical Research*, 2004; 21(4):671–674.
11. Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. *Nature Reviews Immunology*, 2006; 6(2):148–158
12. Singh M, Briones M, Ott G, O'Hagan DT. Cationic microparticles: a potent delivery system for DNA vaccines. *Proceedings of the National Academy of Sciences of the USA*. 2000; 97: 811–816.
13. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Advanced Drug Delivery Reviews*, 2003; 55(3):329–347.
14. Elamanchili P, Diwan M, Cao M, Samuel J. Characterization of poly(D,L,-lactic-co-glycolic acid) based nanoparticulate system for enhanced delivery of antigens to dendritic cells. *Vaccine*, 2004; 22(19):2406–2412.
15. Cui Z, Mumper RJ. Topical immunization using nanoengineered genetic vaccines. *Journal of Controlled Release*. 2002, 81(1–2):173–184.
16. Rieger J, Freichels H, Imberty A, et al. Polyester nanoparticles presenting mannose residues: toward the development of new vaccine delivery systems combining biodegradability and targeting properties. *Biomacromolecules*, 2009; 10(3):651–657.
17. Andrianov AK, Marin A, Roberts BE. Polyphosphazene polyelectrolytes: a link between the formation of noncovalent complexes with antigenic proteins and immunostimulating activity. *Biomacromolecules*, 2005; 6(3):1375–1379.
18. Okada H, Toguchi H. Biodegradable microspheres in drug delivery. *Critical Reviews in Therapeutic Drug Carrier Systems*, 1995; 12(1):1–99.

19. Florindo HF, Pandit S, Lacerda L, Gonçalves LMD, Alpar HO, Almeida AJ. The enhancement of the immune response against *S. equi* antigens through the intranasal administration of poly-[varε]-caprolactone-based nanoparticles. *Biomaterials*, 2009; 30(5):879–891.
20. Freitas S, Merkle HP, Gander B. Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology. *Journal of Controlled Release*, 2005; 102(2):313–332
21. Zheng C, Juhls C, Oswald D, Sack F, Westfehling I, Wittig B, BabiukLA,Hurk SDL: Effect of different nuclear localization sequences on the immune responses induced by a MIDGE vector encoding bovine herpes virus-1 glycoprotein D. *Vaccine,* 2006, 24:4625-4629.
22. 22. Kutzler MA, Weiner DB: DNA vaccines: ready for prime time? *Nature Reviews,* 2008, 9:776- 788.
23. N.Kanaka.D.Devi, N.Narasimha Rao, M.Anuradha, P.Naveena, P.Sravani, Y.Satyasesha Sree. Validation Of Particle Size Distribution In Pharmaceutical Excipients. *Annals of biological research*, 2015, 6(6):1-7.
24. Pokorna D, Rubio I, Müller M: DNA-vaccination via tattooing induces stronger humoral and cellular immune responses than intramuscular delivery supported by molecular adjuvants. *Genetic Vaccines and Therapy,* 2008, 6:1-8.
25. Aravindaram K, Yang NS: Gene gun delivery systems for cancer vaccine approaches. *Methods in Molecular Biology,* 2009, 542:167-178.
26. Bekeredjian R, Kuecherer HF, Kroll RD, Katus HA, Hardt SE: Ultrasound targeted microbubble destruction augments protein delivery into testes. *Urology,* 2007, 69:386-389.
27. http://www.cdc.gov/vaccines.
28. Stephan D.J., Yang,Z.Y., San,H., Simari,R.D., Wheeler,C.J., Felgner,P.L., Gordon,D., Nabel,G.J. and Nabel,E.G. (1996) A new cationic liposome DNA complex enhances the efficiency of arterial gene transfer *in vivo*. *Human Gene Ther*apy, 7, 1803–1812.
29. Baillie AJ, Coombs GH, Dolan TF, Laurie J. Non-ionic surfactant vesicles, niosomes, as delivery system for the anti-leishmanial drug, sodium stibogluconate. *Journal of Pharmacy and Pharmacol*ogy, 1986; 38:502–5.
30. Almeida JD, Brand CM, Edwards DC, Heath TD: Formation of virosomes

from influenza subunits and liposomes. *Lancet,* 1975; 2:899–901.

31. Manns MP, McHutchison JG, Gordon S et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet*, 2001, 358, 958-965.
32. Yap WT, Song WK, Chauhan N, Scalise PN, Agarwal R, Miller SD, Shea LD. Quantification of Particle-Conjugated or Particle-Encapsulated Peptides on Interfering Reagent Backgrounds. *BioTechniques*. 2014; 57:39–44.
33. Wellmann H, Kaltschmidt B, Kaltschmidt C. Optimized protocol for biolistic transfection of brain slices and dissociated cultured neurons with a hand-held gene gun. Journal of *Neuroscience Methods*, 1999; 92:55–64.
34. Rols MP. Mechanism by which electroporation mediates DNA migration and entry into cells and targeted tissues. *Methods in Molecular Biol*ogy, 2008; 423:19–33.
35. Bioject Feedback from the field: Needlefree injection Use in Large Scale Immunization Campaigns, Rockville, MD, 18 December 2003.33.
36. Bos JD, Meinardi MM (2000) The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Experimental Dermatology,* 9: 165–169.

How to cite this article:

Kanaka Durga Devi et al., A potent vaccine delivery system: the promise and the challenge. *Int. J. Adv. Pharm. Biotech.*, 2018; 4(1): 31-47.