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Nanotechnology in vaccine delivery

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ABSTRACT

Success of vaccination in dealing with the communicable diseases can be clearly understood by eradication of smallpox and the imminent decrease of polio, and also the Expanded Program for Immunization (EPI) leaded by The World Health Organization succeeded dramatically to meet its target to furnish six major diseases of children (diphtheria, pertuasis, tetanus, polio, measles, and tuberculosis. Despite these impressive achievements, significant problems are still present for development of a breakthrough vaccine for diseases like cancer, HIV, influenza and even though vaccines has being developed for some diseases its mass commercialization is a big challenge. Nanotechnology, which is a cutting-edge area of present research, gave new dimensions to vaccine world and the results came out as DNA vaccine, conjugate vaccine and many more advancements. This brief review will put some light upon the enormous scope of nanotechnology in vaccine delivery also new advancement in terms of route for vaccine delivery with recent marketed products in clinical use. Briefly, challenges for commercialization of vaccines worldwide are also discussed.

1. INTRODUCTION

Immunization, also called vaccination or inoculation, is development of resistance to specific diseases using microorganisms (bacteria or viruses) that have been modified or killed. These treated microorganisms do not cause diseases, but rather trigger the body's immune system to build a defense mechanism that will protect the body from attack of similar antigen in future [1]. Till now most of the vaccines have been developed using live attenuated organisms, killed whole organisms or inactivated toxins (referred to as toxoids). Live vaccines such as smallpox, polio (oral), measles, mumps, rubella, varicella, adenovirus and others are advantageous in terms of producing both humoral and cellular immunity and often require only one boost but suffer from a serious risk of reverting back to their virulent form in addition to their intrinsic instability which make them difficult to deliver. On the other hand, killed or inactivated whole organism vaccines (such as influenza, hepatitis A and others) as well as toxoid vaccines (including diphtheria and tetanus) although safer than live vaccines, generate a weaker immune response and typically require multiple doses. Moreover, for vaccines against both infectious diseases and cancers, peptide-based vaccines are getting attention which are found to be effective in small animal models but lack of immunogenicity in humans because of size, degradation, non-specific targeting, lack of cross-presentation, and other issues. This reveals serious requirement of new methods which not only accommodate the antigen and co-stimulators but also solve the problem of poor immunogenicity. In this direction adjuvants are found to provide solution which will be leading vaccine science with the aid of nanotechnology [2].

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2. ADJUVANTS: AN OVERVIEW

Adjuvants can be defined as molecules, compounds or macromolecular complexes that boost the potency and longevity of specific immune response to antigens with minimal toxicity [3]. The term "adjuvant" was first used by Ramon in 1926 for a substance used in combination with a specific antigen to enhance its immunogenicity [4]. Their mechanism of action involve [4] (1) epitope stablization, (2) targeting the antigen to antigen-presenting cells by formation of multimolecular aggregates, or by binding antigen to a cell-surface receptor on APCs, (3) directs antigen

presentation by MHC class I or MHC class II pathways, by means of fusion or disruption of cell membranes, or by direct peptide exchange on surface MHC molecules, and (4) stimulation of Th1 or Th2 CD^{4+} T-helper cells or CD^{8+} cytotoxic T lymphocytes. Various adjuvants were tried to improve immunogenicity of antigen and the most common example, alum, was first choice due to safety, cost and wide availablity [5]. However, some limilations of alum as antigen/immune potentiator instability in a liquid medium and inability to co-deliver specific immune potentiators turned the direction of vaccinology towards nanotechnology. Nanotechnology provides multiple platforms such as polymeric nanoparticles, liposomes, self assembling peptides, inorganic nanoparticles, and micro/nanoemulsions (Figure 1) which are being explored as adjuvant in the next generation of subunit vaccines [6; 7].

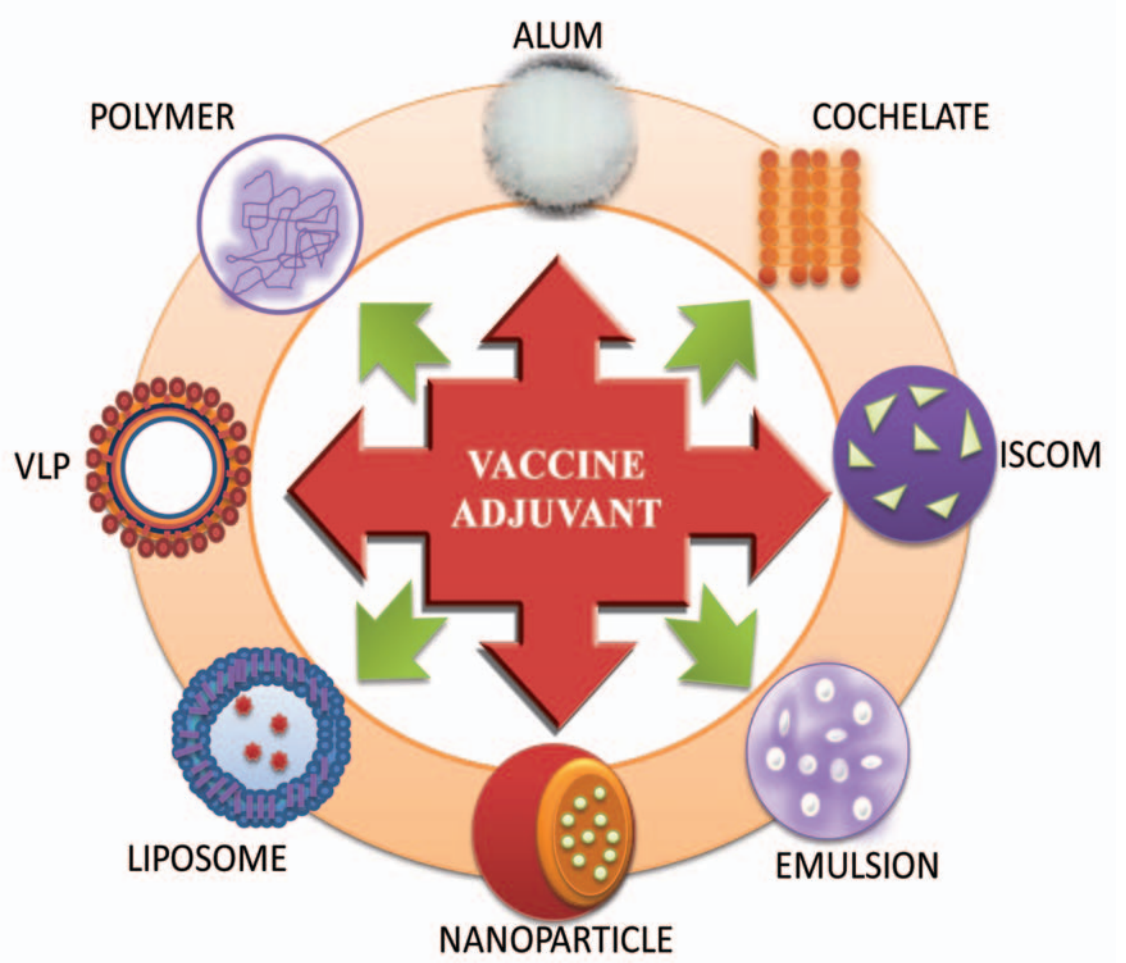

Figure 1 Adjuvants used in vaccine formulation

3. NANOPARTICULATE VACCINE DELIVERY SYSTEMS

Biotechnological advancement such as stem cell therapy, recombinant DNA methods etc. have given a new dimension to biomedical science. In vaccine world also DNA vaccine, subunit vaccines, as well as conjugate vaccines (Prevnar and Menactra®) have received significant attention [8; 9]. Although new vaccines based on recombinant proteins and DNA have several advantages over traditional vaccines but they are less immunogenic. Here nanotechnology supported this emerging field by providing multiple options to improve stability as well as antigencity. These nano-carriers are capable of mimicking the physiological environment along with targeting ability which produces selective and enhanced response than antigen alone. Clinical trials for nanoparticulate vaccine delivery systems are listed in Table 1. Clinically used vaccines based on nanotechnology system are listed in Table 2. Various nanoparticulate vaccine delivery systems are described in details.

Table 1. Clinical trials for nanoparticulate vaccine delivery systems

S No.	Clinical trial	Condition	Antigen	Adjuvant	NCT no.	Phase	Status
LIPOSOME AS ADJUVANT							
1	Decitabine, Vaccine Therapy, and Pegylated Liposomal Doxorubicin Hydrochloride in Treating Patients With Recurrent Ovarian Epithelial Cancer, Fallopian Tube Cancer, or Peritoneal Cancer	Cancer	In this trial the purpose is to study the side effects and best dose of decitabine in combination with pegylated liposomal doxorubicin hydrochloride and vaccine therapy		NCT01673217	I	Completed
2	Trial on the Safety of a New Liposomal Adjuvant System, CAF01, When Given With the Tuberculosis Subunit Vaccine Ag85B-ESAT-6 as Two Injections With Two Months Interval to Healthy Adult Volunteers	Tuberculosis	Ag85B-ESAT-6 antigen	Liposomal Adjuvant CAF01	NCT00922363	I	Completed
3	A Phase I Safety Study of a Cancer Vaccine to Treat HLA-A2 Positive Advanced Stage Ovarian, Breast and Prostate Cancer	Cancer	T Helper peptide and 7 tumor-specific HLA-A2-restricted peptides	Polynucleotide adjuvant and liposome	NCT01095848	I	Unknown
4	A Phase I, Randomized, Double-Blind, Placebo-Controlled, Clinical Trial to Compare the Safety and Immunogenicity of Recombinant Envelope Protein rgp120/HIV-1SF2 (BIOCINE) Combined With Seven Adjuvants in Healthy HIV-1 Uninfected Individuals	HIV infection	Recombinant Envelope Protein rgp120/HIV-1SF2 (BIOCINE)	1) Alum. Hydroxide, 2) Lipid A, 3) Lipid A,Liposome encapsulated monophosphoryl, 4) Syntex, 5) MF59, 6) Threonyl Muramyl Dipeptide, 7) MTP-PE/MF59	NCT00001042	I	Completed
5	Safety and Immunogenicity Study of Intramuscular CCS/C-adjuvanted Influenza Vaccine in Elderly	Influenza	Influenza vaccine	VaxiSomeTM= CCS-Cholesterol [CCS/C]	NCT00915187	II	Completed
6	Study to Assess dHER2+AS15 Cancer Vaccine Given in Combination With Lapatinib to Patients With Metastatic Breast Cancer	Metastatic Breast Cancer	dHER2	AS15 liposomal formulation (GlaxoSmithKline)	NCT00952692	I/II	Completed
7	Phase 1b Maintenance Therapy Study of ONT-10 in Patients With Solid Tumors	Solid Tumors	ONT-10	PET Lipid A	NCT01978964	Ib	Recruiting
8	Phase 1 Study of ONT-10 in Patients With Solid Tumors	Solid Tumors	ONT-10	PET Lipid A	NCT01556789	I	Recruiting
9	Vaccine Therapy Plus Interleukin-2 With or Without Interferon Alfa-2b in Treating Patients With Stage III Melanoma	Stage III melanoma	-	Liposomal interleukin-2	NCT00004104	II	Completed
NANOPARTICLES AS ADJUVANT							
1	RSV-F Vaccine and Influenza Vaccine Co-Administration Study in the Elderly	Respiratory Syncytial Virus	RSV-F Protein	Nanoparticle	NCT01709019	I	Completed
2	RSV-F Vaccine Dose Ranging Study in Young Women	"	"	"	NCT01704365	II	Completed
3	RSV F Dose-Ranging Study in Women	"	"	"	NCT01960686	II	Active
VIRUS LIKE PARTICLES VACCINE							
1	Dose-Ranging Study of Quadrivalent Human Papillomavirus (HPV) (Types 6,11,16,18) L1 Virus-Like Particle (VLP) Vaccine(V501007)	Papilloma virus Infections	Human Papilloma-virus (HPV)	Virus-Like Particle V501-007	NCT00365716	II	Active
2	Safety and Immunogenicity of Norovirus GI.1/GII.4 Bivalent VLP Vaccine (NOR-107)	Norovirus	Norovirus GI.1/GII.4	Monophosphoryl Lipid A, Alum. Hydroxide	NCT02038907	II	Not started recruiting
3	Phase 1 Norwalk Vaccine Study	Norovirus	Norwalk VLP Vaccine	Adjuvant/Excipients	NCT00806962	I	Completed
4	Safety and Immunogenicity Study of MEDI-517 (GSK 580299) With or Without Adjuvant in Healthy Adult Females	Prophylaxis HPV-16/18,	MEDI-517, a Virus-Like Particle Vaccine	Alum. Hydroxide	NCT00693615	II	Completed
5	Safety, Tolerability and Immunogenicity of a Plant-made H7 Virus-like Particle (VLP) Influenza Vaccine in Adults	Respiratory Tract Infections	H7 VLP vaccine	Alhydrogel	NCT02022163	I	Not started recruiting
6	Immunogenicity, Safety, Tolerability of a Plant-made H5 Virus-like-particle (VLP) Influenza Vaccine	Respiratory Tract inf.	H5 VLP vaccine	Alhydrogel	NCT01991561		Not recruiting

7	A/H5N1 Virus-Like Particle Antigen Dose Ranging Study With Adjuvant I/II	Influenza (Pandemic)	A/H5N1 Virus-Like Particle		NCT01594320, NCT01596725	I	Completed
8	H5-VLP + GLA-AF Vaccine Trial in Healthy Adult Volunteers	Influenza A , H5N1 Infection	H5-VLP	GLA-AF	NCT01657929	I	Completed
9	A(H7N9) VLP Antigen Dose Ranging Study With Adjuvant 1	Avian Influenza	(H7N9) VLP Antigen		NCT01897701	I	Ongoing
10	A(H7N9) VLP Antigen Dose-Ranging Study With Matrix-M1™ Adjuvant	Influenza (Pandemic)	(H7N9) VLP	Matrix-M1™	NCT02078674		Recruiting
11	Study to Evaluate the Safety and Immunogenicity of MEDI-517P in Healthy Adult Female Volunteers Who Are HPV-16 or HPV-18 DNA Positive	Healthy	MEDI 517	Alum. Hydroxide	NCT00263744	I/II	Completed
12	Efficacy and Immunogenicity Study of Recombinant Human Papillomavirus Bivalent (Type 16/18) Vaccine	Cervical Intraepithelial Neoplasia	HPV 18 virus-like particle	Alum adjuvant	NCT01735006	III	Recruiting participants
13	Bivalent Norovirus Vaccine Study	Gastroenteritis	GI.1/GII.4 Bivalent Virus-Like Particle(VLP)	Monophosphoryl Lipid A (MPL) and Alum. Hydroxide	NCT01168401	I	Completed
14	A Phase I Safety and Immunogenicity Study of HIV p17/p24:Ty-VLP in HIV-1 Seronegative Subjects	HIV Infections	HIV p17/p24:Ty-VLP	Alum. Hydroxide	NCT00001053	I	Completed
15	ICC-1132 - Candidate Vaccine Against P Falciparum Malaria	P.Falciparum Malaria	ICC-1132 VLP	Alhydrogel	NCT00587249	I	Completed
16	Broad Spectrum HPV Vaccine Dose Escalation Study (V502-002)	Human Papilloma Virus	(HPV) L1 Virus-Like Particle	(AAHS) and ISCOMATRIX™ (IMX)	NCT00851643	I	Completed
ISCOM™ AS ADJUVANT							
1	Study of a Parenterally Administered H5N1 Influenza Vaccine in Healthy Adults (PANFLUVAC)	Healthy	H5N1 Influenza antigen	3rd generation ISCOM™	NCT00868218	I	Ongoing
2	Study of NY-ESO-1 ISCOMATRIX® in Patients With Measurable Stage III or IV Melanoma	Melanoma	NY-ESO-1	ISCOM™	NCT00518206	II	Completed
PROTEASOME							
	Immunotherapy of Melanoma With Tumor Antigen RNA and Small Inhibitory RNA Transfected Autologous Dendritic Cells	Metastatic Melanoma	siRNA and tumor antigen RNA	Proteasome	NCT00672542	I	Completed
MISCELLANEOUS							
3	Vaccine Therapy in Treating Patients With Stage II Melanoma That Can Be Removed by Surgery	Melanoma (Skin)	Tyrosinase/gp100 peptide	Montanide ISA 51 ,a Block Co-Polymer CRL 1005 or With GM-CSF	NCT00003274	II	Completed
	Inactivated Influenza A/H9N2 Vaccine With and Without MF59 Adjuvant in Ambulatory Adults	Influenza	A/H9N2 Vaccine	MF59	NCT00133471	I/II	Completed

Table 2 Clinically used vaccines based on nanotechnology system.

Vaccine	Nanotech system	Company
A. Hepatitis-B		
Shanvac-B®	VLP	Shantha Biotechnics
Revac-B®	VLP	Bharat Biotech International
Hepavax-Gene®	VLP	Crucell
Heberbiovac HB®	VLP	CIGB-Heber Biotec
Gene Vac-B®	VLP	Serum Inst. of India
Euvax B®	VLP	LG Life Sciences
Enivac HB®	VLP	Panacea Biotec
DTP-Hep B®	VLP	P.T. Bio Farma
Bio-Hep-B®	VLP	BTG (SciGen, FDS Pharma)
GenHevac B®	VLP	Pasteur-Merieux Aventis
Engerix-B®	VLP	GSK
Recombivax HB®	VLP	Merck
Fendrix®	AS04	GSK
B. HPV		
Cervarix®	AS04	GSK
Gardasil®	VLP	Merck
C. Hepatitis-A		
Epaxal®	Virosome	Crucell
D. Influenza		
Fluad®	MF 59	Novartis

3.1. Virus-like particles (VLPs) and virosomes

VLPs are self assembled viral envelope proteins devoid of genetic material and essentially non-infective in nature. They form particles of 20–100 nm. While, virosome consist of an envelope of one virus with antigenic material of different source [8]. Key features [10] that underlay their immunogenicity, safety and protective potential are (1) well-defined geometry with uniform, repetitive and ordered surface structures, (2) particulate and multivalent nature, (3) preservation of native antigenic conformation, (4) stability in extreme environmental conditions etc.

VLPs as vaccine adjuvant [11] are known since the late 1980s and many VLP based products are in the commercial market but still growth rate is not up to the mark. This reveals the dark points of VLPs. The most important point to be addressed is that VLP foreign epitope display strategies typically only permit epitopes of a limited size to be targeted. The pathogens usually undergo antigenic variation in response to host immune pressures so vaccines based on VLPs displaying foreign epitopes will only be effective against highly conserved B- or T-cell epitopes [12; 13]. Another challenge is scale up of structurally complex VLPs [14; 15].

3.2. Liposomes

Liposomes are spherical entities composed of a phospholipid bilayer shell with an aqueous core. Allison and his coworkers reported it as a vaccine adjuvant for the first time in 1974 [16]. Antigen can be associated via covalent lipid conjugation (either pre- or post-vesicle formation), non-covalent surface attachment (biotin/antibody-epitope interactions), encapsulation, and surface adsorption [17; 18; 19; 20; 21]. These methods can be selected based upon the complexity of antigen and as the size and complexity of the antigen decreases, surface conjugation becomes more prominent for antibody induction [19; 22]. Immunogenicity of liposomal vaccines is influenced by many parameters as vesicle size and bilayer structure, lamellarity, charge, fusogenicity and lipid transition temperature. Large vesicles (250–700 nm in diameter) showed better response towards TH1 and increase both persistence at the injection site and transit to draining lymph nodes [23; 24; 25]. Further, uni-lamellar large vesicles were found to be more efficient to induce immune response as compared to multi-lamellar vesicles and also the preparation of multi lamellar vesicle varied as per number of lamellae which faced the reproducibility problem [26; 27]. In terms of charge of liposome, cationic vesicles promote stronger antigen-specific serum antibody responses than equivalent neutral or anionic formulations. However, antibody and cell mediated responses are not always correlated [28; 29]. If lipid properties are considered, liposomes of greater rigidity and higher gel-liquid crystal transition temperature elicit higher antibody and cell-mediated responses to a variety of encapsulated and surface-associated antigens [30; 31]. Also, fusogenicity increased the capacity of liposomes to promote immunity to associated antigens [32]. Advantages of liposomes include acceptably low reactogenicity, versatility of carrying diffrent type of antigens (hydrophilic/hydrophobic) and biocompatibility as these formulations are made of lipids that occur naturally in the cell membranes [33; 34]. However, leakage of antigen from liposome is a common problem which questions its stability and site specific release. Inter Bilayer Crosslinked Multilamellar Vesicles (ICMVs) have been developed recently with improved encapsulation efficiency and stability but lack of proper antigen characterization within liposome is still an unreachable goal [27; 35].

3.3. Micro emulsion/ nano emulsion/ multiple emulsion delivery systems

Emulsions as vaccine adjuvant, have been known from a long ago. In 1940, the first emulsion based Freunds adjuvant came into picture but was found to be poorly tolerated due to its non-degradable mineral oils. Later in 1960s, first degradable oil based adjuvant was developed by Merck using peanut oil and in 1997, Novartis came with MF59 which was the first emulsion based adjunct approved for human use (Fluad vaccine) [36]. Likewise, Montanide™ ISA 51 and 720 which composed of metabolizable squalene-based oil with amannide monooleate emulsifier formulations

were developed and entered in phase I and/or II clinical trials for vaccines against malaria, HIV and various cancers [37]. AF03 (for pendamic influenza) was developed by Sanofi and AS04 and AS02A were developed by GlaxoSmithKline that consisted of combinations of MPL® and either aluminum salts or QS-21, a purified component of the Quil A. AS04 was used in the European-licensed HBV vaccine, Fendrix®. Another combination adjuvant DETOX™ made up of MPL® and Mycobacterium phlei cell wall skeletons in a squalene emulsion was included in the Canadian-licensed Melacine® for late-stage melanoma [38]. These emulsion based adjuvants can be formulated by techniques like microfluidization (MF59), phase inversion (AF03) or high pressure homogenization [39; 40].

Although novel adjuvants came into existence with the great efforts of nanotechnology, many challenges are yet to overcome. Stability of emulsion based adjuvant was always a major challenge for formulation development. In addition to adjuvant stability antigen, which most often belong to protein category, can be affected by oil/water interface, glass/water interface, and the water/air interface [38]. Lyophilization could serve this problem but there are less reports available till now [41]. However, some adjuvants were found to be stable for single vial products i.e. MF59 containing Fluad vaccine with 1 year shelf life. Alternatively two vial system was applied for AS03 adjuvant where the adjuvant and antigen were mixed prior to use (Arepanrix™ Pandemic Influenza Vaccine) [42; 43]. Further antigen characterization becomes complicated in the presence of emulsion adjuvants due to interference with many routine assays, including reverse phase HPLC, size exclusion HPLC, dynamic light scattering, DSC, CD, tryptophan fluorescence spectroscopy, and other assays that are sensitive to the scattering and absorbance of light by oil droplets [44]. Moreover, for the performance of these adjuvants, 'adjuvant effect' plays an important role which states that the complete emulsion is responsible for immunogenic effect rather than its individual constituents. Thus formulation factors such as droplet size, stability of the droplets and the ability of the antigen to interact with the surface of the droplets must be paid due attention [45].

3.4. Nanoparticles

Role of nanoparticles in drug delivery is well known and many products are coming into market nowadays. The most exciting aspects of nanoparticles which make them a special class of nano medicine and now in vaccinology are (1) ability to co-deliver antigen and immune potentiator[7] (2) their potential to mimic features of pathogens such as viruses (3) targeting potential [46] and (4) ability of nanoparticles to incorporate new classes of adjuvant components such as TLR and Nod-like receptor (NLR) ligands also makes them attractive adjuvant candidates [47]. In addition biodegradable nature and scalability are the other advantages of this system.

These wonderful properties with a single delivery system has opened new possibilities of delivering subunit antigens to specific antigen presenting cells (APCs) to induce T cell responses [48]. Moreover, the capability of inducing both cellular and humoral responses by attaching both B and T cell epitopes can further enhance the immune response. This could be especially beneficial for AIDS and Malaria vaccine development [49; 50]. As alum already established itself as a safe and effective adjuvant so Alumina (Al_2O_3) nanoparticle-based adjuvant have recently been used to deliver antigens to dendritic cells in mice [51]. Gold nanoparticles are also being studied for this purpose and research is going on to conjugate peptides and proteins to gold nanoparticle surfaces [52; 53]. "CaP technology" which was developed by BioSante Pharmaceuticals, introduced calcium phosphate nanoparticles which have shown immune responses similar to or greater than aluminum salts. Additionally, CaP has also shown promise as a mucosal adjuvant. Vaccines utilizing CaP in preclinical studies include anthrax, HBV, flu (H5N1 avian and seasonal) and HSV-2 [54]. Cholesterol, an important component of lipoid drug delivery system, was conjugated to a variety of carbohydrates including pullulan, dextran and mannose and the resulting amphiphilic molecules were capable of being self-assemble with and without proteins into colloidal stable nanoparticles (30–40 nm size) [8]. These particles were found to be safe and well tolerated upon vaccination [55]. Proteosomes composed of the outer membrane proteins (OMPs) of Neisseria meningitidis, comprise one more class of nanoparticles with hydrophobic nature. They bind to apolar/amphiphilic antigen with noncovalent interaction. During clinical studies they were found to be non-toxic and well-tolerated [9]. Similarily, nanoparticles with biodegradable polymers as (D, L-lactide-co-glycolide) (PLGA) and polylactide (PLA) [56] and non-biodegradable polymers as latex, silica, polystyrene are also under evaluation [57; 58].

With the several opportunities of nanoparticles as a suitable adjuvant for vaccine development, still challenges exist. In the beginning, efforts were made to encapsulate the protein antigen inside particle but harsh manufacturing process resulted degradation of antigen and poor immunogenicity [59]. Later on adsorption of antigen on nanoparticle surface by electrostatic adsorption solved the problem of unnecessary exposure of antigen to manufacturing problems [60; 61]. Another challenge, the co delivery of antigen and immuno stimulants was overcome to some extent by accommodating them inside matrix and adsorbing on surface, respectively. However, the need to lyophilize the product, optimization of lyophilization parameters to ensure antigen stability and increased cost are some bottlenecks of this class of adjuvant.

3.5. ISCOMS

ISCOM and ISCOMATRIX® are ~40 nm cage-like particles produced by combining a protein antigen, cholesterol, phospholipids and the saponin adjuvant Quil A but the latter does not contain antigen. This matrix traps the protein through apolar interactions [62]. The performance of ISCOM appears to be partially dependent on antigen association, which is predominantly by electrostatic attraction forces [63]. Saponin based adjuvant Matrix-M™ was recently used in a Phase I study of seasonal influenza in elderly even though it displayed potent immune activation but GLP-toxicity studies reported a mild to moderate safety profile for this adjuvant [64]. Combination of TLR9 agonist, and ISCOMATRIX adjuvant

was also evaluated to produce better immunogenic response [65]. Although research is going on ISCOM/ISCOMATRIX® adjuvant to design vaccines for HIV, HSV, HPV, HCV and cancer (utilizing NY-ESO-1 as the antigen) but the safety issue due to toxicity of saponins at elevated levels, instability, manufacture and cost are hindering their clinical development.

3.6. Cochleate

Cochleates are highly stable structure consisting of a large tube, roll or spiral shaped lipid bilayers they were first reported by Papahopoulus and co-workers in 1975 [66]. Cochleates are derived from the interaction of anionic lipid vesicles with divalent cations (Ca^{2+} and Mg^{2+}) wherein the function of cation is to establish an ion bridge between two negative charges of lipids from adjacent membranes to stabilize the structure. Antigens can be trapped during the process of roll-up protecting them from environmental exposure and allowing a very slow delivery of antigen to the immune system [67]. Proteoliposome-derived cochleate containing recombinant gD protein were administered to mice intranasally against genital herpes [68]. AFCo1, a meningococcal B-derived cochleate adjuvant, was also reported to strongly enhance antibody and T-cell immunity against *Plasmodium falciparum* merozoite surface protein 4 and 5 [69].

3.7 Polymers

Polymer and co-polymer adjuvant were also evaluated for their potential to enhance immunogenicity. Pluronic was the most extensively used copolymer for this purpose. Protein antigens (tetanus toxoid, diphtheria toxoid and anthrax recombinant protective antigen) were formulated with pluronic F127 in combination with CpG motifs or chitosan and it was found that IgG antibody response was significantly enhanced by the F127/CpG and F127/chitosan combinations compared to antigens mixed with CpGs or chitosan alone or aluminum salts [70]. Optivax®, a flexible, linear, active nonionic block copolymers with a core of hydrophobic polyoxypropylene flanked on both ends by hydrophilic polyoxyethylene was also evaluated for this purpose [71]. Optivax® oil formulation (OF) and Optivax® aqueous formulation (AF), were compared for induction of immunity to encephalitogenic and regulatory T-cell receptor V-gene determinants. In studies performed in lewis rats immunized with myelin basic protein, Optivax® OF was found to be more efficient than Optivax® AF for inducing delayed type hypersensitivity, T-cell proliferation, antibodies, and autoimmune encephalomyelitis (mild sign) while Optivax ® OF was more efficient for inducing inflammatory T-cell and antibody responses to immuno regulatory Vβ8.2 proteins and peptides which induced a non-inflammatory Th2 response. These data suggest the differential adjuvant effects of Optivax® OF versus Optivax® AF for induction of Th1 versus Th2 responses [72].

4. ADVANCEMENTS IN ROUTES OF ADMINISTRATION OF VACCINE

Till now we are dependent upon intravenous route to administer vaccine which, in general, fails to induce a pathogen-specific mucosal immunity because of mucosal invading nature of most of the pathogens. Second important hurdle in case of injectable vaccine is Cold-chain management, failing which adequate response of vaccine cannot be expected although lyophilized vaccines are available but they require reconstitution in diluents at the time of use under sterile conditions which may sometime affect it adversely. Along with these challenges universal fear of needle sticks and difficult administration has attracted the attention towards vaccine delivery through other routes.

4.1. Oral delivery

Polio vaccine Sabin is the successfully commercialized product through oral route but progress in oral vaccination is rather slow due to the many hurdles posed by the gastrointestinal tract [73]. Dilution during the transport of the vaccine through the gastrointestinal tract which requires a higher concentration for the vaccine to be administered and pH instability are some of them [74].

4.2. Nasal delivery

Dry powder formulations can afford better stability characteristics for a vaccine and potentially reduce the requirements for cold-chain management or the addition of preservatives. Intranasal vaccine (FluMist®) is a licensed product for nasal route at the same time dry powder inhaler brought lots of innovative way for vaccine delivery [75]. The GelVac technology developed by DelSite Biotechnologies (Irving, Texas) consists of dry powder formulations of a vaccine with a natural plant-derived acidic polysaccharide material. On contact with the nasal mucosa, DPI generates a muco-adhesive gel with entrapped antigen and provides a mechanism for the prolonged exposure of the antigen to the nasal mucosal tissue. This method of vaccine delivery is potentially adaptable for inactivated antigens, live attenuated viruses, and DNA vaccines. Other recent technologies are VersiDoser (Mystic Pharmaceuticals), Dry Powder Inhaler (Becton Dickinson) and Optimist an exhalation-actuated device (Optinose, Ltd) for bidirectional intranasal drug and vaccine delivery [76].

4.3. Transdermal delivery

Mark Kendall, a biomedical engineer recently presented a Nanopatch which was coated with dry vaccine. It was a one-centimeter-square silicon patch with around 20,000 invisible micro projections on its surface. It was smaller than a postage stamp and provided ease to application as well as transport. Another advantage of Nanopatch over needles based system is better immune response as it makes thousands of small projections into the skin where immune cells are abundant. In terms of cost also they are more effective as they require less vaccine. BD Soluvia™ is an example of licenced intradermal vaccine using microinjection system [75; 77].

5. CHALLENGES FOR COMMERCIALIZATION

In this section major challenges faced by world, in implementing these scientific advances to improve effectiveness of vaccination and technical challenges faced by research team are discussed. Starting from technical challenges, the mechanism of cellular entry for various nanomaterials is still a mystery [78]. Studies have also raised concern regarding the toxicity of some nanomaterials such as carbon nanotubes [79]. Process of scaling

up and building manufacturing facilities for successful clinical applications is also not fully developed. Although lithography and microfluidic chip technology can support in this aspect but still much research is required to make them commercialized at large scale [80; 81; 82].

In relation to medical and scientific challenges, many developing countries face lack of awareness regarding existence of problem, limited data on disease burden, and a weak scientific basis. Furthermore, in developing countries many children who are the prime target for vaccine administration, suffer from malnutrition (including zinc, vitamin A, and selenium deficiencies), parasitic infection, multiple infections with more than one pathogen, and mucosal abnormalities thus targeting a particular disease becomes difficult. Structural and demographic obstacles include poor infrastructure, logistic problems, expanding populations, and diversity. Under societal and cultural issues, the major obstacles are poverty, illiteracy (especially among women), religious taboos, superstition, influence of traditional healers/shamans, and an overemphasis on curative, rather than preventive medicine. Along with these problems, economic issues like limited resources, high cost of vaccines, competing priorities, national pride and fear of dependence on industrialized countries also hinder the mass commercialization of vaccines in developing world [83].

6. CONCLUSIONS

Entry of nanotechnology into vaccine world explored a bright research area which along with development in terms of route of delivery of vaccine, will solve many problems regarding safety and stability of vaccine. So from the future perspective, development of vaccines using combined strategic approach like nanocarriers delivered by mucosal route of delivery can play a major role in the treatment of infectious diseases but along with scientific advancements there is a need to pay serious attention and take effective steps for mass immunization of vaccine in developing countries then only a dream of fully immunized future world will come into reality.

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