**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) led 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].

**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 CD4+ T-helper cells or CD8+ 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 availability [5]. However, some limitations 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].

![Adjuvants used in vaccine formulation](image)

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>
<thead>
<tr>
<th>No.</th>
<th>Clinical trial</th>
<th>Condition</th>
<th>Antigen</th>
<th>Adjuvant</th>
<th>Phases</th>
<th>NCT no.</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Decitabine, Vaccine Therapy, and Puromycin Liposomal Doxorubicin in Treating Patients With Recurrent Ovarian Epithelial Cancer, Fallopian Tube Cancer, or Peritoneal Cancer</td>
<td>Cancer</td>
<td>Decitabine, Vaccine Therapy, and Puromycin Liposomal Doxorubicin</td>
<td>Liposomal Adjuvant, CAF01</td>
<td>I</td>
<td>NCT01673217</td>
<td>Completed</td>
</tr>
<tr>
<td>3</td>
<td>A Phase II, Safety, Tolerability and Immunogenicity of a Plant-made H7 Virus-like Particle (VLP) Influenza Vaccine in Adults</td>
<td>Respiratory Tract Infections</td>
<td>H7 VLP vaccine</td>
<td>Alhydrogel, Alhydrogel, Alhydrogel, Alhydrogel, Alhydrogel, Alhydrogel, Alhydrogel, Alhydrogel</td>
<td>I</td>
<td>NCT01951654</td>
<td>Not started recruiting</td>
</tr>
<tr>
<td>4</td>
<td>Study to Assess dHER2+AS15 Cancer Vaccine Given in Combination With Lapatinib to Patients With Metastatic Breast Cancer</td>
<td>Metastatic Breast Cancer</td>
<td>dHER2</td>
<td>AS15 liposomal formulation (GlaxoSmithKline)</td>
<td>I/II</td>
<td>NCT00952692</td>
<td>Completed</td>
</tr>
<tr>
<td>5</td>
<td>Phase II, Maintenance Therapy Study of OncoVacc in Patients With Stage III Melanoma</td>
<td>Stage III Melanoma</td>
<td>OncoVacc</td>
<td>CAF01</td>
<td>I/II</td>
<td>NCT0015187</td>
<td>Completed</td>
</tr>
<tr>
<td>6</td>
<td>Vaccine Therapy Plus Interleukin-2 With or Without Interferon Alpha-2b in Treating Patients With Stage IA to IA-II Melanoma</td>
<td>Stage IA to IA-II Melanoma</td>
<td>Interleukin-2</td>
<td>Liposome</td>
<td>I</td>
<td>NCT02991038</td>
<td>Active</td>
</tr>
<tr>
<td>7</td>
<td>Study of Quadrivalent Human Papillomavirus (HPV) Virus-Like Particle (VLP) Vaccine in the Prevention of HPV 6, 11, 16, 18 Infection</td>
<td>Papilloma virus infections</td>
<td>HPV 6, HPV 11, HPV 16, HPV 18</td>
<td>V501-007</td>
<td>I</td>
<td>NCT00365716</td>
<td>Active</td>
</tr>
<tr>
<td>8</td>
<td>Safety and Immunogenicity Study of Intramuscular Cys/C-Adjuvanted Influenza Vaccine in the Elderly</td>
<td>Respiratory Syncytial Virus (RSV)</td>
<td>RSV-F Protein</td>
<td>Liposome</td>
<td>I</td>
<td>NCT01709019</td>
<td>Completed</td>
</tr>
<tr>
<td>9</td>
<td>A Phase I Safety Study of a Cancer Vaccine to Treat HLA-A2 Positive Advanced Stage Ovarian, Breast and Prostate Cancer</td>
<td>Cancer</td>
<td>Cancer Vaccine</td>
<td>Liposome</td>
<td>I</td>
<td>NCT01095848</td>
<td>Unknown</td>
</tr>
<tr>
<td>Study ID</td>
<td>Study Title</td>
<td>Disease/Condition</td>
<td>Pathogen/Agent</td>
<td>Adjuvant</td>
<td>Clinical Trials</td>
<td>Study Status</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-----------</td>
<td>----------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>A/H5N1 Virus-Like Particle Antigen Dose Ranging Study With Adjuvant I/II</td>
<td>Influenza (Pandemic)</td>
<td>A/H5N1 Virus-Like Particle</td>
<td></td>
<td>NCT01594320</td>
<td>Completed</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>H5-VLP + GLA-AF Vaccine Trial in Healthy Adult Volunteers</td>
<td>Influenza A, H5N1 Infection</td>
<td>H5-VLP</td>
<td>GLA-AF</td>
<td>NCT01657929</td>
<td>Completed</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>A(H7N9) VLP Antigen Dose Ranging Study With Adjuvant 1</td>
<td>Avian influenza</td>
<td>(H7N9) VLP</td>
<td></td>
<td>NCT01897701</td>
<td>Ongoing</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>A(H7N9) VLP Antigen Dose-Ranging Study With Matrix-M1™ Adjuvant</td>
<td>Influenza (Pandemic)</td>
<td>(H7N9) VLP</td>
<td>Matrix-M1™</td>
<td>NCT02078674</td>
<td>Recruiting</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Study to Evaluate the Safety and Immunogenicity of MEDI-517P in Healthy Adult Female Volunteers Who Are HPV-16 or HPV-18 DNA Positive</td>
<td>Healthy</td>
<td>MEDI 517</td>
<td>Alum. Hydroxide</td>
<td>NCT00263744</td>
<td>I/II Completed</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Efficacy and Immunogenicity Study of Recombinant Human Papillomavirus Bivalent (Type 16/18) Vaccine</td>
<td>Cervical Intraepithelial Neoplasia</td>
<td>HPV 18 virus-like particle</td>
<td>Alum adjuvant</td>
<td>NCT01735006</td>
<td>Recruiting participants</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Bivalent Norovirus Vaccine Study</td>
<td>Gastroenteritis</td>
<td>GI.1/GI.4 Bivalent Virus-Like Particle(VLP)</td>
<td>Monophosphoryl Lipid A (MPL) and Alum. Hydroxide</td>
<td>NCT01168401</td>
<td>I Completed</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>ICC-1132 - Candidate Vaccine Against P Falciparum Malaria</td>
<td>P. Falciparum Malaria</td>
<td>ICC-1132 VLP</td>
<td>Alhydrogel</td>
<td>NCT00587249</td>
<td>I Completed</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Broad Spectrum HPV Vaccine Dose Escalation Study (V502-002)</td>
<td>Human Papilloma Virus</td>
<td>(HPV) L1 Virus-Like Particle</td>
<td>(AAHS) and ISCOMATRIX™ (IMX)</td>
<td>NCT00851643</td>
<td>I Completed</td>
<td></td>
</tr>
</tbody>
</table>

**ISCOM™ AS ADJUVANT**

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Study Title</th>
<th>Disease/Condition</th>
<th>Pathogen/Agent</th>
<th>Adjuvant</th>
<th>Clinical Trials</th>
<th>Study Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Study of a Parenterally Administered H5N1 Influenza Vaccine in Healthy Adults (PANFLUVAC)</td>
<td>Healthy</td>
<td>H5N1 Influenza antigen</td>
<td>3rd generation ISCOM™</td>
<td>NCT00868218</td>
<td>Ongoing</td>
</tr>
<tr>
<td>2</td>
<td>Study of NY-ESO-1 ISCOMATRIX® in Patients With Measurable Stage III or IV Melanoma</td>
<td>Melanoma</td>
<td>NY-ESO-1</td>
<td>ISCOM™</td>
<td>NCT00518206</td>
<td>Completed</td>
</tr>
</tbody>
</table>

**PROTEASOME**

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Study Title</th>
<th>Disease/Condition</th>
<th>Pathogen/Agent</th>
<th>Adjuvant</th>
<th>Clinical Trials</th>
<th>Study Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immunotherapy of Melanoma With Tumor Antigen RNA and Small Inhibitory RNA Transfected Autologous Dendritic Cells</td>
<td>Metastatic Melanoma</td>
<td>siRNA and tumor antigen RNA</td>
<td>Proteasome</td>
<td>NCT00672542</td>
<td>I Completed</td>
</tr>
</tbody>
</table>

**MISCELLANEOUS**

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Study Title</th>
<th>Disease/Condition</th>
<th>Pathogen/Agent</th>
<th>Adjuvant</th>
<th>Clinical Trials</th>
<th>Study Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Vaccine Therapy in Treating Patients With Stage II Melanoma That Can Be Removed by Surgery</td>
<td>Melanoma (Skin)</td>
<td>Tyrosinase/gp100 peptide</td>
<td>Montanide ISA 51 , a Block Co-Polymer CRL 1005 or With GM-CSF</td>
<td>NCT0003274</td>
<td>II Completed</td>
</tr>
<tr>
<td></td>
<td>Inactivated Influenza A/H9N2 Vaccine With and Without MF59 Adjuvant in Ambulatory Adults</td>
<td>Influenza</td>
<td>A/H9N2 Vaccine</td>
<td>MF59</td>
<td>NCT00133471</td>
<td>I/II Completed</td>
</tr>
</tbody>
</table>
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 and essentially non-infective in nature. They form particles of 20–100 nm. 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 different 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 Freund’s 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 adjuvant 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 pandemic 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 Fluarid 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 (Al₂O₃) 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 (HSN1 avian and seasonal) and HSV-2 [54]. Cholesterol, an important component of lipid 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]. Similarly, 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 (Ca²⁺ and Mg²⁺) 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]. AFC01, 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 VJβ8.2 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.

7. REFERENCES


Dear Readers,
Curr Trends Pharm Sci, welcomes comments and suggestions on any of the topics covered in the issue.

Letters, E-mails should be addressed to:

Editorial Office:
National Institute of Pharmaceutical Education and Research
Balanagar, Hyderabad - 500 037, Andhra Pradesh, INDIA
Telefax: +91-40-23073751, Phone: +91-40-2307340
E-mail: ctps@niperhyd.ac.in; website: www.niperhyd.ac.in