Health Biotechnology and Biopharma (2017), 1(2): 5-24

Review Article

Chemical aspects of antigen nano carriers for vaccine delivery

Shahrbano Moradpour [1], Soroush Sardari [1]*

[1]Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

***Corresponding author:** Soroush Sardari, Department of Medical Biotechnology, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran. Email: ssardari@hotmail.com*

DOI: 10.22034/HBB.2017.08

Received: August 5, 2017; **Accepted:** September 2, 2017

ABSTRACT

Scientists could take many approaches to design and classify vaccines against a microbe. These choices could be typically based on fundamental information about antigen carriers of vaccines, how to produce them synthetically and which chemical reactions involved for attaching antigen to carriers. This review briefly describes design and development of novel vaccine carriers.

Keywords: Vaccine delivery, nano carriers, liposome, dendrimer, cellular immune response

INTRODUCTION

Recently attention has been directed toward the aspect of antigen carriers as a tool for delivering vaccine to target cells. Antigen carriers as nanoparticles (NPs), polysacharids or proteins could be conjugated into antigens. The NPs, as antigen carriers could be acted by encapsulating antigenic materials. Antigens conjugated onto antigen carriers especially NPs increase immunogenicity to the immune systems that it would be presented by the pathogen. In addition, The NPs not only guide antigens to the targeted sites, but also it could increase release of antigens. This review has paid attention to the chemical aspects of virus like particles (VLPs), liposomes, Immunostimulating complex (ISCOMs), polymeric NPs, and non-degradable NPs, proteins and polysacharids as delivery

systems. The vaccines prepared by these methods will be given antigen-specific cellular immune responses to many infectious diseases. The following classification of antigen carriersystems might be considered by researchers.

Nanoparticles for vaccine delivery

In the past decade nanoscale materials such as virus-like particles, liposomes and nondegradable nanospheres have delivered vaccine antigens which can play as stabilizers and adjuvants. In addition, antigens are entered by a number of nanoparticles (NPs) through various pathways, with modulating antigen's immune response. As a consequence, induction of protective Th1-type immune responses to intracellular pathogens might be critical. Nanoparticles are suitable for antigen delivery at mucosal surface and intradermal administration due to their features. NPs are applied for the delivery of vaccines such as virus like particles (VLPs), liposomes, ISCOMs, polymeric NPs, and non-degradable NPs for microbial proteins.

The Virus-Like Particles (VLPs) and their modification

VLPs compared to NPs systems could be produced easily and are able to stimulate strong immune responses [1-3]. VLPs are formed in the size range between 20-150 nm from a single protein to construct a complex as a self-assembled viral envelope indicating a prominent epitopes density [2, 4]. The assembly of VLPs occurs without encapsulation for any viral RNA illustrating the replication does not give rise to VLPs. As a result, the VLPs are noninfectious.

In order to create T and B-cells epitopes considerable attention has been to evolve antigen carrier systems. Among, the evolution of hybrid VLPs has validated that non-replication vectors introduce an efficient and safe approach for showing immune responses. Some of the recombinant VLPs including HBs Ag (hepatitis B surface antigen), Ty-VLPs (Ty viruslike particles), PPV-VLPs (porcine parvovirus virus-like particles) and PV-VLPs (papillomavirus virus-like particles) operate powerful in antigen delivery systems [5].

The hepatitis B virus (HBV), small envelope protein (HBsAg-S) is able to self- assemble VLPs. For example particles with 22 nm size are comprising about 100 HBsAg-S molecules effecting in priming cellular and humoral responses even without adjuvant, thus nanoparticles can be used in hepatitis B vaccination [5, 6]. For chemical modification of virus-like particles, protein capsids derived from viruses may be modified. The employed methods are based on the modification of lysine, cysteine, and tyrosine side chains, the installation of unnatural amino acids and the specific challenges involved by the polyvalence and size of virus-based scaffolds [7].

Virus-like particles could be modified by chemical techniques. These techniques are Traditional Bioconjugation Strategies, Tyrosine-Selective Bioconjugation Strategies and Copper (I)-Catalyzed Azide-Alkyne Cycloaddition.

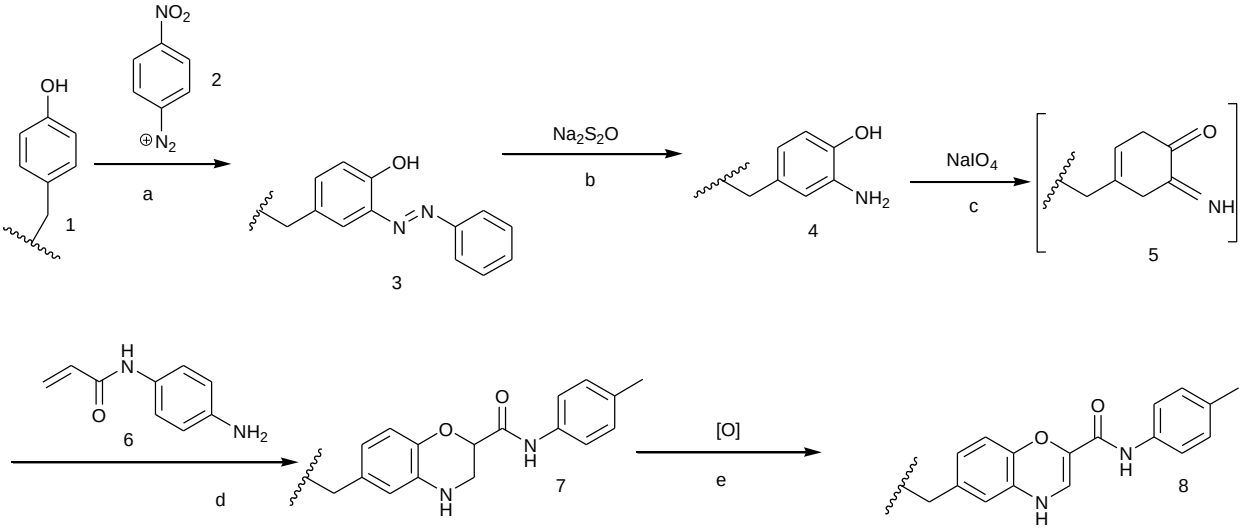
Traditional Bioconjugation Strategies

Traditional bioconjugation methods are used for covalent modification of virus- like particles are listed as follow: Amino groups of lysine side chains and the *N* -terminus usually are acylated with *N*-hydroxysuccinimide esters or isothiocyanates. Thiol groups of the sulfhydryl group of cysteine are usually alkylated with maleimides or bromo/iodo acetamides. Carboxylic acid residues are activated and captured using carbodiimides (usually 1-ethyl-3-(3-dimethyll aminopropyl)carbodiimide hydrochloride, *EDC*) and amines [7, 9].

Tyrosine-Selective Bioconjugation Strategies

The aromatic groups of tyrosine and tryptophan provide the opportunity for chemical modification by bioconjugation similar to amines and thiols groups in lysine and cysteine, respectively (Scheme 1) [7,10,11].

The exploitation of tyrosine on virus particles could be performed by three methods. The oxidation of phenol groups of tyrosine can be occured by one electron using persulfate reagents, and mediated by the nickel complex of the gly-gly-his (GGH) tripeptide or by the photochemical action of tris (2,2Î-bipyridyl) ruthenium (II) [12-14].



Scheme 1. Modification of Tyrosine

This observation was achieved by the addition of disulfide trapping agents to thio ether derivatives of surface-exposed tyrosine residues on Cowpea Mosaic Virus (CPMV) as well as to dityrosine crosslinks within the capsid [7, 15].

Francis *et al.* reported a number of methods for bioconjugation and used them for virus modification [7, 16].

Copper (I)-Catalyzed Azide-Alkyne Cycloaddition

The modification of virus surface in order to position joined structures could be occured by incorporation of unnatural amino acids carrying azide and alkyne side chains into capsid proteins under genetic regulation [7]. The incorporation of azidohomoalanine in place of methionine in both the hepatitis B virus (HBV) particle and the bacteriophage Qß capsids expressed in *E. coli* was performed by the auxotroph technology conducted by Tirrell [7, 17-19].

Labeled materials are resulted from rigid control over the expression of protein and next cycloaddition reaction between azide and alkyne gives rise to these positions straightforward.

Immunostimulating complex (ISCOMs)

ISCOMs are a collection of colloidal saponin including micelles of approximately 40 nm as self-adjuvanting vaccine delivery systems. There are two kinds of ISCOMs and whichever consisting of saponin (most often Quil A form the tree *Quillaia saponaria*), cholesterol, phospholipid (phosphatidylethanolamine or phosphatidylcholine) [20-22].

Viral envelope proteins such as from herbs simplex virus type 1, hepatitis B, and influenza could be entrapped using ISCOMs. On the other hand, the assembly of ISCOMs could be resulted from the protein derived from a type of bacteria and parasites plus *E. coli*, *Brucella aborus*, and *Plasmodium falciparum* [23-25].

These complexes without viral proteins also could be utilized and known as ISCOM matrices and their ratio of cholesterol, phospholipid and saponin are 1:1:5.Then non-ionic detergent was eliminated using dialysis or ultracentrifugation [26]. As a result, the complex has been arranged in a pentagonal dodecahedron structures of micelles consisting of saponin and lipid joined with together by hydrophobic interactions and stabilized through its negative surface charge [20, 27].

Construction and Modification of liposomes as antigen carrier

Approximately 35 years ago, liposomes were used as vaccine adjuvants [28]. Liposomes are comprised of an aqueous core surrounded by a hydrated phospholipidbilayer, typically neutral lipids like phosphatidylcholine (PC), and cholesterol. In addition, they could be prepared

by different construction approaches and their composition could be altered. Due to existing hydrophobic and hydrophilic parts in liposomes, they are appropriate for delivering antigens and immune-stimulatory molecules with physiochemical features. Liposomes could be applied as vesicles [29] and it is notable for the intracellular delivery of an antigen or a vaccine. The surface modification of liposomes can be performed by covalently coupling the phospholipid head groups and reactive groups on liposomes and the targeting molecules. The biocompatibility and non-toxicity of liposomes can be considered because their material composition is extremely indistinguishable to the composition of mammalian cell membranes.

General methods of liposome preparation are passive and active loading techniques. Passive preparation of liposome methods include mechanical and solvent dispersion techniques [30, 31]. In addition, all the preparation of liposome methods contain four essential steps:

1. Removal of organic solvent.
2. Hydration of the lipidic film in an aqueous media.
3. Centrifugation of the resultant liposome.
4. Analyzing the liposome.

Therefore, lipid bilayers of liposomes should be formed consisting of functional groups that are able to be chemically altered or cross-linked by those macromolecules [32-34].

Generally, the conjugation of liposomes efficiently and selectively occurs by three major reactions, reaction between activated carboxyl groups and amino groups yielding an amide bond.

Reaction between pyridyldithiols and thiols could result in disulphide bonds, and reaction between maleimide derivatives and thiols yielding thio ether bonds [32]. Another approach is the reaction of an activated ester groups such as N-succinimidyl or p-nitrophenyl carbonyl located onto liposomes's surface with amino groups [35]. A further example of liposomes surface modification is the reaction between carboxylic groups of immunoglobulins and water-soluble carbodiimide. Therefore, this activated protein could be bound to the surface with free amino groups [36]. The exposure of liposomes surface with carboxylic groups can provide the possibility for a directed activation with water-soluble carbodiimide before adding various lignds [37].

Another example of liposomes modification is the modification of reactive groups on liposomes surface by using heterobifunctional cross-linking reagents. Therefore, the synthesis of a lipid derivatives was performed using N-succinimidyl-3-(2-pyridyldithi) propionate (SPDP) coupling to SH-containing proteins for further modification [38].

In addition, it is possible to use thiol groups to react to a ligand with maleimide-containing phospholipid molecules [39]. This method is extensively used for formation of liposomal conjugate where maleimide carring phospholipids are prepared using various maleimide reagents in a reaction [39]. Furthermore, pyridyl-dithiopropionyl or maleimide cross-linking reagents can provide the possibility of different high and lowmolecular weight compounds attached to liposomes [40, 41].

Moreover, SH groups such as thiol groups are positioned on immunoglobulin species, which are far from antigen-binding sites, can be used in this reaction. Whereas, it enables the liposome attached antibody fragments to retain their particular interaction with antigens [40, 41]. Virosomes as liposomal vaccine based on viral proteins are used as vaccine for hepatitis A and influenza [42].

Liposomes are prepared under buffer conditions which can provide an opportunity for proteins incorporation without any denaturation. On the other hand, traditional difficulties on encapsulated protein and stable release of associated antigen could deficiently be adjusted.

Dendrimers conformation and their aspects in vaccine delivery

A new type of synthetic macromolecules are branched and nanoscale dendrimers with high functionality. The structures of the dendrimer has a great impact on their physical and chemical properties. These molecules have important application in nanotechnology and pharmaceutical chemistry. Also dendrimers are suitable for biomedical applications.

Vogtle *et al.* reported the first application of dendrimer as a new group of monodispersed branched polymers in 1978 [45, 46].

Subsequently, the initial foundational constitutions of small hyper branched molecules for the next model of polypropylene imine (PPI) dendrimers were developed by the Meijer and Muhlhaupt groups in the early 1990s [47, 48]. Two synthetic approaches, divergent and convergent methods (Fig. 1), involve in dendrimer production in divergent method, the polymeric structure occurs from the central core toward the surface. Whereas, the convergent ones start to grow according to top-down approach from the edge or boundary of dendrimers [49-51]. Both methods have advantages and disadvantages and the designed structure determines what appropriate route to use in dendrimer production. Although, the construction of high generation denrimers is conceivable using divergent method. While, the steric resistance existed in higher generation dendrimers gives rise to defects on the surface of dendrimers. In addition, growing effects are suppressed by product purification after each

repetitive series of synthesis steps. In comparison with divergent method, the convergent one is easily purified, characterized and capable to attach different types of dendrons to one dendrimer. On the other hands, the attachment of large dendrons to the central core will create steric constraints when one tries convergent method [49-51].

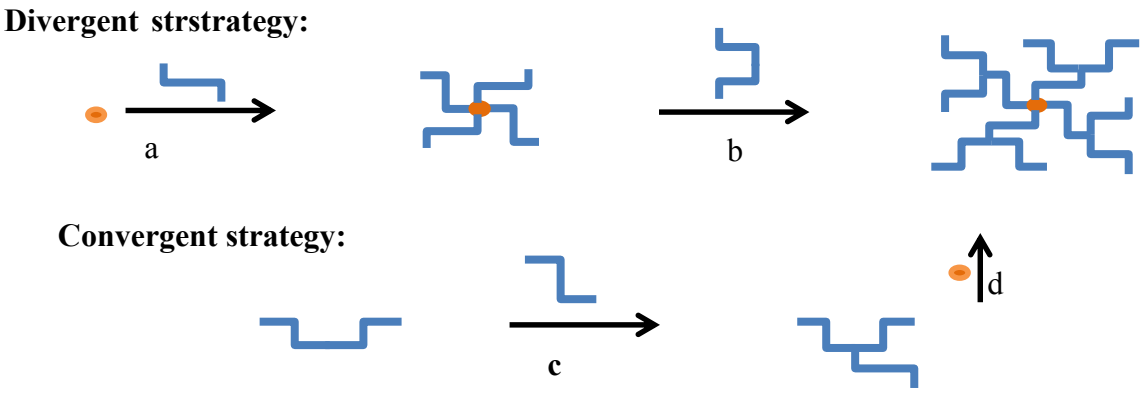


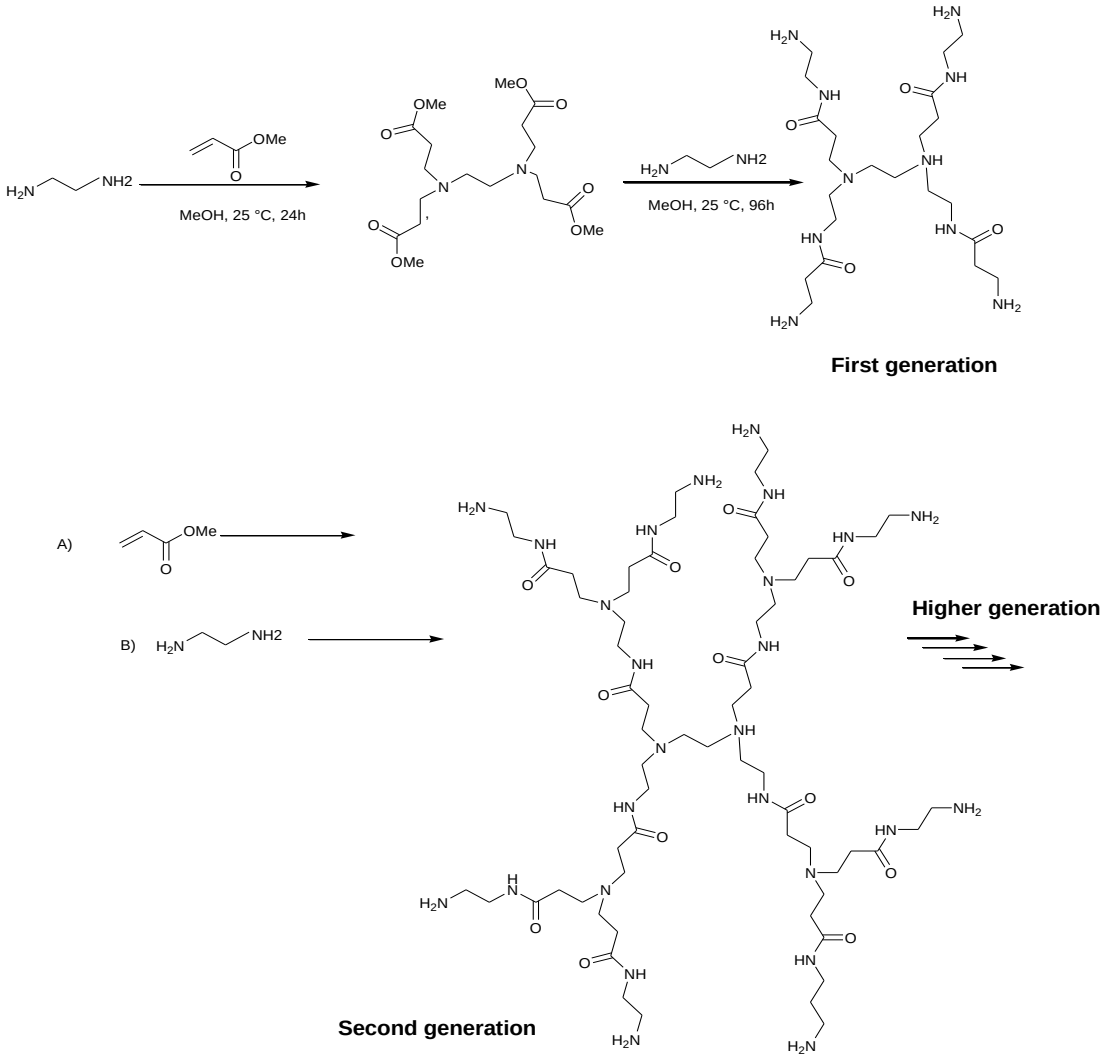
Figure 1. Schematic representation of dendrimers conformation.

At the present polydiamido amine (PAMAM) and polypropylenimine (PPI) dendrimers are commercially available and synthesized using the divergent method [50, 52]. PAMAM dendrimers are synthesized by Michael addition between the multifunctional central core (ammonia or ethylene diamine) and methyl acrylate. The resulted product consisting of three or four ester groups reacts with an excess amount of ammonia or ethylene diamine in order to amidation and form a molecule containing three or four reactive amine groups. The repetition of the Michael addition and amidation reaction results in high-generation dendrimers. These achieved products, full-generation dendrimers (G2, G3, G4), and half-generation dendrimers (G2.5, G3.5, G4.5) with primary amine and carboxylate groups as terminal functional groups, respectively [53-55].

Table 1. Advantages, disadvantages, preparation and classification methods of liposomes [30, 31, 43-44]

Advantages of liposome	**Disadvantages of liposome**	**General methods of liposome preparation**	**Method of liposome preparation and drug loading**	**Passive loading techniques**	**Mechanical dispersion method**	**Classification of liposomes**	**Unilamellar vesicles classification**
Liposomes increased efficacy and therapeutic index of drug (actinomycin-D)	Low solubility	Drying lipids from organic solvent	Passive loading techniques	Mechanical dispersion method	Sonication.	multilamellar vesicles (MLV)	large unilamellar vesicles (LUV)
Liposomes increased efficacy and therapeutic index of drug (actinomycin-D)	Short half-life	Dispersing the lipid in aqueous media	Active loading technique	Solvent dispersion method	French pressure cell: extrusion	unilamellar vesicles. Unilamellar vesicles	small unilamellar vesicles (SUV)
Liposomes are non-toxic, flexible, biocompatible, biodegradable, and non-immunogenic	Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction	Dispersing the lipid in aqueous media		Detergent removal method	Freeze-thawed liposomes		
Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol)	Leakage and fusion of encapsulated drug	Analyzing the final product			Lipid film hydration by shaking or freeze drying		
Liposomes help reduce the exposure of sensitive tissues to toxic drugs	Production cost is high				Micro-emulsification.		
Site avoidance effects	Fewer stables				Membrane extrusion		

In conventional liposomes, a single phospholipid bilayer vesicle is enclosed to the aqueous media. In multilamellar liposomes, phospholipid vesicles have an onion structure. Also, several vesicles may be formed inside the other vesicles with greater size, making a structure of concentric phospholipid vesicles.



Scheme 2. Formation of PAMAM (polydiamido amine) dendrimers [53-55]

Dendrimers are synthesized by divergent method through additional reaction between nitrile groups and primary amino groups [13, 14]. Although, the divergent method has fasilated the availability and the synthesis of dendrimers. Due to the specific structure of dendrimers which are able to display molecules at their surface. As a result, they will be potentially effective adjuvants for epitope-based vaccines. In addition, the particular features of dendrimers such as

molecular weight, particle size and lipophilicity could be modulated through slight modifications in the preparation conditions [46, 56, 57]. For example, a synthetic derivative of poly amidoamine dendrimers consisting of the PADRE peptide, a repetitive series of aminoacid sequence reported by Daftarian *et al.* which showed immunostimulant features for the delivery of a genetic vaccine with antitumor activity [58]. These systems accompanied to Tyrosinase-related protein 2 (TRP-2) as an antigen are applied for subcutaneous immunization of B16 melanoma tumor-bearing mice. In addition, a protective immune response, the delay in tumor growth and reduction in the size of the tumor were demonstrated by the animals receiving the antigen-loaded PADRE-dendrimers. In contrast to responses obtained upon injection of the antigen-loaded dendrimers without the PADRE peptide and antigen alone, this response was notably higher. Furthermore, these carriers (PADRE) could associate and deliver different plasmids and proteins, which are applicable in cancer prophylaxis.

The generation of polymeric NPs and some of their applications as antigen carriers

Polymeric NPs have the ability of drug delivery and are biodegradable [25, 59]. In addition, the alterations of copolymer composition regulate the release kinetics of loaded drugs from polymeric NPs [25, 59]. A series of polymers containing poly (hydroxy acids), poly (amino acids), or poly saccharides forms polymeric NPs creating a vesicle which can accommodate or display antigens.

Polymeric NPs are constructed from poly (hydroxy acids) and divided into polylactic-coglycolic acid (PLGA) and polylactic acid (PLA). The synthetic method for synthesizing these polymeric NPs is double emulsion-solvent evaporation technique [22, 25, 60, 61].

First, a selected polymer was dissolved in an organic solvent like ethyl acetate or methylene chloride and then the antigen was added. Second, the mixture was vortexed to obtain a primary emulsion. In order to emulsify, an emulsifying agent such as polyvinyl alcohol or polyvinyl pyrrolidine was added to form an emulsion. As a result, the polymer was precipitated around the antigen. In addition, the solvent was evaporated and dried to prevent degradation of polymer due to the hydrolysis of water-catalyzed ester [25, 62, 66].

There is a limitation to utilize this technique because of antigen entrapment efficiency is low and proteins might be denatured at the oil-water interface [67].

For stabilizing protein and preventing from denaturation, the stabilizers such as surfactants or sugars, including trehalose and sucrose are added. In contrast, the participation of poly

amino acids such as poly γ-glutamic acid (γ-PGA) or poly L-histidine eliminates the requirement of an emulsion step in the synthesis reaction. Therefore, encapsulated protein retains stable [68-71].

The polymeric structures are formed by self-assembling amphiphilic copolymers through hydrophobic interactions. These structures are containing a hydrophobic core and a hydrophilic outer shell [22, 72]. In addition, the lack of γ-linked glutamic acids recognition in γ-PGA by means of normal proteases results in increased stability. These polymeric NPs are formed by dissolving poly amino acid in dimethyl sulfoxide (DMSO) and NaCl solution. NPs size is regulated through NaCl concentration terminating in monodisperse with a size range between from 30 to 200 nm in diameter [73].

Protein encapsulated γ-PGA could be prepared by adding the antigen to γ-PGA and then are centrifuged. The efficiency of encapsulation is between 30 to 60 % and it is stable under an acidic pH even after 10 days [74].

Among polymeric NPs, Hydrophilic polysaccharide polymers prepared with both dextran and chitosan could be outstanding candidates for vaccine delivery. One method for producing nano particles is self-assembling by chemical modification [75].

The resulting encapsulation is achieved with 30 and 60 % efficiency and it is stable under an acidic pH after 10 days [74]. Hydrophilic poly saccharide constructed from dextran and chitosan are also chosen to prepare NPs as appropriate candidate for vaccine delivery [76].

There are several methods to prepare chitosan NPs. One method is based on chemical modification in order to self-assembly particles with a mean diameter of 160 nm [75].

Another method to produce particles as two hydrophilic colloids spontaneously are mixed together and after with chitosan precipitate around plasmid to form particles with a size range between100 to 250 nm [77]. As a result, DNA is protected from nuclease degradation by these particles.

Tokumitsu developed the emulsion-droplet coalescence method for intra-tumoral injection [78]. This method progresses to produce particles with 450 nm size by chitosan emulsion cross linking and precipitating around the drug, gadopentetic acid and these NPs are evaluated as an outstanding delivery vehicle due to slow release and long-term retention inside the tumor.

Chitosan NPs can be formed in the range of 20 to 400 nm by interaction between the positive charge of amino groups in chitosan and the negative charge of tripolyphosphate [25, 79, 80]. In addition, further modification can be occured by adding poly ethylene glycol to help absorption or to decelerate release.

There are non-degradable NPs such as gold, carbon, and silica for vaccine delivery [25, 81-84]. These nanoparticles could carry antigens through the encapsulation of antigens or by providing a surface for covalent attachment with antigens [25, 81-84].

Nanospheres such as Gold NPs as antigen carrier

Gold NPs with rigid structure are non-biodegradable and are provided by controllable synthesis methods [85]. Chloroauric acid is used as starting solution in the gold NPs production process. Therefore, depending on how strong reducing agent is, gold NPs size can be changed. Gold NPs could have a size range from 2 to 150 nm [86]. However, mainly gold NPs with a range size between from 2 to 50 nm are used [25]. These particles could be fabricated typically monodisperse and uniform in shape which is essential for maintaining antigen loading consistency between groups. The smaller particles are formed in reducing agent condition and then they can be extended to organize larger particles with a desired aspects ratio using cetyl trimethyl ammonium bromide and silver acetate [25, 83, 87-89]. The surface modification of gold NPs can occur using carbohydrates [90, 91]. Gold nanorods can conjugate to an antigen derived from respiratory syncytial virus to be as a carrier. In addition, other kinds of gold NPs could be used as carriers for antigens derived from variant virus such as Influenza and foot-and mouth disease or as a DNA vaccine adjuvant for human immunodeficiency virus (HIV) [89, 92-94].

Carbon nanotubes as antigen carrier

Carbon NPs could be used in vaccine delivery including oral delivery [25, 95]. In carbon NPs production, silica NPs are used as a template, the carbonization of particles can occur at high temperature under nitrogen gas and using sucrose as a carbon source to form particles with a range size about 450 nm while the inside of the particle surface has been embedded with 50 nm mesopores. Using carbon NPs for antigen could be secured from the harsh environment, resulting management to increase mucosal immunity [95]. In addition, these NPs delivery systems are able to bring into focus on small molecular antigens produced by the pathogen instead of whole microbes as with traditional vaccines to increase mucosal immunity [95].

The expression of these components occurs on the microbial membrane while these components might be included polysaccharides, proteins, lipoproteins and glyco proteins. The application of membrane antigens instead of whole microbes for vaccination often decrease immunogenicity. On the other hand, carbon NPs with an

appropriate safety identification are appealing and engaged for future licensing of vaccines [91].

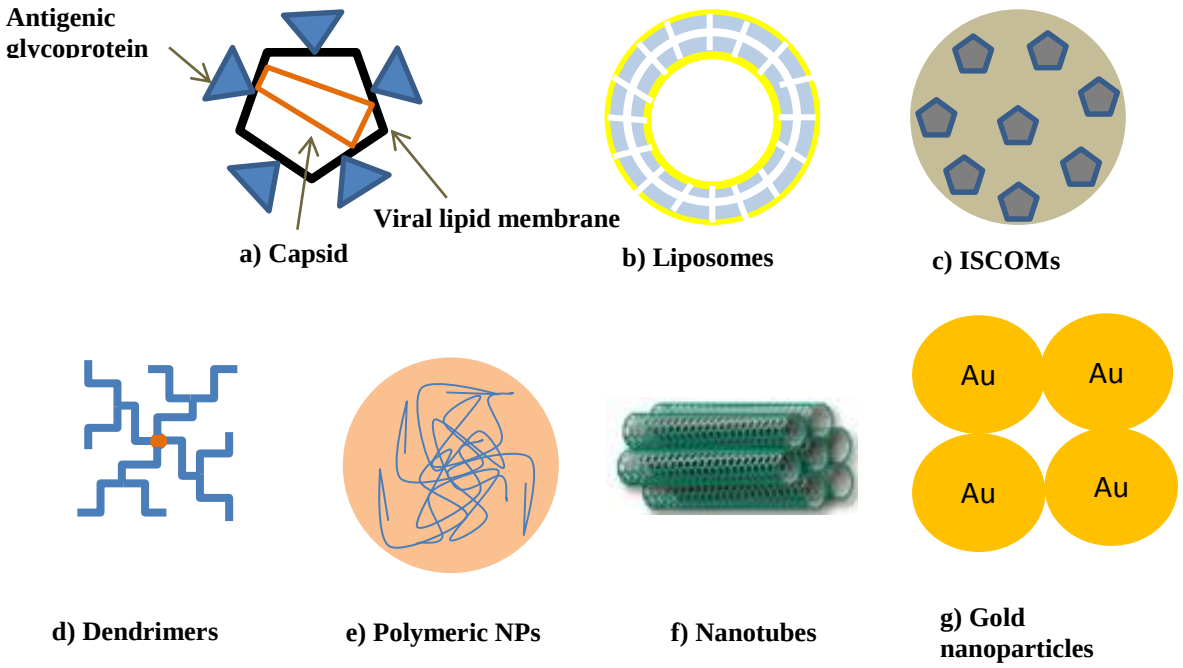


Figure 2. The kinds of typical nanocarriers.

a) virus like particles (VLPs). **b)** Liposomes are vesicles formed from a phospholipid bilayer mimicking a cell membrane structure. **c)** Immunostimulating complex (ISCOMs). **d)** Dendrimers are branched molecules the usually present the associated small drug molecule at their extremities **e)** Polymeric nanoparticles are solid polymeric matrices, Cyclodextrins are cyclic oligosaccharides with an inner lipophilic cavity for drug **f)** Carbon nanotubes. **g)** Inorganic nanoparticles such as gold nanoparticles

CONCLUSION

There are many methods to design and classify vaccines. The classification could typically be relied on fundamental information about antigen carriers for vaccinesand involved for attaching antigen to carriers. This review briefly described the progression in the design and development of novel vaccine carriers. The vaccines prepared by these methods will be given antigen cellular immune responses to many infectious diseases.

REFERENCES

[1]. Kingsman SM, Kingsman AJ. Polyvalent recombinant antigens: a new vaccine strategy. *Vaccine,* 1988; 6(4): 304-06.

[2]. Kalnciema I, Skrastina D, Ose V, Pumpens P, Zeltins A. Potato virus Y-like particles as a new carrier for the presentation of foreign protein stretches. *Mol Biotechnol*, 2012; 52(2): 129-39.

[3]. Roldão A, Mellado MC, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. *Expert Rev Vaccines*, 2010; 9(10): 1149-76.

[4]. Grgacic EV, Anderson DA. Virus-like particles: passport to immune recognition. *Methods*, 2006; 40(1): 60-65.

[5]. Boisgérault F, Moron G, Leclerc C. Virus-like particles: a new family of delivery systems. Expert Rev. *Vaccines*, 2002; 1(1): 101-09.

[6]. Khodabakhsh F, Norouzian D, Vaziri B, Ahangari Cohan, Sardari S, Mahboudi F, Behdani M, Mansouri K, Mehdizadeh A. Development of a novel nano-sized anti-VEGFA nanobody with enhanced physicochemical and pharmacokinetic properties. *Artif Cells Nanomed Biotechnol*, 2017; 1-13.

[7]. Strable E, Finn MG. Chemical modification of viruses and virus-like particles. *Virus Nanotech*, 2009: 1-21.

[8]. Cannon JG. Bioconjugate Techniques. *J Med Chem*, 1997; 40: 631.

[9]. Wong SS. Chemistry of protein conjugation and cross-linking. *CRC Press*, 1991.

[10]. Hooker JM, Kovacs EW, Francis MB. Interior surface modification of bacteriophage MS2. *J Am Chem Soc*, 2004; 126(12): 3718-19.

[11]. Tilley SD, Francis MB. Tyrosine-selective protein alkylation using π-allylpalladium complexes. *J Am Chem Soc*, 2006; 128(4): 1080-81.

[12]. Brown KC, Kodadek T. Protein cross-linking mediated by metal ion complexes. *Met Ions Biol Syst*, 2001; 38: 351-84.

[13]. Amini F, Denison C, Lin HJ, Kuo L, Kodadek T. Using oxidative crosslinking and proximity labeling to quantitatively characterize protein-protein and Protein-Peptide complexes. *Chem Biol*, 2003; 10(11): 1115-27.

[14]. Strable E, Finn MG. Chemical modification of viruses and virus-like particles. *Virus Nanotech*, 2009: 1-21.

[15]. Meunier S, Strable E, Finn MG. Crosslinking of and coupling to viral capsid proteins by tyrosine oxidation. *Chem Biol*, 2004; 11(3): 319-26.

[16]. Antos JM, Francis MB. Transition metal catalyzed methods for site-selective protein modification. *Curr Opin Chem Biol*, 2006; 10(3): 253-62.

[17]. Kiick KL, Weberskirch R, Tirrell DA. Identification of an expanded set of translationally active methionine analogues in Escherichia coli. *FEBS Lett*, 2001; 502(2): 25-30.

[18]. Kiick KL, Saxon E, Tirrell DA, Bertozzi CR. Incorporation of azides into recombinant proteins for chemoselective modification by the

Staudinger ligation. *Proc Natl Acad Sci*, 2002; 99(1): 19-24.

[19]. Kiick KL, Tirrell DA. Protein engineering by in vivo incorporation of non-natural amino acids: Control of incorporation of methionine analogues by methionyl-tRNA synthetase. *Tetrahedron*, 2000; 56(48): 9487-93.

[20]. Kersten GF, Teerlink T, Derks HJ, Verkleij AJ, Van Wezel TL, Crommelin DJ, Beuvery EC. Incorporation of the major outer membrane protein of Neisseria gonorrhoeae in saponin-lipid complexes (iscoms): chemical analysis, some structural features, and comparison of their immunogenicity with three other antigen delivery systems. *Infect Immun*, 1988; 56(2): 432-38.

[21]. Lovgren K, Morein B. The requirement of lipids for the formation of immunostimulating complexes (iscoms). *Biotechnol Appl Biochem*, 1988; 10(2): 161-72.

[22]. Lü JM, Wang X, Marin-Muller C, Wang H, Lin PH, Yao Q, Chen C. Current advances in research and clinical applications of PLGA-based nanotechnology. *Expert Rev Mol Diagn*, 2009; 9(4): 325-41.

[23]. Claassen IJ, OsTERHAus AD. The iscom structure as an immune-enhancing moiety: experience with viral systems. *Res Immunol*, 1992; 143(5): 531-41.

[24]. Morein B, Simons K. Subunit vaccines against enveloped viruses: virosomes, micelles and other protein complexes. *Vaccine*, 1985; 3(2): 83-93.

[25]. Gregory AE, Titball R, Williamson D. Vaccine delivery using nanoparticles. *Front Cell Infect Microbiol*, 2013; 3.

[26]. Kersten GF, Spiekstra A, Beuvery EC, Crommelin DJ. On the structure of immune-stimulating saponin-lipid complexes (iscoms). *Biochim Biophys Acta*, 1991; 1062(2): 165-71.

[27]. Özel M, Hλlund S, Gelderblom HR, Morein B. Quaternary structure of the immunostimulating complex (iscom). *J Ultrastruct Mol Struct Res*, 1989; 102(3): 240-48.

[28]. Allison AG, Gregoriadis G. Liposomes as immunological adjuvants. *Nature*, 1974; 252: 252.

[29]. Copland MJ, Rades T, Davies NM and Baird MA. Lipid based particulate formulations for the delivery of antigen. *Immunol Cell Biol*, 2005; 83: 97-105.

[30]. Himanshu A. Sitasharan P and Singhai AK. Liposome as drug carriers. *IJPLS*, 2011; 2(7): 945-51.

[31]. Riaz M. Liposomes preparation methods. *Pak J Pharm Sci*, 1996; 9(1): 65-77.

[32]. Cevc G. Phospholipids Handbook. ed., New York; Taylor & Francis: 1993.

[33]. Torchilin VP, Levchenko TS, Lukyanov AN, Khaw BA, Klibanov AL, Rammohan R, Samokhin GP, Whiteman KR. P-

nitrophenylcarbonyl-PEG-PE-liposomes: fast and simple attachment of specific ligands, including monoclonal antibodies, to distal ends of PEG chains via p-nitrophenylcarbonyl groups. *Biochim Biophys Acta*, 2001; 1511(2): 397-411.

[34]. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*, 2005; 4(2): 145-60.

[35]. Chonn A, Cullis PR. Ganglioside GM1 and hydrophilic polymers increase liposome circulation times by inhibiting the association of blood proteins. *J Liposome Res*, 1992; 2(3): 397-410.

[36]. Dunnick JK, McDougall IR, Aragon S, Goris ML and Kriss JP. Vesicle interactions with polyamino acids and antibody: in vitro and in vivo studies. *J Nucl Med*, 1975; 16: 484.

[37]. Kung VT, Redemann CT. Synthesis of carboxyacyl derivatives of phosphatidylethanolamine and use as an efficient method for conjugation of protein to liposomes. *Biochim Biophys Acta*, 1986; 862(2): 435-39.

[38]. Leserman LD, Barbet J, Kourilsky F, Weinstein JN. Targeting to cells of fluorescent liposomes covalently coupled with monoclonal antibody or protein A. *Nature*, 1980; 288(5791): 602-04.

[39]. Martin FJ, Papahadjopoulos D. Irreversible coupling of immunoglobulin fragments to preformed vesicles. An improved method for liposome targeting. *J Biol Chem*, 1982; 257(1): 286-88.

[40]. Thomas M, Klibanov AM. Conjugation to gold nanoparticles enhances polyethylenimine's transfer of plasmid DNA into mammalian cells. *Proc Natl Acad Sci*, 2003; 100(16): 9138-43.

[41]. Torchilin VP, Lukyanov AN. Peptide and protein drug delivery to and into tumors: challenges and solutions. *Drug Discov Today*, 2003; 8(6): 259-66.

[42]. Saroja CH, Lakshmi PK, Bhaskaran S. Recent trends in vaccine delivery systems: a review. *Int J Pharma Investig*, 2011; 1(2): 64.

[43]. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. *Nanoscale Res Lett*, 2013; 8(1): 102.

[44]. Sharma A, Sharma US. Liposomes in drug delivery: progress and limitations. *Int J pharm*, 1997; 154(2): 123-40.

[45]. Buhleier EW, Wehner W and VÖGtle F. Cascade and Nonskid-Chain-Like Syntheses of Molecular Cavity Topologies. *Synthesis*, 1978; 2: 155-58.

[46]. Heegaard PM, Boas U, Sorensen NS. Dendrimers for vaccine and immunostimulatory uses: A review. *Bioconjugate Chem*, 2009; 21(3): 405-18.

[47]. de Brabander-van den Berg EMM, Meijer EW. Poly (propylene imine) dendrimers: large-scale synthesis by hetereogeneously catalyzed hydrogenations. *Angew Chem Int Ed*, 1993; 32(9): 1308-11.

[48]. Wörner C, Mülhaupt R. Polynitrile-and polyamine-functional poly (trimethylene imine) dendrimers. *Angew Chem Int Ed*, 1993; 32(9): 1306-08.

[49]. Grayson SM, Frechet JM. Convergent dendrons and dendrimers: from synthesis to applications. *Chem Rev*, 2001; 101(12): 3819-68.

[50]. Abbasi E, Aval SF, Akbarzadeh A, Milani M, Nasrabadi HT, Joo SW, Hanifehpour Y, Nejati-Koshki K and Pashaei-Asl R. Dendrimers: synthesis, applications, and properties. *Nanoscale Res Lett*, 2014; 9: 247.

[51]. Hawker CJ, Frechet JM. Preparation of polymers with controlled molecular architecture. A new convergent approach to dendritic macromolecules. *J Am Chem Soc*, 1990; 112(21): 7638-47.

[52]. Szymański P, Markowicz M, Mikiciuk-Olasik E. Nanotechnology in pharmaceutical and biomedical applications: Dendrimers. *Nano*, 2011; 6(06): 509-39.

[53]. Cheng Y, Xu Z, Ma M, Xu T. Dendrimers as drug carriers: applications in different routes of drug administration. *J pharma Sci*, 2008; 97(1): 123-43.

[54]. Dufes C, Uchegbu IF, Schätzlein AG. Dendrimers in gene delivery. Adv. *Drug Deliv Rev*, 2005; 57(15): 2177-202.

[55]. Wu LP, Ficker M, Christensen JB, Trohopoulos PN, Moghimi SM. Dendrimers in medicine: therapeutic concepts and pharmaceutical challenges. *Bioconjugate chem*, 2015; 26(7): 1198-211.

[56]. Cordeiro AS and Alonso MJ. Recent advances in vaccine delivery. *Pharm Pat Anal*, 2015; 5: 49-73.

[57]. Mahmoudi M, Shokrgozar MA, Sardari S, Moghadam MK, Vali H, Laurent S, Stroeve P. Irreversible changes in protein conformation due to interaction with superparamagnetic iron oxide nanoparticles. *Nanoscale*, 2011; 3(3): 1127-38.

[58]. Li X, Deng X, Yuan M, Xiong C, Huang Z, Zhang Y, Jia W. In vitro degradation and release profiles of poly-DL-lactide-poly (ethylene glycol) microspheres with entrapped proteins. *J Appl Polym Sci*, 2000; 78(1): 140-48.

[59]. O'Donnell PB, McGinity JW. Preparation of microspheres by the solvent evaporation technique. *Adv Drug Deliv Rev*, 1997; 28(1): 25-42.

[60]. Sahoo SK, Panyam J, Prabha S, Labhasetwar V. Residual polyvinyl alcohol associated with poly (D, L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. *J Control Release*, 2002; 82(1): 105-14.

[61]. Sales-Junior PA, Guzman F, Vargas MI, Sossai S, González CZ, Patarroyo JH. Use of biodegradable PLGA microspheres as a slow release delivery system for the Boophilus microplus synthetic vaccine SBm7462. *Vet Immunol Immun*, 2005; 107(3): 281-90.

[62]. Feng L, Qi XR, Zhou XJ, Maitani Y, Wang SC, Jiang Y, Nagai T. Pharmaceutical and immunological evaluation of a single-dose hepatitis B vaccine using PLGA microspheres. *J Control Release*, 2006; 112(1): 35-42.

[63]. Kasturi SP, Qin H, Thomson KS, El-Bereir S, Cha SC, Neelapu S, Kwak LW, Roy K. Prophylactic anti-tumor effects in a B cell lymphoma model with DNA vaccines delivered on polyethylenimine (PEI) functionalized PLGA microparticles. *J Control Release*, 2006; 113(3): 261-70.

[64]. Florindo HF, Pandit S, Gonçalves LM, Videira M, Alpar O, Almeida AJ. Antibody and cytokine-associated immune responses to S. equi antigens entrapped in PLA nanospheres. *Biomaterials*, 2009; 30(28): 5161-69.

[65]. Harikrishnan R, Balasundaram C, Heo MS. Poly d, l-lactide-co-glycolic acid (PLGA)-encapsulated vaccine on immune system in Epinephelus bruneus against Uronema marinum. *Exp Parasitol*, 2012; 131(3): 325-32.

[66]. Sah H. Stabilization of proteins against methylene chloride/water interface-induced denaturation and aggregation. *J Control Release*, 1999; 58(2): 143-51.

[67]. Lee ES, Shin HJ, Na K, Bae YH. Poly (l-histidine)–PEG block copolymer micelles and pH-induced destabilization. *J Control Release*, 2003; 90(3): 363-74.

[68]. Holowka EP, Sun VZ, Kamei DT, Deming TJ. Polyarginine segments in block copolypeptides drive both vesicular assembly and intracellular delivery. *Nat mat*, 2007; 6(1): 52.

[69]. Matsusaki M, Fuchida T, Kaneko T, Akashi M. Self-assembling bionanoparticles of poly (ε-lysine) bearing cholesterol as a biomesogen. *Biomacromolecules*, 2005; 6(4): 2374-79.

[70]. Matsusaki M, Hiwatari KI, Higashi M, Kaneko T, Akashi M. Stably-dispersed and surface-functional bionanoparticles prepared by self-assembling amphipathic polymers of hydrophilic poly (γ-glutamic acid) bearing hydrophobic amino acids. *Chem Lett*, 2004; 33(4): 398-99.

[71]. Letchford K, Burt H. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *Eur J Pharm Biopharm*, 2007; 65(3): 259-69.

[72]. Kim H, Akagi T, Akashi M. Preparation of size tunable amphiphilic poly (amino acid) nanoparticles. *Macromol Biosci*, 2009; 9(9): 842-48.

[73]. Akagi T, Baba M and Akashi M. Biodegradable nanoparticles as vaccine adjuvants and delivery systems: regulation of immune responses by nanoparticle-based vaccine. *Adv Polym Sci*, 2012; 247: 31-64.

[74]. Lee KY, Kwon IC, Kim YH, Jo WH, Jeong SY. Preparation of chitosan self-aggregates as a gene delivery system. *J Control Release*, 1998; 51(2): 213-20.

[75]. Ghavami GH, Sardari S. New chimeric anti-tubercular dendrimers with selfdelivering property. *African J Microbiol Res*, 2011; 5(18): 2550-54.

[76]. Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, August JT, Leong KW. Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. *J Control Release*, 2001; 70(3): 399-421.

[77]. Tokumitsu H, Ichikawa H, Fukumori Y. Chitosan-gadopentetic acid complex nanoparticles for gadolinium neutron-capture therapy of cancer: preparation by novel emulsion-droplet coalescence technique and characterization. *Pharm Res*, 1999; 16(12): 1830-35.

[78]. Fernández-Urrusuno R, Calvo P, Remuñán-López C, Vila-Jato JL, Alonso MJ. Enhancement of nasal absorption of insulin using chitosan nanoparticles. *Pharm Res*, 1999; 16(10): 1576-81.

[79]. Xu Y, Du Y. Effect of molecular structure of chitosan on protein delivery properties of chitosan nanoparticles. *Int J Pharm*, 2003; 250(1): 215-26.

[80]. Calvo P, Remuñan-López C, Vila-Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. *Pharm Res*, 1997; 14(10): 1431-36.

[81]. Saupe A, McBurney W, Rades T, Hook S. Immunostimulatory colloidal delivery systems for cancer vaccines. *Expert Opin Drug Deliv*, 2006; 3(3): 345-54.

[82]. Bhumkar DR, Joshi HM, Sastry M, Pokharkar VB. Chitosan reduced gold nanoparticles as novel carriers for transmucosal delivery of insulin. *Pharm Res*, 2007; 24(8): 1415-26.

[83]. Lee PW, Hsu SH, Tsai JS, Chen FR, Huang PJ, Ke CJ, Liao ZX, Hsiao CW, Lin HJ, Sung HW. Multifunctional core-shell polymeric nanoparticles for transdermal DNA delivery and epidermal Langerhans cells tracking. *Biomaterials*, 2010; 31(8): 2425-34.

[84]. Kalkanidis M, Pietersz GA, Xiang SD, Mottram PL, Crimeen-Irwin B, Ardipradja K, Plebanski M. Methods for nano-particle based vaccine formulation and evaluation of their immunogenicity. *Methods*, 2006; 40(1): 20-29.

[85]. Gregory AE, Titball R, Williamson D. Vaccine delivery using nanoparticles. *Front Cell Infect Microbiol*, 2013; 3.

[86]. Turkevich J, Stevenson PC, Hillier J. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Disc Faraday Soc*, 1951; 11: 55-75.

[87]. Zhou X, Zhang X, Yu X, Zha X, Fu Q, Liu B, Wang X, Chen Y, Chen Y, Shan Y, Jin Y. The effect of conjugation to gold nanoparticles on the ability of low molecular weight chitosan to transfer DNA vaccine. *Biomaterials*, 2008; 29(1): 111-17.

[88]. Chen YS, Hung YC, Lin WH, Huang GS. Assessment of gold nanoparticles as a size-dependent vaccine carrier for enhancing the antibody response against synthetic foot-and-mouth disease virus peptide. *Nanotechnology*, 2010; 21(19): 195101.

[89]. Marradi M, Chiodo F, García I, Penadés S. Glyconanoparticles as multifunctional and multimodal carbohydrate systems. *Chem Soc Rev*, 2013; 42(11): 4728-45.

[90]. Zhao L, Seth A, Wibowo N, Zhao CX, Mitter N, Yu C, Middelberg AP. Nanoparticle vaccines. *Vaccine*, 2014; 32(3): 327-37.

[91]. Stone JW, Thornburg NJ, Blum DL, Kuhn SJ, Wright DW, Crowe JE. Gold nanorod vaccine for respiratory syncytial virus. *Nanotechnology*, 2013; 24(29): 295102.

[92]. Tao W, Ziemer KS, Gill HS. Gold nanoparticle-M2e conjugate coformulated with CpG induces protective immunity against influenza A virus. *Nanotechnology (Lond)*, 2014; 9(2): 237-51.

[93]. Xu L, Liu Y, Chen Z, Li W, Liu Y, Wang L, Liu Y, Wu X, Ji Y, Zhao Y, Ma L, Shao Y and Chen C. Surface-engineered gold nanorods: promising DNA vaccine adjuvant for HIV-1 treatment. *Nano Lett*, 2012; 12: 2003-12.

[94]. Wang T, Zou M, Jiang H, Ji Z, Gao P, Cheng G. Synthesis of a novel kind of carbon nanoparticle with large mesopores and macropores and its application as an oral vaccine adjuvant. *Eur J Pharm Sci*, 2011; 44(5): 653-59.