

**Review Article**

## **Chitosan-based Nanoparticles in Mucosal Vaccine Delivery**

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### **ABSTRACT**

Most infectious diseases are caused by pathogenic infiltrations from the mucosal tract. Nowadays, the use of vaccines has been widely investigated for the prevention of different infectious diseases, infertility, immune disorders, malignancies, and allergies. Broad-spectrum adjuvant substances have been studied for immune system stimulation with a greater efficiency against specific antigens. Various adjuvants have been developed such as inorganic, oil-based, and emulsion adjuvants, bacterial products and their derivatives, cytokines, cytosine-guanine dinucleotide (CpG) motifs, and particulate systems. Mucosal vaccine delivery is an alternative route to induce both humoral and cellular immune responses. Applying nanoparticles in vaccine formulations allows not only improved antigen stability and immunogenicity, but also targeted delivery, and consequently, more specific release of the agent of interest. Chitosan nanoparticles have immunological activity and mucoadhesive properties. They have been used as a mucosal vaccine delivery system for many antigens. This review provides an overview of the recent advances in chitosan nanoparticles as a novel mucosal vaccine delivery system.

**Keywords:** Adjuvant, Chitosan Nanoparticle, Mucosal Vaccine Delivery

### **Nanoparticules à Base de Chitosan dans l'Administration de Vaccins Muqueux**

**Résumé:** La plupart des maladies infectieuses sont causées par des infiltrations pathogènes provenant du tractus muqueux. De nos jours, l'utilisation de vaccins a été largement étudiée pour la prévention de différentes maladies infectieuses, de l'infertilité, des troubles immunitaires, des tumeurs malignes et des allergies. Des substances adjuvantes à large spectre ont été étudiées pour la stimulation du système immunitaire avec une efficacité accrue contre des antigènes spécifiques. Divers adjuvants ont été développés, tels que des adjuvants inorganiques, à base d'huile et d'émulsion, des produits bactériens et leurs dérivés, des cytokines, des motifs cytosine-guanine dinucléotide (CpG) et des systèmes particuliers. L'administration du vaccin muqueux est une voie alternative pour induire des réponses immunitaires humorales et cellulaires. L'application de nanoparticules dans les formulations de vaccins permet non seulement d'améliorer la stabilité et l'immunogénicité des antigènes, mais également de cibler la délivrance et, par conséquent, la libération plus spécifique de l'agent d'intérêt. Les nanoparticules de chitosan ont une activité immunologique et des propriétés mucoadhésives. Ils ont été utilisés comme système d'administration de vaccins muqueux pour de nombreux antigènes. Cet examen fournit un aperçu des avancées récentes dans les nanoparticules de chitosane en tant que nouveau système d'administration de vaccins muqueux.

**Mots-clés:** Adjuvant, Nanoparticule de Chitosane, Administration de Vaccin Muqueux

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## INTRODUCTION

For years, vaccines have been widely used and investigated for the prevention of different infectious diseases, fertility, immune disorders, malignancies, and allergies. In recent years, some methods have been developed to boost the efficiency of vaccines. One of the main approaches in this regard is the use of pharmaco-immunological agents called adjuvants. Adjuvants are incorporated in the formulation of approximately all vaccines to induce immune response and generate a considerable potent immune reaction (Perrie et al., 2008). Aucouturier et al. (2001) observed that the addition of special compounds such as saponin, lecithin, metallic salts, and agar in the formulation of related antigens led to a significant increase in tetanus and diphtheria antitoxin titers in blood of immunized animals. Currently, a broad range of adjuvant substances are being studied and utilized for efficient stimulation of antigen-specific immune cells. Adjuvant systems that have been widely investigated nowadays are summarized in Table 1. Conventional vaccine delivery technologies are based on injection into the body that often lacks enough stimulation at the site of interest, where most pathogens enter the body through mucosal surfaces. The most effective way to induce mucosal immunity (secretory IgA) is to administer a vaccine directly to the mucosal surface. There are specialized cells in mucosal surfaces that are capable of antigen uptake and presenting it to the professional antigen presenting cells (APCs) that can stimulate both local and systemic immune responses. M cells that are placed within the epithelium of Peyer's patches are considered a specialized route for the delivery of particles. The transverse section of Peyer's patches is schematically shown in Figure 1A. Peyer's patches are composed of follicles containing B cells and interfollicular areas containing T cells. Each follicle extends into a domed region, the dome villus, which is covered by a specialized epithelium, the follicle-associated epithelium (FAE). Figure 1B shows uptake of antigens by M cells and stimulation of immune system (des

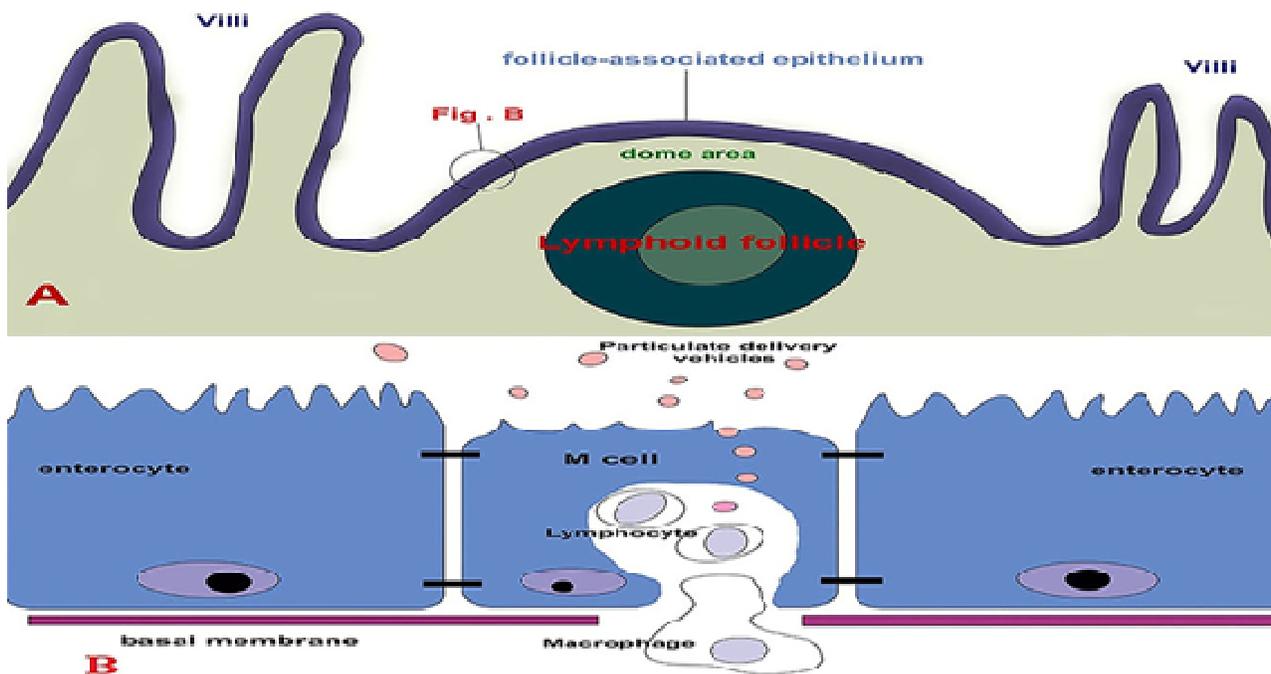
Rieux et al., 2006). In this study, we aimed to review the recent advancements in chitosan (CS) nanoparticles as a new vaccine delivery or adjuvant system.

## NANOPARTICLES AS VACCINE ADJUVANTS

So far, various vehicles have been investigated to increase the efficacy of vaccines that finally lead to the employment of nanoparticle-based pharmaceutical vehicles as successful systems for the delivery of vaccines. Currently, different particle-based adjuvants have been introduced such as liposomes, virus-like particles, emulsions, micro/nanoparticles, and virosomes (Akagi et al., 2007). Nanoparticles constructed from biodegradable polymers have attractive for biomedical applications for the delivery of various therapeutic agents such as vaccines, genes, and drugs. Commonly, particles with sizes range from 1 to 100 nm are known as nanoparticles that are categorized into two classes in pharmaceutical texts. One of these categories is nanocapsules that have a central cavity, and the second is nanospheres that have an internal matrix texture (Letchford and Burt, 2007). Among various polymers, poly- $\gamma$ -glutamic acid ( $\gamma$ -PGA), polylactic acid (PLA), poly(lactide-co-glycolide) (PLGA), poly(hydroxybutyrate) (PHB), poly(glycolic acid) (PGA), poly( $\epsilon$ -caprolactone) (PCL), and (CS) are the polymers that have been mostly investigated for the fabrication of polymer-based nanoparticles (Rahimian et al., 2015). Among these biodegradable polymers, PLA and PLGA are Food and Drug Administration (FDA) approved agents. The particles prepared from these polymers are biodegradable and biocompatible, which are highly capable of DNA, peptide, and protein delivery (Nandedkar, 2009; Mohammadpour dounighi et al., 2010). Antigens encapsulated in polymeric nanoparticles induce antigen-specific cellular and humoral immune responses via specialized delivery of the antigen to the target. This induction occurs during the processing of antigens by modulators and initiators of the immune system and antigen-presenting cells (APCs) through both major histocompatibility complex

**Table 1.** Type of adjuvants that have been studied in vaccine technology

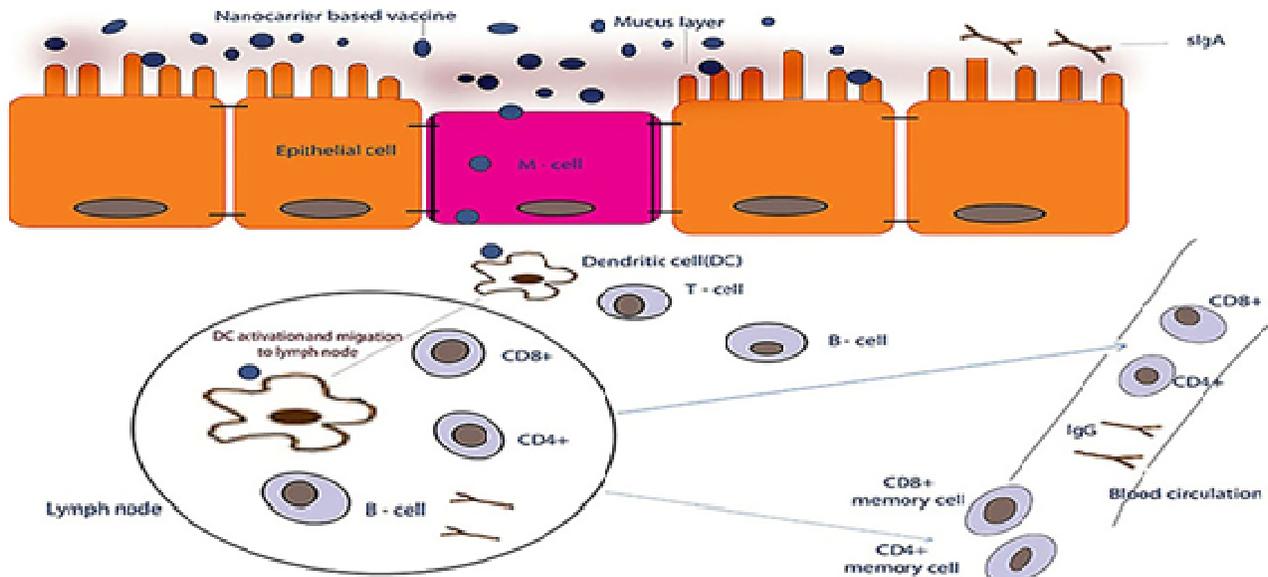
References	Immune response	Antigens	Adjuvants	
(Kool et al., 2012)	Humoral immunity /rarely induce cellular immune responses	Diphtheria, tetanus, pertussis (DTP), <i>Haemophilus influenzae type b</i> , pneumococcal conjugates, hepatitis A , B and polio virus	Aluminium phosphate or hydroxide	
(Jiang et al., 2004)	High levels of IgG/ does not increase IgE production.	Diphtheria, tetanus, pertussis vaccines	Calcium phosphate	
(Schwarz and Leo, 2008)	Humoral and cellular immunity	Human papillomavirus (HPV)(1)	Adjuvant System 04 (AS04) (consists of aluminum hydroxide and monophosphoryl lipid A [MPL])	
(Jiao et al., 2010)	Humoral and cellular immunity	<i>Edwardsiella tarda</i> , veterinary vaccines	Freund's incomplete adjuvant (FIA)	
(Reed et al., 2009)	Cellular immunity	Malaria, HIV and cancer vaccine trials	Montanide	
(Leroux-Roels et al., 2007)	Eliciting both humoral and cellular immune responses	Pandemic flu (GSK)	Squalene	
(Jansen et al., 2006; Kool et al., 2012)	Cellular immunity	New Castle Disease virus (NDV), infectious bronchitis virus (IBV)	Emulsions (e.g., MF59)	
(Mishra et al., 2007)	Cellular and humoral immunity	<i>M. tuberculosis</i> , Tetanus toxoid (TT), Diphtheria toxoid (DT)	Liposomes	
(Sjölander et al., 2001)	Induced a mixed Th1/Th2 response	Influenza virus antigens	ISCOMs	
(Reed et al., 2009; Sun et al., 2009)	Unique ability to stimulate cell-mediated immunity and to enhance antibody production	Veterinary vaccines	Saponins	
(Zheng et al., 2014)	Enhance cellular immune responses through a variety of mechanisms/ humoral immunity	Plasmid DNA	Cytokines	
(Marchetti et al., 1998; Reed et al., 2009)	Cellular / humoral immunity	<i>H. pylori</i> antigens	Bacterial lipopeptide Lipopolysaccharide, Bacterial toxins (CT LT)	Bacterial products
(Mohammadpourounighi et al., 2010; Wen et al., 2011; Mohammadpour Dounighi et al., 2012; Farhadian et al., 2015; Mohammadpour Dounighi et al., 2016)	Enhanced mucosal and humoral immunity / cellular immunity	HBsAg – Snake and scorpion venom	Chitosan	Polymeric nanoparticle
(Chong et al., 2005)	Cellular immunity	Against hepatitis B virus core antigen	PLGA	
(Ataman-Önal et al., 2006)	Cellular and humoral immunity	HIV-1 p24 protein	PLA	
(Cooper et al., 2005)	Cell-mediated immune and humoral responses	Hepatitis B virus, ovalbumin	CpG-motifs (CpG-ODNs)	



**Figure 1.** A: Schematic transverse sections of a Peyer's patch lymphoid follicle and overlying follicle-associated epithelium (FAE), B: depicting M cell transport of particulate delivery vehicles

(MHC) class I and II pathways. Exposure of immature dendritic cells (DCs) with particles, antigens, and pathogens triggers the phagocytosis process, which in return, leads to the uptake of foreign bodies into DCs and exhibition of antigens on MHC class II or even on MHC class I. Therefore, the antigen delivery process plays an important role in the design of efficient vaccines (Akagi et al., 2012). The employment of particulate adjuvants in vaccine formulation increases vaccine efficiency via preserving the antigen from destruction, impacts distress throughout intramuscular and subcutaneous injections, and induces T cytotoxic cells (TCC) and other cellular immunity against special pathogenic agents such as viruses (Nandedkar, 2009). Between various delivery routes, oral consumption due to higher compliance is a favorable administration approach. In the oral administration of vaccines, antigens have poor bioavailability. To improve the bioavailability of oral antigens, one of the most beneficial ways is the combination of antigens within

colloidal vehicles. Former studies have confirmed that the size of particulate antigens plays an important role in the induction of antibody production by the immune system. It is approved that particulates with lower particle size create superior immune responses (Chikaura et al., 2016). Generally, M cells uptake particles ranging from below  $1\ \mu$  to above  $5\ \mu$  in size. Particles smaller than  $1\ \mu$  are transferred into the basal medium, while the particles above  $5\ \mu$  are delivered to Peyer's patches. The optimum size of nanoparticles for transcytosis by M cells is proposed to be smaller than  $200\ \text{nm}$ . The formulation of negatively charged and hydrophobic nanoparticles is favorable because of the optimal absorption by M cells. Various absorption parameters that play an important role in nanoparticle uptake via M cells include particle size, hydrophobicity/hydrophilicity balance, and the existence of a targeting molecule at the surface of nanoparticles (des Rieux et al., 2006). Because of the special structure of nasal mucosa, it is a promising immunization route. The nasal administration route is



**Figure 2.** Development of cellular and humoral immune responses following mucosal administration of nanocarrier-based vaccines (following nasal/oral administration of particulate antigens, both cellular and humoral immune responses against pathogens can be induced). The cellular immune response of the adaptive immune system is comprised of both T helper (Th) lymphocytes (CD4+) and cytolytic T lymphocytes (CTL), also known as killer T lymphocytes (CD8+). Humoral immunity at the mucosal surface is principally mediated by secretory IgA (SIgA) antibodies

easily accessible and non-invasive. Moreover, it creates both systemic and mucosal immune responses and leads to slight antigen degradation compared to other routes such as oral administration. Further, there is a significant number of reports on the efficacy of different biomolecules and vaccines such as peptides, proteins, and nonpeptide drugs that are sensitive to acidic and enzymatic destruction and first-pass hepatic metabolism through the nasal route. It is believed that the nasal route can be accounted as an alternative to parenteral and oral routes. High accumulation of APCs in the nasal mucosal linings mediates both systemic and mucosal immune reactions against foreign agents that attempt to attack the human body via the respiratory system. The absorption of proteins and peptides through the nasal mucosa could be improved via different approaches such as the use of bioadhesive microspheres, bioadhesive polymers, absorption enhancers, and enzyme inhibitors. Hydrophilic

mucoadhesive nanoparticles have received great attention for the delivery of antigenic proteins through the nasal route. Mucoadhesive nanoparticles tightly attach to the mucosa and enhance the viscosity of mucin, and lead to the improvement of mucosal absorption (Zhang et al., 2008). M cells present under the nasal epithelium covering the nasal-associated lymphoid tissue (NALT) and are usually responsible for the uptake and delivery of antigens to the submucosal lymphoid tissues (Amidi et al., 2007). It is proved that M cells uptake macromolecules and particles through adsorptive endocytosis phenomenon as a vesicle, and then they create humoral and cellular immune reactions (Figure 2). Particle size is a main parameter in immunogenicity by differential interactions with APCs (Akagi et al., 2012). Pulmonary vaccine delivery has been the center of attention during the last decade due to the advantages of this method like the fact that it rules out the use of needles and may elicit immunity at the portal of entry for many

pathogens. Immunization via the pulmonary route induces both systemic and mucosal immune responses against airborne pathogens. Previous studies showed antigen uptake by DCs, which are located in the respiratory tract (Tonnis et al., 2012). Sublingual and buccal are the other routes used for mucosal vaccine delivery. Many low molecular weight drugs have been delivered through this approach for many years. These routes have become one of the main interesting sites among researchers for vaccine delivery during the past few years. Prominent advantages of these routes over the oral route include reducing enzymatic activity and preventing low gastric pH effect, which cause antigen degradation. Researchers have found that sublingual vaccine delivery does not redirect to the olfactory bulb and it seems a safer alternative to nasal vaccine delivery (Shim et al., 2013). The vaginal mucosa is covered with multi-layered squamous epithelia. Lamina propria that is located underneath epithelial layers of the vagina comprise B cells, CD4+, CD8+ T cells, and antigen presenting cells. In recent years, the vaginal route has been rediscovered as a potential route for the delivery of peptides and macromolecules. Mucoadhesive vaginal vaccine delivery system has emerged as a potential route for inducing humoral and cellular immunity systems (Neutra and Kozlowski, 2006). Recently, rectal route has been suggested for immunization against several pathogens such as *mycobacterium bovis*, hepatitis A virus, rotaviruses (RV), and human immunodeficiency virus (HIV). Rectal mucosa includes lymphoid nodules in the small intestine like Peyer's patches. This structure is covered by M cells that play an important role in antigen uptake (Parez et al., 2006). Among the various routes of mucosal vaccine delivery, nasal and oral routes are more favorable due to patient compliance and induction of immune responses (mucosal and systemic) more effectively.

## CHITOSAN

For the first time in 1859, Rouget prepared CS through the deacetylation of chitin through boiling in

concentrated potassium hydroxide solution. CS is a cationic polyelectrolyte, which is well-known as a natural-based structure. CS can be easily fabricated from the deacetylation of chitin. This natural polymer has attracted wide attention from researchers for use as an agent in pharmaceutical compounds because of its particular specifications such as low cost, low toxicity, biodegradability, biocompatibility, low immunogenicity, and tissue compatibility (Mohammadpour dounighi et al., 2010). Studies have demonstrated toxicity of CS in mice (LD50: ~ 16 g/kg). CS is available in various forms depending on the degree of deacetylation (DDA) and molecular weight (Mw) (Mohammadpour dounighi et al., 2010). This diversity is dramatically enhanced by the wide range of chemical modifications. The degree of deacetylation significantly affects parameters such as biodegradability, immunological activity, solubility, and physico-chemical characteristics. The pKa of various amine groups of CS varies within the range of 5.5 to 6.5 depending on the origin of the polymer. The dissolution of polymer is achieved by the protonation of functional groups of -NH<sub>2</sub> on the C-2 position of D-glucosamine residues that leads to the conversion of polysaccharides into polycation in acidic condition. This polymer has low solubility in physiological condition and pH  $\geq$  7.4. CS is solved easily in dilute solutions of various organic acids such as tartaric and citric acid, whereas its solubility in inorganic acids is weak (Bansal et al., 2011). CS is classified into three main types based on difference in molecular weight, high molecular weight CS (700 to 1000 kDa), low molecular weight polymer (less than 150 kDa), and medium molecular weight polymer falling between low-molecular and high-molecular weight. Deacetylation degree of commercial CS polymers is within the range of 66-95%. CS exhibits polymorphism in solid form and has a semicrystalline structure (Wong, 2009). The interaction between negatively charged sialic acid groups on the mucin and positively charged groups on CS result in the mucoadhesive characteristics of CS. The mucoadhesive properties of polymer lead to prolonged contact time between the antigen and the



**Figure 3.** Methods of preparation of chitosan-based nanoparticles

surface of absorption, thereby increase in absorption generally (Dyer et al., 2002). CS progressively has been favored as a potential nanoparticulate vehicle due to its particular preferences. However, the low solubility of CS in water is the main challenge of employing it as a biomaterial. CS is outlined by its mucoadhesive property and capability of opening the tight junctions guarding the paracellular pathway, hence promoting permeability of cells to biopharmaceutics (Kammona and Kiparissides, 2012).

### CHITOSAN STRUCTURE AND CS NANOPARTICLES PREPARATION METHODS

CS polymer is a N-deacetylated derivative of chitin and most of its glucopyranose residues are 2-amino-2-deoxy- $\beta$ -D-glucopyranose. Structure of chitin differs from CS in an acetamido group at the position of C-2. To synthesize CS, this acetamido group is deacetylated and replaced with an amine group. The NH<sub>2</sub> group on the C-2 position of D-glucosamine repeating units of CS is protonated in acidic condition and leads to polymer dissolution. During the past 30 years, various methods have been developed for the preparation of CS nanoparticles and microparticles for different purposes.

Until now, at least seven techniques have been introduced for the synthesis of CS particles such as polyelectrolyte complexation, ionotropic gelation, solvent evaporation/co-precipitation, microemulsification, complex coacervation, and emulsification solvent diffusion (Sonaje et al., 2010). The disadvantages and advantages of each method are illustrated in Figure 3. Among the above-mentioned techniques, ionic gelation technique is broadly applied for the formation of CS nanoparticles because of its non-complexity and non-invasiveness. The electrostatic interaction between negatively charged group of polyanion such as tripolyphosphate (TPP) and positively charged amine group of CS leads to the formation of CS nanoparticles (Mohammadpour dounighi et al., 2010). In the ionic gelation technique, nanoparticle formation occurs instantaneously by the rapid mixing of TPP solution into the CS solution under homogenization at room temperature. The antigen-encapsulated nanoparticles are fabricated by dissolving of different amounts of antigen into TPP solution before mixing with CS solution. The encapsulated antigen release from particles formed in this process can be explained by both main phenomena of erosion and diffusion. After

the penetration of water into the nanoparticle matrix, the antigen could be distributed outside CS nanoparticles as the swelling of CS-TPP matrix. In erosion, the structure of CS polymer is hydro-degraded in smaller molecular weight units, and the matrix is disintegrated and the antigen is released from CS particles (Deng et al., 2006) (Figure. 4). Size, zeta potential, and antigen loading efficiency of chitosan nanoparticles are dependent on factors such as CS and crosslinker concentration, pH of media, antigen concentration, and homogenization speed. This technique is non-invasive, thus, it does not impose any probable hazards and cytotoxicity. However, protein instability results from chemical crosslinking agents and not using of organic solvents in the particle formation medium (Makhlof et al., 2011).

#### **CS NANOPARTICLES IN VACCINE DELIVERY**

CS nanoparticles have been broadly studied and applied as antigen delivery systems and immunoadjuvants in the parenteral and mucosal administration of vaccines. CS-based nanoparticle antigen delivery system enhances the absorption of the agent from the nasal and intestinal mucosa through particular interactions between nanovehicles and epithelium. CS nanoparticles increase immune stimulation via enhancing antigen exposure time with mucous membranes via bioadhesion and cellular delivery. Studies have clearly shown that due to bioadhesive properties of CS, the clearance half time of CS nanoparticles is enhanced (Abruzzo et al., 2015). CS nanoparticles can increase the paracellular delivery of agents via interactions between the positive charges of the polymer and negatively charged cell membrane or through complexation of calcium ion complicated in the tight junction structures. The interaction between CS nanoparticles and tight junctions results in the uptake of nanoparticulates and it has been expected that CS nanoparticles are most probably transferred by adsorptive endocytosis (Figure 5). This phenomenon is saturable depending on temperature and energy (Sandri et al., 2015).

#### **ORAL VACCINE DELIVERY**

Antigen delivery through the oral administration route is one of the important objectives in vaccinology. The gastro-intestinal pH differs from very acidic gastric condition (pH: 1.2–3.0) to gently basic in the gut (pH: 6.5– 8.0). The various pH conditions can lead to deamidation, pH-induced oxidation, and hydrolysis of peptide and protein agents, subsequently resulting in destruction activity of agents. Enzymes present in the gastrointestinal tract, such as chymotrypsin, trypsin, pepsin, pancreatic amylase, pancreatic lipase, and procarboxy peptidase and pH of this tract can change the activity of agents. Various strategies have been investigated to develop an effective oral vaccine delivery formulation. One of such strategies is enteric coatings that have been investigated widely in efforts for the oral delivery of protein and vaccine; this strategy has been applied in the pharmaceutical industry as well. By enteric coating delivery system engineered and modified as a vehicle, a stable structure against gastric acidic condition can be created and then antigens can be released in the basic intestinal pH condition. Besides, CS polymer has been mostly used for coating the surface of nanoparticulates to increase the affinity of particles on mucosa of the intestinal lumen (des Rieux et al., 2006). Recently, pH-sensitive nanoparticulate systems coated with CS have been developed for vaccine administration via the paracellular pathway. CS due to its cationic charge can attach to epithelium surfaces and lead to a temporary opening of the tight junctions between adjacent cells (Sonaje et al., 2010). Immunity from oral route is induced following antigens embodiment and uptake from the intestinal lumen and their delivery by M cells into dendritic cells, macrophages, and lymphocytes (des Rieux et al., 2006). M cells that are called FAE are mainly placed within the epithelium of Peyer's patches in ileum; these cells have some noticeable features for the uptake of particles. However, lower percentage of M cells in the gastrointestinal tract (1% of the total surface of the intestine) causes significant problems in humans. To enhance the efficiency of oral vaccine

delivery systems, it is necessary to employ the targeted nanoparticles to M cells. There is increasing attention to finding a particular marker of the apical pole of M cells in humans, or even better, a common set to various species of conserved apical membrane target proteins of M cells and FAE. Despite presenting a low percentage of intestinal cells, M cells revealed an attractive interest in vaccine and drug delivery (Zhang et al., 2016).

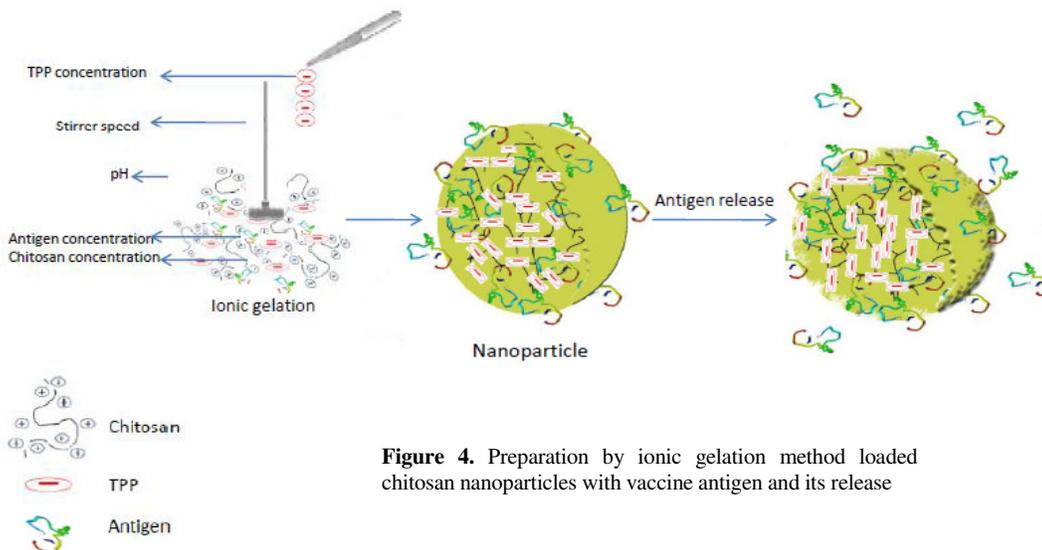
### NASAL VACCINE DELIVERY

Nasal mucosa is the earliest site of exposure and is considered as one of the very efficient protection systems against inhaled antigens and NALT at the nasal cavity surface that plays an important role in the defense of mucosal surfaces. The NALT epithelial cell layer like immune cell types presented in the Peyer's patches of GALT contains M-cells and is placed just below the surface of epithelium, which contains lymphoid follicles (mostly B-cells), dendritic cells, macrophages, and intra follicular areas (mostly T-cells) (Islam et al., 2012). Furthermore, another characteristic of the nasal epithelium is that it is leaky and there are underlying blood vessels, cervical lymph nodes, and lymphoid cells to which antigens may have direct access if they can be adequately transported across the epithelium. If the suitable particulate delivery system is employed as a vehicle for antigens, an efficient humoral and cellular immune response can be obtained via nasal delivery of vaccines. However, there are some problems to achieving this aim including fast clearance of particulate vaccine or drug formulation from mucosal surfaces, limited absorption of encapsulated antigens across the mucosal barrier, and enzymatic destruction because of particulate carrier instability. To overcome these drawbacks, CS nanoparticulate systems are considered as an opportunity for the nasal administration of vaccines because of their capability to enhance retention time by binding to mucosal membranes (Islam et al., 2012). The ability of CS nanoparticulates to promote the nasal delivery of

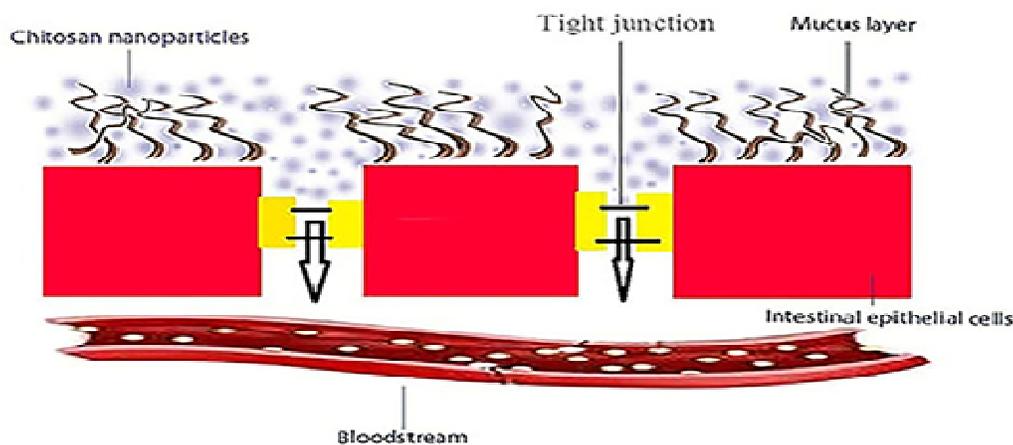
macromolecules has been shown using insulin as a model peptide (Fernandez-Urrusuno et al., 1999). In that work, two types of formulations including CS nanoparticles and CS solution were studied. The levels of plasma glucose following nasal administration of insulin-entrapped nanoparticles to conscious rabbits were significantly lower than those corresponding to the same dose of CS solutions. Very recently, another study showed evidence regarding the ability of CS nanoparticulates as nasal vehicles for macromolecules using tetanus toxoid (TT) as a model antigen (Vila et al., 2004). In this work, TT was employed as a model antigen for nasal vaccine delivery. TT-entrapped CS nanoparticulates result in a stronger and more long-lasting humoral immune response (IgG concentrations) as compared to the fluid vaccine. Furthermore, the IgA level in the mucosal response was significantly higher than that achieved for the fluid vaccine. Results have shown that the mode of action of CS nanoparticulates is not significantly related to the Mw of CS polymer. The greater immune response observed for low Mw CS nanoparticles at earlier times as compared to higher Mw CS nanoparticles at later times could be attributed to the inherent immunostimulatory properties of CS or to a different release pattern of TT from low versus high Mw CS particles. In nasal delivery route, CS possesses good bioadhesive characteristics and can decrease the fast clearance of vaccine from the nasal cavity where it could be delivered to NALT, the effective and introducing places for vaccine induced immune responses. Another benefit of CS solution is that when exposed to nasal mucosa, it does not change the morphology of mucosal cells. Generally, CS solution has minimal side effects and toxicity, which makes it a suitable choice for use in vaccine delivery as a biocompatible and safe material.

### DISCUSSION

CS has particular specifications, thus, it has attracted wide attention from researchers for use as a delivery system due to its low cost, low toxicity,



**Figure 4.** Preparation by ionic gelation method loaded chitosan nanoparticles with vaccine antigen and its release



**Figure 5.** Schematic diagram of the mechanisms through which chitosan nanoparticles improve proteins and peptides absorption

compatibility. Currently, various types of adjuvants have been developed such as inorganic compounds, oil-based or emulsion adjuvants, bacterial products and their derivatives, cytokines (interleukins and interferons), oligodeoxynucleotides containing CpG motifs, and particulate delivery systems. CS nanoparticles have been used extensively in the field of medicine regarding the interesting properties of CS and the simplicity of its preparation, antigen protection capability, biodegradability, biocompatibility, and tissue the ability of immune response induction,

and its appropriate size for transference and absorption through mucosal membranes. Researchers have established the ability of CS nanoparticles to deliver different antigens for inducing immunity against encapsulated antigens from oral (follicle-associated epithelium M-cells) and nasal (Peyer's patches M-cells) routes. Therefore, it can be concluded that CS nanoparticles can be considered as a vehicle for vaccines and can be used as an adjuvant in vaccine delivery systems, especially in mucosal vaccine delivery. It is worth mentioning that the degree of

deacetylation and molecular weight of CS nanoparticles play a major role in CS physico-chemical and vaccine delivery properties.

### Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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