International Journal of Nanomedicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

The Mucoadhesive Nanoparticle-Based Delivery System in the Development of Mucosal Vaccines

Kai Zhao[1,2], Yinzhuo Xie[2], Xuezheng Lin[1], Wei Xu[3]

[1]Taizhou Central Hospital (Taizhou University Hospital), Taizhou University, Taizhou, Zhejiang, 318000, People's Republic of China; [2]Institute of Nanobiomaterials and Immunology, School of Life Science, Taizhou University, Taizhou, Zhejiang, 318000, People's Republic of China; [3]College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, People's Republic of China

Correspondence: Kai Zhao, Tel +86 576 88660338, Email zybin395@126.com, Xuezheng Lin, Taizhou Central Hospital (Taizhou University Hospital), Taizhou University, Taizhou, Zhejiang, 318000, People's Republic of China, Email linxz@tzzxyy.com

Abstract: Mucosal tissue constitutes the largest interface between the body and the external environment, regulating the entry of pathogens, particles, and molecules. Mucosal immunization is the most effective way to trigger a protective mucosal immune response. However, the majority of the currently licensed vaccines are recommended to be administered by intramuscular injection, which has obvious shortcomings, such as high production costs, low patient compliance, and lack of mucosal immune response. Strategies for eliciting mucosal and systemic immune responses are being developed, including appropriate vaccine adjuvant, delivery system, and bacterial or viral vectors. Biodegradable mucoadhesive nanoparticles (NPs) are the most promising candidate for vaccine delivery systems due to their inherent immune adjuvant property and the ability to protect the antigen from degradation, sustain the release of loaded antigen, and increase the residence time of antigen at the administration site. The current review outlined the complex structure of mucosa, the mechanism of interaction between NPs and mucosa, factors affecting the mucoadhesion of NPs, and the application of the delivery system based on mucoadhesive NPs in the field of vaccines. Moreover, this review demonstrated that the biodegradable and mucoadhesive NP-based delivery system has the potential for mucosal administration of vaccines.

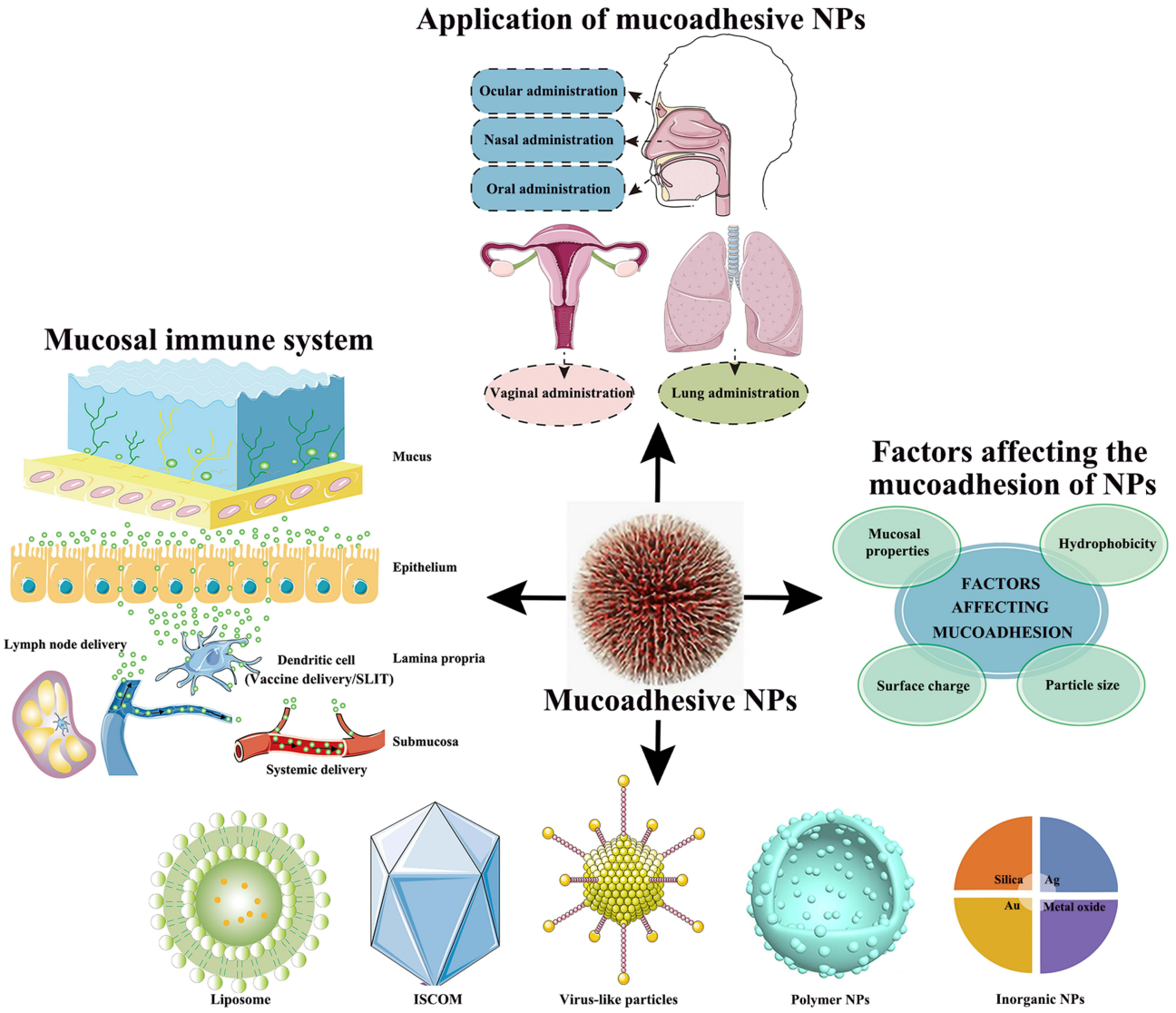
Keywords: delivery system, nanoparticles, mucosal vaccine, mucosal immunization, mucosal adhesion

Introduction

Vaccine immunization can prevent and control the spread of infectious diseases by inducing an effective protective immune response, leading to a great improvement in the health of humans and animals.[1] Most pathogenic microorganisms invade the body through the mucosal surface. Therefore, mucosal immunization is one of the most effective strategies against pathogenic microbial infection.[2] However, only very few mucosal vaccines have been developed and approved for clinical use in humans and animals, including intranasally administered live attenuated influenza vaccines FluMist/Fluenz® and Nasovac™, and orally administered microbiological vaccines.[3] The complexities of mucosal immune regulation and the lack of appropriate antigen delivery systems to access mucosal inductive sites have remained substantial obstacles.[4] A vaccine can work most efficiently if it can elicit both humoral and cellular immune responses. However, conventional vaccines often induce antibody responses with only limited cellular immunity. Many vaccines currently in use are directed toward systemic pathogens or toxins and are administered by intramuscular injection. The intramuscular injection requires using a needle or micro-needle, well-trained medical professionals, and a sterile dosage form, which have apparent shortcomings of high production costs, poor patient compliance (especially children), and lack of mucosal immune response.[5] Therefore, strategies for eliciting the mucosal and systemic immune response are being developed to replace the traditional intramuscular vaccine and improve the immune response of mucosal vaccines, such as appropriate vaccine adjuvant, delivery system, and bacterial or viral vectors.[6,7] Among them, the development of safe and effective mucosal adjuvant and the delivery system remains the focus of mucosal vaccine development. It is well known that mucosa is covered with mucus, which is a viscous gel layer found on various mucosae, such as the eyes, nose, respiratory and gastrointestinal tract, protecting the underlying cells.[8] The mucus layer provides the possibility of

Received: 21 January 2022
Accepted: 14 September 2022
Published: 28 September 2022

Graphical Abstract



mucosal adhesion for delivery systems. Mucoadhesive nanoparticle (NP)-based delivery system has attracted much attention in the field of mucosal vaccines because it can not only protect the loaded antigen from degradation, achieve the optimal sustained release of antigen encapsulated in the NPs, and prolong the antigen delivery time through biological adhesion, but also significantly enhance humoral, cellular, and mucosal immune responses.[9]

The mucoadhesive NP-based delivery system has received extensive attention in the development of mucosal vaccines and promises to become a safe and effective vaccine adjuvant and delivery system. This mandates successful vaccine delivery to the mucosal site of interest to produce a strong protective effect, which introduces several distinct challenges in developing an efficacious vaccine. The present report discussed the latest progress in the vaccine delivery system based on mucoadhesive NPs. This review provided valuable insights into designing more effective mucoadhesive NP-based delivery systems to enhance their effectiveness in mucosal vaccine development.

Mucosal Immunity

The mucosal surface is the portal of entry to many pathogenic microorganisms and the initial transmission site of most infectious diseases. Mucosal immunity plays a crucial role in preventing the invasion of pathogenic microorganisms infected through the oral cavity, respiratory tract, and reproductive tract. Mucosal immunity is a complex network composed of innate and adaptive immunities.[10] The mucosal epithelial barrier provides multiple layers of protection for innate immune cells and minimizes the risk of potential pathogen infection. Based on innate immune protection, the adaptive immune system can be immunized through the cytoplasmic fluid depending on the specificity of the pathogen. These two immunizations interact and work together to protect the mucosa.[11]

Mucosa

The mucosa is the main portal for most pathogens to enter the body. Mucosa, especially mucus, is an essential part of mucosal adhesion. The mucosa is a moist layer wall that attaches to the surfaces of various body cavities, including the nasal cavity, oral cavity, eyes, gastrointestinal tract, and reproductive tract. Unlike systemic immunity, mucosal immunity occurs at the mucosal level, an antigen is derived from the mucosal cavity, and antigen-presenting cells (APCs) are macrophages and M cells located between epithelial cells or dendritic cells in the submucosa.[12]

The mucosa consists of the lamina propria, above which is the epithelium. The number of layers of epithelial cells is usually monolayer, such as in the large intestine, small intestine, and stomach, while there are multiple layers in certain parts (such as the cornea and esophagus). The surface of the epithelial cell layer is moist due to the presence of a mucus layer. The mucus components are relatively complex, mainly including proteins, lipids, inorganic salts, water, bacteria, and cell debris. It is usually secreted by goblet cells and specific glands, such as the salivary, cardiac, and pyloric glands. Mucosa protects the human body and forms a closed system to the outside world. When harmful substances invade the human body, mucosa and mucus prevent external pathogenic factors in the body. Therefore, the mucosa is the first line of defense of the human immune system.

Mucosal Immune System

The mucosal immune system is an essential part of body's immune system. It primarily removes pathogenic microorganisms that invade the body through the mucosal surface. The mucosal immune system is the body's first line of defense against local mucosal infection, and it is widely distributed in the mucosal tissues of the digestive system, respiratory system, urogenital system, and some exocrine glands.

The mucosal immune system is divided into the mucosal inductive and effector sites (Figure 1).[13] At the mucosal inductive site, antigen absorption triggers an initial immune response. After the antigen is processed and presented, it provides a constant source of memory cells and T cells, which are then transported by the blood to distant mucosal effector sites. The mucosal effector site is diffuse lymphoid tissues widely distributed in the LP. After the antigen is presented to the inductive sites, activated T and B lymphocytes are induced to migrate to LP and glands to produce specific immune responses at the mucosal effector site. For example, B lymphocytes differentiate into plasma cells and secrete many IgA, cytotoxic T lymphocytes (CTLs) produced by T lymphocytes initiate cellular immunity, or IgG produced by T cells initiates humoral immunity.[14] In addition to inducing antigen-specific mucosal IgA and serum IgG responses, mucosal immunity can also induce the opposite type of immune response, systemic unresponsiveness (such as oral tolerance). These properties protect the host from pathogens. On the other hand, it causes the body to develop immune tolerance to common food antigens and normal microorganisms.[10]

Routes of Mucosal Immunization

Vaccine mimics the natural route of infection by inoculating at the mucosal sites, directly stimulating the abundant lymphoid tissues to produce many immune-active cells and antibodies, and even cutting off the pathway of pathogenic microorganisms invading the human body before infection.[15] In addition, delivery of antigen to mucosal surfaces can induce systemic immunity.[16] Many different mucosal immunization routes are have been developed, mucosal immunization routes include digestive tract immunization (oral, sublingual, and rectal), respiratory tract immunization (nasal

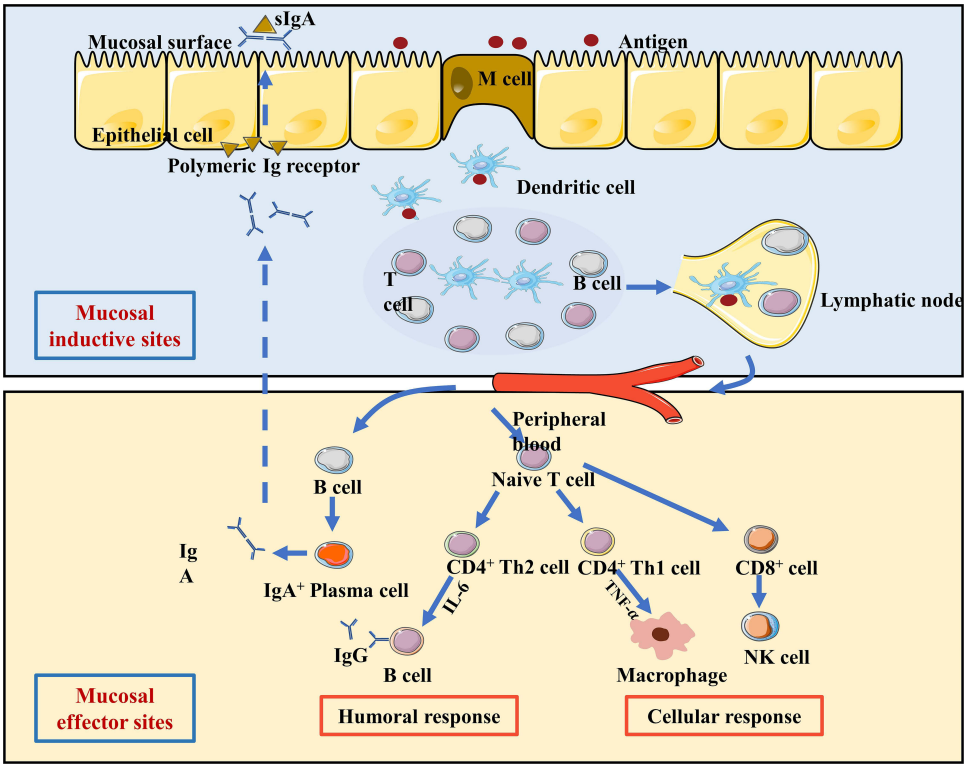


Figure 1 Induction and regulation of antigen-specific mucosal immune response in inductive and effector sites.

drop, spray, and intrapulmonary), genital tract immunization (vaginal), and ocular immunization.[17] Table 1 shows the routes, advantages, and disadvantages of mucosal immunization.[17,18] Strategies for eliciting mucosal and systemic immune responses should be developed, considering the advantages and disadvantages of various mucosal immune routes.

Mucosal Adhesion

Mucosal adhesion is a particular case of adhesion, and it is defined as a state in which two materials establish an interface and adhere to each other for a long period of time. When one of the materials is essentially mucosa, this phenomenon is called mucosal adhesion.[30] Mucosal adhesion is considered the most effective method for the selective release of drugs to mucosal tissues. In order to enhance local drug delivery or transfer "difficult" molecules into the systemic circulation, including proteins, peptides, and oligonucleotides, mucosal adhesion is of interest for pharmaceutical research.[31] In the mucosal adhesion, two essential steps have been identified to describe the interaction between mucoadhesive material and mucosa. 1) Contact stage: intimate contact between the mucosal adhesion material and the mucosa (wetting and swelling); 2) Consolidation stage: the mucosal adhesive material penetrates the tissue or mucosal surface, and various physical and chemical interactions occur to consolidate and strengthen the adhesive joint, resulting in prolonged adhesion (Figure 2).[32]

When the drug reaches the mucosal site, it will pass through the mucus according to its adhesion and diffusion properties. Larger drugs (usually in the micron range) cannot diffuse in the mucus layer due to their steric hindrance, while they can interact with the mucosal tissue lumen via the mucus protein chain. Small and non-mucoadhesive drugs with diameters below the cutoff pore size of mesh structure can readily diffuse in mucus via Brownian motion.[33] However, smaller drugs that are mucoadhesive, even in small sizes, tend to get trapped in mucus. For smaller drugs, the mucin network in the "dead corner" pocket may affect the diffusion rate. The mucous membrane has a natural clearance mechanism, which gradually removes particles from the mucosa. Because of these particle elimination mechanisms, researchers have developed corresponding strategies to overcome the mucus barrier and promote the diffusion and penetration of NPs for enhanced therapeutic effects. The main strategy to achieve mucosal penetration is a hydrophilic but neutral surface, which prevents hydrophobic and

Table 1 Advantages and Disadvantages of Different Mucosal Immunization Routes

Mucosal Site	Immunization Route	Advantages	Disadvantages	Examples	References
Respiratory tract	Intranasal	Easy to administer, convenient mode of remote immunization, longer lasting, inducing distant mucosa to produce immune responses.	Moderate levels of cellular and humoral responses, risk of entering nerve tissue through olfactory nerves, may exacerbate nasal or respiratory inflammation.	Film, spray, gel, nanoparticles, microparticles	[19,20]
	Endotracheal	Fast immunity, large immune effect, wide range, painless.	Equipment requirements are large, easy to waste in air.	Aerosol, dry powder, nanoparticles, microparticles	[21]
	Intrapulmonary	Larger size, longer lasting.	Vaccination requires facilities and trained professionals.	Aerosol, dry powder, nanoparticles, microparticles	[22]
Digestive tract	Oral	Easy to administer, convenient mode of remote immunization, pass through the small intestine and selectively target the large intestine to induce immunoprotective immunity comparable to that in the colon.	The extremely low pH of the stomach, the presence of proteolytic enzymes and bile salts as well as low permeability in the intestine, extra manufacturing procedures required but not significant.	Liquid drugs, capsules, tablets, hydrogels, nanoparticles, microparticles	[23,24]
	Sublingual	Need less antigen, high safety	High medical equipment requirement, injection may cause secondary infections.	Film, tablets, spray, gel, nanoparticles, microparticles	[25]
	Rectum	Inducing strong immune responses in the rectum and colon	Vaccination requires facilities and trained professionals, discomfort and accidental trauma.	Polymer implants, nanoparticles, microparticles; hydrogels	[26,27]
Reproductive tract	Vaginal	Better effect on prevention of genital tract infection	Affected by menstrual cycle, hormone changes	Film, spray, gel, Polymer implants, nanoparticles, microparticles	[28]
Other	Ocular	Good tissue immunity, high antibody titers	Unorganized local immunity	Eye drops, injection, hydrogel	[29]

electrostatic interactions. When the drug reaches the inner membrane of epithelial cells, it can further take effect through cell absorption or tissue penetration.[8] Understanding the interaction between mucoadhesive material and mucosa is critical to explain mucosal adhesion. The adhesion mechanism and action of mucosa have attracted much attention in vaccines and medicine. Many different adhesion theories have been proposed, while only a combination of them can provide a satisfactory explanation. The different theories involved in mucosal adhesion are described in Table 2.[31–34] In addition, by combining different theories of mucosal adhesion, we can design the corresponding mucoadhesive NP-based delivery system as the adjuvant of the mucosal vaccine.

Interaction Between NPs and Mucosa

The application of nanotechnology in mucosal delivery has achieved significant success. Materials with a particle size ranging from 1 nm to 1000 nm are called NPs.[35] In nanomedicine research, NPs have been used to design and develop delivery systems for vaccines and drugs. The NP-based delivery system has obvious advantages, such as improving the targeting of antigen/drug, protecting antigen/drug from degradation during in vivo transport, and controlling drug release at specific sites or cells in response to specific signals.[36,37] A variety of NPs, including polymer NPs, inorganic NPs,

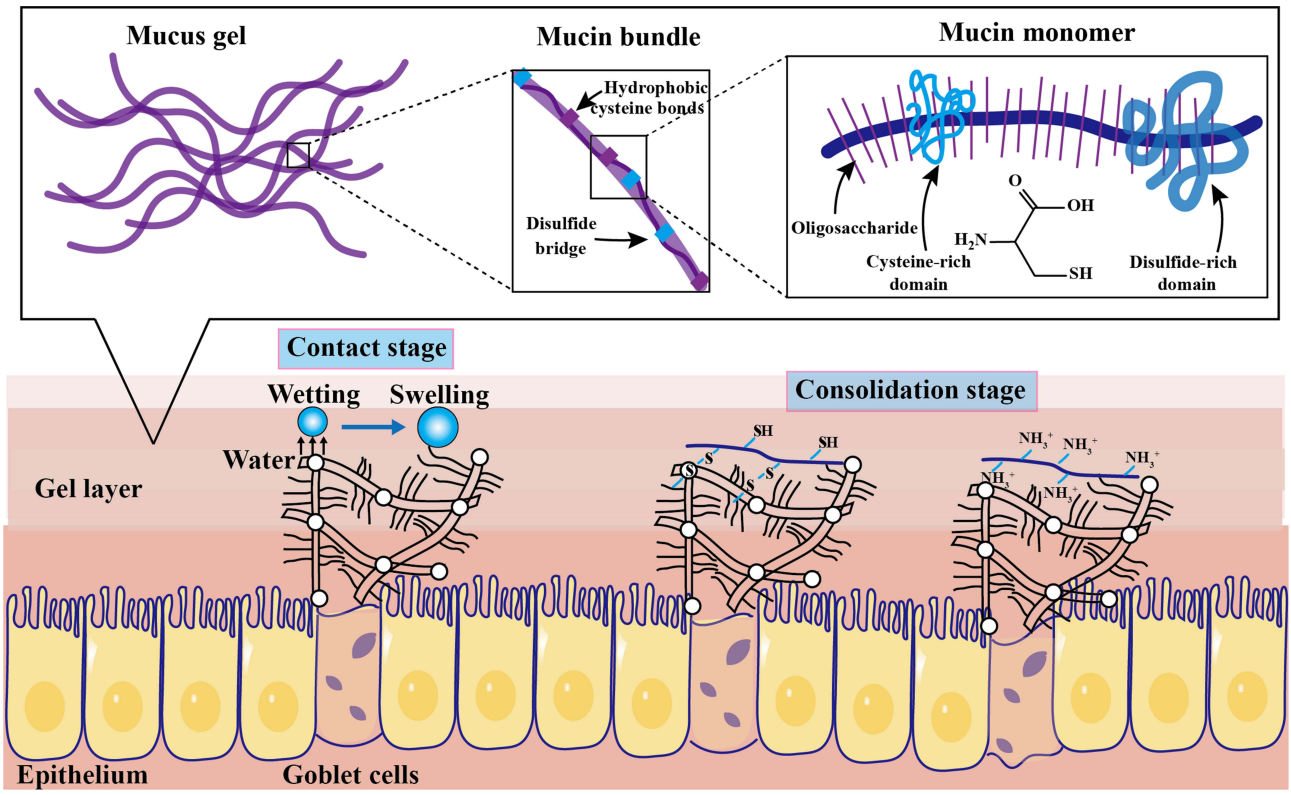


Figure 2 Schematic diagram of the interaction between mucoadhesive material and mucosa.

liposomes, immune-stimulating complexes (ISCOM), virus-like particles (VLPs), and self-assembled proteins, have been developed and become potential vaccine adjuvants and delivery carriers.[38]

Types of NPs

Liposomal NPs

Since liposome was first described in the 1960s, many liposomal vaccines/drugs have been approved for various diseases, such as cancer, COVID-19, influenza, hepatitis, and fungal infections.[39] Liposome is a nanocarrier composed of a lipid bilayer and a water core.[40] They can be either monolayer vesicles composed of a single phospholipid bilayer or multilamellar vesicles composed of a multilayer concentric phospholipid shell.[41] Liposome-based NPs realize vaccine/

Table 2 Theories of Mucosal Adhesion

Theory	Short Description	References
Adsorption theory	Interaction between mucosa and mucoadhesive material is related to the establishment of hydrogen bonds and van der Waals bonds. Hydrophobic bonds and chemisorption may also contribute to mucosal adhesion.	[31]
Electron theory	Adhesion is established due to electrostatic attraction between negatively charged mucin and positively charged material.	[32]
Fracture theory	Fracture theory is probably the most commonly used theory for the measurement of mucosal adhesion mechanics. Mucosal adhesion is related to the force required to interface the two previously joined solid surfaces.	[32]
Diffusion theory	Mucoadhesive polymers are driven by concentration gradient differentiation and interpenetrate with mucin fibers to form mucosal adhesion.	[33]
Wetting theory	Describes the ability of mucoadhesive polymers (liquid or low viscosity forms) to diffuse in the mucus layer. Mucosal adhesion can be measured by contact angle.	[8]
Mechanical theory	Adhesion is dependent on the roughness of two different surfaces	[34]

drug delivery by fusing liposomes into the lipid bilayer so that the vaccine/drug is delivered to the cytoplasm. Lipid NPs (LNPs), such as nanostructured lipid carriers, solid lipid NPs, liposomes, mixed micelles, and nanoemulsions, have been used in vaccine/drug delivery, especially oligonucleotides, DNA, and mRNA antigens.[42] The exciting results of the COVID-19 mRNA vaccine have incredibly aroused people's interest in the development of LNPs.[43]

ISCOM

ISCOM is mainly composed of antigen, cholesterol, phospholipid, and saponin adjuvant Quil A. ISCOM is a 40 nm NP used as a delivery system for vaccine antigens, targeting the immune system both after parenteral and mucosal administrations.[44] It has been proved that ISCOM has excellent safety and tolerance in humans and animals, and it can induce humoral and cellular immune responses.[45] Using murine models and a model cancer antigen, ISCOM® vaccines have been shown to induce potent CD8+ T lymphocytes responses to mediate protection in three different tumor models, promote Th1-biased immunity, and induce CD8+ T lymphocytes responses in the absence of CD4+ T lymphocytes.[46] Lipophilic ISCOM containing Quil A is active by both parenteral and mucosal routes. Subcutaneous immunization of guinea pigs with diphtheria toxoid (DT) has shown that ISCOMS-based vaccines prime protective immunity against challenges with diphtheria holotoxin more efficiently than the equivalent doses of DT in the conventional alum vaccine.[47] ISCOMS-based vaccine adjuvant may provide a novel mucosal and systemic immunization strategy.

VLPs

VLPs are self-assembled from viral structural proteins. It is a multi-protein supramolecular structure with many characteristics of viruses. VLPs lack the viral genome that cannot replicate, and VLP-based vaccines have the advantages of most traditional vaccines. Therefore, VLPs have become a safe template and have been widely used in the vaccine field. Many VLP-based vaccines are available on the market, such as hepatitis B virus vaccine (Recombivax), human *Papillomavirus* vaccine, and hepatitis E virus vaccine.[48] Some studies have indicated that VLP-based vaccines have excellent immune effects and poor immunostimulatory activity,[49] for example, SVA VLPs vaccine has good immune effect as same as inactived virus,[50] FMDV VLPs vaccine has same protection efficiency as conventional FMD inactived vaccine.[51] Additionally, to improve poor immunostimulatory activity of VLP-based vaccine, vaccine adjuvant is included in most VLP-based vaccines formulations.[52,53] Novel classes of adjuvants, such as liposomes, agonists of pathogen recognition receptors, polymeric particles, emulsions, cytokines, and bacterial toxins, can be used to further improve the immunostimulatory activity of most VLP-based vaccines.[54]

Polymer NPs

Synthetic, semi-synthetic, and natural polymers, such as polylactic acid (PLA), poly (d, l-lactide-co-glycolide) (PLG), poly (d, l-lactic-coglycolic acid) (PLGA), pullulan, polycaprolactone (PCL), and chitosan,[14] have been used to prepare NPs to deliver vaccine antigen or drug. Polymer NPs have a size range of 1–100 nm and have been widely used in the vaccine field due to their biocompatibility, biodegradability, non-toxicity, and ability to be easily modified into desired shapes and sizes.[38,55] Polymer NPs encapsulate or capture vaccine antigen and deliver it to particular cells or maintain slow antigen release. Therefore, polymer NPs have been extensively studied in vaccines.[16] Currently, many polymer NPs have been studied and reported for mucosal vaccine advances, and studies have shown that polymer NPs can effectively deliver envelope antigen directly to APCs and significantly enhance cellular and humoral immune responses.[56]

Inorganic NPs

Inorganic NPs have the advantages of non-toxicity, good biocompatibility, and high stability. In addition, inorganic NPs have unique physicochemical properties, such as high specific surface area and unique optical and magnetic properties, and can be modified by various specific ligands to enhance their binding force to target cells or molecules. In addition to the excellent controlled release effect, inorganic NPs can also protect the loaded antigen/drug from degradation and reduce the use of antigen/drug, thereby significantly reducing the drug toxicity.[38] Common inorganic NPs include silica NPs, gold NPs, silver NPs, and metal oxide NPs.

AuNPs are chemically inert and non-toxic, making them attractive candidates as vaccine carriers. AuNPs are made by reducing gold salt in the presence of a suitable stabilizer, and the size is controllable from 2 nm to 150 nm. Many reports

have described the superior properties of gold NPs to provide a solid foundation for intracellular delivery of various substances.

Silver NPs have been widely used in the medical field due to their excellent antibacterial effect. Silver NPs have been used as molecular imaging agents, drug delivery system, diagnosis and treatment of vascular diseases, and wound healing. In addition, silver NPs are easily absorbed by cells and have been used in dentistry due to their antibacterial effects. However, there is currently no FDA-approved silver-based nanocarrier for drug delivery systems.

Silica nanomaterials, especially mesoporous silica NPs, have attracted people's interest due to their potential for drug solubility and sustained release effects. Mesoporous silica NPs are nano-silica particles with a honeycomb structure of the hollow channel, making them have a large surface area, adjustable size, easy surface modification, biocompatibility, low toxicity, and potential for potential sustained and controlled release. In addition, metal oxide NPs, such as Ag_2O, ZnO, TiO_2, MgO, CaO, Fe_3O_4, and Fe_2O_3 NPs, are widely used in various applications including healthcare and cosmetics. The application of inorganic NPs in vaccines may be a strategy and platform for achieving more effective immunity.

Mucosal Adhesion of NPs

The high specific surface area of NP results in a sharp increase in the interface that establishes the bond with the mucosa. The adhesion between the NP and mucosa usually shows a longer interaction time. Therefore, the NPs have different mucosal adhesion behaviors on the mucosal surface (Figure 3).[57,58] When the NPs reach the mucous membrane, they interact with the mucous membrane or diffuse through the mucus according to the characteristics of the NPs. Studies

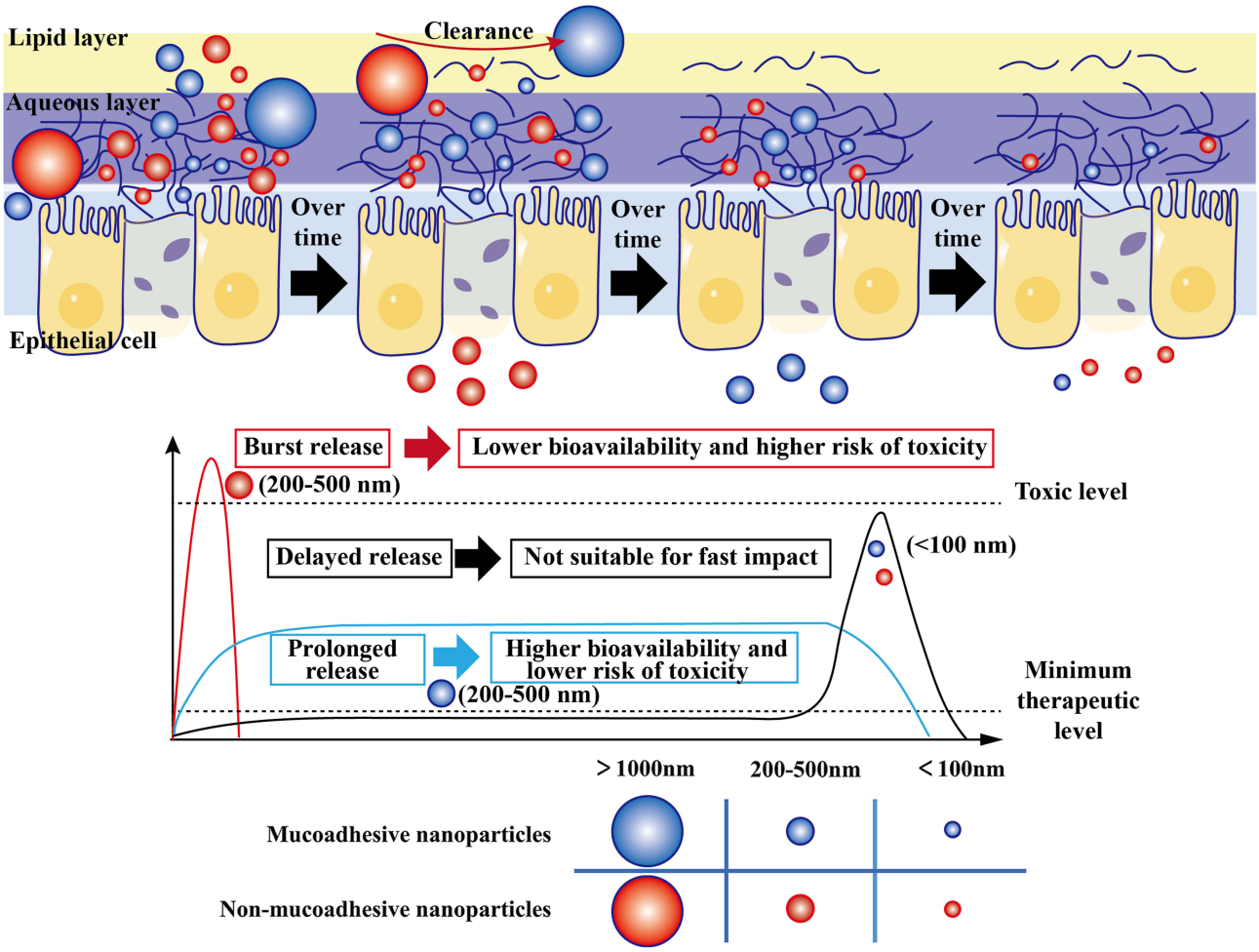


Figure 3 Mucosal adhesion behavior of nanoparticles on the mucosal surface.

have shown that mucus generally does not significantly reduce the diffusion rate of small molecules. However, for particles of 100 nm or larger, mucus can reduce the diffusion rate by several thousand times.[31] The particle size is essential for the adhesion of the NPs to the mucosa, and larger NPs (>1000 nm) cannot diffuse through the mucus layer due to steric hindrance. For smaller NPs (200–500 nm), non-mucoadhesive NPs can quickly pass through the mucus to reach the epithelial lining, while mucoadhesive NPs (200–500 nm) remain in the mucus due to their interaction with the lumen layer via mucus binding. Thus, they release drugs at optimal concentrations (in the therapeutic window) for a prolonged time. This nanoparticle delivery achieves high bioavailability with little risk of toxicity. The smaller NPs (<100 nm) may be retained in the dead zone due to the spatial network structure of mucin, affecting the rate of diffusion through mucus. This results in the drug being released with a considerable lag time or delay. In addition, its release properties are uncontrollable. The mucoadhesive NP-based delivery system will play an essential role in future mucosal vaccine research to achieve sustained antigen release and desirable immune responses.

When the NPs pass through the mucosal cavity and reach the mucosal tissue cells, the NPs face new challenges that ultimately determine their fate. The engineered NPs, such as metal NPs, inorganic NPs, and polymer NPs, can penetrate the cell membrane and be transported to various cells (intestinal epithelial cells, macrophages, and endothelial cells).[59] NPs are taken up by cells mainly through phagocytic pathways, including phagocytosis, clathrin-mediated endocytosis, caveolae-dependent endocytosis, receptor-mediated absorption, and macropinocytosis, and some other non-endocytic mechanisms, such as passive diffusion, cavitation, direct microinjection, and electroporation.[60] In phagocytosis, macrophages, dendritic cells, and other phagocytic cells form phagosomes, which engulf solid particles, such as pathogens and NPs (Figure 4).[61] Receptor-mediated endocytosis is based on the interaction of specific receptors, which improves the efficiency of cell interactions with NPs that recognize specific receptors. Therefore, receptor-mediated endocytosis is an advantageous approach for many surface-engineered NPs.

On the other hand, pinocytosis is subdivided into clathrin-mediated endocytosis, pit-mediated endocytosis, and macrocytosis. Once the NP is taken up by the cell, the fate of the NP will become more complicated. Some NPs in the cytoplasm or trapped in vesicles enter the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus through unknown mechanisms. It is worth noting that many questions about the actual process and the mechanism of NP uptake by cells are still unknown. Understanding the actual processes and mechanisms of NP uptake by cells can help us design NPs to overcome the epithelial barrier.

Factors Affecting the Mucoadhesion of NPs

Mucosal Properties

The mucosal surface is generally an essential barrier between the body and the external environment. Mucosal barriers and their epithelial cells have a highly complex and dynamic structure that protects the body from pathogens and toxins. Mucus is the most distinctive feature of mucous membranes and is the main part of mucosal adhesion. The adhesion of mucus is related to its chemical properties and natural clearance mechanism.[62] Mucus distribution varies in different mucosal sites, as shown in Table 3.

In the nasal passage, nasal mucus is renewed every 5–10 min.[86] The renewal time of eye mucus is very fast (5–10 s), which can remove deposited particles and toxic substances.[87] In contrast, the turnover rate of gastrointestinal mucus is much slower. The relationship between secretion and clearance affects the thickness of different mucosal locations. In addition, the pH of mucus also affects mucosal adhesion behavior. The pH of mucus may vary greatly depending on the location of the mucosa, and the aggregation of mucin fibers will increase with the acidity of the environment, thereby greatly increasing the viscoelasticity of mucus.[88] Eye mucus is generally weakly alkaline, while the pH of nasal, airway, and lung mucus is generally neutral. Vaginal mucus is acidified due to the presence of *Lactobacilli*, and the pH is usually between 3.5 and 4.5.[89] Mucosal adhesion is related to its clearance rate. Understanding the thickness and clearance time of the mucous layer on different mucosal surfaces is very important for the development of mucoadhesive NPs, because they must overcome the mucosal clearance or penetrate the mucus faster than mucus renewal and clearance in order to carry out mucosal adhesion. Natural mucosal clearance is a challenge for mucosal adhesion-based nano delivery systems targeting the mucosal release of vaccine/drug. However, attention should also be paid to the usefulness of the nano delivery system for mucus penetration

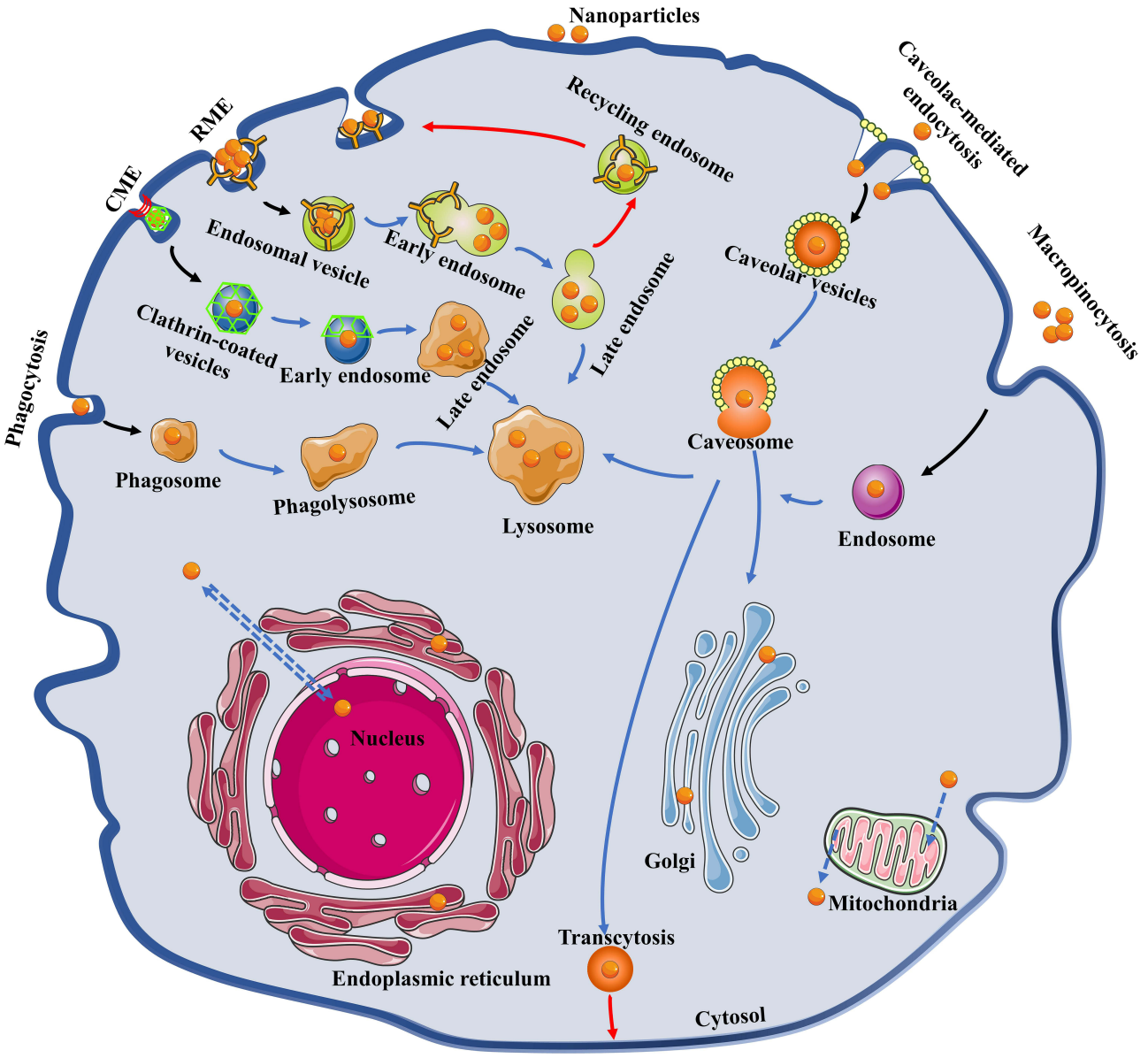


Figure 4 Schematic diagram of the nanoparticle entry process: phagocytosis of nanoparticles (black arrow), intracellular transport (blue arrow) and cellular exocytosis (red arrow).

when designing delivery systems for mucosal adhesion. Furthermore, overcoming the mucus barrier implies incompatible requirements on NP surface properties.[90] For example, mucus-penetrating NPs always exhibit neutral and hydrophilic surface properties. However, hydrophilic/neutral surfaces also reduce interactions with cell membranes.[91] In contrast, positive or hydrophobic surfaces are preferable for efficient cell internalization.[92] Therefore, it is a challenge to develop a mucoadhesive NP-based delivery system that can effectively penetrate the mucus layer and mucosal immunity.

Surface Charge

The electrostatic interaction between the charged NPs and mucosa has important biological significance. In most cases, the presence of sialic acid and ester sulfate makes the mucus negatively charged.[93] When the positively charged and negatively charged NPs are in contact with mucus, electrostatic attraction and repulsion are observed, respectively. Chitosan is the most widely used positively charged mucosal adhesion material.[94,95] The elimination rate of chitosan-modified PLGA nanospheres encapsulating calcitonin is about one-third of that of unmodified nanospheres, indicating

Table 3 Features of Different Mucosal Sites

Type of Mucus	pH Value	Clearance Time or Rate	Mucins Concentration	Mean Layer Thickness	References
Ocular	7.5–7.8	5–10 s	0.01%	3–5 μm	[63,64]
Nasal	6.3–6.7	5–10 min	2–3%	10–15 μm	[65,66]
Buccal	6.8–7.4	0.1–1.85 mL/min	0.1–0.5%	10–100 μm	[67,68]
Airway	7.0	1 mm/min	2–4%	15 μm	[69,70]
Lung	7.0	5–10 cm/min	2–4%	5–55 μm	[71,72]
Esophageal	4.0–7.0	0.2–0.3 mg/cm^2/min	0.1–0.3%	95 μm	[73,74]
Gastric	1.0–3.0	4–5 h	3%	170 μm	[75,76]
Small intestinal	5.9–7.5	47–270 min	1%	0–37 μm	[72,77,78]
Colonic	6.2–7.6	270–300 min	<5%	100 μm	[75,79,80]
Rectal	6.8–7.9	3–4 h	<5%	125 μm	[81,82]
Vaginal	3.5–4.5	6 mL/d	1–2%	20 μm	[83,84]
Cervical	4.0–4.5	1.5 mL/d	5%	200 μm	[85]

that chitosan-modified PLGA nanospheres can adhere to the epithelial cells of trachea and lung due to their mucosal adhesion properties.[96] In contrast, negatively charged or uncharged NPs can easily move through the mucus.[97] The positively charged NPs have the ability to increase mucosal adhesion, and the positively charged particles show higher cellular uptake through endocytosis than negatively charged particles.[53,98] Therefore, in order to penetrate the mucus to reach epithelial cells, it is a promising strategy to make NPs negatively charged.

Particle Size

The particle size of NPs plays an important role in mucosal adhesion. Generally, mucin fibers can form a cross-linked and interpenetrating network structure, which affects the mucus permeability of the NPs. As mentioned above, NPs larger than the mesh size in the mucus are blocked and cannot pass through the mucus barrier. In contrast, NPs smaller than the mesh size and non-sticky can diffuse through mucus.[99] The effective diffusion rate of carboxylated polystyrene NPs in the entire extracted natural porcine intestinal mucus is decreased with the increase of particle size (20–500 nm).[100] Similarly, the low-molecular-weight polystyrene NPs are prepared, and the transport of NPs in human respiratory mucus has been compared. The results have found that the polystyrene NPs with a particle size of 100 nm and 200 nm can quickly penetrate into respiratory mucus, and the polystyrene NPs with a particle size of greater than 500 nm are fixed in the mucus.[101] Therefore, it is important to control the particle size of NPs within 200 nm.

Hydrophobicity

Hydrophobic interaction is also one of the basic mechanisms for NPs to diffuse through mucus. There are a large number of hydrophobic segments in mucin fibers, that is, in low glycosylation regions. Therefore, the hydrophilicity of NPs plays an important role in mucus penetration and transport. Hydrophilic NPs possess many hydrophilic functional groups, which enables the NPs to bind to mucin through hydrogen bonds and facilitates mucus permeability. Increasing the hydrophobicity of NPs helps the adhesion of NPs to mucus. The interaction between several NPs (silica NPs, PEGylated PLGA NPs) with different physicochemical properties and mucin has been examined by dynamic light scattering, showing that positively charged hydrophobic NPs can interact with mucin, while negatively charged hydrophilic NPs cannot interact with mucin.[102]

Increasing the interaction between NPs and mucin can enhance mucosal adhesion. Most strategies use mucoadhesive polymers to modify the surface of NPs. Commonly used mucosal adhesion polymer NPs, such as polyethylene glycol (PEG),[103,104] polycarbophil, carbopol, and[105–107] polymethacrylate, can achieve mucosal adhesion through hydrogen bonding, hydrophobic interaction, and entanglement with mucin.[108] In addition, thiolated polymers that can form disulfide bonds with mucin are increasingly used in mucosal delivery systems because of their ability to significantly enhance mucosal adhesion. Acetylcysteine functionalized chitosan-vitamin E succinate nanomicelles have been synthesized for the oral delivery of paclitaxel, the nanomicelles significantly enhance the mucosal adhesion and permeability, and the intestinal absorption of paclitaxel is increased by 4.5 folds.[109]

NPs Adhered to Mucosa

NPs, as the vaccine adjuvant and delivery systems, have several advantages to stimulate the host's immune system. Firstly, the particle size of NPs can be adjusted to prepare the optimal particle size, which is suitable for the particles to be transported into the body and then absorbed by APCs.[110] Secondly, NPs can be easily functionalized with various ligands or peptides and be targeted for administration. Third, NPs can be formulated to control the release of antigen at a specific site of the mucosal surface. The most common mucoadhesive NPs include natural cellulose,[111] natural polymers,[112–114] and other mucoadhesive groups or polymers.[115–117] Polymer NPs have been widely used in the delivery of mucosal vaccines and mucosal drugs.

Natural Polymer NPs

Natural polysaccharide polymers include chitosan, alginate, inulin, dextran, pullulan, hyaluronic acid, and cyclodextrin. Protein-based polymers include gelatin and collagen. Compared with synthetic polymers, natural polymers have ideal properties, including biocompatibility, biodegradability, and low toxicity or non-toxicity. Studies have proved that polymer NPs can effectively deliver antigens to APCs and significantly improve cellular, humoral, and mucosal immune responses.[118,119] Therefore, natural polymers and polymer NPs have been widely used for the delivery of mucosal vaccines and mucosal drugs.

Due to good adhesion and permeability, chitosan has become one of the most promising natural polysaccharide polymers. Positively charged primary amino group on chitosan will establish an ionic bond, hydrogen, and hydrophobic bond with the negatively charged sialic acid and sulfonic acid in mucus, making chitosan a mucoadhesive biomaterial suitable for the delivery of vaccines and drugs through mucosal administration. Chitosan NPs have been widely used in mucosal vaccination. Inactivated avian infectious bronchitis virus (IBV) vaccine encapsulated in chitosan NPs has been prepared by the ionic gel method, which has been administered to chickens via the eye-nose route, and the nano vaccine induces a stronger mucosal immune response and increases the levels of IFN-γ, IgA antibody, and anti-IBV IgG antibody.[120] Newcastle disease virus (NDV) F gene plasmid DNA encapsulated in chitosan NPs has been prepared in our laboratory. The NPs induce stronger cellular, humoral, and mucosal immune responses than those of naked plasmid DNA, indicating that the chitosan NPs can be used as adjuvant and delivery carriers for the DNA vaccine.[121] Alginate is non-toxic, biodegradable, low-cost, and readily available, and it has the characteristics of mucosal adhesion, biocompatibility, and non-immunogenicity. Therefore, it has been used in the delivery of vaccines.

Synthetic Polymer NPs

Currently, a variety of synthetic polymers have been used to prepare NPs. These synthetic polymer NPs are used to protect the antigen, help the antigen reach the specified site, increase the retention time of the antigen at the target site, and sustain slow release. *N*-2-Hydroxypropyl trimethyl ammonium chloride chitosan (N-2-HACC), a water solubility chitosan derivative, has been synthesized, and the N-2-HACC NPs loaded with NDV have been prepared. The nano vaccine has very low toxicity, high safety, and sustained release effects, and it can induce stronger cellular, humoral, and mucosal immune responses.[122]

As one of the most successfully developed biodegradable polymers, PLGA is a copolymer synthesized by the polymerization of PLA and PGA, and is one of the most successfully developed biodegradable polymers. Due to its biodegradability and biocompatibility, PLGA has considerable flexibility in targeted modification or functionalization and has been used in mucosal drug delivery systems. Similarly, surface modification of anionic PLGA with mucoadhesive polymers (N-[1-(2,3-dioleoyloxy) propyl]-N, N, N-trimethyl ammonium, chitosan, N-trimethyl chitosan, and glycolchitosan) can enhance mucoadhesion and lead to a stronger mucosal immune response.[123]

PEG is a polyether compound, also known as polyethylene oxide (PEO). The mucoadhesive property of PEG itself is questionable because it lacks side groups (such as amines and carboxylic acids) that can specifically interact with mucin components. However, PEG can form hydrogen bonds with sugar residues on glycosylated proteins, making an excellent mucosal adhesion. The performance of PEG depends on the route of administration and the biological fluid flux. Several attempts have been made to improve the adhesion.[124,125]

Polyacrylic acid (PAA), also known as carbomer, is a synthetic polymer of acrylic acid. Due to its good biocompatibility, PAA has been approved for non-parenteral drugs. The mucosal adhesion of PAA is based on the entanglement of physical chains between polymer and mucin, followed by hydrogen bonding or ionic interaction.[126] Compared with other polymers, there are a large number of carboxyl groups in the side chain of PAA. Therefore, PAA may adopt a more favorable macromolecular conformation to increase the accessibility of hydrogen bonds. Compared with the unmodified sample, the thiolation of PAA with 1-cysteine in the presence of the cross-linking agent EDC can increase mucosal adhesion 140 times.[127] In addition, the mucosal adhesion of PAA-cysteine NPs is increased by six times compared with the unmodified ones.[128] The thiolation of PAA improves the bonding properties, which can be explained by the formation of disulfide bonds between the thiol group of polymer and cysteine-rich subdomain in mucus.[109]

Application of Mucoadhesive NPs in Mucosal Vaccine

Mucoadhesive nanosystems with different compositions and biological properties have been extensively studied for vaccine, drug, protein, and gene delivery applications.

Oral Vaccine

Compared with the traditional vaccine, the mucosal vaccine avoids the pain and risk of infection caused by injections. In general, mucosal vaccination, especially oral vaccination, can reduce costs and make immunization of large numbers of people more feasible. Vaccines, such as protein vaccines and DNA vaccines, may be degraded when passing through the gastrointestinal tract or mucosal layer, resulting in reduced biological activity and immune effect of the vaccine. Antigen loaded or encapsulated in NPs can avoid being degraded by an acidic environment and metabolic enzymes in the gastrointestinal tract. In addition, NPs have attracted scores of interest because of their small size and high surface area to volume ratio. Therefore, NPs can easily penetrate the barriers and interact with cell membranes.[129] NPs have been widely studied in the application of vaccines because of their capability of desirable antigen release, targeting specific immune cells, and inducing desirable immune responses.[129,130]

Over the past few years, because chitosan has the ability to adhere and reversibly destroy the tight junction of epithelial cells, it has become a research hotspot in the field of mucosal adhesion materials.[131] Sodium alginate is an anionic polysaccharide with good biological properties, it can easily interact with cationic chitosan NPs through electrostatic interaction to form an electrolyte complex. A novel oral vaccine carrier based on alginate-coated LMWC NPs has been prepared, and the carrier effectively protects the antigen from degradation in acidic media and induces mucosal immunity and systemic immune response.[132] Using chitosan and water-soluble snail mucin as natural polymers, insulin-loaded mucoadhesive NPs based on mucin-chitosan complexes for oral delivery have been prepared by a self-gelling method, and the results show that the complexes have a significant hypoglycemic effect on diabetic rats after oral administration.[133] Additionally, HBsAg-loaded trimethyl chitosan/hydroxypropylmethylcellulose phthalate (HPMCP) has been administered orally. HPMCP NPs not only improve the stability of acid resistance but also protect the loaded HBsAg from degradation, indicating that HPMCP NPs can be used for oral delivery of HBsAg.[134] However, although studies on mucoadhesive NPs as an oral delivery system of vaccine seem encouraging, to date, there are still very few oral vaccines on the market.

Nasal Vaccine

The intranasal vaccine has recently become an attractive alternative to subcutaneous, intradermal, and intramuscular vaccines. Intranasal vaccination is needle-free and non-invasive, and it can be used for mass immunization without the need for a trained medical professional. The nasal mucosa is easily accessible and highly vascularized, and it has abundant immune cells with the potential to induce an effective immune response. In addition, nasal administration has been reported to induce systemic and mucosal immune responses. Despite these advantages, only a few commercially available intranasal vaccines have been developed for clinical use in humans and animals, including intranasally administered live attenuated influenza vaccines FluMist/Fluenz® and Nasovac™.[3] Large molecules have very low nasal absorption, so it is necessary to develop strategies to improve absorption.[135] Since NPs can serve as a delivery system and immunomodulator for vaccine applications, they have been successfully applied to the field of vaccines.[136] Interestingly, through the nasal route, NPs can also bypass mucus and directly interact with mucosal cells to trigger immune responses.[137] Using the protective antigen of anthrax as a model antigen,

the mucosal adhesion chitosan NPs loaded with C48/80 have been prepared and evaluated, and the results show that the NPs significantly prolong the residence time of the antigen in the nasal cavity and produce a strong systemic and mucosal immunity.[138] The PLGA, chitosan-coated PLGA (C-PLGA), and glycol chitosan-coated PLGA (GC-PLGA)) NPs loaded with HBsAg are synthesized, respectively, and GC-PLGA NPs have a long nasal cavity retention time and trigger a relatively strong immune response.[139] Another attractive aspect of nasal administration is the ability to enter the brain through the nasal cavity, and the route is often referred to as nasal-to-brain administration.[140] Moreover, in the fight against COVID-19, a new nasal spray recombinant NDV vector vaccine is currently in clinical research, bringing us one step closer to controlling the pandemic. The study of naturally derived nasal sprays has found that some known naturally derived ingredients have antiviral properties. Therefore, their topical use as a nasal spray is effective in reducing the symptoms of respiratory infections.[141]

Ocular Administration

In ophthalmic applications, the NP delivery system is convenient to avoid irritation, foreign body sensation, and patient discomfort.[142] Mucoadhesive NPs are an emerging therapeutic tool that can release and maintain levels of active substances for a long time and can extend the dosing interval to several months. At present, different biomaterials, including polyacrylates, PLA, PLGA, ALG, hyaluronic acid, and chitosan and its derivatives, have been used in ocular drug delivery.[143]

Chitosan-based NPs can be absorbed by the cornea and conjunctival epithelium, thereby enhancing drug delivery to the extraocular tissues and minimizing the toxicity of the drug to the eye tissue and blood flow (through systemic absorption).[144] Chitosan-based NPs have broad prospects in the treatment of extraocular diseases. Chitosan NPs loaded with hyaluronic acid have been developed for topical ocular delivery of dexamethasone, and in vitro cumulative drug release studies have shown that the dexamethasone can be sustained for 12 h.[145] However, the delivery system used for ocular administration is not mucoadhesive, indicating a limitation of ocular administration. It is now recognized that mucoadhesive polymer NPs can deliver the required drug levels to specific anterior and posterior parts of the eye in a safe and reproducible manner at the right time. In this case, it can be ensured that better mucoadhesive NP delivery systems will be explored in the next few years.

Lung Administration

Due to the large alveolar surface area, the low thickness of the epithelial barrier, and extensive vascularization in the alveolar region, pulmonary delivery has become a popular method to deliver therapeutic or diagnostic drugs.[146] The use of NPs as pulmonary delivery carriers has gained significant interest because of their ability to enter the intracellular compartments and increase bioavailability. Although pulmonary mucosal vaccination is a promising method to induce protection against respiratory infections, there are still many technical challenges.

NP delivery to the lungs suffers from two major drawbacks: 1) NPs, with the exception of NPs with a particle size of <50 nm, are exhaled from the lungs; 2) NPs exhibit formulation instability due to their high surface energy, leading to NP aggregation and/or particle-particle interaction. To overcome these issues, NPs are often applied to the lungs in the form of suspensions. However, in this case, the size of the generated droplets will vary with the nebulizer technique, and the applied stress during nebulization can affect the formulation stability.[147] Inhalation is the primary immune method for pulmonary mucosal vaccination. A powder formulation for inhalation of CAF01 adjuvant has been prepared, and the adjuvant activity is retained after drying.[148] Then, a dry powder formulation based on the tuberculosis subunit vaccine H56/CAF01 has been prepared by spray drying, and the results show that the subunit vaccine induces humoral immunity and cell-mediated immune response.[149]

Vaginal Administration

Vaginal administration can achieve local and systemic administration.[150] Compared with conventional oral administration, vaginal administration has several advantages, including avoiding the drug degradation in the gastrointestinal tract and liver first-pass effect.[151] Conventional drugs, such as gels, tablets, and capsules, lead to poor retention and low efficacy due to the self-cleaning effect of the vagina.[152] In the past few years, mucoadhesive NP delivery systems for vaginal treatment have received increasing attention. Natural and synthetic polymer NPs, including polysaccharides, polycaprolactone, polyacrylate, and PLGA, have been used in vaginal drug delivery due to their stability, mucosal

adhesion, and mucus penetration.[54] Clotrimazole encapsulated in the PLGA-CTS NPs is used to treat vaginal infections caused by *Candida albicans*, and the results show that the nano clotrimazole has good biocompatibility with vaginal epithelial cells and can interact with mucin, indicating the NPs can be used as a vaginal drug delivery carrier.[153]

Future Perspectives

Most pathogenic microorganisms enter the body at mucosal surfaces. Therefore, mucosal immune responses function as a first line of defense. Protective mucosal immune responses are most effectively induced by mucosal immunization through oral, nasal, rectal, or vaginal routes. In recent years, significant progress has been made in the field of mucosal vaccines. New vaccine delivery systems, adjuvants, and immune strategies provide strong support for the development of mucosal vaccines. However, in order to obtain the optimal vaccine mucosal immune delivery system, novel and efficient vaccine delivery systems and alternative solutions need to be explored, indicating that the development and commercialization of mucosal vaccines require more effort and a long time. In order to develop a safe and effective mucosal vaccine, a variety of influencing factors need to be considered, including antigens, adjuvants, delivery systems, routes of administration, and animal models for safety and efficacy assessment; Additionally, to obtain an effective protective mucosal immune response, we believe that the key biological and technical aspects of mucosal vaccine design also need to be considered.

Protective mucosal immune responses are most effectively induced by mucosal immunization through oral, nasal, rectal, or vaginal routes. However, the vast majority of vaccines in use today are administered intramuscularly or subcutaneously. The design of mucosal vaccines requires a comprehensive understanding of the structure of the mucosal immune system and the physical and chemical barrier properties of the mucosa. Nowadays, our understanding of mucosal immunity and the development of mucosal vaccines have lagged behind, in part because the administration of mucosal vaccines and the measurement of mucosal immune responses are more complicated. The dose of mucosal vaccine that actually enters the body cannot be accurately measured because antibodies in mucosal secretions are difficult to capture and quantity. However, research and testing of mucosal vaccines are currently accelerating, stimulated by new information on the mucosal immune system and by the threat of the mucosally transmitted virus.

In the present review, we discussed emerging strategies that are expected to contribute to the development of a new generation of mucosal vaccines, the main purpose of which is to maximize the effectiveness and safety of mucosal immunity. Compared with conventional delivery systems, delivery systems based on mucoadhesive NPs have shown great advantages. The delivery system based on mucoadhesive NPs is a promising, feasible, and safe choice for targeted delivery and mucosal release of vaccine/drug, and these NPs are superior to traditional formulations in terms of improved vaccine efficacy, controlled release, targeted delivery, and therapeutic effects. Moreover, the mucoadhesive NPs can also significantly enhance the stability of the vaccine, prolong the duration of immunity, and reduce the frequency of administration. However, the design and delivery of vaccines based on mucoadhesive NPs remain a technical challenge. It requires the careful formulation and dosage design, combined with a suitable and efficient delivery carrier, to achieve the requirements of crossing the physiological barrier and inducing a mucosal immune response. Here, we look forward to an early breakthrough in the synthesis technology of vaccine delivery systems and vaccines for mucosal administration, and more mucosal vaccines will be used in clinical practice.

Acknowledgments

This work was supported in part by the "Pioneer" and "Leading Goose" R&D Program of Zhejiang (2022C02031), National Natural Science Foundation of China (32170844), General Scientific Research Project of Education Department of Zhejiang Province (Y202146992) and Taizhou Science and Technology Plan Project in 2022 (22nya04).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, design, execution, acquisition of the literature search, analysis and interpretation; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no other potential conflicts of interest in relation to this work and no competing financial interest.

References

1. Harandi AM. Systems analysis of human vaccine adjuvants. *Semin Immunol*. 2018;39:30–34. doi:10.1016/j.smim.2018.08.001
2. Hellfritzsch M, Scherliess R. Mucosal vaccination via the respiratory tract. *Pharmaceutics*. 2019;11(8):375. doi:10.3390/pharmaceutics11080375
3. Pavot V, Climent N, Rochereau N, et al. Directing vaccine immune responses to mucosa by nanosized particulate carriers encapsulating NOD ligands. *Biomaterials*. 2016;75:327–339. doi:10.1016/j.biomaterials.2015.10.034
4. Strober W, Coffman RL. Tolerance and immunity in the mucosal immune system. Introduction. *Res Immunol*. 1997;148(8–9):489–599. doi:10.1016/S0923-2494(98)80141-7
5. Lycke N. Recent progress in mucosal vaccine development: potential and limitations. *Nat Rev Immunol*. 2012;12(8):592–605. doi:10.1038/nri3251
6. Mansouri S, Jin L. Evaluation of mucosal and systemic vaccine responses by cyclic di-GMP (CDG)-adjuvanted protein subunit vaccines. *Bio Protoc*. 2019;9(8):e3217. doi:10.21769/BioProtoc.3217
7. Herbst-Kralovetz MM. Overcoming barriers in the mucosal delivery of virus-like particle-based vaccines. *Ther Deliv*. 2014;5(7):741–744. doi:10.4155/tde.14.52
8. Lai SK, Wang YY, Hanes J. Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv Drug Deliv Rev*. 2009;61(2):158–171. doi:10.1016/j.addr.2008.11.002
9. Hou CS, Yi B, Jiang JK, et al. Up-to-date vaccine delivery systems: robust immunity elicited by multifarious nanomaterials upon administration through diverse routes. *Biomater Sci*. 2019;7(3):822–835. doi:10.1039/c8bm01197d
10. Borger JG, Le Gros G, Kirman JR. Editorial: the role of innate lymphoid cells in mucosal immunity. *Front Immunol*. 2020;11:1233. doi:10.3389/fimmu.2020.01233
11. Hickey DK, Patel MV, Fahey JV, Wira CR. Innate and adaptive immunity at mucosal surfaces of the female reproductive tract: stratification and integration of immune protection against the transmission of sexually transmitted infections. *J Reprod Immunol*. 2011;88(2):185–194. doi:10.1016/j.jri.2011.01.005
12. Woodrow KA, Bennett KM, Lo DD. Mucosal vaccine design and delivery. *Annu Rev Biomed Eng*. 2012;14:17–46. doi:10.1146/annurev-bioeng-071811-150054
13. Kim SH, Jang YS. The development of mucosal vaccines for both mucosal and systemic immune induction and the roles played by adjuvants. *Clin Exp Vaccine Res*. 2017;6(1):15–21. doi:10.7774/cevr.2017.6.1.15
14. Singh B, Maharjan S, Cho KH, et al. Chitosan-based particulate systems for the delivery of mucosal vaccines against infectious diseases. *Int J Biol Macromol*. 2018;110:54–64. doi:10.1016/j.ijbiomac.2017.10.101
15. Tlaxca JL, Ellis S, Remmele RL. Live attenuated and inactivated viral vaccine formulation and nasal delivery: potential and challenges. *Adv Drug Deliv Rev*. 2015;93:56–78. doi:10.1016/j.addr.2014.10.002
16. Shakya AK, Chowdhury MYE, Tao WQ, Gill HS. Mucosal vaccine delivery: current state and a pediatric perspective. *J Control Release*. 2016;240:394–413. doi:10.1016/j.jconrel.2016.02.014
17. Mato YL. Nasal route for vaccine and drug delivery: features and current opportunities. *Int J Pharm*. 2019;572:118813. doi:10.1016/j.ijpharm.2019.118813
18. Jin Z, Gao S, Cui XL, Sun DJ, Zhao K. Adjuvants and delivery systems based on polymeric nanoparticles for mucosal vaccines. *Int J Pharm*. 2019;572:118731. doi:10.1016/j.ijpharm.2019.118731
19. Pabst R. Mucosal vaccination by the intranasal route. Nose-associated lymphoid tissue (NALT)-Structure, function and species differences. *Vaccine*. 2015;33(36):4406–4413. doi:10.1016/j.vaccine.2015.07.022
20. Chatterjee B, Gorain B, Mohananaidu K, et al. Targeted drug delivery to the brain via intranasal nanoemulsion: available proof of concept and existing challenges. *Int J Pharm*. 2019;565:258–268. doi:10.1016/j.ijpharm.2019.05.032
21. Bivas-Benita M, Zwier R, Junginger HE, Borchard G. Non-invasive pulmonary aerosol delivery in mice by the endotracheal route. *Eur J Pharm*. 2005;61(3):214–218. doi:10.1016/j.ejpb.2005.04.009
22. Khan MS, Roberts MS. Challenges and innovations of drug delivery in older age. *Adv Drug Deliv Rev*. 2018;135:3–38. doi:10.1016/j.addr.2018.09.003
23. Morales JO, Fathe KR, Brunaugh A, et al. Challenges and future prospects for the delivery of biologics: oral mucosal, pulmonary, and transdermal routes. *AAPS J*. 2017;19:652–668. doi:10.1208/s12248-017-0054-z
24. Marasini N, Skwarczynski M, Toth I. Oral delivery of nanoparticle-based vaccines. *Expert Rev Vaccines*. 2014;13:1361–1376. doi:10.1586/14760584.2014.936852
25. Kraan H, Vrieling H, Czerkinsky C, et al. Buccal and sublingual vaccine delivery. *J Control Release*. 2014;190:580–592. doi:10.1016/j.jconrel.2014.05.060
26. Melo M, Nunes R, Sarmento B, Das Neves J. Rectal administration of nanosystems: from drug delivery to diagnostics. *Mater Today Chem*. 2018;10:128–141. doi:10.1016/j.mtchem.2018.09.001
27. De Temmerman ML, Rejman J, Demeester J, et al. Particulate vaccines: on the quest for optimal delivery and immune response. *Drug Discov Today*. 2011;16(13–14):569–582. doi:10.1016/j.drudis.2011.04.006
28. Sofi HS, Abdal-hay A, Ivanovski S, Zhang YS, Sheikh FA. Electrospun nanofibers for the delivery of active drugs through nasal, oral and vaginal mucosa: current status and future perspectives. *Mater Sci Eng C*. 2020;111:110756. doi:10.1016/j.msec.2020.110756
29. Imperiale JC, Acosta GB, Sosnik A. Polymer-based carriers for ophthalmic drug delivery. *J Control Release*. 2018;285:106–141. doi:10.1016/j.jconrel.2018.06.031
30. Morbe UM, Jorgensen PB, Fenton TM, et al. Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. *Mucosal Immunol*. 2021;14(4):793–802. doi:10.1038/s41385-021-00389-4

31. Kharenko EA, Larionova NI, Demina NB. Mucoadhesive drug delivery systems. *Pharm Chem J*. 2009;43(4):200–208. doi:10.1007/s11094-009-0271-6
32. Smart JD. The basics and underlying mechanisms of mucoadhesion. *Adv Drug Deliv Rev*. 2005;57(11):1556–1568. doi:10.1016/j.addr.2005.07.001
33. Newby JM, Seim I, Lysy M, et al. Technological strategies to estimate and control diffusive passage times through the mucus barrier in mucosal drug delivery. *Adv Drug Deliv Rev*. 2018;124:64–81. doi:10.1016/j.addr.2017.12.00232
34. Peppas NA, Sahlin JJ. Hydrogels as mucoadhesive and bioadhesive materials: a review. *Biomaterials*. 1996;17(16):1553–1561. doi:10.1016/0142-9612(95)00307-X
35. Kim ED, Han SJ, Byun YH, et al. Inactivated eyedrop influenza vaccine adjuvanted with poly (I:C) is safe and effective for inducing protective systemic and mucosal immunity. *PLoS One*. 2015;10(9):e0137608. doi:10.1371/journal.pone.0137608
36. Zhao L, Seth A, Wibowo N, et al. Nanoparticle vaccines. *Vaccine*. 2014;32(3):327–337. doi:10.1016/j.vaccine.2013.11.069
37. Rizzo LY, Theek B, Storm G, Kiessling F, Lammers T. Recent progress in nanomedicine: therapeutic, diagnostic and theranostic applications. *Curr Opin Biotechnol*. 2013;24(6):1159–1166. doi:10.1016/j.copbio.2013.02.020
38. Sadeghi S, Lee WK, Kong SN, Shetty A, Drum CL. Oral administration of protein nanoparticles: an emerging route to disease treatment. *Pharmacol Res*. 2020;158:104685. doi:10.1016/j.phrs.2020.104685
39. Chang D, Ma YY, Xu XX, Xie JB, Ju SH. Stimuli-Responsive polymeric nanoparticles. *Macromol Rapid Commun*. 2017;9:1700030. doi:10.3389/fbioe.2021.707319
40. Bulbake U, Doppalapudi S, Kommineni N, Khan W. Liposomal formulations in clinical use: an updated review. *Pharmaceutics*. 2017;9(2):12. doi:10.3390/pharmaceutics9020012
41. Raj S, Khurana S, Choudhari R, et al. Specific targeting cancer cells with nanoparticles and drug delivery in cancer therapy. *Semin Cancer Biol*. 2021;69:166–177. doi:10.1016/j.semcancer.2019.11.002
42. Shah MAA, He NY, Li ZY, Ali ZS, Zhang LM. Vaccine delivery using nanoparticles. *J Biomed Nanotechnol*. 2014;10(9):2332–2349. doi:10.1166/jbn.2014.1981
43. Fan YC, Marioli M, Zhang K. Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. *J Pharm Biomed Anal*. 2021;192:113642. doi:10.1016/j.jpba.2020.113642
44. Morein B, Hu K-F, Abusugra I. Current status and potential application of ISCOMs in veterinary medicine. *Adv Drug Deliv Rev*. 2004;56(10):1367–1382. doi:10.1016/j.addr.2004.02.004.48
45. Sun HX, Xie Y, Ye YP. Iscoms and Iscomatrix. *Vaccine*. 2009;27(33):4388–4401. doi:10.1016/j.vaccine.2009.05.032
46. Lenarczyk A, Le TTT, Drane D, et al. ISCOM® based vaccines for cancer immunotherapy. *Vaccine*. 2004;22(8):963–974. doi:10.1016/j.vaccine.2003.09.014
47. Aguila A, Donachie AM, Peyre M, et al. Induction of protective and mucosal immunity against diphtheria by a immune stimulating complex (ISCOMS) based vaccine. *Vaccine*. 2006;24(24):5201–5210. doi:10.1016/j.vaccine.2006.03.081
48. Gao Y, Wijewardhana C, Mann JFS. Virus-like particle, liposome, and polymeric particle-based vaccines against HIV-1. *Front Immunol*. 2018;9:345. doi:10.3389/fimmu.2018.00345
49. Marin A, Chowdhury A, Valencia SM, et al. Next generation polyphosphazene immunoadjuvant: synthesis, self-assembly and in vivo potency with human papillomavirus VLPs-based vaccine. *Nanomedicine*. 2021;33:102359. doi:10.1016/j.nano.2021.102359
50. Mu S, Sun S, Dong H. Potent protective immune responses to senecavirus induced by virus-like particle vaccine in pigs. *Vaccines*. 2020;8(3):532. doi:10.3390/vaccines8030532
51. Guo HC, Sun SQ, Jin Y, et al. Foot-and-mouth disease virus-like particles produced by a SUMO fusion protein system in *Escherichia coli* induce potent protective immune responses in Guinea pigs, swine and cattle. *Vet Res*. 2013;44(1):48. doi:10.1186/1297-9716-44-48
52. Jain NK, Sahni N, Kumru OS, Joshi SB, Volkin DB, Russell Middaugh C. Formulation and stabilization of recombinant protein based virus-like particle vaccines. *Adv Drug Deliv Rev*. 2015;93:42–55. doi:10.1016/j.addr.2014
53. Fontana D, Marsili F, Etcheverrigaray M, et al. Rabies VLPs adjuvanted with saponin-based liposomes induce enhanced immunogenicity mediated by neutralizing antibodies in cattle, dogs and cats. *J Virol Methods*. 2020;286:113966. doi:10.1016/j.jviromet.2020.113966
54. Cimica V, Galarza JM. Adjuvant formulations for virus-like particle (VLP) based vaccines. *Clin Immunol*. 2017;183:99–108. doi:10.1016/j.clim.2017.08.004
55. Sharifianjazi F, Irani M, Esmaeilkhanian A, et al. Polymer incorporated magnetic nanoparticles: applications for magnetoresponsive targeted drug delivery. *Mater Sci Eng B*. 2021;272:115358. doi:10.1016/j.mseb.2021.115358
56. Singh RK, Kim HW. Inorganic nanobiomaterial drug carriers for medicine. *Tissue Eng Regen Med*. 2013;10(6):296–309. doi:10.1007/s13770-013-1092-y
57. Sabet S, Rashidinejad A, Melton LD, McGillivray DJ. Recent advances to improve curcumin oral bioavailability. *Trends Food Sci Tech*. 2021;110:253–266. doi:10.1016/j.tifs.2021.02.006
58. Das Neves J, Bahia MF, Amiji MM, Sarmento B. Mucoadhesive nanomedicines: characterization and modulation of mucoadhesion at the nanoscale. *Expert Opin Drug Deliv*. 2011;8(8):1085–1104. doi:10.1517/17425247.2011.586334
59. Zhao F, Zhao Y, Liu Y, Chang XL, Chen CY, Zhao YL. Cellular uptake, intracellular trafficking, and cytotoxicity of nanomaterials. *Small*. 2011;7(10):1322–1337. doi:10.1002/smll.201100001
60. Behzadi S, Serpooshan V, Tao W, et al. Cellular uptake of nanoparticles: journey inside the cell. *Chem Soc Rev*. 2017;46(14):4218–4244. doi:10.1039/c6cs00636a
61. Fornaguera C, Castells-Sala C, Borros S. Unraveling polymeric nanoparticles cell uptake pathways: two decades working to understand nanoparticles journey to improve gene therapy. *Adv Exp Med Biol*. 2020;1288:117–138. doi:10.1007/5584_2019_467
62. Wu L, Shan W, Zhang ZR, Huang Y. Engineering nanomaterials to overcome the mucosal barrier by modulating surface properties. *Adv Drug Deliv Rev*. 2018;124:150–163. doi:10.1016/j.addr.2017.10.001
63. Zhao H, Jumblatt JE, Wood TO, Jumblatt MM. Quantification of MUC5AC protein in human tears. *Cornea*. 2001;20(8):873–877. doi:10.1097/00003226-200111000-00019
64. Schmoll T, Unterhuber A, Kolbitsch C, Le T, Stingl A, Leitgeb R. Precise Thickness measurements of bowman's layer, epithelium, and tear film. *Optom Vis Sci*. 2012;89(5):E795–E802. doi:10.1097/OPX.0b013e3182504346

65. Ozsoy Y, Gungor S. Nasal route: an alternative approach for antiemetic drug delivery. *Expert Opin Drug Deliv*. 2011;8(11):1439–1453. doi:10.1517/17425247.2011.607437
66. Beule AG. Physiology and pathophysiology of respiratory mucosa of the nose and the paranasal sinuses. *Laryngorhinootologie*. 2010;89:S15–34. doi:10.3205/cto000071
67. Dziemianczyk D, Grabowska SZ, Balicki R. Evaluation of secretory mucin concentration of patients with squamous cell carcinoma oral cavity. *Rocz Akad Med Bialymst*. 2005;50:334–338.
68. Dawes C. How much saliva is enough for avoidance of xerostomia? *Caries Res*. 2004;38(3):236–240. doi:10.1159/000077760
69. Clunes MT, Boucher RC. Cystic Fibrosis: the mechanisms of pathogenesis of an inherited lung disorder. *Drug Discov Today Technol*. 2007;4(2):63–72. doi:10.1016/j.ddmec.2007.09.001
70. Donaldson SH, Corcoran TE, Laube BL, Bennett WD. Mucociliary clearance as an outcome measure for cystic fibrosis clinical research. *Proc Am Thorac Soc*. 2007;4(4):399–405. doi:10.1513/pats.200703-042BR
71. Huang ZH, Ma AJ, Lei JL. Progress in study on the skin mucus lectin in fish. *Zool Res*. 2013;34(6):674–679.
72. Ensign LM, Schneider C, Suk JS, Cone R, Hanes J. Mucus penetrating nanoparticles: biophysical tool and method of drug and gene delivery. *Adv Drug Deliv Rev*. 2012;24(28):3887–3894. doi:10.1002/adma.201201800
73. Majewski M, Jaworski T, Sarosiek I, et al. Significant enhancement of esophageal pre-epithelial defense by tegaserod: implications for an esophagoprotective effect. *Clin Gastroenterol Hepatol*. 2007;5(4):430–438. doi:10.1016/j.cgh.2007.01.002
74. Villa N, Vela MF. Impedance-pH testing. *Gastroenterol Clin North Am*. 2013;42(1):2667–2669. doi:10.1016/j.gtc.2012.11.003
75. Wu L, Liu M, Zhu X, Shan W, Huang Y. Modification strategies of lipid-based nanocarriers for mucosal drug delivery. *Curr Pharm Des*. 2015;21(36):5198–5211. doi:10.2174/1381612821666150923103000
76. Bansil R, Celli JP, Hardcastle JM, Turner BS. The influence of mucus microstructure and rheology in Helicobacter pylori infection. *Front Immunol*. 2013;4:310. doi:10.3389/fimmu.2013.00310
77. Luissint AC, Parkos CA, Nusrat A. Inflammation and the intestinal barrier: leukocyte-epithelial cell interactions, cell junction remodeling, and mucosal repair. *Gastroenterology*. 2016;151(4):616–632. doi:10.1053/j.gastro.2016.07.008
78. Lehr CM, Poelma FGJ, Junginger HE, Tukker JJ. An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop. *Int J Pharm*. 1991;70(3):235–240. doi:10.1016/0378-5173(91)90287-X
79. Brownlee IA, Havler ME, Dettmar PW, Allen A, Pearson JP. Colonic mucus: secretion and turnover in relation to dietary fibre intake. *Proc Nutr Soc*. 2003;62(1):245–249. doi:10.1079/PNS2003206
80. Copeman M, Matuz J, Leonard AJ, Pearson JP, Dettmar PW, Allen A. The gastroduodenal mucus barrier and its role in protection against luminal pepsins: the effect of 16,16 dimethyl prostaglandin E2, carbopol-polyacrylate, sucralfate and bismuth subsalicylate. *J Gastroenterol Hepatol*. 1994;9(Suppl 1):S55–59. doi:10.1111/j.1440-1746.1994.tb01303.x
81. Ferguson A. Mucosal immunology: from bench to the bedside and beyond. *Immunol Today*. 1996;89(4):475–482. doi:10.1046/j.1365-2567.1996.d01-791.x
82. Tao W, Zwischenberger JB, Nguyen TT, et al. Gut mucosal ischemia during normothermic cardiopulmonary bypass results from blood flow redistribution and increased oxygen demand. *J Thorac Cardiovasc Surg*. 1995;110(3):819–828. doi:10.1016/S0022-5223(95)70116
83. Wang X, Liu S, Guan YY, Ding J, Ma C, Xie ZG. Vaginal drug delivery approaches for localized management of cervical cancer. *Adv Drug Deliv Rev*. 2021;174:114–126. doi:10.1016/j.addr.2021.04.009
84. Karn PR, Vanic Z, Pepic I, Skalko-Basnet N. Mucoadhesive liposomal delivery systems: the choice of coating material. *Drug Dev Ind Pharm*. 2011;37(4):482–488. doi:10.3109/03639045.2010.523425
85. Witten J, Samad T, Ribbeck K. Selective permeability of mucus barriers. *Curr Opin Biotechnol*. 2018;52:124–133. doi:10.1016/j.copbio.2018.03.010
86. Habesoglu M, Demir K, Yumusakhuylu AC, Yilmaz AS, Oysu C. Does passive smoking have an effect on nasal mucociliary clearance? *Otolaryngol Head Neck Surg*. 2012;147(1):152–156. doi:10.1177/0194599812439004
87. Partenhauser A, Bernkop-Schnurch A. Mucoadhesive polymers in the treatment of dry X syndrome. *Drug Discov Today Technol*. 2016;21(7):1051–1062. doi:10.1016/j.drudis.2016.02.013
88. Celli JP, Turner BS, Afdhal NH. Rheology of gastric mucin exhibits a pH-dependent sol-gel transition. *Biomacromolecules*. 2007;8(5):1580–1586. doi:10.1021/bm0609691
89. Owen DH, Katz DF. A vaginal fluid simulant. *Contraception*. 1999;59(2):91–95. doi:10.1016/S0010-7824(99)00010
90. Soderholm AT, Pedicord VA. Intestinal epithelial cells: at the interface of the microbiota and mucosal immunity. *Immunol*. 2019;158(4):267–280. doi:10.1111/imm.13117
91. Gao YK, He Y, Zhang HT, et al. Zwitterion-functionalized mesoporous silica nanoparticles for enhancing oral delivery of protein drugs by overcoming multiple gastrointestinal barriers. *J Colloid Interface Sci*. 2021;582:364–375. doi:10.1016/j.jcis.2020.08.010
92. Hu S, Yang Z, Wang S, et al. Zwitterionic polydopamine modified nanoparticles as an efficient nanoplatform to overcome both the mucus and epithelial barriers. *Chem Eng J*. 2022;428:132107. doi:10.1016/j.cej.2021.132107
93. Netsomboon K, Bemkop-Schnurch A. Mucoadhesive vs. mucopenetrating particulate drug delivery. *Eur J Pharm Biopharm*. 2016;98:76–89. doi:10.1016/j.ejpb.2015.11.003
94. Mazzarino L, Travelet C, Ortega-Murillo S, et al. Elaboration of chitosan-coated nanoparticles loaded with curcumin for mucoadhesive applications. *J Colloid Interface Sci*. 2012;370:58–66. doi:10.1016/j.jcis.2011.12.063
95. Mohammed MA, Syeda JTM, Wasan KM, Wasan EK. An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. *Pharmaceutics*. 2017;9:53. doi:10.3390/pharmaceutics9040053
96. Yamamoto H, Kuno Y, Sugimoto S, Takeuchi H, Kawashima Y. Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions. *J Control Release*. 2005;102(2):373–381. doi:10.1016/j.jconrel.2004.10.010
97. Crater JS, Carrier RL. Barrier properties of gastrointestinal mucus to nanoparticle transport. *Macromol Biosci*. 2010;10(12):1473–1483. doi:10.1002/mabi.201000137
98. Li Y, Gu N. Thermodynamics of charged nanoparticle adsorption on charge-neutral membranes: a simulation study. *J Phys Chem B*. 2010;114:2749–2754. doi:10.1021/jp904550b

99. Huckaby JT, Lai SK. PEGylation for enhancing nanoparticle diffusion in mucus. *Adv Drug Deliv Rev*. 2018;124:125–139. doi:10.1016/j.addr.2017.08.010
100. Yildiz HM, McKelvey CA, Marsac PJ, Carrier RL. Size selectivity of intestinal mucus to diffusing particulates is dependent on surface chemistry and exposure to lipids. *J Drug Target*. 2015;23(7–8):768–774. doi:10.3109/1061186X.2015.1086359
101. Schuster BS, Suk JS, Woodworth GF, Hanes J. Nanoparticle diffusion in respiratory mucus from humans without lung disease. *Biomaterials*. 2013;34(13):3439–3446. doi:10.1016/j.biomaterials.2013.01.064
102. Griffiths PC, Cattoz B, Ibrahim MS, Anuonye JC. Probing the interaction of nanoparticles with mucin for drug delivery applications using dynamic light scattering. *Eur J Pharm Biopharm*. 2015;97:218–222. doi:10.1016/j.ejpb.2015.05.004
103. Yoncheva K, Gomez S, Campanero MA, Gamazo C, Irache JM. Bioadhesive properties of pegylated nanoparticles. *Expert Opin Drug Deliv*. 2005;2(2):205–218. doi:10.1517/17425247.2.2.205
104. Furst T, Dakwar GR, Zagato E, et al. Freeze-dried mucoadhesive polymeric system containing pegylated lipoplexes: towards a vaginal sustained released system for siRNA. *J Control Release*. 2016;236:68–78. doi:10.1016/j.jconrel.2016.06.028
105. Garipova VR, Gennari CGM, Selmin F, Cilurzo F, Moustafine RI. Mucoadhesive interpolyelectrolyte complexes for the buccal delivery of clobetasol. *Polymers*. 2018;10:225–231. doi:10.3390/polym10010085
106. Abu-Rumman A, Abu-Huwaij R, Hamed R. Development and appraisal of mucoadhesive tablets of hydralazine using Isolated mucilage of *Annona squamosa* seeds. *Int J Pharm Sci Nanotechnol*. 2016;9(4):3349–3356. doi:10.1080/00218464.2020.1864337
107. Asati S, Jain S, Choubey A, et al. Bioadhesive or mucoadhesive drug delivery system: a potential alternative to conventional therapy. *J Drug Deliv Ther*. 2019;9(4–A):858–867.
108. Mouftah S, Abdel-Mottaleb MMA, Lamprecht A. Buccal delivery of low molecular weight heparin by cationic polymethacrylate nanoparticles. *Int J Pharm*. 2016;515(1–2):565–574. doi:10.1016/j.ijpharm.2016.10.039
109. Lian H, Zhang TH, Sun J, et al. Enhanced oral delivery of paclitaxel using acetylcysteine functionalized chitosan-vitamin E succinate nanomicelles based on a mucus bioadhesion and penetration mechanism. *Mol Pharm*. 2013;10(9):3447–3458. doi:10.1021/mp400282r
110. Kang ML, Cho CS, Yoo HS. Application of chitosan microspheres for nasal delivery of vaccines. *Biotechnol Adv*. 2009;27(6):857–865. doi:10.1016/j.biotechadv.2009.06.007
111. Javanbakht S, Shaabani A. Carboxymethyl cellulose-based oral delivery systems. *Int J Biol Macromol*. 2019;133:21–29. doi:10.1016/j.ijbiomac.2019.04.079
112. Onuigbo E, Iseghohimhen J, Chah K, Gyang M, Attama A. Chitosan/alginate microparticles for the oral delivery of fowl typhoid vaccine: innate and acquired immunity. *Vaccine*. 2018;36(33):4973–4978. doi:10.1016/j.vaccine.2018.05.087
113. Nayak AK, Pal D, Santra K. Development of calcium pectinate-tamarind seed polysaccharide mucoadhesive beads containing metformin HCl. *Carbohydr Polym*. 2014;101:220–230. doi:10.1016/j.carbpol.2013.09.024
114. Sandri G, Motta S, Bonferoni MC, et al. Chitosan-coupled solid lipid nanoparticles: tuning nanostructure and mucoadhesion. *Eur J Pharm Biopharm*. 2017;110:13–18. doi:10.1016/j.ejpb.2016.10.010
115. Surassmo S, Saengkrit N, Ruktanonchai UR, et al. Surface modification of PLGA nanoparticles by carbopol to enhance mucoadhesion and cell internalization. *Colloids Surf B Biointerfaces*. 2015;130:229–236. doi:10.1016/j.colsurfb.2015.04.015
116. Bravo-Osuna I, Vauthier C, Farabollini A, Palmieri GF, Ponchel G. Mucoadhesion mechanism of chitosan and thiolated chitosan-poly (isobutyl cyanoacrylate) core-shell nanoparticles. *Biomaterials*. 2007;28(13):2233–2243. doi:10.1016/j.biomaterials.2007.01.005
117. Haltner E, Easson JH, Lehr C-M. Lectins and bacterial invasion factors for controlling endo- and transcytosis of bioadhesive drug carrier systems. *Eur J Pharm Biopharm*. 1997;44(1):3–13. doi:10.1016/S0939-6411(97)00096-9
118. Maity S, Mukhopadhyay P, Kundu PP, Chakraborti AS. Alginate coated chitosan core-shell nanoparticles for efficient oral delivery of naringenin in diabetic animals-An in vitro and in vivo approach. *Carbohydr Polym*. 2017;170:124–132. doi:10.1016/j.carbpol.2017.04.066
119. Romano CL, De Vecchi E, Bortolin M, Morelli I, Drago L. Hyaluronic acid and its composites as a local antimicrobial/antiadhesive barrier. *J Bone Jt Infect*. 2017;2(1):63–72. doi:10.7150/jbji.17705
120. Lopes PD, Okino CH, Fernando FS, et al. Inactivated infectious bronchitis virus vaccine encapsulated in chitosan nanoparticles induces mucosal immune responses and effective protection against challenge. *Vaccine*. 2018;36:2630–2636. doi:10.1016/j.vaccine.2018.03.065
121. Zhao K, Zhang Y, Zhang XY, et al. Preparation and efficacy of Newcastle disease virus DNA vaccine encapsulated in chitosan nanoparticles. *Int J Nanomedicine*. 2014;9:389–402. doi:10.2147/IJN.S54226
122. Zhao K, Sun YW, Chen G, et al. Biological evaluation of N-2-Hydroxypropyl trimethyl ammonium chloride chitosan as a carrier for the delivery of live Newcastle disease vaccine. *Carbohydr Polym*. 2016;149:28–39. doi:10.1016/j.carbpol.2016.04.085
123. Kolesova O, Simonetti G, Donati L, et al. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. *Polym*. 2011;3:1377–1397. doi:10.3390/polym3031377
124. Kolesova O, Simonetti G, Donati L, et al. Surface-modified gemcitabine with mucoadhesive polymer for oral delivery. *J Microencapsul*. 2012;29(5):487–496. doi:10.3109/02652048.2012.665086
125. Cleary J, Bromberg L, Magner E. Adhesion of polyether-modified poly (acrylic acid) to mucin. *Langmuir*. 2004;20(22):9755–9762. doi:10.1021/la048993s
126. Cook MT, Khutoryanskiy VV. An in vitro assessment of mucus/mucoadhesive interactions. *Int J Pharm*. 1995;124:173–182. doi:10.1016/j.ijpharm.2015.09.064
127. Bernkop-Schnurch A. Thiomers: a new generation of mucoadhesive Polym. *Adv Drug Deliv Rev*. 2005;57:1569–1582. doi:10.1016/j.addr.2005.07.002
128. Dunnhaupt S, Barthelmes J, Hombach J, Sakloetsakun D, Arkhipova V, Bernkop-Schnurch A. Distribution of thiolated mucoadhesive nanoparticles on intestinal mucosa. *Int J Pharm*. 2011;408:191–199. doi:10.1016/j.ijpharm.2011.01.060
129. Yang Y, Xing RG, Liu S, et al. Chitosan, hydroxypropyl trimethyl ammonium chloride chitosan and sulfated chitosan nanoparticles as adjuvants for inactivated Newcastle disease vaccine. *Carbohydr Polym*. 2020;229:115423. doi:10.1016/j.carbpol.2019.115423
130. Smith JD, Morton LD, Ulery BD. Nanoparticles as synthetic vaccines. *Curr Opin Biotechnol*. 2015;34:217–224. doi:10.1016/j.copbio.2015.03.014
131. van der Lubben IM, Verhoef JC, Borchard G, Junginger HE. Chitosan for mucosal vaccination. *Adv Drug Deliv Rev*. 2001;52(2):139–144. doi:10.1016/S0169-409X(01)00197-1

132. Biswas S, Chattopadhyay M, Sen KK, Saha MK. Development and characterization of alginate coated low molecular weight chitosan nanoparticles as new carriers for oral vaccine delivery in mice. *Carbohydr Polym*. 2015;121:403–410. doi:10.1016/j.carbpol.2014.12.044
133. Mumuni MA, Kenechukwu FC, Ofokansi KC, Attama AA, Diaz DD. Insulin-loaded mucoadhesive nanoparticles based on mucin-chitosan complexes for oral delivery and diabetes treatment. *Carbohydr Polym*. 2020;229:115506. doi:10.1016/j.carbpol.2019.115506
134. Farhadian A, Dounighi NM, Avadi M. Enteric trimethyl chitosan nanoparticles containing hepatitis B surface antigen for oral delivery. *Hum Vaccin*. 2015;11(12):2811–2818. doi:10.1080/21645515.2015.1053663
135. Dombu CY, Betbeder D. Airway delivery of peptides and proteins using nanoparticles. *Biomater*. 2013;34(2):516–525. doi:10.1016/j.biomaterials.2012.08.070
136. Ilinskaya AN, Dobrovolskaia MA. Understanding the immunogenicity and antigenicity of nanomaterials: past, present and future. *Toxicol Appl Pharmacol*. 2016;299:70–77. doi:10.1016/j.taap.2016.01.005
137. Anselmo AC, Mitragotri S. Impact of particle elasticity on particle-based drug delivery systems. *Adv Drug Deliv Rev*. 2017;108:51–67. doi:10.1016/j.addr.2016.01.007
138. Bento D, Staats HF, Goncalves T, Borges O. Development of a novel adjuvanted nasal vaccine: C48/80 associated with chitosan nanoparticles as a path to enhance mucosal immunity. *Eur J Pharm Biopharm*. 2015;93:149–164. doi:10.1016/j.ejpb.2015.03.024
139. Pawar D, Mangal S, Goswami R, Jaganathan KS. Development and characterization of surface modified PLGA nanoparticles for nasal vaccine delivery: effect of mucoadhesive coating on antigen uptake and immune adjuvant activity. *Eur J Pharm Biopharm*. 2013;85(3):550–559. doi:10.1016/j.ejpb.2013.06.017
140. Piazzini V, Landucci E, D'Ambrosio M, et al. Chitosan coated human serum albumin nanoparticles: a promising strategy for nose-to-brain drug delivery. *Int J Biol Macromol*. 2019;129:267–280. doi:10.1016/j.ijbiomac.2019.02.005
141. Hoseini-Tavassol Z, Ejtahed H-S, Soroush A-R, et al. Natural derived nasal spray; a proposed approach for COVID-19 disease control. *Infect Disord Drug Targets*. 2021;21(8):e160921191568–e160921191568. doi:10.2174/1871526521666210218201113
142. Nagarwal RC, Kant S, Singh PN, Maiti P, Pandit JK. Polymeric nanoparticulate system: a potential approach for ocular drug delivery. *J Control Release*. 2009;136:2–13. doi:10.1016/j.ijbiomac.2019.02.005
143. Diebold Y, Calonge M. Applications of nanoparticles in ophthalmology. *Prog Retin Eye Res*. 2010;29:596–609. doi:10.3390/molecules26092485
144. Janagam DR, Wu LF, Lowe TL. Nanoparticles for drug delivery to the anterior segment of the eye. *Adv Drug Deliv Rev*. 2017;122:31–64. doi:10.1016/j.addr.2017.04.001
145. Kalam MA. Development of chitosan nanoparticles coated with hyaluronic acid for topical ocular delivery of dexamethasone. *Int J Biol Macromol*. 2016;89:127–136. doi:10.1016/j.ijbiomac.2016.04.070
146. Foged C. Thermostable subunit vaccines for pulmonary delivery: how close are we? *Curr Pharm Des*. 2016;22(17):2561–2576. doi:10.2174/1381612822666160202141603
147. Ali ME, Lamprecht A. Spray freeze drying for dry powder inhalation of nanoparticles. *Eur J Pharm Biopharm*. 2014;87(3):510–517. doi:10.1016/j.ejpb.2014.03.009
148. Ingvarsson PT, Schmidt ST, Christensen D, et al. Designing CAF-adjuvanted dry powder vaccines: spray drying preserves the adjuvant activity of CAF01. *J Control Release*. 2013;167(3):256–264. doi:10.1016/j.jconrel.2013.01.031
149. Thakur A, Ingvarsson PT, Schmidt ST, et al. Immunological and physical evaluation of the multistage tuberculosis subunit vaccine candidate H56/CAF01 formulated as a spray-dried powder. *Vaccine*. 2018;36(23):3331–3339. doi:10.1016/j.vaccine.2018.04.055
150. Wong TW, Dhanawat M, Rathbone MJ. Vaginal drug delivery: strategies and concerns in polymeric nanoparticle development. *Expert Opin Drug Deliv*. 2014;11(9):1419–1434. doi:10.1517/17425247.2014.924499
151. Marciello M, Rossi S, Caramella C, Remunan-Lopez C. Freeze-dried cylinders carrying chitosan nanoparticles for vaginal peptide delivery. *Carbohydr Polym*. 2017;170:43–51. doi:10.1016/j.carbpol.2017.04.051
152. Leyva-Gomez G, Pinon-Segundo E, Mendoza-Munoz N, Zambrano-Zaragoza ML, Mendoza-Elvira S, Quintanar-Guerrero D. Approaches in polymeric nanoparticles for vaginal drug delivery: a review of the state of the Art. *Int J Mol Sci*. 2018;19(6):1549. doi:10.3390/ijms19061549
153. Martinez-Perez B, Quintanar-Guerrero D, Tapia-Tapia M, et al. Controlled-release biodegradable nanoparticles: from preparation to vaginal applications. *Eur J Pharm Sci*. 2018;115:185–195. doi:10.1016/j.ejps.2017.11.029