REVIEW

Mucosal vaccine delivery: A focus on the breakthrough of specific barriers

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Abstract Mucosal vaccines can effectively induce an immune response at the mucosal site and form the first line of defense against microbial invasion. The induced mucosal immunity includes the proliferation of effector T cells and the production of IgG and IgA antibodies, thereby effectively blocking microbial infection and transmission. However, after a long period of development, the transformation of mucosal vaccines into clinical use is still relatively slow. To date, fewer than ten mucosal vaccines have been approved. Only seven mucosal vaccines against coronavirus disease 2019 (COVID-19) are under investigation in clinical trials. A representative vaccine is the adenovirus type-5 vectored COVID-19 vaccine (Ad5-nCoV) developed by Chen and coworkers, which is currently in phase III clinical trials. The reason for the limited progress of mucosal vaccines may be the complicated mucosal barriers. Therefore, this review summarizes the characteristics of mucosal barriers and highlights strategies to overcome these barriers for effective mucosal vaccine delivery.

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1. Introduction

Mucosal vaccines are applied directly to the mucosal surface, such as the sublingual tract, digestive tract, respiratory tract, and urogenital mucosa, to induce an immune response. Compared with vaccines injected intramuscularly or subcutaneously, mucosal vaccines can effectively mimic the natural infection of microorganisms, stimulate the lymphatic tissues under the mucosa to recruit various immune cells and secrete antibodies, which directly block the path by which microorganisms invade the host. Therefore, inducing mucosal immunity is the best way to prevent infection by pathogenic microorganisms that invade the body through the mucosa. In addition, a “homing” phenomenon exists in mucosal immunity. Specifically, under the mediation of specific homing receptors, most of mature immune cells will home to the mucosal propria or epithelium of the sensitized site for an immune response, and the rest will reach other mucosal sites to form an extensive common mucosal immune network. This means that T cells and B cells that are activated at one mucosa site can migrate to other distal mucosal sites to produce a similar immune response.

Thanks to these advantages, mucosal vaccines have attracted widespread attention. However, only a very small number of mucosal vaccines are approved for use worldwide, mainly include live attenuated, inactivated and adenovirus vector vaccines (Table 1). Some vaccines (e.g., rotavirus vaccine) have been temporarily suspended due to their toxicity, which shows that there are still many difficulties in the design and development of mucosal vaccines. Currently, seven COVID-19-related mucosal vaccines have entered clinical evaluation (Table 2), including two live attenuated vaccines and five adenovirus vector vaccines. The fastest progress has been achieved for an adenovirus vector vaccine (Ad5-nCoV) developed by Chen and co-workers, which has been demonstrated to trigger the same level of immune effect as intramuscular injection at a dose of 1/5, providing great encouragement to mucosal vaccine development.

In addition to viral vectors, several biodegradable and safer nonviral vectors are also used for mucosal vaccine delivery. These nonviral vectors are usually designed to protect antigen components from degradation, increase the residence time of antigens in mucosal sites and increase the uptake of antigens by antigen-presenting cells (APCs), etc. (Fig. 1). Various mucosal vaccines based on different vectors are undergoing clinical evaluation or clinical research (Table 3). Strategies and delivery systems that target different cell types and promote the penetration of vaccines through the mucosal (including mucoadhesive, mucopenetration and mucolytics) have been proposed and summarized previously. Due to the significant differences in the structure and physiological environment of specific mucosae, there are different requirements for vaccine delivery. However, few articles have

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### Table 1  Licensed mucosal vaccines.

| Type of vaccine | Name | Antigen | Formulation | Disease | Administration | Approved time | Approved |
|-----------------|------|---------|-------------|---------|----------------|--------------|----------|
| Live attenuated | OPV (b/m/OPv) | Poliovirus | Aqueous | Poliomyelitis | Oral | 1961 | FDA |
| Live attenuated | Dukoral® | Vibrio cholerae | Aqueous | Cholera | Oral | 2003 | Canada |
| Live attenuated | Fluenz™/Flumis® | Influenza A and influenza B viruses | Spray | Influenza | Nasal | 2003 | FDA |
| Live reasortant | RotaTeq® | Rotavirus | Aqueous | Infant diarrhea | Oral | 2006 | FDA |
| Live attenuated | Rotarix | RIX4414 strain | Aqueous | Infant diarrhea | Oral | 2008 | FDA |
| Live attenuated | Shanchol® | Vibrio cholerae | Aqueous | Cholera | Oral | 2013 | WHO |
| Live attenuated | Vivotif® | Salmonella typhimurium | Capsule | Acute gastroenteritis | Oral | 2013 | FDA |
| Adenovirus vector | Adenovirus Type 4 and Type 7 Vaccine | Adenovirus Type 4 and Type 7 | Oral capsule | Febrile acute respiratory disease | Oral | 2011 | FDA |
| Live attenuated | Vaxchora™ | Vibrio cholerae | Aqueous | Cholera | Oral | 2015 | FDA |

### Table 2  The candidates of COVID-19 mucosal vaccine in clinical.

| Type of vaccine | Name | Antigen | Carrier | Administration | Status | NCT |
|-----------------|------|---------|---------|----------------|--------|-----|
| Ad-vectored vaccine | Ad5-nCoV | Spike protein | Ad | Inhal | Phase III | NCT04540419 |
| Ad-vectored vaccine | hAd5-S-Fusion | Spike protein | Oral capsule | p.o. | Phase I/II | NCT04845191 |
| Live attenuated vaccine | DelNS1-2019-nCoV-RBD0PT1 | RBD domain of S protein | Influenza virus (CA4-DeiNS1) | Inhal | Phase II | ChCTR2000039715 |
| Live attenuated vaccine | COVI-VAC | SARS-CoV-2 | / | Inhal | Phase I | NCT04619628 |
| Ad-vectored vaccine | BBV154 | RBD domain of S protein | Ad5 | Inhal | Phase I | NCT04679909 |
| Ad-vectored vaccine | ChAdOx1 nCOV-19 | Spike protein | Ad | i.m. | Phase I | NCT04751682 |
| Ad-vectored vaccine | AdCOVID | Spike protein | Ad | i.m. | Phase I | NCT04816019 |

Ad, adenovirus; p.o., oral administration; i.m., intranasal administration.
summarized in detail the vector design requirements for breaking through different mucosas. Therefore, we provide a detailed summary of the existing delivery strategies for overcoming different mucosal barriers.

2. Mucosal immune response

Unlike systemic immunity, antigens that evoke mucosal immunity originate from the mucosal surface of various cavities and are captured by M cells or DCs in the mucosal inductive site and induce immune responses at the mucosal effector site. M cells and DCs have different mechanisms of antigen uptake. Specifically, in addition to having antigen phagocytosis similar to DC cells, M cells can also capture antigens by ingesting IgA-antigen complexes. An antigen can bind to IgA and induce a conformational change in the IgA molecule, enabling the antigen-secretory IgA (sIgA) complex to bind to M cells through specific receptors. In addition, M cells can also provide a transcellular antigen uptake pathway for DCs. However, due to the lack of protein-degrading lysosomes in M cells, they do not have the ability to process antigens but instead transfer the captured antigens to nearby APCs (e.g., DCs and B cells). Only after undergoing this necessary APC processing can the captured antigens effectively induce antigen-specific immune responses. Nonetheless, in order to induce stronger mucosal immunity, researchers are not only interested in enhancing DCs uptake, but also targeting M cells with vector design to enhance the antigen uptake capacity of M cells.

The production of sIgA is the main distinguishing feature compared with the systemic immune response. Concurrently, sIgA is the main effector of mucosal immunity, which can prevent viral invasion by combining with the virus. Compared with neutralizing Abs (nAbs, the focus of vaccine development), sIgA has the potential advantages of preventing the spread of viruses through the mucosa: sIgA (1) can prevent bacteria from adhering to the surface of epithelial cells; (2) can effectively neutralize harmful substances such as pathogens that enter the mucosal epithelium; and (3) can be discharged without the combination of complement and virus to form immune complexes. This immune complex can stimulate goblet cells to secrete mucus, which prevents the adherence of microorganisms. In contrast, nAbs bind to foreign pathogens and prevent them from entering target cells by blocking receptor binding or cellular uptake. Additionally, complement fixation of nAbs can also kill the virus. However, the different escape mechanisms of viruses, including the arming of pathogens and the rapid mutation of surface glycoproteins expressed by viruses, limit the application of vaccines for inducing nAbs in this case. The induction of sIgA production through mucosal immunity is a promising measure to prevent viral spread.

3. Barriers to mucosal vaccine delivery

The mucosal delivery of various drugs must break through multilayer barriers. The factors that affect the mucosal delivery of drugs are summarized into the following categories (Fig. 2):

### 3.1. Common barriers

#### 3.1.1. Structural barriers

As shown in Fig. 2A, numerous characteristics clearly differ between type I mucosa (e.g., gastrointestinal, respiratory, and upper female reproductive tracts) and type II mucosa (e.g., corneal, oral, esophageal, and lower female reproductive tracts). Type I mucosa is typically covered with columnar epithelial cells, and the mucus layer is secreted by goblet cells. More importantly, antigens are transported from the lumen to DCs through M cells and provide a place for antigen presentation in mucosa-associated lymphoid tissues (MALTs). In addition, the production of IgG and sIgA can be induced via transport by neonatal Fc receptor (FcRn) and polymeric immunoglobulin receptor (pIgR), respectively. However, type II mucosa with a multilayer squamous epithelium composed of multilayer keratinocytes lacks M cells, goblet cells, MALTs and polymeric immunoglobulin A (pIgA). Thus, other tissues (nearby glands) are needed for mucus secretion, and the DCs that take up the antigens must also migrate to
| Barrier Type of vaccine | Carrier | Antigen/epitope | Disease | Ref./NCT |
|------------------------|---------|----------------|---------|----------|
| Oral mucosa Subunit vaccine | Mucoadhesive wafers | HIV gp140 protein | HIV | 69 |
| Microneedle arrays | HBSAg | HBV | 132 |
| SIMPL tablet | OVA | / | 67 |
| Nanoﬁbrous mucoadhesive films | / | / | 68 |
| Gastrointestinal mucosa Inactivated vaccine | Chitosan and alginate delivery carriers | HEV71 | Hand-foot-and-mouth disease | 133 |
| Attenuated vaccine | Albumin–chitosan matrix microsphere | Typhoid Vi® antigen | Typhoid | 134 |
| Ad-vectored vaccine | Adenovirus type-4 | Hemagglutinin from H5N1 virus | Influenza | NCT02918006, Phase II |
| | Adenovirus type-5 | Hemagglutinin from H1N1 virus | Influenza | NCT04845191, Phase I/II |
| | Adenovirus type-5 Spike protein | COVID-19 | | |
| | Adenovirus type-5 Spike protein and nucleocapsid | COVID-19 | | NCT04732468, Phase I |
| | Chimpanzee Adenovirus | Hepatitis B virus | Hepatitis B | NCT04297917, Phase I |
| Subunit vaccine | Pollen grains or ragweed pollen | OVA | / | 135,136 |
| | Flagellin and mannosamine coated poly (anhydride) NPs | OVA | / | 137 |
| | Porous silica NPs | BSA | / | 138 |
| | CPP-rich PEGylated NPs | Recombination urease subunit B (rUreB) | Helicobacter pylori infection | 77 |
| | PMMMA-PLGA | Surface immunogenic protein (SIP) from group B Streptococcus agalactiae (GBS) | Streptococcus agalactiae infection | 139 |
| DNA vaccine | Poly (lactide-co-glycolide) microparticle | A rotavirus VP6 DNA | Infant diarrhea | 140 |
| | Chitosan NPs | LTB (L), STXB (S) and CTXB (C) | Diarrhea | 141 |
| | PLGA NPs | Hepatitis B virus (HBV) HBSAg gp160 | Hepatitis B | 140,142 |
| | Poly(t,a-lactide-co-glycolide) (PLG) | / | HIV | 143 |
| Respiratory tract mucosa Ad-vectored vaccine | Adenovirus vector RBD domain of Spike protein | COVID-19 | | NCT04679909, Phase I |
| | Adenovirus vector Spike protein | COVID-19 | | NCT04751682, Phase I |
| | Adenovirus vector 85A antigen | Tuberculosis | | NCT04121494, Phase I |
| | Adenovirus vector Spike protein | COVID-19 | | NCT04552366, Phase I |
| | Adenovirus vector Spike protein | COVID-19 | | NCT04816019, Phase I |
| | Adenovirus vector Influenza Vietnam 1194 | H5N1 Influenza | | NCT01443936, Phase I |
| | Adenovirus vector Spike protein | COVID-19 | | NCT05043259, Phase I/II |
| Recombinant virus Subunit vaccine | CaP nanoshell | Recombinant dengue virus | Dengue | 144 |
| | Polysaccharidic lapidated NPs | OVA | / | 145 |
| | Bacterium-like particle N’-Trimethyl chitosan NPs | RSV fusion (F) protein | RSV | 146 |
| | Poly (gamma-glutamic acid) NPs | OVA | / | 147 |
| | PCL-PEI and PCL-PEG polymeric hybrid micelle | Citra conic anhydride-modified OVA | / | 149 |

(continued on next page)
adjacent lymph nodes. Furthermore, the absence of pIgA means that it can only induce an IgG response without a sIgA response.

3.1.2. Mucus barriers

The mucus layer is the first barrier to invaders entering the body through the mucosa. Briefly, in the mucus layer, drug delivery is affected by the composition of the mucus (more attention is given to the structure of the mucin), renewal frequency, and flow rate. As an important part of mucus, high-molecular-weight mucins form a network with size exclusion function through hydrophobic interactions and chain entanglement, which implies that the pores formed by the mucin network demand customized drug sizes. Simultaneously, the mucus may fall off through mechanical action, or it may be degraded by certain enzymes and, thereby, undergo constant renewal. In addition, the penetration efficiency of the drug is significantly affected by the thickness of the mucus. The thickness of the mucus varies within and between different organs, which makes it difficult for vaccines to penetrate the mucus layer. For example, the thickness of the mucus layer in the cornea or

| Table 3 (continued) | Barrier | Type of vaccine | Carrier | Antigen/epitope | Disease | Ref./NCT |
|---------------------|---------|----------------|---------|----------------|---------|---------|
| Adeno-associated virus type 12 | Nanoemulsion | Influenza A nucleoprotein | / | Influenza | 150 |
| Porous maltodextrin-based lipid core NPs | Nanogel | N-Acetyl-neuraminylactose-binding hemagglutinin protein | / | Helicobacter pylori infection | 151 |
| Porous maltodextrin-based lipid core NPs | Nanogel | Toxoplasma gondii antigens | / | Toxoplasma gondii infection | 152 |
| Porous maltodextrin-based lipid core NPs | Nanogel | Clostridium botulinum type-A neurotoxin BoNT | / | BoNT | 153 |
| Hybrid NPs | Thermal-sensitive hydrogel | BSA | / | Influenza | 154 |
| DNA vaccine | Mannosylated protamine sulphate | Model DNA: anti-GRP DNA | / | / | 157 |
| mRNA vaccine | PEG12KL4 | Luciferase mRNA gp120 | RSV infection | 97 |
| mRNA vaccine | Cationic cyclodextrin-polyethylenimine 2k conjugate (CP 2k) | HIV-1 CN54 gp140 | HIV | 160–162 |
| mRNA vaccine | Hyperbranched poly (beta amino esters) (hPBAEs) | HIV-1 CN54 gp140 | HIV | 163 |
| Vaginal mucosa | Inactivated vaccine | Polystyrene nanospheres | HIV-1 | HIV | 158 |
| Subunit vaccine | Calcium phosphate (CAP) NPs | HSV-2 protein | HSV | 159 |
| Subunit vaccine | Poly-acrylic acid (Carbopol) gel | HIV-1 CN54 gp140 | HIV | 160–162 |
| Subunit vaccine | Hydroxyethylcellulose (HEC) gel | HIV-1 CN54 gp140 | HIV | 163 |
| Subunit vaccine | Thermo-sensitive poloxamer | HIV-1 CN54 gp140 | HIV | 164,165 |
| Subunit vaccine | Liposome-loaded HEC gelling rods | HIV-1 CN54 gp140 | HIV | 108,166 |
| Subunit vaccine | Liposome-loaded microneedle array | HSV-2 gD | HSV | 29 |
| Ad-vectored vaccine | Ad | HIV-1 gp140CF | HIV | 167,168 |
| Ad-vectored vaccine | rAd5 | HIV gag | HIV | 169 |
| Ad-vectored vaccine | Pseudovirion | Recombinant HPV-SIV gag | HPV | 170 |
| Ad-vectored vaccine | Recombinant virus | HIV gp160 and gag192-208 | HIV | 35 |
| VLP vaccine | Recombinant virus | HIV gag p24 | HIV | 171 |
| VLP vaccine | Virus-like NPs | HIV-1 gag | HIV | 172 |
| VLP vaccine | Virus-like NPs | HIV-1 gag p55 | HIV | 173 |

Ad, adenovirus; ALG, alginate; CMC, carboxymethylcellulose; COPD, chronic obstructive pulmonary disease; ETEC, Escherichia coli; ETSD, enhanced T-cell stimulation domain; LAIVs, live attenuated influenza vaccines; MVA, modified vaccinia ankara; PMMA-PLGA, Poly[(methyl methacrylate)-co-(methyl acrylate)-co-(methacrylic acid)]-poly(ε-caprolactone-co-glycolide); rPA, recombinant protein; RSV, respiratory syncytial virus; UTI, urinary tract infection.
conjunctiva is 1–7 μm, while it is approximately 10 μm in the trachea. In the same tissue, such as the digestive tract, the mucus layer gradually increases from 200 μm in the upper digestive tract to 800 μm in the lower digestive tract, which is much thicker than the eyeball mucosa and the mucosae of other organs.

3.2. Special barriers

3.2.1. Barriers of the oral mucosal

There are some unique barriers to oral mucosal inoculation. In comparison to other mucosal tissues, there are no specialized epithelial-associated follicles and no existing organized lymphoid structures in oral mucosal tissues. There are no signs of M cells in the oral mucosal tissue; in contrast, several dendritic cell subsets have been demonstrated to appear in epithelial tissue. DCs in the oral mucosa include (1) CD11b+CD11c- and CD11b+CD11c+ myeloid DCs, which are mostly located at the mucosal/submucosal interface; (2) B220+120G8+ plasma cell-like DCs, which are mainly present in submucosal tissues; and (3) CD207+ Langerhans cells (LCs), which are present only in the mucosa itself. When the antigen penetrates the mucus and reaches the epithelial tissue, it can be presented by LCs and trigger a specific immune response. Moreover, there are three large salivary glands and hundreds of small salivary glands in the mouth, which will continuously secrete saliva (Fig. 2B). The saliva consists of 99% water, which leads to dilution of the antigen. In addition, the oral cavity is the main site of sustenance ingestion and is constantly exposed to the external environment.
avoid triggering an immune response against harmless microorganisms and bacteria, immune tolerance is triggered in the oral tissues\textsuperscript{31,32}. Immune tolerance is triggered mainly by increased expression of IL-10, allergen-specific IgG4 and programmed cell death ligand 1 (PD-L1), together with decreased expression of IL-4, CD86 and CD80\textsuperscript{33}.

3.2.2. Barriers of the gastrointestinal tract mucosa
To effectively induc mucosal immunity in the GI tract, various barriers need to be overcome by oral vaccines (Fig. 2B). First, the structure of the intestinal epithelium and mucus layer limit the penetration of antigenic substances. Second, the pH gradient of the GI tract for resisting foreign invaders (pH changes from 1.0 to 2.0 in the stomach, and increases to 2.5–6.0 in the duodenum and 7.0–8.0 in the colon) will also have a significant impact on the activity of antigen molecules\textsuperscript{34}. Third, a high concentration of enzymes in the entire GI tract requires that the design of vaccines be strictly considered to avoid the degradation and denaturation of biomolecules. More importantly, vaccines that target the mucosa of the GI tract require higher antigen doses to induce an effective immune response. However, uptake of vaccine antigens by intestinal mucosa is relatively limited, which leads to an attenuated immune response. Against these barriers, nanocarriers are usually designed to protect antigens from extreme pH and proteases and to increase the uptake of antigens by targeting APCs\textsuperscript{35}.

3.2.3. Barriers of the respiratory tract mucosa
To effectively induce local immune response of the respiratory mucosa, an immunogenic vaccine must break through several mucosal barriers (Fig. 2B) and effectively transit to adjacent MALTs, such as nasal-associated lymphoid tissues (NALT) and bronchial-associated lymphoid tissues (BALT). The mucus layer comprising heavily glycosylated and sialylated mucins covers the respiratory tract and can efficiently block the foreign matter, including pathogens. However, this same layer also impedes the valid delivery of mucosal vaccines targeting the respiratory tract. The foreign matter trapped in the mucus layer will be removed from the organ cavity by the cilia to promote excretion of the mucus layer, which means that drugs adhering to the mucous must spread as quickly as possible. Otherwise, even if vaccines successfully adhere to the mucus layer, antigens may not reach the epithelial surface before being expelled by mucociliary clearance (MCC)\textsuperscript{36}.

3.2.4. Barriers of the vaginal mucosa
Unlike other mucous membranes, the physiological state of the vaginal mucosa is significantly regulated by age and hormone levels, and thus, drug delivery to vaginal mucosa will be restricted by additional factors (Fig. 2B). As a type II mucosal, the vaginal mucosa is composed of uncornified and pluristratified epithelial cells with a thickness of 200–300 μm and lacks goblet cells\textsuperscript{37}. In addition, lactobacilli were found uniquely in human vaginas with an abundance of more than 70%, while other mammals have only ~1%. The high density of lactobacilli creates an acidic environment (pH 3.5–4.5), which effectively protects women from pathogens\textsuperscript{38}. Factors such as hormones and microorganisms jointly regulate the formation of mucus, thereby affecting the delivery of drugs.

3.2.5. Barriers of ocular mucosa
The anatomical structure of the eye can be divided into different tissues, such as conjunctiva, cornea and vitreous body. Among these tissues, the conjunctiva and lacrimal glands contain IgA\textsuperscript{+} plasma cells that secrete sIgA and various immune cells that produce cytokines and chemokines\textsuperscript{39}, indicating their role as effector sites. In contrast, the cornea contains DCs and macrophages but no apparent lymphocytes\textsuperscript{40}, which indicates that the cornea is an immune inductive site rather than an effective site. Different mucosas require tailored delivery strategies, and some drugs may have to reach the posterior segments of the eye, whereas there is no such need for ocular vaccines.

Eyedrops represent the most common drug formulation among all ocular drugs. However, the efficacy of eyedrop drugs is limited by rapid tear film turnover, quick tear drainage and the obstacle of multiple bilayers. The tear film contains multiple layers, including the mucus layer as the innermost layer. The outer mucus layer comprises secreted mucins, which can be reset quickly by blinking and tear film turnover. The inner layer is formed by epithelium-tethered mucins (glycocalyx) with lower clearance frequency\textsuperscript{41}. Frequent instillation of eyedrops is needed because of the rapid reset of the mucus composition and the multilayers protecting the cornea. However, repeated instillation may result in worse patient compliance and ocular surface toxicity\textsuperscript{42}.

Eyedrops must travel a long distance and pass through various ocular barriers to reach the back of the eye, leading to minimal drug levels in the posterior segment. Moreover, the blood–retinal barrier (BRB) prevents effective drug and vaccine delivery via the systemic route to the eye. Direct injection into the eyeball (e.g., intravitreal) or periorcular injection (e.g., subconjunctival) may address these problems\textsuperscript{43}. Subconjunctival delivery penetrates the eye through transscleral diffusion\textsuperscript{44}, and soluble drugs exhibit better penetration capability but worse sustained release than hydrophobic drugs. As a result, conventional subconjunctival delivery requires frequent injection\textsuperscript{45}. Intravitreal (IVT) injections can directly deliver drugs to the back of the eye, but repeated injections are also required because of vitreous humor turnover. Repeated injections might increase the risk of retinal detachment, hemorrhage and endophthalmitis\textsuperscript{46}. Moreover, direct delivery of drugs or vaccines to the retina may induce neurotoxicity, and the safety profile of drugs and vaccines should be seriously assessed.

4. Nanocarriers for mucosal vaccine delivery
Against the various barriers to mucosal vaccine delivery, a variety of excellent delivery systems have been designed to promote mucosal immune effects. An ideal mucosal delivery system should have the following characteristics: First, it must be safe and non-toxic. Second, antigen uptake and presentation can be successfully achieved. In mucosal immunity, M cells and DCs are the major players in the process of antigen uptake, so targeting M cells and DCs may be a potential strategy. Third, the mucus barrier is an important factor limiting vaccine uptake. Therefore, the structure of the mucus layer can be disrupted by vector design to facilitate vaccine uptake. Fourth, the design of the carrier must adequately protect the antigenic components from enzymatic or chemical degradation so that the vaccine components can be safely delivered to target cells. However, to truly obtain a mucosal vaccine with strong protection and durability, it is necessary to design the vaccine according to the characteristics of different mucosas, so as to realize the specific immune response of vaccine antigens in the mucosa. Therefore, we summarize the barrier characteristics of different mucosal membranes and enumerate existing representative work.
4.1. Oral mucosa

There are two main sites for oral mucosal inoculation: the sublingual and buccal mucosae (with upper and lower inner lip mucosa)\(^6\). As with other mucosal tissues, the surface of the oral mucosa is covered with mucus\(^7\). The buccal epithelial tissue which is 500–800 \(\mu\)m thick, contains 40–50 layers of cells\(^8\). The sublingual epithelial tissue, which is 100–200 \(\mu\)m thick, contains 8–12 layers of cells\(^9\). The oral mucosa allows rapid antigen passage because of its limited thickness. A study has shown that OVA antigen requires only approximately 20 min to cross the oral mucosa and then accumulates at the mucosal/submucosal junction. OVA is captured and processed by antigen-presenting cells at 1 h after administration\(^10\). The oral mucosa contains minimal amounts of enzymes (compared with the intestine) and possesses a neutral pH environment (compared with the stomach)\(^11\). In addition, antigens can be accessed directly by APCs or follow paracellular/pericellular pathways to avoid reaching the submucosal vasculature, thus avoiding first-pass effects\(^12\). It was also found that the risk of antigen redirection to the olfactory bulb observed after sublingual immunization is significantly lower than that after intranasal immunization\(^13\), indicating the safety of vaccination via the oral mucosa.

4.1.1. Advanced carriers for oral mucosal vaccine delivery

Current vaccines for oral mucosal inoculation recruit various forms of delivery systems to deliver the relevant antigens, such as virus-like particles, adenoviral vectors, polymers, proteins, and microneedles. Various properties of the delivery system have an impact on the antibody level induced by vaccines.

4.1.1.1. Microneedles. To deliver sufficient antigen to APCs deep in the epithelium and lamina propria, microneedle patches with various forms of antigens (e.g., DNA, peptides, etc.) are designed to breach the mucosal barrier through nanoneedle tips. For example, McNeilley et al. produced a microneedle array with a size of 16 \(\text{mm}^2\) (Nanopatch). This array contains 3364 protrusions, each with a length of 110 \(\mu\)m. This delivery system can directly deliver vaccines to a depth of 50 \(\mu\)m in the oral mucosa of mice, thereby facilitating antigen uptake by APCs\(^14\). Similarly, Zhen et al. constructed microneedle arrays of proMMA fillers (proMMA) using mannose-PEG-cholesterol (MPC)/mannosylated lipid A-liposomes encapsulated with bovine serum albumin (BSA) as a model antigen (Fig. 3C). As a solid, proMMA is non-lysable lipid A-liposomes encapsulated with bovine serum albumin (BSA) as a model antigen (Fig. 3C). As a solid, proMMA is stable under storage conditions. In contrast, proMMA can dissolve in water rapidly and revert to MLL. Administration through the oral mucosal (o.m.) or oral mucosal administration under anesthesia (a.e./o.m.) significantly increased IgA levels in mouse saliva, intestinal and vaginal washings, which were not achieved by subcutaneous (s.c.) or intradermal (i.d.) immunization. In addition, storage at 40 °C for three days did not affect the activity of proMMA (Fig. 3D)\(^15\).

4.1.1.2. Gels. To prolong the residence time of antigens at the mucosal sites, reduce antigen loss due to swallowing, White et al. used a thermoresponsive gel (TRG) combined with a trivalent inactivated poliovirus vaccine (IPV) for sublingual immunization\(^16\). TRG is in fluid-like liquid formation at 2–8 °C but transforms into a gel at physiological temperatures (\(\approx 37 \degree C\)) within seconds. This feature helps the retention of vaccine at the delivery site, thus allowing prolonged contact between the antigen and the mucosa\(^17\). As a result, TGR-assisted IPV induced higher levels of serum IgG and sIgA than IPV without TGR.

4.1.1.3. Polymer-based nanofibers. Another oral mucosal delivery system with enormous attention is nanofibers. In order to reduce antigens interaction with mucin, leading to a robust, long-lived antibody and T cell responses, Collier et al. developed an epitope-bearing peptide—polymer conjugate (OVAQ11-PEG) that has the ability to self-assemble into nanofibers (Fig. 3E)\(^18\). When used for sublingual immunization, OVAQ11-PEG can synergize with cholera toxin (CT) adjuvant to increase the titer of anti-pOVA IgG in mice (Fig. 3F). Furthermore, Collier et al. transformed peptide-polymer nanofibers into tablets (SIMPLs), which are heat-stable and easily-administrable. There was no obvious difference in the sublingual antibody responses after heating SIMPLs for 1 week at 45 °C compared to the conventional carrier vaccine.

Josef Mašek et al. have invented a mucoadhesive film based on nanofibers that enables prolonged release of NPs into submucosal tissue (Fig. 3G). This platform is composed of a variety of nanofibers with different functions, such as an electrospray nanofibrous reservoir layer, mucoadhesive film layer and protective backing layer. The electrospray nanofibrous reservoir layer could absorb a variety of antigens such as lipovirus bodies, virus-like particles and protein macromolecules. This mucoadhesive film could achieve prolonged antigen release by attaching to the oral mucosa\(^19\). This mucoadhesive film effectively ensures the local NPs concentration, which is beneficial to realize NPs-mediated delivery to take place. Furthermore, the platform effectively avoids the losses of NPs due to saliva washout.

4.1.1.4. Other delivery systems. Liquid formulations are the predominant form of sublingual vaccines, but are easily cleared during activities such as swallowing, speaking, and eating, resulting in poor immunogenicity. To break through this predicament, Wang et al.\(^20\) developed a biopolymer platform of mucoadhesive wafers blended with carboxymethyl cellulose (CMC) and alginate (ALG) binary polymer to enable effective sublingual delivery of lyophilized protein vaccines (Fig. 3H). The screened wafer is loaded with HIV gp140 protein and combined with an adjuvant (αGalSer) for sublingual inoculation of mice. A greater immune response was generated in immunized mice than in the control group.

Furthermore, virus-like particles are also commonly used oral mucosal delivery vehicles. For example, researchers found that sublingual administration of human papillomavirus-like particles could protect the genitalia from human papillomavirus pseudovirus attack with or without CT adjuvant\(^21\). Seth et al.\(^22\) developed a sublingual lyophilized (FD) formulation of VLPs carrying J8 peptide (J8-VLP). Through tracking of J8-VLPs in vivo after sublingual inoculation, J8-VLPs rapidly entered the circulation, triggering high serum IgG antibody levels along with high levels of IgA antibodies in saliva.

4.2. Gastrointestinal mucosa

In addition to the oral cavity, the gastrointestinal (GI) tract has other potential mucosal immune induction sites for oral vaccines, such as tonsils, Peyer’s patch (PP) lymph nodes and submucosal lymphoid tissues. Although tonsils are the first lymphoid tissue to contact oral vaccines, most of the vaccines are designed for small intestinal PP lymph nodes because of the short residence time of
vaccines in the upper GI tract. However, due to the complexity of the GI tract, the delivery of oral vaccines targeting the GI mucosa will be limited by a variety of obstacles.

4.2.1. Advanced carriers for gastrointestinal mucosal vaccine delivery

4.2.1.1. Adenoviral vectors

Replicable (live) vaccine vectors combine the improved efficacy of live attenuated vaccines with the safety of inactivated vaccines or subunit vaccines, which promotes its theoretical advantages in vaccine development. For example, the safety of an oral hemagglutinin-encoded vaccine based on adenovirus serotype 4 (Ad4-H5-Vtn, Fig. 4A) was evaluated in phase I studies (NCT01006798)\(^72\). The data showed that Ad4-H5-Vtn induced a cellular immune response with a promising safety profile. Kim et al. developed an influenza hemagglutinin vaccine based on an adenovirus vector\(^73\). To protect the adenovirus from degradation by stomach acid, they used radiocontrolled capsules (RCCs) to deliver the adenovirus vaccines to either the jejunum or the ileum. Antigen-specific mucosal and systemic immune responses were both induced. However, compared with jejunum delivery, ileum delivery induced more antibody (e.g., IgG and IgA isotypes)-secreting cells and increased not only mucosal-homing B cells but also the number of vaccine responders.

4.2.1.2. Lipid-based vehicles

Lipid-based nanoparticles (LNPs) are widely used as oral drug delivery systems\(^74\). They are usually able to effectively wrap the drug inside the particles and protect the drug from the external environment. For example, to prevent antigen and adjuvant degradation under acidic conditions...
in the stomach, a composite microparticle formulation was described by Chiriva-Internati’s group\textsuperscript{75}. \textit{Aleuria aurantia} lectin (AAL, a high-affinity ligand targeting M cells) was added to the composition to impart microparticles with M cell-targeting ability (Fig. 4A). After oral administration of microparticles with an immunodominant peptide epitope from sperm protein 17 (SP17), SP17-specific antitumor effects were induced.

To develop subunit vaccines capable of targeting APCs, Wang et al. designed a multivalent liposome that has the ability to target APCs through the mannose-PEG\textsubscript{1000}-cholesterol conjugate (MPC)\textsuperscript{76}. The system is composed of an emulsifier dissolved in the oil phase (O) with MPC, soy phosphatidylcholine, stearylamine and monophosphoryl lipid A, and the water phase (W) with sucrose and BSA. In addition, the O/W emulsions containing BSA (as a model antigen) administered to mice via oral mucosal administration induced an effective immune response, as evidenced by the secretion of IgG in the sera and IgA in the saliva, intestine and vagina (Fig. 4B).

4.2.1.3. Polymer-based nanoparticles. To effectively improve mucus permeation and transepithelial uptake, Zhang et al. synthesized an anionic and hydrophilic PEG derivative (PEG-Suc) coated on NPs enriched with the cell-penetrating peptide (CPP) poly-L-arginine (Fig. 4C)\textsuperscript{77}. By analyzing the uptake of NPs by cells in the presence or absence of the mucus layer secreted by E12 cells, the results confirmed that the internalization of R12/OVA/PEG-Suc (18.24%) was significantly higher than that of R12/OVA and FITC-OVA (7.51% and 4.37%) in the presence of mucus (Fig. 4D). The mucus penetrating ability of the NPs may be derived from the slight negative surface charge generated by PEG-
Succinctly, PEG can enable NPs to effectively penetrate the mucus layer and promote the transport of antigens to the submucosal APCs.

Since APCs overexpress mannose receptors, Xu et al.78 encapsulated BSA, a model protein vaccine, into mannosylated chitosan nanoparticles (MCS NPs) for targeting APCs. Subsequent coating with Eudragit® L100 of the MCS NPs (MCS/BSA/Eud) protected the antigen from the harsh acid environment (Fig. 4E). Since Eudragit® L100 is an enteric coating polymer, MCS/BSA/Eud NPs are dissolved by intestinal fluid after entering the intestine, thereby exposing MCS/BSA NPs. This auxiliary coating retained the ability of MCS NPs to target DCs in the PP through mannose and finally effectively induced systemic IgG antibodies and mucosal IgA reactions.

4.2.1.4. Other strategies for GI mucosal immunity. To promote the penetration of hydrophilic antigens into the intestinal mucosa, Chen et al.79,80 designed pH-responsive bacterial nanocarriers. These nanocarriers usually have similar basic characteristics to the intestine, thereby exposing MCS/BSA NPs. This auxiliary coating retained the ability of MCS NPs to target DCs in the PP through mannose and finally effectively induced systemic IgG antibodies and mucosal IgA reactions.

4.3. Respiratory tract mucosa

The respiratory tract, which is composed of the upper respiratory tract (URT) and lower respiratory tract (LRT), is constantly exposed to various bacteria and viruses, which makes it an underprivileged site to the respiratory mucosa were summarized.

4.3.1. Advanced vectors for respiratory tract mucosal vaccine delivery

To effectively overcome the barriers of the respiratory mucosa and reach adjacent MALT, nanocarriers are used to simulate the size and characteristics of respiratory viruses, with the aim of inducing humoral and cellular immune responses and improving the effectiveness of various vaccines. There are many types of nanocarriers for respiratory mucosa delivery, such as adenovirus, lipid NPs, protein- or peptide-based nanocarriers, and inorganic nanocarriers. These nanocarriers usually have similar basic characteristics. For example, the size of NPs is generally between 20 and 200 nm (average diameter of most respiratory viruses), and they have a positive charge to enhance adhesion to nasal mucus and avoid elimination by cilia.2,83 Since nanocarriers are important for vaccines to overcome mucosal barriers, the vaccine delivery vehicles in respiratory mucosa were summarized.

4.3.1.1. Adenovirus-based vaccines. Several adenovirus-based vaccines have been developed against COVID-19 over the past two years,84–86 all of which confirmed that the ad-vectorized vaccine encoding the antigen protein of SARS-CoV-2 protected mice from infection. Chen et al. developed the first aerosolized inhaled adenovirus type-5 vector-based vaccine that possesses the potential to prevent COVID-19 (Ad5-nCoV) worldwide.87 They demonstrated that one dose of Ad5-nCoV, which is 1/5 the dosage of an intramuscular vaccine, could induce a strong cellular response, achieving protection of the URT and LRT against SARS-CoV-2 infection. In comparison to intramuscular injection, mucosal vaccination induces not only pathogen-specific systemic immunity but also mucosal immunity. Moreover, the mucosal vaccine shows an important advantage that protects against SARS-CoV-2 replication in URT, which further blocks person-to-person transmission. Vaccine inoculation by intramuscular injection does not exhibit this property.

4.3.1.2. Lipid-based nanocarriers. To deliver STING agonists into the cytoplasm of alveolar epithelial cells (AECs) without disrupting the integrity of the lung surfactant (PS) layer, Wu et al. screened a series of biomimetic liposomes that are modified by PS to encapsulate 2,3-cyclic guanosine monophosphate-adenosine monophosphate (cGAMP, an agonist of STING)88 (Fig. 5A). Augmentation of the recruitment and differentiation of DCs as well as activation of CD8+ T cells and humoral immune responses to the influenza vaccine were observed after intranasal immunization with the combination of PS-GAMP and influenza vaccine (Fig. 5B). Irvine et al. developed an interbiliary-crosslinked multilamellar vesicle (ICMV)89 and further used it for encapsulation of antigens and two Toll-like receptor (TLR) agonists (poly I:C and monophosphoryl lipid A)90. After immunization via the intratracheal route, ICMVs primed 13-fold more T cells than equivalent protein- or peptide-based soluble vaccines and generated long-lived T cells that were biased toward an effector memory (Tem) phenotype in the lung and distal mucosae (for example, vaginal). Notably, Tem cells play an important role in protection against therapeutic tumors and prophylactic viruses. Additionally, Jin et al. demonstrated that mannose-conjugated cationic lipid nanoparticle (LNP-Man) is more efficient than LNP in delivering mRNA encoding hemagglutinin (HA) antigens against the H1N1 influenza virus intranasally91 (Fig. 5C). Humoral and cellular immune responses were elicited in C57BL/6 mice that were immunized through intranasal administration.

4.3.1.3. Peptide-based nanocarriers. Peptide-based nanocarriers are also used in the development of mucosal vaccines. Chong and Collier et al. demonstrated a nanofiber that consists of an epitope from influenza acid polymerase (PA) and β-sheet forming peptide Q11 (a self-assembling peptide)92. After intranasal immunization, the nanofibers showed enhanced uptake by DCs in lung-draining mediastinal lymph nodes and further generated a stronger CD8+ T cell response compared with subcutaneous immunizations. Moreover, the soluble antigen peptide of the nanofibers can be easily replaced with other antigens, such as Ee,93 OVA,94,95 and PADRE,96 and a similar immune effect can be achieved after administration via the same route. To develop a platform for robust mRNA transfection in the airways, Lam et al. introduced novel mRNA inhalation preparations carrying PEG2KL4, in which the cationic KL4 peptide was chemically linked to the linear PEG of 12-mers97 (Fig. 5D). Pulmonary delivery of the PEG12KL4/luciferase mRNA complex resulted in luciferase expression in the deep regions of mouse lungs (Fig. 5E).
This mRNA delivery capability was not compromised after formulation into dry powders by spray drying (SD) and spray freeze drying (SFD) techniques. These results demonstrate the application potential of PEG12KL4 in mRNA inhalation vaccines. In addition, various strategies have been used to deliver mRNA into the respiratory mucosa to induce mucosal immunity. 

4.3.1.4. Other carriers. Additionally, many types of vectors are used in the development of vaccines, which help to induce respiratory tract mucosal immunity. For example, Wang et al. developed an influenza vaccine nanoplatform (GO-PEI, Fig. 5F) based on graphene oxide (GO) NPs functionalized by polyethylene (PEI). Combined with recombinant influenza hemagglutinin (HA), the influenza GO-PEI NPs (HA/GO-PEI) were found to elicit powerful humor and cellular immune responses, thereby protecting the intranasally immunized mice from influenza virus challenge.

To assist inactivated virus (WIV) vaccines in breaking through the barrier of the respiratory tract mucosa, Gao et al. introduced a chitosan (CS)-functionalized iron oxide nanozyme (IONzyme), which actually increased the adhesion of WIV to the mucosa and further triggered an 8.9-fold increase in IgA production.
of strong adaptive mucosal immunity and thereby successfully protected immunized mice from the virus.

4.4. Vaginal mucosa

The lower reproductive tract, composed of the vagina and cervix, is a unique place for pathogens to enter and spread between individuals. The pathogen-specific immune response of the female reproductive tract is an important strategy to protect the health of women and control sexually transmitted infections. Compared with other mucosal delivery methods, vaginal delivery seems to be hindered by more factors. Thus, vaccine inoculation through other mucosal sites is preferred over vaginal mucosal inoculation when aiming to induce mucosal immunity in the lower reproductive tract, although the vaginal mucosa is certified to be a more effective inoculation site\(^{105}\). However, many outstanding researchers have developed potential vaginal mucosal delivery systems for the delivery of various drugs\(^ {47,106,107}\), including vaccines.

4.4.1. Advanced vectors for vaginal mucosal vaccine delivery

To effectively induce vaginal mucosal immunity, microneedles or nanoemulsions are used to improve the immune effect of vaccines. Wang et al.\(^ {108}\) synthesized a microneedle array (proSMMA) composed of two types of liposomes, mannosylated lipid A-liposomes (MLLs) and stealth lipid A-liposomes (SLLs), which were both loaded with antigens and NH\(_4\)HCO\(_3\) and had sizes of 200 and 50 nm, respectively (Fig. 6A). The proSMMA revert to two types of liposomes when they are rehydrated, which leads to vaginal mucosal DC uptake of MLLs and SLLs followed by direct drainage into the lymph nodes. Their results showed that under the action of lysosomes, NH\(_4\)HCO\(_3\) reacted with CO\(_2\) and NH\(_3\) and further generated ROS (Fig. 6B). These reactions effectively promoted the mixed Th1/Th2-type

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**Figure 6** Advanced strategies for vaginal mucosal delivery. (A and B) A microneedle array (proSMMA) was designed for vaccine delivery to the vaginal mucosa. Reprinted from Ref. 108. Copyright © 2017, Elsevier. (C) A squalene-assisted PEG-b-PLACL nanoemulsion was used to induce an antigen-specific vaginal mucosal immune response. CD11b/c\(^+\) and F4/80\(^+\) cells in vaginal mucosa were stained by (D) HE and (E) IHC after OVA/SQ@NE immunization. (F) OVA-specific IgG titers in serum and OVA-specific IgA levels in vaginal washes. Reprinted from Ref. 109. Copyright © 2021, Elsevier.
response. Mice vaccinated with proSMMAs via vaginal mucosa patching were protected from the virus. Huang et al. developed a squalene-assisted PEG-b-PLACL nanoemulsion (~200 nm) containing OVA antigens (OVA/SQ@NE)\(^{109,110}\) (Fig. 6C). Administered intravaginally into Balb/c mice, the retention time of protein antigens in the genital tract is prolonged, which further results in increased infiltration of CD11b/c\(^+\) cells and F4/80\(^+\) macrophages in the vagina (Fig. 6D) and increased secretion of antigen-specific serum IgG and mucosal IgA (Fig. 6E), suggesting strong antigen-specific cellular and humoral immune responses.

In addition, other drug delivery systems targeting the vaginal mucosa are also promising for future vaccine development. For example, propylene glycol-embodying deformable liposomes (FDL, ~185 nm) effectively improve the permeability of fibronectin (FN) through the mucosa of the approach channel because propylene glycol, as an edge activator, can significantly increase the fluidity and flexibility of the phospholipid bilayer, thereby enhancing the permeability of the mucous membrane\(^{111}\).

4.5. Ocular mucosa

The eye, one of the most delicate organs in the human body, is where optesthesia occurs. When exposed to the external environment, the ocular mucosa could be a route of potential entry for various pathogens, including infectious pathogens. SARS-CoV-2 RNA was detected in the conjunctival scrapings and tears of COVID-19 patients, indicating that SARS-CoV-2 could be transmitted through ocular secretions\(^{112}\). These findings suggest that the valid induction of ocular mucosal immunity might contribute to controlling the pandemic. However, drug delivery or vaccination through the ocular mucosa remains challenging.

4.5.1. Advanced strategies for ocular mucosa delivery

To elevate the penetration ability and retention time of eyedrop formulation drugs, the physicochemical properties of NPs must be modified. Size, surface charge and surface coating are the three nanoparticle properties most focused on by researchers (Fig. 7A). NPs which have smaller sizes than the mucus meshwork pore show better penetration ability. The mucus layers are negatively charged due to the presence of carboxyl and sulfate groups on the mucin proteoglycans. Thus, NPs with a neutral or slightly negative surface charge could prevent electrostatic interactions and achieve proven penetration\(^{113}\). Polymeric coating of NPs could change the surface properties of NPs, reduce nonspecific binding to the NPs and improve the penetration capacity even further. Xu et al.\(^{114}\) demonstrated that a dense PEG coating on the surface of PLGA NPs (a dense brush PEG conformation) contributes to significant penetration. The fast penetration through the tear film reaching the cornea prevents the NPs from being eliminated by the quick tear film turnover and fast tear drainage, thus prolonging the retention time.

Regarding the injection formulation of drugs and vaccines targeting the ocular mucosa, the utilization of NPs remains effective. Soluble drugs exhibit great transcleral diffusion after subconjunctival injection; however, rapid elimination of the drug occurs. Drug encapsulated in PLGA NPs has been demonstrated to have sustained release for more than a week\(^{115}\), reducing the injection frequency. Another commonly used injection method is intravitreal injection. The vitreous is composed of a delicate mesh network of gel-forming collagen fibrils and hyaluronan molecules. Specific characteristics are needed for NPs to diffuse in the vitreous. NPs with particle sizes larger than 1000 nm would be trapped in the vitreous gel, while NPs with particle sizes smaller than 500 nm show excellent diffusion patterns\(^{117}\). The surface charge and coating of NPs are also important for drug delivery\(^{53}\).

To date, several ocular drugs for humans have been approved for clinical use, but fewer attempts at ocular vaccination have been reported. Among those vaccines, eyedrops are the main drug formulation. Barisani et al. used bacterial ghosts to construct a subunit vaccine against Chlamydia trachomatis (Ct), successfully inducing mice to produce specific IgG and sIgA\(^{118}\) (Fig. 7B). Gu

![Figure 7](image-url)

**Figure 7** Advanced strategies for ocular mucosal delivery. (A) The size, surface charge and surface coating of NPs have a decisive impact on the delivery abilities of eyedrop vaccines. (B) Membrane protein encapsulated in bacterial ghosts (BGs) could induce the production of Chlamydia trachomatis (Ct)-specific sIgA in tears by eyedrop inoculation. (C) The Fe\(_3\)O\(_4\) NPs coated with glutamic acid, DNA vaccine pRSC-gD-IL-21 and PEI were developed against HSV-1 infection.
et al. studied an ocular vaccine against herpes stromal keratitis (HSK)\textsuperscript{10}. Glutamic acid-coated Fe\textsubscript{3}O\textsubscript{4} NPs combine with the DNA plasmid expressing the HSV glycoprotein D (gD) antigen and interleukin-21 (IL-21) (Fig. 7C). This vaccine shows great transfection efficacy and induces not only mucosal but also systemic cellular immunity in mice. Wechsler et al. and Yasuo et al. constructed subunit vaccines (glycoprotein) and DNA vaccines (DNA plasmids) against HSV, respectively\textsuperscript{12,13}. These two vaccines both require subconjunctival injection. Research on injection vaccines targeting the ocular mucosa is limited by the need for anesthesia before injection, precision of the injection and side effects following injection.

5. Summary and prospects

Mucosal vaccines exhibit promising potential in the treatment of some diseases as well as the prevention and interruption of disease transmission. However, since the first mucosal vaccine OPV was approved by the FDA in 1961, the development of mucosal vaccines has been slow and limited to traditional dosage forms, such as inactivated vaccines and attenuated vaccines. The specific barriers of different mucous membranes may be responsible for this limitation.

Since the first step toward successful mucosal vaccines is the effective uptake of antigen by APCs, a variety of advanced strategies have been designed to promote antigen delivery. Targeting M cells, DCs, etc. is considered an effective way to promote antigen presentation. However, no M cells exist in the type II mucosa, and methods including M cell targeting are not applicable in addition to the traditional APC strategy. Moreover, a mucus layer appears on the surface of various mucosae, and many mucosal drug delivery strategies focus on the breakthrough of the mucus layer. At present, many attempts to use NPs for mucoadhesion, mucopenetration and mucolytics have been conducted. Notably, different parts of the mucosa possess their own unique environmental and structural characteristics. Therefore, the design of drugs should fully consider the characteristics of the specific mucosa.

The COVID-19 outbreak in 2019 has attracted increased attention to the development of various types of vaccines, such as mRNA vaccines and mucosal vaccines. The mucosal vaccine represented by Ad5-nCoV has been developed and is promising to fill the gap in COVID-19 mucosal vaccines. However, most types of mucosal vaccines are developing slowly. As summarized in this review, numerous mucosal vaccines based on subunits and mRNA have been demonstrated to be valid against various diseases. We believe that more types of mucosal vaccines will be approved for use in the near future.

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Author contributions

Mengwen Huang collected relevant research articles and completed the manuscript. Miaomiao Zhang and Hongbin Zhu made additions and revisions to the manuscript. Xiaojiao Du and Jun Wang provided the idea and revised the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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