Mucoadhesive and Mucopenetrating Polymer-Based Adjuvants

Subjects: Polymer Science

Contributor: Nathaly Vasquez-Martínez, Daniel Guillen, Silvia Andrea Moreno-Mendieta, Sergio Sanchez, Romina Rodríguez-Sanoja

Mucus is a viscoelastic gel that acts as a protective barrier for epithelial surfaces. The mucosal vehicles and adjuvants need to pass through the mucus layer to make drugs and vaccine delivery by mucosal routes possible. The mucoadhesion of polymer particle adjuvants significantly increases the contact time between vaccine formulations and the mucosa; then, the particles can penetrate the mucus layer and epithelium to reach mucosa-associated lymphoid tissues.

Keywords: mucoadhesion ; polymeric particles ; immune response ; mucosal vaccines ; mucosal adjuvants

1. Introduction

Several materials commonly used in the pharmaceutical industry are also used as mucosal vaccine adjuvants; however, few studies have been devoted to evaluating the direct influence of mucoadhesion and mucopenetration on the strength and quality of the antigen-specific immune responses stimulated after mucosal vaccination. Consequently, researchers provide an overview of polymer-based particles in the following sections. Based on the available experimental findings, researchers analyzed the association between increased mucoadhesive strength, mucosal penetrability, and enhanced immune response quality after mucosal vaccination.

2. Chitosan and Chitosan Derivatives

Chitosan (CS) is a natural cationic polysaccharide obtained by the deacetylation of chitin. CS has been widely used in various biomedical applications due to its biodegradability, biocompatibility, low toxicity, immunogenicity, and mucoadhesive properties [1][2][3]. The mucoadhesive properties of CS are attributed to the protonation of the amino groups in weakly acidic media, which interact with the negatively charged sialic acid moieties of mucin. However, different chemical processes have been applied to CS to improve its application limitations, such as high hydrophilicity, low solubility from pH 7.4, high degree of swelling, and thermal stability [4][5]. These modifications, in turn, are favorable to promoting adhesion to mucosal surfaces and, as a result, enhance mucosal contact time.

For example, the chemical conjugation of CS with hydrophilic ethylene glycol branches improves solubility in water at neutral and acid pH values and its steric stability [6][7]. Pawar and Jaganathan (2016) compared the immunogenicity of CS NPs and glycol chitosan (GC) NPs loaded with a hepatitis B surface antigen (HBsAg) after nasal administration in Balb/c mice. While the anti-HBsAg antibody titer induced by HBsAg alone was minimal, HBsAg conjugated with GC NPs significantly increased serum IgG and IgA antibody titers in nasal, saliva, and vaginal secretions, compared to the CS-conjugated group. Splenocytes isolated from mice immunized with GC NPs and CS NPs secreted significantly higher amounts of IL-2 and IFN-γ than the control mice immunized with Alum-adsorbed HBsAg. Nasal clearance studies of radiolabeled particles in rabbits showed a nasal cavity retention time of up to 240 min for GC NPs (20% radioactivity) compared to 180 min for CS NPs (20% radioactivity) and 90 min for HBsAg alone (<20% radioactivity). In confirming nasal deposition after nasal administration in mice, only formulations with GC and CS NPs were retained in the NALT at 30 min, with higher fluorescence intensity for GC NPs than FITC-BSA [8].

Similarly, adding cross-linking agents, such as tripolyphosphate (TPP), improves the encapsulation efficiency during the elaboration of CS NPs [9]. Co-crosslinked vanillin/TPP was used for developing a trivalent oral vaccine (DwPT). Studies of the adhesion behavior of the microspheres were related to the ζ-potential of the groups, the electrostatic interaction between the positively charged CS and the negatively charged sialic acid of mucin, and the degree of cross-linking. Thus, the highest swelling index was for the group with the lowest degree of cross-linking. Batches with electropositive charge (placebo CS microspheres, diphtheria toxoid (DT) CS microspheres, and tetanus toxoid (TT) CS microspheres: ~+30 mV) showed a higher adhesion to mucin than those with ζ-potential around +10 mV (whole-cell pertussis (wP) CS microsphere and trivalent (DwPT)). Antibody response in serum corresponded to the mucoadhesion of the microspheres, developing a

higher IgG antibody titer in TT and DT batches on days 28 and 35 after immunization, followed by batches with a lower adherence (PT: pertussis toxin). This response was consistent with that observed in saliva and intestinal secretions [10].

Other derivatives of CS have been developed to improve, specifically, absorption and bioadhesion properties. Currently, the most used are obtained by quaternization, acylation, thiolation, and carboxymethylation [5]. Trimethyl chitosan (TMC), a quaternized derivative of CS with polyampholytic properties, improves CS solubility without affecting its mucoadhesive cationic nature, reduces cytotoxicity, and enhances absorption on mucosal surfaces in a wide range of pH values, increasing the carrying capacity [11][12][13].

In 2010, Vyas laboratory used PLGA microparticles (MPs) coated with CS and TMC for the intranasal administration of HBsAg to mice. While unmodified PLGA MPs had a negative ζ-potential (−14.4 ± 1.2), the coating with CS and TMC increased the ζ-potential to values between +5 mV and +10 mV for PLGA/CS MP and +10 mV and +20 mV for PLGA/TMC MP. The authors also indicated that the ζ-potential directly influenced the adsorption capacity of MPs to mucin, i.e., PLGA MPs showed insignificant mucin retention, while CS-PLGA and TMC-coated MPs had significantly higher mucoadhesive properties. Remarkably, this increase in mucoadhesion improved the immunogenicity of the formulation. However, PLGA/TMC MPs induced substantially higher antibody IgG titers throughout the study than PLGA/CS MPs, both in serum and distal mucosal sites [14]. A second study found the same results with PLGA/TMC NPs and demonstrated the adjuvanticity effect of TMC through the stimulation of dendritic cell maturation. Furthermore, TMC-coated MPs were selectively taken up by M cells in the NALT following nasal administration compared to the FITC-BSA solution, which would substantially explain the enhancement of vaccine formulations' immunogenicity [15].

Another quaternized CS derivative is N-[(2-hydroxyl-3-trimethyl ammonium) propyl] CS or HTCC. HTCC polymers have different degrees of quaternization or extent of positive charge [12]. Zhang et al. prepared OVA-loaded curdlan sulfate-O-HTCC NPs as an intranasal vaccine system. Although the inclusion of curdlan, a β-glucan capable of activating innate immune cells via Dectin-1 receptors and TLR-4 [16], could promote the antigen-specific immune response, its negative surface charge was considered a limitation for mucosal application. For this reason, O-HTCC was added, which, in addition to conferring a positive ζ-potential on the particle, improved its adhesion and subsequent internalization by epithelial cells due to its high viscosity. The OVA–curdlan–O-HTCC complex led to higher OVA-specific CD4+ T-cell, CD8+ T-cell, and B-cell proliferation when nasally administered to mice, compared with the proliferation induced by OVA, OVA–curdlan, OVA–CS, or CS–curdlan [17].

Carboxymethyl chitosan (CMCS) is another water-soluble CS derivative with an improved degradation rate, a desired characteristic for its use in vivo [18]. Recently, CMCS was also used to coat the surface of calcium phosphate (CaP) NPs. The electrostatic interactions and hydrogen bonds between mucin and CaP–CMCS–BSA allowed in vitro adhesion close to 90% compared to CaP–BSA adhesion (60%). Additionally, the diffusion efficiency was higher for CaP–CMCS–BSA than for CaP–CS–BSA, CaP–BSA, and BSA alone. The coating with CMCS and CS improved the apparent permeability coefficient in the mucus layer at 2 h, an index of apical to basolateral transport. Ex vivo biodistribution in a rat study showed that CaP–CMCS–BSA/FITC absorption was improved in the small intestine at 2 h compared to CaP–CS–BSA/FITC, attributed to the change in surface charge caused by coating with CS and its derivative (CaP–CMCS–BSA, ζ-potential: −4.7 mV vs. CaP–CS–BSA, ζ-potential: 8.5 mV). These findings are correlated with the efficacy of oral vaccination since the levels of IgG and sIgA antibodies in sera and feces, respectively, increased after each boost in the animals that received CaP–CMCS–OVA compared to OVA alone [19].

For their part, methyl CS has been studied for diverse biological activities, including as tissue regeneration activator, absorption enhancer, and mucoadhesive [20]. Suksamran et al. evaluated methylated CS MPs for entrapping OVA. Calcium alginate MPs–OVA, calcium alginate–yam starch microparticles (YMP)–OVA, and (YMP)–OVA coated with methylated N-(4-N, N-dimethylaminocinnamyl) CS ($TM_{65}CM_{50}CS$) were used in this work. The evaluation of swelling showed that the degree and rate of swelling of the $TM_{65}CM_{50}CS$-coated MPs were higher than those uncoated, both in HCL pH 1.2 and in PBS pH 7.4. Similarly, the in vitro mucoadhesion study using the everted gut sac with porcine jejunum showed that, while the adherence percentages of calcium alginate MPs and YMP MPs were low (29.62% and 11.29%, respectively), the coating with $TM_{65}CM_{50}CS$ of both particles increased mucosal adhesion during the first hour (45.64% and 43.38%, respectively). Oral immunization resulted in significantly higher IgG and IgA levels in mice receiving OVA-loaded $TM_{65}CM_{50}CS$-coated MPs, which again confirms the role of mucoadhesive polymers in immunogenicity [21].

The ζ-potential of the CS-based vaccines significantly influences the induction of an immune response affecting more than one mechanism. Jesus et al. demonstrated that. after the intranasal administration of polycaprolactone/CS (PCL/CS) NPs in C57BL/6 mice, the lowest dose of adsorbed antigen (1.5 µg HBsAg) induced antibody titers comparable to the dose containing six times more adsorbed antigen (10 µg HBsAg). Furthermore, this group had the highest number of

responding animals. However, serum IgG titers were significantly low compared to previous studies with the same dose of antigen (1.5 µg HBsAg), so the authors suggested that the decrease in ζ-potential (CS: +30 mV) to values close to neutrality generated by antigen interaction (PCL/CS: +26 mV; PCL/CS: 1.5 µg HBsAg: +22 mV; PCL/CS:10 µg HBsAg: +5.7 mV) leads to a reduced uptake in the epithelial barrier. These observations were independent of the mucoadhesive behavior of the particles without antigen evaluated in vitro. Therefore, the authors suggested that the antigen on the particle's surface reduces the ζ-potential and hinders the interaction with mucin in vivo, avoiding particle–cell interactions and ultimately impacting the immune response [22]. Although this finding contradicts what was observed for other CS-based particles reviewed, it highlights the importance of assessing the mucoadhesion of the polymeric system alone, as well as the particle-entrapped antigens of interest.

3. Cellulose Derivatives

Carboxymethylcellulose (CMC), an anionic and water-soluble cellulose derivative [23], has been successfully used as a mucoadhesive polymer to enhance immune responses. Hanson et al. developed CMC and alginate (ALG) wafers loaded with the HIV gp140 protein and with α-GalSer as an adjuvant. In ex vivo tests with porcine sublingual mucosa, wafers with a higher CMC content withstood intense mucosal washings and had a higher tissue penetration of the coupled protein (fluorescently labeled bovine serum albumin (BSA)) compared to wafers with a higher ALG content and the free protein. However, the presence of ALG in the formulation was necessary to maintain protein stability on the wafer. Following sublingual administration in mice, most mucoadhesive wafers generated a greater T-cell response in the lungs and cervical lymph nodes [24]. In other studies, it has been suggested that CMC's viscosity and anionic structure allows the formation of ionic bonding and hydrogen bonds with mucin layers [25][26][27].

4. Mannan-Decorated Polymeric Particles

Similar results have also been achieved using the dual immunostimulant and mucoadhesive capacity of mannan isolated from the cell wall of *Saccharomyces cerevisiae* [28]. Mannans present immunostimulatory activity via pathogen recognition receptors (PRRs) in APCs. An in vivo optical imaging system, following the intranasal administration of thiolated hydroxypropylmethylcellulose phthalate microspheres (Man-THM), showed that mannan decoration increased the residence time of Cy5.5-conjugated OVA-loaded Man-THM in the respiratory mucosa compared to OVA alone or OVA-loaded THM. Subsequently, the mucosal immune response was evaluated following the nasal immunization of the ApxIIA toxin from *Actinobacillus pleuropneumoniae* loaded in the MPs groups. The findings also demonstrated that the microspheres reached the lungs and secondary lymphoid tissues and induced systemic IgG and secretory IgA responses to the ApxIIA in bronchoalveolar lavage (BAL) and nasal and vaginal washes. Although the immunostimulatory role of mannosylation in enhancing immunogenicity has been reported [29][30], in this work, the authors highlighted the mucoadhesion of the mannosylated microspheres to explain the improved immunogenicity in vivo.

5. Alginate Coating

Vyas and his team (2014) assessed the coating of CS MPs with alginate (A-CSMp). In contrast to most of the works reviewed up to this point, where the positive surface charge plays a fundamental role in adhesion to mucin, alginate as an anionic polyelectrolyte changes the ζ-potential of the particle to an electronegative value (−29.7 mV). FITC-BSA was rapidly washed from rat jejunal tissues; however, the in vitro retention time in the mucosa was prolonged when FITC-BSA was associated with A-CSMp. In the same way, in the in vivo assays, only A-CSM loaded with FITC-BSA successfully generated uptake by M cells in Peyer's patches. When evaluating the efficacy of the particulate system in an oral anthrax vaccination model, high-titer anti-PA serum IgA and IgG antibodies were observed in animals receiving particles loaded with antigens compared to the free *Bacillus anthracis* protective antigen [31].

Similarly, Saraf et al. loaded alginate-coated CS NPs (ACNPs) with HBsAg anchored to E. coli EH-100 lipopolysaccharide (LPS) (LPS-HBs-ACNPs) as an adjuvant for oral administration. As expected, the alginate coating changed the ζ-potential of the NPs from +45.2 mV (0.5% CS-0.1% TPP) to −26.2 mV (0.5% CS-0.1% TPP-2% alginate-2% LPS) due to the negatively charged -COO- electrostatic interaction of the alginate on the positively charged -NH3 of the CS. Despite the ζ-potential's more negative values, in vitro mucoadhesion studies showed that alginate-coated NPs were more mucoadhesive than CS NPs alone. Although anti-HBsAg serum IgG titers were higher for HB-ACNPs after oral administration, sIgA antibody titers in mucosal secretions were higher for LPS-HBs-ACNPs. The anchoring of LPS targeted the NPs to M cells, conferring immunogenicity to the system [32] independently of the mucoadhesive properties of ACNPs. As in the case of LPS, any ligand can be anchored to the particulate system to target it and to allow specific binding to M cells or mucosal epithelial cells. Excellent reviews have been conducted on this topic [33][34][35].

On the other hand, sodium alginate protects the NPs from the hostile environment of the gastrointestinal tract, the same as the introduction of hydrophilic groups, such as hydroxyalkyls, carboxyalkyls, succinyls, and thiols, or polymer grafts, such as PEG. In this way, Amin and Boateng (2022) proposed a system based on OVA-loaded CS NPs coated with sodium alginate or PEG for oral vaccine administration. Both sodium alginate and PEG coatings increased the stability of NPs upon exposure to gastric fluids with the protection of the encapsulated protein (4 h and 1 h, respectively), compared to uncoated NPs (<30 min). After transfer into simulated intestinal fluid, both coatings showed stability for 120 h, although with different release profiles of OVA. Increased alginate concentrations were related to a higher level of mucin binding. According to the authors, the alginate coating ensures stability, allows a higher antigen load to reach the site for mucosal immune response, improves mucoadhesive properties, and enhances the sustained release of antigen-loaded NPs [36].

6. Xyloglucan

Xyloglucan (XG), a non-anionic polysaccharide and the main hemicellulose component, has been applied with *Quillaja* saponins to vaccine formulations against brucellosis. While *Brucella* LPS was weakly immunogenic, when *B. abortus* LPS-loaded XG NPs were administered nasally to Balb/c mice, higher systemic and mucosal IgG antibody levels and mucosal IgA were induced. Increased immunogenicity was associated with a greater mucoadhesion force of the XG and the LPS-XG NPs compared to the LPS alone, as well as the ex vivo retention of LPS-XG NPs over 24 h in goat mucosa [37].

As in the case of CS, XG has been previously used in pharmaceutical applications in different formulations and by different routes, including mucosal, transdermal, and intraperitoneal, due to its biodegradability, cost-effectiveness, and non-toxicity. Some authors have suggested that the XG molecular structure, “mucin-like,” is responsible for mucoadhesive properties, including swelling capacity and increasing concentration-dependent viscosity [38][39]. All these characteristics, taken together, expand the possibility of the future use of XG in mucosal vaccinations [40].

7. Poly (Acrylic Acid) and its Derivatives

Poly (acrylic acid) and its derivatives have excellent mucoadhesive capacity compared to cellulose, polycarbophil, chitosan, and pectin [41][42][43]. An example is Carbopol®, a highly cross-linked hydrophilic polymer, which provides it with mucoadhesive and viscoelastic properties. Coucke et al. used spray-dried powders of amylopectin (Amioca®) with polyacrylic acid (Carbopol® 974P) in different proportions (SD 0/100, 25/75, 50/50, 85/15, and 100/0) for the intranasal administration of H3N2-inactivated influenza virus and in combination with the LTR192G adjuvant in rabbits. The formulation SD25/75 induced the highest serum response of IgG anti-haemagglutinin compared to the formulation SD100/0, thus highlighting the importance of polyacrylic acid. Despite this, neither SD25/75 nor SD0/100 induce a local mucosal response. The immune response was directly related to the negative ζ-potential of Carbopol® 974P and the mucoadhesive properties of the formulations. The reticulated, predominantly elastic, or highly structured characteristics of SD25/75 (G’ >> G”) increased the residence time in the nasal cavity. In contrast, the lowest viscosity and cross-linking of SD100/0 were associated with a low mucosal retention [44].

8. γ-PGA

The poly-γ-glutamic acid (γ-PGA)-based vaccine adjuvant, an anionic biopolymer, was used for the intranasal delivery of the influenza fusion protein sM2HA2 and OVA, co-administered with 3-O-desacyl-4′-monophosphoryl lipid A (MPL) and QS21 in a system denominated γ-PGA/MPL/QS21(CA-PMQ). Using in vivo single-positron-emission computed tomography imaging, it was possible to determine that γ-PGA increased the OVA residence time by up to 12 h in the nasal cavity. This signal decreased at 6 h when OVA was administered alone. This result is correlated with the higher serum IgG, IgG1, and IgG2a antibody responses in the groups vaccinated with OVA/CA-PMQ and sM2HA2/CA-PMQ compared to the groups that received OVA and sM2HA2 alone, as well as being superior to that induced by the cholera toxin used as a mucosal adjuvant. Likewise, animals vaccinated with the antigen/CA-PMQ induced more IL-4 and IFN-γ–secreting cell populations in the spleens stimulated with OVA, sM2, and HA2 protein than mice immunized with proteins alone or the control group. Additionally, the CA-PMQ induced high titers of sM2HA2-specific IgA antibodies at the administration and distal sites, along with an increased survival time (80–100%) following the challenge with influenza A subtypes and cleared pulmonary viral titers [45]. The presence of carboxyl groups within γ-PGA can provide a strong interaction with the mucus layer.

The anionic model (−35.5 mV) of Kurosaki et al. with benzalkonium chloride (BK) and γ-PGA NPs in a complex with OVA (OVA/BK/γ-PGA) was used for pulmonary administration. They observed an increased fluorescence intensity in the lung (Alexa647-OVA/BK/γ-PGA) indicative of lung deposition compared to Alexa647-OVA. OVA/BK/γ-PGA increased the levels

of specific IgG antibodies, while in the animals that received OVA or the vehicle (BK/γ-PGA), anti-OVA IgG was not detected. The induction of immune responses at the mucosal site was also significantly higher in the OVA/BK/γ-PGA group [46]. Their study did not discuss the role of γ-PGA mucoadhesion in the results obtained. However, the authors suggest the uptake efficiency of BK/γ-PGA NPs by the antigen-presenting cells in the alveolar region. Due to the high capture efficiency of particles <2 μm in the lung [47], the adhesion phenomenon could favor the increased particle residence time in the lung mucosa. Evaluating bioadhesive properties in these systems could help to improve rational vaccine design using polymeric particles.

9. Thiolated Polymers

The previously reviewed polymer-based adjuvants could be thiolated to improve mucoadhesion. In the past two decades, important research has been conducted using thiolated polymers or so-call “thiomers”, mainly in excipients for drug delivery. Thiomers can interact with mucin through disulfide bonds with the cysteine-rich subdomains of mucus glycoproteins [48]. These covalent bonds are supposed to have stronger binding than the non-covalent interactions that are formed between the polymers and the sialic acid of the mucus layer [49], improving the mucoadhesive properties of the polymers.

Using a tensile test and rotating cylinder method to obtain compressed tablets, Roldo et al. demonstrated that increasing the number of thiol groups covalently attached to chitosan-4-thio-butyl-amidine conjugated significantly improves mucoadhesion compared to unmodified CS. Thiolation increased the total adhesion work (TWA, μJ) up to 100 times [50]. Similarly, thiol reactivity impacts mucopenetration. When the thiol reactivity is medium to low, extensive interpenetration occurs in the mucus layer, with a larger interface for disulfide bond formation. Conversely, highly reactive thiols have difficulty penetrating through the mucus because they form disulfide bonds with the mucins on the surface of the mucus layer, facilitating their rapid removal through mucus turnover [51].

In a recent study, Sinani et al. immunized Balb/c mice with BSA-loaded NPs prepared using aminated CS (aCS) and aminated and thiolated CS (atCS) polymers; mice were nasally immunized at 14-day intervals. At the end of the experiment (day 253), the nanoparticles (aCS and atCS) induced a more robust systemic response, resulting in an almost two orders of magnitude higher systemic IgG titer than the BSA/CpG ODN control, with atCS being the best. These results are correlated with the increased mucoadhesion observed in the aCS and the atCS. Both aCS and atCS modulated the Th2 immune response and enabled immune response at distal mucosal sites [52].

Cellulose acetate phthalate (CAP) is widely used as an enteric coating for pharmaceutical dosage forms due to its solubility at pH values above 6 (such as in the intestines) but poor water solubility at a low pH (such as in the stomach). After exposure to intestinal fluids, the polymer swells, with the subsequent softening or complete dissolution of the phthalate, allowing the release of the biologically active compounds [53]. Lee et al. orally immunized mice with M5BT, a chimerical multi-epitope recombinant protein of foot-and-mouth disease virus (FMDV), alone, loaded in thiolated CAP MPs (T-CAP), or loaded in non-thiolated MPs (CAP). In ex vivo studies in the porcine intestinal mucosa, T-CAP mucoadhesion was 1.48-fold higher than CAP. The improvement in the mucoadhesion properties was reflected in the highest production of antigen-specific IgG antibodies in animals that received M5BT/T CAP. Similarly, this group of animals had significantly higher levels of anti-M5BT IgA in fecal samples at 2 and 4 weeks due to the longer transit time of antigens in the mucosa and increased MHC class II- expression on APC in PPs, related to IgA production [54].

For cationic thiomers such as atCS, the interactions are predominantly driven by electrostatic forces. In contrast, for anionic thiomers, such as T-CAP, interaction with the mucus occurs through hydrogen bonds, van der Waals interactions, and chain entanglement. In both cases, the bioavailability is improved by the extension of the residence time [55]. Notably, regardless of the surface charge of the polymer particles and resulting surface forces, the thiolation of both polymers improved in vivo immune response.

Further evidence has shown that thiomers are susceptible to thiol oxidation at pH ≥ 5, with their effectiveness being reduced following oral administration. Typically, thiol groups (R-SH) can form disulfide bonds with mercaptopyridine substructures, whereby thiol groups are stabilized against oxidation and increase their reactivity. S-protected thiomers, so-called “pre-activated”, have shown greater mucoadhesion than unprotected thiomers, according to Iqbal et al. (2012) [49]. In this work, Iqbal et al. synthesized a polymer with improved mucoadhesive, cohesive, and in situ gelling properties. For this purpose, poly (acrylic acid) (PAA), PAA-cysteine (PAA-cys), and 2-mercaptonicotinic acid (2MNA) coupled with PAA-cys (PAA-cys-2MNA) were compressed into tablets, and the mucoadhesion strength was determined by the rotating cylinder method. Adding thiol groups improved the mucoadhesive properties 456-fold, while the S-protected thiomer

increased the contact time to 960-fold compared to unmodified PAA. These thiolated nanosized carriers and others, such as thiolated cyclodextrins [51][56], are research fields that may be explored further for mucosal vaccine development.

10. Enhancement of Epithelial Permeability by Polymer-Based Adjuvants

Although mucoadhesive molecules improve the bioavailability of drugs and antigens administered via the mucosa, the mucus layer still limits passage into the epithelium. The transit time of particles in the mucosa is determined by the physiological renewal time of the secreted mucus layer [57].. Mucus turnover reduces the mucosal residence time of particulate delivery systems because they can be trapped by the mucus and rapidly eliminated [58], which could compromise their effectiveness as mucosal adjuvants.

Therefore, polymer-based adjuvants are expected to adhere to the mucous layer, penetrate the epithelium, and reach the inductive sites for mucosal immune responses before being removed. Hence, this section briefly describes the strategies to facilitate mucus barrier penetration and improve the permeability of polymer-based adjuvants once they are in the mucosa.

10.1. Mucus-Penetrating Particles

Particles with a low adhesion and small size, thus with few steric hindrances to the mucin network, are often referred to as mucus-penetrating particles. Unlike mucoadhesive particles, mucus-penetrating particles seek to minimize the strength of electrostatic and hydrophobic interactions with the mucin. Polymers with neutral or low positive charges are generally included in the design of mucus-penetrating formulations. Several studies have reported the surface coating of particles with PEG. PEG is used as an adhesion promoter acting at the interface to improve adhesion. Hence, PEG chains tethered or grafted are covalently attached at one end on the polymer surface while the other is free, allowing PEG to diffuse from the polymer network to the mucus and enhancing interpenetration [163]. Wang et al. further demonstrated the formation of hydrogen bonds between the ether oxygen atoms of the PEG chain and glycosylated proteins of mucins. Additionally, they reported PEG with a low molecular weight (2 and 10 kDa), near-neutral surface charge (ζ-potential of 2 ± 4 and 1 ± 3 mV, respectively), minimized mucoadhesion by reducing hydrophobic hydrogen bonding, and electrostatic interactions to have better mucus-penetrating properties. The authors even proposed that PEG-covered particles between −10 and −7 mV are within the interval that defines mucoadhesive vs. mucoinert characteristics [59].

Despite its widespread use in over-the-counter drugs and vaccines, recent approaches suggest that PEG is not immunologically inert [60][61][62]. Several authors demonstrated that introducing PEG to mucosal vaccine formulations increases their protective efficacy [63][64]. Similarly, an extensive recent review explained the impact of PEGylation in terms of biodistribution for anticipating safety and efficacy [65]. Therefore, it is essential to study the tolerability and safety profile of PEG, despite being an alternative to increased mucopenetration.

Some works have also raised doubts about coating particles with PEG due to surface modifications that can alter the linked polymers' physical and biological properties. Bamberger et al. evaluated the effects on APC response after functionalizing spermine NPs with acetylated dextran (Sp-Ac-DEX) through a process called DEXylation and PEGylation. The average particle size was considerably increased by DEXylation, with subsequent aggregation. PEGylation and DEXylation decreased the primary amines and, therefore, the ζ-potential. This was reflected in the 20% reduction in the cell viability of bone-marrow-derived dendritic cells and macrophages treated with DEXylated NPs, whereas PEGylation treatment increased viability by 10–20% compared to unmodified NPs. However, the binding and cellular uptake of surface-modified NPs was lower in PEGylated particles [66].

Other polymers with mucopenetration ability are poloxamers, also known as Pluronic®. These block copolymers consist of hydrophilic poly (ethylene oxide) (PEO), and hydrophobic block-poly (propylene oxide) (PPO) ordered in an A-B-A triblock structure: PEO-PPO-PEO [67][68][69]. Díaz et al. demonstrated that the addition of mucoadhesive and thermosensitive poloxamer 407(F127)-based hydrogels to CS microspheres in a formulation for nasal and conjunctival ram immunization improved both local and systemic humoral immune responses against the BLSOmp31 antigen, an outer membrane protein of *Brucella* spp., along with the reduced excretion of *Brucella ovis* [70]. Pastor et al. proposed a Pluronic® (PF127) and Gantrez® AN119 thermally sensitive hydrogel for intranasal vaccine delivery since the hydrogel increases the residence time of the antigens in the nasal epithelium, allowing their penetration into the deep skin layers of the nose thus reaching the submucosa, where they can trigger an immune response [71].

Another type of mucopenetrant includes nanoemulsions (NEs). Di Cola et al. evaluated PEG-coated O/W NEs with emulsified, added CS as a proposal for the nasal administration of drugs or vaccines. They observed that CS-added NE led to a local shrinking of the mucin gel network, forming larger pores between the mucin bundles. This phenomenon does

not occur in the absence of CS. The SAXS (small-angle X-ray) monitoring of the penetration of solute CS-added NE into the PGM showed a higher diffusion over time (20 min) through the mucus mesh. SANS (neutron scattering) confirmed that, unlike the steric hindrance caused by the pore-like size of mucus caused by mucoinert NPs, the CS-added NE based on Solutol® mucopenetrates by the collapse of the mucus mesh [72].

Coating dextran particles with mucopenetration properties have also been explored to improve drug administration performance [73][74][75][76] and enhance immunoadjuvant activity in vivo [77]. Other strategies, such as coating polymeric particles with polydopamine (PDA) [78] or cell-penetrating peptides [79] used in drug delivery, might be explored and characterized in mucosal vaccines, as well as continuing the search for new adjuvants with mucopenetrating properties.

10.2. Permeation of Polymeric Particles via the Mucus Layer

An additional consideration for the design of polymer-based particles is passing through the second barrier, the epithelial cell membrane. The permeability of peptides, proteins, and drugs is often deficient. In this sense, absorption enhancers have been developed, which, in addition to preventing enzymatic degradation, facilitate the opening of the epithelial barrier and improve absorption through intracellular or paracellular mechanisms [80]. Absorption and permeation enhancers include surfactants, such as bile salts, fatty acids, phospholipids, tight junction modulators, cyclodextrins, and detergents [81][82][83][84]. This group also includes mucolytic agents, such as acetylcysteine or enzymes, which can decrease the elastic properties and dynamic viscosity of the mucus, influencing the integrity of the mucus layer [85]. For example, Zhang et al. reported the oral administration, in mice, of self-assembled nanoparticles with recombinant urease subunit B from *Helicobacter pylori*, coated with a cell-penetrating peptide, and coated with PEG derivative. NPs were transported transepithelially, improving the systemic and mucosal antibody response and the protection against *H. pylori* after the challenge [86]. It will be essential to continue studying absorption enhancers in mucosal vaccine formulations to improve the immune response.

11. Challenges and Opportunities

For several decades, many polymer-based particles have shown promise as potential human mucosal vaccine adjuvants due to their biodegradability, biocompatibility, and nontoxicity characteristics. Added to this is the extensive study of the adjuvant mechanisms of particulate systems. However, in the mucosa, the mucin networks that cover the compartments are often considered a barrier for the particles, so the mucoadhesive and mucopenetrating capacity of the polymer-based particles often defines their adjuvant mechanism of action.

The search for polymers with better mucoadhesive properties, regardless of the polymer's source, but focusing on the physicochemical characterization of polymeric particles and the contribution of these properties to mucoadhesion, will allow the rational design of mucosal vaccines. However, it is not an easy task because, on the one hand, the smallest nanoparticles are the most mucopenetrating. Still, on the other, there is a lack of studies that suggest an ideal surface charge or a hydrophobicity that favors adhesion. At the same time, it cannot be ignored that there are multiple other cellular mechanisms to elicit the immune response triggered by the polymeric particles, i.e., enhanced antigen uptake, immune cell presentation and recruitment, and traffic to lymph nodes.

Studies demonstrating the correlation between the observed immune response, the physicochemical characteristics, the mucoadhesion, and the mucopenetration ability are scarce. More studies that examine all these factors simultaneously are required to position mucoadhesion as another immune response mechanism necessary for designing more efficient polymer-based particulate adjuvants.

12. Conclusions

The COVID-19 pandemic highlighted the need for mucosal vaccination as an effective strategy to eradicate infectious diseases that have the mucosa as a natural route of infection. Mucoadhesion is probably the most important feature to improve local and systemic immune responses since, by prolonging the residence time of particulate polymers in mucosal tissues, the absorption and sometimes penetration through the mucosal epithelia are allowed and improved. In this sense, studying the physicochemical characteristics of the polymeric particles used as mucosal vaccine adjuvants and how they affect mucoadhesion is crucial to developing new mucosal vaccines.

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