Natural Polymeric Composites Derived from Animals

Subjects: Polymer Science

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The special property of polymeric-based systems is that they serve as adjuvants, may trigger an antigen-mediated immune response, and also substantially deliver the antigen or vaccine to the desired anatomical or physiological location. Moreover, the benefit of polymeric-based delivery methods is that they may be made using natural polymers, which reduces the risk of tissue cytotoxicity.

Keywords: natural polymer ; vaccine delivery ; adjuvant

1. Introduction

Among the most practical, economical, and long-lasting ways to protect and treat contagious illnesses is vaccination, which also slows the spread of infectious diseases. The significance of immunization by vaccination is that it trains the immune system to identify and defend against contagious diseases and provides passive community-level protection by promoting herd immunity [1][2]. Different vaccines such as DNA vaccines, synthetic peptide vaccines, nanovaccines, and recombinant protein vaccines have been created using contemporary biotechnological methods [3][4][5][6][7]. The main goal of these vaccines is to create an immunological response mediated by an antigen against the disease. Many approaches have been created to transport the vaccine to the desired place and boost the system's effectiveness by making the vaccine more immunogenic [8]. There has been evidence of the effectiveness of a variety of delivery strategies, including polymeric, nanoparticles (NPs), and three-dimensional scaffolds [9][10][11][12]. These technologies enable the delayed release and administration of antigen molecules in a manner that does not need booster doses. Additionally, it ensures that harmful antigen molecules are effectively presented to immune cells [13]. Furthermore, an excellent vaccine delivery technology has demonstrated its capacity to function as an "adjuvant," which interacts with the human immune system and induces an immunogenic response. Adjuvants boost the effectiveness of vaccines and decrease the number of antigens and the number of immunizations needed to produce protective immunity by enhancing the immunogenicity of immunogens that are less potent [14].

2. Animal-Based Polymers

2.1. Chitosan

Chitosan is derived from chitin, the second highly abundant polysaccharide. Chitin comprises β-(1–4)-poly-N-acetyl-D-glucosamine and can be discovered in a wide range of organisms, mostly in the exoskeletons of animals (e.g., insects, shrimp, lobsters, and crabs) [15]. The major source of chitosan was derived from removing the acetyl group (CH3-CO) mainly from chitin (poly-(β-1→4)-2-amino-2-deoxy-D-glucopyranose).

Chitosan benefits from its cationic characteristics and is a great immunomodulator in vaccine delivery, especially in the nucleic with negatively charged DNA through electrostatic interaction to produce polyplexes (2). It has been known to be used in anti-viral vaccine strategies that allow favorable interaction with the negatively charged virus. Immunotherapeutic effects of chitosan have been proved by inducing the production of cytokines (e.g., TNF-α, IL-1β, IFN-γ, and IL-10) which become a great indicator in the initiation of humoral immunity [16][17][18] and cellular immunity through stimulation of IgG antibodies [18]. Strong responses through both humoral and cellular immunity are crucial in developing a more efficient vaccine therapy.

Extensive research has been conducted on chitosan as a potential biopolymer in biomedical applications; however, chitosan presents some drawbacks such as poor solubility in physiological environments (pKa value around 6.3–6.4), and its intrinsic properties may be affected through crosslinking with other materials and potential toxicity, depending on its molecular weight and types of chitosan [15][19]. Chitosan's anti-viral activities have been known to be impacted by these

key factors: molecular weight and degree of acetylation and substitution [20]. Recently, a novel formulation of NPs of chitosan-tripolyphosphate (TPP) using the ionotropic gelation method to deliver plasmid DNA (pDNA) vaccines for cervical cancer was investigated [21]. The results showed good stability of NPs, high pDNA encapsulation efficiency rate, improved rate of cell viability during NPs cytotoxicity test in vitro, and an increase in E7 antigen transcription, the oncoprotein of high-risk human papillomavirus (HPV). This study suggested a good formulation for nucleic acid vaccine vehicles for viral infection and others.

The issue of muti-drug resistance in tuberculosis (TB) [22] adds additional urgency to developing a new vaccine formulation, which is currently restricted to the BCG vaccine only. Several studies have attempted to produce new formulations for TB vaccines by using chitosan-based NPs. A novel nanosphere of chitosan (CHT)- or trimethyl chitosan (TMC)-coated PLGA (i.e., containing HspX, EsxV, and PPE44 *Mycobacterium Tuberculosis* (*Mtb*) antigens) was designed [23]. Hspx–PPE44–EsxV (HPE), together with the adjuvant of resiquimod (RC), HPERC, was loaded in chitosan in a study regarding the TB booster vaccine to overcome waning immunity in TB [24]. Both studies showed a Th1-dominant response in groups incorporated with chitosan polymers and groups with single BCG immunization and HPERC vaccine booster groups compared to control groups. These studies present a good vaccine candidate for TB vaccination. Delivery of vaccine through a transdermal route using microneedle (MN) patch aid with the iontophoresis technique can monitor the transportation of vaccine molecules. However, MN serves a limitation such as a low load of vaccine; therefore, a polyacrylamide/chitosan iontophoresis-driven MN system was designed to deliver ovalbumin, and it resulted in enhanced immune response compared to conventional intramuscular injection in vitro [25].

Moreover, mucosal vaccine administration (e.g., through oral, nasal, and vaginal routes) is more favorable compared to parenteral administration due to several factors such as local immune response activation and eliciting epigenetic memory, which can induce greater immunogenicity during second exposure [26][27].

Chitosan was used in various applications as a basis for mucosal vaccine delivery vehicles due to its mucoadhesive characteristic. Goblet cells in the large intestines release mucus so that chitosan is able to hydrogen bond with the mucin of the mucus by retaining OH and NH2 groups when at physiological pH (6 to 7.5), serving an excellent deal in mucosal vaccine delivery.

Chitosan also can be applied as an adjuvant for vaccines to boost the immune system. Chitosan derivatives are more favorable compared to an unmodified form of chitosan. Some recent studies on chitosan adjuvants are as follows. Chitosan-modified as the carrier for adjuvant nanographene oxide (GO-CS) and P239 vaccine for hepatitis E virus (HEV) treatment [17]. Adding chitosan as an adjuvant improved graphene oxide toxicity and increased bioavailability. In comparison with the P239 vaccine alone, the GO/CS/P239 vaccine induced more production of IgG antibodies and activation of cytokines.

A combined adjuvant system was achieved by encapsulating chitosan and *Salmonella Typhi* porins in micro (MicroAS) and nanoparticulate (NanoAS) forms to carry rGRA1 and rBAG1 vaccine antigen against *T. gondii* in Toxoplasmosis disease was presented [18]. The new formulation resulted in higher cellular and humoral response in the microsystem compared to the nanosystem. Thus, this formulation is more efficient in microsystems by profoundly increasing the protection against *T. gondii.* Readers are directed to a more detailed explanation of the application of chitosan as an adjuvant in the following article [28].

In summary, chitosan has shown great efficacy in vaccine formulation, both as a carrier and adjuvant. These easily available natural polymeric materials are foreseen as excellent biomaterials in the COVID-19 vaccine delivery platform [29], exploiting various capabilities in mucosal vaccine delivery in various viral infectious diseases. In addition, chitosan's unique properties as a natural mucoadhesive material are critical to prolonging antigen retention and tissue penetration through its ability to temporarily open intercellular tight junctions that are beneficial to amplify both humoral and cellular immunity in chitosan-based vaccines where broad studies have been conducted to apply this biomaterial as vaccine delivery and adjuvants for various diseases. Overseeing the potential of chitosan application in mucosal vaccination presents a good approach for COVID-19 vaccination as its viruses enter the host through the mucosal as the main route.

2.2. Gelatin

Gelatin is a form of soluble protein that is derived from insoluble fibrous protein, collagen (i.e., main component of extracellular matrix in animal tissues such as skin cartilage and bone) [30]. Pre-treatment of collagen using acid treatment and alkaline treatment resulted in type A and type B gelatin, respectively. This biopolymer is characterized by a variety of accessible functional groups, hydrophilic biodegradable polypeptide polymers, that serve a promising window in the

encapsulated high payload of antigens and have been known to be successfully used in the vast application of vaccine delivery systems [31].

Apart from that, gelatin also can be modified into coupling with many cross-linkers [31][32] and low antigenicity [33], making it a well-established vaccine delivery vehicle. The potential of gelatin can be seen through a study that showed human macrophages were successfully targeted when observed during the application of gelatin hydrogel nanofiber where this system provided great immunomodulatory effects by upregulating the pro-healing gene of M2 macrophage (CD206) and markedly reducing the pro-inflammatory gene (IL-1β and IL-8) expression [34]. Macrophage-mannose receptor CD206 was characterized as a negative-prognostic biomarker for many malignancies where this biomarker improves the overall survival (OS) of the patients [35][36][37]. This indicates a positive impact of gelatin to induce CD206 to be incorporated as a vaccine for cancers.

The malaria (i.e., one of the top three infectious diseases worldwide) vaccine is not commercially available due to the vast development stages of *Plasmodium* in host and immune responses making malaria vaccine production a major challenge [38][39]. Plus, effective vaccine delivery tools are demanded, especially in endemic areas with poor healthcare facilities. Surface protein P47 of *Plasmodium falciparum* and adjuvant CpG was fabricated into gelatin-based MN, where this system triggered toll-like receptor (TLR) 9 (TLR9) signaling and dendritic cells (DCs) at the same levels achieved by the native vaccine, thus showing the potential of the P47 MN-gelatin-based malaria vaccine that could be deployed [40]. In another study, aminated nanoparticulate MN-based gelatin resulted in a higher production of antibodies against tetanus toxoid compared to gelatin NPs [41]. For the synthesis of gelatin NPs, Khramtsov et al. [42] proposed a one-step modified desolvation method to overcome the limitations of the common two-step desolvation method associated with poor reproducibility and low yields. This method presented the reproducible synthesis of gelatin NPs, with yields of 62–82%.

The result of a study showed that gelatin NPs were convenient carriers for the delivery of antigens OVA and adjuvant polyinosinic: polycytidylic acid in various administrations, such as through mucosal delivery, where they both can modulate systemic and mucosal immune responses, including EG7 tumor growth inhibition in C57BL/6 mice [43]. Gelatin NPs have been studied to simultaneously deliver messenger RNA (mRNA) and plasmid DNA (pDNA) [44]. Transfection efficiency was observed in polynucleotides indicating a potential system using gelatin in vaccination deployment. Mannosylated gelatin NPs encapsulated inactivated porcine reproductive and respiratory syndrome virus (PRRSV)-induced activation of T cells in vitro through triggering DC maturation and activation, consequently inactivating PRRSV-infected cells via the improved T-cell signaling cascade [45].

A vaccine against botulinum toxin was fused into gelatin-based MN vaccines, resulting in good immunogenicity and protection efficacy of the AHc vaccine when stored at room temperature for 6 months, which indicates a brilliant vaccine strategy, especially in overcoming the limitation of the vaccine in terms of the cold-chain problem [46]. Taking advantage of various accessible functional groups that can be targeted by gelatin serves as a great opportunity in new vaccine development, especially for uncommercially available vaccines such as the malaria vaccine, which is still scarce and poorly accessible. Plus, MN-based gelatin has great potential to deliver high-molecular-weight vaccine antigen or adjuvant because they enable large molecules to pass the stratum corneum via micropores, thus overcoming the limitation of the thick skin barrier in delivering the vaccine through the skin.

Based on the above-mentioned studies on gelatin-based vaccines, it is suggested that gelatin has great potential in the development of MN vaccines where it is known as having several advantages such as being less invasive than traditional injections, easy to self-administer, and not requiring cold storage [47]. This is essentially important, especially for the distribution of vaccines such as for malaria and TB vaccines, where the prevalence is usually daunting in poor countries with poor healthcare facilities. This might shift the vaccination program landscape in poor countries in the future.

2.3. Albumin

Albumin is a highly abundant serum protein in the bloodstream similar to IgG, accounting for about 80–90% of the overall protein pool. Albumin has been recognized to be a potential polymeric biomaterial that has serum stability and longevity due to its extraordinarily extended serum half-life, which amounted to 19 to 21 days in humans [48]. Albumin binds to the neonatal Fc receptor (FcRn), which has broad biodistribution in many types of cells such as endothelial, epithelial, and DCs. “Albumin trafficking” enables active endosomal escape and lymph node drainage to elicit various immune signaling cascades through FcRn-mediated transport [49]. This mechanism leads to many developing therapeutic vaccines benefiting from albumin’s immunomodulatory properties.

Several studies on albumin as a nanocarrier were taken advantage of through albumin trafficking to increase vaccine efficiency through the activation of APCs. Matrix-2 protein virus-like particle (M2e VLP), an influenza ectodomain, was

loaded in bovine serum albumin (BSA) MPs and showed enhanced stimulation of APCs and a further increase in APCs and M2e-specific IgG antibodies in a combination of adjuvant Alhydrogel® and monophosphoryl lipid-A (MPL-A®) high levels in vivo [50]. Therefore, this system can potentially improve the influenza vaccine. A vaccine targeting *P. aeruginosa* PA14 strain infection using BSA-NPs containing *P. aeruginosa* ATCC 27853 antigens was used in a murine model [51]. The subjects incorporated with this system showed a high clearance of bacteria in the lungs, indicating a promising window for *P. aeruginosa* vaccines using albumin-based NPs.

The efficacy of *Pseudomonas aeruginosa* antigens in BSA-NPs was assessed after administration in vivo upon microbial infection. Macrophagic RAW 264.7 and BHK-21 cells uptake the NPs with no elicit cytotoxicity [52]. Histology assessment on a tissue section of mice treated with BSA-NPs resulted in improved skin conditions by enhancing the thickness of the skin, eliciting follicular hypertrophy, vascular crowding, and significant collagenases as well significant cellular infiltration. Moreover, BSA-NPs encapsulating rNS1 (i.e., recombinant non-structural protein 1) from Dengue virus 1 in mice presented an elevated seroconversion rate compared to rNS1 without BSA-NP immunized subjects, suggesting a good achievement in incorporating this vaccine into BSA-NPs [52]. Transdermal immunization of the measles vaccine benefited through a novel ablative laser was formulated into BSA microparticles (MPs) [53], where this novel system showed profoundly higher proliferation of MHC I/II along with CD80 and CD40, thus implying a higher immunization response was achieved by this system compared to the control group.

Albumin has also been incorporated into vaccines for cancer. The anti-tumor effect on HPV-induced cervical cancer was evaluated using NPs coated with human serum albumin (HSA) loaded with a modified and positively charged specific epitope of HPV16 E7 MHC-I followed in significantly higher E7-specific IL-10, IFN-γ, and CTL responses [54]. A stimuli-sensitive vaccine delivery system aiming to produce more targeted therapy to a target site through photothermal, near-infrared irradiation (NIR) was accomplished by encapsulating hydrophilic tumor vaccine peptide into HAS-gold NPs [55]. Both studies in vitro and in vivo showed augmentation of the anti-tumor immune response and achievable tumor ablation. Lastly, the addition of albumin to IFNβ adjuvant expands its short half-life, and its co-administration with OVA or HPV E7 long peptides heightens the specific immunity of CD8 +T cells. A profound anti-tumor effect was observed in a TC-1 tumor model [56].

In short, utilizing “albumin trafficking” through FcRn-mediated to target epithelial cells has shown a promising tool for common transdermal administration of vaccines harnessing the complexity of epithelial immunity that acts as the first-line defense in innate immunity and is key to be linked with adaptive immunity, thus enhancing the therapeutic efficacy of albumin as a vaccine carrier.

2.4. Hyaluronic Acid (HA)

The natural mucopolysaccharide of hyaluronic acid (HA) is a long chain of sugar molecules that are abundant in the body such as in mucus or joint fluid. This polymer is characterized as an anionic comprising disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine that are connected by glycosidic bonds of β (1, 4) and β (1, 3). HA has specific CD44 receptor-mediated targeting, which is usually found on the cell-surface membrane-bound proteins of various cancer cells [57][58]. CD44 is vital in integrating cellular microenvironment cues with generating various gene expressions such as cell survival, which is crucial in vaccine cancer platforms. Cancer vaccine targeting CD44 biomarkers by using HA shows a therapeutic window for life-threatening cancer, where it serves as a critical player in self-restoration, tumor induction, metastasis, and chemoradioresistance [59]. Several studies on cancer vaccines loaded in HA were designed to target CD44.

Exploiting the ability of HA to reprogram tumor-associated macrophages (TAM) transfection of miR-125b as antigen into HA-poly(ethylenimine)-NPs to target CD44 able to drive TAM to lung tissues with increasing (>6-fold) M1 and M2 macrophages were observed in the administered mouse model in comparison to the untreated control group [60]. This indicates the capability of HA in reprogramming TAM for vaccine anticancer therapy. However, tumor vaccines have been associated with poor immunogenicity and high heterogeneity that lowers clinical efficacy. A study was conducted to overcome this drawback by using stimuli-responsive NIR light, which was a good approach. HA-functionalized polydopamine NPs in combination with Imiquimod as adjuvant and doxorubicin (DOX) were prepared into a thermal-sensitive hydrogel [61]. Taken together, this system showed potential in theranostic tumor vaccine through prolonged retention in the tumor site along with the maturation of DC, CTL, and memory T cells in lymph nodes and spleen.

In a separate study, tumor vaccine limitation was improved by loading implantable blood clots into liposome-protamine-HA NPs (LPH NPs) carrying LPH-vaccine and in LPH NPs containing siRNA (LPH-siRNA) aiming to synergistically recruit and activate DCs [62]. Remarkably, LPH-siRNA that specifically targets programmed death-ligand 1 (PD-L1), mucin-containing

molecules 3, and T-cell immunoglobulin act to reduce immunosuppressor effect on DCs that leads to increased T-cell priming that tailored with tumor therapy strategy.

Next, micelles comprising HA successfully carrying OVA antigen and CpG-DNA adjuvant in the nasal vaccine were able to induce MHC II in the bone marrow DCs of a mouse to produce IgG in the blood. Indicating an active immune cascade achieved by this system [63]. HA nanocapsules were made to carry OVA antigen in an ex vivo study [64]. The interaction of immune responses was presented through the activation of macrophage, and the ability of this nanocapsule to retain OVA represents an interesting approach in needle-free vaccination.

HA has also been studied as an adjuvant in vaccine formulation in two separate studies. HA-glycine cholesterol conjugate was developed as an excipient for OVA antigen, and the antigen-specific immune response was observed in a mouse model [65]. In addition, wide T-cell-mediated immunity was activated such as CTL activation, cytokines proliferation, and induction of IgG antibodies, showing the capability of HA-derived conjugate in vaccine formulation. Lastly, modified-HA tetraglycine-l-octaarginine to be used as a mucosal vaccine adjuvant for HINI vaccines on A/Puerto Rico/8/34 (PR8) strain represents a good move, especially in the current research toward the new emerging infectious disease COVID-19 [66]. Interestingly, this modified-HA adjuvant was able to produce cross-protective capabilities by inducing IgG and IgA with PR8, and less proliferation of PR8 resulted in no profound weight loss in mice observed in experimental groups compared to control groups.

In summary, HA-based vaccine delivery has extensively been studied in tumor vaccines owing to its capability to target the CD44 receptor that is abundant in the surface protein of tumor cells [47].

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