Review Article

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Applications of polymer-based nanoparticles in vaccine field

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Abstract: Polymer-based nanoparticles have good solubility, stability, safety, and sustained release, which increases the absorption of loaded drugs, protects the drugs from degradation, and prolongs their circulation time and targeted delivery. Generally, we believe that prevention and control of infectious diseases through inoculation is the most efficient measure. However, these vaccines including live attenuated vaccines, inactivated vaccines, protein subunit vaccines, recombinant subunit vaccines, synthetic peptide vaccines and DNA vaccines have several defects, such as immune tolerance, poor immunogenicity, low expression level and induction of respiration pathological changes. All kinds of biodegradable natural and synthetic polymers play major roles in the vaccine delivery system to control the release of antigens for an extended period of time. In addition, these polymers also serve as adjuvants to enhance the immunogenicity of vaccine. This review mainly introduces natural and synthetic polymer-based nanoparticles and their formulation and properties. Moreover, polymer-based nanoparticles as adjuvants and delivery carriers in the applications of vaccine are also discussed. This review provides the basis for further operation of nano vaccines by utilizing the polymer-based nanoparticles as vaccine adjuvants and delivery systems. Polymer-based nanoparticles have exhibited great potential in improving the immunogenicity of antigens and the development of nano vaccines in future.

Keywords: polymer-based nanoparticles; vaccine adjuvant and delivery system; nano vaccine

1 Introduction

Vaccines are biological preparations that can afford actively acquired immunity for a peculiar illness. Vaccines can prevent the effects of infection of many pathogens. There are several types of vaccines in development and in use, including attenuated vaccine, inactivated vaccine, toxoid vaccine, protein subunit vaccine, DNA vaccine, live attenuated vaccine, and vector vaccine [1–4]. However, these traditional vaccines are susceptible to degradation, have a short duration of action, and present serious side effects and inflammatory reactions at the injection site during vaccination. Furthermore, developing countries are lagging behind in vaccine research, making it extremely difficult and challenging for multi-dose conventional vaccines to activate the required immune response in these developing countries [5]. Some diseases caused by intracellular pathogens don't have the effective prevention or therapeutic vaccines. These diseases are required vaccine formulations that not only promote strong cellular or Th1-based immune responses, but also induce humoral or Th2 immune responses and mucosal immune responses [6]. Therefore, to enhance the vaccines immune efficacy and achieve sustained release and mucosal immunity, several strategies was carried out: utilizing an efficient adjuvant, implementing an efficient delivery system, and using appropriate effective antigen presentation targets [7–10].

Alum compounds adjuvants and Freund's adjuvants are traditionally used as vaccine carriers and adjuvants. However, paraffin oil present in Freund's adjuvant is non-

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degradable and causes toxicity problems, and the injection of alum compounds adjuvant produces a local inflammatory response [5, 11]. Compared to traditional adjuvants, polymer-based nanoparticles are non-toxic, safe, and biodegradable. Although there have been many experiments in which polymer nanoparticle adjuvants were validated using animal models, there is still a lack of clinical trials and lack of convincing treatment for human diseases. We should further research and continue to conduct clinical trials. In recent decades, innovations in the design and development of novel biomaterials for biomedical applications have shown that polymers have high physiochemical properties, biocompatibilities, and biodegradability. Their application as vaccine adjuvants and delivery carriers may be a useful alternative to conventional bacterial adjuvants and virus vectors [5]. This review mainly introduces natural and synthetic polymer-based nanoparticles, the formulation and properties of these nanoparticles, and the applications of polymer-based nanoparticles in the vaccine field.

2 Polymer-based nanoparticles

Polymer-based nanoparticles are valuable in the treatment and prevention infectious diseases, because their small particle size enables them to cross the biological barrier when administered parenterally. They are also able to improve cellular uptake [12]. Polymeric nanoparticles also provide a sustained release system and improve the stability of labile drugs from *in vivo* enzymatic degradation [13]. Presently, a variety of natural and synthetic biodegradable polymer-based nanoparticles can be used to enhance immunogenicity for vaccine delivery. In addition, vaccine antigens encapsulated in the polymer-based nanoparticles administered through the mucosal pathway can protect antigens from degradation and ensure that the encapsulated antigen is released at the action site to stimulate efficient immune responses [14]. Polymer-based nanoparticles have also exhibited great value and potential in improving the immunogenicity of vaccines and the development of nano vaccines. However, in order to achieve higher productivity and desired morphology, further research is needed on the clear mechanism of nanoparticle synthesis. For future therapeutic applications, it would be highly desirable to expand nanoparticle production [15].

2.1 Natural and synthetic polymer-based nanoparticles

Natural polymers such as chitosan, dextran, hyaluronic acid, β -cyclodextrin, alginate, and gelatin pullulan have been explored for preparation nanovaccines. Some natural and synthetic polymers used in vaccination can be seen in Table 1. Chitosan-based nanoparticles have been widely applied in vaccines due to their biodegradability, biocompatibility, non-toxicity, and easy modification in terms of shape and size [16–18]. Synthetic polymers mainly include chitosan derivatives, poly(lactic acid-glycolic acid) (PLGA), poly(ϵ -caprolactone) (PCL), dendrimers, poly(lactic acid) (PLA), poly(alkyl cyanoacrylates), polyanhydride, poly(ethylene glycol) (PEG), and so on. Some chitosan derivatives, such as *O*-2'-hydroxypropyl trimethyl ammonium chloride chitosan (*O*-2'-HACC), *N*-2-hydroxypropyl trimethyl ammonium chloride chitosan (*N*-2-HACC), and *N*-2-hydroxypropyl dimethylethyl ammonium chloride chitosan (*N*-2-HFCC), have been synthesized and applied in animal vaccines in our previous studies [19–21]. Due to the introduction of epoxypentyl dimethylethyl ammonium chloride (EEA) on its $-NH_2$ group, these chitosan derivatives have higher water solubility compared to chitosan. Because of their biocompatibility, low virulence, high biodegradability, and controlled and sustained release mode of PLGA and PLA, they are major synthetic polymers for mucosal delivery of antigens [22–24].

2.2 Formulation of polymer-based nanoparticles

Numerous techniques are available for the formulation of polymer-based nanoparticles. Commonly used techniques including solvent evaporation, emulsification-solvent diffusion, nanoprecipitation, etc [25]. The different preparation methods were discussed, and the advantages and disadvantages of formulation methods are shown in Table 2.

Solvent evaporation methods for the preparation of polymer-based nanoparticles can be seen in Figure 1. Polymer solution is prepared in a volatile solvent and an emulsion is formulated. The emulsion is converted to suspension as it evaporates the solvent of the polymer, allowing it to diffuse through continuous phase of the emulsion [25]. The single emulsion method is O/W that prepares the emulsion oil (O) in water (W) by adding water and a surfactant to the polymer solution [26]. The double emulsion method is also known as the W/O/W method [27]. The method utilizes high speed homogenization or sonication, and then evaporate the solvent by continuous magnetic stirring at

Table 1: The essential information of some natural and synthetic polymers used in vaccination as adjuvants and delivery system

Materials	Name	Advantages	Disadvantages	Polymers can loaded	Preparation method
Natural polymers	Chitosan	Biocompatibility, biodegradability, non-toxicity, easily modified, low allergenicity	Lower immunogenicity	Protein, DNA, RNA, peptides	Emulsification, nanoprecipitation, solvent evaporation, ionic gelation
	Pullulan	Biocompatibility, biodegradability, non-toxicity	Lower immunogenicity	Protein, DNA, RNA, peptides	Solvent evaporation, nanoprecipitation
	Alginate	Biodegradability	Lower immunogenicity, soluble in high pH, insoluble in low pH	Protein, peptides	Emulsification, ionic gelation
	Lentinan	Enhanced antigen presentation	Lower immunogenicity	Protein, peptides	Emulsification
	Inulin	Well tolerated by the body and cause minimal reactogenicity	No immunological activity insoluble form, not able to stimulate DC maturation <i>in vitro</i>	Protein	Emulsification, ionic gelation
Synthetic polymers	Mannan	Enhanced phagocytosis of antigen	Lower immunogenicity	Protein	Ionic gelation
	Albumin	Enhanced antigen presentation	Lower immunogenicity	Protein	Solvent evaporation, emulsification, ionic gelation
	Heparin	Biocompatible, biodegradable	Lower immunogenicity	Protein	Solvent evaporation
	Poly(lactic-co-glycolic acid) (PLGA)	Biocompatibility, biodegradability, low toxicity	Low drug loading	Protein, peptides, DNA	Solvent evaporation, emulsification
	Poly(ϵ -L-lactide-co-glycolide) (PLG)	Biocompatibility, biodegradability	Use of organic solvents, not very stable, and often not effective for mucosal administration	Protein, peptides	diffusion, nanoprecipitation, salting-out
	Poly(ethylene glycol) (PEG)	Flexibility	Exhibit poor drug loading	Protein, peptides	Solvent evaporation, nanoprecipitation
	Polyphosphazenes	Film-forming nature, biodegradable	Not very stable	Protein, peptides	Solvent evaporation, nanoprecipitation
	Polyelectrolytes	Self-assembled, water-soluble, non-toxic	Low drug loading	Protein	Emulsification diffusion
	Polyanhydrides	Biodegradable, non-toxic	Exhibit poor drug loading	Protein, peptides	Emulsification diffusion
	Polymethacrylates	Enhance immune responses	Exhibit poor drug loading	Protein, peptides, DNA	Emulsion polymerization
	Poly(ϵ -caprolactone) (PCL)	Biocompatibility, biodegradability, low toxicity	Lack of intrinsic immunogenicity	Protein, peptides	Nanoprecipitation
	Poly(D, L-lactide) (PLA)	Biocompatibility, biodegradability, low toxicity	Low drug loading	Protein, peptides, DNA	Solvent evaporation, nanoprecipitation, salting out
	Polyethylenimine (PEI)	Intrinsic immunostimulatory property	Low transfection efficiency of APCs <i>in vivo</i>	Protein, peptides, DNA	Emulsification diffusion

Table 2: Advantages and disadvantages of formulation of polymer-based nanoparticles

Formulation methods	Advantages	Disadvantages
Emulsification-solvent diffusion	High encapsulation efficiency, no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, narrow size distribution	High volumes of water to be eliminated from the suspension, leakage of water-soluble drug into the saturated-aqueous external phase during emulsification
Salting out	Minimizes stress to protein encapsulants	Exclusive application to lipophilic drugs, extensive nanoparticles washing steps
Dialysis	Simplicity and common	Not an efficient means to encapsulate water soluble drugs
Solvent evaporation	Simplicity, versatile	Mainly applied to liposoluble drugs, having possibility of coalescence of nanoparticles during evaporation
Nanoprecipitation	Simplicity, quickness and reproducibility	Need to find a suitable drug/polymer/solvent/non-solvent system, poor encapsulation efficacy of hydrophilic drugs
Supercritical fluid	Easy and reproducible scaleup, good control of structural homogeneity Production of high purity nanomedicines	Most polymers exhibit poor solubility or even non-solubility
Ionotropic gelation	Simple in practice	More complex to optimize

Figure 1: Schematic representation of the solvent-evaporation technique for the production of nanoparticles

room temperature or under reduced pressure [25]. After the solvent evaporates, the solidified nanoparticles can be washed and collected by centrifugation and lyophilized for long-term storage [28]. The method can be easily performed in the laboratory, but it is only suitable for liposoluble drugs, which takes time and requires too much energy during homogenization [29].

Emulsification-solvent diffusion method (seen in Figure 2) is a technique to transform the salting out process [27]. A conventional O/W emulsion is formed between a partially water-miscible solvent that contains a polymer, a drug and an aqueous solution containing a surfactant. The polymer solvent and water are saturated with each

Figure 2: Schematic representation of the emulsification-solvent diffusion method for the preparation of nanoparticles

other at room temperature and afterwards diluted with a large amount of water to induce the diffusion of the solvent from dispersed droplets into the external phase. This results in the formation of particles [30]. The advantages of this method include enhanced encapsulation efficiency, easy enlargement, narrow size distribution, and simplicity [27]. However, the disadvantage is that a large amount of water and water-soluble drugs are removed from the suspension during the emulsification process and leak into the outer phase of the saturated aqueous phase, thereby reducing the encapsulation efficiency [31].

Nanoprecipitation is also called solvent displacement method (seen in Figure 3) [25]. The organic phase is formed by dissolving the polymer and drug in a polar solvent. The solution is then added dropwise to an aqueous solution

Figure 3: Schematic representation of the nanoprecipitation method for the preparation of nanoparticles

containing emulsifier or surfactant and rapid diffusion of solvent occurs, resulting in the formation of nanoparticles [27, 32, 33].

The salting out method relies on the emulsification of the organic phase (polymer solution in acetone) into an aqueous phase by adding a high concentration of salts (electrolytes) (magnesium chloride, calcium chloride and magnesium acetate) or sucrose (non-electrolyte). However, in this technique it is necessary to wash the nanoparticles in large quantities [34]. The advantages and disadvantages of nanoprecipitation and salting out method are shown in Table 2 [28, 33].

Dialysis provides a simple and effective method for preparing small, narrowly distributed polymer-based nanoparticles. Dialysis is performed on a miscible non-solvent [35]. The basic prerequisites are the miscibility of the solvent and the presence of the diluted polymer solution. Displacement of the solvent within the membrane results in a gradual reduction of the mixture's ability to dissolve the polymer [28]. Based on the use of physical barriers, in particular dialysis membranes or common semi-permeable membranes, the passive transport of solvents is able to slow the mixing of polymer solutions with non-solvents. The dialysis membrane contains polymer solutions [25].

The characteristics of the supercritical fluid method are that it has very fast mass transfer, near zero surface tension, and effective solvent elimination. This permits innovative processing applications that can overcome the classic limitations of liquid-based processes [36]. The principles of the nanoparticles preparation, including rapid expansion of supercritical solution (RESS) and rapid expansion of supercritical solution into liquid solvent (RESOLV) in the supercritical fluid method, have been developed [25]. RESS has been used for the micronization of compounds that exhibit good solubility in supercritical CO_2 . It is particularly attractive because it does not contain organic

solvents. However, it is only suitable for substances that are soluble in supercritical CO_2 small molecules and compounds containing a few polar bonds, which are only a small quantity of those of biomedical interest [37]. To minimize coalescence and have better control over particle size distribution, significant improvements in RESS using liquid solvents or solutions at the receiving end of the supercritical solution expansion have been proposed, under the name RESOLV. When the receiving solution is water, nanoparticles produced in this manner can prevent aggregation by the presence of a polymer or another protective agent in the receiving solution [36, 38].

Ionotropic gelation involves the spontaneous aggregation of natural polymers with multivalent counterions, the most common being sodium tripolyphosphate (TPP). The process begins by dissolving the polymers in a dilute acidic solution which is further diluted with water. TPP was then added while constantly stirring, resulting in the formation of nanoparticles [39]. Upon mixing the two phases, Nanoparticles are formed immediately through intermolecular and intramolecular ligation between the TPP phosphate and the amino group of the chitosan [40].

2.3 Physiochemical properties of polymer-based nanoparticles

Chitosan is easy to combine with DNA to form polyelectrolyte complex, which makes DNA a highly resistant to nuclease degradation. The chitosan backbone is abundant in hydroxyl and amino groups, making it easy to chemically modify and increase its targeting [41, 42]. PLGA is the commonly used polymer in vaccines [26]. PLGA nanoparticles are internalized in cells by fluid phase pinocytosis and by clathrin-mediated endocytosis [43]. Low molecular weight polymers with higher glycolide content have shorter degradation times due to their hydrophilicity and amorphous nature [44]. PEG has been used to coat PLA nanoparticles, and it usually aggrandizes the solubility of nanoparticles, decreasing their aggregation and interaction with proteins in physiological fluids. This produces spatial stability on the surface of the gene carrier to enhance transfer efficiency [45]. Compared to the bulk materials and microparticles, nanoparticles exhibit different mechanical properties. Moreover, whether the nanoparticles will fall into the surface or become distorted under immense pressure is controlled by the comparison of the stiffness between nanoparticles and the contacting exterior surface [46], which may reveal the performance of nanoparticles. Understanding the mechanical characteristics of nanoparticles and their interactions with different

types of surface is essential to improve surface quality and material removal [47].

2.4 Proteins encapsulated into polymer-based nanoparticle

Protein administration alone represents a real challenge because the oral bioavailability is limited by the gastrointestinal tract epithelial barrier. The digestive enzymes are susceptible to gastrointestinal degradation as they have short half-life in the body—in fact, most of them can't diffuse across some biological barriers [48]. Encapsulation therapeutic proteins in polymer-based nanoparticles are prospective alternatives to break through these shortcomings. To prevent hydrolysis and enzymatic degradation in the body, proteins can be incorporated into a polymer matrix, which helps them maintain their activity and integrity. Sometimes it can target therapeutic proteins to target site and increase their bio-availability [49]. For drug delivery, disparate proteins and peptides are encapsulated in chitosan nanoparticles. Water-soluble chitosan-alginate carriers are made from spherical nanoparticles in order to overcome enzymatic and absorption barriers to orally administered BSA [50]. One method of encapsulating hesperidins is to utilize chitosan nanoparticles to stabilize oil-in-water (O/W) emulsion with lecithin as a low cost food grade surfactant [51]. Lately studied insulin loaded PLGA nanoparticles for keeping insulin integrality during preparation and delivery. Using these solvent evaporation techniques to prepare nanoparticles, a higher entrapment efficiency can be observed at the target load. The yields of nanoparticles prepared by different methods differ significantly [52]. Solving the problem of targeting and bioavailability of peptidic drugs such as desmopressin, the encapsulation into nanoparticles has become standard in pharmaceuticals [53].

2.5 Nucleic acids encapsulated into polymer-based nanoparticles

Due to their size and negative charge, nucleic acids are weak and have undesirable inherent transfection consequence. Nucleic acids can be embedded in a polymer matrix and electrostatic adsorption on the surface of nanoparticles by a suitable surfactant or the addition of a cationic polymer to the matrix [54, 55]. Chitosan can concentrate and encapsulate plasmid DNA to form nanoparticle complexes that can adhere to intestinal epithelial cells, transport through M-cells across mucosal bound-

aries, and transfect epithelial cells and/or immune cells in the gut-associated lymphoid tissue [56, 57]. Our group developed the plasmid DNA of NDV F gene encapsulated in the N-2-HACC/CMCS nanoparticles, and found that intranasally immunized chickens with the nanoparticles produced higher anti-NDV IgG and sIgA antibody than the chickens in other groups did. They also had significantly stimulated lymphocyte proliferation and higher levels of induced IL-2, IL-4 and IFN- γ levels, which demonstrated that the nanoparticles could serve as efficient DNA delivery systems and vaccine adjuvants for stimulating humoral, cellular, and mucosal immune [58].

3 Application of polymer-based nanoparticles

3.1 Natural polymer-based nanoparticles

3.1.1 Chitosan-based nanoparticles

Chitosan is especially suitable for nasal delivery [59]. Chitosan nanoparticles have mucoadhesive properties that prevent rapid nasal clearance and therefore increases the antigen retention time in the nasal mucosa. This may prolong the contact time between nose-associated lymphoid tissue and formulation due to a reduction in mucociliary clearance. Standard nasal residence time for dosing solution can be increased by a factor of four due to use of adherent adhesive chitosan particles [60]. Chitosan-based nanoparticles have been widely used in vaccine development—examples include vaccines against *Clostridium botulinum* type A neurotoxins, *Neospora*, hepatitis B virus, and Newcastle disease [16].

Chickens inoculated with inactivated avian influenza virus (AIV)-chitosan-nanoparticles showed the highest haemagglutination inhibition (HI) antibody titer weeks after the inoculation phagocytic activity, and phagocytic index in the AIV-chitosan nanoparticles group significantly increased [61]. To prevent bronchitis caused by respiratory syncytial virus (RSV), chitosan-DNA nanospheres administered intranasally induced the expression of corresponding protein, and improved the levels of anti-RSV-specific IgG, sIgA, cytotoxic T lymphocytes, and IFN- γ [62]. The chitosan-DNA nanoparticles were effective against diseases of the *T. pyogenes* infection and provided strategies for further development of novel vaccines encapsulated in chitosan nanoparticles [63].

3.1.2 Alginate

Sodium alginate nanoparticles are natural polymers made of sugar monomers that significantly increase the bioavailability of encapsulated drugs. Due to its anionic nature, alginate is known for its mucoadhesive properties, which allow them to alternatively stick on mucosal membranes. This is profitable for the development of drug delivery systems for using transmucosal. In addition, studies show that alginate-based substances are sensitive to pH and may be useful to “mechanically” control the releasing mechanisms that encapsulate biomolecules from alginate drug delivery vehicles [64]. Studies have demonstrated that antigen adsorption to chitosan particles, and coated with sodium alginate, that sodium alginate-coated chitosan nanoparticle carrier can inhibit rupture release of ovalbumin originally in artificial gastric acid (pH 1.2). In addition, the cytotoxicity test results of chitosan and sodium alginate polymer nanoparticles showed that cell viability was close to 100% [65].

3.1.3 Hyaluronic acid

Hyaluronic acid (HA), also known as hyaluronan, is a natural bioadhesive polymer. HA is a mucopolysaccharide made up of *D*-glucuronic acid and *N*-acetyl-*D*-glucosamine residues discovered in vertebrates, connective tissues, synovial fluid, and extracellular tissue matrix in the eye [66]. Vaccine delivery systems based on bioadhesive hyaluronic acid produce a substantial immune response against LKT63 influenza antigens following intranasal administration [67]. Combining trimethyl chitosan with HA to form a more stable and robust nanoparticle to contain antigen increased IgG antibody compared to both unconjugated nanoparticles and free antigen [68].

3.1.4 Pullulan

Pullulan is composed of a peculiar connection of α (1 $\rightarrow$ 4) glycosidic bonds and α (1 $\rightarrow$ 6) connected maltotriose residues and natural non-ionic water-soluble linear polysaccharide. Because it has special properties including non-toxicity, non-mutagenicity, non-immunogenicity, and non-carcinogenicity, pullulan-based nanoparticles have significant research value in creating excellent vaccine transmit systems [69]. More recently, a pullulan nanogel-based PspA nasal administration delivery system based on cationic cholesteryl groups was exploited. The experiment was aimed at *Streptococcus pneumoniae*

Xen10 bacteria and tested for efficacy in a mouse pneumococcal airway infection model. The results showed that nasal and bronchial secretions have higher PspA-specific serum IgG and IgA antibodies [70].

3.1.5 Inulin

Inulin is a complex polymeric carbohydrate (polysaccharide) that is natural and hydrophilic. It is an inexpensive dietary fiber compound that is a linear natural plant-derived polysaccharide and is linked by a fructose unit chain and linked by glycosidic bonds [71, 72]. Recently, inulin has been characterized as a more potent adjuvant as it enhances humoral and cellular immune responses when administered with antigens [73]. Studies have shown that high molecular weight inulin nanoparticles can be used as novel drug delivery systems or other molecules of interest [74]. Zhang *et al.* prepared methylprednisolone loaded ibuprofen modified inulin based nanoparticles have no significant cytotoxic effects under all conditions of use and have great potential for the treatment of synergistic effects of spinal cord injury [75].

3.1.6 Dextran

Dextran is a highly biocompatible and biodegradable neutral bacterial exopolysaccharide with a simple repeating glucose subunit. Its simple and unique biopolymer properties make it ideal for nanomedicine, nanomedicine carriers, and cell imaging systems or nanobiosensors. Most importantly, it is very soluble in water and does not show cytotoxicity after drug delivery [76]. Prepare various dextran coated nanoparticles and optimize for drug delivery and targeting [50]. Studies have shown that the high negative zeta potential of Zidovudine-stearic acid/Dextran nanoparticles (AZT-SA/Dex Nps) exhibits higher stability, better zidovudine controlled release, and more efficient cellular uptake [77]. Dextran and albumin-derived iron oxide (Fe_3O_4) nanoparticles have improved stability, biocompatibility, and blood circulation time [50].

3.2 Synthetic polymer-based nanoparticles

3.2.1 Polypeptides

Poly-L-lysine (PLL), poly(γ -glutamic acids) (γ -PGA), and polyaspartamide P[Asp(DET)] are all polypeptides. Concentrated of plasmids encoding chicken ovalbumin (OVA)

with PLL peridiumed polystyrene nanoparticles. The acquired compounds can stimulate higher levels of OVA-specific antibodies and CD8⁺ T cells in mice and significantly inhibit tumor increase following attack with the OVA expressing EG7 tumor cells [78]. The carrier system based on a mixture of a homopolymer of a DNA polymer with a cationic polyaspartamide P[Asp(DET)] and block copolymer PEG-b-P [Asp(DET)] are presented [79]. Optimization of homopolymers and block copolymers for DNA complexes, against non-specific eliminate stability and cells transfection efficient. The complexes are concluding DNA encoding tumor antigen SART3, CD40 and GM-CSF, are immunity intraperitoneally and by inhibiting tumor growth and metastasis to extend the life span of animals carrying CT26 colon cancer [80].

3.2.2 PLGA

PLGA-PEG nanoparticles has been prepared to encapsulate amphotericin B to improve its solubility and to target the macrophages of tissues infected with visceral leishmaniasis—the nanoparticles exhibited higher efficacy compared to free amphotericin B [81]. Pigs vaccinated with the PLGA-nanoparticles peptide did not contract fever and influenza symptoms, and swine influenza viruses (SwIV) were not detected in bronchoalveolar lavage [82]. Tetanus toxoid (TT) has been extensively studied for the controlled release from TT-PLGA microspheres [83]. PLGA/PLA-based nanoparticles are capable of delivering encapsulated antigens to antigen presenting cells in controlled and sustained means [84, 85]. Studies have shown that PEG strengthens the PLA nanoparticles in gastrointestinal (GI) fluid stability and promotes the transmit of nanoparticles to the lymphatic system through the GI mucosa [86]. Mice immunized by PLGA nanoparticles encapsulating the bivalent H1 antigen showed significant effects against tuberculosis, as the immunization provides long-term protection [87]. The limitations of PLGA nanoparticles can be overcome through extensive and comprehensive assessments of pharmacokinetics, biodistribution, and toxicity [27].

3.2.3 PCL

PCL is a semi-crystalline, biodegradable, hydrophobic, biocompatible, and inexpensive polymer. Due to *in vitro* stability, hydrophobicity, and slow degradation pattern, PCL-based nanoparticles have the potential to be candidates for mucosal antigen delivery and DNA delivery

in the future [88]. Mucoadhesive chitosan-PCL nanoparticles also have potential to combat H1N1 as a carrier for nasal vaccinations. Compared to other antigenic preparations, chitosan-coated PCL nanoparticles cause considerable cell and humoral immune responses [89]. Different amounts of antigen were adsorbed into the nanoparticles in fixed amounts, and these different doses induced the same humoral and mucosal antibodies in the nasal secretion of mice [90]. Methoxy polyethylene glycol-polycaprolactone is an ecotropic diblock copolymer for the delivery of hydrophobic drugs [91]. The quercetin-biapigenin mixture is encapsulated in PCL-loaded nanoparticles and has synergistic antioxidant and hepatoprotective effects [92].

3.2.4 Polyanhydride

Polyanhydride is a polymer formed by the polymerization of carboxylic acids, and its monomers are formed by anhydride linkage. Polyanhydride has good water instability, biodegradability, and safety [93, 94]. Various polyanhydrides have been reported containing 1,6-bis (p-carboxyphenoxy) hexane (CPH), sebacic anhydride, and 1,8-bis (p-carboxyphenoxy)-3,6-dioxaoctane (CPTEG), peculiar combinations of CPH and CPTEG copolymer microparticles have been serve as antigens. The particles are validly phagocytosed through antigen-presenting cells and located in phagosomes. The amphiphilic polyanhydride nanoparticles can excite antigen-presenting cells that mimic pathogens [95].

3.2.5 Dendrimers

A dendrimers is a type of highly branched, symmetrical, and radial macromolecule similar to a dendrite. Dendrimers have many features, including a large number of branch points, three-dimensional and spherical with monodisperse, and fixed nanometer size range. The characteristic three dimensional structures of dendrimers enable them to pass through cell membranes; therefore, they are better nano delivery materials than the classical polymers [96]. Due to these unique properties, cationic dendrimers have been widely used for drug and gene delivery [97–99]. However, to date, there is no report regarding whether dendrimers can deliver a DNA vaccine in the field of veterinary vaccines.

3.2.6 Poly(lactic acid)

PLA has been widely used in biomedicine, and it is considered to be a good material for preparing micron and nanoparticles because the particle size and shape can be controlled to meet the preparation requirements [100]. Using PEG-PLA-PEG nanoparticles as a delivery carriers for oral immunization enhanced mucosal uptake results in an effective immune response that preserves the antigenicity of the encapsulated HBsAg [101]. Surfactant-free anionic PLA nanoparticles coated with HIV-1 p24 protein that induces Th1 responses were also confirmed in the macaque model; this protein delivery system demonstrates the potential of charged nanoparticles in the field of vaccine development [102].

3.3 Targeting of the immune system

Targeting nanoparticles to immune cells can enhance immune responses. CpG is a ligand of TLR9 expressed by immunizing cells. Using CpG to activate immune cells leads to an induction of various responses such as the generation of cytokines and chemokines that help to enhance the immune response [103]. Studies have shown that TLR ligands targeted to deliver to human and mouse DCs immensely increases adjuvantation. According to these studies, intracellular TLRs (TLR3 and TLR7/8) ligands were encapsulated in PLGA nanoparticles and peridium with anti-DC-SIGN antibodies that recognize DC-specific receptors [104]. Hunsawong *et al.* found that a dengue virus serotype-2 nanovaccine induced robust humoral and cell-mediated immunity in vaccinated mice [105]. In addition, mice immunized with amino-functionalized polymeric nanoparticles that accept human papillomavirus antigens exhibited an improved immune response compared to mice that received naive nanoparticles [106]. Nanoparticles are also used to target HIV-infected cells, and it has showed that the superparamagnetic iron oxide nanoparticles have significant potential to improve cytotoxic T lymphocyte (CTL) function and served as an adjunctive therapy in both the imaging and eradication of latently HIV-infected cells [107].

4 Future perspective

Polymer-based nanoparticles have great potential as adjuvants for delivery systems and vaccines [108]. In recent years, the main goal of developing polymer-based

nanoparticles is to improve the efficacy of treatment while minimizing the development of drug resistance [109]. However, there will be many challenges that come with the manipulation of size, shape, and other properties [110]. The local toxicity of polymer-based particle vaccines can be a detrimental problem that hinders their clinical application. Furthermore, the difficulty of synthesizing non-aggregated nanoparticles with consistent and ideal properties and considering how these properties affect their interactions with the biological system at all levels from cell through tissue and to whole body are still obstacles that we must work to overcome in the future. Ideally, the adjuvants or delivery vehicles we prepare will have the ability to stimulate humoral, cellular, and mucosal immune responses simultaneously or discretely. In the future, we can incorporate new attractive vaccine systems by incorporating some other attractive features such as sustained release, target and alternative delivery methods, and delivery routes [111, 112].

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