

Nanoparticles in Vaccine Development

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Abstract

Vaccination has greatly improved human health. Despite of all improvements in this field, there is not an efficient vaccine for many diseases, and of the available ones, some could not produce a long-term immunity. Recently, there have been many researches on the applicability of nanostructures as an efficient system for vaccine delivery, and the initial results have been promising. Their potential adjuvanticity, capability of the stimulation of both humoral and cellular immunity responses, more stability in environmental conditions, possible targeted vaccine delivery, the need for low quantity of proteins (in the case of subunit vaccines), etc., are of the main reasons that this area has gained many interests. Here, we try to review the main nanostructures that could be act as a delivery vehicle in vaccine delivery.

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Introduction

Since the invention and introduction of vaccines by Edward Jenner in 1796 [1], vaccination has had a significant role in societies public health. Some diseases that were nightmares for human one day and used to cause deadly outbreaks, were controlled, and in some cases, were eradicated, thoroughly, by vaccines. Nothing, but drinking healthy water, has had this astonishing influence on human health and population growth, even antibiotics [2]. Despite the great mortality reduction and health improvements, and, also, despite all the researches done in this field, vaccine development has not had an acceptable growth. For some deadly diseases, such as tuberculosis, AIDS, malaria, etc. there are not an efficient vaccine. The first generation vaccines (i.e. live attenuated vaccines) are still the most efficient ones, but unfortunately they are not safe enough. On the other hand, the newer generations of vaccines are safer, but they are not able to produce a long life immunity and there is a need for repeated administrations. Indeed, these types of vaccines need adjuvants to induce immune responses [3]. All in all, there is a real need for the next generation of vaccines that are both efficient and safe.

The emergence of nanotechnology and its development has had a great influence on all aspects of human life. Clearly, it has caused a great improvement in all fields of science,

including medicine [4, 5]. Drug delivery [6, 7], controlled gene delivery [8, 9], pathogen diagnosis and identification [10, 11], DNA extraction and purification [12, 13], tissue engineering [14, 15], bio- and molecular imaging [16, 17], are some examples of the applications of nanotechnology in life and medical sciences. Exploiting nanotechnology in vaccine development is a new area and the initial results have been so promising.

It should be added that some nanoparticles, such as virosomes, virus-like particles (VLPs), and MF59 have been used for years, while the use of the other nanoparticles are in early stages. In this manuscript, we try to introduce the major types of nanoparticles, the main nanostructures that have been used in vaccine delivery.

Nanostructures and vaccines

According to the dimensions of the structural elements, nanostructure materials are categorized in three main types: nanofibers (that are one-dimensional), nanotubes (which are two-dimensional), and nanoparticles (with three-dimensional structures) [18].

Generally, nanostructures play two main roles in vaccine formulations: firstly, they are used as a vehicle for the delivery of vaccines, and, secondly, due to their intrinsic adjuvanticity, they could improve the immune responses [19, 20]. Of nanostructure materials, nanoparticles are the mostly used ones in vaccine studies. Here we discuss some



of the main nanoparticles used in vaccine delivery, including polymeric nanoparticles, inorganic nanoparticles, liposome, virus-like particles, and virosomes.

Nanoparticles

Nanoparticles (NPs) have been used more than any other types of nanomaterials in vaccine formulations. The nanoparticles used for vaccine delivery usually have three different parts: the material (s) that the nanoparticle is composed of, including, natural polymers, synthetic polymers, inorganic substances, lipids, etc.; immunogen or immunomodulatory agents such as antigens, DNA vaccines, siRNA, cytokines, etc.; and, finally, targeting and immunostimulatory ligands that are added to the particle surface, like immune specific ligands, tissue specific ligands, pathogen associated molecular patterns (PAMPs), etc. The NP material composition has important roles in transport, cellular uptake, and intracellular trafficking of the NPs and, also, its biodegradability and biocompatibility.

Furthermore, it has a role in pharmacokinetic properties of NPs, the release rate, biodistribution, and bioavailability of the immunogen. The immunogen, which is the main part of a nanovaccine, could be attached to the NPs in three different ways: conjugation (covalent binding), adsorption (on the surface of the NPs), and encapsulation (within the NPs). The incorporation of PAMP ligands to the vaccine formulations can elicit inflammatory responses, by stimulating pathogen recognition receptors (PRRs).

These receptors are expressed, mainly, on immune cells including macrophages, dendritic cells, and B cells. Toll like receptors (TLRs) are a main group of PRRs. TLR ligands, such as CpG DNA, lipopolysaccharide (LPS), monophosphoryl lipid A, and muramylpeptides are strong adjuvants that are applied in a variety of vaccine formulations [21, 22].

Nanoparticle characteristics, such as size, surface hydrophobicity, charge, surface modification, and addition of ligands, have a great impact on vaccine efficacy. The size of the nanoparticles determines the cellular uptake mechanism (endocytosis, phagocytosis, macropinocytosis, clathrin dependent and/or caveolae mediated). Endocytosis (via clathrin-coated vesicles, caveolae or their independent receptors) is used for the uptake of the particles of 20-200 nm, and mainly ingested by dendritic cells. Larger particles (0.5–5 μm) are, usually, taken up by macropinocytosis, and particles greater than 0.5 μm are taken up by phagocytosis, and mainly ingested by macrophages [23].

Size is a critical parameter that influences the immunogenicity, smaller particles (<500 nm), in particular 40-50 nm, could promote CD8 $^{+}$ and CD4 $^{+}$ Type 1 T cell responses and the larger particles (>500nm) are able to induce appropriate CD4 $^{+}$ Type 2 T cell and antibody responses [24]. The surface charge of the nanoparticles has a great influence on the phagocytosis by APCs, and therefore, on immune responses. Macrophages and

dendritic cells have negatively charged surfaces, and due to this, they could effectively uptake the cationic particles [25, 26].

It is observed that DNA adsorbed onto cationic polystyrene nanoparticles enhances both humoral and cellularimmune responses, compared to naked DNA [27]. Increasing positive charges on particles surfaces may induce maturation of dendritic cells [28, 29].

It seems that the surface modification of nanoparticles with chemicals could enhance their cellular uptake and trafficking [30]. Nanoparticles for vaccine delivery can be fabricated from a variety of substances, including natural and synthetic polymers, lipids, virus components, and inorganic materials.

Polymeric Nanoparticles

Polymeric NPs have some interesting properties, such as their high loading capacity, their stability, easy surface modification, and their safety [31]. Both natural and synthetic polymers have been exploited for the fabrication of nanoparticles for vaccine delivery, including proteins, polysaccharides, amino acids, lipids, poly (lactic acid), poly (lactic-co-glycolic acid), poly (ethyleneimines), and polyesters. Generally, by the selection of a polymer and its copolymer, and also changing the concentration ratio of these compounds, it is possible to fabricate an appropriate nanoparticles for vaccine delivery.

Morphologically, there are three different types of nanoparticles. The first type is nanosphere, in which the vaccine agent (protein, peptide, DNA, siRNA,...) is dispersed throughout the matrix, adsorbed on the surface, or is covalently attached to the surface of the nanoparticle. The second type is nanocapsule, in which the vaccine agent is located within the nanoparticle. Finally, the third type is nanomicelle, in which amphiphilic co-polymers assembles spontaneously to entrap the vaccine component [32]. As mentioned before, there are a variety of polymers that can be used for the fabrication of the polymeric nanoparticles and could be categorized into two main groups: natural and synthetic polymers.

Natural Polymeric Nanoparticles

Many polymeric nanoparticles have been used for vaccine delivery, including gelatin [33], alginate [34, 35], hyaluronic acid [36], and chitosan [37, 38], which the latter is the most used polymer in the field of vaccine formulations. Appropriate properties, such as high stability in gastrointestinal tract [39], the possibility of surface modification [40], easy fabrication, biocompatibility, biodegradability, lack of immunogenicity and toxicity, and finally, solubility in water, have made these nanoparticles as a good choice for vaccine formulations. Gelatin has been used for years as a preservative substance in vaccine formulations. However, for the first time, in 2006 Coester and colleagues, investigated the possibility of the gelatin NPs for vaccine delivery and showed that the antigens was efficiently taken up by DCs [33]. There have been some researches on the use of gelatin NPs for vaccine delivery, but some challenges, such as the need for purification after

fabrication, low safety, and low encapsulation efficiency [41] has restricted their use as a vaccine delivery vehicle. Alginate-based nanoparticles are another group of NPs that have attracted the attention of researchers for vaccine delivery. Alginate, which is extracted from cell walls of brown algae, is an anionic polysaccharide. The excellent properties of alginate NPs, such as naturality, biocompatibility, biodegradability, high loading capacity for proteins, lack of toxicity, mucoadhesion capability, and adjuvanticity, made it a suitable choice for vaccine delivery. Indeed, in contrast to gelatin NPs, it has some good characteristics, such as the ease of fabrication, easy to scale up and the lack of need for further purification after fabrication [41].

For the first time, in 1950, Slavin propounded the alginate as a natural adjuvant [42]. Although, for many years later it was exploited as an adjuvant in vaccine formulations [43-45], antigen encapsulation by alginate microparticles for active immunization was performed in the late twentieth century [46, 47].

The use of alginate nanoparticles has become a new growing field and, specially, alginate NPs are used widely in combination with the chitosan NPs for vaccine delivery. Chitosan, as a natural polymer, consists of D-glucosamine and N-acetyl-D-glucosamine. Commercially, it can be derived through the alkaline deacetylation of chitin, the major compound of crustaceans' exoskeletons. Because of some excellent properties, such as anti-inflammatory, antimicrobial, antiviral, and antitumor activity, and, also, wound healing property, chitosan has gained many applications in medical and pharmaceutical fields [48]. Bodmeier and colleagues in 1989, for the first time, exploited chitosan as a carrier for enteric drug delivery [49].

In 1997 Calvo et al. showed the efficiency of chitosan nanoparticles in vaccine delivery [50]. Since then, the use of chitosan nanoparticles in the delivery of vaccines, especially through the mucosal routes, has been extensively investigated. Physically, there are four different formulations for chitosan: solution [51-53], powder [54, 55], gel [56], and particulate [50, 57, 58]. All four formulations have their own advantages, however, the latter is more efficient in induction of immune responses and, for this reason, is widely investigated for vaccine delivery. Because of mucoadhesion property of chitosan nanoparticles, mucosal routes, including oral, nasal, and pulmonary, are preferred for nanoparticle administration. However, there have been many studies in which other routes, such as transdermal [59], intradermal [60], and parenteral [61] routes have been used and the results have been promising. Among these entry routes, the nasal route seems to be the most appropriate, particularly because of the absence of harsh conditions, safety, and the ease of administration. High bioavailability, biocompatibility, biodegradability, mucoadhesion capability, easy and inexpensive fabrication, easy to scale up, and the lack of need for purification after fabrication, are unique

properties that make the chitosan nanoparticles as an excellent system for vaccine delivery. However, chitosan nanoparticles are so sensitive to acidic conditions [49], so in oral delivery route they are readily degraded and will lose their efficiency. To overcome this limitation, chitosan nanoparticles, which already loaded with the desired vaccine, are coated by alginate. Alginate is stable in acidic conditions [49] and does not allow the release of chitosan nanoparticles, but it is sensitive to the pH of the small intestine and will be degraded there, and the chitosan NPs and their cargoes will be released. These is a common strategy that is used frequently for oral vaccine delivery by chitosan nanoparticles [62].

Synthetic Polymeric Nanoparticles

Biodegradable synthetic polymeric nanoparticles have a great potential in drug and vaccine delivery applications. Poly (lactic acid) (PLA), Poly (lactic-co-glycolic acid) (PLGA), poly (glycolic acid) (PGA), Poly (isobutyl cyanoacrylate) (PIBCA), poly (-caprolactone) (PCL), and poly (ethylene imine) (PEI) are the main synthetic polymers used in biomedical applications. Among them, PLA and PLGA, has gained the most attention. At first, PLA was used widely for drug delivery, however, because of some limitations, such as crystalline nature, poor flexibility and slow biodegradation rate of the molecule, its usage as delivery vehicle was limited [63], instead, the use of PLGA increased greatly. PLGA is a biocompatible and, mechanically, strong polymer that has approved by FDA. The first reports on the use of PLGA for vaccine delivery comes back to the late 20th century. Kim et al. used of PLGA NPs for oral vaccination of mice against *Helicobacter pylori* in 1999 and found that the mucosal and systemic responses against this agent was properly induced [64]. In 2001, Conway and coworkers investigated the protective effect of antigens entrapped in PLGA nanoparticles, on immunization against *Bordetella pertussis* infection following parenteral or oral immunization. The results showed that immunization with two parenteral doses of 1 μ g or three oral doses of 100 μ g of pertussis toxoid (PTd) and filamentous haemagglutinin (FHA) encapsulated in PLGA conferred a high level of protection against B. pertussis challenge [65]. Since then, in many studies, the different characteristics of the encapsulation conditions, the amounts of loaded protein, the route of administration, and other parameters have been investigated. Because of significant properties, such as biocompatibility, biodegradability, sustained and tunable release of the cargo, the capability of the co-encapsulation of antigens and immunopotentiators, good mechanical strength, and long clinical experience, the use of PLGA for vaccine delivery is so promising and, hopefully, we will have commercially available PLGA-based vaccines in near future.

Although, PLGA NPs have excellent properties, there are, still, some limitations for the use of these NPs. For example, because of the hydrophobicity and acidity of PLGA, the cargoes (DNA, proteins, peptides, etc.) are

unstable when coupled with PLGA NPs and, also, during the NPs degradation invivo [66].

The fast burst release of the cargo from the PLGA matrices is another problem that reduces the efficiency of the NPs as a vehicle for vaccine delivery. There have been many attempts for overcoming these problems: by controlling the parameters, such as molecular weight of the polymer, ratio of lactide to glycolide and drug concentration, it is possible to tune the overall physical properties of the polymer-cargomatrix [67, 68].

Inorganic Nanoparticles

Inorganic nanoparticles, as an alternative to organic ones, could be used for the delivery of vaccines.

Gold, silica, carbon, calcium phosphate, and magnetic nanoparticles are the main inorganic nanoparticles used in drug and vaccine delivery. In 2001, Hillyer and Albercht investigated the gold micro- and nanoparticles' uptake by gastrointestinal cells and showed that the uptake of these particles is done by small intestine enterocytes [69]. Gold nanoparticles have been used for the delivery of DNA vaccines [70], protein [71, 72], peptide [73] and conjugate vaccines [74].

All of these studies have shown that both arms of adaptive immune system (humoral and cellular responses) are efficiently induced. Gold NPs, due to biocompatibility, biodegradability, lack of immunogenicity [75], and the ease of fabrication and surface modification [76], represent an appropriate inorganic nanocarrier for vaccine delivery [72, 73, 77].

Silica nanoparticles are other inorganic nanoparticles that have been used for vaccine delivery. Mercuri and colleagues in 2006 showed that silica particles are a potent adjuvant and stimulate the humoral responses effectively [78]. In 2009, this team investigated the immunological parameters of the adjuvanticity effect of these particles and showed that the particles were efficiently taken up by macrophages and an acceptable immune response was induced [79]. Guo and coworkers in 2012, used silica nanoparticles for the immunization of the mice against porcine circovirus type II and showed that both arms of adaptive immunity were activated properly and an effective immunity were established [80]. Mody and coworkers in 2013 showed that albumin-loaded silica nanoparticles were able to induce both humoral and cellular immunity [81]. Silica nanoparticles, due to properties like biodegradability, biocompatibility, possibility of surface modification, possibility of fabrication in any desired size, and, of course, due to promising results, seem to be potent nanocarriers for vaccine delivery. Magnetic nanoparticles are another group of the inorganic nanoparticles that have been used for vaccine delivery [82-84].

These nanoparticles are biocompatible and FDA-approved [85]. For *in vivo* applications, paramagnetic nanoparticles are needed, and also for the inhibition of aggregation and oxidation, they should be coated by other substances, such as silica, heparin, chitosan, or protamine. Carbon [86, 87]

and calcium phosphate [88, 89] nanoparticles, which are biocompatible and nontoxic, have been used for vaccine delivery.

Liposomes

A liposome is an artificially-prepared spherical vesicle composed of a lamellar phase lipid bilayer. Liposomes were discovered by Bangham et al. in 1965. Allison and Gregoriadis in 1974 used of liposome for the delivery of vaccines [90, 91] and nowadays, there are some liposome-based adjuvants in the market. Among the liposome properties that exert dramatic effects on the resulting immune responses, the size, surface charge, bilayer fluidity, lipid composition, and method of antigen attachment could be mentioned. These properties have been reviewed in a paper by Watson and coworkers [29], where, the authors have presented a comprehensive review of the physicochemical properties of liposomal vaccines and the way they could influence immune responses.

Liposomal vaccines have several advantages including: safety [92, 93], biodegradability [94], the possibility of the incorporation of different antigens (i.e. proteins, peptides, carbohydrates, nucleic acids, and haptens) enhanced bioavailability, the possibility of the customization to target specific tissues (by the incorporation of ligands, such as TLR ligands) [95], the possibility of the preparation in different sizes and charges [96]. Despite their obvious advantages, development of the liposome formulations for vaccine delivery has some problems. Stability during storage, scaling up, and sterilization, are of the liposomal formulation problems that should be addressed [97]. There are several liposome formulations for vaccine delivery that have been commercialized or are in various stages of clinical trials. Epaxal [98], that is a virosomal vaccine against hepatitis A, and Infexal V [99], also a virosomal vaccine against influenza, are commercialized nanovaccines that have been used for many years in different countries all over the world. There are, also, several liposome formulations as therapeutic vaccines against diseases like, malaria, hepatitis A, influenza, tuberculosis, colorectal cancer, and prostate cancer that are at different clinical stages [100].

Virus-like Particles

Virus-like particles (VLPs) are excellent recombinant vaccine antigens which are made of viral capsid proteins. These proteins are self-assembled into particulate structures that resemble the viruses from which they are derived, but they do not have the genetic material of the original viruses [101]. Capsid proteins of a broad spectrum of viruses, such as papillomaviruses [102, 103], parvoviruses [104, 105], polyomaviruses [106, 107] and RNA viruses [108, 109] are used for the construction of VLPs.

First report on naturally occurring VLPs was in 1965 by Blumberg et al. [110], however, it took more than a decade for experimentally generation of a VLP [111]. Nowadays, there are near twenty VLP-based vaccines that are licensed for human use and are commercially available, and, also,

there are near 30 others that are in various stages of clinical trials. For a comprehensive review on VLP-based vaccine see the paper by Kushmir *et al.* [112]. Since VLPs have the shape and size of the native viruses, they could present different pathogen associated molecular patterns (PAMPs) to the innate immune system and are taken up by antigen presenting cells (APCs), therefore, they are potent immunogens and could efficiently induce both innate and adaptive immune responses. Furthermore, because of their size and particulate shape, they have the ability to escape from endosomes and act as an endogenous antigen and be presented by MHC I complexes, so they could activate both arms of adaptive immune system. Due to the lack of viral genetic material, these nanoparticles are non-replicable and, therefore, so safe. Indeed, the production of VLPs is not a great deal and could be done through recombinant DNA technology in different expression systems, including *E. coli*, mammalian cells, insect cells, yeasts, plants, etc. [113, 114]. Despite of the all mentioned advantages, there are some obstacles related to these NPs such as the slow rate of biodegradability, potential of spanning the host cell membrane, potential toxicity for human body due to high surface area and reactivity, potential danger for young children, elderly, and immunocompromised people are the main obstacles [115]. Still, VLPs are among the best formulation for vaccine delivery.

Virosomes

Virosomes are spherical, unilamellar vesicles consisting of reconstituted viral envelopes, lacking viral genetic material [116]. Virosome particles are generated in vitro from a variety of enveloped viruses through a three step process: detergent solubilization of the virus; removing of genetic material and internal proteins; and, finally, reconstitution of hollow membrane vesicles [117]. It was in the 1970s that the virosomes were introduced and their potential as vaccines was proposed [118, 119].

Virosomes closely resemble the native virus' size and shape and because of their surface organization, they are closer to the virus structure than VLPs. These particles could efficiently interact with the immune system and activate both arms of adaptive immunity.

So far, four virosome-based vaccines are licensed for human use, and are commercially available. The first vaccine, that was on the basis of the influenza virosomes, was Epaxal® (Crucell), which was approved in 1994 for commercial use. In this vaccine, the virosomes were generated from influenza strain A/Singapore/6/86 (H1N1) and, after assembly of virosomes, they were coated with the inactivated hepatitis A virus. Inflexal® V (Crucell) was the second virosomal vaccine that launched to the market in 1997. This vaccine is a mixture of virosomes from three influenza strains A(H1N1), A(H3N2) and B/Yamanashi. The vaccine has showed an appropriate immune response in all age groups, including, elderly, adults and children, and even immunocompromised patients [120]. Nasalflu (Berna Biotech) was the third virosomal vaccine which

was launched in 2000. This vaccine was consisted of virosomes from three influenza strains A/Beijing/262/95-like, H1N1; A/Sydney/5/97-like, H3N2; and B/Beijing/184/93-like.

Indeed it contained the heat labile toxin from enterotoxigenic *E. coli* as an additional mucosal adjuvant. This vaccine was available on the market for just one year and because of some unwanted side effects [121], it was withdrawn in 2001. Invivac® was the fourth virosomal vaccine that was launched by Solway in 2004. This vaccine was consisted of Virosomes from three influenza strains: A(H1N1), A(H3N2) and B [122]. However, this vaccine was only commercially available during the season 2004/2005. There are also other virosomal-based vaccines against diseases, such as AIDS, hepatitis C, malaria, candida, breast cancer, etc. that are in clinical development [123].

Conclusions

Regarding to the importance role of the vaccines in the societies' health status, the improvement of the vaccines is inevitable. Although new generations of the vaccines are safer than the old ones, they are not so efficient in provoking of immune responses.

For overcoming this obstacle, there have been many researches on the capability of nanoparticles as a carrier for vaccine delivery in recent years. Nanoparticles could be exploited not only as a carrier for the delivery of vaccines, but also for their intrinsic adjuvant property. By this property, they could stimulate immune responses more efficiently.

In association with NPs, vaccine candidates could stimulate both humoral and cellular arms of the adaptive immunity. The capability of NPs in inducing cellular immunity, which arises from the ability of these particles from escaping from phago-lysosomes and entering the cytosol, is of a great importance. By this the antigen could be presented on the MHC I by cross presentation pathway and induce the cellular immunity. This is especially important when the goal is the delivery of subunit protein vaccines, which naturally go through the exogenous pathway and just could induce the humoral immunity.

The more stability of vaccines is another advantage of the NPs in the delivery of vaccines. This is so important, especially in warm out-of-the-way areas, where there must be cold chain for the transportation of the vaccines. The association of the vaccine components with the NPs not only could protect them from the environmental conditions, but also protect them from the harsh conditions of the body, like cleavage by proteases and nucleases or degradation in the gastrointestinal tract.

Hence, the resident time of the vaccine components in the body will increase. The slow release of the vaccine components is another advantage of the nanovaccines. By releasing slowly, the presentation time of the vaccine will be increased and this could reduce the administration times.

The possibility of targeting is a great feature of the delivery of vaccines by NPs. By the addition of appropriate ligands, it is possible to conduct the nanovaccines to desired cells and tissues. It is also possible to add immunomodulators, like CpG motifs, TLR ligands, etc. to the nanovaccines, for a better stimulation of the immune responses. As discussed in the text, many different NPs have been used in the delivery of vaccines. Scientists, in different laboratories, have exploited different NPs for this aim. Some NPs have gained more attentions and some have not. For example, chitosan NPs, for some intrinsic properties (such as high bioavailability, biocompatibility, biodegradability, mocoadhesion ability, etc.) and, also, simple preparation, have been widely used for this purpose, while the use of gelatin NPs as a vaccine carrier has restricted due to the difficulties in preparation. The interaction of the NPs and the immune system depends on the NPs' features, such as size, surface charge, and hydrophobicity and these features have effect on the NPs uptake by immune cells, their adjuvanticity, antigenicity, and inflammatory responses. There have been many researches on these parameters, but still there are many un-answered questions: aggregation problem of NPs in the body, the poor knowledge about the interaction of the NPs and the cells and the distribution of the NPs after entering the body, are main problems that must be answered. By addressing these problems we could have expect to have the next generation of the vaccines in the future: nanovaccines.

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