Nanoparticle-Based Vaccines: From Design and Immune Modulation to Clinical Translation

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Abstract

Vaccines are one of the most notable developments in modern medicine, and their role in combating and even eradicating diseases cannot be overlooked. Through an immunological cascade, vaccines produce an immunological memory response evident by memory cells and antibodies formation. Ever since the introduction of the first vaccine against smallpox, great efforts have been made to further improve vaccine efficacy, overcome limitations, and develop vaccines against emerging pathogens. The utilization of nanotechnology in vaccines has many benefits, as nanotechnology can address most of the limitations of traditional vaccines. Moreover, it has paved the path for the development of several vaccinations and is expected to continue contributing to future advancements. Nanoparticles have several types, with each type having its advantages and drawbacks. The physicochemical characteristics of these nanoparticles, namely particle size, particle shape, particle rigidity, surface charge, and hydrophobicity, affect the immune response potency. Optimization of these properties can allow the manufacture of vaccines that trigger strong immune responses. This strategic optimization permitted the production of effective vaccine formulations against several major pathogens in the market. Notable nanoparticle vaccines include the human papillomavirus, malaria, hepatitis B, and severe acute respiratory syndrome coronavirus 2 vaccines. This comprehensive review explores the vaccine immunological cascade, the platforms of nanoparticles, their advantages and drawbacks, and the impact of their physicochemical characteristics on vaccine efficacy. Moreover, this review highlights the nanovaccine formulations that have been approved for use.

Keywords: nanovaccines; lipid nanoparticles; virus-like particles; vaccine delivery; immune modulation.

1. Introduction

Vaccines are a powerful tool in medicine, contributing to a critical role in preventing infectious diseases [1]. They exert their protective and preventive action by training the immune system to resist the infectious microorganism subsequent to encounter through the formation of immunological memory. This immunological memory permits rapid antibody production and memory lymphocyte activation consequent to pathogens encounter [2]. The importance of vaccines cannot be overstated. Diseases like smallpox have been eradicated thanks to widespread vaccination efforts [3]. Polio, another disabling disease, is about to be completely eradicated due to global vaccination programs[4]. Vaccines also protect against a multitude of other serious illnesses, including whooping cough, tetanus, measles, mumps, diphtheria, rubella, and influenza [5]. By preventing these diseases, vaccines not only safeguard individuals from potentially life-threatening illnesses but also reduce healthcare costs and ensure a healthier, more productive population [6].

Vaccines contain substances called antigens and adjuvants. An antigen is a substance that triggers the immune response, while an adjuvant is an added substance that potentiates this immune response [7]. Vaccines are available in different types, including live attenuated vaccines, which use a non-virulent weakened form of the live virus, and inactivated vaccines, which contain a non-replicating form of the virus or bacteria [8]. The live attenuated and inactivated vaccines have the disadvantages of switching to virulence and low efficacy, respectively. Toxoid vaccines, formulated from bacterial toxins, have the drawbacks of low immunogenicity and multiple dose requirements. Protein subunit vaccines manage to be less immunogenic when compared to live attenuated vaccines.

Recombinant vector vaccines are created by incorporating the antigen sequence into an attenuated virus or bacterium and have the drawback of low efficacy due to mutation possibility. Nucleic acid vaccines are the most recently developed vaccine type that offers many advantages, such as rapid production and good stability [9, 10]. **Figure 1** illustrates the different types of vaccines.

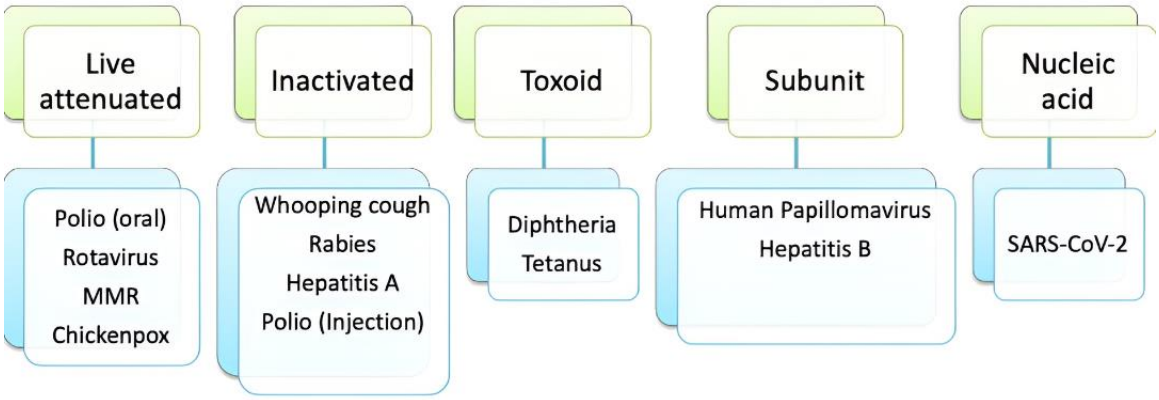

Figure 1. Types of vaccines and their examples

Nanotechnology has revolutionized the field of vaccine development. In essence, nanotechnology vaccines utilize particles engineered at the nanoscale to deliver antigens, the parts of a virus or bacteria that trigger the immune response, into the body. These nanoparticles (NPs) can be crafted from several different materials, including lipids or polymers [11]. There are several types of nanotechnology vaccines being explored. Some encapsulate the antigen itself, preventing its degradation and enhancing its delivery to immune cells. Others can be engineered to target certain immune cells to produce a more potent response. Additionally, researchers are developing nanotech vaccines that can combine antigens from multiple pathogens, potentially offering broader protection in a single shot [12].

The potential advantages of nanotechnology vaccines are significant. Compared to traditional vaccines, they may offer improved stability, allowing for easier storage and transport, particularly in regions with limited resources. Additionally, nanocarriers can enhance the immune response, potentially leading to longer-lasting protection with fewer doses. Furthermore, the targeted delivery of antigens with nanocarriers could minimize side effects by reducing exposure to unnecessary components (**Figure 2)** [13, 14].

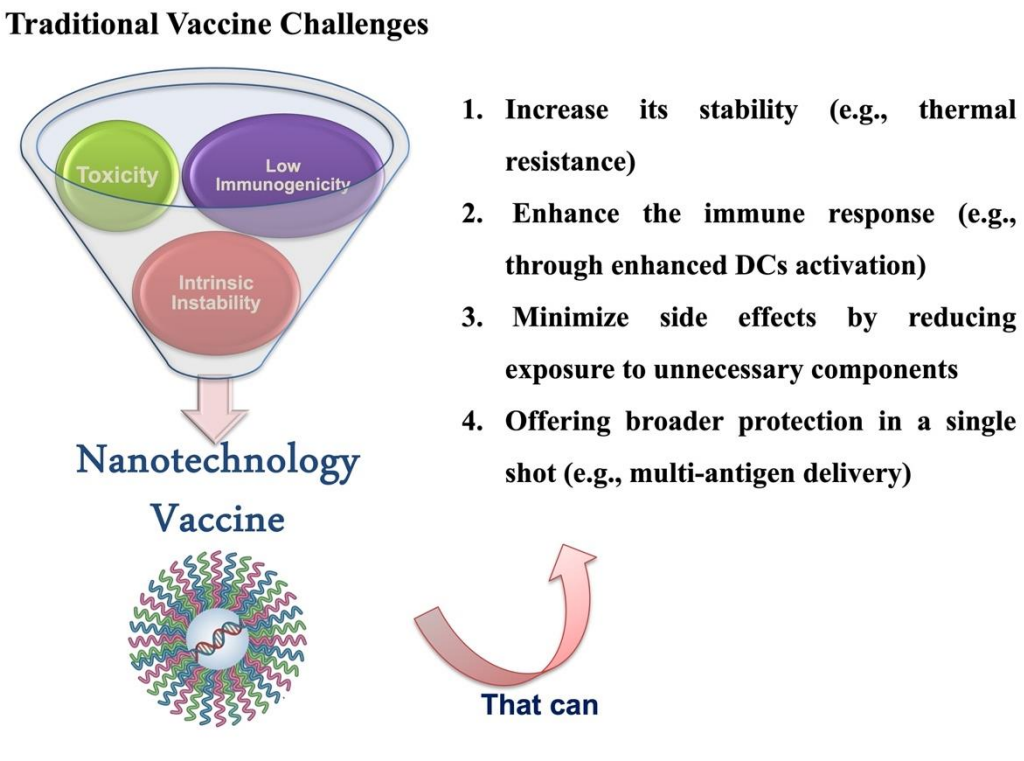

Figure 2. The disadvantages of the currently available vaccines that can potentially be overcome through nanotechnology

In this review**,** the vaccine immunological cascade is thoroughly discussed, along with the various nanoparticle platforms, their advantages and drawbacks, and the influence of their physicochemical properties on vaccine efficacy. Moreover, approved applications of nanotechnology in vaccine formulation are highlighted.

2. Vaccine immunological cascade

Vaccines aim to protect against pathogen infection through antibodies and memory cell formation. Through stimulation of the immune response, vaccines form immunological memory that protects upon pathogen encounter [15]. There are different routes for vaccine administration. These routes include oral, intranasal, subcutaneous, intradermal, and intramuscular (IM) routes. Oral and intranasal are considered mucosal routes that provide needle-free vaccine administration and also stimulate immunological responses on the mucosa and systemic levels [16]. Despite these advantages of mucosal vaccines, few mucosal vaccines are currently licensed [17]. The formulation and administration of antigen subunit mucosal vaccines remain challenging. That is because the physiological barriers of mucosal tissue degrade and reduce the cellular uptake of the vaccines [18]. Otherwise, the IM route is the most frequently utilized for vaccine administration. The IM route has the advantages of good tolerability and minimal injection site unfavorable reaction, which is an advantage that is desirable for vaccine licensure [19]. Once the vaccine is injected, the injection site muscle injury initiates the required immune response of the vaccine. The skeletal muscle cells and muscle-resident immune cells start to induce a local inflammatory response. This local inflammation is essential for initiating the cascade of immunological events that activate the adaptive immunity required for effective vaccine response [20]. The injury of skeletal muscle causes the damaged cells to release endogenous danger molecules called danger-associated molecular patterns (DAMPs) extracellularly and into the blood circulation. The circulatory immune cells recognize the DAMPs by pattern-recognition receptors (PRRs), prompting their recruitment to the injury site [21, 22]. Furthermore, the

damage-activated resident immune cells release protein substances called cytokines, which trigger migration and more immune cell recruitment to the injury site [21]. A subset of immune cells uptakes the vaccine antigen. The immune cells can uptake the antigen through a diversity of mechanisms, namely receptor-mediated endocytosis, phagocytosis, and macropinocytosis [23]. Subsequently, these cells degrade the antigen and proceed to load and display the antigen fragments on a molecule expressed on their surface called the major histocompatibility complex class II (MHC-II) molecule [24]. These immune cells are the antigen-presenting cells (APCs) and function to present the antigens to the T lymphocytes (T cells) to activate adaptive immunity [25]. The APCs consist of macrophages, B lymphocytes (B cells), and dendritic cells (DCs), which are the specialized and proficient APCs [26]. Following the antigen uptake, DCs mature and migrate to the zone of T cells in the secondary lymphoid tissues to present the antigen [27].

Antigen presentation is essential to mount the adaptive immunological response [25]. B and T lymphocytes are the cells mediating the response. The B cells undergo differentiation into plasma cells that release antibodies. There are two subsets of effector T cells: cytotoxic (Tc) T cells and helper (Th) T cells. Cytotoxic T cells, also called CD8+ T cells, exterminate the infected cells through direct contact, while T helper cells, or CD4+ T cells, secrete cytokines to augment both B- and T-lymphocyte reactions [28, 29]. Secondary lymphoid organs (SLOs) are antigen presentation sites and the tissues of immune surveillance [30]. The SLOs comprise the lymph nodes (LNs), tonsils, spleen, and intestinal Peyer's patches [31]. The LNs are found all over the body at strategic locations to maximize the sampling of foreign antigens [32]. Moreover, the LNs are anatomically compartmentalized into three compartments: the cortex, the paracortex, and the medulla. These compartments each contain distinct immune cell subsets. The cortex, as the B cell

zone, harbors B cells arranged in follicles; the paracortex, as the zone of T cells, contains T cells and DCs. Moreover, the medulla harbors macrophages and plasma cells [33]. The LNs are surrounded by a channel lined with macrophages and DCs called the subcapsular sinus (SCS) [34].

After migrating from the administration site, the DCs and soluble antigens drain from the lymph into the LNs via afferent lymphatic vessels [35]. Upon entering the lymph node, DCs depart the subcapsular sinus toward the zone of T cells [36]. The soluble antigens enter the subcapsular sinus (SCS) and, according to their size, can diffuse into B and T cell zones or be engulfed by SCS macrophages. Small antigens can enter the B cell zone, where B cells capture and present the antigens to the T cells. Alternatively, the antigens can enter through fibroblastic reticular conduits into the T cell zone, where lymph node–resident DCs present the antigens to the T cells. On the other hand, for large antigens, SCS macrophages internalize and transport the antigens to the B cell zone, where the B cells recognize the antigens or transport the antigens to the zone of T cells for presentation [37, 38].

In antigen presentation to Th cells, lymph node T cell zone resident naïve CD4+ T cells recognize the antigen presented by the DC's MHC-II molecule and interact with the antigen via the T cell receptor (TCR) [39]. Then, the T cells with the co-stimulatory receptor CD28 engage with the ligands CD80 and CD86 on DCs to increase TCR activation, thereby promoting T cell proliferation and effector cell differentiation. Finally, DCs secrete a mixture of cytokines to regulate immune activation [40]. After the presentation, T cells are differentiated into effector Th cells, which function to secrete cytokines that mediate the response of other immune cells [41].

In comparison, in the antigen presentation to Tc cells, CD8+ T cells first identify the antigen presented on the major histocompatibility complex class I (MHC-I) molecule

by host cells and DCs [42, 43]. DCs can cross-present antigens via both the MHC class II to CD4+ T helper cells and the MHC-I to CD8+T cells [42]. Then, TCs engage with antigen fragments via the TCR and the co-stimulatory signal from the CD28 receptor is stimulated to activate the killing machinery and the T cells' differentiation into effector cytotoxic T cells. TCs eliminate the cells presenting the antigen through granzymes and perforin secretion [43, 44]. Following antigen elimination, most effector CD8+ T cells undergo apoptosis and die, but a few cells survive and further differentiate into long-lived memory T cells [45]. Consequent to antigen re-encounter, memory T cells promptly differentiate into effector cells to effectively and promptly eliminate the antigen [46].

To mount the antibody response, the B cells bind with the antigen using the B cell receptor, and as APCs digest and display the antigen through MHC-II. On the border of the B and T zones, the antigen is recognized by CD4+ T cells *via* the TCR. CD4+ T cells subsequently secrete cytokines that signal the activation and proliferation of B cells [47]. Once activated, some B cells undergo differentiation into short-lived plasma cells or memory B cells [48]. While some B cells return to the follicle to form germinal centers (GCs). In the GCs, B cells proliferate and differentiate into long-lived memory B cells and plasma cells [49]. Consequently, GCs' plasma cells migrate to the bone marrow and carry on secreting antibodies to protect long-term [48]. Antibodies produced by plasma cells are primarily IgM antibodies for initial acute exposure to the antigen and later on, through class switching and affinity maturation, IgG antibodies and IgA antibodies [50]. These antibodies protect against antigens through antigen neutralization by preventing antigens from binding and entering the host cell, pathogens, or infected cells, complement-mediated lysis, and antibody-dependent cellular cytotoxicity [51]. Upon subsequent contact with the antigen,

memory B cells differentiate into plasmablasts to generate a rapid and effective antibody response [47].

The DCs, in particular, are recognized as the most effective activators of naive T cells [52]. Even though recent research has indicated that macrophages can also be crucial for T-cell priming by particle vaccinations [53]. Cross-presentation, a step in the processing of antigens for loading onto MHC-I molecules (to prime CD8+ T-cells, crucial for viral and cancer vaccines), is induced by DCs internalizing particulate immunogens [54]. Particle vaccines are frequently injected through subcutaneous or intradermal routes, and they are either drained *via* the lymphatic system to LNs or rapidly at the injection site engulfed by DCs. The APCs engulfed vaccine nanoparticles must successfully unload their antigen in order to trigger immune cells to present antigen and prime T-cells [55]. **Figure 3** demonstrates the immunological cascade following the IM administration of vaccines.

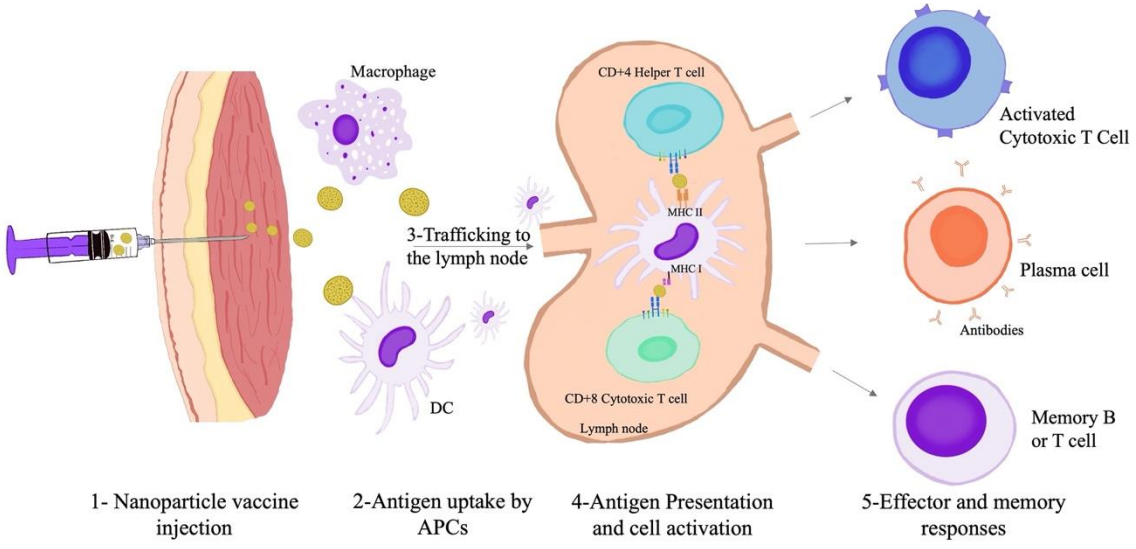

Figure 3. Immunological cascade after intramuscular administration of vaccines.

3. **Nanoparticles and vaccine delivery**

In recent years, nanoparticles have received attention for their potential as vaccine delivery vehicles. The antigen of the vaccine can be attached to the surface of the nanoparticles or encapsulated within them. Antigen encapsulation within nanoparticles prevents degradation upon administration and thereby improves immunogenicity. On the other hand, when antigens are conjugated onto the nanoparticles, these antigens can be presented to the immune system, considerably like the pathogen, thereby eliciting a comparable reaction. Additionally, nanoparticles made from some composites can sustain the release of the antigen to ensure maximum immune system exposure. Researchers are currently investigating the prospects of using nanoparticles to administer vaccines through non-traditional routes such as transdermal and inhalational administration, as well as formulating a single nanoparticle-based vaccine incorporating multiple antigens capable of eliciting immunity against multiple pathogens[12, 56, 57]. **Figure 4** represents the advantages of various nanoparticles in vaccine delivery.

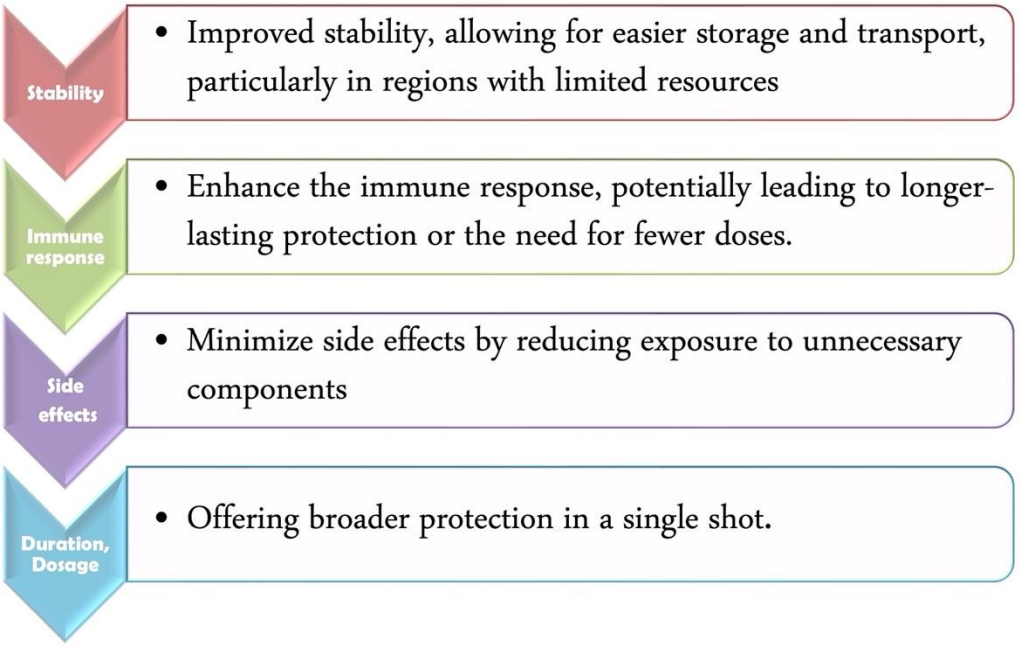

Figure 4. The advantages of various nanoparticles in vaccine delivery

NPs used as vaccine delivery systems can be constructed from several materials, encompassing natural and synthetic polymers, lipids, viral components, and inorganic compounds. In the following sections, the various nanoparticle platforms used to deliver vaccines will be covered, along with their advantages and limitations. Moreover, the influence of the physicochemical characteristics of nanocarriers on vaccine efficacy will be discussed.

3.1.Platforms of nanoparticles used in vaccine delivery

3.1.1. Inorganic nanoparticles

Inorganic nanoparticles are constituted of inorganic materials (*i.e.,* gold, mesoporous silica, and iron oxide). These nanoparticles possess several advantages as high drug loadings, stability, small particle size, and controlled tunability. However, despite their advantages, inorganic nanoparticles are not biocompatible or non-biodegradable, and have high cellular toxicity [58].

3.1.2. Polymeric nanoparticles

The polymeric nanoparticles are formulated from polymers in which the active compound is either entrapped or surface-adsorbed onto the core of the polymers. Polymeric nanoparticles comprise nanospheres and nanocapsules, which differ in structure [59]. Polymeric nanoparticles can be comprised of naturally occurring polymers as chitosan, inulin, albumin, heparin, pullulan, gelatin, and mannan. Additionally, synthetic polymers, including poly (lactic-co-glycolic acid) (PLGA), poly (D, L-lactide-co-glycolide), poly (ethylene glycol), polyphosphazene, polyanhydrides, polymethacrylates, and poly (D, L-lactide), can be utilized to create polymeric nanoparticles [60]. Polymeric nanoparticles are versatile, biocompatible, and offer sustained release. However, polymeric nanoparticles

have drawbacks. The drawbacks of polymeric nanoparticles include preparation solvent toxicity, difficult large-scale production, and biphasic release of material [61].

3.1.3. Liposomes

Liposomes are spherical vesicles comprised of phospholipid bilayers separated by water-containing spaces. Cholesterol and other natural phospholipids form phospholipid bilayers of the nanoparticle. The choice of the component of phospholipid bilayers affects the liposome characteristics such as size, stiffness, fluidity, and surface charge. Phosphatidylcholine, phosphatidylglycerol, dipalmitoylphosphatidylcholine, distearoylphosphatidylcholine, cholesterol, stearylamine, and dicetyl phosphate are examples of phospholipids used in liposome preparation [62]. Liposomes have several advantages, including non-toxicity, non-immunogenicity, biocompatibility, and biodegradability. However, liposomes also have several disadvantages as elevated production costs, poor solubility, and short half-life [63].

3.1.4. Immune-stimulating complexes (ISCOMs)

The ISCOMs are micelles containing colloidal saponin, cholesterol, and phospholipids. The main saponin used to prepare ISCOMs is Quil A and its purified components. While the main phospholipids used are phosphatidylcholine and phosphatidylethanolamine. The ISCOMs are self-adjuvanting vaccine delivery vehicles. Moreover, ISCOMs can be used as adjuvants without the addition of viral protein, and in this case, such complexes are known as ISCOMATRIX or empty ISCOM [64].

3.1.5. Virus-like particles (VLPs)

Virus-like particles (VLPs) are self-assembling viral structural protein nanoparticles that serve as vaccine antigen delivery vehicles. They are molecularly and morphologically similar to native virions. Nevertheless, VLPs don’t replicate and infect due to the genetic

material absence [65]. Moreover, VLPs are classified based on the existence of an envelope into enveloped VLPs and non-enveloped VLPs. VLPs are biocompatible and biodegradable and can be manufactured in mammals, plants, insects, and bacteria. VLP preparation has the drawbacks of low yield and a complex purification process [66, 67].

3.1.6. Lipid nanoparticles (LNPs):

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are the two kinds of LNPs. The SLNs are the first-generation lipid nano-spheres which are constituted of room-and human-body temperature solid lipids. SLNs can be comprised of waxes, glycerides, or triglycerides [68]. SLNs have the strengths of liposomes and polymeric nanoparticles while overcoming their downsides; they are easy to prepare, can be prepared without organic solvents, are inexpensive, and are suitable for large-scale use. SLNs also have good biodegradability and compatibility, great physical stability, a good release profile, chemical versatility, and a nontoxic lipid system. Additionally, SLNs are less toxic and easier to obtain approval for than polymeric carriers [69]. On the contrary, SLNs have limitations such as low drug entrapment efficiency attributable to the SLNs' perfect crystalline structure and the likelihood of drug expulsion during storage [70]. To tackle these limitations, the second-generation lipid nanoparticle NLCs were designed [71]. The NLCs are formed of a combination of both solid and liquid lipids and have a higher capacity for drug loading compared to SLNs as a result of their imperfect crystal structures. Moreover, through the avoidance of lipid crystallization during the manufacture and storage and the inclusion of liquid lipids, the NLCs have minimized the expulsion of the loaded materials. Furthermore, NLCs have more controlled release and increased solubility of loaded cargo in a lipid matrix compared to SLNs [70, 72].

3.2. Physicochemical characteristics that influence the potency of nanoparticle vaccines

3.2.1. Particle size

The two main objectives of vaccination are to imitate a dangerous infection and to establish an immune memory for possible future exposures. Before a vaccine can trigger an immune response, it must successfully transfer antigen from the injection site to secondary lymphoid tissue, which is home to APCs, B cells, and T cells. Through the interstitial fluid and the collecting lymphoid arteries, antigens can go straight to lymphoid organs (like the spleen or LNs). Nevertheless, particle vaccines can enter the lymphatic system and interact with the T and B cells found in the LNs after being absorbed by APCs at the injection site [73, 74]. For this reason, the vaccines' ability to spread to the LNs is greatly influenced by their particle size.

The transmission of the vaccines to the LNs is significantly influenced by their particle size. Large vaccine particles must be actively transported by immune cells known as DCs in order to reach the LN. Conversely, smaller particles are thought to move through the interstitial flow much more easily. As a result, Small, non-liposomal nanoparticles (less than 50 nm) are more effectively transported into lymphatic capillaries and drain LNs than particles larger than 100 nm due to interstitial flow, or the drainage of fluids from the interstitial space [75, 76]. Significantly, the efficiency of DC migration towards the draining LNs increases retention in the LNs for smaller particles and adds a parameter for larger particles (> 50 nm) that may change the amount of antigen that can reach the LNs [77].

The antigen must overcome many obstacles before it can enter secondary lymphoid tissue via various administration routes. Consequently, different DC subsets are found in different tissues, such as the skin's Langerhans cells, connective tissue's CD103 + DCs, and

the gut's mucosal DCs [78]. Orally administered biodegradable polylactic acid (PLA) microparticles with a diameter of 1–26 μm were used in one investigation. These particles' absorption into intestinal lymphoid structures called Peyer's patches rose until 11 μm, at which point it fell once more. Larger particles remained in the spleen, but smaller particles were later carried from the Peyer's patches in the jejunum by the lymphatic system [79]. The decrease in splenic localization when the size of the microparticles surpasses threshold is explained by the authors' hypothesis that phagocytes are less likely to take up particles larger than 10 μm [80, 81]. It has been demonstrated that nanoparticles are more effective than microparticles for oral distribution because they are absorbed more effectively by the intestinal epithelial cells and can reach deeper into Peyer's patches [82]. Hence, it makes sense that particle vaccinations should be created with a size in the nanometer range for the best gut barrier crossing.

Regarding nasal administration, it has been demonstrated that when particle size decreases, non-liposomal particle movement across the rat nasal mucosa increases, resulting in increased IgG and IgA responses—indicators of the mucosal and general immune responses, respectively [83].

A crucial element in the internalization process of particles is their size. The range of nanoparticle sizes, typically thought to be between 10 and 100 nm, is based on how well the nanoparticles are distributed and cleared *in vivo*. Although small particles may speed up the process of cellular entrance, there appears to be no upper size limit for cellular internalization up to 5 μm. A group of polyethylene glycol hydrogels ranging in size from 100 nm to 5 μm was studied for their ability to penetrate HeLa cells [84]. Large microparticles were generally observed to have slower internalization rates than small nanoparticles. These particles are internalized through clathrin- and caveolae-mediated processes as well

as non-clathrin- and non-caveolae-mediated pathways. Taking everything into account, particles smaller than 50 nm can instantly drain and more thoroughly enter the LNs. Nonetheless, the LNs retain larger particles more effectively, highlighting the necessity of research to determine the ideal particle size for both lymphatic drainage and retention. Additionally, the dispersion might be impacted by the mode of administration, which should be considered while designing vaccines.

3.2.2. Particle shape

Along with particle size, shape is a crucial characteristic that affects the immunological response. The aspect ratio (AR), defined as the ratio of particle height to width, is commonly used to describe particle shape. Huang and associates. assert that the way mesoporous silica nanoparticles are transported throughout the body after being injected into mice is influenced by their shape [85]. It was noted that only the liver and spleen were able to capture the long-rod particles (720 nm, high AR) and the short-rod particles (185 nm, low AR). But in the spleen, the long-rod particles were more obvious than the short-rod ones. Additionally, the body eliminated short-rod particles more rapidly through urine and feces than long-rod particles. It is clear that the shape of the particles influences their absorption by tissue-resident cells, which in turn impacts their ability to be distributed and retained in the tissues. Yet, in this experiment, a size impact cannot be completely ruled out [85]. In another study, tubular-shaped filomicelles with a high AR were observed to persist in the bloodstream of rats and mice for up to one week following injection [86]. Within two days, short tubular micelles were taken out of circulation. Human macrophages were able to phagocytose filomicelles shorter than 3 μm, but not those longer than that. Researchers suggest that the extended circulation time of long filomicelles is due to their reduced frequency of interaction with phagocytes and the blood vessel wall. Because they

will contact with phagocytes more and be less affected by blood flow, shorter cylinders will be more effectively absorbed and removed from circulation more quickly. However, because these particles were administered intravenously, the focus of this investigation was on circulatory uptake rather than lymphatic trafficking [86]. Following oral administration of mesoporous silica nanoparticles, a number of effects were observed; lowering ARs (5, 1.75, and 1) increased absorption in the small intestine while lowering urine production [87]. Interestingly, the particles with the lowest aspect ratio showed the highest number in the spleen, while the other particles were mostly deposited in the liver, lungs, and kidneys. These results underscore the importance of the manner of administration in deciding how the characteristics of the particle would affect immunogenicity, indicating that the biodistribution profile of spherical particles will be more advantageous following oral administration than non-spherical ones.

3.2.3. Particle rigidity

Along with size and form, rigidity can affect how quickly particles are eliminated and biodistributed. Hydrogels containing particles known as red blood cell mimics (RBCMs) that mimic red blood cells (RBCs) in terms of shape, size (6 μm), and hardness, were created by Merkel et al. [88]. They found that rigidity had an inverse relationship with circulation time, with the most rigid RBCMs being removed significantly more quickly than the least rigid ones. This was probably brought on by the ability of the less stiff particles to puncture regions with limited blood flow, extending circulation periods. Hydrogel nanoparticles based on "soft" polyethylene glycol (PEG) were produced to resemble hard particles. Compared to rigid particles, these particles showed longer elimination and distribution half-lives, respectively [89]. After 30 minutes and 12 hours, tissues were examined and it was found that nearly every tissue, including the heart, lungs,

brain, blood, kidney, and spleen had higher quantities of soft particles, except for the liver. It was believed that the alterations in biodistribution were caused by the soft nanoparticles' prolonged circulation duration, which cause organs with high blood flow to retain more. Their decreased retention in this tissue could be explained by the degradation of soft nanoparticles in the liver. It was also discovered that the less rigid particles survived lung filtration and were mostly located in the spleen, whereas the harder particles gathered in the capillaries of the lungs. According to this study, when the more rigid particles first come into contact with microvasculature, they lodge in that tissue [88].

Compared to liposomes carrying PC with a low transition temperature, it has been shown that liposomes carrying PC with a high transition temperature—that is, high stiffness—stay in the bloodstream better. There was also a decrease in the uptake by the spleen and liver [90, 91]. Senior et al. reported similar findings and proposed that the lengthier circulation was caused by reduced contact between the blood's high-density lipoprotein and the liposomes [92]. There is proof that high-density lipoprotein's ApoA-I and ApoA-II interact with PC and liposomes that carry cholesterol, causing a faster clearance [93]. Rigid cationic liposomes remained at the injection site longer than less rigid ones following IM injection. This was consistent with the higher number of non-rigid liposomes in the draining LNs [94]. Kaur et al. found that the cholesterol content of cationic liposomes, which also influences stiffness, had no effect on transport to LNs or drainage from the injection site after IM injection [95]. It seems that particles with increasing rigidity may find it more difficult to pass through tiny lymphatic capillaries, which will impede their lymphatic trafficking, much as particles of increasing size.

3.2.4. Surface Charge

For use as vaccinations, systems of polymeric particles with varying surface charges—both positively and negatively charged—have been developed. Protein absorption in circulation and nonspecific cellular internalization are significantly influenced by surface charge. Zeta potential, which has an absolute value less than 100 mV, is commonly employed to calculate the average surface charge of particles. One of the main benefits of charged particles is their ability to enhance adsorption through electrostatic interactions with other vaccine ingredients, especially the antigen and adjuvant. Protein antigens can occasionally be preserved more effectively by adsorption via electrostatic interactions than by encapsulation. Adding a surface charge to injectable polymeric particle vaccines may also boost the immune response, which has been investigated both *in vitro* and *in vivo*.

In general, cationic particles function better than anionic ones when it comes to the absorption and activation of APCs, especially macrophages and DCs [96, 97]. Yet, there are several qualifications to this generality. First, many studies have shown that the effect is amplified by a higher surface charge, and that, in contrast to particles with a neutral charge, particles with a strong enough negative charge may improve absorption by APCs and subsequent activation [98]. Second, in addition to differences in absorption and reaction, anionic and cationic particles may interact with APCs in different ways. Catalytic polystyrene particles, for example, which were produced by cationic surface modification, were associated with lower phagosome acidification than more neutral polystyrene particles or particles with anionic surface modifications [99]. Last but not least, cationic PLGA particles produced with PEI and plasmid DNA containing CpG did not raise CD83 in human DCs, but treatment of anionic PLGA particles or plasmid alone did. However, DC activation by activating either CD83 or CD86 was not possible in a comparable

experiment that employed PLA particles instead of PLGA particles [100]. The impact of charged particles may vary depending on the type of polymer used to make them, and the charge may not be sufficient to activate APCs [101]. It may not be immediately applicable to biodegradable polymers because most of the early research that concluded cationic particles were superior for APC interactions was conducted using non-degradable polystyrene particles. Enhancing antigen presentation may be another benefit of the "proton sponge" effect in polymeric particles containing polycations. Endolysosome disruption is thought to be aided by increased Cl ion input from polycationic polymers such as PEI, which have a considerable H+ ion buffering capacity. It is believed that this disruption will increase the presentation of MHC-I and cause cross-presentation in different particle systems [102-104]. Notably, however, studies of adaptive immune responses have focused on a narrower range of particle surface charge magnitudes. Antigen is not always necessary for *in vitro* tests that only look at particle interactions with APCs, and antigen adsorption reduces the size of the particle surface charge.

3.2.5. Hydrophobicity

In general, hydrophobic particles are phagocytosed by cells more quickly and effectively than hydrophilic ones [105]. Liu et al. investigated the impact of surface chemistry on immune activation by synthesizing microparticles from poly (D, L-lactic acid), poly (D, L462 lactic-co-glycolic acid), and poly (monomethoxypolyethylene glycol-co-D, L-lactide). Despite having uniform sizes and shapes, these particles varied in their surface hydrophobicity. Results indicated that the most hydrophobic particles prompted the highest expression of MHC-II and CD86 in DCs, as well as peak cytokine production in splenocytes. This enhanced response is attributed to the fact that hydrophobic surfaces adhere more robustly to cell membranes, streamlining the internalization of both the particles and their associated

antigens. They concluded that the hydrophobicity of microparticles is one of the main parameters in optimizing the immunogenicity of particulate vaccine delivery systems [104]. According to the hazard theory, which holds that hydrophobic patterns serve as harm signals that activate the immune system, particles with more hydrophobicity have improved immunogenicity [105]. In order to investigate the complex immunological effects of polyanhydride nanoparticles with different hydrophobicities, the Narasimhan group used copolymers based on 1,6-bis(p-carboxyphenoxy) hexane (CPH), 1,8-bis(p-carboxyphenoxy)-3,6-dioxaocatane (CPTEG), and sebacic acid (SA). The less hydrophobic, SA-rich particles were found to be more efficiently absorbed by DCs than the more hydrophobic ones (i.e. CPH-rich) [106]. Additionally, the more hydrophobic particles expressed MHC-II and CD86 but did not induce DCs to produce IL-6, IL-1, or TNF, while the less hydrophobic particles expressed more secreted cytokines but did not express any surface markers. The particles that were least hydrophobic (20:80 CPH/SA) broke down and triggered an immune reaction the fastest. Formulations with a high CPH content (20:80 CPTEG/CPH, 50:50 CPTEG/CPH) maintained in the lungs for at least 63 days, decomposed more slowly and generated bigger antibody titers with a wider range of epitope specificity [107]. The delayed, continuous release of antigen was thought to be the cause of the induction of longer-lasting plasma cells and the enhanced inflammation brought on by the hydrophobicity of the particles. **Figure 5** summarizes the physicochemical characteristics of nanoparticles for ideal vaccine delivery

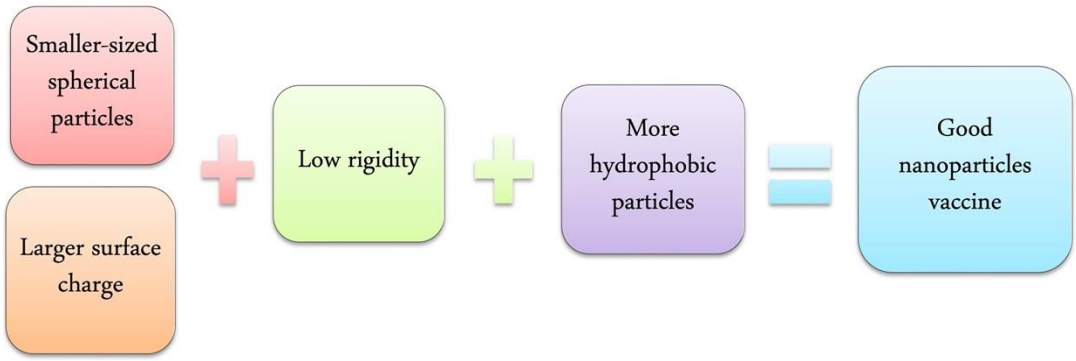

Figure 5. Physicochemical characteristics of nanoparticles for ideal vaccine delivery

3.3.Clinically approved nanovaccines

There are many nanovaccines that passed the clinical trial phase and received authorization for use. The most notably known nanoparticle vaccines are those developed for hepatitis B, human papillomavirus (HPV), malaria, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These vaccines are categorized according to the type of nanoparticles used, including lipid nanoparticle vaccines, virus-like particle vaccines, and immune-stimulating complex vaccines. **Table 1** summarizes the currently approved nanovaccines and their corresponding nanoparticle platforms.

Table **1.** Summary of clinically approved nanovaccines and their corresponding nanoparticle platforms

Pathogen	**Vaccine**	**Type of NP**	**Antigen**	**Particle size** (nm)	**Reference**
Severe acute respiratory syndrome coronavirus 2	*Comirnaty*	LNP	spike protein	~100	[110]
	Spikevax				
	Covifenz	VLP		100-150	[108]
	Nuvaxovid or Covovax	ISCOM		27.2	[109]
Hepatitis B virus	*Recombivax HB*		small S antigen	20	[110-112]
	Engerix–B				

	Heplisav-B	VLP			
	PreHevbrio		pre-S1, pre-S2, and S		
Malaria	*Mosquirix*		*Plasmodium falciparum circumsporozoite* protein, hepatitis B surface antigen	~ 20	[113, 114]
Human papillomaviruses	*Cervarix*		L1 protein of virus types 16 and 18	55	[115-117]
	Gardasil		L1 protein of virus types 6, 11,16 and 18		
	Gardasil 9		L1 protein of virus types 6, 11, 16, 18, 31, 33, 45, 52, and 58		
	Cecolin		L1 protein of virus types 16 and 18	50-60	[118]
Hepatitis E Virus	*Hecolin*		genotype 1 antigen	20-30	[119, 120]

3.3.1. Lipid nanoparticle vaccines

Lipid nanoparticles are utilized to transport nucleic acid cargos such as messenger RNA (mRNA) encoding the vaccine antigens [65]. The mRNA cargo encapsulation in LNPs protects it from RNase degradation and enhances its cellular transport. [121]. Pfizer-*BioNTech's Comirnaty*™ and *Moderna's Spikevax*™ are Food and Drug Administration (FDA)-approved mRNA-lipid nanoparticle-based vaccines against SARS-CoV-2 [122]. The mRNA encodes the virus' spike (S) protein, which assists the viral attachment and entry into host cells. The LNPs are approximately 100 nm and demonstrate potent immune-stimulatory properties with mRNA [123], eliminating the necessity for an adjuvant [124]. Furthermore, mRNA containing modified nucleosides is used to minimize the mRNA immunogenicity and enhance the translational capacity [65, 125]. The LNP facilitates the

uptake via endocytosis by disrupting endosomal membranes and the mRNA delivery inside the host cell cytosol [126]. Inside the ribosomes, the mRNA sequence is translated into the S protein [127]. The unprocessed S protein is presented as a membrane-bound antigen at the cell surface as an antigen for B cells. Consequently, the S protein is degraded by lysosomes in APCs and presented *via* MHC-II to T cells [128]. It is also degraded by the proteasome to be presented via MHC-I to cytotoxic T cells [129, 130].

3.3.2. Virus-like particle vaccines

Virus-like particle-based vaccines are the most commercially available type of nanoparticle vaccine. VLP-based vaccines comprise the hepatitis B vaccine, malaria vaccine, human papillomavirus (HPV) vaccine, hepatitis E vaccine, and SARS-CoV-2 vaccine.

The first generation of hepatitis B vaccines were plasma-derived vaccines, which have now been effectively replaced by recombinant VLP vaccines. Hepatitis B vaccines are enveloped recombinant subunit vaccines against the hepatitis B virus (HBV) surface antigen (HBsAg) with a particle size of 20 nm [110, 111]. Hepatitis B surface antigen (HBsAg) is comprised of three envelope proteins: small hepatitis B surface antigen or S antigen, middle hepatitis B surface antigen or Pre-S2 antigen, and large hepatitis B surface antigen or Pre-S1 [131]. Recombivax*HB*® and *Engerix*–B® are second-generation HBV vaccines. Both are yeast-derived recombinant vaccines comprising the small S antigen and are used worldwide for the vaccination of neonates and adults [112]. They differ in the antigen concentration and the type of aluminum adjuvant [132]. *Heplisav*-B™ is another small S antigen yeast-derived vaccine that differs from the other two vaccines in the type of adjuvant used. It uses a CpG sequence 1018, which is a toll-like receptor 9 agonist adjuvant to improve the immune response and decrease the required number of doses, two doses compared with three doses with *Engerix*–B®. *Heplisav*-B is FDA-approved for adults

eighteen years of age and above [133, 134]. A third-generation vaccine, *PreHevbrio*® (Sci-B-Vac) is a vaccine against all three HBsAg envelope proteins (pre-S1, pre-S2, and S). It is derived from mammalian cells and is approved by the FDA for individuals ages 18 and older. It elicits a more rapid and better immune response than that of second-generation HBV vaccines; hence it showed greater efficacy in high-risk groups, immunocompromised, and non-responders to conventional second-generation vaccines. Furthermore, it has been suggested that it could be used as a therapeutic vaccine to treat persistent HBV infection [110, 112].

Mosquirix® is a yeast-derived World Health Organization (WHO) approved vaccine against malaria and is currently the only vaccine approved for it. It has a particle size of approximately 20 nm and contains the causative mosquito *Plasmodium falciparum circumsporozoite* protein fused to and combined with the HBsAg [113, 114].

The human papillomaviruses (HPV) vaccines are against the virus' major capsid protein (L1). *Cervarix*® vaccine is an insect cell-derived vaccine containing capsid protein of HPV types 16 and 18. Unlike *Cervarix*®, *Gardasil*® and *Gardasil* 9® are yeast-derived vaccines that contain proteins of four (*i.e.* 6, 11, 16, and 18) and nine (*i.e.* 6, 11, 16, 18, 31, 33, 45, 52, and 58) HPV types, respectively [117]. These vaccines have a particle size of 55 nm [115, 116] and are FDA-approved for females aged nine to twenty-five for *Cervarix*®, to twenty-six for *Gardasil*®, and to forty-five years old for *Gardasil* 9® [122]. Another bivalent type 16 and 18 vaccine, *Cecolin*™ is authorized for use in China and is prepared using the expression system of *Escherichia coli* [110, 135]. It has a particle size varying from 50 to 60 nm [118].

Hecolin® is a Hepatitis E vaccine (HEV) genotype 1 with a particle size of 20 to 30 nm. It is prepared using the *Escherichia coli* expression system and is approved for use in China [119, 120].

Covifenz® or CoVLP is a plant-based SARS-CoV-2 vaccine measuring approximately 100–150 nm in size. It was developed using *Nicotiana benthamiana* and consists of recombinant spike proteins derived from SARS-CoV-2. The vaccine was authorized in Canada for adults aged 18–64 years in 2022; however, its authorization was voluntarily cancelled by the sponsor in 2023 [108, 136].

3.3.3. Immune-stimulating complexes vaccines

Nuvaxovid® or *Covovax*® is a recombinant subunit SARS-CoV-2 vaccine with protein-protein micellar nanoparticles based on the virus S protein. It is the latest SARS-CoV-2 vaccine to be WHO-approved and is approved and used in many countries worldwide. The vaccine is 27.2 nm in size and has a polydispersity index (PDI) of 0.25–0.29 [109]. It uses Matrix-M™ 40 nm adjuvant nanoparticles with *Quillaja saponins*, cholesterol, and phospholipid composition to enhance the immune response to spike glycoprotein [137].

4. Conclusion

Vaccines are key healthcare tools in maintaining global health and preventing outbreaks. Nanoparticles, through their physicochemical properties, greatly influence the immune response induced by vaccines; therefore, the optimization of these properties can enhance vaccine efficacy. In this review, we addressed the vaccine-elicited immune response, different nanoparticle types, and how the immune response of nanovaccines is influenced by their properties—namely size, shape, rigidity, surface charge, and hydrophobicity. Through the examination of multiple pieces of evidence, this review concludes that these properties should be carefully considered during vaccine development. Nanotechnology

has contributed to significant advancements in vaccinology, evident by the various approved for use vaccines highlighted in this review, and is anticipated to contribute substantially to future developments.

Author contributions

Conceptualization: N.E.; Writing—original draft preparation: N.E. and M.K.; Visualization (graphical abstract and figures): N.E. and M.K.; Writing—review and editing: N.M., H.F., S.H., and O.O.; Supervision: G.F., S.K., and M.E. All authors have read and agreed to the published version of the manuscript.

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The authors declare that they have no conflict of interest.

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List of Abbreviations

APCs: Antigen-presenting cells

AR: Aspect ratio

B cells: B lymphocytes

DAMP: Danger-associated molecular patterns

DCs: Dendritic cells

FDA: Food and Drug Administration.

GCs: Germinal centers

HBsAg: Hepatitis B virus surface antigen

HBV: Hepatitis B virus

HPVs: Human papillomaviruses.

IgA: Immunoglobulin A

IgG: Immunoglobulin G

IM: Intramuscular

ISCOMs: Immune stimulating complexes

LNPs: Lipid nanoparticles

LNs: Lymph nodes

MHC-I: Major histocompatibility complex class I

MHC-II: Major histocompatibility complex class II

mRNA: Messenger RNA

NLCs: Nanostructured Lipid Carriers

NPs: Nanoparticles.

PDI: Polydispersity index

PEG: Polyethylene glycol.

PLA: Polylactic acid

PLGA: Polylactic-co-glycolic acid

PRRs: Pattern-recognition receptors

RBCMs: Red blood cell mimics

RBCs: Red blood cells

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2

SCS: Subcapsular sinus

SLNs: Solid lipid nanoparticles

SLOs: Secondary lymphoid organs

T cells: T lymphocytes

Tc: Cytotoxic T cells

TCR: T cell receptor

Th: helper T cells

VLPs: Virus-like particles

WHO: World Health Organization

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