Chapter 8
Recent Advancement of Nanostructured Materials for Clinical Challenges in Vaccinology

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8.1 Introduction

Despite the surprisingly impressive success of vaccines in controlling and eradicating communicable diseases, there persists numerous globally catastrophic diseases without fully preventive vaccines, especially malaria, flu, AIDS (human immunodeficiency virus, HIV), hepatitis, and tuberculosis. Nanotechnology-based strategies have been developed both to fabricate advanced vaccines to control and eliminate these diseases and to clear the way for their worldwide administration. The limitations of why a particular pathogen may create difficulties for designing and developing vaccines are distinctive and connected to the coexisting history of humans and pathogens; however, there are usually issues that could be successfully addressed through the effective implementation of nanotechnology products such as nanostructured materials. Due to technological advancement, conventional materials, as well as bulk materials, have been replaced by advanced nanostructured and nanoengineered materials such as nanocomposite gels (Chowdhury et al., 2015; Harun-Ur-Rashid & Imran, 2019; Rezaul Karim et al., 2020), nanomedicine (Yang et al., 2022; Guo et al., 2021; Sun et al., 2021; Zheng et al., 2021; Cheng et al., 2021; Wei et al.,

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2021), molecular machines (Imran et al., 2019), nanobiosensors (Harun-Ur-Rashid et al., 2022), and polymer nanocomposite drugs (Díez-Pascual, 2022; Shen et al., 2021). These structurally engineered nanomaterials have been introduced and investigated extensively to find out solutions for currently existing problems and to address and overcome the present challenges and limitations in many different sectors, including building and construction (Janczarek et al., 2022; Quazi & Park, 2022), automobile (Harun-Ur-Rashid et al., 2023a, b; Imran & Susan, 2022), aviation and space (Pathak & Dhakate, 2022), optics (Halali et al., 2020), packaging (Dey et al., 2022), textiles (Perera et al., 2022), electronics (Mo, 2022), energy (Adegoke & Maxakato, 2022), catalysis (Zeng et al., 2022), agriculture (An et al., 2022), food (Wu & Mu, 2022), cosmetics (Fauzi et al., 2022), environment (Wang et al., 2022), pharmaceuticals (Sridharan et al., 2022), biomedical, and health (Derakhshi et al., 2022).

A successful vaccine will require uplifting immunologic reactions that vary from immunologic reaction raised by natural infection. Nanostructured materials, with their specific compositions, basic adaptable construction, and nanoscale size allowing the involvement of major immunologic routes, unitedly facilitate the repeated design procedures essential to detect such preventive immunologic responses and attain them with expected reliability. Nanostructured materials also serve as approaches for engineering the transfer of the major vaccine components to specific immune cells and key tissues such as lymphoid tissues. They might be highly polyvalent, enhancing their involvement in the immune response system (Fries et al., 2021). Vaccines, prepared from nanostructured materials or nanoparticles (NPs) that serve as antigen transfer vehicles composed of lipidic, proteic, polymeric, metallic, or graphene, are termed nanovaccines. In nanovaccines, such NPs are commonly functionalized with the antigen through surface modification or encapsulation treatment. Covalent bonds or intermolecular forces of attractions arbitrate the unification of the antigen to the incorporated NPs. When the nanomaterials are intended for biomedical purposes, as illustrated in Fig. 8.1, some of the issues are to be taken care of such as drug toxicity, bioavailability, organ specificity, drug stability and solubility, and entire safety. The development and advancement of nanostructured materials as drug carriers have attracted researchers and commercial communities because of their outstanding characteristics such as better chemical and biological stability, greater carrier capacity, suitability for incorporating both hydrophobic and hydrophilic substances, and regulated drug delivery ability.

Nanostructured materials are widely used in various sectors of nanovaccines, cancer therapy, biomolecule detection, and regenerative medicine because of their biological, physical, and chemical characteristics, including flexibility, strength, performance, durability, surface morphology, surface zeta potential values, surface charge, and potential antimicrobial activity, which has been schematically represented in Fig. 8.2 (Foyez & Imran, 2022; Rudramurthy & Swamy, 2018). The rapid success of nanomaterials in biomedical applications has created a perception that nanomedicine is the "savior" of mankind. Nonetheless, the successful global deployment of nanomedicine or nanovaccines that we observe is the consequence of extensive research, design, improvement, and optimization of products, which must be commemorated with additional funding for further development. Conversely, the

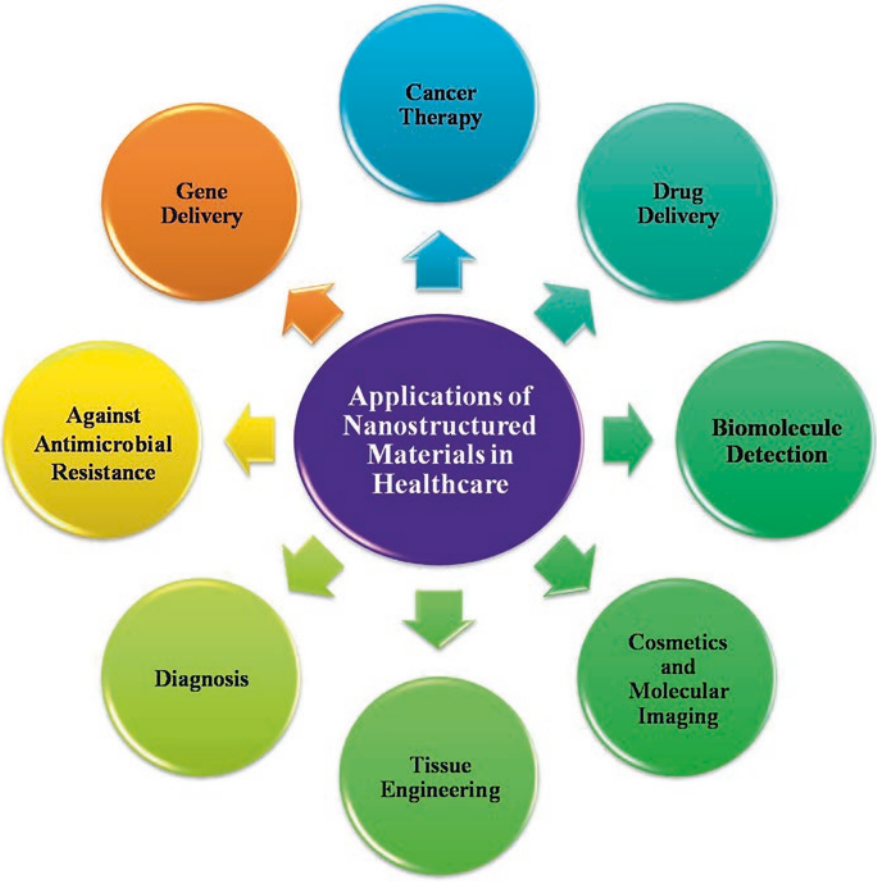

Fig. 8.1 Applications of nanostructured materials in various biomedical and healthcare sectors. (The figure has been reproduced with permission from ref. Rudramurthy and Swamy (2018). Copyright@2018, Springer)

oversimplified aggrandizement of nanomedicine or nanovaccines needs to be circumvented. Deliberation, awareness, and prospective thinking must triumph in managing the pandemic situation. This chapter will especially focus on the achievements of nanostructured materials in nanovaccine applications and the remaining clinical challenges in nanovaccinology.

8.2 Nanostructured Materials-Based Nanovaccines

The immunologic reaction system is an integrated network of cells, tissues, and organs that act as the safeguard of the body against diseases. The immune system comprises inborn and adaptive immunities. Adaptive immunity is capable of detecting a pathogenic component and evolving a durable impression of it. The aim of

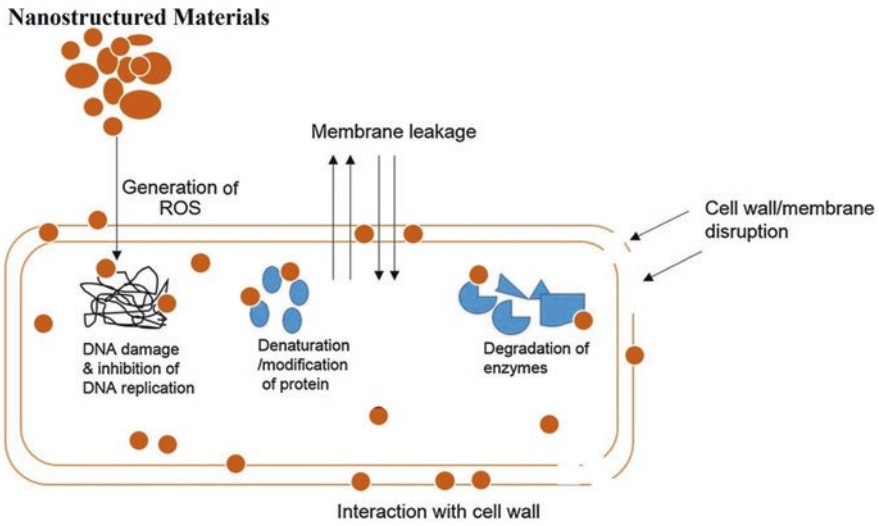

Fig. 8.2 Schematic representation of the antimicrobial activity of nanostructured materials. (The figure has been reproduced with permission from ref. Rudramurthy and Swamy (2018). Copyright@2018, Springer)

vaccination is to teach the adaptive immune system to either create immunological reminiscence before infection or to detect ongoing disease (Koff, 2016). Although the development of prophylactic vaccines in the case of deadly communicable diseases such as anthrax, smallpox, and plague has made a very remarkable contribution to healthcare. Recently, modern vaccines have shown great effectiveness in the treatment of incurable diseases like HIV infection, cancer, and type I diabetes (Greenwood, 2014). Nanovaccines have been designed and developed to overcome the limitations of conventional vaccines as well as to provide smart modulation to facilitate superior efficacy by increasing the stability of antigens, improving immunogenicity, specifying targeted delivery, and delaying the release of drugs (Azharuddin et al., 2022). Nanostructured materials present in nanovaccines provide effective protection to antigens and adjuvants against proteolytic and enzymatic degradation (Bishop et al., 2015). Nanostructured materials can induce both antibody-dependent and cell-arbitrated immune responses due to their idiosyncratic physicochemical properties (Fig. 8.3). In addition, they assist in transferring the drug in targeted areas and can promisingly load multiple antigenic components into a single scaffold. Nonetheless, an excellent adjustment of NPs physical characteristics, for instance, size, shape, and surface charge may lead to a great improvement in the durability of antigen that enhances cell-regulated immunity.

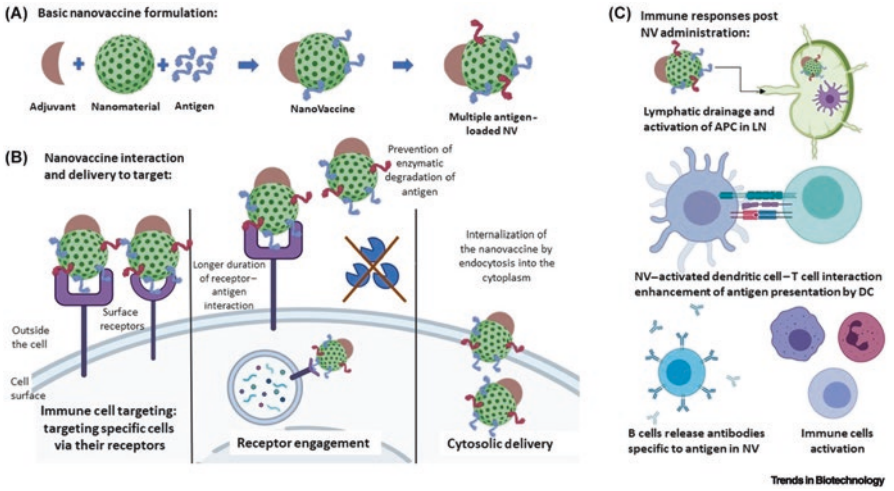

Fig. 8.3 The basic mechanisms of nanovaccines and their importance. Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; LN, lymph node; NP, nanoparticle; NV, nanovaccine. (The figure has been reproduced from the ref. Azharuddin et al. (2022). Copyright@2022, Elsevier)

8.2.1 Key Features of Nanovaccines

Traditional vaccines based on weakened or deactivated pathogens may have the potential risk of incorporating live pathogens and the incompetence to bring out a satisfactory level of an immune response, thus motivating the introduction of novel vaccines. With the advancement of nanotechnology, nanostructured materials-based vaccines (nanovaccines) have been fabricated to overcome the limitations of conventional vaccines as well as to provide advanced-level treatment. A nanovaccine should have some key features (illustrated in Fig. 8.4), such as enhanced immunogenicity, extended antigen stability, sustained release capability, and targeted delivery. Antigens present in the vaccine will be protected from enzymatic degradation since the NPs have a protective nature. NPs are immunogenic and capable of enhancing the immune response against the targeted antigen. One of the major features of nanovaccines is targeted delivery, which facilitates the transfer of antigen to specific sites and thereby minimizes harmful side effects. Enhanced activation of both humoral and cell-mediated immune responses can be achieved by the application of nanovaccines. Different types of antigens can be effectively loaded into a single NP that creates the opportunity to treat a wide range of pathogens as well as diseases. Nanovaccines can persist for an extended period of time without any change or degradation and thus offer enough opportunity for APCs to trigger the immune response.

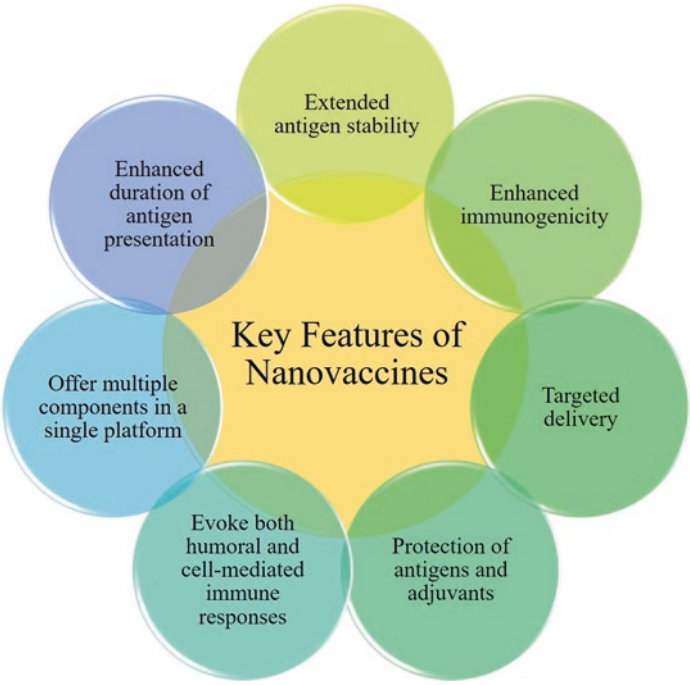

Fig. 8.4 The expected key features of nanovaccines

8.2.2 Types of Nanostructured Materials Used in Nanovaccines

Nanostructured materials such as metallic NPs, carbon nanomaterials, liposomes, silica and magnetic NPs, micelles, polymeric nanocomposites, dendrimers, protein NPs, and so on, utilized in nanovaccines preparation, act as suitable vehicles for antigens due to their nanoscale size that is comparable to the size of pathogens. They are also capable of loading and delivering active biomolecules. Gold NPs (AuNPs) have been employed in nanovaccines against influenza (Tao et al., 2014), malaria (Kumar et al., 2015), and cancer (Ahn et al., 2014). Though the gradual accumulation of nanostructured materials is a safety concern, which required more specific investigations, inorganic NPs such as carbon nanotubes (Hassan et al., 2019), silica NPs (Bancos et al., 2014), and magnetic NPs (Guo et al., 2015). Polymeric nanostructured materials including polylactide-co-glycolic acid (PLGA) copolymers, micelles, dendrimers, chitosan, protein, and liposomes are widely employed in the formulation of nanovaccines.

8.2.3 *Impact of NPs Size on Immunogenicity*

The size of NPs employed in nanovaccines effectively controls the activation of the immensity of immune response and thereby affects the performance of the vaccine. Commonly, it is found that smaller particles are more potential for selected drug delivery systems due to their greater ability to overcome biological barriers (Mumper et al., 2003). However, this trend is not true for all conditions, for example, 1000 nm size bovine serum albumin (BSA)-loaded PLGA particles elicit stronger serum IgG response than 200–500 nm size BSA NPs (Gutierro et al., 2002). Table 8.1 summarizes the effect of NPs size on the immune response.

Table 8.1 The effect of NPs size on immunological reactions (Azharuddin et al., 2022)

Size (nm)	Material	Context	Immunological reactions
1.5	Gold	*Listeria*	AuNP–LLO (listeriolysin O peptide) plus Advax™ adjuvant induced LLO-specific T cell immunity and protection against *Listeria* challenge
2–50	Gold	Foot and mouth diseases	Specific antibodies were induced by 2, 5, 8, 12, and 17 nm FMDV plus cysteine (pFMDV)–AuNP conjugates. Maximal antibody titer was generated with 8–17 nm conjugates
10–100, 60–350, 400–2500	Bilosome	`Influenza	Larger bilosome particles with influenza A antigens elicited immune responses that had a significantly greater Th1 bias than the small particles
12	Gold	`Influenza	Matrix 2 protein (M2e)–AuNP conjugates induced M2e-specific IgG serum antibodies
20–123	Polystyrene	Respiratory syncytial virus (RSV)	IFN-γ induction from CD8 T cells was limited to 40–49 nm beads, whereasCD4 T cell activation and IL-4 were induced by 93–123 nm beads
30–200	Polystyrene	Tumor	Nanobeads of 40–50 nm effectively induced cellular responses by activating$CD8^+$ T cells with IFN-γ production
40	Gold	Tetanus toxoid	Enhanced tetanus toxoid (TT)-specific IgG (34.53×) and IgA (43.75×) was elicited by TT-ARE-CsAuNPs
100, 500	PLGA	Nicotine	The 100 nm particles induced significantly higher antibodies than the 500 nm particles
200, 500, 1000	PLGA	Bovine serum albumin	A greater IgG response was elicited by 1000 nm particle than by 200–500 nm particles
200–600	PLA	Hepatitis B virus	Hepatitis B virus surface antigen (HBsAg) encapsulated in 2–8 μm particles generated more antibodies than 200–600 nm particles
220, 660, 1990	PMMA Eudragit®	HIV	HIV TAT protein modified NPs of 220 or 630 nm elicit strong TAT-specific cellular immune response but weaker anti-TAT antibody response than NPs of1.99 μm

Antigens transferred by NPs are embodied through multiple endocytic routes. In addition, the charge and functionalization techniques of selected molecules can make the transfer of antigens to APCs for antigen dispensing. Cationic NPs are incorporated by APCs more quickly and assist the transfer of antigens inside the cells by way of endosomal escape (Gao et al., 2019). Some of NPs such as cationic dendrimers loaded with antigens exhibit improved delivery performance of antigens to dendritic cells (DCs), and stimulate DCs including the discharge of cytokines such as IL-12 and IL-1β at the same time (Lu et al., 2015). DCs perform a critical role in the harmonization of the natural and accommodative immune system by antigen uptaking, processing, and dispensing of epitopes to naive T cells (illustrated in Fig. 8.5). Currently used vaccines are exogenic to the cells, which is why DCs play an important role in vaccine-mediated immune responses shown by cell against any diseases.

Different organic nanostructured materials are used to formulate nanovaccines for the safe keeping and transporting of active ingredients. For example, two vaccines for COVID-19 utilize lipid nanoparticles (LNP) for transferring the mRNA that systematizes to detect S-protein (spike protein) of SARS-CoV-2 where NPs function as nanocarriers (NCs) having the size ranging from 50 to 200 nm (Guerrini et al., 2022). Nucleic acid or protein-based nanovaccine, illustrated in Fig. 8.6a, comprises several components like polyethylene glycol (PEG)-lipids, ionizable lipids, structural lipids, and cholesterol. These nanovaccines are designed and developed to bring out functional and dynamic immune responses capable of generating specific antibodies against pathogens. The intramuscular (IM) administration of COVID-19 nanovaccines confirms an effective biodistribution and builds local reactogenicity that provides entire immunogenicity. After IM injection, the nanovaccine reaches the lymph nodes (Fig. 8.6b, (1) and (2)). Eventually, the objective of all nanovaccines is the well-controlled delivery of the antigen inside the cell (Fig. 8.6c) for triggering T-cell to support B-cell antibody generation, illustrated in Fig. 8.6b, (6).

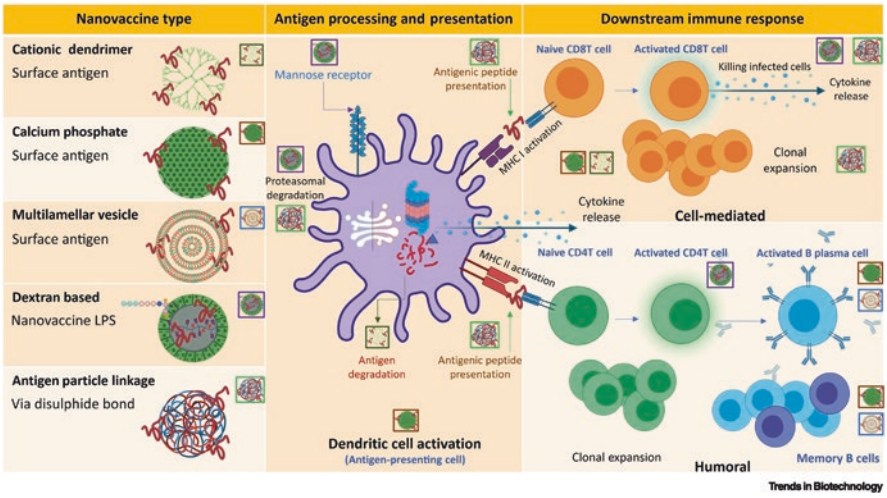

Fig. 8.5 The mode of action of nanovaccines. (The figure has been reproduced from the ref. Azharuddin et al. (2022). Copyright@2022, Elsevier)

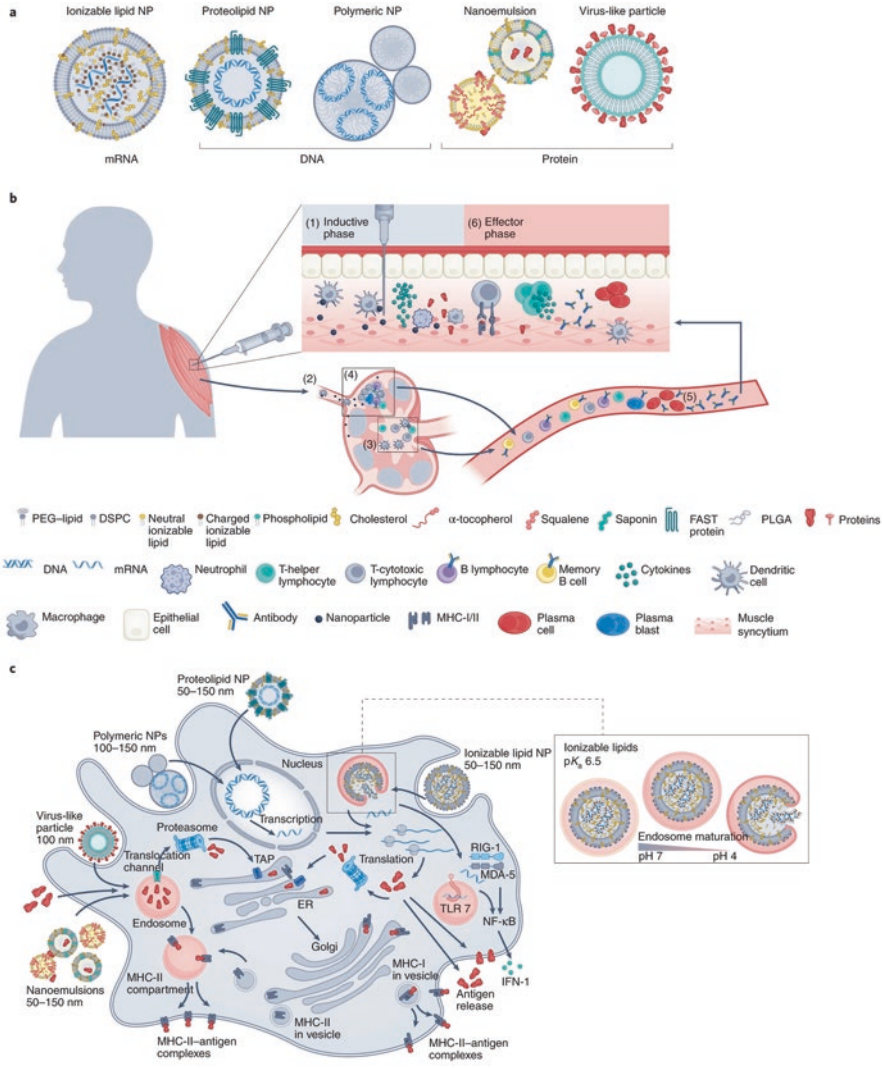

Fig. 8.6 Sketch of the nanovaccine components essential for the treatment of COVID-19, immunomodulatory features, and intracellular destination. (The figure has been reproduced with permission from ref. Guerrini et al. (2022).Copyright@2022, Springer Nature)

The modified metal oxide-based nanostructured materials are used for formulating antitumor vaccines (Chattopadhyay et al., 2016). The cobalt oxide (CoO) nanomaterials, carefully modified by N-phosphonomethyliminodiacetic acid (PMIDA), induce an antitumor immune response (illustrated in Fig. 8.7). The metal oxide nanovaccine can activate macrophage (MΦ) evidenced by tumor necrotic factor alpha (TNF-α) and interferon-gamma (IFN-γ)-level increment.

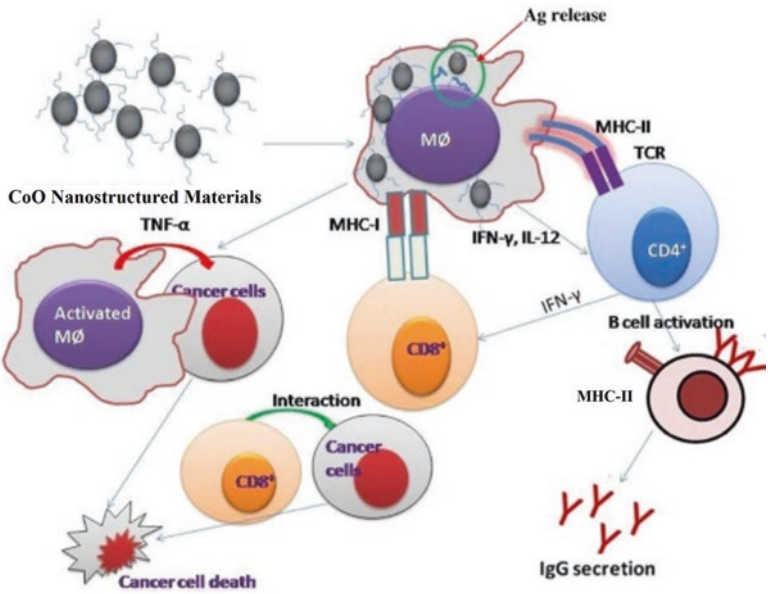

Fig. 8.7 Probable mechanism of PMIDA-modified CoO-based nanostructured materials as anti-tumor vaccine. (The figure has been reproduced with permission from ref. Chattopadhyay et al. (2016). Copyright@2016, Elsevier)

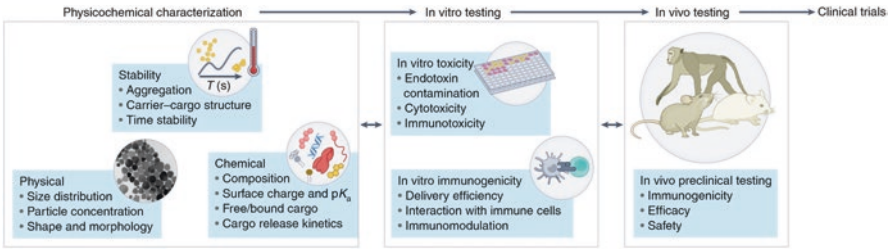

Fig. 8.8 Characterization strategy for nanovaccines. Combination of assays: physical, chemical, stability, in vitro immunogenicity, in vitro toxicology, and in vivo preclinical testing. (The figure has been reproduced with permission from ref. Guerrini et al. (2022). Copyright@2022, Springer Nature)

8.3 Characterization of Nanostructured Materials for Nanovaccines

The characterization of nanostructured materials should be conducted thoroughly in order to evaluate the properties, efficacy, and safety of nanovaccines before clinical practice by assessing stability, physical, and chemical characteristics. Before clinical trials, all the assessments must be performed by in vivo and in vitro testing (Fig. 8.8). Particle-size distribution of nanomaterials influences and determines

biodistribution and immunomodulation of the nanocarriers and active ingredients of nanovaccines. LNP-mRNA NPs of 64 nm and 146 nm both are able to activate immunoglobulin G (IgG) titer in mice. However, the activation performance of 146 nm particles is better than that of particle size 64 nm. Dynamic light scattering (DLS) or multiangle light scattering (MLS) methods are preferably employed to determine particle size, which is very effective for primary screening. Other approaches like nanoparticle tracking, ultracentrifugation, tunable resistive pulse sensing, and transmission electron microscopy (TEM) may provide the required data for the optimization and selection of suitable nanostructured materials for effective nanovaccine formulation. Chemical characterization of nanostructured materials is essential for safe and successful nanovaccine preparation. This process requires meticulous sample preparation and analytical procedures. Commonly used liquid chromatography (LC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy can provide the required data to evaluate chemical characterization. Electrophoresis coupled with capillary electrophoresis and MS or MS detector can provide information on protein integrity and molecular weight, concentration, disulfide bonds, aggregation, and glycosylation of nucleic acid for accomplishing protein sequence and post-translational modifications.

8.4 Stability Testing of Nanostructured Materials

The transportation and storage of nanovaccines are a great concern. Advanced technology is applied to manufacture stable nanovaccine that can withstand higher temperatures. Protein-based nanovaccine may lose its potency due to protein antigen unfolding. To avoid this degradation, the thermal decomposition and stability of protein antigens can be tracked by differential scanning calorimetry (DSC) and circular dichroism. So all the qualities of the nanomaterials must be evaluated and monitored at different timeframes by putting the samples under practical transportation, storage, and application conditions. The transformation from laboratory to practical applications proceeds through batch-to-batch constant assessment. Various nanostructured materials and their diverse physicochemical characteristics may alter the efficiency of nanovaccines. So, it is essential to appropriately select nanomaterial for serving specific quality for manufacturing nanovaccine with reproducible potency, safety, and bioavailability.

8.4.1 In Vitro Immunostimulation and Toxicology Testing

The composition, modification, and optimization of nanostructured materials affect the in vitro delivery effectiveness, immune cell interaction, and immunomodulatory characteristics of nanovaccines. Nanomaterials play a vital role in activating antigen-presenting cells (APCs) that regulate induction and initiation of

immunologic reactions. The antigen transportation and interaction of NPs are crucial and must be examined to optimize the nanovaccine for initiating cellular and humoral responses. Currently, unique methods are used to investigate the immunologic reaction in vitro. For example, tissue engineering can provide the platform to regenerate an in vitro model of human organs that replace the living models to study the working principles of nanoparticle aggregation and to conduct a toxicological assessment (Cupedo et al., 2012) and immunological investigation (Wagar et al., 2021).

Toxicological profiling of nanostructured materials proposed for nanovaccines formulation is an essential step in biocompatibility assessment. Toxicological estimation can be conducted in vitro by applying the approved standards (ISO 29701:2010, ISO 10993-22, and ASTM E2526-08), though specific and appropriate target organs or cells are highly appreciated. Specific and appropriate target cells, including immune cell subtypes (T cell, B lymphocyte, and human monocyte), blood cells (peripheral mononuclear), and entire blood cells, should be recommended to establish clear exposure–response relationships (Crist et al., 2013; Haile et al., 2017; Camera et al., 2021). More specifically, peripheral blood cells (especially mononucleates) are felicitous cells for a micronucleus study that is a prerequisite for the risk estimation of any kind of nanostructured materials selected as components and excipients for nanovaccines formulation. Usually, nanovaccines are administered IM, and they interact with blood. So, blood immunotoxicity and hematotoxicity of nanomaterials are required to be estimated by the following standard in vitro test techniques (ISO/TR 10993-22:2017, ISO 10993-4, and ASTM E2524-08) developed for nanostructured materials used for biomedical and healthcare purposes. Systematic dose-response assessment is typically worthy for bioformulations because of prospective hypersensitivity responses (Szebeni & Moghimi, 2009).

8.4.2 In Vivo Preclinical Testing

The tenacity and biodistribution of nanostructured materials are determined by in vivo imaging methods that facilitate the optimization of nanovaccines (Pardi et al., 2015; Tan et al., 2020; Ciabattini et al., 2021). The potency of nanovaccine depends on antibody neutralization. In vivo preclinical testing is conducted in mice to investigate the safety and protective role since no united safety gateway is designed and constructed for humans. Challenge-protection investigations for tracking the impact of the defection of the pathogen in aimed organs and any changes that occur in the body linked to pathological conditions need to be studied.

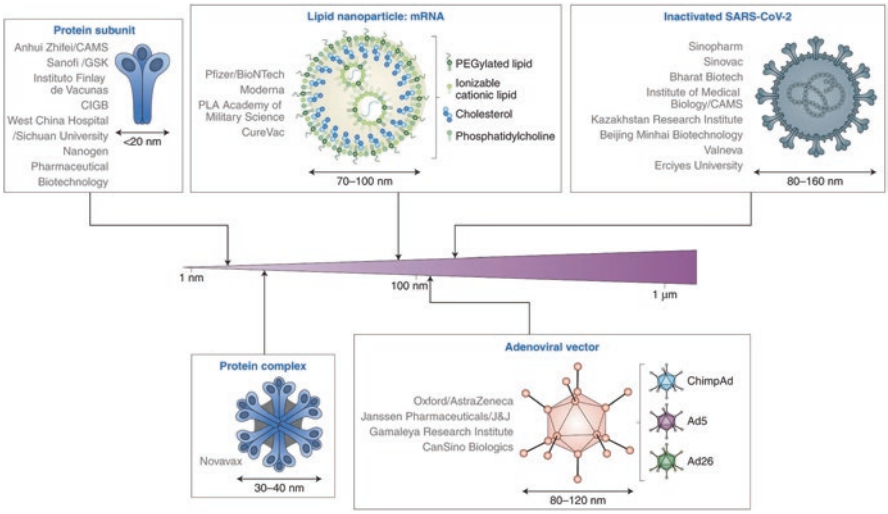

Fig. 8.9 Some nanovaccines for COVID-19 treatment. (The figure has been reproduced from ref. Kisby et al. (2021).Copyright@2021, Springer Nature)

8.5 Achievements of Nanovaccines and Remaining Challenges

Notably, most vaccines depend on natural or synthetic vector systems composed of nanostructured materials (Kisby et al., 2021). Almost all vaccine candidates in Fig. 8.9 fall in between the nanosize range. The advancement of nanostructured materials has created the opportunity to produce an endurable and efficient mRNA transfer mechanism composed of complete and perfect LNPs from decade-old liposome research (Kon et al., 2022; Higuchi et al., 2022; Albertsen et al., 2022). Now, the mRNA-LNPs based nanovaccines are more versatile, powerful, stable, and effective. Adenovirus particles stay persistently within the nanostructured materials and can be engineered to facilitate a suitable platform with intrinsic immunogenicity for effective vaccination. This type of vector's thermal stability is superior to that of the mRNA systems (Ripoll et al., 2022; Li et al., 2021).

Though there is a remarkable advancement in nanotechnology-based vaccines, some challenges still exist and need to be addressed. The thermal stability of mRNA cargo is poor. So, additional optimization of the nanostructured materials is required to be most pertinent for administration, create target-oriented immune activation, and extend the effect's duration. Sometimes, higher production cost is an issue for underdeveloped or developing countries. So, effective but low-priced alternatives should be introduced to the market. Ambiguous durability, duration of immunoprotection, and the reason for hypersensitivity of nanovaccines are required to be addressed and clarified wherever necessary. Further development is essential for selecting and optimizing adjuvants and antigens to improve the efficacy of nanovaccines (Fries et al., 2021).

8.6 Nanovaccines in Clinical Use and in Clinical Trials

Very few nanovaccines have been successfully transformed from the laboratory version to the clinical version. Among these clinical versions mostly trigger humoral responses only; however, it is critical to design and develop vaccines that are able to produce robust cellular responses against cancer and other infectious diseases. Vaxfectin® is a cationic liposomal nanovaccine that is under clinical trials at present. Vaxfectin® has been successfully employed for the treatment of herpes simplex virus type 2 (HSV-2) as well as influenza virus (H5N1). One more clinical version of nanovaccine is Inflexal® V, which has been utilized to treat influenza. For the treatment of cancer, another nanovaccine called Stimulax® is currently administered. Recently, substantial focus has been given to nanostructured materials for the development of potential vaccines for the control and eradication of COVID-19 (shown in Fig. 8.10).

In addition to COVID-19, the utilization of nanovaccines in the treatment of various diseases is quite common indeed. Many of such vaccines have been approved

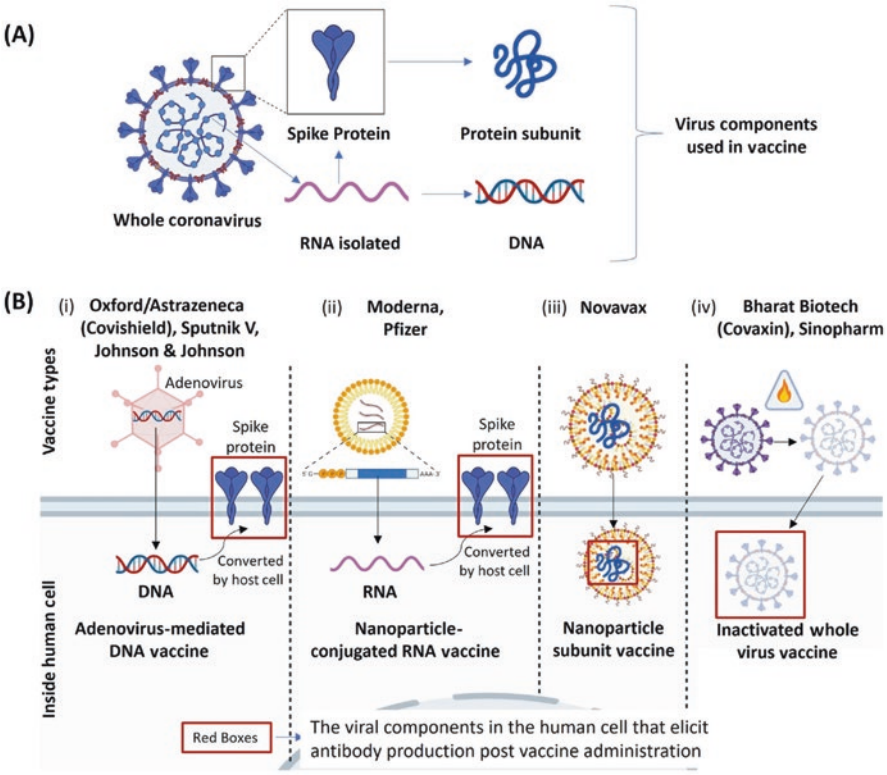

Fig. 8.10 Strategies for the development of nanovaccines against SARS-CoV-2. (The figure has been reproduced from the ref. Azharuddin et al. (2022). Copyright@2022, Elsevier)

by the respective authorities including the European Medicines Agency (EMA), FDA, and so on while many are under clinical trials at present. A list of such vaccines is provided in Table 8.2.

8.7 Future of Nanovaccines

Variations in the effectiveness of immunization have been observed across different demographic groups, including different age groups (such as young or adult individuals), patients with diabetes or without diabetes, males and females, and other categories during the development of various COVID-19 vaccines. The involvement of nanotechnology can offer an arrangement of the latest strategies to potentially develop a periodic vaccine where one infection may potentially facilitate other infections. For instance, influenza infection can induce bacterial super-infection and pneumonia. Similarly, coinfection with influenza A virus can intensify the infection created by SARS-CoV-2. Still, there are scopes to design and develop more effective and versatile nanovaccines with multiple epitopes and/or adjuvants to elicit a wide range of immune responses. Nanotechnology products may offer the best possible non-viral strategy to enclose and transfer nucleic acids. However, the thermal instability of vaccines remains an unsettled issue. It is undeniable that the natural immune system is uniquely composed of different individuals, and a general purpose approach is not a sustainable solution, where nanovaccines may play a pivotal role in the development of a new candidate of personalized vaccines for multifaceted and sustainable protection against catastrophic diseases. Figure 8.11 schematically illustrates the idea of future nanovaccines.

8.8 Comparative Study of NPs Suitable for Vaccine Development

The key features of the nanostructured materials used for the fabrication of nanovaccine have been condensed in Table 8.3 (Rosales-Mendoza & González-Ortega, 2019). The following table summarizes the required information such as the ease of synthesis, price, biocompatibility, FDA approval for medical use, and potentiality for utilization in clinical trials regarding the nanostructured intent to use in nanovaccine formulation. Gold NPs (AuNPs) have been potential applications in drug delivery, sensing, and imaging since they were first synthesized in 1951. PLGA NPs are basically utilized in drug delivery systems since their approval by the US FDA for biomedical implementations. In the vaccinology field, PLGA NPs as drug delivery systems in parenteral administration have been accepted by the US FDA and EMA (Nimesh, 2013). AuNPs-based vaccines are manufactured for the treatment of tumors (Trabbic et al., 2021). Chitosan-functionalized AuNPs (CsAuNPs) have

Table 8.2 The list of nanovaccines that are already approved or under clinical trial phase (Azharuddin et al., 2022)

Institution	Vaccine	Antigen	NPs	Trial stage
Moderna and NIAID	mRNA-1273 LNP	mRNA-1273 mRNA	LNP with mRNA encapsulated	Phase I/II/III
BioNTech and Pfizer	mRNA BNT162b2	mRNA encoding the trimerized RBD ofSARS-CoV-2	LNP with mRNA encapsulated	Phase I/II (UTRN) Phase I/II (Germany) Phase II/III (USA) Phase I (Japan)
Novavax	NVX-CoV2373	Full-length SARS-CoV-2 S glycoprotein	Recombinant glycoprotein NP saponin-based Matrix-M1 adjuvant	Phase I/II/III
Imperial College, London Acuitas Therapeutics, Vancouver	LNP-nCoV saRNA ARCT-021	saRNA and pre-fusion stabilized SARS-CoV-2 S protein	LNP with saRNA encapsulated	
Suzhou Abogen Biosciences Walvax Biotechnology and People's Liberation Army	ARCoV	mRNA encoding RBD of SARS-CoV-2 S glycoprotein	LNP with mRNA encapsulated	Phase I
Novavax		ARS-CoV S protein and influenza M1 protein	SARS-CoV VLP nanovaccine	Preclinical
Imophoron and Bristol University		Multiepitope display	VLP ADDomer™	Preclinical
Crucell	Inflexal®V	Influenza	Virosome with influenza virus surface antigens (hemagglutinin and neuraminidase)	Phase III completed
Crucell	Epaxal®	Hepatitis A	Virosome with inactivated virus particles	Phase III completed
Merck	Gardasil®9	HPV	Capsomere (major capsid protein L1)	Completed
Dendreon Pharmaceuticals	Provenge (Sipuleucel-T)	Prostate cancer	Each dose of contains a minimum of 50 million autologous CD54* cells activated with PAP-GM-CSF	Phase III completed

(continued)

Table 8.2 (continued)

Institution	Vaccine	Antigen	NPs	Trial stage
Novavax	NanoFlu™	Influenza	Recombinant HA protein on Tween 80 NP with Matrix-M adjuvant	Phase III
Novavax	EBOV GP Vaccine	Ebola	2014 Guinea Ebola virus recombinant glycoprotein on Tween 80 NP with/without Matrix-M adjuvant	Phase I
DAIDS/NIAID/NIH	MPER-656	HIV	HIV-1 gp41 membrane proximal external region (MPER) with liposomes	Phase I
BioNTech	W_ova1	Ovarian cancer	Liposome-formulated mRNAs. Three ovarian cancer tumor-associated antigens in combination with (neo-) adjuvant chemotherapy	Phase I
ImmunoVaccine Technologies	DPX-0907	Ovarian, breast, and prostate cancer	Liposomes with seven tumor-specific HLA-A2-restricted peptides, a universal T helper peptide, and a polynucleotide adjuvant in Montanide ISA51 VG	Phase I
Merck	Tecemotide	Multiple myeloma	Liposomes with tecemotide lipopeptide and 3-*O*-deacyl-4′-monophosphoryl lipid adjuvant	Phase II
Cascadian Therapeutics	ONT-10	Solid tumor	Liposomal MUC1 cancer vaccine	Phase I
XEME Biopharma	Oncoquest™	Follicular lymphoma Chronic lymphocytic leukemia	Liposomes containing autologous tumor-derived antigen and IL-2	Phase I/II
Lipotek Pty	Lipovaxin-MM	Metastatic melanoma	Multicomponent liposomes containing tumor antigens (gp100, tyrosinase, and melanA/MART-1) with DC-targeting moiety DMS-5000	Phase I

Abbreviations: *ARE Asparagus racemosus* extract, *CsAuNPs* chitosan-functionalized AuNPs, *F* RSV fusion protein, *HA* influenza virus hemagglutinin, *HPV* humanpapillomavirus, *LNP* lipid nanoparticle, *melanA/MART-1* melanoma antigen recognized by T cells, *MUC1* mucin 1, *NSCLC* non-small cell lung cancer, *PAP-GM-CSF* pulmonary alveolar proteinosis granulocyte macrophage colony-stimulating factor, *RBD* receptor-binding domain, *RSV* respiratory syncytial virus, *S* SARS-CoV-19 spikeprotein, *saRNA* self-amplifying mRNA, *VLP* vaccine-like particle

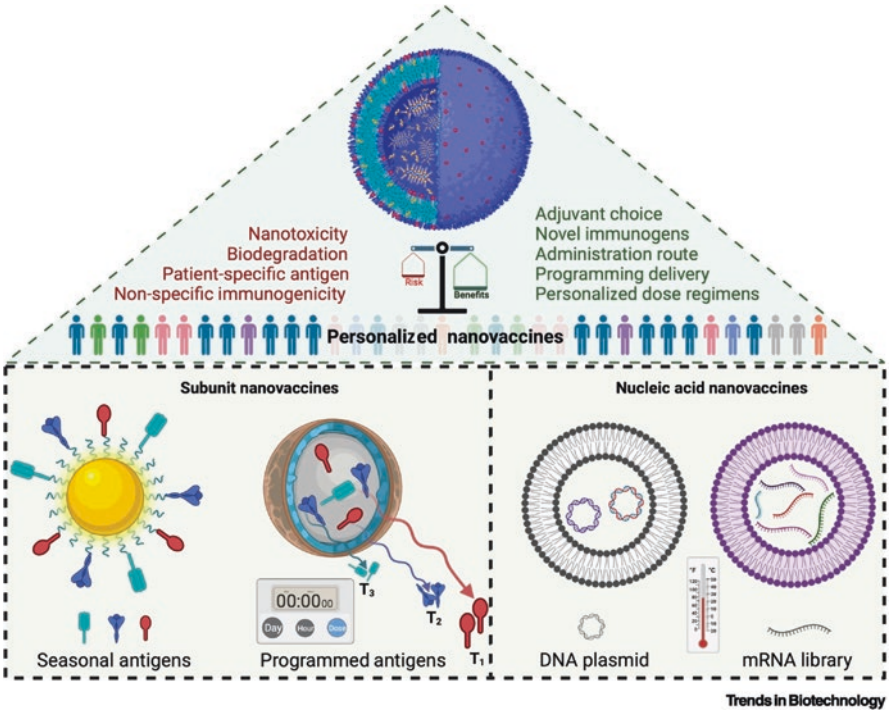

Fig. 8.11 The future of nanovaccines: personalized vaccines from nanostructured materials. (The figure has been reproduced from the ref. Azharuddin et al. (2022). Copyright@2022, Elsevier)

Table 8.3 Comparative analysis of the nanostructured materials employed in the design and development of nanovaccine (Rosales-Mendoza & González-Ortega, 2019)

Nanomaterial	Ease of synthesis	Cost	Biocompatibility	FDA approval for medical use	Used in clinical trials
Gold	Simple	High	Moderate	No	No
PLGA	Moderate	High	High	Yes	Yes
Silica	Moderate	Medium	Moderate	No	No
Carbon Nanotubes	Moderate	Low	Moderate	No	No
Chitosan	Simple	Medium	High	No	Yes
Liposomes	Hard	High	High	Yes	Yes
Nanogels	Simple	Medium	High	No	Yes
Virus-like particles	Hard	Low	High	Yes	Yes

been used for the oral delivery of tetanus toxoid (TT) where the NPs are 40 nm in diameter (Barhate et al., 2014). In this case, soluble triterpene glycosides processed from *Quillaja saponaria* (QS) have been used as adjuvants for the treatment of tetanus. AuNP-based nanovaccine has been formulated for the treatment of influenza

(Wang et al., 2018). Recombinant trimetric influenza A/Aichi/2/68 (H3N2) hemagglutinin (HA) has been combined with 18 nm AuNPs through a metal-chelating chemical process.

Most nanovaccines adsorbed in PLGA NPs are intended to treat human and animal diseases (Gu et al., 2019; Chudina et al., 2015). PLGA NPs containing HBsAg having trehalose and $Mg(OH)_2$ as stabilizers have been incorporated in vaccines for oral immunization (Mishra et al., 2011). PLGA NPs have been utilized to formulate the *Helicobacter pylori* vaccine (Tan et al., 2017).

Silica NPs can be altered chemically or physically to incorporate antigens or adjuvants. The toxicity of silica NPs is still a controversy. Hollow mesoporous silica nanoparticles (HMSNPs) have been used to formulate vaccines for cancer (Lee et al., 2020) and tuberculosis treatment (Montalvo-Quirós et al., 2020). Currently, a vaccine candidate against enterohemorrhagic *E. coli* O157:H7 is being developed by utilizing the EspA protein produced in recombinant *E. coli* as the selected antigen. rEspA has been entrapped onto Silica NPs having a diameter of 96 nm (Hajizade et al., 2018).

Carbon nanomaterials especially carbon nanotubes have been used as drug-delivery vehicles since these materials can be functionalized to introduce carboxylic (–COOH) or amino ($–NH_2$) groups for attaching antigens, adjuvants, or ligands to formulate nanovaccines (Holmannova et al., 2022; Bavandpour et al., 2020; Sawutdeechaikul et al., 2019).

Chitosan is a polymer that contains positively charged moieties derived from the d-glucosamine units. It is produced commercially by deacetylating chitin, which is the structural component of the exoskeleton of shrimp and crab, using NaOH (Rinaudo, 2006). Chitosan is nontoxic, biodegradable, and biocompatible since it is derived from natural sources; however, it is not approved by the US FDA. Chitosan NPs (CsNPs) have been extensively studied as drug delivery systems, especially for protein and gene delivery purposes. CsNPs containing nanovaccines have been investigated for the delivery of antigens and proteins especially for intranasal and oral immunization. Multiple attempts have already been taken and CsNPs-composed vaccines have been developed for the treatment of COVID-19 (Safer & Leporatti, 2021), E. coli involved diseases (Mohammed et al., 2021), Rift Valley Fever or tetanus (Gao et al., 2021), and Avian Coronavirus (Lopes et al., 2021).

Nanogels are common hydrogel NPs that are stimuli-responsive and smart nanostructured materials. They have been employed for diverse biomedical applications including nanovaccinology applications to transfer proteins or oligonucleotides-based antigens (Basu et al., 2021). Injectable sustained-release hydrogel NPs based vaccines have been formulated for COVID-19 treatment from Cowpea mosaic virus (CPMV), a plant virus (Nkanga et al., 2022). CPMV is a potential immunogenic adjuvant that is very much promising for the development of nanovaccines against infectious diseases and cancers. Hydrogel NPs modified with polyethylenimine functionalized graphene oxide (GO)-based RNA nanovaccines have been reported for sustainable cancer immunization (Yin et al., 2022). Supramolecular polymer hydrogel NPs have been employed to enhance the performance of influenza vaccines (Roth et al., 2021).

Virus-like particles (VLPs) are considered common platforms for vaccine development. Currently, multiple vaccines including hepatitis B virus (HBV) and human papillomavirus (HPV) vaccines are produced and marketed worldwide. An expandable, durable, and highly immunogenic VLP-based nanovaccine effective against SARS-CoV-2 has been developed by genetically fusing the receptor-binding motif (RBM) of the spike protein from SARS-CoV-2 into cucumber mosaic virus ($CuMV_{TT}$) (Mohsen et al., 2022).

8.9 Perspectives and Opportunities for Nanostructured Materials in Vaccine Arena

In general, each nanostructured material has both pros and cons and an extensive study of each offers a major advance in vaccine development. For the appropriate implementation of nanostructured materials as a successful candidate for vaccine formulation, adequate information such as exploiting immunology advances, employing bio-nanofabrication methods, and expanding the use of nanocomposites is crucial to select the best-fitting nanomaterial that is capable of serving the intended objectives. Understanding the immunologic reaction mechanism is essential to design and develop a successful vaccine. The immune system associated with protective and therapeutic effects is a complex biological network. The recent advancements in our understanding of regulatory T-cells have established a reliable platform for vaccine development.

The implementation of bio-nanofabrication may facilitate the vaccine formulation pathways with the help of nanostructured materials obtained through biocompatible synthetic routes. Numerous methods already have been established for the fabrication of metallic NPs using bioextracts from plants and algae. In the case of bio-nanofabrication approach, plant extracts act as natural reducing agents for biocompatible NPs synthesis that may be suitable for nanovaccine formulation. Bio-nanofabrication is successfully implemented to synthesize multiple metal and metal oxide NPs including Se, Ag, Au, ZnO, and TiO_2 (Agarwal et al., 2019). Microorganisms like fungi, bacteria, and yeast are also potential candidates for the synthesis of NPs applicable in vaccine formulation (Ahmed et al., 2017; Hulkoti & Taranath, 2014).

Expanding the utilization of nanocomposites (NCs) composed of complex combinations of nanostructured materials can result in extraordinary physicochemical features, leading to novel functional characteristics. In vaccine sectors, several composites have been explored, for instance, AgNPs/silica (Zhao et al., 2016), AuNPs/silica (Nguyen & Shen, 2016), and poly(glycerol adipate-co-ω-pentadecalactone) (PGA-co-PDL) polymeric nanoparticles (NPs) within L-leucine microcarriers (Rodrigues et al., 2018). Synthesis of polymer NCs may open possibilities to create multifunctional nanostructured materials having expected immunogenic activity, which may establish a strategy for future nanovaccines.

8.10 Concluding Remarks

Majority of the population is not familiar with the utilization of nanotechnology products. As nanovaccines are prepared using nanostructured material vectors, it may be challenging for people to readily accept multiple doses. The unfamiliarity with nanovaccines may lead to immoderate perspectives and conspiracy theories. That is why awareness, rumination, comprehension, and easy transmission of the scientific and clinical data produced from unparalleled and deliberate manifestation of nanostructured materials to the public are required. It should always be remembered and practiced that the safety of patients should never be compromised. The development of nanovaccines relies on the perspectives of regulators, ethics reviewers, inventors, and investors in nanotechnology for its introduction in biomedical applications. The outstanding development in the designing of antigen may provide multifunctional platforms of nanostructured materials for specifying immune responses that are effective and appropriate for protection against cancer, HIV/AIDS, malaria, TB, COVID-19, and many other infectious diseases. Once the proper and specific immunogens are identified, multiple platforms may provide nanovaccines with better thermal stability and environment friendly to facilitate distribution throughout the most resource-limited areas of the world.

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