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Review article

Bioinspired nanocarriers for viral nanovaccines: A review on recent research, challenges, and future trends

Amira M. Heniedy[1,*], Nabila A. El-Sheridy[2] and Nessrin Saleh[3]

[1] *Egyptian Ministry of Agriculture and Land Reclamation, El-Beheira, Egypt*
[2] *Research and Development Section, European Egyptian Pharmaceutical Industries, Alexandria, Egypt*
[3] *Alexandria Directorate of Health Affairs, Ministry of Health and Population, Alexandria, Egypt*

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Corresponding author*:
Amira M. Heniedy
Email: Amiraheniedy@hotmail.com

Abstract: Bioinspired nanocarrier-based viral vaccines represent a rapidly developing vaccine technology. During the COVID-19 pandemic, multinational companies competed to develop safe and effective vaccines within a short timeframe, leveraging nanocomponents that could induce both humoral and cellular immunity for a broad immune response with minimal adverse effects. Notably, many bioinspired nanocarrier-based vaccines developed during the pandemic period surpassed the efficacy of traditional vaccines. Bioinspired nanocarriers have been clinically designed to be loaded with genetic materials, antigenic proteins, and antiviral drugs using various molecular and nanotechnological strategies. After enduring the lengthy development timelines of traditional viral vaccines, scientists have utilized machine learning and artificial intelligence tools to minimize the development time and cost of nanovaccines. Nevertheless, there is currently no clear vision of how multidisciplinary in vivo/in vitro research studies can enhance the efficiency of bioinspired nanocarrier-based viral nanovaccines to meet the standards of health technology assessment. This review article demonstrates how laboratory-produced and genetically engineered natural-based nanocarriers are ready to penetrate vaccine markets and achieve the delicate balance between efficiency and cost in emergent crises. The merits and demerits are discussed insightfully, providing futuristic perspectives on the safety, acceptability, and competitiveness of nanotechnology-based next-generation vaccines.

List of frequent abbreviations

ADA	Anti-drug antibody
AI	Artificial intelligence
BGs	Bacterial ghosts
BSA	Bovine serum albumin
ChAdOx1 nCoV-19	Chimpanzee Ad-based vector
cccDNA	Covalently closed circular DNA
CMC	Critical micelle concentration
CRISPRs	Clustered regularly interspaced short palindromic repeats
HBV	Hepatitis B virus
HA	Hemagglutinin
HSA	Human serum albumin
HC	Hemolysis confident
HIV	Human immunodeficiency virus

HPV	Human papillomavirus
H. pylori	Helicobacter pylori
HVJ	Hemagglutinating virus of Japan
Ig-HIV	Immunoglobulin-based HIV biologics
LAB	Lactic acid bacteria
L-HSA	Lactosaminated-human serum albumin
IM	Intramuscular
IN	Intranasal
ML	Machine learning
MERS-CoV	Middle East respiratory syndrome coronavirus
NPs	Nanoparticles
NA	Neuraminidase
NAb	Neutralizing antibodies
NDV	Newcastle virus
RNAi	RNA interference
RV	Reverse vaccinology
TPP	Target product profiles
VoCs	Variants of concern
VSV	vesicular stomatitis virus
VLPs	Virus-like particles
WHO	World Health Organization

1. Introduction

Viral infections cause numerous mortalities worldwide each year [1]. Although all efforts of the WHO to control viral infections through vaccine programs, epidemiological studies, and healthcare funding have been successful, there are limitations in providing effective protection against viral mutations and complications [2]. Scientists have found that traditional artificial immunizations cannot keep up with the spread of viruses. Many biotechnologies, such as nanomedicine and genetic engineering, should be exploited to produce efficient delivery systems to provide long-lasting immunity and increase the specificity of vaccines [3]. Eco-friendly vaccination development is the next generation of vaccination, aiming to use natural-based techniques for the production and coverage of vaccines to protect people and the environment from the adverse effects of vaccines [4, 5]. In addition, vaccine cost and cost-effectiveness are key socioeconomic factors that affect the availability and acceptability of vaccines in developing countries [6]. During emergencies, vaccine development should be fast, efficient, affordable, and widely accepted and commercialized globally to manage disease outbreaks [7].

Traditional vaccines are less efficient and more challenging to produce, require more time to prepare, and can cause hazardous post-vaccination reactions [8]. Canadian researchers conducted a comprehensive analysis of the TPP to compare the COVID-19 vaccine strategy. They developed a comprehensive profile addressing safety, efficiency, logistics, and cost of goods sold. Inactivated virus vaccines had a lower TPP score (71/100), while protein-based vaccines and viral vectors showed higher scores of 80/100 and 74/100, respectively [9]. Most conventional vaccines cannot provide long-lasting immunity and require booster doses to provide effective protection. Moreover, they are produced in low yield and may cause allergic reactions after immunization, in contrast to virus-like particle vaccines that have a faster manufacturing process, are produced in higher yields, and contain no allergens [10]. From a market perspective, traditional vaccines are affordable and competitive in emergencies, particularly given the need for frequent doses. For instance, viral vector-based COVID-19 vaccines, such as AstraZeneca and Johnson & Johnson, received substantial public finding by being sold globally at low prices per dose [11].

Bioinspired nanocarriers are nanomaterials derived from biological sources, and have been recently used in vaccine development. These natural products induce the immune response and protect drugs from being wiped out by

macrophages or destroyed by body conditions, such as pH and temperature [12]. They can be prepared in the laboratory as biosurfactants and bacterial ghosts, naturally extracted as animal and human proteins, or as genetically engineered nanosystems (Figure 1) [13]. Bioinspired nanocarriers and nanoadjuvants offer a novel approach to next-generation vaccine technology, providing lower hazards compared to traditional vaccines, which can be fatal in some cases [14]. Their use in viral vaccine preparation encompasses a range of mechanisms, including the delivery of antigenic proteins or viral genes, self-assembly with NPs or liposomes, enhancement of antiviral drug stability and solubility, enabling active targeting, prolonged circulation time, controlled drug release, and inducing broad immunity [15]. These technologies have demonstrated efficacy in targeting mutations and strain variants in a single vaccine, controlling several viruses with a single vaccination dose, and inducing humoral immunity as nanoadjuvants while minimizing side effects [16, 17]. Nanovaccine formulation relies on nanocarriers to enhance the bioavailability, specificity, and biocompatibility of these nanoformulations with minimal nano-hazard [18]. According to the Brighton Collaboration Viral Vector Vaccines Safety Working Group, no significant adverse effect has been identified following the administration of the recombinant adenovirus vaccine [19].

Furthermore, using specific ligands with nanocarriers enhances vaccine biocompatibility and reduces rapid clearance from the body by macrophages [20]. For instance, adding a receptor-binding domain to the SARS-CoV-2 spike ferritin NP vaccine induces post-vaccination immunity that lasts for 7 months in mice [21]. In comparison with the recombinant adenovirus-vectored COVID-19 vaccine, which reached peak immune response 28 days after immunization, the ligated nanocarrier is more advantageous [19]. Recently, researchers reported that ML and AI can be utilized to predict more efficient antiviral drugs, candidate proteins, and potential epitopes [22]. Robots and AI-based models play a critical role in designing less toxic delivery carriers for next-generation vaccines [23]. This review article provides an insightful discussion of the use of laboratory-produced and genetically engineered natural-based nanocarriers that are penetrating vaccine markets as alternatives to traditional vaccines. Furthermore, the benefit-to-risk ratio of nanotechnology-based next-generation vaccines is thoroughly examined, offering a futuristic vision of safety, acceptability, and cost-competitiveness in vaccine markets.

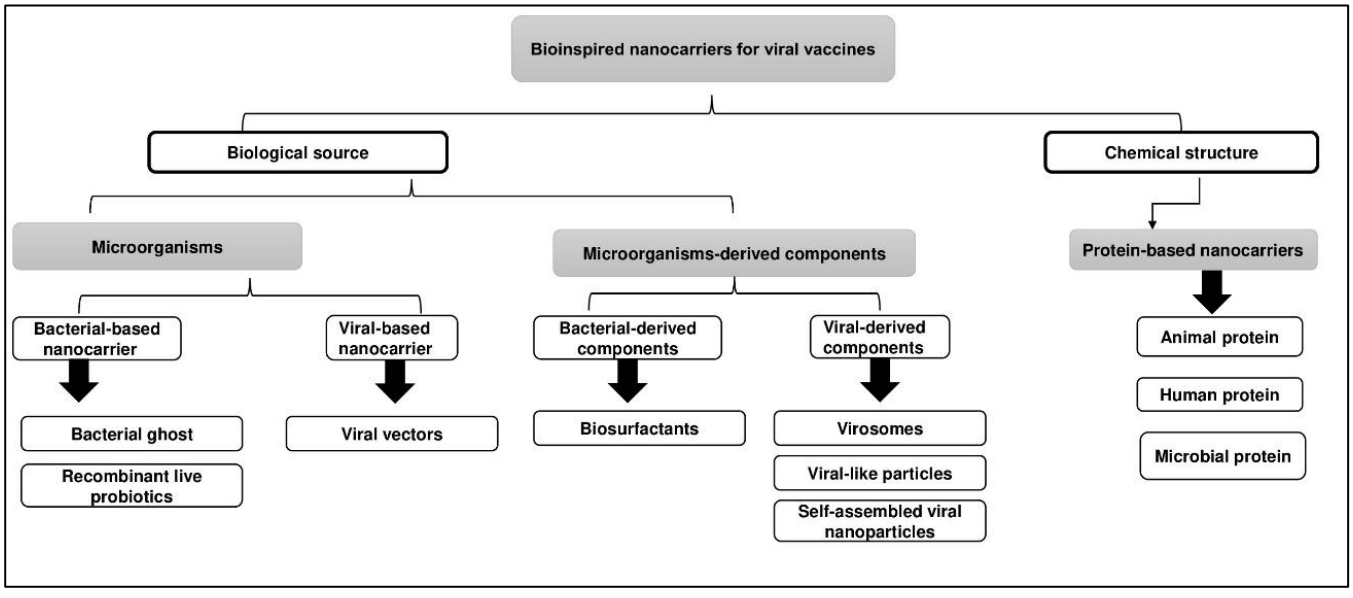

Figure 1. Schematic diagram illustrating the classification of bioinspired nanocarriers for viral vaccination.

2. Microorganisms as nanocarriers and eco-friendly viral vaccine

2.1. Bacterial-based nanocarrier

2.1.1. Bacterial ghost

Recent studies have shown that cell envelopes derived from Gram-negative bacteria called bacterial ghosts (BGs) could be implemented as nanocarriers for viral genetic material [24], viral protein subunits, and viral antigens [25]. Additionally, it could be used as a vaccine adjuvant to induce immunity. BGs deliver protein vaccines in two ways. First, the antigen and protein targets are surface displayed on BGs via genetic engineering, which enhances their immunogenicity and targeting. Second, by co-incubating BGs and the antigen in a non-recombinant BGs mixture. In BGs, outer membrane, periplasmic space, and inner membrane proteins can elicit immunogenicity due to their antigenic properties. DNA vaccines were delivered using *Lactobacillus casei* bacterial ghosts. These bacterial ghosts presented DNA to antigen-presenting cells, specifically macrophages, thereby initiating and regulating immune responses. Loading the antigen protein of porcine rubulavirus into *Lactobacillus casei* BGs significantly increased the expression of tumor necrosis factor-α, inducible nitric oxide synthase, and interleukins in macrophages [26]. In another aquatic study, immunization involved using an *E. coli* bacterial ghost carrying the pEG-G plasmid to combat spring viremia of the carp virus, known for its high mortality rates (nearly 90%) in juvenile carps. Compared to the naked pEG-G plasmid, the bacterial ghost containing the plasmid triggered a more robust immune response (Figure 2).

Furthermore, the fish exhibited enhanced immunity against the carp virus [24]. The poultry industry faces significant challenges from two viral infections: Newcastle disease and avian influenza viruses. A genetic construct comprising the external domain of the matrix protein 2 and the nucleoprotein gene of the avian influenza virus, along with fusion epitopes and hemagglutinin-neuraminidase proteins of the Newcastle disease virus, was delivered using two distinct strains of *Escherichia coli* BGs (pathogenic and non-pathogenic). Results indicated that while both types of BGs effectively transferred the construct to chicken macrophage-like monocytes, the BG derived from the pathogenic strain demonstrated notably superior performance as a stimulant and delivery system [27]. *Salmonella enteritis* ghosts, which carried the hemagglutinin globular head domain from the H1N1 virus, caused both humoral and cellular immunity in poultry. This approach resulted in dual protective effectiveness against H1N1 influenza and salmonellosis (Figure 2) [28]. In humans, *E. coli* ghosts demonstrated antiviral and antibacterial activity against hand-*E. coli* O157:H7 and foot and mouth disease by eliciting the super immune response (Figure 2) [29].

Sai Gong *et al*. utilized the outer membrane protein of *E. coli* O157:H7 to express antigenic proteins from enterovirus 71 and coxsackievirus, developing a vaccine against hand, foot, and mouth disease. This vaccination induced mucosal immunity by boosting IgG and IgA production. Furthermore, the vaccine candidate protected mice from contracting infections caused by *E. coli* [30]. A *Salmonella typhimurium* bacterial ghost, modified with an antigen delivery and expression vector, was genetically engineered to carry the envelope protein domain III from all four dengue virus serotypes. In a mouse model, these bacterial ghosts with plasmid expression significantly enhanced both humoral and cellular immunity. Upon exposure to all dengue virus serotypes, the vaccinated mice showed reduced viremia levels [31, 32].

2.1.2. Recombinant live probiotics

Mucosal immunity is vital in respiratory viral infections, as it can prevent viral recognition and invasion from the initial point of entry. Probiotic bacterial strains have proved to induce potent mucosal immunity responses, making them promising delivery systems to potentiate the effect of expressed antigens from these recombinant probiotics. This suggests the potential for developing novel vaccines that influence the mucosal immune system, thereby inhibiting virus replication on mucosal membranes [33].

The creation of genetically engineered LAB has been the main focus of research over the past 20 years, as they are live vectors that can be exploited in the production of mucosal vaccines. Respiratory and non-respiratory virus-derived antigens of these mucosal vaccines can be generated by species like *Lactobacillus plantarum* and *Lactobacillus casei* [34]. This type of vaccine is termed a subunit vaccine, which is known for its safety compared to other types, such as inactivated or attenuated vaccines. The use of strong adjuvants

as a probiotic delivery vehicle and careful selection of probiotic species is crucial to elicit the appropriate immune response, as subunit vaccines have limited immunogenicity. Three critical aspects must be studied in this type of vaccine to evaluate its efficiency and applicability: (a) the cellular and molecular pathways engaged in mucosal antiviral defense enhancement, (b) the expression system of heterologous proteins, and (c) the efficient expression of viral antigens [33]. Pre-immunity against SARS-CoV-2 must be induced in the mucosal surfaces, where the virus first replicates, besides the ability to induce both the humoral and cellular defenses. This can be achieved using LAB as both mucosal adjuvants and delivery systems. For example, *Lactobacillus plantarum* develops effective oral vaccines due to its ability to survive the acidic environment of the gastrointestinal tract and colonize the intestinal tract transiently. It positively modulates immunological responses at mucosal tissue sites, both locally in the intestinal mucosa and distantly in the respiratory mucosa through the gut-lung axis [33]. In Golden Syrian hamsters, the oral vaccine expressed omicron antigens successfully, and the expressed antigen was demonstrated to stimulate a strong IgG immune response against the omicron spike protein with no remarkable adverse side effects [35]. Influenza viruses cause the infectious disease commonly referred to as "flu." The annual influenza vaccination is the primary approach to reducing death and morbidity [36]. The NA genes from the influenza virus were integrated into the probiotic strain *Enterococcus faecium* L3 through genetic engineering. When administered orally to test mice, this vaccine elicited a notable boost in mucosal immunity and specific local IgA and serum IgG levels. Additionally, three recombinant *L. plantarum* strains expressing the hemagglutinin antigenic protein from the swine influenza A virus were tested in mice via oral and intranasal administration routes [37]. In mice, the intestinal and upper respiratory tracts, all three strains induced potent mucosal, cellular, and systemic immune responses that offered tolerance against the fatal influenza virus challenge [38].

2.2. Viral-based nanocarriers

2.2.1. Virus vectors

Viral vector vaccines have gained significant attention recently, especially due to their role in combating the COVID-19 pandemic. They use a modified virus (the vector) to deliver the genetic material into cells to elicit an immune response. Their application in vaccine delivery is attributed to some valuable characteristics, including the rapid induction of immune response, single-dose protection, durable protection, rapid development, and flexible administration [39, 40]. Viral vectors are genetically engineered, bioinspired delivery systems that can direct particular virus antigens to particular tissues. They can trigger durable immunological and therapeutic cellular and humoral responses without the need for an adjuvant. "One shot against multiple diseases" is possible when two or more infections are included in a single vector to immunize against multiple diseases. In order to reduce the toxicity of specific replicating viral vectors, viral vectors can be genetically altered to make them replication-deficient [41]. Vesicular stomatitis viruses, adenoviruses, and adeno-associated viruses are frequently employed as viral vectors. A further tactic involved the expression of the immunomodulatory NS5A peptide of the hepatitis C virus using an adeno-associated viral vector. Expression of this peptide decreased T cell activation, which was undesirable, and mitigated the vector's immunogenicity [42]. The adenovirus vector is the most commonly utilized non-replicating viral vector for COVID-19 vaccinations and other vaccines, including influenza and HBV. There are around 300 distinct serotypes of adenovirus, a double-stranded, non-enveloped DNA virus that can infect cells in various tissues [39].

Ad vector vaccines against SARS-CoV-2 have been approved in four different platforms innovated by different companies: Sputnik V, Ad26.COV2-S, Ad5-nCOV, and ChAdOx1 nCoV-19. Ad26.COV2-S is an intramuscularly delivered vaccine comprising a non-replicating human adenovirus type 26 vector containing the SARS-CoV-2 spike protein gene. Preclinical studies have demonstrated its efficacy in protecting rhesus macaques and hamsters against SARS-CoV-2. Similarly, the ChAdOx1 nCoV-19 adenovirus vector vaccine employs a modified non-replicating ChAdOx1 encoding the optimized S protein of SARS-CoV-2 [43].

Based on adenovirus vectors, the Sputnik V vaccine achieved an efficacy of 91.6% in preventing symptomatic infections, as reported in phase-III clinical trials.

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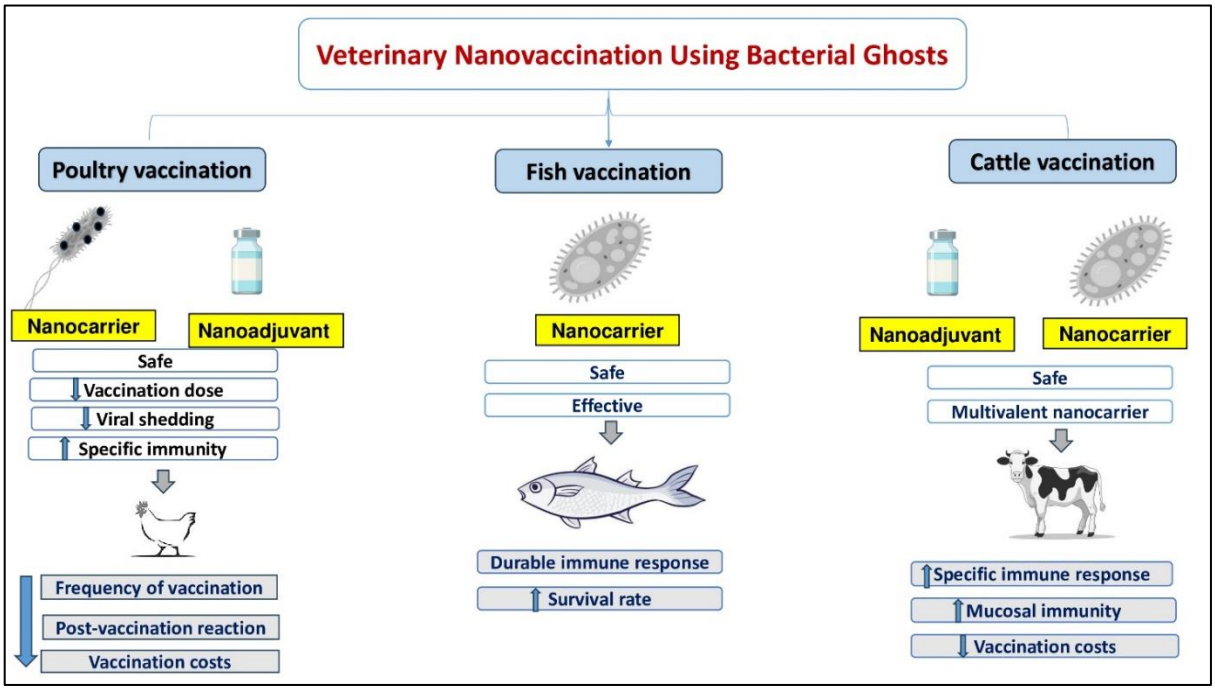

Figure 2. Diagram illustrating the application of bacterial ghosts in veterinary nanovaccine development.

This vaccine utilizes two distinct human adenovirus vectors, Ad5 for priming and Ad26 for boosting, enabling a more robust immunological response by overcoming pre-existing immunity. The Ad5nCOV vaccine, utilizing an Ad serotype 5 viral vector, is administered as a single dose and has shown a phase-III trial efficacy of 65.7% in preventing mild COVID-19 symptoms and 91% in preventing severe disease [39, 43]. Adenovirus vector vaccines can serve as platforms for both replication-competent and replication-incompetent designs. Clinical trials involving a replication-incompetent Ad5 vector expressing hemagglutinin and ChAdOx1 expressing nucleoprotein and matrix protein-1 have shown promising results for adenovirus vector vaccines against the influenza virus. Furthermore, a replication-competent Ad serotype 4 carrying the HA protein has demonstrated sustained mucosal and systemic immunity in humans without adverse side effects [43].

BriLife is a potential COVID-19 vaccination candidate utilizing a recombinant vesicular stomatitis virus viral vector capable of replication. It replaces the glycoprotein gene of the vesicular stomatitis virus with the spike protein of the SARS-CoV-2 virus. Research on hamsters has shown that a single dose of this vaccine is safe and effective in protecting against COVID-19. Another innovative vaccine is the nasal spray SARS-CoV-2 vaccine, DelNS1-2019-nCoV-RBD-OPT, which uses the influenza virus as its vector and generates dual antibodies against SARS-CoV-2 and the influenza virus. When administered as a nasal spray, this vaccine can stimulate mucosal immunity in the upper respiratory tract. Apart from providing cross-protection against H1N1 and H5N1, studies have found that it has broad efficacy against omicron and other variants of concern [39].

AdVs are attractive for developing viral vector vaccines targeted to HBV due to their high liver tropism. They can play an innovative role as an antiviral therapy by acting as a viral vector vaccine or gene therapy for HBV. The Ad5-derived TG1050 vaccine is a novel anti-HBV immunotherapeutic that encodes a fusion protein of altered HBV core, polymerase, and some envelope protein domains. A single dosage of TG1050 enhanced the production of cytokines and cytolysis by HBV-specific T cells and decreased viral replication [44, 45]. In another study, immunocompetent uninfected mice that received a heterologous modified vaccinia Ankara-boost vaccination following ChAdOx1 administration, which encodes an HBV immunogen containing three full-length HBV antigens, exhibited enhanced T cell responses [46].

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Table 1. Clinical trials showing different viral vector vaccine development and routes of administration.

Viral disease	Viral vector	Vaccine name	Delivery route	Clinical status	Outcomes	Registry number
HIV	pDNA /rVSV	HIV-1 Nef/Tat/Vif, Env pDNA Vaccine then HIV-1 rVSV envC (boost)	Electroporation IM, IM	Phase 1	-Env-specific CD4 + and CD8 + T-cell responses. - Secreted Env-specific IgG binding antibodies. - Antibody-dependent cell-mediated cytotoxicity response.	NCT02654080
	DNA - Modified Vaccinia Virus Ankara	DNA vaccines priming then a MVA -CMDR vaccine boost	IM	Phase 1	-Induced higher concentrations of specific CD4+ T cells. -Significant increase in the number of epitopes recognized by Env-specific T cells due to boost doses. - Heterologous variants cross-recognition.	NCT02296541
	Ad26	Tetravalent Ad26.Mos4.HIV	IM	Phase 1/2a	-Increased the humoral and $CD4^+$ T-cell immune responses. - preserved vaccine's safety combined with tolerability properties.	NCT02935686
	AVV	AAV8-VRC07	IM	phase 1	-Induced biologically active broad neutralizing antibodies.	NCT03374202
COVID-19	Ad5	Ad5-nCoVO-IH, Ad5-nCoV/O-IH, Ad5-nCoV-IH	Nebulized inhalation device for oral inhalation	Phase III	-Geometric mean titer Nab which are anti-Omicron-specific >= 4-fold increase in comparison to pre-immunization.	ChiCTR2200063996
	live-attenuated influenza virus	dNS1-RBD	IN spray	Phase III	-Protection against omicron variants. -Acceptable safety profile. - Vaccine tolerance.	ChiCTR2100051391
	Ad 5	Ad5-nCoV	IM	Phase IV	-High concentration of specific NAb against SARS-CoV-2. -Induced anti-RBD antibodies.	NCT04916886
	ChAd	ChAdOx1 nCov-19	IM	Phase IV	- High number of generated Memory-B cell. -After booster vaccination, anti-spike IgG levels were elevated.	CTRI/2021/08/035648
	ChAd	ChAdOx1 nCoV-19	IM	Phase Ib-IIa	-A single dose promoted the quantity and quality of antibody responses to SARS-CoV-2.	NCT04444674
	ChAd	ChAdOx1 nCoV-19	IN	Phase I	-Acceptable tolerance in participants. -No consistent mucosal antibody response was stimulated. -No potent systemic response.	NCT04816019
Influenza virus	Ad 4	AD4-H5-VTN	Oral capsule or IN spray	Phase I	-Increase in specific $CD4^+$ and $CD8^+$ T cells in the peripheral blood. -Increase in IgA and IgG in nasal, and rectal secretions. -Induced high levels of serum NABs.	NCT01806909 And NCT01443936
	MVA	MVA-H5-sfMR	IM	Phase I/IIa	-Immune system stimulating vaccine which elevated antibodies levels. -Tolerated side-effects in participants.	NTR3401

Antivirals effectively reduce viral infections but are limited in achieving a complete virological cure because they do not target stable covalently closed cccDNA. Targeting this HBV replication intermediate is crucial for achieving a permanent cure for HBV infection, as the viral lifespan relies on the presence of functional cccDNA [45]. Many clinical trials have been conducted to improve desired outcomes and reduce adverse effects of these types of vaccines by testing different aspects of vaccine design, adjusting dosing intervals, and using various routes of administration, as demonstrated in Table 1.

Different strategies for HBV gene therapy may help get rid of cccDNA. These approaches include the use of CRISPRs, CRISPR/Cas systems, Transcription-activator-like effector nucleases, and RNAi [45]. Helper-dependent AdVs were utilized to administer anti-HBV RNAi activators, resulting in a sustained therapeutic effect in vivo. An AdV expressing a CRISPR/Cas9 system combined with eight guide RNAs targeted the HBV X gene, identifying a common sequence in the HBV genome and significantly reducing viral protein release [45, 47].

The possible replacement of antiretroviral medications for HIV infection treatment is demonstrated by the capacity of Ig-HIV, particularly broadly neutralizing antibodies, to reduce viral replication in clinical investigations. Transgene expression can continue for the duration that the AAV-transduced cell survives. AAV vectors are non-pathogenic; their genomes remain in the cell nucleus as episomes. Using AAV-based viral vectors, myocytes and skeletal muscle's long lifespan can be utilized to provide constant administration of Ig-HIVs [48]. Four rhesus monkeys were used in a therapy study; they were infected with simian-HIV and AD8 for 86 weeks before being administered three distinct monoclonal antibodies encoded with AAV. One monkey sustained remarkable levels of two out of three encoded monoclonal antibodies for over two years despite ADA responses, restricting the delivery of monoclonal antibodies. This led to an abrupt decline in plasma viremia, which remained undetectable for three years. This indicates that if the anti-drug antibody is effectively suppressed against antibodies delivered by AAV in monkeys, it will impart a broad application with functional cure in humans [48, 49].

3. Microorganism-derived components as therapeutic agents and nanocarriers

3.1. Bacteria-derived components

3.1.1. Biosurfactants

Microbial biosurfactants are amphipathic components with a hydrophilic head and a hydrophobic tail [50, 51]. Biosurfactants begin to reversibly self-assemble, forming spherical-shaped molecules after reaching CMC. Microbial sources of biosurfactants are bacteria [52], fungi [53], and yeast [54]. Biosurfactants are therapeutic agents with antiviral activity against enveloped viruses such as coronavirus [55], NDV, HIV [56], hepatitis C, and herpes simplex virus by an adsorption/penetration process that involves creating ion channels in the virus's membrane [57]. Probiotic biosurfactants produced from *L. plantarum* using agrifood waste as a production medium showed anti-NDV laSota activity at concentrations of 3.75 mg/mL and 7.5 mg/mL that differed according to the class of biosurfactant [58].

Similarly, the anti-enveloped virus activity of rhamnolipids was assessed in a concentration-dependent manner, demonstrating rapid inactivation and destruction of the virus in both the liquid phase and on the surfaces. This research presents promising outcomes for controlling the COVID-19 pandemic or MERS-CoV by incorporating rhamnolipids into face masks [59, 60]. Furthermore, biosurfactants serve as environmentally friendly adjuvants in viral vaccination, enhancing the immunogenicity of human and animal vaccines (Table 2). They achieve this by fusing with antigens, forming a micotube on the cell membrane to facilitate antigen uptake by antigen-presenting cells, and modulating innate immunity through the induction of macrophage apoptosis [61, 62]. *Bacillus cereus* biosurfactants are used as immune adjuvants prepared with the inactive avian influenza virus H9N2 to produce a safe oil-based vaccine for layers and broilers [63]. Delivery-wise, biosurfactant micelles could increase the water solubility of an antiviral drug such as curcumin to show antiviral activity against the influenza virus [64].

3.2. Viral-derived components

3.2.1. Virosomes

Virosomes are vesicles composed of phospholipids derived from viral envelopes, from which the nucleocapsid has been removed. HA and NA, two envelope glycoproteins

produced from influenza viruses, are typically reassembled with liposomes to generate virosomes for vaccine delivery [71]. Moreover, virosomes can be produced from other enveloped viruses, including the vesicular stomatitis virus, respiratory syncytial virus, and HVJ [72].

NPs derived from the human hepatitis B virus were integrated with liposomes to create particles resembling virosomes. The inclusion of lipoproteins provides structural stability to virosomes and facilitates disease targeting, as well as endolysosomal escape post-internalization [49]. In mice, a SARS-CoV-2 Wuhan spike virosome vaccine produced a more comprehensive range of neutralization to the challenged virus than one that used the Beta spike [41]. A polyvalent virosome influenza vaccine was developed based on the swine influenza A virus subtypes H1N1, H1N2, and H3N2, incorporating hemagglutinin and neuraminidase proteins. Intramuscular immunization resulted in elevated hemagglutination inhibition titers, with antibodies lasting for eight months post-immunization, indicating sustained protection [73].

Table 2 . Advantages of biosurfactants in vaccine development.

Biosurfactant formulation	Application	Mechanism of action	Advantages	Reference
Surfactin-free polylactic acid NPs	Nanocarrier & nanoadjuvant	- Induce immunogenicity against HIV - Delivery of vaccine Ag	- Good biodegradable - Good biocompatible	[65]
WH1fungin	Nanoadjuvant	- Increase in CD4 and CD8 T cells in mice	- Induction of durable immune response - High effectiveness without side effects, allergic reactions, and cytotoxicity	[61]
Rhamnolipid nanomicelles	Antiviral drug	- Disruption of viral membrane permeability - Solubilization and fragmentation of viral lipid envelop	- Safe as hand sanitizer against SARS-CoV-2 - No dermal or eye toxicity in rabbits	[66]
Emulsan	Nanocarrier & nanoadjuvant	- Activation of macrophage - Induction of IgG2a increase - Ag carrier by forming microsphere	- Low toxicity -Immunomodulation effect - Improve injectable and intranasal vaccines	[67]
Trehalose lipids	Nanoadjuvant	- Induce high Ab level	- Improve antigenicity and immunogenicity of vaccine - Protect the liposome-base vaccine during the freeze-drying process from disruption and damage.	[68]
Polymexin	Nanoadjuvant	- Induce mucosal IgA antibodies	- Safe for use against mucosally transmitted viral infections as SARS-CoV-2 and influenza A virus	[69]
Gramicidin S	Antiviral drug	- Disruption of viral membrane permeability - Solubilization and fragmentation of viral lipid envelope	- Could be formulated to be a therapeutic agent for corona virus treatment. - Low side-effects	[70]

3.2.2. Viral-like particles

VLPs are non-infectious particles resembling native viruses but lacking the viral genes responsible for infection [74]. VLPs have precise structures, can encapsulate diverse medications, and display functional moieties on their surfaces. Packaging medicinal molecules within VLPs can be achieved through chemical conjugation or non-covalent interaction [49]. In humans, the surface (HBsAg) and core (HBc) antigen structural proteins of the hepatitis B virus were used in VLPs as the first recombinant vaccine against HBV [75]. Currently, various FDA-approved vaccines for HBV based on VLPs are available on the market, such as Engerix-B® and PreHevbrio® (Sci-B-Vax) [76, 77]. Two other vaccines produced against human papillomavirus (HPV), which causes cervical cancer, are Gardasil® (quadrivalent vaccine) and Gardasil9® (nonavalent vaccine) [78].

In a recent phase I/II clinical trial, a COVID-19 VLP vaccine incorporating spike and structural proteins from emerging SARS-CoV-2 variants demonstrated a good safety profile and induced sustained antibody titers with broad T cell-mediated immunity, including a T-helper-1 biased response [79].

The most effective approach to prevent HIV-1 transmission is to induce broad neutralizing antibodies and enhance T cell responses through the presentation of virion-associated envelope glycoprotein. Replication-incompetent HIV-1 virus-like particles offer both benefits. By incorporating specific mutations that remove the reverse transcriptase domain while maintaining safety features, VLPs closely mimic infectious virions. These VLPs serve as valuable immunogens capable of eliciting antibody responses targeting the envelope glycoprotein on fully infectious HIV-1 virions [80]. In a Phase I (NCT00001053) clinical trial, the HIV p17/p24\:Ty-VLP vaccine induced potential T-cell immunity and mucosal antibody responses against HIV-1. Chimeric VLPs containing both SARS-CoV-2 spike (S) proteins and influenza virus A matrix proteins were used to vaccinate mice intramuscularly. This resulted in the production of high titers of S-specific IgG and neutralizing antibodies, leading to a significant reduction in viremia [81].

4. Protein-based nanocarriers

4.1. Animal proteins

Recently, nanotechnologists have been interested in using eco-friendly proteins as drug delivery carriers in the form of biopolymers, nanoparticles, nanocapsules, and nanomicelles. Human proteins, animal proteins, and plant proteins are used as a promising technology for the treatment of infectious diseases such as *Brucella abortus* [82], cytomegalovirus [83], COVID-19 [84], and HIV-1 [85]. Natural animal proteins possess a lot of advantageous aspects, such as availability, low toxicity, stability in different pH conditions, high capability and bioactivity, specificity, and good solubility in water [86]. Moreover, they protect the native drug from being degraded by gastric enzymes or immune cells and control drug release inside the body as they reduce the amount of released drug before reaching the target organ [85].

Animal proteins such as bovine serum albumin and milk proteins have numerous therapeutic applications and can serve as non-pharmaceutical carriers for antiviral drugs or immune-stimulant agents. For example, β-casein nanomicelles were employed as a pH-stable nanoencapsulation system for the oral administration of anti-HIV drugs, with the support of Eudragit® microparticles to facilitate controlled drug release in the gastric environment. This is particularly beneficial as the gastric environment is known to pose challenges for anti-HIV drugs [85]. Ritonavir-casein micelles demonstrated that a milk-based nanoformulation could be a safe, cost-effective, and appropriate option for HIV-infected children. This formulation provides efficient oral bioavailability in gastric conditions, rapid ritonavir release, and serves as a non-immunogenic drug delivery system [87].

Antiretroviral therapy aims to prolong the lifespan of HIV patients by utilizing bovine-albumin-based NPs. These nanoparticles enhance the solubility of poorly water-soluble antiviral drugs such as efavirenz and stabilize water-soluble drugs such as tenofovir. They facilitate controlled drug release and targeted delivery to infected immune cells [87, 88]. Acyclovir is highly effective against ocular viral infections; however, formulating it poses challenges. Acyclovir-loaded BSA NPs can address these challenges by enhancing the drug's solubility in aqueous solutions and improving corneal permeability [89]. Animal protein-based nanocarriers play a dual role in pharmaceutical applications, serving as drug nanocarriers for antiviral agents and as components of eco-friendly vaccination strategies, acting as nanoadjuvants, immunomodulators, and stabilizers. For instance, artificial immunization against the Zika virus necessitated the

utilization of BSA as an adjuvant, a double-stranded RNA analogue as an antigen, and NPs as a targeted delivery system [90]. COVID-19 vaccination strategies can benefit from self-assembled zein NPs encapsulated with BSA, aiming to improve drug stability, enhance drug release, and target infected cells specifically [84].

4.2. Human proteins

Human serum albumin (HSA) is one of the tiniest plasma proteins (66.5 kDa) produced in the liver and released into the blood circulation. It is a natural nanocarrier with strong drug-binding characteristics with cellular receptors, excellent biocompatibility, active targeting ability, high specificity, and controlled biodistribution. HSA protects loaded drugs from elimination from the bloodstream and provides longer half-life drug delivery, extending to 19 days [91]. The primary mechanisms by which albumin transports drugs include direct combination through covalent or non-covalent binding, fusion technology, and drug-HSA NPs [92]. HSA is now recognized as an eco-vaccination technology that safely enhances immunogenicity by serving as a stabilizer and protein-based carrier for viral vaccines (Figure 3). Loading the M2 protein of the influenza virus onto the HSA nanocarrier maximizes the humoral immune response against the virus, helps control viral load, and prevents viral outbreaks resulting from viral mutations [93].

Active targeting drug delivery is the essential advantage of HSA in antiviral drug formulations, as it is very beneficial in reducing viral replication, avoiding adverse effects of drugs, and maintaining a high blood drug concentration for a relatively long time [94]. For instance, albumin nanoparticles increase the accumulation of anti-HBV drugs in the liver due to the targeting effect of albumin NPs [95]. Moreover, researchers began to add ligand-specific sites to increase the specificity and targeting efficiency of HSA because of the presence of species of non-specific albumin binding sites on human hepatocytes [96]. The conjugation of L-HSA ligand in a polymeric nanoparticle-HSA formulation loaded with lamivudine contributed to HBV control by enhancing the targeting efficiency of L-HSA nanoparticles to specific receptors on the hepatocyte surface while avoiding other organs [97].

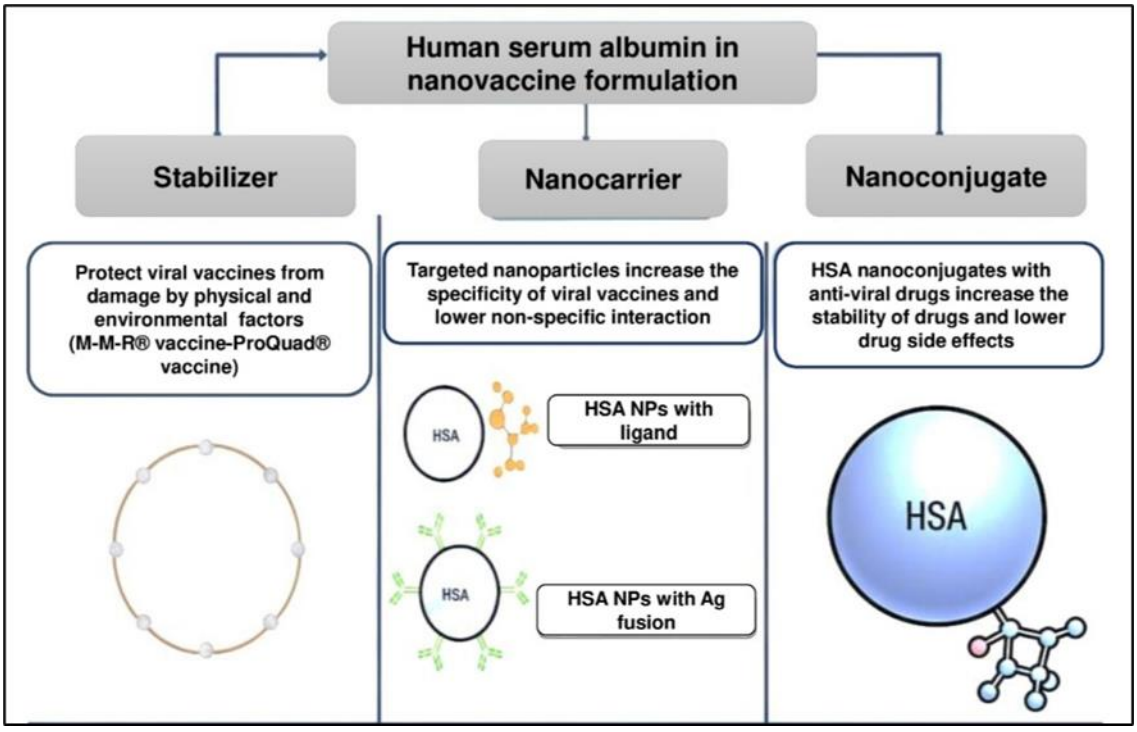

Figure 3. Schematic illustration of the role of human serum albumin in nanovaccine development.

The long half-life of HSA in blood circulation makes it an excellent bio-carrier for antiviral drugs that need sustained drug release. The abacavir-loaded albumin NPs slowly released after the first 15 minutes, a very low amount of drug before reaching the target organ [98]. The conjugation of anti-HIV-loaded HSA with liposomes by covalent bond improved the controlled drug release in blood stream after intravenous injection and represented an active drug carrier system for modulating resistance time in the bloodstream [99]. Similarly, HSA can be added to the surface of nevirapine, another poorly water-soluble anti-HIV therapy, to modify it and create an intravenous injection-ready nevirapine nanosuspension [100].

4.3. Microbial proteins

Ferritin is an iron-storage protein consisting of 24 protein subunits self-assembled to form hollow spherical nanocages, which are used to encapsulate metal-based NPs [101]. The self-assembling of ferritin lowers its toxicity in vivo and in vitro studies, activates humoral and cellular immunity, and acts as a perfect thermostable nanocarrier for therapeutic agents (Table 3 and Table 4) [102, 103]. Un-glycosylated ferritin produced by *E. coli* and *H.pylori* allows antigenic protein to retain a native immunogenicity and protect it from undesired interactions (Figure 4) [104]. For these reasons, scientists leverage these advantages as nanocarriers for eco-friendly vaccination against various infectious viruses, including swine fever, foot and mouth disease, hematopoietic necrosis virus, influenza virus, and COVID-19. For example, *Helicobacter pylori* ferritin nanoparticles loaded with recombinant infectious HNV glycoprotein enhanced the anti-IHNV innate immunity, protected the viral glycoprotein from degradation, making it suitable for oral vaccine administration, and maintained vaccine stability under various storage conditions [105]. Hpf NPs were developed as an antigen nanocarrier for the rotavirus VP6 protein. This formulation aims to allow the VP6 protein to be excreted in breast milk, presenting a promising technology for safeguarding animal offspring from fatal viral infections through maternal immunity [106].

Combining recombinant vaccine technology and ferritin nanotechnology was employed by utilizing a pseudoviral vector containing the *H. pylori* ferritin gene fused with the influenza HA gene to produce a recombinant protein. This protein was subsequently loaded onto NPs using transient transfection technology. Following the purification of ferritin-HA NPs, their immunogenicity was tested in mice, revealing a broader immunity against the influenza virus that surpassed the efficacy of other commercial vaccines [107]. Utilizing the same technology, the single-chain glycoprotein genes of the Epstein-Barr virus and *H. pylori* ferritin genes were fused to create a recombinant immunogenic vaccine. This vaccine successfully surpassed expectations during in vivo studies [108]. The most significant challenge faced by scientists working on the COVID-19 vaccine is the emergence of VoCs. To address this issue, they aim to develop next-generation COVID-19 vaccines based on genetically engineered nanoproteins and viral proteins. These vaccines aim to provide durable and broad immunization against VoCs, including the omicron virus [109, 110]. Ferritin nanoparticle vaccine (phase 1 clinical trial, NCT03186781) demonstrated high safety, targeting activity, and immunogenicity against the H2 influenza virus, making it the most effective vaccine technology against the influenza virus, which causes thousands of illnesses and deaths in animals and humans worldwide [111].

Moreover, bacterial ferritin NPs can serve as carriers to enhance the immunogenicity of antigen epitopes. For instance, they are utilized in epitope vaccines against the canine distemper virus by coupling the ferritin sequence with viral glycoprotein sequences to produce ferritin with multivalent antigenic epitopes on the surface. Similarly, in the respiratory syncytial virus vaccine, genetically engineered *H. pylori* ferritin is used to carry eight viral proteins inside the nanocage [112, 113].

5. Limitations of microbial-based nanocarrier for nanovaccines development

A bacterial ghost can be used as a DNA nanocarrier and a nanoadjuvant for nanovaccine development to induce specific immunity [24]. However, it is not widely applied in medical practice due to hyperimmune responses against bacterial cells, which may lead to autoimmune reactions, genetic mutations in bacterial ghosts, low-controlled drug release, and limited loading efficiency [122]. Another drawback of bacterial ghosts is their restricted bacterial lysis and low production yield.

Table 3. Preclinical studies showing the efficiency of ferritin NPs in viral nanovaccine development.

Formulation	Application	Laboratory animal	Route of administration	Adjuvant	Advantages	Reference
Ferritin-based NPs	Nanocarrier for Zika virus protein	Mice (A129)	Subcutaneous injection	None	-Induce very high humoral and innate immunity against the lethal Zika virus	[114]
Ferritin-based NPs	Nanocarrier for COVID-19 virus protein	Non-human primate	IM injection	Aluminum hydroxide	- Elicitation of broad-spectrum antibody response - Effective and affordable vaccine for infants and worldwide vaccination against SARS-CoV-2 - Provide a less frequent dose (only one dose/ year) - High stability for at least two weeks at 37°C	[109]
Ferritin-based NPs	Nanocarrier for H1N1 hemagglutination protein	Mice & pigtail macaque	IM injection	AddaVax™	- Induce higher specific immunity in comparison with Ag dose - Dose sparing effect - Efficient protection	[115]
Self-assembled Ferritin-conjugated NPs	Nanocarrier of African swine fever proteins with multiple epitopes	BALB/c mice	IM injection	None	- Robust antibody response against African swine fever virus - Elicitation of broad spectrum antibody response till 231 day - Effective and safe vaccine	[116]
Self-assembled Ferritin-conjugated NPs	Nanocarrier of SARS-CoV-2 proteins with multiple epitopes	hACE2 transgenic mice	IN vaccination	None	- Enhance long-lasting immunity against divergent SARS-CoV-2 variants - Induce very high humoral, cellular, and mucosal immunity	[117]
Ferritin nanocage	Nanocarrier of Human enterovirus 71 proteins with multiple epitopes	BALB/c mice	Subcutaneous injection	Freund's adjuvant	- Induce a high immune response against Human enterovirus 71 - 100% natural passive immunity	[118]
Self-assembled Ferritin-conjugated NPs	Nanocarrier of two different Ag (HIV-1 and Influenza HA)	Guinea pigs	IM injection	None	- Elicitation of antibody response against HIV and Influenza virus	[119]
Self-assembled Ferritin-conjugated NPs	Nanocarrier for Dabie bandavirus protein	Naïve ferrets & BALB/c mice	IM injection	MF59	- Induce very high immunity against Dabie bandavirus - Efficient protection with a small dose	[120]
Self-assembled Ferritin-conjugated NPs	Nanocarrier of HPV capsid proteins with multiple epitopes	Guinea pigs & BALB/c mice	IM injection	AddaVax™	- Provide high immunogenicity against oncogenic and non-oncogenic HPV types	[121]

Table 4 . Summary of the most recent clinical studies that depend on ferritin nanocarriers for viral nanovaccine development.

Vaccine	Phase	NCT number	Study completion year	Study type	Importance
EBVgp350-ferritin vaccine + Matrix-M1™	Phase I	NCT04645147	2025	Interventional	- Clinical studies have commenced by formulating an Epstein-Barr glycoprotein-ferritin nanoparticle vaccine with a saponin-dependent adjuvant
EBVgp350-ferritin vaccine + Matrix-M1™	Phase I Phase II	NCT05683834	2026	Interventional	- Evaluation of the safety and immunogenicity of Epstein-Barr glycoprotein-ferritin nanoparticle vaccine when administered to seronegative adults
SpFN.1B-06-PL+ALFQ	Phase I	NCT04784767	2023	Interventional	- Evaluation of the safety, tolerability, and immunogenicity of adjuvanted ferritin nanoparticle vaccine carrying immunogenic SARS-COV-2 spikes on the surface in adults ages 18-55.

Therefore, researchers are developing new preparation strategies for large-scale BG production and efficient lysis through the incorporation of lysis genes in genetically engineered plasmids that enable bacterial cell lysis during expression (European Patent Office, EP3107992B1). The loading efficiency of BG for antigenic proteins or DNA delivery can be enhanced by anchoring the inner membrane or lysis hole with streptavidin or by genetically engineering BG for massive expression of Ags in the periplasmic space [30, 123]. The large-scale production of biosurfactants requires specific materials and multiple purification steps, which increase production costs [124]. Using affordable carbon sources for biosurfactant production and AI-machine systems could enhance the effectiveness of scientific equipment and reduce production costs [125].

Viral vector vaccines have several advantages, as well as specific drawbacks and constraints. Thus, recognizing these obstacles is critical for making rational decisions and advancing vaccine development and implementation strategies. Viral vector vaccines have the following notable drawbacks:

I) Pre-existing immunity to the vector: Pre-existing immunity to the viral vector itself, when it is a common serotype among the population, is a limiting factor in vaccine efficacy. Antibodies can neutralize these common serotypes before releasing their antigenic genetic material, thereby diminishing vaccine efficacy. For example, pre-existing Ad5 antibodies in the serum can attenuate the immune protection effect by lowering NAb levels [126].

II) Lower levels of NAbs: Viral vector vaccines based on certain viral strains produce lower levels of NAbs than inactivated and mRNA-based vaccines, which can reduce the success rate of viral infection prevention. Although such adverse events are rare, they are serious and potentially life-threatening [127].

III) Mild adverse effects: Guillain-Barré syndrome and thrombosis, along with thrombocytopenia or immune system overactivation, have been associated with some viral vector vaccines. Occasionally, vaccination with viral vectors may cause an unusually vigorous immune response, which may be fatal in rare cases [128, 129].

IV) Sophisticated production technique: Compared with other vaccine formats, the manufacturing of viral vector vaccines is more complicated and expensive. The production process requires growing viruses in cell cultures, which demands specialized equipment and expertise [130].

V) Restricted frequent dosing: Repeated dosing with the same vector may be less effective due to the possibility of the immune system generating a strong response against the vector, thereby rendering the vaccine ineffective [131].

VI) Concerns with public perception and ethics: The nature of these vaccines may be misunderstood, leading to resistance to vaccination and impeding public health initiatives.

To increase the yield of neutralizing antibodies against the desired antigen encoded by viral vector vaccines, the following aspects should be considered:

I) **Improved vector and viral antigen design:** Enhancing the design of viral vectors can help evade pre-existing immunity. Viral vector design can be optimized through several strategies, including directed evolution. Creating viral vector libraries with targeted mutations forms the basis of the receptor-agonistic technique known as directed evolution. For example, the cap AAV viral vector may result in new properties that facilitate targeting specific cells, decrease vector self-immunity, increase transduction efficiency, and generate a broad spectrum of neutralizing antibodies against infections caused by various viral strains. One approach for implementing these genetic engineering alterations is AAV-targeted evolution based on a Cre-recombination system, which employs a state-of-the-art technique to improve AAV qualities for medicinal use. Using the Cre-Lox recombination system enables the creation and selection of AAV variants with the required traits [132]. To improve antigen design, such as capsid proteins, two strategies can be employed: epitope optimization and the development of mosaic antigens. Epitope optimization can enhance immune system recognition, antigen presentation, and subsequent processing, thereby increasing the quality and quantity of neutralizing antibodies. Mosaic antigens, particularly for viruses such as HIV, can combine epitopes from several viral strains, thereby boosting the production of neutralizing antibodies [133].

II) Using vaccine combinations: Combining viral vector vaccines with other vaccine types, such as vector-like particles (VLPs), which have a unique repetitive surface structure that enables them to display "conservative" epitopes from a variety of antigens, can help induce broad-spectrum neutralizing antibodies. VLP-based vaccines against influenza and HIV have been shown in recent trials to elicit stronger immune responses against various circulating virus variants [127].

III) Enhancing vaccine delivery approaches: A promising strategy to increase vector accumulation at the target tissue, evade immune system responses, and prevent inappropriate interactions with phagocytic cells or complement present in serum is to modify the viral surface using nanomaterials [134].

IV) Optimizing the route of administration and dosing intervals: Oral or nasal routes are more favorable to the public due to their pain-free administration and safety, in addition to their ability to confer mucosal immunity. Mucosal vaccines can elicit immune responses at mucosal sites as well as systemic immune responses with relatively low antigen doses, evading first-pass metabolism and reducing the risk of anaphylactic shock. Compared with the IM route, IN inoculation with a chimpanzee adenovirus (Ad)-vectored SARS-CoV-2 vaccine produces larger quantities of NAbs [135]. Determining the optimal intervals between doses in a prime-boost regimen combined with different routes of administration can maximize the antibody response for a longer duration [136].

V) Host immune system modulation: Administering cytokines or cytokine-inducing agents can modulate the host immune system to enhance antibody production [137]. Applying genetic adjuvants is another approach to modulate the immune system. By incorporating genes of stimulatory agents for the immune system, such as GM-CSF, into the viral vector, the immune response can be improved, thereby boosting the generation of neutralizing antibodies [138].

Avoiding serious adverse effects, such as Guillain–Barré syndrome and thrombosis with thrombocytopenia resulting from viral vector vaccines, requires several strategies. These include careful vaccine design, rigorous clinical testing, post-marketing surveillance, and testing in a diverse population to monitor rare adverse events. Conducting thorough risk-benefit analyses and ensuring effective communication to educate patients helps mitigate these events. The benefits of vaccination must outweigh potential risks, especially for individuals with a higher risk of adverse effects, such as those with pre-existing conditions, including low platelet count and autoimmune disorders. Patients should be educated about the signs and symptoms of serious adverse effects so they can seek appropriate treatment promptly [139].

To overcome pre-existing immunity to viral vector vaccines, alternative, less prevalent serotypes such as Ad26 and Ad35, or vectors derived from non-human sources such as chimpanzee adenoviruses, can be employed [140, 141]. Vector and antigen engineering methods, including the use of chimeric viral vectors that swap variant serotypes or epitope mask vectors such as Ad5, can be effective in overcoming pre-existing immunity to this common serotype among the population [142].

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Overcoming the limitation of repeated dose administration requires heterologous prime-boost viral vector regimens, which may prove effective, or the use of viral vectors such as AAV that induce a long-lasting and strong immune response, thereby eliminating the need for booster doses [143].

6. Limitations of protein-nanocarriers for nanovaccine development

I. Immunogenicity issues: HAS can exhibit low or mild immunogenic responses, as the human immune system can recognizes them as foreign bodies [144]. Although such responses are generally weak, they still represent a limitation when these proteins are used as vaccine nanocarriers.

II. Stability challenges: Studies on ferritin-based nanovaccines have indicated that slight alterations in the C-terminal E-helices of the ferritin structure can impact both stability and immunogenic performance. These structural mutations tend to affect how well the protein maintains its integrity and antigens [145].

III. Folding and post-translational modifications concerns: Bioengineered ferritin produced through *E. coli* expression system showed aggregation or misfolding due to the absence of natural post-translational modifications. The addition of NaCl to the lysis buffer has been shown to mitigate these effects by improving ferritin solubility, promoting proper folding, and enhancing self-assembling behavior [146].

IV. Limited administration routes: Injectable formulations containing BSA may provoke autoimmune reactions , causing rapid elimination of BSA without eliciting any systemic immune response [147]. A single oral dose administration of BSA has shown tostimulate humoral immunity in commercial strain chickens [148]. However, nasal delivery of BSA NPs improves bioavailability and allows for controlled drug release [149].

V. High production costs: The large-scale purification of bioengineered ferritin remains technically demanding. It often requires highly advanced gel electrophoresis and chromatographic procedures (patent number CN102127166A). For instance, according to the MyBiosource.com site, one milligram of recombinant ferritin costs around $290. Consequently, the high costs of isolation and purification of bioengineered ferritin pose challenges for nanovaccine development [150]. Computational optimization of histidine-tag positions on the ferritin NP surface has recently been proposed to streamline purification process and reduce costs [151].

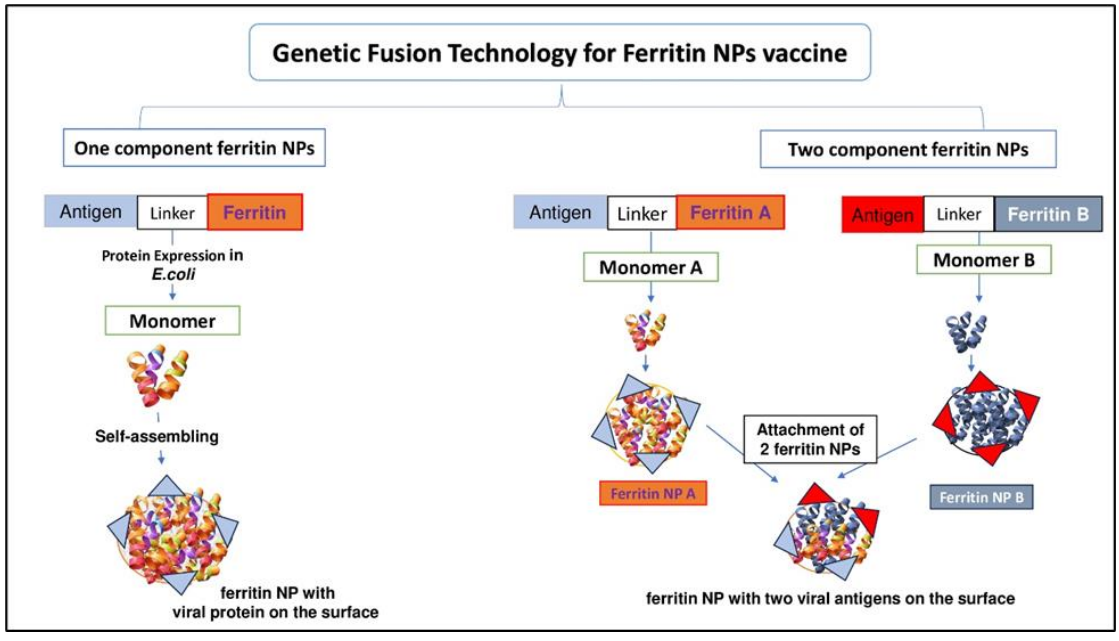

Figure 4. Schematic figure illustrating ferritin nanoparticle-based nanovaccine development through genetic fusion technology.

7. Discussion

Synthetic component-based vaccine systems face many challenges and drawbacks in clinical applications. For example, synthetic surfactants are used for the solubilization of water-in-oil emulsion vaccines, such as Tween 80, in the preparation of the water-in-oil Newcastle vaccine. Tween 80 has side effects, including skin burning, irritation of the skin and eyes, and harmful effects when inhaled [152]. Consequently, anaphylactic and post-vaccination reactions might be associated with the use of Tween 80. Currently, Tween 80 has been incorporated into COVID-19 vaccines under clinical trial, but has not yet been released [153]. Moreover, HC percentages of Tween-silver NPs from different blood donors were 27.988 and 36.465, compared with human serum albumin-silver NPs, which showed lower HC percentages (0.389 and 0.850) after a 24-hour test. These results indicated that HSA-based formulations exhibited less toxic and hemolytic effects [154]. On the other hand, biosurfactants act as safe and non-toxic immunogenic nanoadjuvants due to their biocompatibility. Preclinical studies showed promising results regarding the use of biosurfactant micelles as nanocarriers for targeted delivery in COVID-19 vaccines by enhancing bioavailability and antigen stability [155].

The side effects of pharmaceutical drugs can adversely influence drug consumption, reducing the acceptability of these drugs as well as viral vaccines, in both animal and human vaccine markets [15]. For example, post-vaccination reactions following vaccination campaigns in poultry farms affected birds' appetite, body weight gain, and egg production, which could last for approximately two weeks after vaccination [156]. In cattle farms, milk yield decreased by 1.4 liters per cow after double vaccination with the bovine herpes vaccine at a four-week interval, with a greater adverse effect observed after the second vaccination [157]. Moreover, significant economic losses and allergic effects have been reported following the vaccination of animals with traditional inactivated or killed vaccines [158]. These adverse outcomes include injection-site sarcoma in cats, anaphylaxis, collapse, and cyanosis in dogs, as well as hypersensitivity signs and early embryo loss in cattle after the administration of killed vaccines [159–161]. Accordingly, multivalent vaccines could be a solution for post-vaccination reactions in poultry and animal farms [154]. Multivalent vaccines carried by bioinspired nanoproteins may provide longer-lasing immunity, reduce the frequency of vaccination, and elicit humoral immunity with minimal side effects [162].

The COVID-19 post-vaccination reactions in females in the MENA region were analyzed by Gordian researchers using social media surveys. This cross-sectional study confirmed that menstrual abnormalities were associated with the COVID-19 vaccine [163]. In 2024, researchers demonstrated that the Pfizer COVID-19 vaccine BNT162b2 adversely affected menstrual regularity in females by influencing the protein that regulates the menstrual cycle [164]. The use of bioinspired nanocarriers minimized systemic toxicity, regulated drug release, and enhanced vaccine effectiveness [120]. Low to moderate side effects, including local, systemic, and allergic reactions, were reported following COVID-19 vaccination with AstraZeneca, which used a non-replicant chimpanzee adenovirus to enhance immunogenicity. This made it the most widely used vaccine during the COVID-19 pandemic. The AstraZeneca vaccine was licensed by the WHO in 2021 for universal access as a safe and effective vaccine without serious adverse effects [165]. Moreover, the Newcastle virus vaccine, based on a turkey herpesvirus vector as a nanocarrier for commercial layer immunization, proved effective in reducing oronasal and cloacal viral shedding to non-detectable levels, thereby lowering post-vaccination viremic infections [166].

The production and development of traditional vaccines are more expensive than those of next-generation vaccines and require specific storage conditions. For example, the AstraZeneca/Oxford vaccine has lower production costs and a lower price per dose compared to Moderna and Pfizer/BioNTech, making it more suitable for low- and middle-income countries [167]. Vaccination against various virus strains in a single dose or the use of combination vaccines with multivalent and multipathogen viral vectors provides a novel approach for minimizing vaccination costs and improving accessibility in developing countries [168]. Moreover, traditional vaccination is less suitable for controlling epidemic disease, which requires rapid development and stable formulations [9]. In contrast, next-generation vaccines offer improved stability and an extended half-life. Many viral vector vaccines remain stable at typical

refrigerator temperatures (2-8°C), which facilitates storage and transport, thereby improving accessibility in regions with limited cold chain infrastructure [39]. Adenovirus vectors are more stable than enveloped viruses for vaccine development, which explains their extensive use in the manufacturing of COVID-19 vaccines [169]. The stability of virus-vector vaccines (COVID-19 and rotavirus vaccines) at 2-8°C has positively influenced global vaccine acceptance [120].

Vaccine design technology offers a valuable tool for developing more stable, less toxic, and more efficient vaccines in a shorter time, compensating for multiple stages of translational research and reducing development costs [170]. Moreover, it supports the selection of the most effective antiviral drugs and appropriate nanocarriers. For instance, immunoinformatics enables the in silico design of engineered ferritin nanocages to be metadynamically tested with multiple SARS-CoV epitopes, allowing for the selection of the most stable multivalent vaccine. Similarly, in silico prediction of adenovirus capsid proteins from different species enhances the immune response against SARS-CoV epitopes in a coordinated manner [171]. Computational modeling of histidine-tag insertions at four optimal positions on the surface of ferritin NPs demonstrated efficient and rapid purification of hemagglutinin and COVID-19 vaccines [151].

Genetic engineering techniques are further applied in nanocarrier vaccine development through molecular cloning. Recently, engineered hyper-producing strains have been created for biosurfactant production, offering higher quality and yield [24]. Recombinant biosurfactants can enhance the effectiveness of nanovaccines by serving as nanoadjuvants or nanomicelles for gene delivery [25]. while there are safety concerns associated with vaccines due to the risk of endotoxin contamination produced by biosurfactant-producing bacteria, most fermentation-derived biosurfactants present greater challenges related to impurity and heterogeneity [172]. Using transgenic silkworms for the production of recombinant human serum albumin combined with advanced extraction and purification techniques provides a large-scale and cost-effective product for market economics [26]. Genetically engineered ferritin NPs are designed as efficient nanocarriers for multivalent nanovaccines, maximizing immunity and reducing the frequency of immunization [173]. Furthermore, viral vectors can be genetically manipulated to carry genetic material from multiple strains of the same virus or even different virus types. This property is particularly valuable for the development of vaccines against rapidly mutating viral strains, especially single-stranded RNA viruses such as HIV, or for targeting multiple viral infections within the same body system, such as the respiratory tract, for example, co-vaccination against influenza and COVID-19 infections simultaneously [174, 175].

Research studies have shown that low-dose vaccination reduces vaccine side effects and has a beneficial impact on the manufacturing process, making it more suitable for emergency vaccination [176]. In 2024, South African researchers conducted a systematic review and meta-analysis on the effects of frequent doses and sex on the prevalence of pericarditis and myocarditis following COVID-19 BNT162b2 vaccination. They reported that the risk was maximized after the second dose [177]. In effective vaccines, the less frequent the dosage, the more efficient the vaccine. For example, a single vaccination dose of the influenza vaccine, designed to use vesicular stomatitis virus as a vector, provided rapid and strong protection against various influenza infections caused by different virus serotypes and could be a promising technique for emergency vaccination [16].

Although next-generation vaccine technology offers advantages for global vaccine development, many nanovaccine strategies are still in preclinical studies, such as biosurfactants and bacterial ghosts, due to insufficient safety data and challenges in production and scale-up. However, some ferritin nanocarrier and viral vector vaccines have demonstrated preclinical safety and the ability to be manufactured under good manufacturing practice [29, 178]. For instance, viral vector platforms such as Ad26.COV2.S (Johnson & Johnson) and ChAdOx1 (AstraZeneca/Oxford) have reached advanced clinical phases, including Phase III and efficacy evaluations [179]. Nevertheless, all clinical and preclinical studies have reported numerous challenges and limitations, not only regarding vaccine safety in humans and animals, but also in the successful design and administration of vaccines. Various scientific strategies are employed to address these issues, such as combination vaccine approaches, viral vaccine design technology, and targeted vaccine delivery. For example, a combination vaccine against the Chikungunya virus, which incorporates both VLP and DNA

viral vaccines, was developed to enhance cellular immunity and mitigate adverse vaccine effects [180].

Additionally, adenovirus engineering through hexon protein modification and PEGylation has been utilized to design vaccines that target the specific viral capsid while minimizing immune activation [180]. Furthermore, some viral vaccines are more suitable for emergencies because they can serve as delivery systems for multiple antigens or drugs and can be administered through different routes without inducing anaphylactic reactions. Bacterial ghosts have the potential to be administered orally, intravenously, nasally, or as ocular drops without eliciting undesirable immune responses, making them particularly suitable for emergency vaccination [30].

8. Future directions

The combination of synthetic polymers, natural biopolymers, and nanoproteins could be advantageous for developing efficient future vaccines. For example, a novel bioengineered design of PEGylated ferritin nanocages provides efficient targeting and prolonged circulation time of the nanoformulation [181]. Entrapment of HSA in a nanoformulated system of biodegradable poly(ε-caprolactone)-polyethylene glycol and non-biodegradable hydrogel enhances the biocompatibility and targeting of HSA [182]. Biodegradable microparticles, such as poly(ε-caprolactone) and poly(lactic-co-glycolic acid), regulate drug release and the immune response to vaccines in booster dose mucosal vaccines, as demonstrated in a preclinical study on guinea pigs with diphtheria [183].

Genetic engineering of biosurfactants using CRISPR-Cas9 technology is considered a promising solution for large-scale manufacturing challenges, as it reduces production and purification costs. Cost analysis data shows that pure, genetically engineered biosurfactants cost between $50 and $100 per kilogram. Although this cost is relatively affordable, it remains higher than that of synthetic surfactants due to the complex purification process and the high cost of the substrates. The use of computational biology, ML tools, and cost-effective downstream processes would make production more sustainable and economically competitive [184]. On the other hand, recombinant BG is regarded as a futuristic solution for mucosal animal immunization against the influenza virus by inserting influenza genes to enhance mucosal immunity and specific immunoglobulin production. Scientists have incorporated the lysis gene into genetically engineered BG to facilitate bacterial lysis and improve the loading efficiency of antigenic proteins and genetic material [30].

Synthetic polymers and biosurfactants could serve as alternative adjuvants to aluminum adjuvants, which cause hypersensitivity, erythema, granuloma, and cutaneous nodules [185]. Moreover, aluminum accumulation in the human body may lead to adverse effects, such as neurodegeneration and renal dysfunction. The development of a biodegradable and biocompatible polyphosphoester-based polymer adjuvant vaccine can enhance the immune response in a virus-like particle vaccine against hepatitis B after booster dose immunization [186]. The combination of the synthetic surfactant dodecyl-dimethyl-ammonium bromide and poly(lactic acid) as a nanoadjuvant formulation demonstrated high safety standards for the clinical application of nanovaccines [187]. Additionally, some biosurfactant nanoadjuvants, such as polymyxin, proved effective and safe in mucosal vaccinations due to their high LD_{50} (790 mg/kg) when administered orally in mice [188].

The risk-to-benefit ratio of next-generation nanovaccines is carefully evaluated by the WHO before vaccine development, particularly for use in low- and middle-income countries. WHO issues guidelines for preclinical evaluation, good manufacturing practice, and clinical trial conduct to ensure vaccine safety, efficacy, and quality. The WHO prequalification program (TRS978, Annex 6) and emergency use listing criteria serve as critical regulatory pathways that allow vaccines to be procured by United Nations agencies such as, UNICEF and Gavi, after sufficient access to clinical data. For novel vaccine formulations, such as viral vector-based vaccines, the WHO and the Brighton Collaboration emphasize the importance of thorough risk-benefit assessment and post-marketing surveillance to facilitate global access [189].

Future clinical vaccine trials are expected to move toward adaptive models that allow early-phase safety, dosing, and preliminary efficacy to be assessed within the same framework. Such approaches can shorten development timelines and reduce the number of participants [190]. At the same time, AI-driven trial design is anticipated to accelerate

the development and prediction of immune responses, identify potential safety signals earlier, and guide adaptive analysis [191]. Trials for multivalent formulations are expected to be designed for head-to-head testing to address the challenges of emergency viral variants through single-dose vaccination, while leveraging novel platforms such as viral vectors and mRNA vaccines to accelerate the production of safe, effective, and accessible vaccines by shortening Phase I timelines due to platform familiarity [192].

Computationally aided techniques, such as RV integrated with ML algorithms, have been employed to accelerate vaccine development by predicting possible vaccine constructs, active binding sites on nanocarriers, antigen immunogenicity, and antigen specificity. The accessibility of high-throughput genomic and proteomic data, enabled by advanced sequencing techniques of infectious pathogens, has catalyzed the vaccine design approach known as RV. An ML prediction model requires genomic and proteomic data preprocessing, followed by feature extraction from preserved antigenic characteristics, after which learning and prioritization of antigenic targets for vaccine development are performed [193]. For example, the use of RV to develop a vaccine against the Nipah virus by selecting the most antigenic viral proteome could enhance the immune response [194]. Finally, the validation and evaluation of the selected potential antigens are conducted to confirm the success of the developed ML model and to enable its large-scale application [193]. Vaxijen is one of the AI-driven prediction systems for antigen detection, based on the assumption that a protein amino acid sequence can represent antigenicity through the chemical properties of its residues [195]. Vaxign-ML RV methods have been applied to identify potential COVID-19 vaccine candidates based on conserved, adhesive, and antigenic proteins within SARS-CoV strains [196]. To optimize future vaccination efficacy, ML can continuously track alterations in the SARS-CoV-2 genome by integrating data validated from laboratory experiments, leading to continuous improvements in selected vaccine targets alongside emerging mutations among microorganisms [197].

9. Conclusions

- According to health technology assessment studies, bioinspired nanocarrier-based nanovaccines represent the most optimal, cost-competitive, and ethical approach for next-generation vaccination.
- The exceptional immune response elicited and the favorable safety profile of bioinspired nanocarriers position them as a distinctive and promising alternative to traditional vaccine components.
- Combination therapy involving viral particles and natural nanoproteins or viral NPs and liposomes enhances the effectiveness, durability, stability, and immunogenicity of nanovaccines.
- Bioengineered bioinspired nanocarriers, such as bacterial ghosts and recombinant live probiotics, provide an effective delivery system for viral mucosal nanovaccines.
- Active targeting of protein nanocarriers increases the circulation time of the vaccine, controls antiviral drug release, and enhances the specificity of nanovaccines, particularly when combined with ligands.
- One-shot vaccination against multiple diseases or variants could be achieved by engineering advanced nanovectors.
- The application of ML algorithms to optimize the match between nanocarriers and loaded antigens could reduce vaccine development costs and improve effectiveness within a shorter time frame.
- Bioinspired nanoadjuvants demonstrate superior safety thresholds for vaccine development compared to synthetic ones.

Nevertheless, despite the low side effects of bioinspired nanocarriers, extensive studies are required to advance these delivery systems for commercial application.

Authors' contributions

Conceptualization: Amira M. Heniedy; Writing – original draft preparation: Nabila A. El-Sheridy, Nessrin Saleh; Writing – review and editing: Amira M. Heniedy, Nessrin Saleh; Supervision: Amira M. Heniedy.

Declaration of interest statement

The authors declare that this work has not been published before and has no competing interests of any type.

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I declare that AI-assisted technologies were used only for formatting and grammar checking. No AI system was used to

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Author information

Corresponding author: Amira M. Heniedy*

Email: Amiraheniedy@hotmail.com

ORCID iD: 0009-0006-3524-3194

Data availability

Data sharing is not applicable to this article as no datasets are generated or analyzed during the current study.

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