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Review

Nano-Vaccines: Pioneering the Future of Immunization

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ABSTRACT

Nanotechnology has revolutionized vaccine development by offering precise control over antigen delivery, adjuvant properties, and immune response modulation. This review examines recent nano-vine design advancements, focusing on their potential applications in infectious diseases and cancer disorders. This review systematically examines the various types of nano vaccines, elucidating their unique properties and applications in vaccine development. Furthermore, the review explores the versatility of nano-vaccine delivery routes and discusses their respective advantages and challenges in eliciting potent immune responses. Despite their promise, nano-vaccines encounter multifaceted difficulties, ranging from biocompatibility and immunogenicity to manufacturing scalability and regulatory hurdles. Nano-vaccines have opened an entrance to boundless hopes in efficiently preventing pathogenic, cancerous, and non-infectious diseases in immune-tolerant individuals. Despite the formidable challenges in toxicity, scaling up, and regulations in nano-vacinnes, more research focused on collaboration with commercial industries can lead to the rapid commercialization of nano-vaccines. Future directions in nano-vaccine research are proposed to optimize their clinical translation and maximize their therapeutic impact.

Keywords: Nanovaccines, types of vaccines, innovative techniques, future outlook, and Challenges in nanotechnology.

1. Introduction

Vaccines, centuries after their invention, are now indispensable technology in our day-to-day life. Since saving lives and protecting oneself has always been humanity's goal, vaccinations have made this dream nearly a reality. Vaccinations not only prevent diseases but also cure them as a therapeutic approach, and their impact on life makes them a valuable advancement in public health (1). Vaccines are biological preparations that work by providing our bodies with an active acquired immunity. Traditionally, there are different forms of vaccines, such as subunit, live attenuated, inactivated, DNA, and toxoid vaccine (2,3). Even though it was an outstanding invention, traditional vaccines alone don't elicit promising immunity; thus, innovation in vaccinology was an emerging strategy. The blossom of Nanotechnology opens the door for evolving vaccines through using nanomaterials (with a diameter ranging from 1 to 1000 nm) as delivery vehicles or carriers for vaccines that elicit significant adjuvants more than being solid particles and enhance antigenicity, which we call Nanovaccines (3,4). A nanovaccine is based on nanosized particles that serve as antigen delivery vehicles, whose composition can be proteic, lipidic, metallic, polymeric, or based on graphene. Such particles are typically functionalized with antigens using surface modification or encapsulation. Covalent or physical interactions mediate the conjugation of the antigen to the nanoparticles (1). The enhanced efficacy of nano vaccines concerning those based on soluble antigens is linked to 1-the increased antigen uptake by antigen-presenting cells (APCs), 2-cytokine secretion stimulation by APCs or lymphocytes (e.g., induction of inflammatory responses), 3-increased antigen stability, 4-decreased antigen degradation, and 5-action as antigens inducing humoral responses against the nanoparticle (5).

2. History of vaccination up to Nanovaccine development

Since the fifteenth century, the earliest reliable vaccination record against smallpox has been based on variolations from China and India. The risk of this intervention outweighed the benefits as it costs losses in lives (1,6). Posteriorly, in the late eighteenth century, Edward Jenner used the cowpox virus (zoonotic disease) to prevent smallpox, an outbreak achievement in vaccinology. Jenner hypothesized that if we inoculated smallpox patients with the material of cowpox pustules, it would protect against the disease. This hypothesis came from his observation that milkmaids infected by the cowpox virus don't contract smallpox or the typical smallpox lesions. He tested his hypothesis in a smallpox-infected 8-year-old boy, who got—after

variolation—a prompt recovery. This was the first known vaccine trial (England-1796). The smallpox virus was declared eradicated in 1977, with the last case reported in Somalia, following years of debates on Jenner's concept. Consequently, vaccination became the accepted method of preventing smallpox in England in 1840. The earliest concealer for the "attenuated vaccines" theory was initially established (7). Edward Jenner is considered a pioneer in vaccinology. In the West, Jenner is often called “the father of immunology”, and his work is said to have saved “more lives than any other man” (8).

Decades later, Louis Pasteur—a French researcher—became a major figure in vaccinations. Pasteur invented procedures for culturing and attenuating microbes via passage in atypical host species, heat, desiccation, and oxygen exposure in an in vitro setting (7). These techniques made it possible to apply the principle of attenuated pathogen-based vaccinations, which were initially evaluated for rabies virus and anthrax bacilli cases. This ground-breaking experiment gave rise to attenuation, which states that microorganisms grown under less-than-ideal conditions or treated with specific chemicals exhibit a decrease in virulence and can, therefore, be utilized as a vaccine to induce immunity rather than disease. Later, the concept's applicability for Bacillus anthracis came with positive findings in 1881 (1).

Subsequently, in the first half of the twentieth century, significant advancements came to light with the development of the Bacille Calmette Guérin (BCG) tuberculosis vaccine. Developed in 1912 by Albert Calmette and Camille Guérin using Mycobacterium bovis grown in artificial culture medium, the first observation involved the protection of cattle following immunization with a bovine tuberculosis bacillus attenuated by its culture on bile-glycerol medium. Despite debates regarding its effectiveness, the BCG vaccination was approved to prevent human tuberculosis in 1921 and is currently employed in the fight against the disease (1). Finding a way to separate the toxins from the bacilli that cause tetanus and diphtheria was another significant advancement in vaccination. This allowed for the development of vaccines that are based on toxins that have been formalin-inactivated, or toxoids, providing a potent defense against tetanus and diphtheria. Moreover, the discovery of cell growth techniques made it possible to cultivate viruses, which in turn enabled the creation of vaccinations against important human infections, including rotavirus and zoster, as well as live and inactivated polio, measles, mumps, rubella, adenovirus, and varicella (1).

The 1987 US FDA approval of Haemophilus influenzae type b (Hib) for use in humans was a remarkable modeling of the clinical use of a conjugate vaccine. The effectiveness of this vaccine type against invasive Hib disease in children prompted its development against other encapsulated dangerous bacteria, including Streptococcus pneumoniae and Neisseria meningitidis serogroup (1,9).

Particularly noteworthy are the attempts to generate recombinant subunit antigens using innovative recombinant DNA technology. The introduction of recombinant vaccines into immunization schedules marked a significant advancement with the release of the recombinant hepatitis B vaccine in 1986 and, more recently, the human papilloma virus (HPV) vaccine in 2006 (10,11).

Although subunit vaccines have been successfully introduced in clinical settings and for diseases such as hepatitis B or human papillomavaccines, their use has not yet become widespread. The low immunogenicity and efficacy of these vaccines have hindered future development because they require the introduction of supplemental adjuvants. That is why there is a demand for effective and safe adjuvants (12).

In recent years, the production of vaccines has benefited greatly from the design of adjuvant formulations based on vaccine delivery methods, which provide considerable advantages over other adjuvants. A more thorough understanding of the impact of antigen-carrier properties on vaccine delivery systems' efficacy has resulted from their logical design. Specifically, particle size and surface composition are important for achieving sufficient carrier uptake by the antigen-presenting cells (APCs). Designing vehicles for vaccine administration of polymer-based delivery systems, such as microparticles, nanoparticles, and nanocapsules, is an interesting application (13).

The late 1970s witnessed the first reports indicating polymeric nanoparticles had the potential to be used as vaccine delivery systems. This first report was by Birrenbach and Speiser, who demonstrated how polymeric micelles, when administered subcutaneously, can boost the immune response against the corresponding antigen. The concept of controlled antigen delivery was initially introduced in the same decade by Preis and Langer, who proposed the use of polymeric microparticles in the production of single-dose vaccinations. These two seminal works paved the way for the creation of novel synthetic adjuvants and vaccination delivery strategies

(12). In the same period, the modified PLA-PEG nanoparticles were presented as an intriguing method for transmucosal vaccine administration. Since then, the use of polymeric nanoparticles for controlled vaccine delivery and release has grown, creating a wide spectrum of nanosystems using various biomaterials and manufacturing methods (12,14).

Several adjuvant formulations based on forming nanoemulsions with certain oils and surfactants, immunostimulants, and non-polymeric nanostructures are on the market or undergoing clinical trials. However, how PLGA microspheres are currently being evaluated in clinical trials shows how useful this method is for delivering vaccines, especially DNA vaccines against HIV and immunotherapy for breast cancer (9). Furthermore, there are encouraging findings in the preclinical stages of several particular prototypes of polymeric nanocarriers (12).

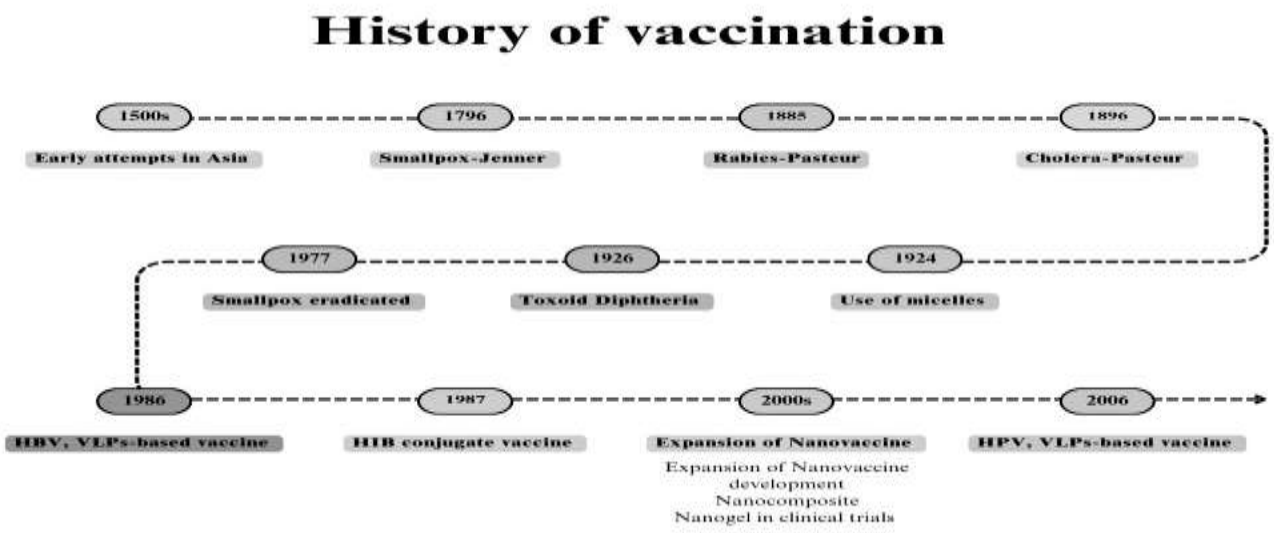

Figure 1: Summary of vaccine development over centuries.

3. Types of NPs applied for vaccine delivery

Nano particulate delivery systems for carrying drugs and vaccines are classified into inorganic (gold, metal oxides, silica, etc.), while organic NPs include (hyaluronic acid, alginic acid, ferritin, polymeric nanoparticles, liposomes, VLPs, virosomes, micelles) depending on their chemical components. Inorganic NPs are used in bioimaging and cancer photothermal therapy. Those types like (silica, carbon, aluminum, titanium and gold nanorods, titanium dioxide, and

polystyrene have the advantages for vaccine development because of their immunomodulatory properties (15,16).

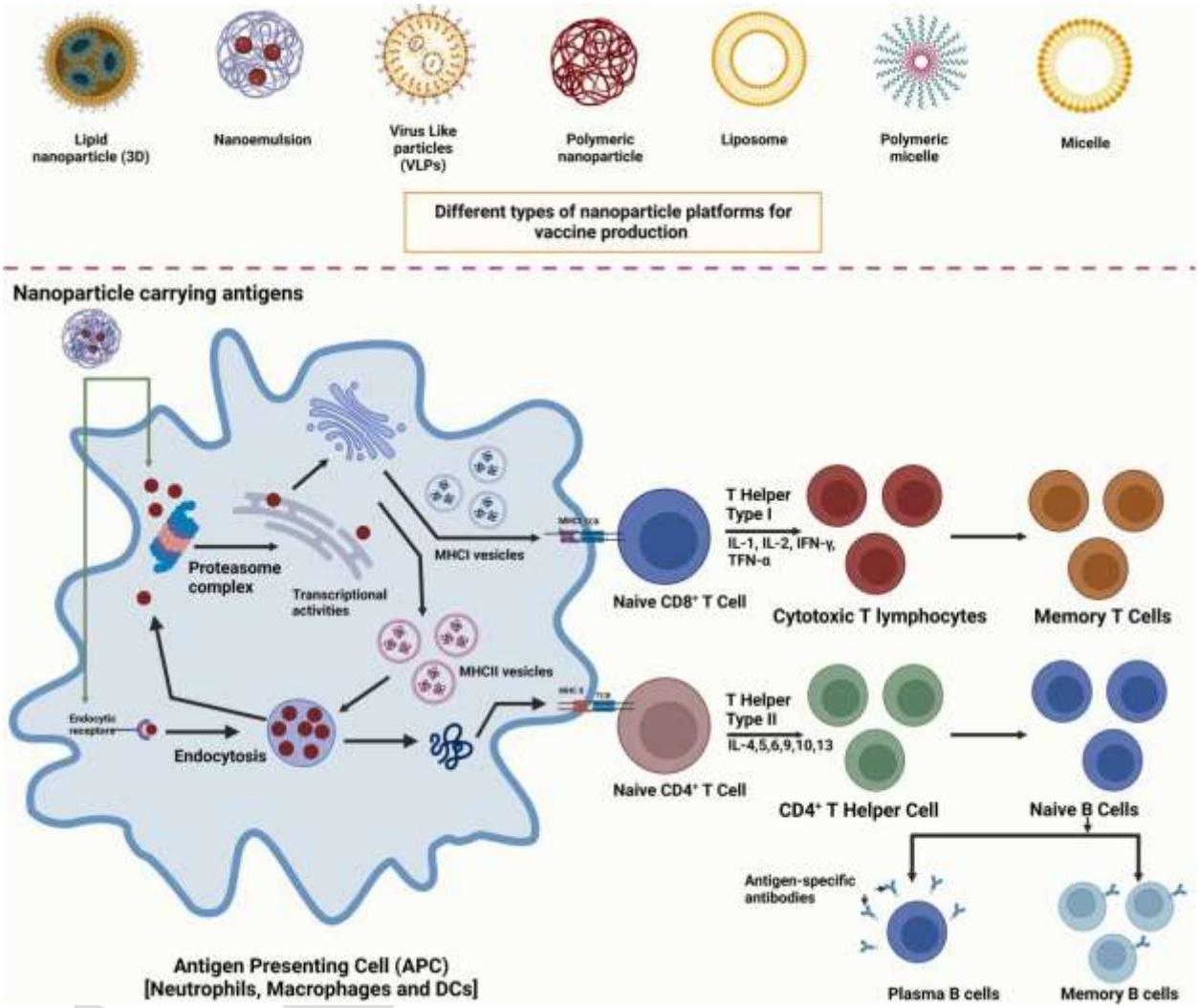

Figure 2: How innate immunity and adaptive immunity—the two immune response components—are triggered by Nanovaccines (17)**.**

3.1. Lipid-based nanovaccines

Lipid-based nanocarriers get recognized by the antigen-presenting cells located in peripheral tissues by either receptor-mediated endocytosis or phagocytosis. The uptake mechanism is determined by the size of nanoparticles, where the particles sized in micrometers undergo phagocytosis. The nanocarriers, which are less than 150 nm in size, are acted upon by clathrin-mediated endocytosis. Phospholipids form nanocarriers, which significantly influence innate

immune response initiation. The physicochemical properties of the vesicles, such as charge, size, type of phospholipid, and surface modification, such as targeting moiety attachment, can contribute to the appropriateness of the nanocarrier composition, resulting in obtaining the desired immune response. Owing to the enhanced electrostatic interaction that takes place between the cationic-lipid-based nanocarrier and negatively charged moieties on the surface of the antigen-presenting cells, cationic lipids are more effective than vaccine adjuvants than anionic and zwitter ionic lipids, resulting in the facility of nanocarriers fusion followed by its cellular internalization and release of the antigen payload. Dimethyl dioctyldecylammonium, 1, 2 -dioleoyl-3-trimethylammonium propane (DOTAP) are the most common cationic lipids (18,19). After recognition, fusion, and cellular uptake, the antigens undergo processing in the antigen-presenting cells and are presented to the molecules of MHC class 1 or class 2. Presentation to MHC class 2 leads to T-helper cell activation, so stimulating antibody production and cellular immunity (20).

3.2. Liposomes

Liposomes have been widely employed in vaccine formulations because of their strong loading capacity, considerable biocompatibility, minimal toxicity, and controlled-release nature (21). They can encapsulate antigens and adjuvants and target specific cells or tissues (17). Commercial liposome production is simple and can provide more encapsulation capacities (17). liposomes can prevent the breakdown of antigens, resulting in an enhanced immunological response (22).

Liposome-based vaccines have two main forms: liposomes conjugated with antigens and liposomes encapsulating antigens. In liposomes encapsulating antigens, antigens are contained in the liposome's lipid bilayer or aq. Within the lipid bilayer or inner aqueous phase of liposomes, bioactive hydrophilic, hydrophobic, and amphiphilic compounds can be encapsulated (21,24,25). BAPCs can selectively take them. Modifications to liposomes' charge, size, lamellarity, and composition, including incorporating cell-specific targeting moieties, allow control over these characteristics (27).

VacciMax® (VM) is a vaccine delivery platform encapsulating adjuvants and antigen sequences in multilamellar liposomes in an oil-in-water emulsion (28). To create liposome formulations known as DepoVaxTM (DPX), HPV-16 E749-57 peptide and adjuvant were

dissolved in an appropriate buffer and combined with a 10:1 (w:w) DOPC/cholesterol mixture (29).

3.3. Polymer-based nano vaccines

In order to develop vaccination platforms for the immunotherapy of different infectious illnesses and cancers, polymers have been thoroughly researched as components and excipients (30). Since polymer-based particles can stop antigen breakdown and clearance while enhancing absorption by professional antigen-presenting cells (APCs), they have been used in various vaccination platforms and adjuvant applications (31). Polymeric nanoparticles with good bioavailability, sustained release, stability, and drug loading have been widely employed to load immunomodulators, vaccine antigens, or both, either conjugated to surface groups or enclosed within the core (15).

The two primary types of polymeric nanoparticles (NPs) are synthetic and natural polymeric NPs, and each has advantages and disadvantages; natural polymeric nanoparticles (NPs) come from biological materials, including proteins, lipids, polysaccharides, and polysaccharides, whereas synthetic NPs are created in the lab by chemical processes (32). Compared to synthetic NPs, natural polymeric NPs offer several benefits, such as reduced toxicity, biocompatibility, and biodegradability. Because of these characteristics, they are used to create vaccines since they are less likely to result in side effects or prolonged toxicity (32).

Natural polymeric nanoparticles (NPs) utilized in the production of vaccines include silk fibroin, alginate, and chitosan (17). A naturally occurring polymer found in crab shells, chitin is the chitosan source, a biodegradable polysaccharide (33). Alginate is a seaweed-derived biopolymer that, when cross-linked with divalent cations, may create a matrix that resembles gel (34). A protein called silk fibroin, which is derived from silk, creates very stable and biocompatible nanoparticles (35).

However, very effective vaccine delivery systems may be created because synthetic polymeric NPs can be carefully modified to regulate their size, shape, and surface features. Still, They could also be more hazardous and have a higher risk of activating an immunological response (36). The most widely used synthetic biodegradable polymers for vaccine delivery against a variety of diseases, including hepatitis, leishmaniasis, and malaria, B (HBV) and Ebola, are PLA (polylactic acid), PGA (polyglycolic acid), and PLGA (poly lacticco-glycolic

acid) (37). To create block copolymers, PLGA can be coupled to polyethylene glycol (PEG) or polyetherimide. These copolymers can then self-assemble into polymeric micelles, including hydrophobic molecules and hydrophobic peptide antigens or proteins, to form micellar nanoparticles (38–40). Synthetic polymeric NPs provide better control over particle characteristics and release patterns, while natural polymeric NPs are often safer and more environmentally friendly (17).

3.4. Dendrimers

They are hyperbranched polymeric 3D trees, including inner cage–like cores of multifunctional properties. They are distinctive and have a lot of properties, such as extensive branching, multi-valency, globular nanostructure, water solubility, and defined molecular weight. Positively charged dendrimers are distinctive in protecting conjugated nucleic acids, proteins, and peptides from being degraded by enzymes through condensing them into Nano particulate formation by electrostatic interactions and enhancing effective antigen delivery (41). A combination therapy using cationic polyamidoamine, which is a functionally modified dendrimer along with anti-PD1 antibody-mediated immune checkpoint blockade, has shown enhanced 0cross-presentation by antigen, prophylactic response, and therapeutic success in a B16-OVA melanoma model. Such synthetic dendrimers keep their property as good photo sensitizers able to produce reactive oxygen species (ROS) to destroy cancer cells (42). Cationic dendrimers such as PAMAM (polyamidoamine) can cause gap junction mutilation and increase permeability on cellular attachment, causing cell lysis (15,43).

3.5. Exosomes

They are membrane-bound, bioactive extracellular vesicles with sizes 30-150 nm, containing cytosolic, transmembrane proteins, lipids, mRNA, DNA, and micro-RNA, which support cell to cell communication without direct contact. Concerning cancer immunotherapy, exosomes play a dual role in anticancer immunity immunosuppression or activation. More exosomal vesicles are loaded with tumor antigens if compared to healthy cells and help to escape immune surveillance. Immune checkpoint proteins like gram death ligand-1 (PD-1) on the surface of budding exosomes from tumor cells have been detected by many studies, which are found to interact with PD-1 receptors on cytotoxic T-cells and prevent them from killing malignant cells. Recently, DC-derived exosomes (DEXs) and modified tumor cell-derived exosomes (TEXs) have got a lot

of attention in generating therapeutic cancer vaccines (44). TEXs preclinical vaccinations have been found to promote anticancer immune response in murine leukemia, metastatic melanoma, colon carcinoma, and breast cancer models. DC-derived exosomes contain the antigen processed with surface-displayed MHC-class 1/2; the peptide-MHC complexes are transported to activate naïve DCs. The antigenic peptide is presented by the naïve DCs on their surface (cross-dressing), or the DCs present the antigen carried on their endogenous MHC class 2 molecules, which allows CD4+ T cells activation. Activation of CD8+ T -cells occurs by peptide –MHC class 1 complexes in a DC-dependent pathway. These DEXs are very stable and can be stored for a very long time (15,45).

3.6. Micro-needle arrays

Several technologies have been used to deliver drugs-needle syringes, liquid jet injectors, micro-needle arrays, and biolistic particle injection. A needle-free system has the advantage of being painless and more effective in delivering plasmid DNA, drugs, and proteins. It's used as an alternative to the needle-syringe route for vaccines targeted delivery systems. Needle-free methods include diffusion patches, liquid jet injections, and microneedle arrays to avoid the pain stimulated by the needle prick. Diffusion through patches is one of the least invasive methods for delivering small molecules (<500 Da). Moreover, electroporation, ablation by laser or heat, radiofrequency high-voltage currents, iontophoresis, liposomes, sonophoresis, and microporation are some of the modern technologies being discovered but are being tested. DNA vaccine in liquid is delivered by the use of a high-speed injector around the Langerhans cells, an approach which is known as a liquid jet injector (46,47)

3.7. Virus-like particles

Because they lack genetic material, virus-like particles (VLPs) are a class of nanoparticles that resemble viruses in structure but are not infectious(17). By showing antigens on their surface, VLPs can function as vaccines by inducing an immune response without causing illness (48–50). VLP vaccinations are superior to traditional vaccinations in several ways, such as safety; VLP vaccinations are safe for humans since they are not infectious and do not contain genetic material efficacy. Vaccines using VLP can produce a potent and long-lasting immune response, as they have a highly structured, repeating presentation of the antigens that resembles the natural structure of viruses, versatility: VLPs can be designed to exhibit a variety of antigens

or epitopes, which can strengthen the immune response and defense against various viral strains or types, stability; Because VLP vaccinations are stable over a broad temperature range, distribution and storage may be made easier (51). VLPs have a diameter ranging from 20 to 200 nm, and like intracellular/endogenous antigens, they can stimulate Th1 and CTL immune responses (26,52). Exogenous antigens preferentially reach the MHC II route and engage with CD4+ T cells, While VLPs can connect to MHC I molecules and be cross-presented, ultimately triggering a powerful CD8+ immune response against infectious illnesses, including cancer(26,53). Like other protein products, VLPs can be made in various cell types, such as mammalian cell lines, yeast, bacteria, insects, and plants (27).

Numerous infectious diseases, such as hepatitis B, coronavirus, influenza, norovirus, and human papillomavirus (HPV), have been vaccinated with vaccines based on vector-like particles (VLPs) (17). One of the most effective VLP-based vaccinations is the HPV vaccine. The primary antigen of the virus, the L1 capsid protein, can be identified by HPV VLPs used in the vaccine; the vaccination offers defense against HPV strain infections that result in cervical cancer and other cancers (17). Another effective VLP-based vaccination is the hepatitis B vaccine; Hepatitis B surface antigen (HBsAg) VLPs, which elicit an immunological response to the virus, make up the vaccine, which shows protection against the hepatitis B virus, which can lead to hepatic cancer (54). Another vaccination that is based on VLP is the influenza vaccine; VLPs displaying the hemagglutinin protein of the influenza virus can be used to produce the vaccine, which stimulate the body's defenses against the pathogen, the vaccination protects against strains of the seasonal and pandemic influenza (55). Lipid nanoparticles that mimic VLPs are used in the COVID-19 vaccines produced by Pfizer/BioNTech and Moderna to deliver mRNA coding for the SARS-CoV-2 spike protein and trigger an immune response against the virus; the prevention of COVID-19 infection is greatly enhanced by these vaccinations (56,57).

4. Routes of Nanovaccine

Historically, parenteral routes have been used to administer vaccines, specifically the intramuscular (IM) and subcutaneous (SC) routes. Most of the time, this makes it possible to create a local depot at the injection site from which the local LN can receive the antigen and any adjuvants that may be present. The mode of action for this process can vary depending on the vaccine composition, either via immune cell capture and transfer to the LN or by passive means.

The advantages that transdermal (TD) and SC injections may offer in terms of vaccine drainage to the lymphatics and overall immunogenicity have led to their presentation as IM immunization alternatives (58). Moreover, it is feasible to administer DNA vaccinations orally and effectively. Oral DNA vaccine delivery benefits from the invention of polymer-based nanoparticles (59). More thorough research in this field may make the use of oral DNA vaccine against illnesses possible.

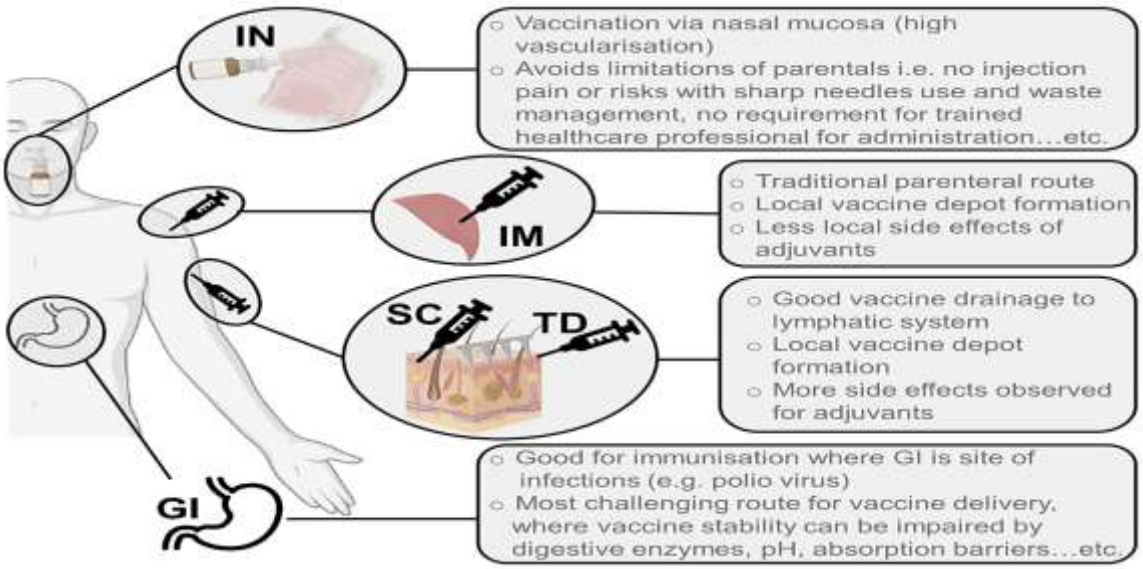

Figure 3: The main administration routes for vaccinations against viruses (60)**.**

4.1. Parenteral vaccination

When draining cancer vaccines to LNs, the first thing to do is to aid in the penetration of vaccinations via the interstitium to lymphatic vessels, which uni-directionally carry interstitial fluid and solutes to lymph nodes (61) As a result, vaccines are only partially delivered to lymphatic vesicles, where they are transported to lymph nodes. Direct injection of LNs can do this like intramuscular, subcutaneous, or intravenous methods. They demonstrated superior targeting of lymph nodes (LNs), draining to both proximal and distal LNs with extended retention times after subcutaneous injection. Co-administration of nano vaccines may compromise immunological (62) tolerance and reinstate immunological responses unique to HBV. In addition to optimizing the nanoparticles' size and loading efficiency, BALB/c mice were used in an immunization

investigation. The mice were administered subcutaneously with phosphate buffer saline (PBS), superoxide dismutase B1 (SODB1) in PBS, and nanoparticles. Additionally, three subcutaneous injections of complete Freund's adjuvant (CFA) and soluble Leishmania Antigens (SLA) were given to each group every three weeks (some only received one dose) (63). We combined PAN with M. paratuberculosis antigens to create nano vaccines that can elicit strong and long-lasting protective immune responses. This investigation used a single subcutaneous (64). We regularly checked inoculated mice to assess the safety of the nano vaccines. Mycopar®-vaccinated animals progressively developed an abscess at the injection site that developed and remained throughout the investigation (65). Parenteral immunization routes are widely used, but they have significant drawbacks; these include the costs and manpower involved in preparing, administering, and discarding injectable materials, as well as the risks related to needle stick injuries and managing sharp waste. Due to these factors, mucosal pathways of The administration has also been investigated for vaccinations, especially in light of the fundamental role that mucosal-associated lymphatic tissue (MALT) plays in locally inducing mucosal immunity (66).

4.2. Nasal route

It has been reported that a safe and effective hepatitis B vaccine is a needle-free nasal vaccination using a nanoemulsion containing the hepatitis B antigen; this vaccination is stable for six months and doesn't need to be refrigerated. The novel nanoemulsion eliminates the possibility of needle-borne infection transmission and is non-toxic and painless. (67) Baker's group affirms the possibility of HIV, anthrax, influenza, and smallpox vaccines using a nasal nanoemulsion approach. Once inside the nasal cavity, NPs remain in the mucus before emerging through the epithelial barrier of the airway. The size of the NPs and other parameters influence how long the nanovaccine remains in the mucus. In general, the 20–80nm nano vaccine can improve immunological reaction in mucosal immunity of the nose (68). Specific nanoparticles (NPs) like liposomes and chitosan can prolong their stay in the nasal mucosa. Different approaches are used to introduce nano vaccines to immune cells in the epithelial cell barrier. Pathway 1 allows nanovaccines to be directly taken up by dendritic cells via the epithelium's synapses. Pathway 2 involves the passive penetration of the nano vaccines across the epithelial cell gap to the underlying DC cells. In pathway 3, M cells ensnared the vaccine within the nanovesicles and deposited it into the

barrier pathway. Pathway 4 allows nano vaccines to deliver antigens to cells using endocytosis ns to cells (69).

There are two main obstacles to intranasal vaccination delivery: the first is precise and repetitive administration of very small amounts of prepared vaccine, and the second. Includes reducing the amount of particles deposited in the lung while applying the developed vaccine to all regions of the nasal mucosa, particularly lymphoid tissues (70).

4.3. Oral nanovaccine

Most vaccines are injectable, which is difficult for patients and necessitates using highly qualified staff for administration. One approach to overcoming such barriers in low-cost vaccinations is to use non-injectable vaccination vials and intranasal and oral routes (71). Oral vaccinations are non-invasive, safe, and simple to administer (needle-free), making them preferable over other immunization routes (71). Oral vaccination provides strong mucosal and systemic immunity and the ability to immunize large populations (71). Unfortunately, inherent demanding situations in oral vaccine improvement must be addressed for vaccines to be effective (71). For example, antigen proteins or peptides must endure the stomach's acidic environment, proteolytic enzymes, and bile salts of the gastrointestinal tract (GI) before interacting with immune cells. Other issues include food (71). Polyanhydride nanoparticle (PNPs) vaccinations are ideal for poultry because they target gut-associated lymphoid tissues (GALT) (72,73). Orally administered powerful PNP-based vaccinations increase levels. In mice, vaccination via the intestinal mucosa resulted in a greater antibody response than other routes. Oral vaccination has limits due to antigen breakdown in the stomach. The mucoadhesive nanoparticle-based delivery technology shields vaccination antigens from degradation in acidic pH settings and delivers them to the small intestine in animal models (72–75). Chitosan-based oral Salmonella nano vaccine targets chicken immune cells, inducing antigen-specific B and T cell responses. This prospective oral Salmonella nano vaccine can potentially reduce salmonellosis in poultry (76).

4.4. Mucosal nanovaccine

Many infections enter the human body through mucosal surfaces, which make them ideal for targeted vaccination delivery. Nanotechnology-based applications provide promise for the tailored delivery of vaccination antigens across mucosal surfaces. Nanotechnology allows for the easy tailoring of the solubility, stability, and surface qualities of vaccine antigens and/or

adjuvants. As a result, nanoparticle-based drug delivery systems have recently sparked a lot of interest in vaccination (77).

Nano-encapsulation of antigens has numerous advantages over traditional antigen delivery methods. For starters, nanoparticle encapsulation may prevent antigen degradation or premature release (78). Nanocarrier surface modification may also enhance the contact between antigens and the mucus layer, allowing for more effective transport to mucosal-associated immune systems (78). Furthermore, rationally developed multi-functionalized nano vaccines can improve immune responses by enhancing immune activation pathways at many levels, such as increasing cellular uptake, targeting APCs or lymph nodes, or improving intracellular endosome escape (78).

Most reported mucosal nano vaccines were designed to prevent viral and bacterial infection. The pathogens studied include viruses transferred by the respiratory tract, such as influenza and coronavirus, and bacteria transmitted through the gut or respiratory tract. Most of these pathogens enter the body through mucosal locations; therefore, creating mucosal vaccines is particularly important for preventing infection. In this COVID-19 outbreak, lipid nanoparticle-based mRNA vaccines have demonstrated outstanding success due to their excellent protective effect across all ages. However, the licensed injectable COVID-19 vaccines primarily activate systemic immune responses, not mucosal ones. Mucosal nano vaccines can trigger strong anti-tumor CD8+ T cell responses, making them effective against cancers situated in the mucosa (78).

4.5. Pulmonary delivery of mucosal nano vaccine:

Unlike parenteral vaccination, pulmonary immunization does not require a needle and syringe, resulting in enhanced safety, lower cost per dose, less pain for patients, and higher feasibility for mass immunizations (79). Nanoparticles delivered the hepatitis B vaccine to the lungs, producing higher humoral, mucosal, and cytokine responses than free antigens (79).

4.6. Transdermal nanovaccine

To develop protective immunity, alternative antigen delivery methods must be efficient, safe, and effective (80) .The transdermal mode of administration has numerous benefits for reaching this goal. It is appealing for needle-free immunization due to its reduced first-pass metabolism and side effects, non-traumatic nature, and self-administration by the patient (80).

Particle-based approaches show much potential for transdermal vaccine delivery through the skin. Compared to soluble antigens, active skin delivery can increase vaccine immunogenicity by identifying and activating more particulate APCs (81).

5. Techniques for the Active Delivery System

The use of various devices to actively promote skin permeability to vaccinations is known as proactive delivery of particle-based systems. Some treatments employed include transdermal electroporation, microneedle patches, sonophoresis, iontophoresis, radiofrequency/thermal and laser skin ablation, and jet or powder injection. These techniques with particle-based vaccinations increase skin permeation, recognition, and interaction with APCs (81).

6. Techniques for Passive Delivery Systems

Non-invasive distribution of submicron and nanomaterials and liposome technologies such as solid nano-emulsions and vesicles are examples of particle-based procedures that do not compromise the skin. Antigen transportation relies on forming a concentration gradient to diffuse particles passively through intact skin and occlusion and trans follicular diffusion to promote skin hydration (81).

Malignant melanoma is a fatal skin cancer with significant morbidity and mortality rates, and its global occurrence has increased in recent years. It resulted from the conversion of melanocytes into malignant cells (82). Conventional therapies for melanoma include surgery, chemotherapy, and radiotherapy; however, the therapeutic advantages are insufficient, resulting in treatment failure. Fortunately, remarkable advancements in immunotherapy have provided new hope for metastatic melanoma patients, revolutionizing melanoma therapy (82). PDM MNs are prepared and characterized for transdermal delivery. We combined the PDM nanosystem onto microneedles (MNs) for melanoma-targeted transdermal distribution to facilitate administration. The MNs were created using a commercially available micro-molding technique in which PDM hyaluronic acid (HA), and polyvinylpyrrolidone (PVP) matrixes were co-loaded into MNs of uniform size and morphology (82).

7. Nanovaccines: pros and cons

The use of conventional methods in the field of vaccination is no longer sufficient, and the incorporation of live or killed microbes to produce an immune response is insufficient to address the severe and occasionally life-threatening medical conditions of today. Due to the properties of nanoparticles, such as their size, shape, charge, inertness, biocompatibility, biodegradability, and many more, many researchers became interested in this new and attractive area of research, which is now known as nanovaccinology (83). Several scientists developed an interest in this intriguing and novel field of study, known as nanovaccinology. This review will address the development of nanovaccines and the various ways the body can absorb them. In addition to the applications, advantages, disadvantages, and types of nanoparticles used in the production of vaccinations for both therapeutic (84).

Benefits and drawbacks of the nanomaterials being utilized in nano vaccines numerous nanomaterials have been employed in biomedicine as adjuvants and antigen carriers in creating nano vaccines. Choosing the right nanomaterial to construct nano vaccines is difficult because there isn't a perfect nanomaterial that can have all the ideal physicochemical and biological properties. Inorganic nanoparticles, for example, give antigens exceptional chemical and thermal stability. However, their biological interaction depends on the particles' size, shape, and surface chemistry; if these are not properly chosen, they might cause toxicity, bioaccumulation, and poor biodistribution (85). Advanced nanovaccine technologies are being researched to elicit a robust, repeatable immune response against challenging diseases. Nanovaccines, as opposed to traditional vaccinations, offer enhanced lymph node access, the best possible antigen packing and presentation, and the stimulation of a long-lasting immune response. The present review offers an overview of the worldwide patterns in developing novel nanoscale vaccines against infectious diseases. It also delineates the biological, experimental, and logistical obstacles linked to their advancement and explains how immunoengineering might be utilized to surmount these obstacles (86).

Pros: there are several benefits to using nanovaccines, ranging from their broad size range to their improved bioavailability. Because this delivery system comes in various sizes, forms, and components, it can be used in a wide range of applications (87). Additionally, the particles can enter the cell readily through a process known as endocytosis because of their

unique sizes, which are extremely similar to the components of the cell (87).

Vaccines in nanoemulsions have a strong affinity for the lymphatic system, which makes it simple to collect them in the areas of lymph nodes. These cutting-edge nanovaccination systems have potential uses in cancer immunotherapy, influenza prevention, and other areas presently being investigated and studied (88). Several advantages are provided by nanoscale additives in these systems, such as aluminum: they are safe, easily obtainable at reasonable costs, and require little processing (88).

Despite developments in conventional vaccines' instability and toxicity are main disadvantage of their use; thus, nanotechnology in vaccine provide the opportunity to enhance the safety and stability via enhancing both cellular and humoral immune systems (89). Interestingly, vaccination with nanopatch technology offers improved antigen bioavailability and antigen site-specific delivery without causing pain, requiring no needles, and being economical (90).

The nanoparticles, which were previously made from poly alky-cyanoacrylate, nevertheless have a short half-life, which results in a shorter therapeutic impact because phagocytes remove them. Despite this, the nanoparticles have new advantages over their previous use. However, methods such as surface modification have made it possible to extend their stay in the body, transport a variety of medications, vaccinations, diagnostic tools, and other biological chemicals, and even allow them to work specifically on different organs.(91) As the field of vaccination develops, new obstacles also arise; for physicians, knowledge is the key to overcoming these challenges (92).

Cons: The drawbacks of using nano vaccines Nano-vaccine technology views the development of new vaccines as a challenging process that calls for a large number of equipment and tools, which drives up the cost of the production process (88). Additionally, various nanomaterials in different sizes and shapes can be employed to create nano vaccines; however, it is difficult to alter the composition of these materials, which could result in some toxicity (88).

Additionally, the idea of a nanovaccine is based on using extremely small particles, known as nanoscale materials, which occasionally clear the body quickly (87). However, certain biodegradable polymers have limited applications in solid particulate vaccines, as they can bind to proteins and lose their immunogenicity over time ,and a significant disadvantage of using

liposomal delivery systems is that they tend to aggregate when stored (87). As many health disorders emerged that could not be treated with the conventional methods of the past, it became imperative to investigate new fields, like nanotechnology, and apply them for the good of humanity. The synthesis of vaccines that can more effectively enhance cellular and humoral immune responses has used the nanoparticles' capacity to traverse biological barriers as well as their preferred size, shape, charge, porosity, efficacy, and stability (93). These recently employed particles demonstrated their significant advantage with the therapeutic plasmids high biocompatibility and its gradual, sustained antigen delivery to increase the immune response intensity (94). Numerous clinical trials have exhibited the promise of nano vaccines in treating and preventing a wide range of infectious disorders (95).

8. Applications for Nanovaccines

Although no one is immune to illnesses, infections, or other health problems in the 21st century, new scientific discoveries make it available to reduce the risk of serious health conditions. 90% of the drugs in this sector are now pills, capsules, tablets, and other dosage forms. Originally, the focus of this field was herbal and alternative remedies. However, the parenteral dosage form significantly impacted human treatment, reducing side effects, increasing drug bioavailability, and improving patient adherence. As a result, scientists were drawn up to develop new technologies for parenteral products, such as nanotechnology, which has proven to be useful in the medical field when dealing with objects that are nanometers in size.

Because of this, they may be the best options for application in biology, science, electronics, biomedicine, and optics. Because of their high surface area to volume ratio and capacity to form different formulations, such as nanocrystals, nanopowders, and nanoclusters, Nanoparticles have become important components in modern medicine in recent years. They are being explored for use as contrast agents in imaging and as carriers for transferring drugs and genes to tumors, among other clinical applications. They can be used to prevent and treat a wide range of illnesses, including cancer and influenza (83,96).

8.1. Cancer

Enhancing the effectiveness of cancer immunotherapy and vaccinations against cancer is made possible by incorporating nanomedicines. The findings show that these platforms can elicit

strong and long-lasting anticancer immune responses. Additionally, by using this therapy approach, unfavorable side effects were reduced concurrently (31,97)

8.2. OMPN nanovaccine

A study on orthotopic melanoma models revealed that a multifunctional nano vaccine called OMPN, which included polydopamine, ovalbumin (OVA), and MnO2, could prevent tumor growth and liver metastasis in a mouse melanoma model. The vaccine was made using a simple one-pot approach. Moreover, MRI tracking demonstrated that DC movement and locomotion in the inguinal lymph node dramatically increased after vaccination, indicating successful DC activation and an anti-tumor immune response. An assessment of the TME after OMPN therapy revealed a considerable increase in CD3+ and CD8+ cell infiltration and IFN-γ release even after laser exposure. Furthermore, the polarization of TAMs from the M2 to M1 phenotype increased more in the OMPN + laser group than in the other groups.

Reduced IL-10 and elevated IL-12p40 levels in the TME after OMPN + laser treatment supported the findings. The authors proposed OMPN as a potential MRI-trackable and efficacious nano vaccine for the treatment of melanoma (98).

8.3. Nanoprodrug

There is proof that MDSCs have a role in the reduced immune response when an immunological checkpoint is blocked. Accordingly, a study using a colon tumor model revealed that a TME-responsive nano prodrug (FIT NPs) designed to deliver tadalafil and indocyanine green (ICG) photosensitizer simultaneously targeted MDSCs in the TME and increased tumor immunogenicity. By inducing immunogenic cell death and reducing MDSC immunosuppressive activity, the results demonstrated a substantial therapeutic efficacy to support photothermal immunotherapy. The late apoptosis/ necrosis rate of MDSCs in the FIT + laser group was 41.0% in vitro. Furthermore, without altering the weight of the mice under study, FIT + L might significantly decrease tumor growth. The authors proposed that these events result in the maturation of DCs and the activation of T cells, enhancing the anti-tumor immune response and immune checkpoint blockade efficacy (99).

8.4. Neoantigen-based cancer nano vaccines

Neoantigen-based cancer nano vaccines are useful therapeutic options that aid in stimulating CD8+ T cell responses, as previously stated. In this case, neoantigen and the acid-activatable polymeric conjugate DMXAA (a STING agonist) in a nanoplatforms were combined

to create an acid-responsive polymeric nanovaccine that would stimulate the STING pathway and improve cancer immunotherapy. The results showed that neo-antigen absorption by DCs was induced by nanovaccines accumulated at the lymph nodes. Furthermore, the STING agonist stimulates IFN-β production and enhances T-cell priming specific to neoantigens by activating the STING pathway in DCs. Furthermore, the B16-OVA melanoma and 4T1 breast tumor mouse models showed significantly reduced tumor growth due to the nano vaccine. Combining anti-PD-L1 antibody treatment with nano vaccines demonstrated increased anti-tumor immune responses in a 4T1 breast cancer model (100).

8.4.1 Hex@Bp

Fascinatingly, studies in this field have shown that serum exosomes (hEX) from tumor-bearing mice treated with hyperthermia showed a variety of TAAs and strong immunoregulatory capabilities in stimulating the differentiation and maturation of DCs. This makes nanotechnology-based photothermal therapy successful in cancer therapy. A cancer nanovaccine called hEX@BP was created using black phosphorus quantum dots and exosomes (hEX) encapsulation to cure murine subcutaneous lung cancer models. In vivo, tumor temperature rise and long-term photothermal therapy performance were demonstrated by hEX@BP and photothermal therapy. Furthermore, after receiving combination therapy, there was an increase in the infiltration of effector T cells into the TME.(101)

8.4.2. SeaMac

A safe, easy-to-use, and reasonably priced way to enhance neoantigen-based cancer immunotherapy is through self-adjuvanted nano vaccines. A study used polymer nanoparticles (NPs) and a neoantigen to design and fabricate a self-adjuvanted nano vaccine that could activate molecules, limit tumor growth, and prolong the tested animals' survival in tumor mouse models of B16-F10 and colon carcinoma 26 (CT26). It works by stimulating DCs to mature, increasing their infiltration into lymph nodes, and ultimately stimulating CD8+ T lymphocytes to destroy tumor cells that express the vaccine's target antigen. However, these are distinct vaccinations since self-adjuvant nano vaccines do not require adjuvant or immune serum to exhibit abscopal effects in B16-F10 and CT26 tumors.

These results may contribute to addressing some of the issues facing nanovaccines, such as their high cost and complex treatment regimens (102).

8.5. Infectious diseases

The properties of nanoparticles, such as their size, shape, and biocompatibility, have contributed to numerous advancements. Nanotechnology is being used in the medical industry to treat infectious diseases that are prevalent worldwide. Many obstacles exist when it comes to treating large populations of people. Using nanoparticles to treat infectious diseases has provided alternative approaches to traditional and alternative medicine. Nanoparticles may target the disease more effectively by entering cells through membranes, taking longer to act than alternative medications, reducing side effects, and saving patients' money using fewer doses (83).

8.6. Influenza

An innovative method of vaccinating against the H1N1 virus involved administering a novel vaccine directly into the respiratory system via nanoparticle delivery. The production of a double-adjuvanted vaccine, including the H1N1 influenza hemagglutinin antigen (HAC1), a drug delivery system based on silica nanoparticles, and the mucosal adjuvant candidate c-di-GMP are all made possible by this novel technique. When the vaccination was given to the lower lungs, it produced results. To guard against the influenza virus, both local and systemic immunity were generated once an antigen response was observed, and T-cells were reactivated. IgG was needed for a wider cross-protective response against the virus, whereas IgA was needed to neutralize the virus in the epithelial cells. It has also been demonstrated that IgA has a role in cross-protection against viral strains that have drifted however this function is not necessary for protection (103,104).

These studies involved mice and demonstrated the vaccine's capacity to trigger an immune response that prevents infection and the virus from spreading (105). Pigs were used in another study to activate the immune system using inactivated antigens encapsulated in PLGA nanoparticles. Water/oil/water double emulsion solvent evaporation is the method utilized for encapsulating. Since PLGA is non-toxic, biodegradable, and biocompatible, it can be effectively used as a nanocarrier for vaccines that have been shown to elicit humoral and cellular immune responses. Cytotoxic T cells were generated by intranasal delivery of the inactivated swine influenza virus in PLGA, which resulted in protection against the illness and a decrease in the virus's spread in the pigs' lungs (106).

The inactivated swine influenza virus was encapsulated in poly anhydride nanoparticles (KAg nano-vaccine) in an experiment. This slowed the disease's progression in the pigs' lungs and produced an antigen-specific cell-mediated immune response. These nano vaccines were able to develop antigen-specific memory T cells in mice, protecting the future. The usage of amphiphilic polyanhydrides based on 1, 6-bis (pcarboxyphenoxy) hexane (CPH) and 1, 8-bis (p-carboxyphenoxy)-3, 6-dioxaocatane (CPTEG) monomers resulted in a delayed release of the antigen, indicating that the immunogenicity was preserved. The vaccine's intranasal delivery produced clinical protection with negligible adverse effects (107).

8.7. Malaria

Any vaccine formulation must ensure a sustained immune response; this is accomplished with gold nanoparticles, recognized for their non-toxic, inert, biocompatible, easily modifiable shape and size and capacity for surface modification. Dendritic cells and other antigen-presenting cells can easily present the vaccine's antigen with the help of these nanoparticles. Therefore, by transmitting blocking antibodies, the immunogenicity of CHrPfs25 supplied with gold nanoparticles increased. Because of the spherical shape of the nanoparticles, less aggregation occurs on the cell surface than when other particles are employed. Studies have shown that the protein used in the vaccine preserved its properties even when connected to the nanoparticles, demonstrating the vaccine's repeatability. Nonetheless, immunogenicity depends on the employed gold nanoparticles' size, shape, and other physical-chemical characteristics (108).

9. Challenges and future prospectives

The development of nano vaccines may be hindered by issues such as the toxicity of nanomedicine, the difficulty of scaling up procedures, and the absence of regulatory requirements. Because pre-clinical and clinical publications have demonstrated their dose-dependent acute and chronic toxicities with preferential bioaccumulation based on the route of administration, the nanoscale size can be a double-edged sword (109).

Another big issue that technical advancements have somewhat lessened is scaling up, although doing so in a sterile setting still presents several difficulties (110).

The regulatory authorities are still confronted with challenges regarding nanomedicines. Using current, clearly defined regulatory frameworks, the regulator's primary responsibility is to guarantee all medical products and devices' efficacy, safety, and quality. Nevertheless, as market

expectations and scientific advancements change, establishing precise regulations is getting harder and harder.

The absence of harmonization and recommendations from these regulatory authorities creates a great deal of ambiguity for product developers, impeding the creation and promotion of innovative products enabled by nanotechnology. Therefore, determining and reaching a consensus on the regulatory standards for the assessed product or device is necessary to ensure an efficient approval procedure. Therefore, the government is frequently involved in developing and scaling up products enabled by nanotechnology (60).

Regulatory guidelines need to thoroughly address aspects like the consistent identification, purity, and strength of the materials used, alongside factors such as particle size, distribution, surface charge, aggregation tendency, interaction with bodily fluids, immune response, distribution in the body, interaction with tissues, and the formulation's pharmacokinetic profile (111,112).

One of the technical issues preventing nanomedicines from being used in clinical settings is that most nanomedicines are less stable due to their large surface area and nanoscale, compared to other dosage forms; also, because of their vulnerability to degradation, amorphous to crystalline transitions, phase transition, hygroscopicity, aggregation, and contamination. Most nanomedicines need stringent storage requirements, which low-income and developing countries cannot readily afford (111,113).

More research in biological models is required to determine the precise biochemical interactions and active ingredients of nano-vaccines that make them a promising option.

In-depth research on molecular pathways is necessary to comprehend the dynamics of the real mechanism underlying the protective immune response brought on by nano vaccines (114).

Certain infections, including those that cause malaria, TB, or HIV, have an extremely intricate infectious cycle or the ability to evade the immune system. Likewise, most tumors have developed to elude immune systems and produce an immunosuppressive environment. Because of this, creating a vaccine is much harder and requires a deeper understanding of the pathogenic process and the immunology of the illness or infection (115).

These nano vaccines' design, production, and administration have limitations, including tumor heterogeneity, T cell exhaustion, inherent RNA instability, decreased or diminished T cell cytotoxicity, decreased or diminished MHC expression by tumor, high costs, and complex

protocols. Nevertheless, these vaccines have shown promise in treating cancer (97). The primary constraints of RNA-based nano vaccines are the intrinsic instability of RNA and the translation efficiency to proteins (116).

Scientists across various disciplines believe that advancements in nanoscale manufacturing technologies and instrumentation, including nanomachines, robotics, nanomedicine, and diagnostic devices, will revolutionize medicine and other industries, not just physicists (117–120).

Nanomaterials have great potential for a range of industrial and biological uses. Their distinct physicochemical characteristics, however, prompt concerns about their possible environmental and public health effects. In such a way, review papers ought to endeavor to give academics, decision-makers, and business experts a thorough grasp of the most current regulatory developments concerning nanomaterials (121). A more comprehensive understanding of lymphatic biology and vaccination mechanisms will continue to influence the development of nanoplatforms, lymph entrance and transfer, and LN imaging methods in the future (122).

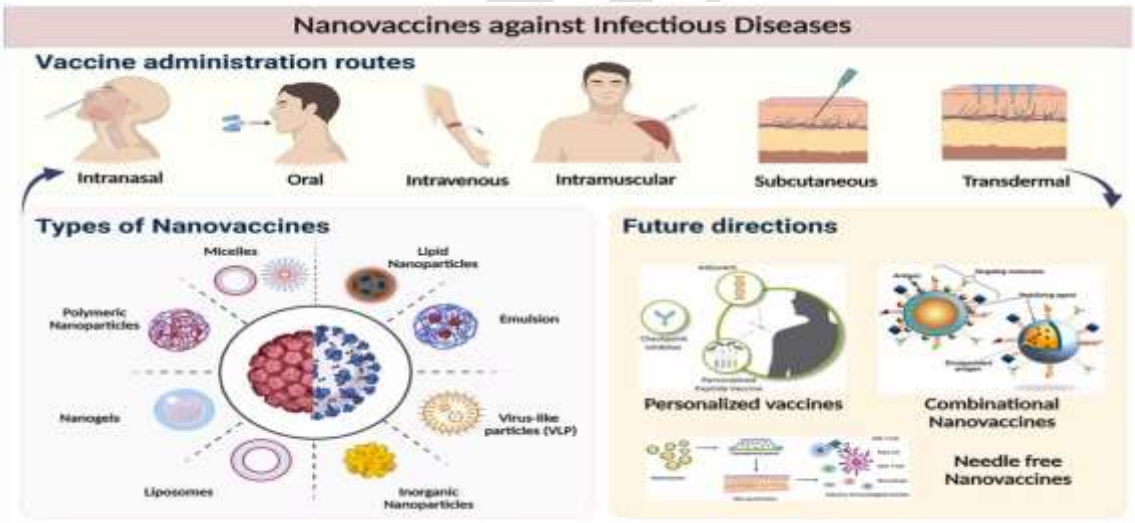

Figure 4. Pioneering the future of immunization (17)**.**

Conclusion

Traditional vaccine development can be time-consuming and high-cost. Nanovaccines have received attention as a new type of vaccine possessing huge potential. Nanotechnology has made the development of nano vaccines possible, and it has unique features that could make

vaccines extremely safe and effective. By dissecting the multitude of nanovaccine types, nano particulate delivery systems for carrying drugs and vaccines are Lipid-based nanovaccines, polymer-based nanovaccines, liposomes, dendrimers, exosomes, and micro-needle arrays depending on their chemical components. They could encapsulate antigens and adjuvants and target specific cells or tissues. The review elucidates their potential applications and benefits in some diseases like Cancer and Infectious diseases, furthermore, by exploring various routes of administration like parenteral vaccination, nasal route, oral, mucosal nanovaccine, and transdermal nanovaccine.

References

1. Rosales-Mendoza S, González-Ortega O. Nanovaccines: An innovative technology to fight human and animal diseases. Nanovaccines: An Innovative Technology to Fight Human and Animal Diseases. 2019. 1–343 p.

2. Liu M, Li Q, Lin J, Lin Y, Hoffman E. Innovative trial designs and analyses for vaccine clinical development. Contemp Clin Trials. 2021;100(July 2020):106225.

3. Pal K, Application C, Medicine T. Nanovaccinology.

4. Sun B, Xia T. Nanomaterial-based vaccine adjuvants. J Mater Chem B. 2016;4(33):5496–509.

5. Zaman M, Good MF, Toth I. Nanovaccines and their mode of action. Methods. 2013;60(3):226–31.

6. Wang L, Wang Z, Ma Q, Fang G, Yang J. The development and reform of public health in China from 1949 to 2019. Global Health. 2019;15(1):1–21.

7. Plotkin SA. Vaccines in the 21 st Century p POLIO VACCINE BY INACTIVATED Herpes simplex. Hybrid Hybridomics. 2002;21(2).

8. Smith KA. Edward Jenner and the small pox vaccine. Front Immunol. 2011;2(JUN):1–6.

9. Peek LJ, Middaugh CR, Berkland C. Nanotechnology in vaccine delivery. Adv Drug Deliv Rev. 2008;60(8):915–28.

10. Harper DM, DeMars LR. HPV vaccines – A review of the first decade. Gynecol Oncol. 2017;146(1):196–204.

11. Street T third, Eua DC. Preventing Disease & Protecting Health. Am. 2004;(596).

12. Vicente S, Prego C, Csaba N, Alonso MJ. From single-dose vaccine delivery systems to nanovaccines. J Drug Deliv Sci Technol. 2010;20(4):267–76.

13. Xiang SD, Scholzen A, Minigo G, David C. Pathogen recognition and development of particulate vaccines : does size matter ? :1–30.

14. Singh M, Chakrapani A, O'Hagan D. Nanoparticles and microparticles as vaccine-delivery systems. Expert Rev Vaccines. 2007;6(5):797–808.

15. Das A, Ali N. Nanovaccine: an emerging strategy. Expert Rev Vaccines. 2021;20(10):1273–90.

16. Small - 2016 - Cheung - Adjuvant-Loaded Subcellular Vesicles Derived From Disrupted Cancer Cells for Cancer Vaccination.pdf.

17. Priyanka, Abusalah MAH, Chopra H, Sharma A, Mustafa SA, Choudhary OP, et al. Nanovaccines: A game changing approach in the fight against infectious diseases. Biomed Pharmacother. 2023;167:115597.

18. Zaky M, Youssef Y, Megahed M. Impact of Surface Design and Coating on The Efficacy of Nano-Carriers as Drug Delivery Systems: A Review. Vol. 2, ERU Research Journal. 2023. p. 415–46.

19. Zaky MF, Hammady TM, Gad S, Alattar A, Alshaman R, Hegazy A, et al. Influence of Surface-Modification via PEGylation or Chitosanization of Lipidic Nanocarriers on In Vivo Pharmacokinetic/Pharmacodynamic Profiles of Apixaban. Pharm 2023, Vol 15, Page 1668. 2023 Jun;15(6):1668.

20. Karunakaran B, Gupta R, Patel P, Salave S, Sharma A, Desai D, et al. Emerging Trends in Lipid-Based Vaccine Delivery: A Special Focus on Developmental Strategies, Fabrication Methods, and Applications. Vaccines. 2023;11(3).

21. Lila ASA, Ishida T. Liposomal delivery systems: Design optimization and current applications. Biol Pharm Bull. 2017;40(1):1–10.

22. of Adult Thymectomy on. 1974;252(Mlc):1974.

23. Tretiakova DS, Vodovozova EL. Liposomes as Adjuvants and Vaccine Delivery Systems. Biochem Suppl Ser A Membr Cell Biol. 2022;16(1):1–20.

24. Nisini R, Poerio N, Mariotti S, De Santis F, Fraziano M. The multirole of liposomes in therapy and prevention of infectious diseases. Front Immunol. 2018;9(FEB).

25. Daraee H, Etemadi A, Kouhi M, Alimirzalu S, Akbarzadeh A. Application of liposomes in medicine and drug delivery. Artif Cells, Nanomedicine Biotechnol. 2016;44(1):381–91.

26. Azmi F, Fuaad AAHA, Skwarczynski M, Toth I. Recent progress in adjuvant discovery for peptide-based subunit vaccines. Hum Vaccines Immunother. 2014;10(3):778–96.

27. Zhang J, Fan J, Skwarczynski M, Stephenson RJ, Toth I, Hussein WM. Peptide-Based Nanovaccines in the Treatment of Cervical Cancer: A Review of Recent Advances. Int J Nanomedicine. 2022;17:869–900.

28. Daftarian PM, Mansour M, Pohajdak B, Fuentes-Ortega A, Korets-Smith E, MacDonald L, et al. Rejection of large HPV-16 expressing tumors in aged mice by a single immunization of VacciMax® encapsulated CTL/T helper peptides. J Transl Med. 2007;5:1–8.

29. Weir GM, Hrytsenko O, Quinton T, Berinstein NL, Stanford MM, Mansour M. Anti-PD-1 increases the clonality and activity of tumor infiltrating antigen specific T cells induced by a potent immune therapy consisting of vaccine and metronomic cyclophosphamide. J Immunother Cancer. 2016;4(1):1–13.

30. Pippa N, Gazouli M, Pispas S. Recent advances and future perspectives in polymer-based nanovaccines. Vaccines. 2021;9(6):1–20.

31. Zhang Y, Lin S, Wang XY, Zhu G. Nanovaccines for cancer immunotherapy. Wiley Interdiscip Rev Nanomedicine Nanobiotechnology. 2019;11(5):1–20.

32. Sathasivam T, Gugler MC, Janarthanan P. Polymers in Nanomedicine. Nanotechnology in the Life Sciences. 2021. 175–198 p.

33. Mohammadpour Dounighi N, Damavandi M, Zolfagharian H, Moradi S. Preparing and characterizing chitosan nanoparticles containing hemiscorpius lepturus scorpion venom as an antigen delivery system. Arch Razi Inst. 2012;67(2):145–53.

34. Li P, Luo Z, Liu P, Gao N, Zhang Y, Pan H, et al. Bioreducible alginate-poly(ethylenimine) nanogels as an antigen-delivery system robustly enhance vaccine-elicited humoral and cellular immune responses. J Control Release. 2013;168(3):271–9.

35. Office E. The Official Journal of Department of Pharmaceutical Sciences.

36. Kim H, Griffith TS, Panyam J. Special Section on Drug Delivery Technologies-Minireview Poly(D,L-lactide-co-glycolide) Nanoparticles as Delivery Platforms for TLR7/8 Agonist-Based Cancer Vaccine. J Pharmacol Exp Ther J Pharmacol Exp Ther. 2019;370(September):715–24.

37. Poon C, Patel AA. Organic and inorganic nanoparticle vaccines for prevention of infectious diseases. Nano Express. 2020;1(1).

38. Liu Y, Guo J, Huang L. Modulation of tumor microenvironment for immunotherapy: Focus on nanomaterial-based strategies. Theranostics. 2020;10(7):3099–117.

39. Chakravarty M, Vora A. Nanotechnology-based antiviral therapeutics. Drug Deliv Transl Res. 2021;11(3):748–87.

40. Yun C heui. Nanoparticles to Improve the E ffi cacy of Vaccines. 2020;10–3.

41. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1 β – dependent adaptive immunity against tumors. 2009;15(10):1170–9.

42. Youse M, Narmani A, Mahdi S. Dendrimers as ef fi cient nanocarriers for the protection and delivery of bioactive phytochemicals. 2020;278.

43. Adams JR, Haughney SL, Mallapragada SK. Effective polymer adjuvants for sustained

delivery of protein subunit vaccines. Acta Biomater. 2015;14:104–14.

44. Admyre C, Johansson SM, Paulie S, Gabrielsson S. Direct exosome stimulation of peripheral human T cells detected by ELISPOT. 2006;1772–81.

45. WIREs Nanomed Nanobiotechnol - 2012 - Hood - A systematic approach to exosome-based translational nanomedicine.pdf.

46. Chen D, Maa Y fun, Haynes JR, Chen D, Maa Y fun, Haynes JR. Needle-free epidermal powder immunization Needle-free epidermal powder immunization. 2016;0584(January).

47. Agency EP. Nanovaccines : recent developments in vaccination. 2009;34(December):995–1003.

48. kheirollahpour M, Mehrabi M, Dounighi NM, Mohammadi M, Masoudi A. Nanoparticles and Vaccine Development. Pharm Nanotechnol. 2019;8(1):6–21.

49. Chavda VP, Yao Q, Vora LK, Apostolopoulos V, Patel CA, Bezbaruah R, et al. Fast-track development of vaccines for SARS-CoV-2: The shots that saved the world. Front Immunol. 2022;13(October):1–20.

50. Gheibi Hayat SM, Darroudi M. Nanovaccine: A novel approach in immunization. J Cell Physiol. 2019;234(8):12530–6.

51. Cai J, Wang H, Wang D, Li Y. Improving Cancer Vaccine Efficiency by Nanomedicine. Adv Biosyst. 2019;3(3):1–18.

52. Arevalo MT, Wong TM, Ross TM. Expression and purification of virus-like particles for vaccination. J Vis Exp. 2016;2016(112):1–7.

53. Mohsen MO, Speiser DE, Knuth A, Bachmann MF. Virus-like particles for vaccination against cancer. Wiley Interdiscip Rev Nanomedicine Nanobiotechnology. 2020;12(1):1–17.

54. Bardiya N. Expression in and purification of Hepatitis B surface antigen (S-protein) from methylotrophic yeast Pichia pastoris. Anaerobe. 2006;12(4):194–203.

55. López-Macías C. Virus-like particle (VLP)-based vaccines for pandemic influenza. Hum Vaccin Immunother. 2012;8(3):411–4.

56. Kuate S, Cinatl J, Doerr HW, Überla K. Exosomal vaccines containing the S protein of the SARS coronavirus induce high levels of neutralizing antibodies. Virology. 2007;362(1):26–37.

57. Chavda VP, Vuppu S, Mishra T, Kamaraj S, Patel AB, Sharma N, et al. Recent review of COVID-19 management: diagnosis, treatment and vaccination. Pharmacol Reports. 2022;74(6):1120–48.

58. Zhang L, Wang W, Wang S, Sciences BP. and Efficacy. 2016;14(11):1509–23.

59. Diseases I mediated. No Title.

60. Cordeiro AS, Patil-Sen Y, Shivkumar M, Patel R, Khedr A, Elsawy MA. Nanovaccine delivery approaches and advanced delivery systems for the prevention of viral infections: From development to clinical application. Pharmaceutics. 2021;13(12).

61. Multi-stage lymphatic delivery system augments lymphoma immunotherapy. 2015;35(2):4987.

62. Bieber K, Autenrieth SE. Dendritic cell development in infection. Mol Immunol. 2020;121(March):111–7.

63. Danesh-Bahreini MA, Shokri J, Samiei A, Kamali-Sarvestani E, Barzegar-Jalali M, Mohammadi-Samani S. Nanovaccine for leishmaniasis: preparation of chitosan nanoparticles containing Leishmania superoxide dismutase and evaluation of its immunogenicity in BALB/c mice. Int J Nanomedicine. 2011;6:835–42.

64. Thukral A, Ross K, Hansen C, Phanse Y, Narasimhan B, Steinberg H, et al. A single dose polyanhydride-based nanovaccine against paratuberculosis infection. npj Vaccines. 2020;5(1):1–10.

65. Sinha S, Kosalai K, Arora S, Namane A, Sharma P, Gaikwad AN, et al. Immunogenic membrane-associated proteins of Mycobacterium tuberculosis revealed by proteomics.

Microbiology. 2005;151(7):2411–9.

66. He W, Sosnik A, Xu C. Editorial of Special Column on Delivery Nanotechnologies to Modulate the Immune System and Combat Inflammation and Infection. Acta Pharm Sin B. 2023;13(6):2296–7.

67. Makidon PE, Bielinska AU, Nigavekar SS, Janczak KW, Knowlton J, Scott AJ, et al. Pre-clinical evaluation of a novel nanoemulsion-based hepatitis B mucosal vaccine. PLoS One. 2008;3(8).

68. Huang J, Ding Y, Yao J, Zhang M, Zhang Y, Xie Z, et al. Nasal Nanovaccines for SARS-CoV-2 to Address COVID-19. Vaccines. 2022;10(3).

69. Kang H, Yan M, Yu Q, Yang Q. Characteristics of nasal-associated lymphoid tissue (NALT) and nasal absorption capacity in chicken. PLoS One. 2013;8(12).

70. Gupta KM, Barnes SR, Tangaro RA, Roberts MC, Owen DH, Katz DF, et al. Temperature and pH sensitive hydrogels: An approach towards smart semen-triggered vaginal microbicidal vehicles (Journal of Pharmaceutical Sciences (2007) 96, (670-681)). J Pharm Sci. 2009;98(3):1198.

71. Angulo C, Sanchez V, Delgado K, Monreal-escalante E, Angulo M, Tello-olea M, et al. Microbial Pathogenesis Oral organic nanovaccines against bacterial and viral diseases. 2022;169(May).

72. Renu S, Markazi AD, Dhakal S, Yashavanth S, Gourapura SR, Shanmugasundaram R, et al. Surface engineered polyanhydride-based oral Salmonella subunit nanovaccine for poultry Surface engineered polyanhydride-based oral Salmonella subunit nanovaccine for poultry. 2018;

73. Salman HH, Irache JM, Gamazo C. Immunoadjuvant capacity of flagellin and mannosamine-coated poly(anhydride) nanoparticles in oral vaccination. Vaccine. 2009;27(35):4784–90.

74. Salman HH, Gamazo C, Campanero MA, Irache JM. Salmonella-like bioadhesive nanoparticles. J Control Release. 2005;106(1–2):1–13.

75. Agüeros M, Areses P, Campanero MA, Salman H, Quincoces G, Peñuelas I, et al. Bioadhesive properties and biodistribution of cyclodextrin-poly(anhydride) nanoparticles. Eur J Pharm Sci. 2009;37(3–4):231–40.

76. Renu S, Markazi AD, Dhakal S, Lakshmanappa YS, Shanmugasundaram R, Selvaraj RK, et al. Oral deliverable mucoadhesive Chitosan-Salmonella subunit nanovaccine for layer chickens. Int J Nanomedicine. 2020;15:761–77.

77. Thakur A, Foged C. delivery. Nanoengineered Biomaterials for Advanced Drug Delivery. Elsevier Ltd.; 2020. 603–646 p.

78. Du G, Qin M, Sun X. Recent progress in application of nanovaccines for enhancing mucosal immune responses. Acta Pharm Sin B. 2023;13(6):2334–45.

79. Tang W, Zhang Y, Zhu G. Pulmonary delivery of mucosal nanovaccines. Nanoscale. 2022;14(2):263–76.

80. Valdivia-Olivares RY, Rodriguez-Fernandez M, Álvarez-Figueroa MJ, Kalergis AM, González-Aramundiz JV. The importance of nanocarrier design and composition for an efficient nanoparticle-mediated transdermal vaccination. Vaccines. 2021;9(12):1–22.

81. Dewangan HK, Tomar S. Nanovaccine for transdermal delivery system. J Drug Deliv Sci Technol. 2022;67(November 2021):102988.

82. Zhu J, Chang R, Wei B, Fu Y, Chen X, Liu H, et al. Photothermal Nano-Vaccine Promoting Antigen Presentation and Dendritic Cells Infiltration for Enhanced Immunotherapy of Melanoma via Transdermal Microneedles Delivery. Research. 2022;2022.

83. Yadav HKS, Dibi M, Mohammad A, Srouji AE. Nanovaccines formulation and applications-a review. J Drug Deliv Sci Technol. 2018;44(January):380–7.

84. Briles DE, Paton JC, Mukerji R, Swiatlo E, Crain MJ. Pneumococcal vaccines. Gram-Positive Pathog. 2019;36(4):362–77.

85. Angulo C, Tello-Olea M, Reyes-Becerril M, Monreal-Escalante E, Hernández-Adame L,

Angulo M, et al. Developing oral nanovaccines for fish: a modern trend to fight infectious diseases. Rev Aquac. 2021;13(3):1172–92.

86. Singh A. Eliciting B cell immunity against infectious diseases using nanovaccines. Nat Nanotechnol. 2021;16(1):16–24.

87. Zhao L, Seth A, Wibowo N, Zhao CX, Mitter N, Yu C, et al. Nanoparticle vaccines. Vaccine. 2014;32(3):327–37.

88. Li XD, Gao JY, Yang Y, Fang HY, Han YJ, Wang XM, et al. Nanomaterials in the application of tumor vaccines: Advantages and disadvantages. Onco Targets Ther. 2013;6:629–34.

89. Kim MG, Park JY, Shon Y, Kim G, Shim G, Oh YK. Nanotechnology and vaccine development. Asian J Pharm Sci. 2014;9(5):227–35.

90. Prow TW, Chen X, Prow NA, Fernando GJP, Tan CSE, Raphael AP, et al. Nanopatch-targeted skin vaccination against West Nile virus and chikungunya virus in mice. Small. 2010;6(16):1776–84.

91. Mahapatro A, Singh DK. Biodegradable nanoparticles are excellent vehicle for site directed in-vivo delivery of drugs and vaccines. J Nanobiotechnology. 2011;9:55.

92. Zhou et al. 基因的改变NIH Public Access. Bone. 2012;23(1):1–7.

93. Hajizade A, Ebrahimi F, Salmanian AH, Arpanaei A, Amani J. All rights reserved for official publication of Baqiyatallah university of medical sciences© Nanoparticles in Vaccine Development. J Appl Biotechnol Reports Rev Artic J Appl Biotechnol Reports. 2014;1(4):125–34.

94. Gregory AE, Titball R, Williamson D. Vaccine delivery using nanoparticles. Front Cell Infect Microbiol. 2013;4(MAR):1–13.

95. Hiremath J, Shyu D liang, Ouyang K, Kang K il, Krakowka S. Version of Record: https://www.sciencedirect.com/science/article/pii/S0264410X17300385. 1925;

96. AZoNano. The Applications & Suppliers of Nanoparticles. 2016;1–8.

97. Fang X, Lan H, Jin K, Gong D, Qian J. Nanovaccines for Cancer Prevention and Immunotherapy: An Update Review. Cancers (Basel). 2022;14(16):1–26.

98. Xiao B, Li D, Xu H, Zhou X, Xu X, Qian Y, et al. An MRI-trackable therapeutic nanovaccine preventing cancer liver metastasis. Biomaterials. 2021;274(September 2020):120893.

99. Zhang T, Xiong H, Ma X, Gao Y, Xue P, Kang Y, et al. Supramolecular Tadalafil Nanovaccine for Cancer Immunotherapy by Alleviating Myeloid-Derived Suppressor Cells and Heightening Immunogenicity. Small Methods. 2021;5(6):1–14.

100. Zhou L, Zhou L, Hou B, Wang D, Sun F, Song R, et al. Engineering Polymeric Prodrug Nanoplatform for Vaccination Immunotherapy of Cancer. Nano Lett. 2020;20(6):4393–402.

101. Liu Q, Fan T, Zheng Y, Yang SL, Yu Z, Duo Y, et al. Immunogenic exosome-encapsulated black phosphorus nanoparticles as an effective anticancer photo-nanovaccine. Nanoscale. 2020;12(38):19939–52.

102. Luo Z, He T, Liu P, Yi Z, Zhu S, Liang X, et al. Self-Adjuvanted Molecular Activator (SeaMac) Nanovaccines Promote Cancer Immunotherapy. Adv Healthc Mater. 2021;10(7):1–9.

103. Brandtzaeg P. Induction of secretory immunity and memory at mucosal surfaces. Vaccine. 2007;25(30 SPEC. ISS.):5467–84.

104. van Riet E, Ainai A, Suzuki T, Hasegawa H. Mucosal IgA responses in influenza virus infections; thoughts for vaccine design. Vaccine. 2012;30(40):5893–900.

105. Neuhaus V, Chichester JA, Ebensen T, Schwarz K, Hartman CE, Shoji Y, et al. A new adjuvanted nanoparticle-based H1N1 influenza vaccine induced antigen-specific local mucosal and systemic immune responses after administration into the lung. Vaccine. 2014;32(26):3216–22.

106. Dhakal S, Hiremath J, Bondra K, Lakshmanappa YS, Shyu DL, Ouyang K, et al. Biodegradable nanoparticle delivery of inactivated swine influenza virus vaccine provides

heterologous cell-mediated immune response in pigs. J Control Release. 2017;247:194–205.

107. Dhakal S, Goodman J, Bondra K, Lakshmanappa YS, Hiremath J, Shyu DL, et al. Polyanhydride nanovaccine against swine influenza virus in pigs. Vaccine. 2017;35(8):1124–31.

108. Kumar R, Ray PC, Datta D, Bansal GP, Angov E, Kumar N. Nanovaccines for malaria using Plasmodium falciparum antigen Pfs25 attached gold nanoparticles. Vaccine. 2015;33(39):5064–71.

109. Bhardwaj P, Bhatia E, Sharma S, Ahamad N, Banerjee R. Advancements in prophylactic and therapeutic nanovaccines. Acta Biomater. 2020;108:1–21.

110. Zhou J, Kroll A V., Holay M, Fang RH, Zhang L. Biomimetic Nanotechnology toward Personalized Vaccines. Adv Mater. 2020;32(13):1–22.

111. Younis MA, Tawfeek HM, Abdellatif AAH, Abdel-Aleem JA, Harashima H. Clinical translation of nanomedicines: Challenges, opportunities, and keys. Adv Drug Deliv Rev. 2022;181:114083.

112. Germain M, Caputo F, Metcalfe S, Tosi G, Spring K, Åslund AKO, et al. Delivering the power of nanomedicine to patients today. J Control Release. 2020;326(July):164–71.

113. Muthu MS. E ditorial Pharmaceutical stability aspects of nanomedicines E ditorial. :857–60.

114. Zaheer T, Pal K, Zaheer I. Topical review on nano-vaccinology: Biochemical promises and key challenges. Process Biochem. 2021;100(August 2020):237–44.

115. Simón-Vázquez R, Peleteiro M, González-Fernández Á. Polymeric nanostructure vaccines: applications and challenges. Expert Opin Drug Deliv. 2020;17(7):1007–23.

116. Yin Y, Li X, Ma H, Zhang J, Yu D, Zhao R, et al. In Situ Transforming RNA Nanovaccines from Polyethylenimine Functionalized Graphene Oxide Hydrogel for Durable Cancer Immunotherapy. Nano Lett. 2021;21(5):2224–31.

117. Modi S, Prajapati R, Inwati GK, Deepa N, Tirth V, Yadav VK, et al. Recent Trends in Fascinating Applications of Nanotechnology in Allied Health Sciences. 2022;

118. Hulla JE, Sahu SC, Hayes AW. Nanotechnology : History and future. 2015;34(12):1318–21.

119. Erkoc P. Nanotechnology-Based Antimicrobial and Antiviral Surface Coating Strategies. 2021;25–52.

120. Rani L, Piotr A. Nanotechnology _ aided advancement in the combating of cancer metastasis. Cancer Metastasis Rev. 2022;41(2):383–404.

121. Malik S, Waheed Y. Emerging Applications of Nanotechnology in Dentistry. Dent J. 2023;11(11):1–30.

122. He A, Li X, Dai Z, Li Q, Zhang Y, Ding M, et al. Nanovaccine-based strategies for lymph node targeted delivery and imaging in tumor immunotherapy. J Nanobiotechnology. 2023;21(1):1–32.