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Review Article

Application of Nanomaterials and Biomaterials in Nanovaccinology

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Abstract

Over the years, the concept of vaccination has encountered great evolution. Most vaccines have been formulated in a way that mimics pathogens in order to activate immune cascades. However, vaccine development was never assumed as a simple task and it involves several studies to obtain detailed knowledge about antigen presentation and recognition by immune system. Nanovaccination has been proposed as one of the most successful break throughs and procurements in health promotion and diseases prevention. Nano vaccines can be classified into various groups based on shape, source, sizes, features and structural constriction. Therefore they are assumed to offer more opportunities and novel approaches to scientists and researches and address unmet needs in vaccine developments. Novel technologies in vaccinology mostly focus on safety-immunogenicity improvements, synergistic immunomodulation, in vivo stability, reduced toxicity and efficient delivery of stimulatory cues. Biomaterials and nano vaccines were proved as promising strategies with optimal safety and efficacy through controlling the release site and pattern for better adjustment of dosing and timing of vaccines and immunotherapies.

In this review, we have summarized future horizons and cutting-edge advances of biocompatible nano biomaterials-based platforms such as liposomes, nanoparticles, carbon based structures and membrane based vaccines. We also described the remaining challenges, limitations, and possible breakthroughs in nano vaccines' formulation and biomaterials application in industrial scale.

Keywords: Vaccine; Carbon nanotubes; Nanotechnology; Biomaterials; Vaccinology

Introduction

Vaccine Strategies

Vaccine design generally consists of four main components: antigen, adjuvant, carrier, and delivery strategy. Antigens are foreign materials that can induce an immune response. Vaccines are categorized into four groups: live attenuated vaccine, inactivated vaccine, subunit vaccine (VLPs), and peptide vaccines based on an antigen-presenting approach. Adjuvants are stimulatory agents of vaccine formulation that exist as independent or conjugate entities and would boost the immune response to antigens. Nanoparticles are viral/non-viral Nano carriers applied to encapsulate or present antigens and/or adjuvants in live attenuated and inactivated vaccines. Adenoviral vectors, proteinaceous nanoparticles, and synthetic nanoparticles are the most common carriers for antigen delivery in vaccine formulations. Vaccines are usually administered through syringes, implants, and microneedle patches [1-10].

Contemporary vaccines would induce active immunization against complete or killed pathogens. This type of vaccine is perspective, specifically in SARS-CoV-2 vaccination. On the other hand, live attenuated (LAVs) and Inactivated Vaccines (IVs) are live a virulent viruses that normally induce immunity in single-dose administration. Nowadays, genetic code expansion has been applied to improve productivity and genetic stability of LAVs to be specifically applied in the production of SARS-CoV-2 vaccines. Inactivated Vaccines (IVs) are consisted of physically or chemically inactivated pathogens or antigen fragments. This type of vaccine is administered in multiple doses to induce sufficient immunity. IVs formulation must include adjuvants and are more stable than LAVs. However, both LAVs and IVs require a cold supply chain. The last vaccine type is called the viral vector vaccine. In this type of vaccine, genetically engineered mammalian viruses such as herpes simplex and non-replicating adenoviral vectors like Ad5-nCoV and ChAdOx1 are used [11-17].

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Next Generation Vaccines

Nanotechnology and nanomaterials play important roles in the development of the next generation of vaccines and immune engineering. Nucleic acid-based (DNA and mRNA vaccines) and subunit vaccines are promising vaccine technologies. These groups are safer, more stable, and easier to scale up but have more potential in terms of risk and failure during clinical phases. Nucleic acid-based (DNA and mRNA vaccines) elicit cytotoxic T cells' responses in addition to antibody production and T helper cells activation [18-20]. *Inovio*, *Ethnos pharmaceuticals*, and *Symvivo* are pharmaceutical companies running clinical trials on Covid-19 DNA vaccine candidates [21]. Meanwhile, *Moderna* and *BioNTech-Pfizer-Fosun Pharma* performed clinical trials on Covid-19 mRNA vaccine candidate. It should be noted that stability, mutagenesis, and antigen half-life are the main obstacles in the development and commercialization of nucleic acid-based vaccines. Nanotechnology has suggested some solutions for the above problems. Nanomaterials such as polymeric nanoparticles, cationic liposomes, nanoemulsions, carbon-based nanostructures, and dendrimers are supposed to facilitate nuclear translocation, antigen delivery, and trafficking to face more immune cells as well as improve formulation stability and scalability [19,22,23]. Protein nanoparticles or Virus-Like Particles (VLPs) are categorized as subunit vaccines. VLPs are stable, scalable, mono-dispersed formulations generated from antigenic subunits and biomaterials. VLPs might root from bacteriophages and mammalian, insect and plant viruses. VLPs are highly visible to immune cells and are defined as immune activators and amplifiers with non-infectious and adjuvant properties [24-30]. *CanSino*, *AstraZeneca, Shenzhen Geno-Immune Medical Institute, Medicago-iBio's* COVID-19 vaccines, and *Johnson & Jonson* influenza virus vaccine *Crucell* are VLP vaccine candidates in the clinical development pipeline. Most of the above vaccine candidates are multivalent platforms that offer simultaneous delivery of antigen and adjuvant to lymph nodes' Antigen-Presenting Cells (APCs) and long-acting immune stimulus. It also facilitates APCs antigen processing and antiviral antibody production by CD8+ and CD4+ T cells in MHC-I and MHC-II pathways [31-33].

Peptide-based vaccines represent the simplest platform in next generation vaccines. They are generally formulated as peptides and T cell /B cell epitopes plus suitable adjuvant or immune-informatics-derived-peptide–nanoparticle conjugates. The efficacy of Peptide-based vaccines is highly dependent on adjuvant and applied nanocarrier. For example, "albumin hitchhiking" is an emerging targeted hepatitis B virus trafficking strategy to lymph nodes' dendritic cells and macrophages. Enhanced viral clearance and stronger humoral and cellular immune responses are pursued by antigen encapsulation and antigen surface presentation through this nanotechnology approach [34-36].

Vaccine Scalability and Manufacturing

Production cost, formulation, and scale-up of vaccine formulation are the main concerns in the development of novel, effective vaccines. The traditional manufacturing process of recombinant proteins using mammalian, bacterial, and yeast cells are still expensive and is susceptible to human contamination. Innovative manufacturing platforms are required to meet high demands during viral disease outbreaks. Plant-based expression systems are a promising production technology that was introduced during the 2014 ebola epidemic. Plant molecular farming is scalable, while fermentation-based technologies are highly sensitive to control parameters. Low production cost and safety are other advantages of molecular farming. Conventional vaccines utilize a cold supply chain, while new technologies of implants and microneedle patches exclude cold chain difficulties in product distribution and moderate to high feasibility for rapid global deployment of vaccines [37-40].

Nanomaterials Improve Vaccine Responses: Mechanisms and Mew Approaches

Nanomaterials and nanotechnology have been applied more specifically in the design and development of new vaccines against HIV (Human Immunodeficiency Virus), TB (Tuberculosis), and malaria. These three pathogens are listed by WHO (World Human Organization) among the top ten reasons for mortality in developing and low-income countries. This notification would highlight the importance of developing more efficient prophylactic strategies and more effective antigen delivery to key immune cells, including APCs, B cells, neutrophils, and macrophages [41-44]. Nano materials' size, shape, blood circulation half-life, adjuvant properties, and complement activation potential are required during vaccine development. Antigen persistence through encapsulation or conjugation with nanostructures would enhance antigen immunogenicity. For example, *Moon et al.* designed ICMVs (Inter bilayer Cross-linked Multilamellar Vesicles) in which malaria antigen has been both encapsulated and conjugated to the vesicles' surface in order to extend antigen persistence in lymph nodes [45]. *Demento et al*. also suggest long acting PLGA ovalbumin encapsulated PLGA nanoparticles would improve APCs' immune response and high-affinity antibody secretion from follicular helper T cells [46]. Long-acting formulation and cross-presentation of HIV, TB, and malaria antigens will potentiate cellular immune response by CD8+ if antigen fragments escape to the cytosol after lysosomal degradation of nanocarrier [47,48]. Nano materials' physicochemical properties, such as charge, size, and flexibility, show a high impact on Lymph Nodes (LN) draining. Nanoparticles within the size range of 10-50 nm are the most suitable for LN draining. Large 50 nm nanoparticles are passively drained to LNs and are acquired by macrophages better [49-51]. Mucosal immune response and mucosal antigen delivery is an attractive field in HIV and TB vaccine design [52,53]. Mucosal mucin permeability and adhesion are also size and charge-dependent [54]. Average pore size cut-offs of 340 nm for vaginal mucus and 200 nm for respiratory mucus must be considered for appropriate antigen traverse [55]. Large (500-5000 nm) anionic nanoparticles are captured by macrophages, while small targeted (20-200 nm)

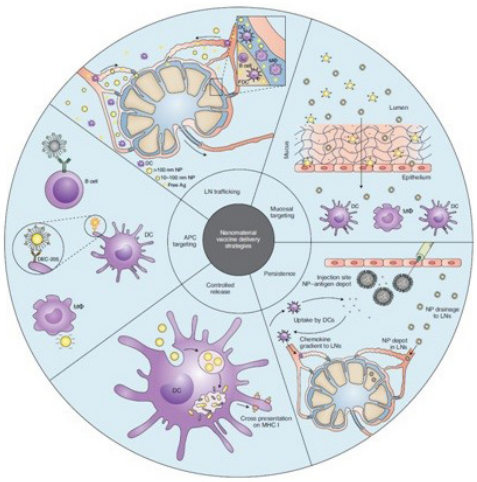

Figure 1: Strategies for engineering nanomaterial vaccine delivery [41].

nanoparticles are endocytosed by DCs (Dendritic Cells) [55-60]. C-Type lectin receptors expressed on Langerhans cells and DCs are highly suggested for targeted follicular dendritic cells that exist in LN [61-,62]. Nanomaterials might also improve adjuvant functionality, minimize their toxicity and decrease their dosing amount [63-65]. *Moon et al*. reported that lipid vesicles with encapsulated malaria antigen and MPLA, as an adjuvant, required adjuvant amount was reduced to 10 times less than free soluble malaria antigen-adjuvant (MPL4) and stronger induced humoral responses were achieved [66] (Figure 1). Lymph Node (LN) trafficking, persistency, controlled release pattern, APC targeting and mucosal targeting are main strategies for engineering nanomaterial vaccine delivery. Trafficking to lymph nodes is largely dependent on size, charge, hydrophobicity and flexibility. During mucosal targeting hydrophilic positively charged mucoadhesive particles create strong entanglment with mucin fiber of mucosal membrane and induce mucosal immunity. Persistency and controlled release patterns would prolongs antigen uptake through endosomal escape and cross presentation of the antigen on MHC I from reservoir systems at site of injections. Administration of anionic nanoparticles or introducing DEC-205 or B cell epitopes on nanoparticles surface would be another engineered strategy which is entitles APC targeting through Dendritic Cells (DCs) and macrophages.

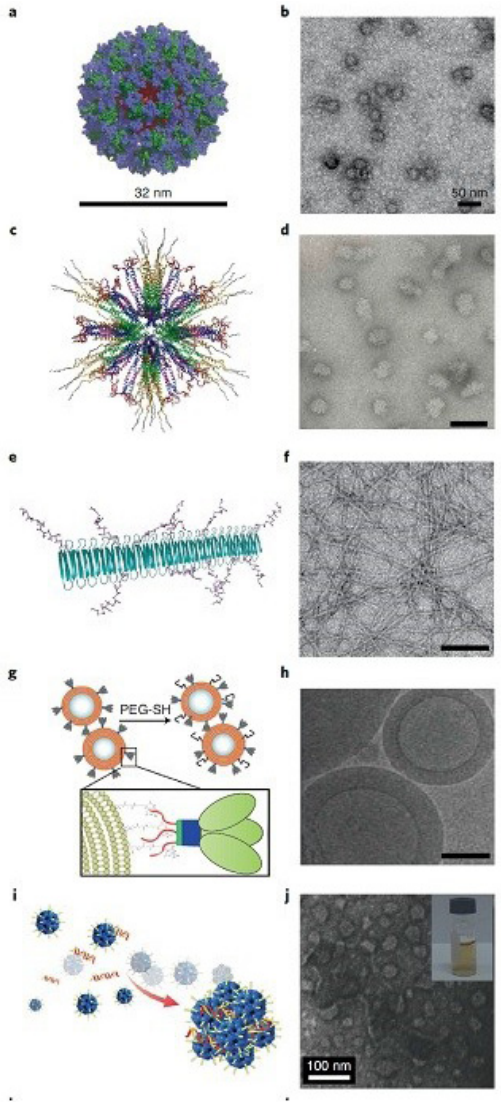

Figure 2: Molecular models and Transmission Electron Microscope (TEM) images of nanomaterial based vaccine against HIV and malaria, Ferritin nanoparticle **(a,b)** Self-assembling protein nanoparticles **(c,d)**. Self-assembled nanofibres **(e,f)**. crosslinked multilamellar vesicles **(g,h),** Fulleren **(I,j)**

Experimentals

Nanotechnology Systems for Vaccines

Low immunogenicity, in-vivo instability, toxicity, and multiple-dose administration are major problems of conventional vaccines. Nanotechnology and nanostructures provide an opportunity for enhanced cellular and humoral immune responses [67]. Higher antigen uptake by macrophages, more efficient antigen presentation and recognition, and specific and selective immunity are the main aims of nano vaccine developments [67,68]. In this review, we are going to summarize recent advances in the application of nanocarriers such as liposomes, emulsions, polymeric systems, peptide nanoparticles, carbon-based materials, and artificial VLPs in the new generation of vaccines. Table 1 summarizes nano carriers' including liposomes, emulsions; natural and synthetic bio/polymer based systems, their composition, antigen types and route of administration. As it is listed in table 1, liposomal vaccines are mostly administrated parenteraly while emulsions, natural/synthetic polymer and carbon based systems would be applicable via parenteral, intranasal, pulmonary and oral routs. The above mentioned systems would be thoroughly explained in the next session.

Liposomes: Liposome formulation, as carrier or adjuvant, has been extensively investigated in vaccine technology, and at least eight liposomal vaccines are launched or undergoing clinical studies for human use [69]. Liposomes' specifications, including fluidity, size, charge, lipid content, lipid types, and surface modifications, could be customized according to antigen properties to achieve optimum immunogenicity. Liposomes' inter-bilayer space and their hydrophilic reservoir are suitable for hydrophobic, hydrophilic, and amphiphilic molecules. A combination of lipid composition and liposome size might affect the type of immune response and cytokine secretion. Small unilamellar liposomes with a size of below 500 nm and cationic lipids such as Dimethyl Dioctadecyl Ammonium (DDA) in their lipid bilayer mostly induce a stronger cellular immune response and interferon-gamma production. Surface antigen, lipid ratio, and surface antigen-lipid ratio are factors that are important in liposomal physical stability and would indirectly affect immune response intensity. There are some excipients that possess immune stimulatory properties. Trehalose Behenate (TDB), di-C14-amidine-based compounds, Monophosphoryl Lipid A (MPL), cationic lipids (e.g. DDA and DOTAP (1, 2-dioleolyl-3-trimethylammonium propane)), cholesterol derivatives and imidazolium compounds are immune stimulatory candidates favorable for a stronger cellular immune response [67,68]. Inter-bilayer cross-linked multilamellar and nickel- chelating liposomes are novel liposomal vesicles designed for stable entrapment of antigen and immune stimulatory molecules within the phospholipid membrane. However, the toxicity of nickel- chelating liposomes still needs to be addressed appropriately. Subcutaneous administration of liposome-in-oil adjuvant formulations of diphtheria toxoids was another solution suggested for reducing antigen transport to LN's draining and continuous antigen presenting to immune cells [70]. *Carroll et al.* also applied cationic liposome consisting of nucleic acid-based toll-like receptor agonist as an adjuvant, imidazolinium chloride and cholesterol as immunomodulator molecules, and a combination of lipoplexes -Fluzone as antigens in a new platform for influenza vaccine [71].

Emulsion and Nano Emulsion

Oil in water and water in oil Emulsions are reported to have a dual function, one as adjuvant and the second as antigen de

Table 1: Nanocarriers composition and application for antigens delivery in vivo [67].

Delivery System	Composition	Antigen	Route
Liposome	DDA, TDB	Ag85B-ESAT-6	Intramuscular
	DDA, TDB	OVA	Intramuscular
	DDA, DODA, TDB	Ag85B-ESAT-6	Intramuscular
	Pegylated DDA, TDBA, TDB	Ag85B-ESAT-6	Subcutaneous
	DDA, DSPC, Cholesterol, TDB	Ag85B-ESAT-6	Subcutaneous
	MPL, DDA, TDB	OVA	Intraperitoneal
	DDA, TDB	Trivalent influenza vaccine	Subcutaneous
	DOPC, DOPG, MPB	OVA	Subcutaneous
	EPC, DOGS-NTA-Ni	His-tagged heat shock protein	Intradermal
	MDMPC, DMPG, Cholesterol, MPL	Polyhistidinylated OVA	Subcutaneous
	Lecithin, Cholesterol	Diphtheria toxoid	Subcutaneous
Emulsion	MF59	Hemagglutinin	Intramuscular
	MF59	Recombinant meningococcal B protein	Intramuscular
	MF59	Recombinant meningococcal B protein	Intramuscular
	W805EC	OVA	Intranasal
	W805EC	OVA	Intranasal
	GLA	Falciparum subunit	Subcutaneous
	GLA-SE	Plasmodium vivax subunit	Subcutaneous
	GLA-SE	Recombinant hemagglutinin	Intramuscular
Synthetic polymer-based system	PLGA	OVA	Subcutaneous
	PLGA, Polylactic acid	Hepatitis B surface antigen	Pulmonary
	Lipid-coated PLGA	OVA	Subcutaneous
	Lipid-coated PLGA	Malaria antigen	Subcutaneous
	Chitosan-coated polycaprolactone	H1N1 hemagglutinin	Intranasal
	Polyanhydrides	Yersinia pestis antigen	Intranasal
	Polylactic acid	Hepatitis B surface antigen	Subcutaneous
	Deacylated cationic polyethleneimine	HIV CN54gp140 antigen	Pulmonary
	PEGylated poly [2- (N, N-dimethylamino) rthylmethacrylate)	HIV gag DNA	Intranasal
Natural biopolymer-based system	N-trimethyl chitosan	OVA	Intranasal
	Chitosan nanoparticles	HBsAg	Intraperitoneal
	Cholesteryl-conjugated pullulan	Clostridium botulinum type-A neurotoxin subunit antigen	Intranasal
Carbon-based system	SWCNT	Tuberculin purified protein derivative	Subcutaneous
	Carbon nanotube	Azoxystrobin	Intraperitoneal
	Carbon magnetic nanoparticles	Hen egg lysozyme	Intravenous
	Carbon nanoparticles	Bovine serum albumin	Oral

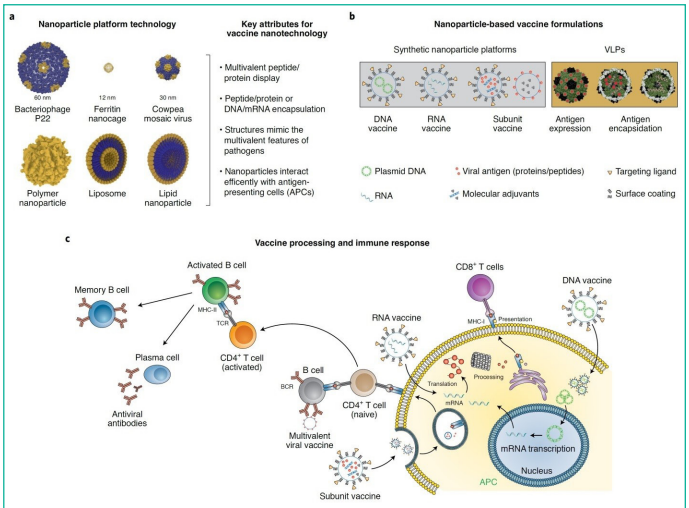

Figure 3: Nanoparticle platform vaccine technologies [1].

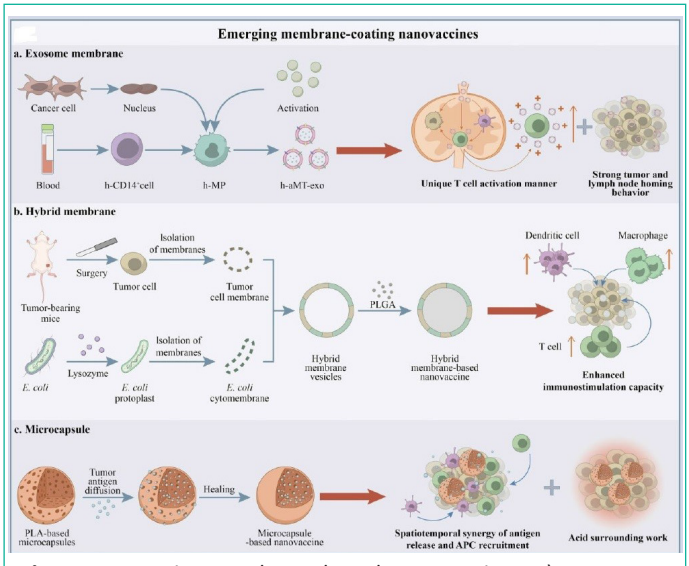

Figure 4: Emerging membrane-based nano vaccines: a) exosome membrane, b) hybride membrane, c) microcapsule [132].

livery system. MF59 is a well-known adjuvant emulsion which is consisted of squalene oil, span 80, tween 80, and citrate buffer.

Fluad was the first flu EMEA-approved vaccine that was formulated in MF59. MF59 was used in the development of meningococcal vaccines and was found to cause a strong humoral immune response after the administration of 3 doses in mice [72-74]. AF03 is another adjuvant emulsion which is consisted of squalene, sorbitan oleate, and cethearet and was used in the formulation of the Humenza TM flu vaccine [75,76].

Squalene-free Nanoemulsions are another group of Nano adjuvants [77]. *Makin et al.* investigated adjuvant properties of water in oil emulsion of W805EC in the intranasal route. Other researchers have announced positive feedback about oil-in-water emulsification of Glucopyranosyl Lipid A (GLA) and GMZ2 in the anti-falciparum aqueous vaccine [78]. Formulation stability and biocompatibility of oil ingredients are critical in the successful commercialization of emulsions in vaccine design and development [68]. Molecular models and Transmission Electron Microscope (TEM) images of nanomaterial based vaccine against HIV and malaria have been summarized in figure 2. As it is shown, ferritin nanoparticles, cross-linked multi lamellar vesicles, self-assembling protein nanoparticles, self-assembling nanofibers and fullerene were biocompatible nanostructures and nanoadjuvants being investigated as carrier and adjuvant in vaccine formulation of HIV envelope protein, HIV trimmers and malaria epitopes [41].

Bio/Polymeric-Based Systems

PLGA (Poly-Lactic-co-Glycolic Acid) is the most extensively used biocompatible, biodegradable polymer in the synthesis of nanoparticles. Surface coating, surface charge, and particle size (>500 nm) are crucial parameters that might affect the release and presentation of antigens and/or adjuvants in oral, mucosal, and systemic delivery of vaccines [67,79]. A number of researches have been performed on encapsulated OVA (ovalbu-

min) in polymeric particles. Surface PEGylation, lipid adjuvants (MLP and alfa-galactosylceramide) entrapments in phospholipid PEGylated particles, and lipid content of DOPC and DOPG were shown to intensify antigen-specific IgG titre in the case of OVA and P. vivax malaria antigen (VMP001) [80,81]. Long-acting PLGA particles were found to provide a more sustained release pattern of antigens in comparison to liposomal delivery systems. This phenomenon would cause higher IgG titre in the same route of administration (e.g., sc.) [46,47]. Poly caprolactone, polyanhydrides, and chitosan are other widely used biocompatible polymers investigated in polymeric nanocarriers for delivery of H1N1 hemagglutinin, Yersinia pestis and recombinant F1-V [82-84]. Electrostatic polyplexes are another polymeric-based system that is under investigation to be applied in the development of subunit antigens (e.g., HBs Ag (Hepatitis B antigen)) or plasmid DNA vaccines [67,85].

Pulmonary administration of electrostatic complexes of cationic polymers and negatively charged plasmids were shown to be effective in the inflation of serum IgG and the production of interferon-gamma in the HIV gag DNA vaccine [86]. N-trimethyl chitosan mucoadhesive particles were administered both intranasal and intramuscular as OVA carriers. Mucosal immunity and higher antigen-specific IgA serum level were the main outcomes reported by *Slutter* and *Sawaengsak* [87,88]. Nanoparticle platform vaccine technologies and their immunization pathways are shown in Figure 3. Some Nanoparticle platform technology includes bacteriophage, ferritin nanocage, cowpea mosaic virus, liposomes, and lipid nanoparticles which varies in physical properties including size, structure and charge. VLPs are classified as non-synthetic particles with expressed or encapsulated antigens, while synthetic nanoparticle might include multi-variant vaccines, DNA vaccines, RNA vaccines, and subunit vaccines [1]. Vaccine processing and nano formulation's fate in inducing immune response has been schematically presented in Figure 3c.

Self–assembled peptide nanoparticles (SAPNs): Self–assembled peptide nanoparticles were expressed and produced in *Escherichia Coli* as 180 repeated peptide constituents forming a scaffold with the immune stimulatory property. These platforms are suitable for a wide range of antigens but has be more specifically studied in the development of seasonal Flu and COVID-19 vaccines [68,89,90].

Carbon-based nanostructure systems: Carbon-based nanostructure systems, including Carbon Nanotubes (CNTs), carbon magnetic nanoparticles, and carbon nanoparticles, are other Nano biomaterials that are interestingly under investigation. Their dual functions of drug/ antigen carrier and adjuvant immune stimulatory potentials are being evaluated by different research teams [67,68,91-106]. The research showed that functionalized MWCNTs using a silicon reaction together with INH drug increase the level of performance and reduces the effective dose of the drug in the treatment of tuberculosis [107]. Another characteristic of functionalized CNTs is penetration into the bacterial membrane. *Sheikhpour et al.* found that CNTs functionalized with carboxylic acid had antimicrobial effects on Staphylococcus aureus by destroying membrane integrity and increasing drug efficiency [108]. Conventional vaccines and nano vaccines are different in activation of the immune response, dose number, cellular uptake, lymph node accumulation, antigen presentation, and migration-activation and cytokines secretion. Lower required dose, increased robust reuptake by DCs, greater accumulation in lymph nodes, increased cellular immunity, and more stimulatory cytokine secretion are shown as the most important advantages of nanotechnological vaccines [102].

CNTs are promising multidisciplinary nanostructures in biomedicine. However, toxicity of CNT and its biocompatibility are important milestones in biomedical administration [92,106]. Long-term exposure to CNTs can cause persistent inflammation, lung cancer, fibrosis, and gene damage in the lung. The presence of MWCNTs inside the body led to the production of cytokines such as TNF-α and IL-1β from immune cells, which play a role in toxicity. SWCNTs also cause acute effects such as inflammation, granuloma synthesis, collagen deposition, fibrosis, and genotoxicity. However, by using new methods such as functionalization, it is possible to produce nanotubes with greater length, greater width, and greater curvature to some extent with less toxicity [109]. Polymeric functionalization with phospholipid PEG derivatives and surfactants would remarkably improve CNTs' biocompatibility; Moreover, functionalization would also facilitate secondary conjugation with drug molecules [94-99]. In the study, it was found that the simultaneous administration of functionalized carbon nanotubes and meropenem in nanofluid conditions caused a significant decrease in the growth of Pseudomonas aeruginosa and antibiotic resistance by increasing the stability of the drug [110]. Functionalized CNTs are not intrinsically immunogenic but are capable of activating immune system cells, including monocytes, macrophages, and DCs, after cellular reuptake. The application of SWCNTs is proposed as immune stimulator candidates and antigen carriers in vaccine studies [67,97,102]. For example, *Meng et al.* showed that SWCNT-conjugated tumor cell lysates resulted in better immune responses than free tumor cell lysate [97,111]. *Zeinali et al.* also reported that immunization with BCG vaccine as PPD-SWCNT induced a higher level of Th1 cell response in comparison to free PPD [97,112]. *Hadidi et al.* also confirmed the immune modulatory properties of PL-PEG-SWCNTs. Their results showed that PL-PEGSWCNTs concentration and PL-PEG-SWCNT–alum-HB vaccine concentration ratio directly affects the expression of activation and maturation markers in MDDC. These data support the idea of the co-adjuvant potential of PL-PEGSWCNT- alum compounds [97]. Different CNTs with different lengths and surface modifications were found to directly affect anti-azoxystrobin IgG antibodies in animal studies [113].

Carbon magnetic nanoparticles are traceable materials, potentially effective in active targeting to DCs. Graphene oxides and fullerene (C60) would potentiate antigen presentation to DC s and MHC- I APCs and T cells, respectively [67].

CNTs internalization is through direct translocation due to its needle-like structure or by endocytic-phagocytic mechanisms. Mechanism of cells reuptake is CNTs type, synthesis method, impurities, size, and surface functional groups [102]. CNTs reuptake by macrophages is mostly mediated by MACROs receptors and would generally end in activation of inflammatory pathways, cytokine secretion, and cell apoptosis /necrosis. PL-PEG-SWNTs smaller than 400 nm internalize non-phagocytic cells, including COS7 and MCF7, through passive diffusion, while larger ones would prefer the endocytosis pathway. The interaction and reuptake of CNTs by phagocytic cells are highly dependent on both natures of phagocytic cells and CNTs functionalization type. So, different cellular signaling pathways might be activated by amine or carboxyl functional groups on the CNTs surface [114-116]. Monocyte-Activating Nanotubes (MA-CNTs) are Oxidate-MWCNT-NH3+ induce maturation of dendritic cells,

cytokines production of IL-6, TNF-Alfa, NFKB signaling activation, and cytokine secretion by T helpers. So, Oxidate-MWCNT-NH3+ would be prospecting immune therapeutics in cancer management [117,118]. Carboxylate Pl-PEG-CNTs activate DC maturation and activation as well as IL6, IL10, and TNF and NFKB production. On the other hand, pure CNTs would activate oxidative stress and caspase-1 pathways, IL-1 production, and cause cytotoxicity [102]. It has been stated that ammonium-functionalized CNTs and ox-CNTs would modulate the immune system without induction of cell apoptosis [119,120]. Allen et al. first describe CNTs' enzymatic biodegradation and elimination by Horseradish Peroxidase (HRP) [102,121]. PEGylated CNTs would experience auto degradation by Myeloperoxidase (MOP), Eosinophil Peroxidase (EPO), and hypochlorous acid in side neutrophils [122]. Macrophage NADPH oxidase-dependent ROS, lignin peroxidase, xanthine oxidases, and manganese peroxidases are responsible for CNTs biodegradation [123,124]. Biodegraded CNTs would finally eliminate by exocytosis through the trans-Golgi complex [102].

CNTs immune modulation capacities include immune stimulation and immune suppression. Immuno stimulation would happen through cellular (macrophage-monocyte) response, DCs, lymphocyte and complement system activation, plus IL-6, IL-12, and Il-2 production. Immunosuppression would occur through the Cyclooxygenase (Cox) pathway, prostaglandin, and Il-10 secretion [102].

It could be concluded that functionalized CNTs might be a potential candidate in vaccine developments. CNTs' physiochemical and structural properties are of great concern in biocompatibility, biodegradability, immunomodulatory, and design of antigen cargos. Further studies on how CNTs physicochemical modification would alter its interaction with immune cells have become necessary in finding the best options for cancer and infectious diseases, including HIV and Covid-19 [102].

***Physicochemical properties of nanomaterial*:** It should be noted that physicochemical properties play important roles in the design and development of nano formulations with improved antigen delivery and presentation that target vaccine molecules to specific sites and induce desired immune responses [125-127]. From this point of view, shape, size, surface charge, surface volume ratio, porosity, hydrophobicity, hydrophilicity, and crystallinity are key factors that affect nanoparticles' pharmacokinetic and pharmacodynamics parameters as well as antigen release and degradation kinetics [127].

The sizes of nanomaterial determine the mechanism of cellular uptake and specificity [127]. It has been reported that large PLGA nanoparticles (1, 7, and 17μm) showed a reduced internalization rate in comparison to smaller ones (300 nm). Smaller nanomaterial (20–200 nm) were readily endocytosed by the resident DCs, whereas larger sizes (500– 2,000 nm) were effectively taken up by the migratory DCs, and particles less than 200 nm size were drained into the lymph nodes. On the other hand, particles up to the 20 nm range were suitably transported to the APCs [127].

Particle shape also affects the cellular interaction, intracellular trafficking, and rate of antigen release inside the host cells, phagocytosis rate and the activation of signaling pathways, and improvement of antigen processing and presentation to T-cells. For example, Spherical gold nanoparticles were actively internalized by bone marrow-derived dendritic cells and were able to induce a stronger immune response in comparison to cube, rod, or worm-shaped particles. Particle shape would also affect localization. It is said that nanorods were practically delivered to the cell nucleus; however, nanosheets have remained in the cytoplasm after endocytosis [127,128].

Surface charge is also effective in antigen internalization. This electrostatic interaction was exemplified by the observation of significantly improved internalization of cationic polystyrene nanoparticles by the APCs in comparison to neutrally charged particles. Surface functionalization and modification with TLR-7, TLR-8, TLR9 agonists, CD47 molecules, TLR2, and TLR4 agonists, and galactose were also reported to activate the complement pathway, increased cytokine production and the expression of immune regulatory genes [127,129].

Hydrophobicity and hydrophobicity also matter in nano vaccines. Hydrophobic polymeric nanostructures are strong inducers of pro-inflammatory cytokines and co-stimulatory molecules than hydrophilic structures. Hydrophobic polymeric nanostructures also facilitate opsonization by increasing the immunoglobulin adsorption on the cell surfaces [127,128].

Nano Vaccines

Nano vaccines are suitable tools for targeting organs or tissue where disease or infection originated from, while conventional vaccines would affect the whole body. Nanoparticles are applicable to improve the solubility of hydrophobic compounds for parenteral administration. They maintain the integrity of antigens against degradation, stabilize peptides, proteins, or nucleic acids and reduce required doses. In addition, mucosal immunity, antigens protection against enzymatic-acidic degradation, and a depot reservoir system with controlled release patterns are listed as other advantages of nano vaccines [130].

Membrane based cancer nano vaccines: Traditional membrane-based cancer nano vaccines are classified as a single-cell type (e.g. erythrocytes, lymphocytes, etc.); however emerging membrane-based cancer nano vaccines are categorized as exoxomes, hybrid cells and microcapsules with outstanding antitumor capacities (Figure 4). The membrane fraction of hybrid cells group might be separated from cancerous cells, dendritic cells and erythrocytes. Hybrid membranes were reported to demonstrate enhanced antigen delivery efficiency and precise targeting via lymph node guiding. There are currently 12 ongoing clinical trials conducting hybrid membrane strategy in vaccine development. *Nie et al.* developed an adjuvant and antigen co-delivery nano vaccine based on Escherichia coli and tumour cell membranes with a potent antitumor activity in vivo [131,132].

Exosomes are one of the most unique nannocarriers from membrane-enclosed Extracellular Vehicle group (EV). Exsomes' size normally varies between 30 to 150 nm. Desirable size, biocompatibility, in vivo stability, and target-specific delivery makes them potential candidate as adjuvant and antigen carriers. Exosomes are also considered as agents for local and systemic cell-to-cell communication through transfering functional substances to recipient cells. The studies' results reveal that exosomes can be exploited as biomarkers and immunotherapeutic agents for nano vaccines development [131,132]. DC-derived Exosomes (DEXs) form a new class of vaccines for cancer immunotherapy which elicit strong immune responses and tumor suppression in animal cancer models. DEXs efficacy have been investigated in patients with advanced melanoma and Hepatocellular Carcinoma (HCC) [133]. Promising outcomes reveal that DEXs can serve as novel cancer nano vaccines due to their in-

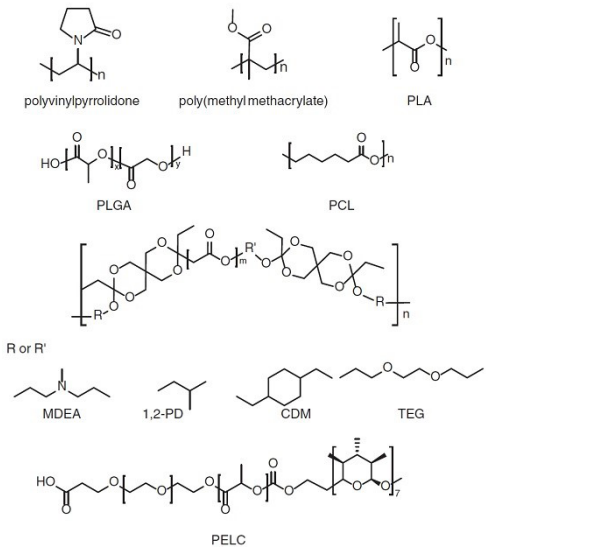

Figure 5: Commonly used synthetic polymers in vaccine formulations [136].

herited antitumor properties. *Schirrmacher and Barz* reported that Tumor-Derived Exosomes (TDEs) displayed antigens similar to their corresponding tumor cells in Cytotoxic Lymphocytes (CTLs [134].

Wei et al. designed a chimeric-membrane nano vaccine based on exosomes from macrophage-tumor hybrid cells. Their customized nano vaccines targeted lymph nodes and T-cells in a unique 'direct exosome interaction' manner with long-lasting induction of tumor regression in various cancer models, especially when combined with anti-PD1 therapy [132,134]. Exosomes' capability as adjuvant was also investigated in combination with genetically improved murine melanoma B16 cells. They successfully induced immunostimulatory signals in mice 7 days after the last immunization. These results reveals the potential benefits of exosomes as adjuvant and carriers for future cancer vaccine development [135]. Exosome-based vaccine candidates for cancer, hepatitis B, AIDS, and other infectious diseases are under investigation by researchers all over the world [131].

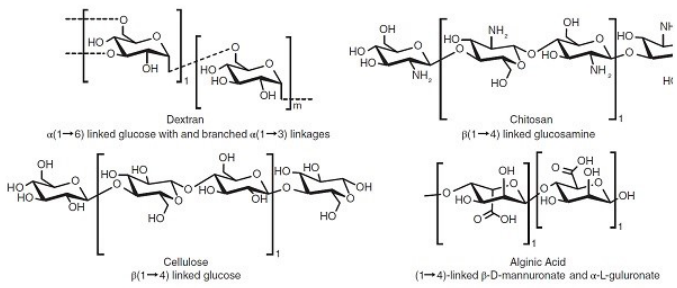

Figure 6: Common polysaccharides in vaccine formulations [136].

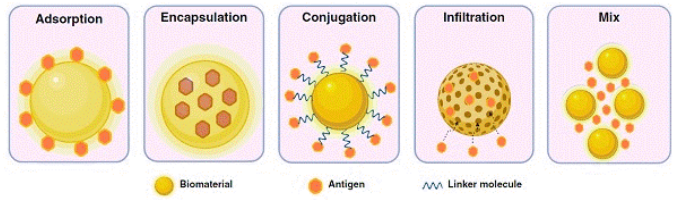

Figure 7: Schematic antigen/biomaterial interaction approaches [136].

As for microcapsule-based nano vaccines, *Wang et al.* prepared a self-healing microcapsule system that can generate a desirable tumor microenvironment in situ, wherein antigen release, APC cells recruitment and acid microenvironment are deigned to work in a synergetic manner to promote anti-tumor activity [132]. Currently, Wei et al. have also designed two microcapsule-based nano vaccines with potent antitumor activity in various hematological cancer and solid tumor models in vivo [132].

New era in vaccinology and Biomaterials' Role: Weak immunogenicity and short-term stability are most common limitations associated with subunit antigens. Biomaterials offer many advantages including biocompatibility, adjustable immunogenicity, low immunological reaction and desirable stability in comparison to conventional vaccine delivery systems. In the recent decade, biomaterial-based platforms such as synthetic and natural polymers, lipids, crystalline scaffolds, microneedles, and other particles have rapidly come out in order to improve vaccine essentials including efficacy, safety and stability simultaneously but only a few of suggested systems provide sustained or controlled release properties. For example, synthetic biodegradable polyesters are suitable for antigen encapsulation (e.g., single / double emulsion solvent evaporation and spray drying). Polyesters are compatible with various administration routes (e.g., dermal, intranasal and subcutaneous) and offer a flexible formulations and platforms for enriched immune response. However, stability and production of a local acidic environment following hydrolysis is their main bottle neck in proteins formulation.

Melt extrusion or co-extrusion with other materials has been investigated to design implantable vaccines for HPV (human papillomavirus) and seems promising. Figure 5 illustrates some of the commonly used synthetic polymers in vaccine formulations [136-139]. Biomaterials application have been suggested as one of the standard protective solutions to overcome these problems and augment immunization. Biomaterials are good in stabilizing host antigens and achieving sustained release pattern. However, there is still a significant challenge in vaccine formulation to achieve optimal therapeutic efficacy. Figure 6 illustrates some commonly used polysaccharides in vaccine formulations that might overcome some of formulation challenges in vaccine developments. Dextran, alginate, chitosan, hyaluronic acid and starch been described as applicable polysaccharides in controlled vaccine delivery.

Distinguished properties of natural polysaccharides that have attracted attention are their desirable water solubility, ease of preparation, simple chemical modification and flexibility of administration in oral and intranasal routs [136].

Chitosan has been highly recommended in vaccine formulation of hydrogels and Metal–Organic Frameworks (MOFs) against infectious diseases owing to its high safety and ease of clearance. This phenomenon might be explained by chitosan's strong electrostatic interaction owing to its positively charged nitrogen that synergistically enhances APC uptake and immune activation.

Figure 7 briefly illustrates several antigen/biomaterial interaction approaches. The interaction might be classified into five categories; surface adsorption, mixing, encapsulation, conjugation. Surface adsorption is completely based on electrostatic or hydrophilic/hydrophobic interactions that lead to the weak antigen attachment and burst release in vivo. However, encapsulation and conjugation through chemical bonding and cross linkage of antigen with selected biomaterials would lead to improved immunogenicity. This assumed to be happened because of gradual degradation of biomaterials intra/extracellular environment. Currently, simultaneous adsorption and encapsulation interactions are the most commonly applied interactions

for improving vaccines sustained release pattern. Pulsatile release seems to be a better alternative in comparison to a single injection followed by several booster shots. This methodology is suggested to avoid immune cell exhaustion and reduced antibody-antigen affinity that normally occur in booster single shots. It might be concluded that biomaterials application in antigen delivery and vaccine development seems effective in improving vaccine stability and performance but there are still questions that need to be addressed by researches [136].

Conclusions

Low rate of patient response and off-target adverse events of nanomaterial's application in vaccine development indicate that many challenges exist and should be addressed to achieve more successful platforms [128]. The key principle is how to trigger appropriate antigen-specific immune responses by stimulating immune cells and inducing innate/adaptive immune responses. The flexible design of nanostructures endows nano vaccines with improved specific immune responses. These vaccine types mainly benefit from their unique drug/antigen delivery, adjuvant properties and customized immunomodulation properties in nano scale [127]. Currently, most vaccines are administered by a parenteral route, which is invasive and has poor patient compliance. However, nanomaterial application in vaccine development provided various options for vaccine administration, including topical, intranasal, inhalation, and oral administration for both therapeutic cancer vaccines and preventive vaccines for infectious diseases [128].

In general, toxicity, scale-up process in sterile conditions, and difficulties in presenting naive antigens are critical limitations in vaccine platforms. However, with the advent of new techniques such as scaled-up methodology for spray drying, some obstacles of scale-up are eliminated, but there is still a long path to omit this millstone [128,129]. Nanotechnology platforms tend to intensify immunogenicity by effective targeted antigen delivery through their immune-modulatory properties; improved formulation stability, controlled release pattern, less immune toxicity and immunosuppression, surface modification, co-delivery of antigens and adjuvants, customized differentiate cellular and humoral immune responses and scalability. Additionally, nanoparticles could be tailored for single dose, non-invasive administration of antigens through immune engineering methods and co-encapsulation with stimulatory molecules.

Although nanotechnology-based vaccines are currently in different stages of clinical trials, these considerations would potentiate ongoing strategies in nanomaterial application, nano vaccines, and anti-infective treatments. More effective vaccines are to be developed by compromising nano vaccines and immune cell interactions. It has been well established that physicochemical properties of nanomaterial, including type, size, shape, surface charge, and hydrophobicity level, are the main factors affecting interactions, antigenicity level, adjuvant properties, and host immune response. Thus it might me concluded that, emerging nano vaccines and nanobiomaterials are beneficial tools for next generation vaccines with optimum efficacy for different route of administrations and targeted immune cells as well as improved safety and more flexible dosing regimen.

Author Statements

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Conflict of Interest

We should acknowledge our team in Pasteur Institute of Iran that shows high attribution in writing this manuscript.

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