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Advances in vaccine delivery strategies to promote effective immunization

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ABSTRACT

Vaccinations are one of the most successful initiatives of the new era in the public health sector. Different types of vaccines are available nowadays depending on the antigen used and the methods of delivery and administration. Conventional vaccines are produced from dead, attenuated, or inactivated pathogens. The antigen activates the body's immune system to recognize the foreign body as a threat and kill the virus in question. Antigen proteins can be administered directly, naturally, to strengthen the immune response along with the adjuvant. However, safety and efficacy remain the two main issues in conventional vaccine production. Therefore, new vaccine formulations have been introduced to target the antigen at the worksite, reduce toxicity, increase the bioavailability of active compounds, and prolong release time, thus improving therapeutic efficacy and safety. Loading the antigen into different delivery platforms such as lipid vesicles, nanoparticles, microparticles, microemulsions, virus-like particles, embedding complex agents, and cyclodextrins is a promising current vaccine strategy. The current review focused on the most recent research on vaccine delivery systems and briefly described the design and development of the COVID-19 vaccine uploaded to these carriers. This paper also discusses the current situation and future scenarios in the field of vaccine production.

INTRODUCTION

The introduction of vaccination into the clinical field at the beginning of the 20th century was a remarkable success story. Vaccinations have efficaciously minimized the spread of diseases and alleviated mortality and morbidity caused by severe infectious diseases. For example, the World Health Organization (WHO) reported that 2–3 million deaths are avoided every year by vaccination against diphtheria, tetanus, pertussis, influenza, and measles (World Health Organization, 2019). However, if global vaccine coverage develops, an additional 1.5 million deaths may be avoided. Indeed, vaccination is an efficient and economical public health strategy for maintaining and improving healthy communities. The introduction of effective global vaccination campaigns in 1979, for instance, allowed the eradication of smallpox, which was one of the most feared diseases at that time (Vincent *et al*., 2003).

Most vaccines are traditionally produced from a dead, attenuated, or inactivated disease-causing pathogen (Lemoine *et al*., 2020). Vaccines are administered via various routes, primarily intradermal, subcutaneous, or intramuscular (IM) injection (Hickling *et al*., 2011). However, there are certain drawbacks faced by these conventional vaccines where the production is time-consuming, and the risk of contaminations is high and undetected (Kurup and Thomas, 2020). Furthermore, safety concerns, philosophical assumptions, religious beliefs, infliction of pain, and

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fear of needles were the main reasons behind the refusal or delay of parenteral vaccines (McKee and Bohannon, 2016).

Recently, novel vaccine designs, including virus-like particles (VLP), conjugate vaccines, cellular vaccines, and nucleic acid vaccines, have been made and showed higher public compliance (Wallis *et al*., 2019). They are also safer substitutes to conventional vaccines due to their complete inability to replicate and enable faster production (D'Amico et al., 2021). For example, DNA vaccines are an excellent example of advanced biological products produced at a low cost on a large scale (Ingolotti *et al*., 2010). Also, various studies with RNA had been performed on oncogenes that are currently in phase I–III clinical trials on specific pathogens like human immune deficiency virus (HIV) (Bobbin *et al*., 2015), Zika (Richner *et al*., 2017), and rabies (Alberer *et al*., 2017). In addition, different biomolecules and synthetic inhibitors against COVID-19 are being tested by worldwide scientists, where nucleic acid-based molecules can be viewed as promising drug candidates (Piyush *et al*., 2020).

Although new vaccine designs are very successful, they require a vector or carrier to be transported to the target sites. Some examples of these carriers are liposomes, polymeric nanoparticles (NPs), inorganic particles, infectious material like bacteria and viruses, immune-stimulating complex (ISCOM) mucosal delivery systems, and microemulsions (Yang *et al*., 2016). Due to the benefits of vaccine delivery systems such as self-administration, biocompatibility, stability, and cost-effectiveness, they have become prominent in delivering vaccines efficiently and improving general immunization (Criscuolo *et al*., 2019). Thus, the current review provides an overview of the emerging new delivery system platforms in vaccine development and their significant roles in managing vaccine formulations and their applications. The review also covers recent research on delivery systems loaded with the COVID-19 vaccines and discusses the current status and prospects in this area.

VESICULAR DELIVERY SYSTEMS (VDSS)

In the last three decades, VDSs have seen exponential development in several clinical areas. Various vesicular devices explored to date could accomplish differing achievements (Kapoor *et al*., 2019). They play an essential role in simulating biological membranes and transport and targeting active agents. The VDSs are highly organized structures consisting of several concentric bilayers formed in water due to self-assembling amphiphilic building blocks. Due to their improved bioavailability and ability to target the drug to a specific site of action, they are chiefly essential for selective vaccine delivery, thus increasing their safety and efficacy (Jain *et al*., 2014b, 2014c). The VDSs possess structural flexibility; therefore, they can enclose hydrophilic drugs in the central aqueous space and are adsorbed at the bilayer's surface.

In contrast, hydrophobic drugs are retained in the lipophilic partitioning of the bilayer. By encapsulating a drug in the vesicular structure, the drug's half-life can be prolonged in the systemic circulation, and toxicity can be reduced. These carriers are also candidates for the development of sustained-release products for rapidly metabolized drugs. They preserve drug activity at a predetermined rate, relatively constant (zero-order kinetics), and reduce unwanted side effects of drugs. Numerous VDSs had been developed like liposomes, virosomes, iccosomes, ethosomes, niosomes, and bilosomes (Jain *et al*., 2014b, 2014c).

Liposomes

They are primary vesicular carriers studied for the delivery of drugs. In 1964, the liposome tale started with an electronic microscopic illustration of multilamellar phospholipid vesicles published in the Molecular Biology Journal by Bangham and Horne of the Cambridge Babraham Institute (Siler-Marinkovic, 2016). A liposome is a spherical-shaped vesicle composed of one or more phospholipid bilayers. Liposomes were initially used as models for the biological membranes due to their similarities in lipid composition and shape (Siler-Marinkovic, 2016). Multiple administration routes have then been thoroughly studied for liposomes as potential carriers for various medications. The basic structure of a liposome and its component is shown in Figure 1 (Kalepu *et al*., 2013).

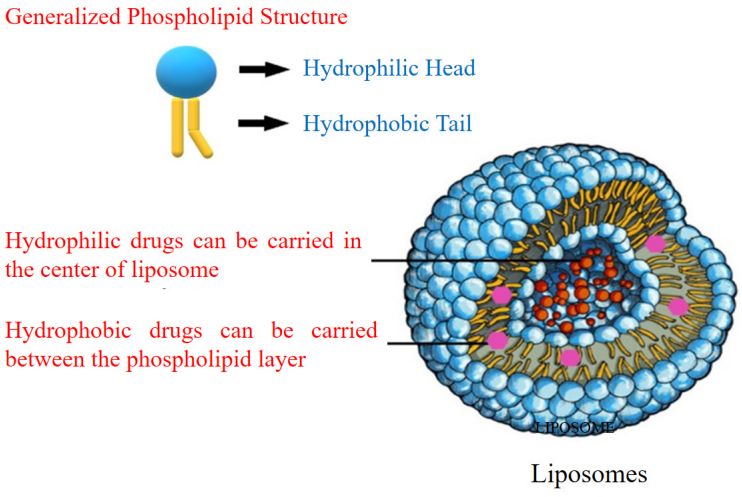

Figure 1. The structural components of the liposomes presenting the location of hydrophilic/ hydrophobic drugs loading into the carrier (Kalepu *et al*., 2013).

Liposomal vaccine carriers

As a vaccine carrier, liposomes have inherent adjuvant properties established as early by Allison and Gregoriadis (1974) and Wang *et al*. (2017). Thus, they have widely been used as vehicles to deliver vaccine antigens. The main advantages of these formulations include antigen stabilization, sustained release of antigens, and promotion of antigen uptake by antigen-presenting cells (APCs). Also, liposomal physicochemical properties are highly adaptable, and their charge, size, and lamellarity can be customized to suit the vaccine requirements (Bernasconi *et al*., 2016).

Liposomes can elicit cell-mediated and humoral immunity against a broad spectrum of antigens such as ovalbumin (OVA), bacterial polysaccharide, influenza subunit vaccines, and bovine serum albumin (BSA) (Wang *et al*., 2019). A previous study approved that strong humoral immune responses were observed in mice after injection with liposomes entrapped diphtheria toxoid. No hypersensitivity reactions were reported in the preimmunized animals when the antigen was incorporated in the liposomes and given by intravenous or intrafootpad injection. In addition, the granulomas were formed at the injection site when adjuvants other than liposomes were used (Gregoriadis and Allison, 1974).

Lipid content affects liposome stability; a more stable formulation can lead to a higher volume of antigen that is bioavailable and with depot benefit. One of these researches was reported by Han *et al*. (1997), who used various combinations of cholesterol, di-palmitoyl phosphatidylserine, di-stearoyl phosphatidylcholine, and di-palmitoyl phosphatidylcholine to prepare the liposomes formulations. Their results showed that liposomes with di-stearoyl phosphatidylcholine were the most stable formula and could better shield the antigen from gastrointestinal tract (GIT) degradation.

Despite the advantages of liposomes, they have shown some limitations as insufficient adjuvant activity, expensive preparation techniques, special storage conditions and handling, and variable purity (Sercombe *et al*., 2015).

Surface-modified liposomes

The first generation of liposomes based on phospholipid bilayer membranes demonstrated inadequate stability and rapid clearance following their injection. Thus, long-circulating liposomes, also known as stealth liposomes, were developed. The liposome shell is coated with hydrophilic polymers like polyethylene glycol (PEG), resulting in decreased blood protein adsorption and prolonged circulation time (Zylberberg and Matosevic, 2016). It was reported that the dose of the PEG-modified liposomes containing antigen is an essential factor for controlling the response of mucosal and systemic immune systems in an oral vaccine. Unmodified liposomes tend to induce a more robust systemic immune response than the PEGylated liposomes, especially at high concentrations. In contrast, the mucosal immune response was more substantial for the PEG-modified liposomes than the unmodified ones at the lower liposome concentration (Minato *et al*., 2003).

Interestingly, the liposomes coupled with IgG showed improved transmucosal transport and were even more immunogenic than the plain liposomes when administrated intranasally. Therefore, studies were performed *in vivo* to determine the potential surface-engineered vesicular carriers for mucosal immunization via the nasal route. For example, the IgG antibodies were immobilized at the liposomes' surface loaded with hepatitis B antigen (HBsAg). Serum containing anti-HBsAg titer collected from the postnasal administration of IgG-coupled liposomes was slightly elevated than that of plain liposomes. Besides, the IgG-coupled liposomes induced both immune responses and humoral (systemic and mucosal) immune responses, while the alum-adsorbed antigen showed neither mucosal nor cellular response (Tiwari *et al*., 2011).

Furthermore, the utility of new mucoadhesive liposomes coated with polymer in conjunction with DNA vaccine showed promising results in mice. The prospective ability of liposomes coated with glycol chitosan as a nasal vaccine delivery system to trigger viral-specific humoral mucosal and cellular immune responses was evaluated and compared with plain liposomes. The adjuvanted chitosan vaccine remained homogeneously dispersed in the mucus following the intranasal administration, demonstrating sufficient mucosal immunity activation (Khatri *et al*., 2008).

Several studies have tested using *Ulex europaeus* agglutinin I (UEA-I) for vaccination through M-cells targeting. When UEA-I was conjugated onto the surface of liposomes, it demonstrated better intestinal absorption of antigen, resulting in robust mucosal and systemic immune responses. In addition, experiments on bovine BSA that encapsulated UEA-I modified liposomes (UEAI-LIP) provided sufficient data supporting UEAI-LIP as a carrier for oral vaccines compared to the unmodified ones (Li *et al*., 2011b).

Gene-loaded liposomes

Another emerging area of liposome research involves inserting RNA- and DNA-encoding antigens into APCs to provide a renewable and continuous source of immunization (McEntee *et al*., 2015). The negatively charged DNA molecules were complexed to the cationic liposomes. Results were promising, yet further studies are required to optimize the liposomes formulations for effective gene delivery. For such tasks, cancer vaccine potency was improved by targeting vaccine antigens to APCs and increasing T cells activation.

Moreover, CD169-expressing splenic macrophages could successfully capture particulate antigens from the blood and deliver them to dendritic cells (DCs), leading to CD8+ T cells activation. In addition, potent immune responses were established when ganglioside M3, a physiological ligand for CD169, was incorporated inside the liposomes because of the enhanced uptake of liposomes by CD169. These results proposed liposomes as a candidate delivery strategy for cancer vaccination (Nijen Twilhaar *et al*., 2020). In another study, the M1 influenza A virus gene was used to create a cationic liposome/DNA vaccine and an M1 encoding plasmid for oral vaccination, which resulted in the expression of the M1 gene in the intestine of the vaccinated mice and showed potent immune responses (Liu *et al*., 2014).

Immune-stimulating complexes

ISCOMs are open cage-like spherical self-assembled particles around 40 nm in diameter. ISCOMs formed spontaneously when phospholipids, cholesterol, and immune-stimulating Quil A saponin were mixed under a particular stoichiometry with the vaccine antigen (Pedersen *et al*., 2012). The antigen-free particle

was recognized as the ISCOMs matrix (Nevagi *et al*., 2018). The Quil A saponin possesses an affinity to cholesterol that causes stabilization in the ISCOM matrix. The technology was vastly evolved where adjuvant-containing vaccines had been tried in clinical trials ever since the primary description of ISCOMs potency as an adjuvant by Hook and Rades (2013).

Following the vaccination with ISCOM adjuvants and antigens, both humoral and cellular immune responses had been documented (Morelli *et al*., 2012). Therefore, various research works are underway to boost the formulation of ISCOMs-based vaccines and adjuvants to control the specificity, type, and effectiveness of the immune response generated. For example, ISCOMs could significantly increase the Th1 type of response, IL-2, and interferon-gamma production, which had been proven to cause a potent immune response in newborns and used as an important delivery mechanism for mucosal administration (Brito *et al*., 2013).

Furthermore, the main advantages of ISCOMs are that they improve the quality of immune responses, activate the humoral and cell-mediated immune response, show an acceptable safety profile with only minor local side effects reported upon injection, provide long-lasting immune responses and permit reduction of antigen dose, and possess flexibility in vaccine design due to the simplicity of the preparation method that involves mixing antigen postmanufacturing. ISCOMs are also vital to future adjuvants and vaccines' success in cancer treatment for veterinary and human use (Nielsen *et al*., 2015). Diphtheria toxin was introduced into the ISCOMs, which was well tolerated and of excellent efficacy when administered mucosally and parenterally. Although oral and intranasal immunizations could lead to systemic responses, the significant IgA response was induced after the nasal administration rather than the oral (McEntee *et al*., 2015).

Current trials had demonstrated positive outcomes when ISCOMs were used as adjuvants and given by injection. One of these trials was a phase I clinical trial in adults that showed the influenza vaccine's loaded ISCOMs effectiveness. Also, chickens were successfully vaccinated by the IM route, resulting in elevated levels of antigen-specific intestinal IgA, CD4, and CD8 positive intestinal intraepithelial T lymphocytes (Nielsen *et al*., 2015). ISCOMs were also used to deliver the mutant V3 loop peptide of HIV-1, nonameric peptides I, II, and III from foot and mouth disease virus capsid VP1 proteins, among many others (Nevagi *et al*., 2018). In addition, when ISCOMs-based vaccines were used in horses and other nonhuman primates, active immunity was produced in the presence of maternal antibodies against equine herpes II virus and measles virus, but this was not the case with the traditional vaccines (Osterhaus *et al*., 1998).

Niosomes

Niosomes were applied as vaccines adjuvants or carriers to assess the immune response elicited by antigen since they possess specific immunological response, decreased toxicity, and better stability than liposomes (Ge *et al*., 2019). The initial studies of niosomes as a vaccine adjuvant were conducted by Brewer and Alexander (1992). They found that niosomes encompassing bovine BSA produced antibody titers of similar concentrations to that produced by the same amount of BSA emulsified in Freund's complete adjuvant (FCA). Niosomes were found to be better IgG2a stimulators than FCA upon examining the IgG subclass specific to BSA stimulated by FCA and niosomes. The efficacy of niosomes as vaccine adjuvants was entirely reliant on their ability to encompass the antigen. Besides, BSA not enclosed in niosomes was found to be ineffective.

Vangala *et al*. (2006) developed a vaccine-loaded vesicular adjuvant system by incorporating dioctadecyl dimethyl ammonium into the niosomal component. The modified niosomes were stable and were suggested for the prevention and treatment of infections. Another promising research suggested the modified polysaccharide O-palmitoyl Mannon (OPM) coated niosomes as a topical vaccine carrier and adjuvant. In addition, the BSA loaded into the mannosylated niosomes was found to possess the ability to elicit humoral and cellular immunity.

Interestingly, niosomes targeted the loaded antigen to the Langerhans cells located below the stratum corneum. For the mannosylated niosomes, the serum IgG levels were significantly higher relative to plain uncoated niosomes (Jain and Vyas, 2005). Another application of mannosylated niosomes is as an oral vaccine carrier and adjuvant. The tetanus toxoid antigen is entrapped within the OPM-coated niosomes. The OPM coat played a significant role in shielding the niosomes from the digestive enzymes present in the GIT and directed the vesicle towards the APCs existing in Peyer's patches. The OPM-coated niosomes induced higher IgG levels than uncoated niosomes and alum adsorbed tetanus toxoid administered orally. They could also evoke humoral and cellular mediated immunity through their combined IgG2a and IgG1 activity (Jain and Vyas, 2006).

In another work, the soluble parasite antigen, *Toxoplasma gondii*, was encompassed in the nonionic surfactant vesicles (NISV) and utilized for vaccination in the murine congenital toxoplasmosis model. As assessed by the lowered abortions and parasite burdens in the vaccinated groups, not only did the niosomes-based vaccine elicit a defensive immune response, but the vaccinated groups' splenocytes also improved the production of antigen-stimulated interferon-γ compared with FCA (Roberts *et al*., 1994).

Moreover, a study of the immune response triggered by type 1 herpes simplex (HSV-1) antigen incorporated within NISV exhibited significant serum antibody production following the vaccination with adjuvant formulations, indicating that NISV effectively enhanced the body's immune response to antigens relevant to disease (Hassan *et al*., 1996).

One more example is that niosomes were evaluated for topical vaccine administration using cholera toxin B as an adjuvant and hepatitis B surface protein as an antigen. The optimal niosomal formulation showed good entrapment efficiency and vesicular size range of 2.83 ± 0.29 μm. In addition, the immune response after three consecutive topical administrations was more successful than the single-dose administration. Thus, cholera toxin B was proved to be a potential adjuvant for topical vaccination when coadministered with the HBsAg encapsulated niosomes, and niosomes are effective vaccine carriers for topical immunization (Maheshwari *et al*., 2011). Further examples of research on niosomes-based vaccines are displayed in Table 1.

Bilosomes

The bilosomes delivery system is a novel vaccine oral carrier formed by altering lipid-based vesicles (niosomes), resulting in greater stability, targeted delivery, and enhanced

absorption through the gut. The niosomal formulation integrates bile salts such as sodium deoxycholate which are known as bilosomes. Incorporating bile salts in the vesicles shields the vaccine molecules from degradation due to bile acids and digestive enzymes present in the GIT, consequently improving the immunological adjuvant property of the antigen encompassed within the vesicles (Rajput and Chauhan, 2017).

In the last few decades, bilosomes potential utilization for oral vaccine delivery has been investigated in several studies (Aburahma, 2016; Thakur and Foged, 2020). It was reported that bilosomes allowed the antigen to be directed to the mucosal tissue without premature release; thus, minor antigens concentrations are required for mucosal immunization with bilosomes (Wilkhu *et al*., 2013). Also, the vesicular size plays an essential role in constructing efficient vaccine vehicles with targeted site delivery to exert therapeutic action. Through experimental studies, this property is displayed, where Peyer's patches absorb a vesicle size of 10 µm or less. In comparison, vesicles with a size of less than 5 µm are transported by the lymphoid drainage method to other cells. In influenza vaccine-bilosomal formulations, the more giant vesicles (6 µm in size) exhibited enhanced uptake within Peyer's patches. In addition, they supported viral cell load reduction better than smaller vesicles (Azuar *et al*., 2019; Wilkhu *et al*., 2013).

Various ligands like lectins, microbial adhesions, and immunoglobulins were conjugated to the vehicles to enhance the targeting efficacy of M-cell receptors. Furthermore, recombinant virus-bilosomes complexes were also examined to increase the transmucosal uptake through M-cells targeting. For example, the recombinant baculovirus (Bac-VP1) was complexed with bilosomes for oral vaccination against human enterovirus 71 in mice (Premanand *et al*., 2013).

Novel surfaced modified bilosomes were developed to improve the stability and entrapment of cholera toxin B subunit complexed to the bilosomes which was favorable to deliver recombinant HBsAg (Shukla *et al*., 2010). Also, glucomannan bilosomes (GM-bilosomes) were developed to improve stability and mucosal immunization of vaccine-loaded bilosomes. The surface-modified GM bilosomes were found as a promising vehicle and adjuvant for oral vaccination (Jain *et al*., 2014a).

Also, Jain et al. (2014b, 2014c) compared three types of vesicular systems, bilosomes, GM-bilosomes, and niosomes, for oral immunization efficiency and functionality of vaccine-loaded vesicular-type nanocarrier. For oral immunization of tetanus toxoid in mice, the GM-bilosomes are superior in their immune response compared to the standard bilosomes and the niosomes obtained. The immunological improvement was attributed to the GM's polymeric nature that increased mannose molecules density at the surface of bilosomes; consequently, the APCs uptake of mannosylated bilosomes was improved. Moreover, the mannosylated bilosomes were found more stable against digestive enzymes.

Ethosomes

Transcutaneous vaccination has several advantages compared to conventional oral or injection immunization such as avoiding hepatic first-pass effect, good compliance, and convenient use. However, the skin's stratum corneum is the main barrier limiting antigen molecules' permeation to activate DCs in the epidermis. Thereby lipid colloidal carries like transfersomes and ethosomes were developed to improve the transdermal permeation of molecules (Al Shuwaili *et al*., 2016).

Ethosomes are flexible, spherical vesicles that deliver drugs into deep tissues and systemic circulation via topical administration. Most ethosomes are composed of ethanol, phospholipids, and water. Ethosomes sometimes contain other excipients, such as surfactants, to better penetrate the membranes and reach the desired target site (Abdulbaqi *et al*., 2016). The ethosomes also interact with the skin's lipid components, resulting in high absorption of lipophilic drugs at drug-specific levels (Sinico and Fadda, 2009). They possess several advantages like extending skin contact time, increasing the therapeutic effectiveness of the enclosed drug, enhancing the final dosage form's consistency and shelf life, and patient adherence. Thereby, novel pharmaceutical formulations such as transdermal patches, gels, and creams were developed by incorporating ethosomes. Besides, ethanol acts as a preservative that enhances ethosomic stability and prolongs its shelf life (Ainbinder *et al*., 2010).

After two decades of extensive research work, ethosomes exhibited efficient drug entrapment, good stability, and improved transdermal drug delivery, hence making them a potential transdermal delivery system for vaccines. In a previous study, ethosomal-based gels were prepared using different solvents for transdermal antigen delivery. The *in vitro* stability analysis indicated that the phosphate buffer solvent-containing gel was more stable than the aqueous preparations. Furthermore, a prompt IgG release and appreciated *in vivo* immunogenic effect were observed with phosphate buffer solvent gel (Zhang *et al*., 2018). Also, ethosomes for transcutaneous immunization against hepatitis B were investigated. The flow cytometric analysis and spectral bioimaging results revealed that the *in vitro* uptake of hepatitis B-loaded ethosomes by murine DCs peaked by 180 minutes.

An *in vitro* permeation study through the dermis of a human cadaver showed an increased transcutaneous delivery

Table 1. Examples of niosomes-based vaccines formulations.

Loaded vaccine	Surfactant	Preparation technique	Administration route	References
Subunit flu antigen, H3N2 antigen (Radio labelling)	Monopalmitoyl glycerol	Aston melt method Strathclyde melt method	Oral, I.M.	(Zhang, 2015)
Diphtheria toxoid	Sorbitan tristearate	Thin-film hydration	Oral	(Shukla *et al*., 2011)
Newcastle disease vaccine	Span 20	Lipid film hydration	Parenteral	(Okore *et al*., 2011)
Protective antigen domain 4 of *Bacillus anthracis*	Span 60	Reverse-phase evaporation	Parenteral	(Gogoi *et al*., 2018)

of ethosomal antigen compared to traditional liposomes. Moreover, a robust systemic and mucosal humoral immune response was obtained from the topical ethosomes following the IM administration compared to the control, alum-adsorbed antigen suspension (Mishra *et al*., 2008). Besides, ethosomes transcutaneous permeation enhancement was significantly higher than other vesicular vectors of a peptide vaccine through the tested piglet skin (Rattanapak *et al*., 2012).

Structurally modified ethosomes were also tried for vaccines delivery. For example, modified hyaluronic acid/galactosylated chitosan ethosomes (HA-GC-Eth) were prepared by layer-by-layer self-assembly techniques and loaded with OVA antigen. The modified ethosomes were stable and showed promising activity *in vitro*. Moreover, the novel (OVA@HA-GC-Eth) loaded silk fibroin nanofibrous mats effectively stimulated the antigen's immune response following the transdermal administration (Yang *et al*., 2020). Also, the structural modification of the deformable vesicles ethosomes with the cationic lipids octadecylamine (Wang *et al*., 2007) or stearylamine (Zhang *et al*., 2017) can evoke immune responses in transcutaneous immunization.

NANOPARTICULATE DELIVERY SYSTEMS

Nanotechnology was first introduced in the 1950s by Feynman, after which successive attempts were made to develop nanomedicine in medical research and treatments. However, nanomedicine was first developed as a modern emerging science only in the early 1990s (Krukemeyer *et al*., 2015). NPs are a colloidal-based drug delivery system that comprises nanometer range polymeric particles ranging in diameter from 1 to 1,000 nanometers (Van *et al*., 2019). In general, NPs are classified into two types, nanocapsules, based on their morphology. The nanocapsules are made up of a core in which the drug is usually dissolved and a polymeric layer that regulates the drug release profile of the core. On the other hand, nanospheres were made up of a continuous polymeric network that allows the substance to be stored within or adsorbed onto its surface (Salatin *et al*., 2017).

The main advantages of NPs are improved drugs bioavailability and therapeutic activity by enhancing aqueous solubility, prolonging of the biological half-life, and drug targeting to a specific body location. One more considerable merit is that NPs have a high binding affinity to small molecules due to their large surface area (Patra *et al*., 2018). The high drug-NPs loading resulted in decreased drug dose, consequently allowing the safe delivery of therapeutic agents to the target and protecting nontarget tissues and cells from harmful side effects. Because of the high drug-NPs storage, the drug dosage is reduced, allowing for safe therapeutic agent distribution to the desired area of action and shielding cells and tissues that are not on target from adverse effects (Mahapatro and Singh, 2011).

Polymeric NPs

During the last decades, polymeric NPs have attracted great interest because of their property, resulting in smaller particle sizes. Polymer-based NPs (50–300 nm in size) were used as adjuvants and vaccine carriers. The loading of vaccines into NPs showed crucial merits in enhancing their immunogenicity via delivering the vaccine to particular intracellular compartments to activate receptors for immune pathways over an extended period (Diaz-Arévalo and Zeng, 2020) stability in the biological fluids and prolong their circulation time in the blood. A particle's size, for example, played a critical role in vaccine efficacy. Particles smaller than 5mm produced more immune responses than larger ones. This is because macrophages take up small nanoparticles more readily than microparticles (Sung and Kim, 2020).

Moreover, the NPs-consisting polymers' characteristics, such as biocompatibility, biodegradability, stability, and toxicity, must be evaluated before their formulation. Several polymers were used for the production of NPs. For example, poly-(lactic-co-glycolic acid) (PLGA) is a biocompatible and biodegradable polymer (Elmowafy *et al*., 2019) that the United States Food and Drug Administration (FDA) newly approved for parenteral administration (Makadia and Siegel, 2011). Polystyrene synthetic is biodegradable, safe, and easy to functionalize (Loos *et al*., 2014). Synthetic poly(propylene)sulfide is a biodegradable and oxidation-sensitive homopolymer (Hirosue *et al*., 2010). Chitosan is a cationic, biodegradable, biocompatible, and not toxic natural biopolymer (Ragelle *et al*., 2013).

One of the challenging tasks is finding an effective method for antigen loading into the NPs, which is highly affected by both antigen and polymer (Lambricht *et al*., 2017). Vaccine molecules loaded NPs can be surface conjugated, encapsulated, or surface adsorbed. Surface adsorption of the antigen at the NPs' surface is mediated by hydrogen bonds, ionic or hydrophobic interaction. For example, dextran sulfate and chitosan polymers were efficiently used to prepare vaccine-loaded NPs. The viral antigen loading into these NPs was mainly by immobilization and hydrogen bond interaction (Pati *et al*., 2018). The antigen molecules must remain intact during the encapsulation process and be adequately released after administration.

Many techniques have been applied to conjugate/incorporate vaccine molecules into the NPs. However, nanovaccine production and manufacturing are still complicated and require modern machines, making this technique costly compared to the conventional one. Furthermore, nanovaccines administration via topical, inhalation, or ocular routes and incorporating multiple antigens into the same particle to defend against more than one disease were also investigated (Deaguero *et al*., 2020). The nanosystem configuration directly influences antigens and adjuvants' release profile from the carrier matrix (Mahapatro and Singh, 2011). Different polymeric nanostructures, varying from particles, nanogels, and micelles to polymerases and core-shell particles, can be synthesized using a wide variety of materials, including natural and synthetic polymers (De Geest, 2020; Moran *et al*., 2018).

Moreover, many studies strongly confirmed that the setting of physicochemical properties of NPs could be used as an essential tool to target vaccine molecules to specific sites and trigger the desired immune responses. Surface modification of NPs altered the interaction extent with APCs and ligand specificity (Pati *et al*., 2018). For example, 47 treelike DCs were anchored to the surface of NPs which could modulate the downstream signaling cascades and reduce NPs engulfment by phagocytic cells (Kawai and Akira, 2011). Other studies used toll-like receptors (TLRs) 7, 8, and 9 that stimulate innate immune responses when identifying pathogenic nucleic acids. Authors found that complexing NPs surfaces achieved increased cytokine production

and the expression of immunoregulatory genes with the TLRs 7, 8, and 9 agonists (Kawasaki and Kawai, 2014).

In a comparative study, Pawar *et al*. (2013) fabricated glycol chitosan-coated PLGA (GC-PLGA) NPs and compared their immunoadjuvant ability compared to chitosan-coated and uncoated PLGA NPs. The results proved that GC-PLGA demonstrated higher systemic and mucosal immune response and lower clearance than chitosan-coated and uncoated NPs. Figure 2 demonstrates loading the drug onto the NP carrier where the antigen is encapsulated inside the particle's core or conjugated to the surface of the NPs (Pati *et al*., 2018). Various targeting molecules were conjugated to the surface of NPs, e.g., antibodies. Fab-fragments, peptides, and PEG or poloxamer surface coating. They could enhance particle distribution to APCs and trigger innate and adaptive immune responses (Parhiz *et al*., 2018). Studies utilizing polymeric NPs for vaccine development against various infective diseases are presented in Table 2.

Inorganic NPs

Recent studies focus on using the inorganic NPs as both carriers and adjuvants in vaccine production and approval preclinical setup. Due to the distinctive features of inorganic NPs such as small particle size, enhanced stability, high loading capacity, and improved permeability, these nanocarriers have occupied a leading place in antigen/vaccine delivery and targeting (Turner *et al*., 2015). Inorganic NPs had been applied in biomedicines to deliver and target drugs to a specific site in the body. They were also used in cancer therapy and are considered as excellent carriers for tumor vaccines (Khan *et al*., 2014). Gold NPs (AuNPs), iron oxide NPs (IONPs), quantum NPs, and carbon nanotubes are typical examples of inorganic NPs.

Gold NPs

In 1996, Demenev and his team were the first to apply AuNPs in designing a vaccine against tick-borne encephalitis. AuNPs are of a wide range of particle sizes (from 1 nm to 8 μm) and display various shapes like spherical, triangular, hexagonal, nanocube, nanoshell, and nanorods (Bansal *et al*., 2020). Owing to their small size, they can cross the cell membrane, interact with the nucleus, and perform their therapeutic action. In addition, AuNPs possess a high degree of stability and biocompatibility. Because of their exciting advantages, AuNPs have been used to prepare antibodies and vaccines against many microorganisms like viruses, bacteria, and parasites. Recently, AuNPs exhibited the best antigen carrier in cancer vaccination (Dykman *et al*., 2018). However, multifunctionalized AuNPs became progressively more complicated, and a balance must be attained between suitable *in vivo* stability, tumor localization, limited cytotoxicity, and efficacy (Nicol *et al*., 2015).

Another point to be considered is that AuNPs are nonbiodegradable; therefore, their pharmacokinetics and elimination fate must be studied and determined. For example, the elimination time of AuNPs from the spleen and liver takes up to 4 months, leading to potential toxicity. Another study reported

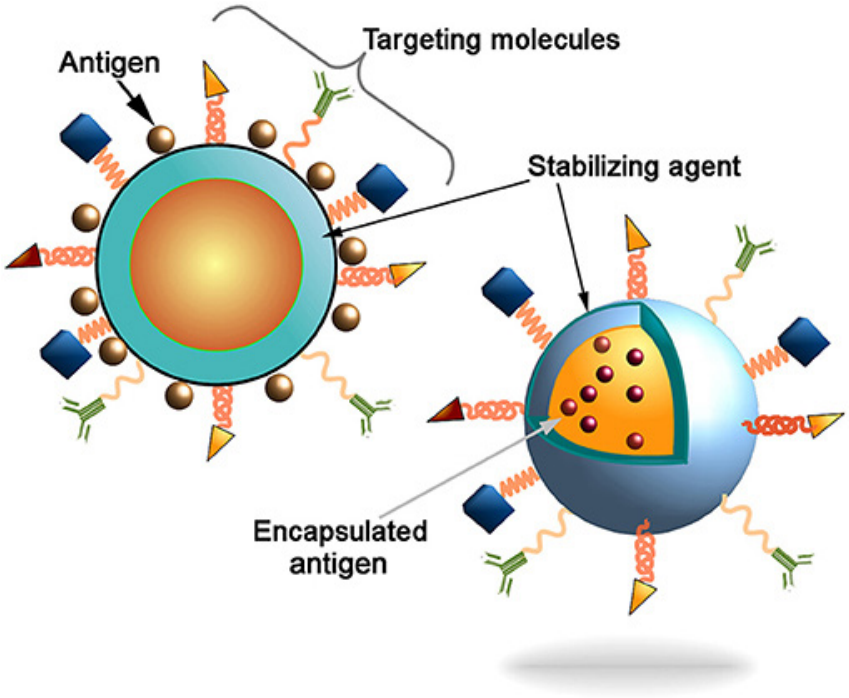

Figure 2. A schematic representation of the nanocarriers illustrates the antigen conjugation to the surface of the NPs or antigen entrapment into the particle's core. The targeting molecules, e.g. antibodies, Fab-fragments, peptides, etc., could further improve the delivery of particles into the APCs to induce characteristic and robust immune responses (Pati *et al*., 2018).

Table 2. Studies utilizing polymeric NPs for vaccine development against various infective diseases.

Vaccine platform	Disease	Administrationroute	Organism model	Humoral response	Characteristics	References
PLGA NP	Influenza virus	SC	Mouse	IgG1, IgG2a, IgG2b, and virus-neutralizing antibody titer	For 14 days after infection, 100% survival was reached. Antigen-specific memory CD4+T cells remained after vaccination for 1.5 years.	(De Geest, 2020)
PS-core protein shell NPs	H1N1 swine influenza virus	IV	Mouse	IgG, IgG1, IgG2a and HA inhibition titers	As shown by the high survival rate and minimal bodyweight loss, protection against the H1N1 virus will last up to 16 days after infection.	(Lee *et al.*, 2018)
Chitosan-PGA NPs	Zika virus (ZIKV)	IM	Mouse	IgG, IgG1, IgG2a	The produced antibody neutralized the ZIKV.	(Wibowo *et al.*, 2021)
PAA-PPI dendrimers and lipid for EBOV	Ebola virus (EBOV)	IM	Mouse	IgG	100% survival was accomplished after mice were immunized with a single dose of polymeric vaccine.	(Blakney *et al.*, 2021)
Mannosylated PEI NPs	HIV	TD	Human (Phase I & II)	---	At week 48 after vaccination, HIV-specific precursor/memory T cells with high proliferation potential were extended dose-dependent.	(Rodriguez *et al.*, 2013)
Chitosan NPs	Hepatitis B	IP	Mouse	IgG, IgG1, IgG2a, IgG2b, IgG3 titer in serum, spleen and bone marrow	Up to 30 weeks after a single dose vaccine, the humoral and cellular responses were sustained, with improved BAFF-R + B cells, CD 138 + plasma cells, and Tfh cells.	(Oliveira *et al.*, 2020)
PHB-core-shell NP	Hepatitis C virus	IM	Mouse	IgG	Reduced virus load titer in ovaries.	(Martínez-Donato *et al.*, 2016)

NPs: Nanoparticles; PLGA: Poly(lactic-co-glycolic acid); LPS: Lipopolysaccharide; PS: Polystyrene; IV: Intravenous; IM: Intramuscular; TD: Transdermal; IP: Intraperitoneal; SC: Subcutaneous; PPI: Poly(propylene imine); EBOV: Ebola virus; PEI: Polyethyleneimine; PHB: Polyhydroxybutyrate.

that AuNPs of 10 nm in size were widely spread in various body compartments where larger particles were detected only in the liver and spleen and blood (De Jong *et al*., 2008).

Also, to design biocompatible and biostable AuNPs, it was necessary to include a hydrophilic excipient to modify the monolayer core's hydrophobic nature. For example, PEG was widely employed to passivate the NPs' surfaces and prevent nonspecific interaction, especially with biomolecules (Fratoddi, 2017). In a previous study conducted by Zhang *et al*. (2009), it was observed that the 20 nm PEG5000 anchored NPs exhibited the lowest uptake by the reticuloendothelial system and showed the most extended biological half-life comparing to the large-sized AuNPs. Another novel delivery system encompassing chitosan-functionalized AuNPs was developed to enhance better systemic and mucosal immune responses and was considered as a sound approach for oral vaccine delivery against various pathogens (Barhate *et al*., 2014).

Iron oxide NPs

Recently, magnetic (Fe3O4 and γ-Fe2O3) IONPs have attracted a lot of attention and become particularly appreciated in medical applications such as thermal therapy, protein immobilization, diagnostic tools, and drug delivery. This is because IONPs possess excellent magnetic properties and a high surface-to-volume ratio for functionalization and are easily prepared. In addition, they are considered safe, bioavailable, and biocompatible (Wu *et al*., 2015).

Various chemical and physical methods were used to prepare IONPs, like dry processes and wet chemical or microbiological techniques (Hasany *et al*., 2013). However, the physical techniques suffered from some problems associated with controlling the particles' size, and they need highly complex and costly machines (Cuenya, 2010).

In 1996, the FDA approved IONPs in the clinical field (Cortajarena *et al*., 2014). The use of IONPs as a diagnostic tool (e.g., magnetic resonance imaging (MRI) and malignant hyperthermia) garnered interest in the healthcare sector. Their wide application was due to some intrinsic parameters such as the small particle size, saturation magnetization, shape, and chemical composition (Blanco-Andujar *et al*., 2016). The currently approved product GastroMARK® (AMAG Pharmaceuticals) is an aqueous dispersion of silicone-coated superparamagnetic IONPs (SPIONs) administered orally before the radiological examination (Cortajarena *et al*., 2014).

Additionally, IONPs showed great promise to serve as both an adjuvant and vaccine delivery platform. It can elicit the immune system as an adjuvant where it induces both humoral and cell-mediated immune responses. Researches had explored the IONPs-immune interactions as vaccines or immune therapies for infectious diseases and cancers (Neto *et al*., 2018). For instance, Zhao *et al*. (2018) reported that OVA-loaded iron NPs produced significant immune responses and tumor inhibition compared to the soluble OVA alone. Thus, they suggested the IONPs as a novel vaccine delivery platform and immune inducer. In another work by Pusic *et al*. (2013), IONPs had been used as a vaccine carrier against malaria. They noticed that vaccination with these NPs induced higher titers of parasite-inhibitory antibodies than immunization with the adjuvant, Montanide.

Fortunately, the surface-modified polymer-coated IONPs were also investigated for antigen delivery. For example, the immunogenic melanoma antigen (hgp10025-33-loaded to dextran-coated SPIONs) displayed an intact homogeneous structure used for functionalization with imaging or drugs or dyes (Kim *et al*., 2012). In addition, chitosan and its derivatives were the most common polysaccharides for producing surface-coated iron NPs (Kumar *et al*., 2010). The surface modification of the NPs was proved via the electrostatic interactions and physical adsorption between iron and the free hydroxyl and amino groups in chitosan molecules (Abarca-Cabrera *et al*., 2021).

A modified version of the nanomagnetic particles was developed and is known as SPIONs with surface functionalization. These NPs were synthesized by different methods to obtain uniform particle size and narrow size distribution (Wallyn *et al*., 2019). As a result, SPIONs exhibit great promise in clinical and preclinical diagnostic testing/imaging and targeted therapy. Currently, the FDA approved using SPIONs clinically as an imaging agent, demonstrating safety and efficacy (Thakor *et al*., 2016). Of note, SPIONs had a distinguished application in vaccine delivery and cancer therapy, and many studies investigated the possibility of delivering antigens by these nanocarriers. For example, the malaria DNA vaccine's transfection efficacy and vector targeting were improved via magnetofection by the SPIONs. In addition, the synthetic SPIONs showed high DNA binding capacity and were considered an excellent gene transfection platform (Balcells *et al*., 2019).

Still, SPIONs have some issues with aggregation and stability and are currently under development to overcome these drawbacks. Furthermore, SPIONs-based immunotherapy is given by intracranial injection, enabling the possibility of controlled magnetic delivery of activated immune cells to treat deep brain tumors and multifocal diseases (Champagne *et al*., 2018). Keeping this in mind, although cancer immunotherapy is undergoing rapid progress, there are still many challenges in this area, and further future studies are required.

Carbon nanotubes

Carbon nanotubes are nontoxic and biocompatible NPs (Hasnain and Nayak, 2019). They are relatively inert, nonimmunogenic, and stable on the shelf and *in vivo* (Scheinberg *et al*., 2010). Nanotubes have a length of up to millimeters and a diameter ranging from 1 to 100 nm. Two types of carbon nanotubes are present: a) single-walled carbon nanotubes and b) multiwalled carbon nanotubes (Divekar, 2020). The high tensile strength of carbon nanotubes mainly accounts for the high-frequency strong carbon-carbon bond, making them thermostable (Saifuddin *et al*., 2013).

Studies reported that carbon nanotubes were appreciated as candidate adjuvants and platforms to target specific antigens and facilitate their delivery to the immune system. Hence high binding capacity is one of the carbon nanotubes delivery systems' critical advantages; therefore, different molecules can conjugate to their surface simultaneously (Scheinberg *et al*., 2010). Based on this, there are still significant studies that have to be conducted to understand the role of carbon nanotubes in vaccine formulations compared to more conventional vectors.

Dendrimers

Dendrimers are NPs of homogeneous, well-defined, and monodispersed structures with a typically symmetric core and inner and outer shells (Abbasi *et al*., 2014). One of the most critical aspects that need to be considered for dendrimers' biomedical application is their pharmacokinetics features (Kaminskas *et al*., 2011). Several trials were conducted in dendrimers' structural modification to function as an effective drug delivery system. For example, dendrimers' peripheral groups' alteration resulted in establishing different antibody-dendrimer (Szymański *et al*., 2011) and peptide-dendrimer conjugates (Kojima *et al*., 2018). Also, dendritic boxes that encapsulate guest molecules were developed (Noriega-Luna *et al*., 2014). Many dendrimers were synthesized based on their structure modifications, although five families are commonly used (Pedziwiatr-Werbicka *et al*., 2019).

The variety of their applications in medicine is leading to an increased interest in this field. Currently, dendrimers are used in drug delivery, photodynamic therapy, imaging, and neutron capture therapy (Abbasi *et al*., 2014). They also have ideal characteristics required for potent adjuvants to enhance vaccines' effectiveness (Yadav *et al*., 2018). A dendrimer-based carrier system was developed to deliver peptide-based vaccines and demonstrated its antimicrobial efficacy in the *Chlamydia trachomatis* mouse model. The dendrimers (G4OH) comprise a peptide mimic of chlamydial glycopeptides antigen and generation 4, hydroxyl-terminated polyamidoamine (PAMAM). The peptide was conjugated through an ester bond. Serum antibodies specific for *Chlamydia* were produced following immunizations with Pep4-conjugated dendrimers via the subcutaneous route.

Moreover, this vaccine formulation provided substantial protection for immunized animals against vaginal infection with *C. trachomatis* by decreasing a load of infection and genital tissue damage. Pep4 conjugated to G4OH showed improved safety relative to Pep4 and adjuvant (alum), indicating a powerful adjuvant effect of the PAMAM dendrimer (Ganda *et al*., 2017). It was reported that the PEG-citrate G2 dendrimer containing several epitopes developed significant cellular immune responses *in vivo* and a more robust Th1 response compared to Th2, thus contributing to the production of potent HIV vaccines (Abdoli *et al*., 2017). PAMAM dendrimers are composed of an ethylene diamine nucleus with their methyl acrylate and ethylene diamine branches (Pedziwiatr-Werbicka *et al*., 2019). Many PAMAM dendrimers have carboxyl groups on their surface, while other generations possess amino groups on their surfaces (Gillani *et al*., 2020). Lysine-conjugated PAMAM dendrimers demonstrated outstanding activity as a vector vaccine, boosting the immunoreactivity and vaccine effectiveness against *Schistosoma japonicum* (helminthic parasites) (Wang *et al*., 2014).

The researchers also designed a vaccine platform based on the dendrimer that embodies the antigen-expressing replicon mRNAs and produces a single-dose protective response toward viruses such as H1N1 and Ebola and other parasites like *T. gondii* (Chahal *et al*., 2016). The vaccine consisted of an ionizable G1-PAMAM dendrimer, the lipid-anchored PEG, and the RNA. These experiments highlight the dendrimers' role in producing new generation vaccines for various infections. It is known that Middle East respiratory syndrome coronavirus (MERS-CoV) and coronavirus disease are developing rapidly. This pandemic's global transmission poses a significant threat to public health and the global economy (Gurunathan *et al*., 2020). Polycationic dendrimers with a primary amine and three forms of polyanionic dendrimers with terminal hydroxyl, succinic acid, and sodium carboxylate were used to test their antiviral function by using the Vero cells model. The most potent cytotoxicity was observed with terminal sodium carboxylate, followed by the cationic dendrimers indicating the polyanionic dendrimers' safety and antiviral activity against the respiratory syndrome coronavirus in the Middle East (Kandeel *et al*., 2020).

In conclusion, dendrimers nowadays are considered as the typical carriers in biomedical applications such as imaging as MRI contrast agents to diagnose diseases, tissue engineering and delivery, and targeting of drugs, vaccines, and other biological products. However, research is underway to create new dendrimers scaffolds that need to be designed with better physicochemical, mechanical, and biological properties (Noriega-Luna *et al*., 2014).

MICROPARTICULATE DELIVERY SYSTEMS

One of the most convenient ways of administering the drug is by mouth. However, some drugs have shown limited oral absorption or a short half-life; therefore, repeated doses were required to achieve its therapeutic effect. This problem created pressure on many patients and led to noncompliance. Thus, drug delivery systems as microparticles were introduced to deliver the drugs to a particular site in the body (Lengyel *et al*., 2019).

Indeed, microparticles can incorporate solid, liquid, or gaseous drugs moieties to form intact entities of particle size ranges between 1 and 1,000 µm. They comprise two main parts, the core and the shell. Usually, the core includes the API Active Pharmaceutical Ingredients (API) and other excipients, while the shell is the coating material surrounding the core. Microparticulate Drug Delivery Systems (MDDSs) were formulated in various systems, including microparticles, microspheres, and microcapsules, which can provide therapeutic activity via different administration routes. The term "microparticles" was used in the literature for the matrix biodegradable microparticles, while the microspheres refer to the shell-enclosed core particles (Lengyel *et al*., 2019).

Microparticles are currently applied in the biomedical field for many advantages regarding the API, such as reducing adverse effects and dosing frequency, masking the unpleasant taste and odor, modifying drugs release profile, avoiding drug interaction to excipients, improving stability, and increasing bioavailability (Jeevana and Jyosna, 2014).

The microparticles improvement journey as an adjuvant and carrier of vaccines began a long time ago. The incorporation of vaccine antigens into such vectors is due to their exciting advantages. For example, the vaccine antigen's thermostability was improved by loading it into the microparticles in a dry powder containing sugar as a stabilizing agent (Foged, 2016). Delayed and controlled drug release from the microparticles was achieved using smart biodegradable polymers (Park, 2014). PLGA is one of the preferred biodegradable polymers for preparing microparticles. PLGA polymer was approved by the FDA to formulate microparticles using various techniques, double emulsion solvent evaporation being the most common.

The polymeric microparticles' size and structure can be modified to resemble the pathogens or deliver the vaccine antigens and trigger a humoral and cell-mediated immune response (Silva *et al*., 2016). This technique paved the way for the preparation of PLGA/polysaccharides or polysaccharides vaccine delivery systems. Mata *et al*. (2011) loaded the malaria peptides into PLGA/sodium alginate microparticles for intradermal injection, which induced a balanced Th1/Th2 response BALB/c in mice compared to the antigen alone or antigen-loaded PLGA microparticles.

Anionic polymers like sodium alginate were employed to formulate live *Mycobacterium*-loaded microparticles as aerosol inhalable vaccine formulations. The coated microparticles were more immunogenic than the liquid aerosols in mice against *Mycobacterium tuberculosis* infection (Nagpal *et al*., 2019). Cationic polymers, such as chitosan, have also been investigated to formulate microparticles. However, these required another preparation technique known as ionotropic gelation and, when combined with PLGA polymer, chitosan showed an adjuvant effect due to its positive charge (Li *et al*., 2016). The novel multifunctionalized dextrans microparticles were also examined as a vaccine adjuvant and carrier, resulting in robust immune responses (Bolandparvaz *et al*., 2020).

The coloading of antigen and adjuvant into microparticles was a new vaccine development strategy in the last decade. The combining systems showed robust immune responses after single-dose vaccination compared to multidose scheduled immunization with the conventional preparations (Zhang *et al*., 2015a, 2015b). Finally, stability is a critical issue in antigens encapsulation which should be optimized for each vaccine formulation. Antigen's stability can be maintained by incorporating stabilizing additives into the core, such as hydrophilic polymers, surface-active agents, sugars, or protein BSA (Han *et al*., 2016). Eudragit E, poly(L-lysine), and branched polyethyleneimine are cationic additives, enhancing immunological response when coencapsulated with attenuated polio vaccine into PLGA microparticles (Tzeng *et al*., 2018). Some examples of antigen vaccines incorporated into MDDSs for better immune response are listed in Table 3.

EMULSIONS AND MICROEMULSIONS

Emulsion-based vaccines

Emulsion adjuvants have a long history of clinical application as vaccines adjuvant. In the 1940s, Freund prepared the first adjuvant emulsion containing killed mycobacteria called complete Freund's adjuvant (CFA). He then developed the incomplete Freund's adjuvant (IFA), similar to CFA but free of the killed mycobacteria. The oil used in the CFA is nonbiodegradable mineral oil with a mannide monooleate emulsifier. Later studies proved that the IFA was also an active adjuvant and had better tolerability than the CFA (Shah *et al*., 2015).

Interestingly, besides their role as vaccines adjuvants, emulsions were employed effectively as a vaccine delivery system. The amphiphilic bioresorbable polymer poly(ethylene glycol)-block-poly(lactide-co-epsilon-caprolactone) (PEG-b-PLACL) was developed by Huang *et al*. (2009) and loaded with OVA as a model antigen. The findings showed that PEG-b-PLACL-emulsified formulations comprised small homogeneous particles and were stable, reproducible, stable, and thus advantageous over the conventional vaccines (Huang *et al*., 2009). In Italy, squalene oil-in-water (O/W) emulsion for influenza vaccine was approved in 1997, and it was approved in various other countries in 2000. Furthermore, squalene-based emulsion with murabutide as an immune potentiator could enhance the antigen's uptake by immune cells and evoke a humoral and cell-mediated immune response in mice (Garçon *et al*., 2012; Kantipakala *et al*., 2019).

Microemulsion-based vaccines

Microemulsions are low viscosity liquid mixtures consisting of oil, water, and surfactant. They are thermodynamically stable and transparent. Based on the phase, microemulsions are present in two forms: O/W and water-in-oil (W/O) (Mohanty *et al*., 2019). Due to their unique properties, these systems have become interesting in pharmaceutical product development and design, including vaccines. For example, a novel ethanol-in-fluorocarbon microemulsion for topical genetic immunization was reported by Cui *et al*. (2003). The pDNA was incorporated into the fluorocarbon-based microemulsion system, and it significantly improved the mouse skin's luciferase expression compared to pDNA in normal saline or ethanol.

The microemulsion preparations had shown promising candidates for adjuvant systems aimed at intranasal immunization (Lee *et al*., 2016). Microemulsion adjuvants have been developed to induce a humoral response in mice and have shown effective *in vivo* protection. Moreover, in a previous study, microemulsion adjuvant had shown to be a better candidate for rabies immunization than other tested adjuvants since it provided strong potency against the virus and did not seem to cause any local reaction (Leclercq *et al*., 2011).

Also, cationic microemulsions could serve as a novel adjuvant to administer a parenteral vaccine (Lamaisakul *et al*., 2020). Influenza vaccine was prepared from Al(OH)3 hydrogel with monophosphoryl lipid A, and dioctadecyldimethyl ammonium bromide and mineral oil are examples of potent and safe microemulsion adjuvants (Lamaisakul *et al*., 2020). The microemulsions and water-in-oil-in-water (W/O/W) multiple emulsions were also investigated as potential adjuvants for the bluetongue vaccine in rabbits. The microemulsion adjuvant displayed a lower titer; however, it was simpler to prepare, stable at 4°C, and did not show any local reactions (Macedo *et al*., 2013).

VIROSOMES

Virosomes or VLP were prepared first by Almeida *et al*. (1975). Virosomes are reconstituted viral envelopes that comprise viral spike glycoproteins and membrane lipids but lack the root virus's genetic material. They can be used as vaccines or adjuvants to deliver nucleic acids, genes, drugs, and macromolecules for therapeutic uses (Asadi and Gholami, 2021; BioPharm International, 2011).

Virosome-based vaccination is successful in regulatory and safety reports and the viability of upgrading development. It has also been approved in more than 40 countries with use in elderly patients and infants. Typically, the virus used to develop virosomes is the influenza virus (Nair *et al*., 2020).

Besides, studies proved that virosomes-enclosed proteins like influenza virus hemagglutinin (HA) and neuraminidase allow the membrane of virosomes to bind with the immune system cells, where it transports the specific antigen to the targeted site, thus

Table 3. Antigen vaccines incorporated into microparticulate delivery systems for better immune response.

Antigen/vaccine	Polymer	Significance	References
Influenza A/PR/34/8	Eudragit-S and Trehalose	To reduce the cost and vaccination frequency as a result of oral mass vaccination.	(Shastri *et al*., 2012)
Imiquimod	Dextran	Increased immune response and providing more protection from disease.	(Bachelder *et al*., 2010)
Vibrio cholera	Cellulose Acetate Phthalate	More significant immune response in the powder form of the oral vaccine.	(Pastor *et al*., 2014)
Protein B of Brachyspira Hyodysentriae	PLGA and Chitosan	Robust immune response and number of vaccine administration are decreased.	(Jiang *et al*., 2014)
DNA vaccine	Mannosylated Chitosan	An intranasal vaccine that targets the antigen into the lung enables robust IgA and cellular response induction and improves protection against pulmonary Mycobacterial.	(Wu *et al*., 2017)
Influenza vaccine	PLGA	A single dose and a long-lasting vaccine (30 days) against pandemic influenza.	(Watkins *et al*., 2017)
HBsAg	Didodecyldimethylammonium bromide/poly(lactic acid (DDAB/PLA) DDAB/PLA-alginate	A single-shot vaccine	(Yang *et al*., 2018)

eliciting an immune response with poor immunogenic antigens, and is fully degraded inside the cells after antigens delivery (Hamilton *et al*., 2012). So virosomal HA greatly enhances cytosolic delivery of vaccines.

Furthermore, the pharmaceutically active compounds are protected from proteolytic degradation and low pH of endosomes until they enter the cytoplasm, which can be considered an essential advantage of virosomes over liposomal delivery systems (Siraj *et al*., 2019). Virosome adjuvants may also enable APCs to acquire antigens, present them in significant histocompatibility complexes, and allow CD4+ T cells and CD8+ T cells to induce cellular and humoral immune responses (Blom *et al*., 2017).

On the other hand, the Sendai virus or hemagglutinating virus in Japan has been documented to be used as a virosome in treating tuberculosis (Kaneda *et al*., 2002). The DNA encoding of the Ag85A antigen is conjugated with the Plasmid Adeno-associated viruses-cytomegalovirus (PAAV-CMV) plasmid. It is then embedded with Sendai virosomes and injected IM into the mice. After the mice immunization, the results obtained showed an elevated Th1 mediated cytokine reaction and activated cytotoxic activity of cytotoxic T lymphocytes and natural killer cells.

Recent research in mice immunized with influenza A virus (H1N1) DNA adsorbed on the surface of cationic influenza virosomes showed complete protection against the homogeneous challenge strain and substantial protection against intrasubtype drift virus challenge (Raska and Turanek, 2015). In the virosomal hepatitis A vaccine, the antigen is bound to virosomes by reacting with phospholipids to be delivered to the normal hepatocyte receptor. The pharmacokinetics, safety profile, and immune responses caused by various hepatitis A virus vaccines, alum-adsorbed, fluid, and virosome vaccines, were recorded in initial experiments, all carrying the same quantity of inactivated hepatitis A virus. Local responses were produced in the virosome-HAV community at a slightly lower rate than alum-adsorbed vaccine recipients (Glück *et al*., 1992). A faster immune response was triggered with the virosome-HAV vaccine with a higher seroconversion rate on day 14, followed by a higher geometric mean titer. Comparable immune responses were observed within 4 weeks of immunization. Interestingly, the levels of antibodies in the groups immunized with alum-adsorbed or fluid vaccinations had declined dramatically. After 1 year of vaccination with virosomes-HAV, all subjects had anti-HAV levels >20 m IU/ml. Thus, compared to other traditional methods like the aluminum-adsorbed hepatitis A vaccine, the virosome-based vaccines can provide increased safety with lower local adverse events. Single-dose immunization successfully controlled outbreaks of hepatitis A and endemic diseases (Poovorawan *et al*., 1995).

Virosomes were also used as carriers and adjuvants for vaccine production, cancer prevention, and immunotherapy. For instance, cervical cancer was treated successfully with virosome-formulated cervical cancer vaccines (Liu *et al*., 2015). In addition, virosome-formulated antimalarial peptides exhibit a strong adaptive immune response and high permissibility in humans (Nair *et al*., 2020). The AMA-1 merozoite and NPNA region are two peptide regions that can act as antigens for a vaccine against malaria (Rodig, 2020).

CYCLODEXTRIN (CD)-BASED VACCINE DELIVERY SYSTEM

For over 100 years, CDs have been known as excipients of significant importance in the pharmaceutical industry (Agrawal and Gupta, 2012). CDs have received tremendous interest in the pharmaceutical field because of their potential to entrap a wide range of drug molecules totally or at least partially, including branched and unbranched toroidal structures (Abdul Rasool and Salmo, 2012). There are three types of CDs: α-, β-, and γ-CDs, also known as parent CDs or first-generation CDs. Chemically modified CDs are developed from the native ones by functionalizing their hydroxyl groups with various substituents and combinations (Braga *et al*., 2021).

Recent research reported that CDs could be altered and used as virucidal agents (Braga, 2019). For example, methylated β-CD had reduced influenza A virus and coronavirus infectivity by sequestering or depleting viral cholesterol from the host cell membranes (Pratelli and Colao, 2015). Meanwhile, Jones *et al*. (2020) developed the idea of CDs modified with mercaptoundecanoic

sulfonic acids to provide essential nontoxic virucidal activity. The results were promising since the modified sugar molecules attract viruses before they are irreversibly inactivated.

Moreover, Daiichi Sankyo, a Japanese pharmaceutical company, developed an advanced influenza vaccine that incorporates hydroxypropyl beta-CD (HPβCD) as an adjuvant agent. During vaccine development studies with various animal models, HPβCD was shown to induce a synergic immune response by interacting with immunoglobulins. It also enhanced antibody production by 30% and induced the production of Th2 cells responsible for long-term immune "memory" and the generation of follicular B helper T cells (Thf), which stimulate B cells (responsible for long-term immune "memory") (Kim *et al*., 2016; Onishi *et al*., 2015). Suvaxyn PCV, an inactivated recombinant porcine circovirus type 1 vaccine, one of the viral proteins containing the adjuvants squalene and sulfolipo-CD (SL-β-CD), was developed Suvaxyn PCV®, an inactivated recombinant porcine circovirus type 1 vaccine, one of the viral proteins containing the adjuvants squalene and sulfolipo-CD (SL-β-CD), was developed by Zoetis (European Medicines Agency Suvaxyn PCV Product Information). SL-β-CD was shown to cause a faster onset of immunity and a more incredible immune response than Carbopol® (polyacrylic acid), used in previous vaccine formulations (Braga *et al*., 2021).

Recently, β-CD and branched polyethyleneimine (CD-PEI) for mRNA vectorization was reported. CD-PEI and mRNA interacted to form nanocomplexes, which were shown to allow mRNA to enter the cell cytoplasm, transfect DCs efficiently, and induce high encoded protein levels expression. The administration of the nanocomplexes was tested in mice through various routes. With an optimized mRNA structure and a very suitable administered route, this CD-PEI-based mRNA vaccine model held promise for future application to specific antigens (Tan *et al*., 2020). Researchers also reported that, with a clinically isolated pandemic H1N1 influenza virus, the HPβCD-adjuvanted influenza HA split vaccine protected against a lethal challenge in cynomolgus macaques. The findings suggested that HPβCD can be used in various vaccines (Onishi *et al*., 2015).

VACCINE DELIVERY SYSTEMS IN COVID-19

COVID-19 is a virus found in China in 2019. In March 2020, the WHO described COVID-19 as a pandemic disease, which has created a dramatic loss of human life globally. It is spread by inhalation or ingestion of the droplets of an affected individual (WHO, 2020).

The spike protein present on the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus's surface binds with the ACE 2 receptor. This binding activity helps the virus to enter into the target cell and causes infection. Therefore, antibodies that can inhibit the binding of spike protein to the angiotensin-converting enzyme II (ACE2) are required (Lan *et al*., 2020 and Wrapp *et al*., 2020). However, to prevent from getting affected by this disease is only by developing an effective vaccine. Many vaccines have already been marketed (Table 4), but those vaccines were not 100% effective. Hence, researchers are still trying to develop an effective vaccine using different delivery systems.

Implementing VDSs in the medical sector would be very promising, especially in developing new therapeutic approaches to COVID-19 (Itani *et al*., 2020). Lipid carriers can be used to deliver the COVID vaccines via the IM route of administration (Fanciullino *et al*., 2021). Also, preclinical data reported systemic distribution of proteins when loaded to the liposomal carriers and administered via IM injection (Li *et al*., 2011a).

A novel mRNA vaccine candidate based on liposome for SARS-CoV-2, EG-COVID, encodes the complete spike protein (S) of the European variant (D614G), 2P-3Q substitution. By using liposome-based technology, lyophilization of the mRNA vaccine platform can be carried out. Strong humoral and cellular immune responses to SARS-CoV-2 were produced with this vaccine, and the infection of SARS-CoV-2 into Vero cells was also inhibited successfully. A clinical trial is supposed to begin soon (Hong *et al*., 2021). Thus, liposomes offer excellent potential in healthcare firms' current clinical trials to treat COVID-19.

On the other hand, the Mymetics corporation, an international biotechnology company, continues to progress its COVID-19 vaccine production plan based on its virosome-vaccine platform. The corporation has received a grant from the Swiss Innovation Agency to begin preclinical studies on Mymetics intranasal virosome-based COVID-19 vaccine candidate for its ability to induce protective respiratory immunity to prevent nasal infection and stop the spread of the virus into the lungs and brain. Since April 2020, the corporation has been striving to design a safe, tolerant, and highly potent virosome-based COVID-19 vaccine administered to individuals of all age groups, especially the immunocompromised and older adults (Bloomberg, 2020). Therefore, virosomes are essential in producing vaccines for intracellular infectious diseases like SARS-CoV-2.

ISCOM is another safe and potent adjuvant called cage-like nanospheres formed when a saponin is mixed with fat. Matrix M is an example of the COVID-19 vaccine designed by the US Biotech company Novavax (Borrell, 2020). The matrix M comprises 40 nm NPs based on saponin extracted from *Quillaja saponaria* Molina's bark with phospholipid and cholesterol. It significantly increased the biological functions and elicited potent, robust, and long-lasting immune responses with vaccine dose sparing properties (Novavax addresses urgent global public health needs with innovative technology, 2021). Preclinical studies conducted in baboons and mice and phase I/II human clinical trials with NVX-CoV2373 revealed high anti-S protein IgG titers with a potent neutralizing effect (Tian *et al*., 2020). The vaccine was also well tolerated among healthy individuals (between 18 and 59 years) (Park *et al*., 2021).

However, recent studies were carried out to assess the *in vitro* SARS-CoV-2 antiviral activity of astodrimer sodium, a dendrimer with a broad spectrum of antimicrobial activity. The astodrimer inhibits the replication of SARS-CoV-2 in Vero E6 cells with a 50% reduction in virus-induced cytopathic effect. Thus researchers suggested the potential use of astodrimer sodium as an antiviral agent, either inhaled or nasally administrated for treatment and prevention of SARS-CoV-2 (Paull *et al*., 2021). When developing a vaccine, the antigen, adjuvant, production method, and distribution strategy are essential considerations.

The genome and structural details of SARS-COV-2 were allocated in record time, allowing for rapid vaccine development (Andersen *et al*., 2020; Benvenuto *et al*., 2020; Hwang *et al*., 2020; Yan *et al*., 2020; Yuan *et al*., 2020). Nanotechnology platforms are beneficial in current vaccine design and have accelerated new candidate vaccines from clinical trials. Evolving nanotechnology

Table 4. COVID-19 vaccines are currently available in the market.

Company/country	Vaccine name	Doses	Vaccine	Efficacy	Adjuvant	References
Bharat Biotech, India	COVAXIN	2	Inactivated SARS-CV 2 virus	81%	Aluminum-based compound	(Ella *et al*., 2021)
Sinovac Biotech, China	CORONAVAC	2	Inactivated SARS-CoV 2 virus	78%	Alum adjuvant	(Xia *et al*., 2021; Zhang *et al*., 2021;)
Novavax, USA	NVX-CoV2373	2	Spike protein	89.3%	Spike NP and matrix adjuvant	(Novavax COVID-19 Vaccine Demonstrates 89.3% Efficacy in UK Phase 3 Trial, 2021)
Pfizer, USA and BioNTech, Germany	BNT162b2	2	mRNA	95.3%	mRNA coding, the spike protein is incorporated in micro liposomes	(Pfizer and BioNTech Achieve First Authorization in the World for a Vaccine to Combat COVID-19, 2020)
Johnson & Johnson, USA	Ad26COVs1	1	Spike protein	74%	Adenovirus carrying a DNA sequence coding the full-length spike protein	(Sadoff *et al*., 2020)
Moderna, USA and US Government	mRNA-1273	2	mRNA	94%	mRNA coding the. Spike protein is incorporate in micro liposomes	(Moderna's Work on our COVID-19 Vaccine, 2021)
AstraZeneca, Sweden-UK UNIV. Oxford, UK	ChAdOx1	2	Spike protein	76%	Adenovirus carrying a DNA sequence coding the full-length spike protein	(Voysey *et al*., 2021)
Medicago, Canada and GSK, Italy	Co- VLP	2	Spike protein	----	Spike proteins aggregated as VLP and GSK adjuvant	(GSK, 2021)

such as mRNA vaccines provided by the lipid NPs and viral vector vaccines have already passed phase II and phase III trials, in addition to inactivated vaccines (Shin *et al*., 2020). To produce an effective COVID-19 therapy, one must first consider the virus's mechanism of action.

The novel SARS-CoV-2 utilizes a "lock and key" system, similar to SARS and Middle East respiratory syndrome coronavirus (MERS-CoV) coronaviruses, where ACE2 acts as a "key" to target specialized cells that retain its "lock" (Yu *et al*., 2020). The lung, heart, arteries, kidneys, and intestine cells all contain these target areas. Once released, the virus can multiply and kill other cells using the organelles of the host cell.

Consequently, a therapy that's top the virus from entering the cell may be helpful (Itani *et al*., 2020). The shape, size, and surface charge of the engineered nanocarriers are essential factors that should be considered when optimizing for intranasal delivery and thus play a crucial role in the procedure's effectiveness. Several experiments were recently analyzed to determine the optimum characteristics of theranostic NPs for pulmonary and intranasal administration (Marasini and Kaminskas, 2019). According to the results, an optimal lung delivery mechanism is smaller than 100–200 nm for better immune responses. The slight positive charges are to increase cell association. It is synthesized with a mixture of NP-loaded and surface-conjugates therapeutic moieties and is hydrophobic (Itani *et al*., 2020).

The inorganic NPs prominent role is targeted toward their potential in diagnostics, therapeutics, and vaccination. The most commonly employed inorganic NPs used are AuNPs. AuNPs are used due to their exceptional characteristic that might be useful in COVID-19 since AuNPs can act as carriers of antigen for SARS-CoV spike S protein and adjuvant. It was found that AuNPs when binding with the S protein through a powerful electrostatic force and high surface energy formed spontaneous protein corona around these NPs. Protein corona loading around the AuNPs increased the size, which remained stable for 7 days, where each particle retained 200–250 spike proteins on the surface. The giant virus-like AuNPs exhibited a more robust antibody response, better stability, and lowered cytotoxicity (Medhi *et al*., 2020). AuNPs are currently used as a viral diagnostic and for inhibition of the viral activity. AuNPs detected the presence of COVID-19 viral antigen through a simple colorimetric shift in a short time using a naked eye assay (approximately within 5 minutes). The anti-spike antibody is attached to the surface of AuNPs to control the viral infection. The AuNPs-antibodies complex were then supposed to bind to pseudo-SARS-CoV-2 and inhibit the virus from binding to the cells' receptors which inhibits viral replication and leads to the destruction of the virus's lipid membrane (Pramanik *et al*., 2021).

At present, researchers have proposed quantum dots NPs to deliver vaccines and fight viral infections. Quantum dots can bind to ligands and consequently prevent the virus from binding to the surface of host cells. The underlying mechanism of action of COVID-19-loaded quantum dots is mainly explained by the ionic interaction between the quantum dots' positive surface charge and the negative charge present of the viral RNA, which leads to the production of oxygen species within SARS-CoV-2, and ultimately

the virus inactivation, as shown in Figure 3 (Manivannan and Ponnuchamy, 2020).

Recent studies on the antiviral activity of astodrimer sodium, a dendrimer with a broad spectrum of antimicrobial activity, *in vitro* against SARS-CoV-2 have been performed. The astodrimer inhibits the replication of SARS-CoV-2 in Vero E6 cells with a 50% reduction in virus-induced cytopathic effect. Thus, according to the researchers, astodrimer sodium is potentially used in inhaled or nasal routes to treat and prevent SARS-CoV-2 (Paull *et al*., 2021).

Microparticles are another carrier that is extensively tried for COVID-19 vaccines. Martin D'Souza is still developing a vaccine against COVID-19 by incorporating the antigen into a microparticulate carrier and administering it via transdermal route microneedle (Beimfohr, 2020). Similarly, antigen-adjuvant-loaded microparticles combination was found to be successful in manufacturing vaccines for the influenza virus. The influenza vaccine showed many benefits. Most notably, long-lasting immunity decreased viral load, and less time was taken for clearing the virus (Ting, 2021). As a result, researchers are trying to manufacture the COVID-19 vaccine using antigen-adjuvant-loaded microparticles to achieve the similar benefits shown in the influenza vaccine.

Emulsions/microemulsions had also been tested preclinically in the coronavirus vaccine, and researches were conducted. Some examples are described in this section. The MERS-CoV receptor-binding domain subunit vaccine (MERS-CoV RBD) induced a detectable level of neutralizing antibodies and T cell responses in mice (Zhang *et al*., 2016). In addition, mice developed a high neutralizing antibody titer upon administering two doses of inactivated SARS-CoV whole virus vaccine. However, the adjuvanted vaccine demonstrated early and robust antiviral responses. In another study, the adjuvanted vaccine provided complete protection against wild-type SARS-CoV infection in hamsters (Roberts *et al*., 2010). In mice, when paired with montanide ISA51, the MERS-CoV RBD domain induced a high neutralizing antibody titer and protected against viral challenge (Du *et al*., 2013).

Lately, a saponin-based microemulsion adjuvant has been investigated for use in the COVID-19 vaccine. A SARS-CoV-2 S1 protein with Fc region of human IgG1 vaccine candidate with saponin-based microemulsion adjuvant had led to the development of high titers of S1- (recombinant protein-) specific neutralizing antibodies. The study was performed using cynomolgus monkeys (Ren *et al*., 2020). Indeed, Roquette has a long history of supplying KLEPTOSE® HPβCD as an excipient. The EU, the US, and Chinese regulatory authorities have all licensed it for oral and parenteral administration. However, subunit vaccines are poorly immunogenic and require an additional adjuvant to stimulate immunity as they are nonliving vaccine antigens. HPβCD, as an adjuvant, induces Th2 cell response, enhances antigen- (vaccine-) specific antibody titers, and provides a more prolonged immune response. Also, Roquette suggested that HPβCD can be a safe and effective adjuvant in producing COVID-19 vaccines (Combating Coronavirus: Cyclodextrins in Treatment & Prevention, 2020).

Johnson & Johnson is developing virus subunit vaccines and has HPβCD as an adjuvant (Administration, no date). The vaccine engineered for the new SARS-CoV-2 pandemic was based on viral DNA. The vaccine was carried by a synthetic adenovirus vector and incorporated HPβCD as a cryopreservative. The new formulation stabilized proteins and prevented them from aggregating and adhering to the container wall (Daily Med, 2021).

Besides, the CDs application's core objective is to enhance drugs dissolution and develop controlled drug release systems (Tan *et al*., 2014, 2020). The formation of β-CD and soluble (sACE2) complex effectively enhances the solubility in water of sACE2 to satisfy inhalation drug atomization criteria. After entering the body by atomization or other drug delivery, the inclusion conjugates release sACE2, which interacts with the SARS-CoV-2 S protein and inhibits the virus's entry into the human cells (Sun *et al*., 2020).

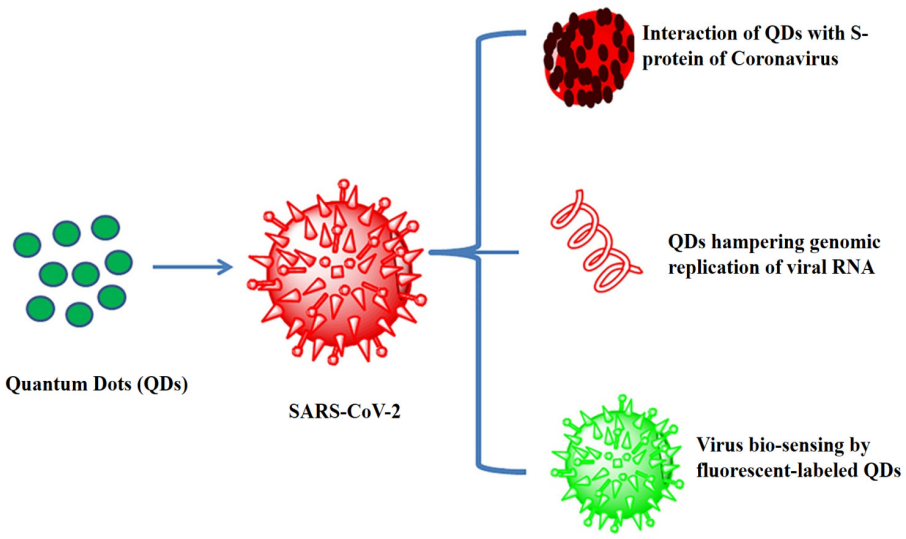

Figure 3. Characteristics and applications of quantum dots loaded with COVID-19 vaccination (Manivannan and Ponnuchamy, 2020).

CURRENT STATUS AND FUTURE PROSPECTS

Currently, two licensed ISCOMs-based vaccines are available for veterinary purposes, and both are used in horses. The first is an influenza vaccine. More than 1 million horses were immunized with this vaccine, and no adverse effects were recorded. The second is a novel peptide vaccine, known as Equity® (Pfizer Australia, Melbourne), which is newly registered to control the estrous activity in mares and fillies (Sanders *et al*., 2005). Some examples of ISCOMs delivery systems in the clinical trial phase for vaccines and veterinary applications are demonstrated in Table 5.

Regarding liposomes drug carriers, they successfully entered the market in 1995 by producing PEGylated liposomal formulation Doxia® (Bulbake *et al*., 2017). Another product for the vaccine adjuvant delivery system is Shingrix®, manufactured by the pharmaceutical company GSK and approved in October 2017 by the FDA to be used in elderly patients aged 50 and above, a prophylactic for shingles (herpes zoster). It has a strong safety profile to induce strong antipathogen immunity compared to conventional microbial vaccines (Wang *et al*., 2017). Further examples of the novel promising, vaccine-loaded liposomal formulations are displayed in Table 6.

Furthermore, hepatitis A (Epaxal®) and influenza (Inflexal® V) vaccines have been used successfully as a carrier device and also as an adjuvant, and it validates the unique features of virosomes (Herzog *et al*., 2009). More than 45 countries have gained approval for the vaccine and have used this to immunize more than 10 million patients worldwide to date (Saroja *et al*., 2011).

Several emulsion formulations had been identified, the prominent of which is MONTANIDE® developed by SEPPIC. Montanides are W/O emulsion adjuvants similar to FIA evaluated in AIDS, cancer, malaria, and other autoimmune diseases for therapeutic application. Another example is MF59; an emulsion adjuvant was initially produced by Ciba Geigy and Chiron Corporation in the 1990s and then developed by Novartis Vaccines and Diagnostics. MF59 has been the most popular emulsion adjuvant in more than 35 countries, with more than 150 million administered doses (O'Hagan *et al*., 2013). Clinical trials confirmed the efficiency of MF59 in the immunogenicity improvement in vaccines for influenza, HIV, hepatitis B/hepatitis C, para-virus, HSV, human papillomavirus, and cytomegalovirus. In addition, MF59 offered enhanced protection and tolerated and lasting immunity to various populations from children to older age groups (O'Hagan *et al*., 2012). Table 7 summarizes emulsion adjuvants in approved vaccines in the preclinical and clinical settings.

However, consistent high-throughput technologies integrated with well-designed analytical tools at all stages of vaccine delivery systems could be a possible approach to obtaining approval for novel vaccines by reducing costs and confirming the highest level of patient safety. The field of vaccine development suffers from a shortage of potent adjuvants and advanced formulations-based delivery systems and carriers (Zielińska *et al*., 2020). Such novel vaccine formulations should be discovered and developed more rationally than in the past and licensed for human use. For example, suppose more challenges and concerns related to polymeric NPs, such as synthetic material protection, expansion manufacturing, and product quality, are resolved. In that case, more polymeric NP-based vaccines will be produced and approved to enter the market (Liu *et al*., 2018 and Nanomedicine and the COVID-19 vaccines, 2020). Also, new techniques such as the reverse microemulsion underwent rapid growth over the past few decades and were applied for NPs manipulating and designing. For example, a bicontinuous reverse microemulsion technique was used to synthesize Al-nano sticks, a new aluminum salt-based material consisting mainly of monodisperse amorphous aluminum (oxy)hydroxide (ALOOH) particles with a nanometer-scale size (80 nm) and sticklike shape. These nanosticks showed favorable physicochemical properties and potent vaccine adjuvant actions (Li *et al*., 2017).

Table 5. ISCOMs delivery system in the clinical trial phase for vaccines and veterinary applications.

Antigen/adjuvant	Disease	Veterinary application or clinical phase	References
HPV16/ISCOM matrix, E6E7 Fusion protein	Human papilloma virus	I (Completed)	(Kaurav *et al*., 2018)
A fusion protein of measles virus	Encephalitis	Mouse	(Varsanyi *et al*., 1987)
HA, fusion protein of canine distemper virus	Viremia	Dog	(De Vries *et al*., 1988)
NY-ESO-1 protein (ISCOM matrix)	Melanoma	II (Completed)	(Smith and Iwenofu, 2018)

Table 6. Novel promising vaccine-loaded liposomal formulations.

Liposome formulation	Lipid(s) and sterol used	Cell line/animal model used	Disease	Route of administration	References
DDA	DDA	BALB/c mice	Respiratory syncytial virus	Intranasal	(Klinguer *et al*., 2001)
DOTMA	DOTMA	HepG2 cells and BALB/c mice	Hepatitis C	Transcutaneous	(Mahor *et al*., 2007)
Adipocyte® (1999)	DOPC, DPPG, Cholesterol and Triolein	----	Neoplastic meningitis	Spinal	(Puangpetch *et al*., 2012)
Maribor® (2012)	DSPC:MPEG-2000:DSPE (3:2:0.015 molar ratio)	----	Acute lymphoblastic leukaemia	Intravenous	(Bulbake *et al*., 2017)

PC: Phosphatidylcholine, SA: Stearylamine; DDA: Dimethyl dioctadecylammonium; DOTMA: 1,2-di-O-octadecenyl-3-trimethylammonium propane; DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane; DC-Chol: Dimethylaminoethane-carbamoyl-cholesterol; DOPC: 1,2-dioleoyl-sn-glycero-3-phosphocholine; PA: Phosphatidic acid; DPPG: Dipalmitoylphosphatidylserine.

Table 7. Emulsion adjuvants in licensed vaccines approved in clinical and preclinical stages.

Formulation	Type of emulsion	Proposed mode of action	Significance	References
MF59	O/W emulsion	Creates an immunocompetent environment and enhances the production of cytokines and chemokines.	First approved squalene oil-based emulsion for flu vaccines Fluad®, Aflunov®, Focetria®, and Celtura®	(O'Hagan *et al*., 2012)
AS03	O/W emulsion	Create an immunocompetent environment by spatial and colocalization of antigen at the site of injection.	Influenza vaccine Prepandrix® (pre-pandemic H5N1) and Pandemrix® (pandemic H1N1).	(Garçon, *et al*., 2012)
AF03	W/O emulsion	No data are available.	Influenza vaccine Humenza® approved in Europe.	(Klucker *et al*., 2012)
Glucopyranosyl Lipid Adjuvant-Stable Emulsion (GLA-SE)	O/W	Activation of DCs and generation of T helper-1 responses.	Combined adjuvanticity by squalene oil and TLR4 agonist GLA.	(Behzad *et al*., 2012)
AS02	O/W	Antibody production and cell-mediated immunity.	In phase 2 Hepatitis B vaccine.	(Surquin *et al*., 2011)
W805EC	O/W	Systemic and mucosal immunity with a T helper-1 partial response.	Hepatitis B, Influenza and Anthrax, H1N1.	(Hamouda *et al*., 2010)

Table 8. Patents published on vaccines loaded to various delivery platforms (cited from patents.google.com).

Patent application	Delivery system	Title	Outcome	References
US20140348906A1	Liposomes	An immunostimulatory composition is comprising liposome-encapsulated oligonucleotides and epitopes.	Strong immune response and increase survival after challenge.	(Kwon, 2014)
US20110165223A1	Liposomes	Antitumor immunization by liposomal delivery of the vaccine to the spleen.	Significant reduction in the tumour size, antibody response triggered by B-cells.	(Sgouros *et al*., 2011)
US20100226932A1	Niosomes	Adjuvant and vaccine compositions	Antigen stability in the vaccine is enhanced by incorporating aluminum salts and entrapping them in niosomes for a particular immunological response.	(Smith *et al*., 2010)
WO2016191553A1	Polymeric NPs	NPs-based vaccine strategy against swine influenza virus.	Inactivates swine influenza A virus, associated with enhanced respiratory disease.	(Renukaradhya *et al*., 2016)
US20120189700A1	Quantum Dots	NPs based immunological stimulation.	The NPs delivery systems elicited an immune response without the use of an adjuvant.	(Aguilar *et al*., 2012)
EP2689785A1	Polymeric microparticles	Method for generating dry vaccine powder formulation for intranasal delivery.	Higher mucosal and systemic IgA and IgG titers than with the common vaccines (for all antigens).	(Nagata and Haruta, 2014)
US20120014991A1	Polymeric microparticles	The novel, non-antigenic mucosal adjuvant formulation modulates the effects of substances, including vaccine antigens, in contact with mucosal body surfaces.	Antibody and cellular responses are increased without the development of tolerance.	(Raa *et al*., 2012)
WO2011020604A1	Virosomes	Multiepitope vaccine for her2/neu-associated cancers.	More efficient in inducing a potent antibody response	(Kammer *et al*., 2011)
WO2016012385A1	CDs	The vaccine composition comprising inactivated poliovirus (IPV) and CDs.	In the presence of thiomersal, CDs or derivatives protects and retains the antigenicity and/or immunogenicity of IPV.	(Chacornac *et al*., 2016)

Several patenting activities and innovations in vaccine research and manufacturing to facilitate access to vaccine technologies that can be used in developing countries are displayed in Table 8.

CONCLUSION

The application of vaccine delivery platforms is gaining importance these days. Owing to the many potential features of these delivery systems, such as reduced dose frequency, improved biocompatibility, compliance, and increased stability, they can be employed to produce vaccines as a carrier or an adjuvant. Furthermore, most of the delivery systems discussed in this review have proven to elicit robust and successful immune responses. Hence these are used to develop more efficient vaccines to fight against various infectious diseases.

Recently many vaccines have been marketed as a preventive measure to curb the infection caused by COVID-19. Although the world has been grappling with the pandemic, researchers and pharmaceutical firms are working around the clock to develop a vaccine that could restore normalcy to everyday life. As a matter of fact, based on a large number of recent publications in this area and the promising clinical results published so far, it is fair to expect that innovative vaccine delivery technologies will become more widely used in the near future, resulting in significant increases in global immunization coverage.

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This study does not involve experiments on animals or human subjects.

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AUTHORS' CONTRIBUTIONS

All authors have made significant contributions in analyzing and interpreting data, collecting references, and writing the article. They agreed to submit to the current journal, approved the final version for publication, and agreed to be responsible for all aspects of the work. The corresponding author was also responsible for designing and reviewing the article for intellectual and scholarly content.

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