



A REVIEW ON NANO/MICROPARTICLES FORMULATION OF VACCINE DELIVERY FOR UNIVERSAL INFLUENZA

**Vivek Patel, Dr. Kantilal Narkhede, Dr. Anuradha Prajapati,
Dr. Sachin Narkhede**

Article DOI: <https://doi.org/10.36713/epra11997>

DOI No: 10.36713/epra11997

ABSTRACT

Worldwide, influenza affects millions of individuals and has the potential to cause serious illness or even death. The most effective approach of prophylaxis is vaccination, but due to strain and viral mutation changes, the seasonal influenza vaccine frequently has limited efficiency and needs to be administered annually. Although they have been employed clinically, more conserved universal influenza antigens like the M2 ectodomain (M2e) and the hemagglutinin stalk region (HA stalk) frequently have poor antigenicity. Universal antigens have been created employing nano/microparticles as influenza vaccine carriers to boost their antigenicity. Indicators of immunity and protection against influenza have been demonstrated in mouse, pig, ferret, and chicken models using polymers, liposomes, metal, and protein-based particles. This review focuses on the physiochemical characteristics, production, characterization, and biologic responses in vivo of the formulations of the seasonal and universal influenza vaccines made from these materials. The assessment concludes with future perspectives for nano/microparticles as carrier systems and other factors to take into account within the perspective of the landscape for the administration of the universal influenza vaccine.

KEYWORDS: *Influenza; Polymeric Nanoparticles; Liposomes; Gold Nanoparticles; Protein Nanoparticles*

INTRODUCTION

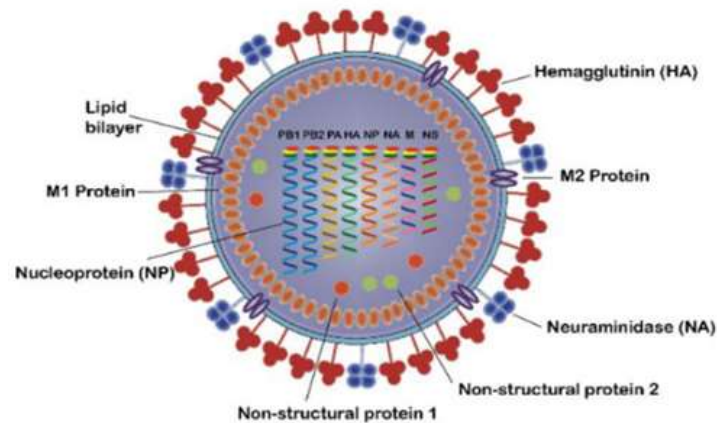
The World Health Organization (WHO) estimates that influenza causes up to 5 million serious cases and 500,000 fatalities annually. The entire annual economic cost of influenza in the USA alone is \$87.1 billion, which includes hospital admissions, outpatient care, lost wages, and fatalities as a result of this pandemic (1). Elderly people, children, pregnant women, and those living in low-income nations are some of those who are most susceptible to contracting severe and fatal influenza infections (2). The use of NPs as a vaccine carrier system can shield vaccine components from early protease degradation, increase stability, induce sustained release, and help with targeted delivery of an immunogen to antigen-presenting cells (APCs). Due to these characteristics, nanoparticles can frequently serve as a vaccination adjuvant (3,4)

INFLUENZA

Flu (influenza) is a highly contagious illness that enters through the respiratory system and causes illness. The influenza viruses can infect both people and animals and have a negative-sense RNA (ssRNA) genome. Influenza complications can result in severe morbidity and mortality. According to recent data from the Center for Disease Control and Prevention (CDC), between 2010 and 2020, there are expected to be 41 million illnesses, 140,000 to 710,000 hospitalizations, and 12,000 to 52,000 fatalities (5,6).



STRUCTURE OF INFLUENZA



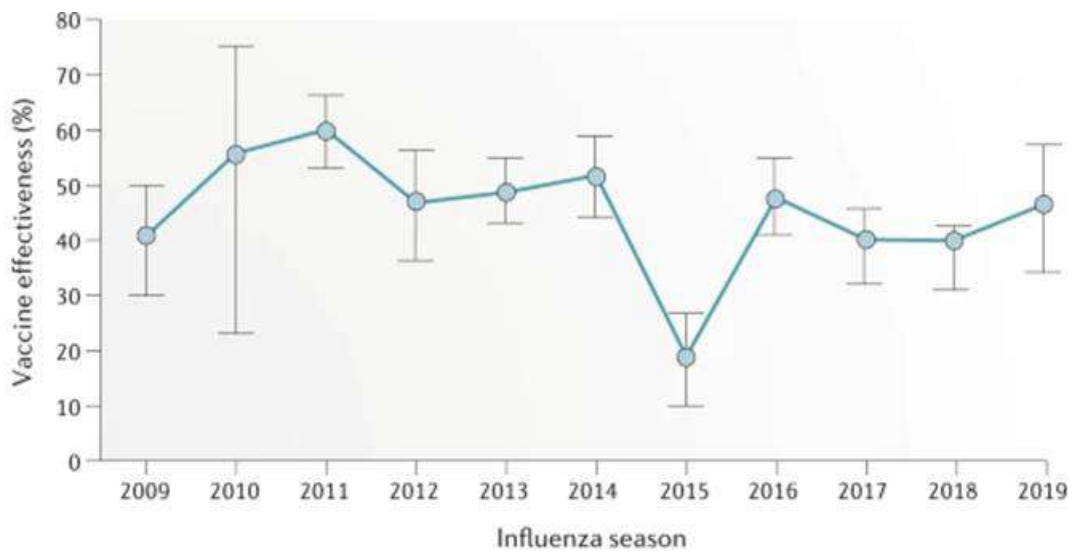
The shape of influenza viruses can be filamentous or spherical. Hemagglutinin (HA) and neuraminidase (NA), two surface lipid membrane glycosylated proteins, are present in the influenza virus. Depending on the antigenicity, various influenza varieties may have a variable number of protein units (7, 8). The nonstructural protein 2 (NS 2), RNA segments coated with nucleoprotein (NP), and lipid envelope membrane proteins are all present in the influenza virus. Based on the surface HA and NA glycoproteins, influenza viruses are classified; there have been reported to be 18 HA subtypes and 11 NA subtypes (9).

TYPES OF INFLUENZA

They have so far identified 4 different influenza virus subtypes (A, B, C, and D) based on changes in the antigens that are present on nucleoprotein and matrix proteins. There are various subtypes of influenza based on the influenza genome. influenza strains of 8 types (A, B) or 7 types (C, D). Out of all of these strains, Type A strain is the one that consistently causes serious respiratory infections that can be fatal. A new influenza pandemic or outbreak is a potential. According to reports, humans can become infected with the influenza B strain. Because B/Victoria and B/Yamagata are influenza B lineages that circulate each year and cause seasonal flu infections, they are employed in the creation of vaccines. Typically, mild symptoms are brought on by influenza C viruses. Small farm animals such as sheep, pigs, and cattle can contract influenza D. On how it infects humans, there is limited data (10,11).

INFLUENZA VACCINE

The use of vaccinations to prevent infections like the flu and other viruses is an effective and cost-effective method of limiting epidemics. Antigenic drift or shift causes annual influenza vaccine efficacy to vary. Vaccine strains need to be updated annually because influenza viruses go through genetic alterations and evade the immune system. The antigenic similarity between the vaccine strains and viruses affects how protective the currently approved vaccines are each year. The host immune system can alter the vaccine's efficacy as well. Young people and the elderly, for instance, are more susceptible to influenza illness (12-14). The antigen affinity between the vaccine strain and the strains that are currently spreading determines how well the annual flu vaccines work. The virus poses a number of challenges, including the potential for new strains of old endemic viruses and the need for potent, cross-protective influenza vaccines. Targeting the conserved sections of the virus could help with the goal in developing a new formulation that successfully produces the neutralising antibodies and offers cross protection (15,16).



Seasonal influenza vaccine effectiveness from 2009 to 2019

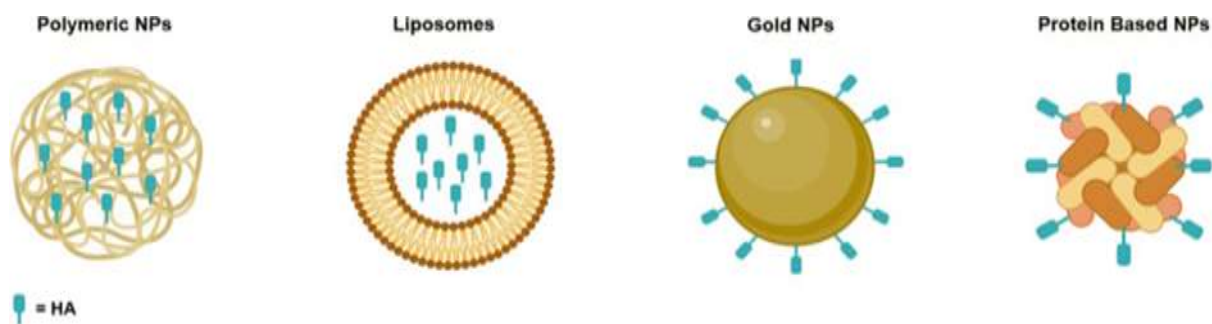
LICENSED VACCINES

Vaccine technology/platform	Vaccine type	Vaccine name (manufacturer)	Target/MOA	Adjuvant used
Inactivated virus	Split virus	Afluria (Seqirus)	HAI	None
		Fluarix (GSK)	HAI	None
		FluLavel (GSK)	HAI	None
		Fluzone, Fluzone HD (Sanofi Pasteur)	HAI	None
		Fluvirin (CLS Limited)	HAI	None
	Subunit	Flucelvax (Novartis)	HAI	None
Live-attenuated	Live, cold-adapted	FluMist (AstraZeneca)	HAI	None
Recombinant protein	Non-purified HA	FluBlok (Sanofi Pasteur)	HAI	None
Inactivated virus	Split virus	Influvac, Imuvac (Abbot)	HAI	None
		Fluarix, Alpharix, Influsplit (GSK)	HAI	None
		3Fluart (Omninvest)	HAI	None
		Afluria, Enzira (Pfizer/CSL)	HAI	None
		Vaxigrip, Vaxigrip Tetra (Sanofi Pasteur)	HAI	None
	Subunit	Agrippal (Seqirus)	HAI	None



NANOPARTICLES AS A VACCINE CARRIER SYSTEM

An effective immunological reaction against the encapsulated antigen has been induced by the nanoparticle (NPs) based vaccine delivery systems. The antigen must be internalised and digested by APCs as the initial step in any successful vaccination. The APCs effectively phagocytose the particulate NPs carrying the antigen because they are particle. NPs can be used as a vaccine carrier system to help transport an immunogen to antigen-presenting cells with precision, prevent vaccine components from being prematurely degraded by proteases, increase stability, and elicit sustainable release (APCs). Nanoparticles can frequently function as a vaccine adjuvant because of these qualities. Protein-, metal-, polymer-, and liposome-containing NPs will be separated out (17).



For influenza vaccinations, various nanoparticle formulations using HA as the model antigen are used. Antigen can also be conjugated to the outside of polymeric nanoparticles, as is the case with the antigen-encapsulated polymer nanoparticles that are shown. Antigens can also be coated on the outside of liposomes, as is the case in the aqueous phase, where liposomes are also seen with encapsulated antigen. Surface conjugation is used to show protein-based nanoparticles (ferritin) and gold nanoparticles (18).

CHARACTERIZATION OF NANOPARTICLES

When comparing NPs, there are a number of physiochemical properties that can vary different platform but also affect how the NPs interact with APCs and other immune cells (19).

- surface charge
- particle size
- loading capacity of the particles
- drug release

VACCINE EFFICACY IN VIVO

In vivo testing is necessary to determine the vaccine's efficacy after the particles have been created and optimised with the antigen and/or adjuvant. Pigs, ferrets, and chickens have also been used to test NP universal influenza vaccines, with ferrets being the most effective larger animal model for influenza. Mouse models are the most frequently used in influenza vaccine testing. Because they are considered to be the most effective for that peptide, BALB/c mice models are the most commonly used for testing NPs with M2e (20).

POLYMERS

In NPs, vaccine components have been encapsulated in a variety of polymers, and the formulation's primary benefit is the controlled release of the antigen and/or adjuvant. The encapsulates are distributed throughout the polymer, including at the polymer surface, in the bulk of matrix devices made of polymeric formulations. Single or double emulsions with solvent evaporation are the most common method for fabricating polymeric nanoparticles. Vaccine NPs are made of the different polymers(21):

- chitosan
- PLGA
- acetalated dextran (Ace-DEX)

CHITOSAN NANOPARTICLES

Chitosan is a cationic, naturally occurring mucoadhesive biopolymer made of glucosamine residues. Chitin, a material found in shells of crustaceans, is partially deacetylated to create this biopolymer. Chitosan has been used as a needle-free vaccine



to mucosal sites including IN, oral, and ocular because of its special mucoadhesive characteristics. Ionic gelation is a common technique for creating chitosan nanoparticles. Chitosan that is positively charged is ionically crosslinked with a salt that is negatively charged (e.g., sodium tripolyphosphate, sodium citrate, sodium sulfate). This is commonly achieved by dripping the chitosan mixture into the salt solution, however microfluidics and simple mixing are other viable options. This method is similar for making NPs from charged polymers like alginate (22).

PLGA PARTICLES

PLGA is one of the most commonly used and explored polymers in vaccine and medicines release due to its biocompatibility and biodegradability. The FDA and European Medicines Agency have currently approved PLGA for controlled drug release. Although PLGA is frequently used as a vaccine carrier in pre-clinical settings, limited research has been done on it in relation to influenza vaccines. Solvent evaporation methods using single and double emulsions are commonly used to make PLGA particles. The antigen or water soluble adjuvant is dissolved in an aqueous phase while the polymer is dissolved in an organic phase in a double emulsion solvent evaporation technique. The polymer and an organic soluble adjuvant may dissolve together in the solvent phase. Typically, an emulsion requires an emulsifying agent or stabilizer, most commonly polyvinyl alcohol (PVA). In the presence of the stabilizer, one phase is suspended in the other and mixed rapidly through homogenization or sonication. A double emulsion is made by mixing the phases twice, each time with the help of a stabilizer. After the second mixing, the emulsion is stirred on a stir plate for a duration of time until the solvent has completely evaporated, at which point the particles are washed and collected via centrifugation in preparation for lyophilization and storage. The methods are the same for a single emulsion, with the exception that the encapsulates would need to be solubilized in the organic phase with the polymer and would only need to be mixed once, in the presence of the stabilizer. This method can be used to a wide range of hydrophobic polymers, and solvent exposure as well as fast mixing can denature protein antigens (24,25).

ACE-DEX MICROPARTICLES

Ace-DEX is a biopolymer synthesized from dextran where the pendant hydroxyl groups are converted into acetal groups. It is acid-sensitive. Once phagocytosed, the polymer's acid sensitivity causes fast intracellular release of cargo in the acidic phagolysosome environment (27). Additionally, Ace-DEX particles have shown to be stable at extremely high temperatures. Ace-DEX has been used in various preclinical applications, such as the delivery of adjuvants to enhance vaccine efficacy, therapies for *Salmonella enterica* infection, and scaffold-based interstitial delivery of chemotherapeutics for glioblastoma. Similar to other biomaterials like PLGA and poly-lactic acid, particulate Ace-DEX has similar cell toxicity and viability (PLA). Ace-DEX can be processed using a variety of techniques, including sonication, homogenization, and electrospray, to make polymeric particles for vaccines. A continuous method that provides simple scalability and better encapsulation efficiencies is electrospray (28).

LIPOSOMES

One of the most common NP formulations for therapeutic delivery is the liposome; however, their use in vaccines was not very common. Inflexal V, a liposomal-based influenza vaccine recognised, comprises an inactivated influenza virus encapsulated in a liposome. This mixture is commonly referred to as a virosome. The virosome formulation of Inflexal V can be delivered to a wider range of ages than some of the influenza vaccines that are currently clinically approved when compared to other influenza vaccines in terms of efficacy. At the laboratory scale, thin-film hydration is commonly used for the production of liposomes. With the use of water-soluble agents present in the solution, a lipid cake is formed for this method and then rehydrated. Then, this solution is mixed through stirring or even more effective methods like sonication. After that, extrusion is used to size and form multilaminar liposomes into unilamellar liposomes. Microfluidics and electrospray can also help this process. At the industrial level, ethanol injection usually involves the introduction of ethanol with suspended lipids into a stirred tank with the encapsulate in an aqueous phase (30-31).

METALS

Chemistry that covalently attaches the antigen or adjuvant to the surface can be used to add antigen and/or adjuvant to metal-based nanoparticle carriers. The use of simple adsorption is also another approach, however it is a dynamic process in which proteins in particular can adsorb to and diffuse off the surface over time. Metal-based particle systems have the advantage of presenting antigen on their surfaces, and gold nanoparticles have been used to study how surface-bound antigen contributes to the development of universal influenza vaccines (32).

GOLD NANOPARTICLES

Gold NPs have been used in a variety of applications due to their small dimensional size, biocompatibility, and good stability. Furthermore, the material offers simple synthesis routes for attaching a wide variety of compounds to the surface. Although they can be synthesized through the reduction of chloroauric acid, gold NPs are most commonly bought commercially.



Whereas polymers and liposomes, which can encapsulate antigen and adjuvant inside their matrix, gold nanoparticles require these components to be functionalized, usually through thiol chemistry (33).

One utilization of gold nanoparticles was for a universal influenza vaccine. An IN M2e vaccine was developed by Tao et al. using CpG as a soluble adjuvant attached to gold nanoparticles. The M2e was covalently connected to the 12 nm gold NPs, however for the vaccination, M2e that was both soluble and covalently attached was used. A dose-response in serum IgG, IgG1, and IgG2a was observed in mice who were vaccinated IN on days 0 and 21 with M2e conjugated NPs and CpG. When mice were challenged with PR8 at day 42, a dose-response was also seen, and 100% of the mice survived the challenge. The authors report titers and survival after the challenge at least 8 months after the initial vaccination to evaluate the vaccination's longevity. In a study that is very similar to this one, the researches indicate that when CpG was included to the vaccination, a stronger immune response was seen. They used the same formulation in a subsequent paper to show survival after challenge from the H1N1 pandemic strains A/California/04/2009, A/Victoria/3/75, and A/Vietnam/1203/2004 (34).

PROTEIN-BASED NANOPARTICLES

To treat influenza, protein-based NPs have been used. These protein carriers have the capacity to come together to form nanoscale structures, including particles. A ferritin-based protein-based nanoparticle is an example of a nanoparticle used for universal influenza applications (32).

FERRITIN NANOPARTICLES

Most living organisms contain ferritin, a protein that binds to and stores iron. It has been shown that the ferritin that can be isolated from *Helicobacter pylori* self-assembles into an octahedron with 24 subunits and a hollow interior. Specific amino acid residues can be used to link proteins in an equivalent manner to the surface of ferritin particles (i.e., aspartic acid). These residues are arranged in groups of three, spaced 28 apart, with eight of the groups outside the NP. The trimeric HA spacing is identical to this spacing (35).

Using ferritin nanoparticles (NPs), Corbett et al. conjugated H3 and H7 HA stalk trimers. Based on electron microscopy imaging, it was determined that the stalk ligated NPs had a diameter of approximately 20 nm. By characterising the stalk regions through antibodies binding, it was determined that the epitopes were conserved when added to the ferritin NPs. Only H3 NPs showed some cross-protective and neutralizing antibodies against H7, however mice vaccinated with H3 or H7 stalk NPs at 0, 4, and 8 weeks in combination with the Ribi adjuvant showed high levels of serum antibody titers against their respective subtypes. Additionally, when challenged with homosubtypic strains 4 to 8 weeks after the final boost, the NP-vaccinated mice had a 100% survival rate. This platform is currently in clinical trials for the evaluation of unadjuvanted H1 stabilized stalk ferritin nanoparticles as an influenza vaccine(35).

CONCLUSIONS AND FUTURE WORK

Both seasonal and universal influenza vaccines have been developed using a wide range of nanoparticle platforms. In comparison to other, more traditional formulations, the platforms increase overall vaccine efficacy in the animal models in which they have been tested. As these platforms advance, it will be important to test them against a variety of heterosubtypic strains to evaluate their real universality. As the field develops, many of the new platforms that have been developed to vaccinate against SARS-CoV-2 will probably also be used to vaccinate against influenza and universal influenza antigens. These include the FDA-approved mRNA-based lipid nanoparticles from Pfizer and Moderna for use in an emergency COVID-19 infection vaccination (36).

Some vaccines for the flu based on NP have entered clinical trials in recent years. For example, NP universal influenza vaccine ACAM-FLU-A conducted a phase I clinical study at Acambis Inc. (now Sanofi Pasteur). The M2e peptide, which creates virus-like particles (VLPs), is fused to the hepatitis B virus's (HBc) capsid protein in the vaccine. 90% of the participants experienced M2e seroconversion after receiving the vaccine, although this protection was short-lived and dropped over the period of ten months (37).

In comparison to non-formulated controls, it has been demonstrated that using nanoparticles in the formulation of universal influenza vaccines increases vaccine efficacy. NP-formulated vaccines provide a number of potential advantages versus non-formulated vaccines. For example, many NP formulations can target immune cells, shield cargo against degradation and clearance, and provide a prolonged release of their cargo. The effectiveness of these formulations might also be increased by new universal antigens and adjuvants. These vaccine formulations have the potential to lessen the financial burden and mortality linked to seasonal influenza outbreaks, while more study is needed to further explore the universality of these vaccines as well as long-term protection.

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