ISSN No:-2456-2165

A Comprehensive Review on Nanoparticle-Based Vaccines

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Abstract:-_Vaccines nowadays have become a huge challenge to develop upon the emergence of infectious and anti-biotic resistant pathogens. Vaccines have proven to save millions of lives throughout the world. In its development process, throughout the years, scientists discovered the use of nanoparticles in vaccines and its benefits. Nanotechnology has emerged out to be a field of science that has transformed the shape of medicine. Through its uses in drug delivery systems and tissue engineering, nanoparticles are now used to develop vaccines too. The field of nanomedicine involves all the uses of nanoparticles in the medicinal world. Because of all of its properties, they are considered suitable in entering the body and targeting specific organs or tissues and providing benefits. In this review article, nanoparticles-based vaccines are fully described along with a brief knowledge of vaccine development and nanoparticle properties.

Keywords:- *Nanoparticles, Vaccines, Pathogen, Nanotechnology, Nanomedicine, Disease, APC, Immunity.*

I. INTRODUCTION

The 21st century has proven to become the century in which many infectious diseases have emerged, not to forget various variants of disease-causing microorganism have also emerged that have the ability to resist drugs administered for their killing [1]. Apart from medications, one of the most rampant approach is the development of vaccine, as it helps in making our immune system strong. Now, vaccine has been a trending topic of discussion and research in this time of COVID-19 pandemic. Vaccines are specially made antigen/medicines that when administered to one's body helps them to build immunity and fight a particular disease [2]. There are 5 broad classifications for vaccine type and they are - live/attenuated vaccines, inactivated vaccines, toxoid vaccines, conjugate vaccines and subunit vaccines [3]. The most in-demand or effective vaccines nowadays are the nucleic acid based and virus-based vaccines. Though the conventional ways of vaccine production are used till now, but they carry with them various risks too of regaining the disease or infection causing immunosuppressive conditions for the human body. More recently, DNA and RNA based vaccines (third-generation) have become in demand due to their various advantages such as they are cost-effective, provide least risk of infection and are able to cause immune response against the particular disease/pathogen [4]. Despite of the advantages these vaccines provide, there were still some hurdles to cross over like - effective transport of the vaccine to the desired area and the need for extra immunogenic drugs in prime-boost immunisation regimens, that includes premature molecular breakdown and an inability to convert into a viable immunogen [5]. When there seemed to be no effective drugs or an efficient vaccine, then one credible approach that came into effect was the application of nanoparticles in vaccine development, making them nanoparticles-based vaccines [6]. Nanomedicine including nanoparticles and microparticles are widely used for drug deliveries, this is because of the various advantages that they offer, like targeting specific organs, preventing damage to other healthy cells, preventing degradation of drugs etc. [7]. By the changes in physicochemical properties and the addition of nanoparticles in vaccines, the vaccine efficacy and stability improves, the delivery of vaccine gets then on the targeted sites, antigen gets easily taken up, humoral and cellular responses are induced [8].

II. REVIEW OF LITERATURE

2.1 All About Vaccines and Vaccinology

As mentioned earlier, vaccines are biological preparation that helps our immunity to gain strength, they help to build immunity in order to fight certain diseases. It is believed that vaccines or vaccination was founded by Edward Jenner because of his discovery of smallpox vaccination in the year 1796, but the truth about its discovery is that from many centuries in various places vaccine discovery had been started, like there are evidences of it in the ancient Chinese texts or beliefs about how in 7th century an Indian Buddhist drank snake venom in order to fight its effects [9,10]. Early methods included variolation, also considered to be the first method of human vaccination. Afterwards, due to the discovery made by Edward Jenner, global eradication of smallpox was officially declared by the year of 1980, but due to the lack of sources and knowledge of the microbial world, the future developments came to a halt, until the last years of the 19th century scientists like Louis Pasteur and Paul Ehrlich discovered the fundamentals and principles of immunology and developed methodologies that led the branch of vaccinology the next level [11]. These developments led to the suppression of various infectious diseases in the world. There is no doubt that vaccine development has saved millions of lives, Viral pandemics and epidemics, like the Ebola virus epidemic and now the infamous COVID-19 pandemic has affected so many lives that led scientists into development of vaccines in order to build immunity and save human kind.

As the developments proceeded the first type of vaccines to be developed were the live/attenuated vaccines. Many new vaccines were made by the early 20th century in order to fight diseases like tetanus, tuberculosis etc. [11,12] then during the middle-years basis of vaccine development to fight viral diseases were made which led to fight off adenovirus, poliovirus, influenza virus etc. [13] The second half of the 20th century was considered to be the golden-age of vaccine development, scientific improvements related to screening and manufacturing were at peak, different types of vaccines were developed, attenuated-virus vaccines against the MMR diseases were a breakthrough [14]. The conjugation of bacterial capsular polysaccharides to proteins began to apply in the 1980s, and because of these bacterial vaccines were developed to fight disease caused by bacteria such as pneumococcus, meningococcus etc. Hence, various types of vaccines and its development technologies were made to fight various diseases [15,16].

Through the years of development vaccine technology has been advanced a lot. The different types of vaccines are shown in the given figure 1: [17]

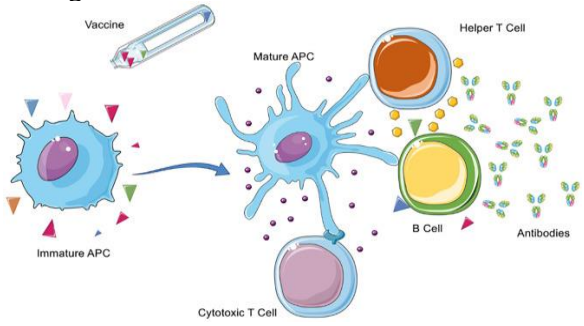
Figure 1: Types of Vaccines

Type of vaccine	Licensed vaccines using this technology	First introduced
Live attenuated (weakened or inactivated)	Measles, mumps, rubella, yellow fever, influenza, oral polio, typhoid, Japanese encephalitis, rotavirus, BCG, varicella zoster	1798 (smallpox)
Killed whole organism	Whole-cell pertussis, polio, influenza, Japanese encephalitis, hepatitis A, rabies	1896 (typhoid)
Toxoid	Diphtheria, tetanus	1923 (diphtheria)
Subunit (purified protein, recombinant protein, polysaccharide, peptide)	Pertussis, influenza, hepatitis B, meningococcal, pneumococcal, typhoid, hepatitis A	1970 (anthrax)
Virus-like particle	Human papillomavirus	1986 (hepatitis B)
Outer membrane vesicle (Pathogen antigen; Gram-negative bacterial outer membrane)	Group B meningococcal	1987 (group B meningococcal)
Protein–polysaccharide conjugate (Polysaccharide; Carrier protein)	*Haemophilus influenzae* type B, pneumococcal, meningococcal, typhoid	1987 (*H. influenzae* type b)
Viral vectored (Viral vector; Pathogen gene; Viral vector genes)	Ebola	2019 (Ebola)
Nucleic acid vaccine (DNA; RNA; Lipid coat)	SARS-CoV-2	2020 (SARS-CoV-2)
Bacterial vectored (Pathogen gene; Bacterial vector)	Experimental	–
Antigen-presenting cell (Pathogen antigen; MHC)	Experimental	–

- Live/attenuated vaccines are formed from the live disease-causing-pathogen itself by reducing its pathogenicity but keeping it alive so that it becomes less virulent. Because of the way they are developed they provide a long-lasting and strong impact. [18,19]
- Inactivated vaccines are developed by killing the previously virulent microorganism by heat or chemical or radiation techniques. It destroys the disease-causing particle from the pathogen. [20]
- Toxoid vaccines are formed from other toxic compounds causing disease instead of the pathogen. [21]
- Subunit vaccines are developed by using a fragment of the pathogen to create the immune response. [22]
- Outer Membrane Vesicles are intrinsically immunogenic and can be altered to make effective vaccinations. [23]
- Viral Vectored Vaccines, viruses that does not produce any harmful effects are used to insert the pathogenic genes to produce specific antigens in order to give immune responses. [24]
- Nucleic Acid Vaccines are developed by inserting bacterial or viral DNA in human cells and mRNA vaccines are novel vaccines which are packaged with vector like substances, for instance, lipid nanoparticles.[25] Many of the COVID-19 vaccines are RNA vaccines only like Pfizer and Moderna.

It takes around 10-15 years to completely develop a vaccine and its budget requirements estimates approximately at 1 billion US dollars. The whole process takes place in the way of phases and many aspects are considered for its development. Vaccine indeed provides a long-term immunization against a specific pathogen/ disease/ disorder. It is created for protection against specific antigen against a pathogen or cancer cells, through the development of cytotoxic T-cells and antibodies.[26] The antigens and the adjuvants present inside a vaccine are taken up by B-cells or antigen presenting cells, it then processes and transmits the signal to B cells, cytotoxic and helper T cells. Antigen-specific antibodies and cytotoxic T cells are made and the vaccination is considered successful.

Figure 2: Vaccine effect on Immature APC

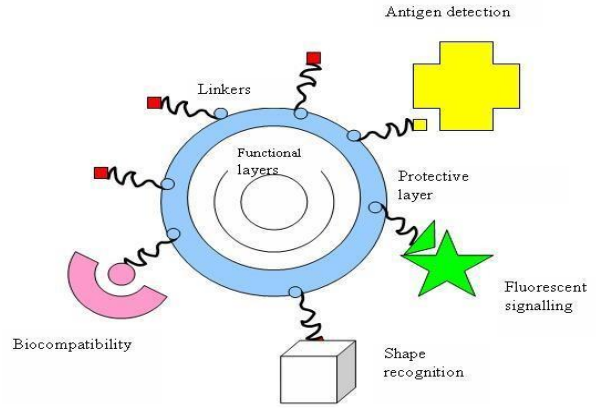


2.2 Nanoparticles and Nanomedicine:

Nanotechnology has emerged as a new field and has worked wonders in the field of medicine. Nano sized particles are used for various clinical purposes like drug deliveries, vaccine developments, regenerative medicines, tissue engineering strategies. Particles of the size ranging between 1 to 100 nano meters are called nanoparticles.

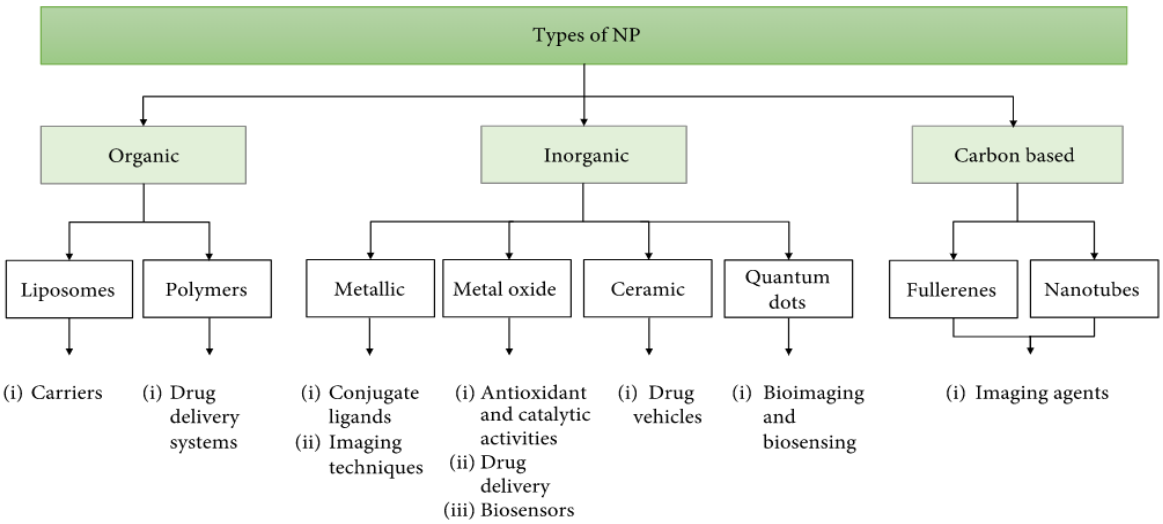
Apart from the various advantages of targeted delivery and enhancing the pharmacological properties of a solution (drug/vaccine), the main reason why it has become such a popular field in medicine is that all the biological processes/ cellular basis of one's body is on a nanoscale level, whether it be proteins or cell parts they all range in the nanoscale dimension which was a strong driving force towards nanomedicine development [27]. For nanoparticles to bio compatibly interact with the living system they require to be coated with biological or molecular coatings that act as the bioinorganic interface; coatings like antibodies, collagen or monolayers of biocompatible molecules are used as coatings [28].

Figure 3: Properties of Nanoparticles



Size is obviously a major factor of all the properties nanoparticles exhibit. Due to that only the other major physicochemical properties arise which makes them more suitable for its use in the medicinal world. Properties include high surface area to volume ratio, zeta-potential, quantum size effect, surface morphology. Antigen is well protected inside the nanomaterials decreases unnecessary immune response. The optical and magnetic properties add up to the advantages NPs provide. These properties justify them as therapeutic, diagnostic and carrier agents in biomedical applications [29-31]. Types of nanoparticles used in nanomedicine of each category including several types of nano formulations are shown in figure 4: [32]

Figure 4: Types of Nanoparticles used in Nanomedicine



2.3 Nanovaccines:

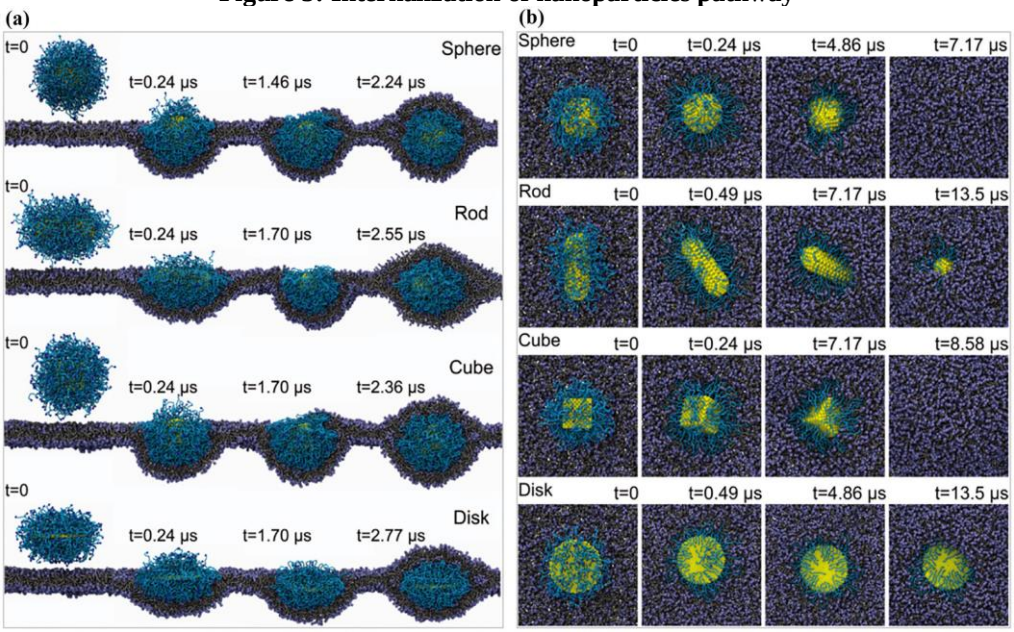
Nanovaccines or nanoparticle-based vaccines is a novel technique to develop vaccines by increasing the vaccine's immunogenicity and its other characteristics. Several types of nanoparticles are used for the development of vaccines like metallic, inorganic, polymer-based nanoparticles, liposomes and composited nanoparticles [33-35]. When it comes to nanovaccines, it is obvious to consider size as an important factor, and so scientists also studied the effect of their size/diameter with the immunogenicity of vaccines [36]. Because of the small diameter they are easily transported through the epithelia and other membranes and so they are found to be internalized by the APCs more efficiently [37]. However, the relationship between the diameter and immunogenicity is a complicated dynamic method and can be influenced by other variables such as particle type, dose of the injection and administration routes. For example, Li et al. administered BALB/c with several zein particles with varied diameters (241.4 to 879.2 nm), dosages (200, 600, 800 g), and routes of administration (intramuscular and subcutaneous). After three intramuscular injections it was deduced that the particle diameter did not really had any effect on immunogenicity. The immunological response that

ISSN No:-2456-2165

was elicited was both long-term and highly targeted and the repetitive treatments produced fast and strong systemic recall responses [38]. One more example is of Gutierro et. al. who produced poly (d,l-lactic-co-glycolic) acid (PLGA) nanovaccines with varied diameters filled with bovine serum albumin (BSA) (200nm, 500nm, 1000nm). BALB/c mice were administered with these nanovaccines intranasally, orally and subcutaneously. The particles had a high total serum ratio of IgG and IgG2a/IgG1. Here in this experiment, it was found out that the small size of the diameter did not have any increasing effect on immunogenicity of the PLGA nanovaccines, and by comparing with the 1000nm vaccine, they caused higher levels of serum IgG antibody levels and similar IgG2a/IgG1 ratios, which might be because of its better access to APCs and a similar antigen-presenting process/mechanism. Particles with sizes of 20nm 50nm are more likely to drain to lymphatic arteries and collect lymph nodes [39].

The 2nd factor of influence is the shape of the nanoparticle, as it is found that it regulates the differentiation of cells like macrophages and can also influence the uptake and biodistribution of the nanoparticles in vivo hence affecting the immunogenicity of nanoparticles-based vaccines. The fastest endocytosis rate was shown by the spherical-shaped ones followed by the cubical- then rod- and then disc shaped nanoparticles. The reason for the shape to attribute to effect can be the membrane-bending energy change. The smallest membrane-bending change was observed in spherical ones and star shaped ones, they both showed similar behaviour in wrapping time and a high efficacy in drug deliveries too, and so these factors can be used as a great tool for developing nanovaccines efficiently. In comparison, the disc-shaped nanoparticles showed the maximum change in the membrane-binding energy. Internalization of nanoparticles pathway is described in the figure 5 [40].

Figure 5: Internalization of nanoparticles pathway



The only conclusion comes out of it is that the shape of the nanoparticles has an effect on the rate of internalization, amount and biodistribution and that it should be considered while designing a nanovaccine.

Surface coating is a really important factor and should be chosen very carefully as it not only effects the hydrophilicity of nanoparticles but also uptake efficacy by the phagocytic cells and the time of circulation. Many studies have been done in order to understand the different types of coatings and their effect, such as poly (ethylene oxide), poly(sarcosine), and hyaluronic acid, and the immune system. It was found out that PEGylation of nanoparticles, such as liposomes and micelles, can cause anti-PEG antibody production in animals when injected repeatedly [41]. Generally, also, upon repeated administration it is found that many PEGylated products show a less therapeutic efficacy and other harmful effects. To study the connection between PEG shedding rate and anti-PEG antibody production, Takuya et. al. created lipid nanoparticles of varied PEG lengths [42]. Wilson et. al. reported that the shorter the PEG acyl chain will be, the faster will be shedding rate of PEG [43]. When compared to lengthy acyl chains, short acyl chains (fast-shedding) produced less anti-PEG antibodies (slow-shedding). Furthermore, rather than hepatocytes, the slow-shedding PEG lipid nanoparticle accumulated mostly in Kupffer cells [39]. The interaction between poly (sarcosine) and hyaluronic acid polymers and immunogenicity was also studied. Cheol et al. discovered that nanoparticles containing a long hydrophilic poly (sarcosine) chain accumulated primarily on B1a cells and induced the synthesis of the class-switched antibody immunoglobulin G 3. (IgG3). Furthermore, the antigenicity of poly(sarcosine) and the characteristics of nanoparticles impacted the production of IgG3 and immunoglobulin M (IgM) by various techniques [44]. Through antibody-dependent cell-mediated cytotoxicity, this result proved useful for therapeutic

applications. Another study showed that in comparison to CS nanoparticles and Alg-CS nanoparticles, HA-CS nanoparticles were the less immunogenic ones. Different surface coatings can cause differences in protein corona's composition, which may contribute further to immunogenicity of nanoparticles like pro-inflammatory surface coating or the anti-inflammatory surface coating.

Various types of nanoparticles have been used for nanovaccine production like, gold, silver, carbon, polymers, and liposomes. For the treatment of different types of diseases, a list of antigens transported by nanocarriers are described in table 1 [45].

Table 1: List of antigens delivered by nanocarriers

Antigen	Nanocarrier used	Disease
Against Bacterial Infection		
Antigenic protein	Poly (D, L-lactic-co-glycolic acid) nanospheres	Anthrax
DNA encoding T cell epitopes of Esat-6 and FL	Chitosan nanoparticle	Tuberculosis
Mycobacterium lipids	Chitosan nanoparticle	Tuberculosis
Polysaccharides	Liposomes	Pneumonia
Bacterial toxic and parasitic protein	Liposomes	Cholera and malaria
Fusion protein	Liposomes	*Helicobacter pylori* infection
Antigenic protein	Nano-emulsion	Cystic fibrosis
Antigenic protein	Nano-emulsion	Anthrax
Mycobacterium fusion protein	Liposome	Tuberculosis
Against Viral Infection		
Antigenic protein	Chitosan nanoparticles	Hepatitis B
Viral protein	Gold nanoparticles	Foot and mouth disease
Membrane protein	Gold nanoparticles	Influenza
Viral plasmid DNA	Gold nanoparticles	HIV
Tetanus toxoid	Poly (D, L-lactic-co-glycolic acid) nanospheres	Tetanus
Hepatitis B surface antigen	Poly (D, L-lactic-co-glycolic acid) nanospheres	Hepatitis B
Hepatitis B surface antigen	Alginate-coated chitosan nanoparticle	Hepatitis B
Live virus vaccine	Chitosan nanoparticles	Newcastle disease
Capsid protein	Virus Like Particles	Norwalk virus infection
Capsid protein	Virus Like Particles	Norwalk virus infection
Influenza virus structural protein	Virus Like Particles	Influenza
Nucleocapsid protein	Virus Like Particles	Hepatitis
Fusion protein	Virus Like Particles	Human papilloma virus
Multiple proteins	Virus Like Particles	Rotavirus
Virus proteins	Virus Like Particles	Blue tongue virus
Enveloped single protein	Virus Like Particles	HIV
Viral protein	Polypeptide nanoparticles	Corona virus for severe acute respiratory syndrome (SARS)
Against Parasitic Infection		
Merozoite surface protein	Iron oxide nanoparticles	Malaria
Epitope of *Plasmodium berghei* circumsporozoite protein.	Polypeptide nanoparticles	Rodent malarial parasitic infection
Surface protein from *Eimeria falciformis* sporozoites	ISCOMs	Diarrhoea

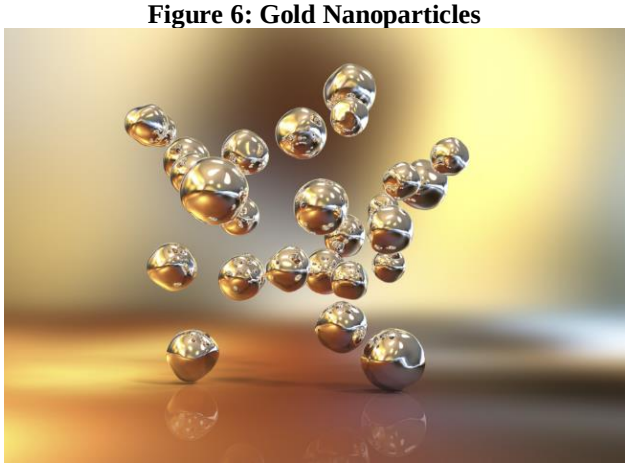
Nanoparticle-based vaccines are produced because of its many advantages and are used extensively for cancer immunotherapy and treatment of infectious diseases.

Types of nanoparticles used in vaccine production:

1. **Liposomes:** They were first used as a delivery system in vaccine for malaria in the 1980s. Liposomes are formed of lipids, having a hydrophobic tail and a hydrophilic head that maintains the hydrophilicity in the biological membranes simulating the vesicles found within the cells [46]. Charge is considered the main factor in liposomes for the activation of cellular and humoral responses, other factors include size and lipid composition.
2. **Virus Like Particles (VLPs):** They are made up of self-assembling viral membranes that keeps the viral surface proteins in place. VLPs can be designed to express additional proteins from other microbes, either by fusing the proteins with membrane antigens or by endogenous production of new antigens [47].
3. **Metal and Non-Metal Inorganic Nanoparticles:** Gold and Silver nanoparticles are highly used as delivering systems. When DNA vaccines are delivered via a gold, silica, or silver NP delivery method, they are found out to be more durable and resistant to degradation. Chito6's covalent attachment to GNPs raises the molecular weight of the NPs, improving DNA binding and stability

without affecting DNA release and transfer. When compared to naked DN, these chimeric NPs with a little amount of DNA produce efficient immune responses [48].

Figure 6: Gold Nanoparticles



4. **Polymeric Nanoparticles:** Polymers such as PLGA, PEG, chitosan, dextran, polycaprolactone are used delivery systems in vaccines. These biodegradable polymers are easy to prepare, are nonimmunogenic and have low levels of cytotoxicity [49].

Nanoparticles have many advantages as extensively discussed but because of their properties they can easily enter any membrane if the body and might affect some sensitive organs too [50]. Harmful effects like apoptosis or necrosis of tissues. It was found that intranasal delivery of nanoparticles caused lung infections causing cytotoxic cellular responses and inflammatory cytokines. One more disadvantage includes, blocking up of vessels because of the aggregation of nanoparticles.

III. CONCLUSION

In this review article, topics like vaccine development and nanoparticle-based vaccine properties and types is briefly described. Vaccine development is the greatest discovery our world has witnessed. Because of it many lives have been saved. Till now, various vaccines are developed and different types of researches are going on and now through the course of developments, nanovaccines or nanoparticle-based vaccines are also developed. Nanoparticles/ nanotechnology/ nanomedicine is a trending and a new topic in the medicinal development world. Because of its many advantages it is used as drug delivery systems and so now, vaccines have also begun to formulate with nanoparticles in it. It provides immense advantage because of their size mainly and other properties follow, but there are still some hurdles to cross over, continuous research goes on in order to fully understand and make use of nanoparticles.

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