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NANOVACCINES

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ABSTRACT
Nano vaccines are a modern and innovative approach to vaccination that uses nanotechnology to improve how vaccines work. These vaccines contain extremely tiny carriers like liposomes, nanoparticles, and virus-like particles that help to deliver the vaccine components more effectively and safely. Because of their small size and smart design, nano vaccines can closely resemble actual viruses or bacteria, which helps the immune system recognize and respond to them more strongly. There are several types of nano vaccines based on the materials used to make them, including lipid-based, polymer-based, protein-based, inorganic, and virus-like particle-based nano vaccines. Each type has its own benefits, like being more stable or triggering a better immune response. Their flexibility and potential, nano vaccines are being developed to protect against infections, fight cancer, and address new health challenges. Researchers are continuing to explore and improve them to make vaccines more effective and easier to use in the future. This review article summarises about the biodegradable and non-biodegradable of nano vaccines.

Keywords: Nano vaccine, Vaccine delivery, Immune responses, Biocompatibility, Targeted delivery, Controlled release, Nanocarriers, Polymer-based, Protein-based, Inorganic nanoparticles, Virus-like particles.

1. INTRODUCTION

The usage of vaccines in the world has been increased drastically during the outbreak of the covid 19 in 2021. Around 80 to 90% of the global population has received at least one dosage of the vaccine in their lifetime. A vaccine is a special medicine that helps our bodies fight off certain diseases. The word “vaccine” comes from the Latin phrase variolae vaccine, meaning cowpox. It was accidentally invented by Edward Jenner in the year 1789. The concept of vaccines was grown beyond cowpox in the later 20th century. Then the vaccine plays a significant role in the human body. They are designed to boost our body’s natural immune system, helping us to find infectious diseases, even cancer.[54]

Vaccine is made from the tiny or specific part of the pathogen (virus or bacteria) that has been weakened or killed or even just a blueprint. When we get vaccinated the immune system of the body starts to recognize it as an antigen and attack them. So that when we are really infected, the memory cells of the immune system recognize it and start to attack against the pathogen.

Vaccines have become one of the safest and most reliable ways to protect the patient, backed by strict testing to ensure safety. The traditional vaccine usually contains weakened or inactive versions of bacteria or viruses, meaning they cannot cause the disease but still helps the body to defend itself. However, in some cases, especially while dealing with viral antigen, the immune response can be weaker than respected. There are lots of researchers going on. In such a way they use nanoparticles in the preparation of the vaccine for the usage of nanoparticles as the vaccine has been experimented. This nanotechnology has various applications in the fields of medicine, agriculture, food industry, cosmetics, construction, textiles, etc. Today the applications of nanotechnology in biomedical science and healthcare have come to be called “Nano medicine" and is considered a hot growth area of nanotechnology [20]. Over the past few decades, the US FDA has approved commercialisation of one hundred medicine applications and products [19].

Nanotechnology has an important and significant role in the field of medicine and drug delivery, due to the major problems in the conventional pharmaceutical agents and the older formulations in the delivery system. The major problem or disadvantages in conventional drug delivery systems is the difficulty in removing the residual parts of such systems, thus leaving nonbiodegradable material in the patient's body that can cause toxicity [7]. Nanoparticles play a leading role. They help to carry the vaccine straight to where it is needed and stimulate the immune system more effectively.

Today's scientists have developed several types of Nano vaccines including lipid nanoparticles, polymeric nanoparticles, virus linked particles, cell-membrane-coated, self-adjuvating Nano vaccines etc. By applying these Nano vaccines, it provided an enhanced immune response which induced both strong antibody(humoral) and cellular immunity with long-lasting effects. It protected the antigen from degradation to increase the shelf life and reduce the cold storage. It is constructed for site specific delivery for prolonged immune memory; they require fewer booster doses, it can be altered for infection, disease, cancer, and autoimmune condition. The nano vaccines are used to develop various diseases like HIV, malaria, influenza and even covid 19. It enhances the anti-tumour immune responses and overcomes tumour induced immune suppression. In this paper it thoroughly discusses the types and materials of Nano vaccines used.

1.1 Nanovacccine

Because bioactive agents and macromolecules like viruses, proteins, and membranes have natural mano structures, nano vaccines are nanoscale carriers that enhance the interaction with cell membranes and proteins. For the diagnostic and remedial purposes the nanoparticle shape and size allow the carrier to be incorporated into a number of biomedical devices[50].Innovative developments in nanotechnology are currently being developed as therapeutic agents in biocompatible, biodegradable, and nanocarriers such as nanoparticles, nano capsules, conjugates of some magnetic nanoparticles, different micellar formation systems, and super paramagnetic iron oxide nanoparticles [63].

The materials used to create the nano Carriers—the microscopic particles that deliver the vaccine—were recently used to categorise the nanovaccines. They are both non-biodegradable and biodegradable. PLGA (polylactic-co-glycolic acid), chitosan, gelatine, alginate, dendrimers, and lipid-based systems are examples of biodegradable materials. Golden nanoparticles, silica nanoparticles, quantum dots, carbon nanotubes, and iron oxide nanoparticles are examples of non-biodegradable materials. The degradation of these careers could be used as an element for the release of therapeutic agents and plasmid DNA into the cytosol.[8].

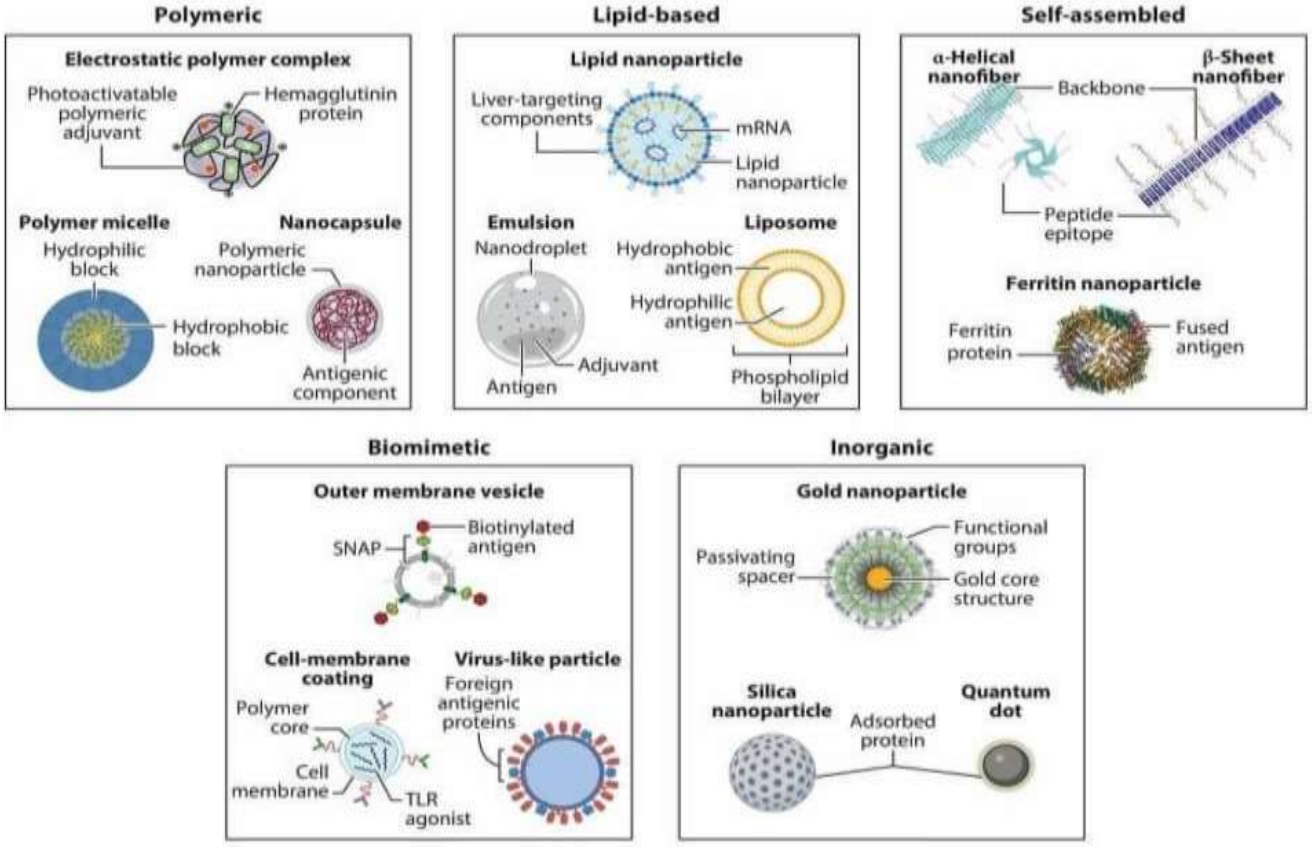

Figure 1: Types of nano vaccine

Whereas carbon silica and most commonly gold were used to encapsulate the antigens and frequently provide the covalent attachment of antigens [6]. But these modern vaccines are safe and have clearly defined components, but their antigens provoke weaker immune responses, showing they require adjuvants and perfect delivery methods to increase their immune responses [51].

Adjuvants are substances that, when combined with a specific antigen, elicit a stronger immune response than when used alone.[8]. Adjuvants such as calcium and aluminium hydroxide and phosphate salts are being approved for human use. However, using adjuvants of the alum type for vaccination has certain drawbacks [6]. So, the effective and secured adjuvants to achieve the prolonged and high immune responses in the delivery of vaccines are easily done by the development of the Nano carrier [2]. Even conjugated weak antigens can become more antigenic due to the antigenic nature of the nanocarriers. The characteristics of nanocarriers include their size and surface characteristics for the creation of higher-quality vaccine formulations.[20]. Antigen can be delivered by nano carriers in a number of ways to stimulate different parts of the immune system. They can enter through unusual channels to express various immune responses, and because of their size, they are both biodegradable and biocompatible.

2. BIODEGRADABLE NANOVACCINE

Biodegradable nanoparticles or nanomaterials are used in biodegradable nanovaccines to deliver antigens and adjuvants that boost the immune system's ability to combat diseases. Vaccine immunogenicity is enhanced by the nanoparticle carriers that are made up of biodegradable nanoparticles. Organic nanovaccines (polymer-based, liposome-based, virus-like particles, protein-based, and self-adjuvating nanovaccines) are among the various types of biodegradable nanovaccines.

Table 1 Types of Biodegradable and Non-Biodegradable Vaccine

TYPES OF NANOVACCINE	EXAMPLES	BIODEGRADABLE OR NON-BIODEGRADABLE	ARE THEY PARTICLES?
Lipid Nano vaccines	Liposomes, lipid nanoparticles (LNPs), SLNs, virosomes.	Biodegradable	Yes
Polymer based nano vaccine	PLGA, PLA, chitosan etc	Biodegradable	Yes
Inorganic based nano vaccine	Gold NPs, silica NPs, Silver NPs, iron oxide etc.	Non-biodegradable	Yes
Virus like particle (VLPs)	HPV VLP, hepatitis B, VLP	Biodegradable (protein)	Yes
Protein/peptide based nano vaccine	Self-assembling, protein, or peptide NPs	Biodegradable	Yes
Self-adjuvating Nanoparticle	Cationic, lipids, polymeric Carriers	Biodegradable (if lipid/polymer based)	Yes

2.1. TYPES OF NANOVACCINES

2.1.1. Organic Nano vaccines.

Organic nano vaccines are vaccines that are made up of organic nanoparticles such as polymers, lipid protein, and other parts to fight against a particular disease by stimulating the immune system.

2.1.1.1 Polymer-Based nano vaccines

To prepare a polymer-based nano vaccines, variety of polymers have been used such as Poly Lactate glycolic acid (PLGA), Poly lactic acid (PLA), Pol412-oxazoline (POx), Polyethylene Glycol (PEG) and natural polysaccharide. In this variety of polymers **PLGA** nanoparticles are the most widely studied because of their safety and biodegradability which has been approved as nano vaccine carriers by FDA [69].

To improve the efficacy of vaccines and subunit vaccines, the self-assembled micellar nanoparticles from amphiphilic biomacromolecules have been characterized [65]. Based on polymer on peptide, the micellar nanoparticles are classified into two types such as polymeric micelles and antigenic peptide micelles. Polymeric micelles are obtained by self-assembly of amphiphilic block copolymers in aqueous or buffer media while the antigenic peptide is incorporated or attached by chemical reaction into the surface. The peptide based micellar are also obtained from self-assembly of peptide antigen amphiphiles in water media.

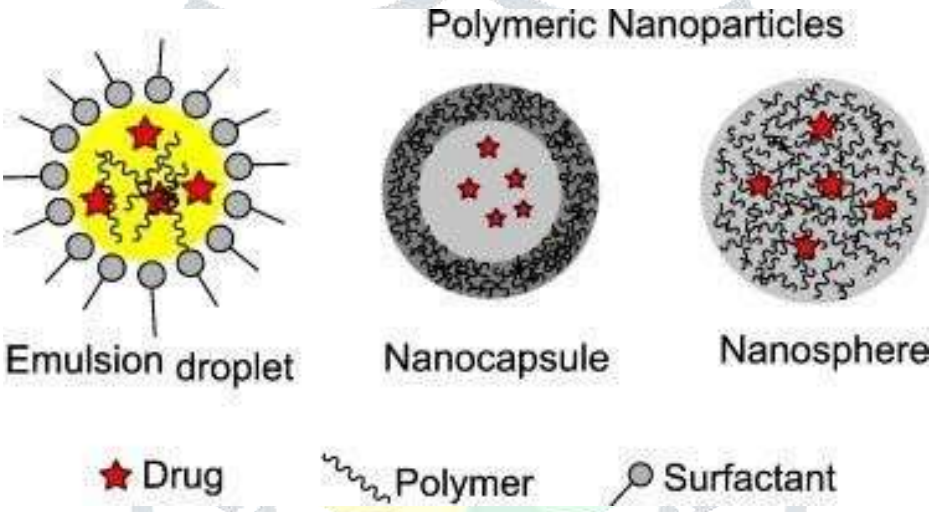

Figure 2: Different Forms of Polymeric Nanoparticles

The main advantage of the PLGA-based particulate vaccine delivery system. They are biodegradable, widely available & approved by FDA.

- They protect antigen from degradation & elimination.
- Those particles enhance antigen uptake by mimicking size and shape of pathogen.
- PLGA particles allow concomitant delivery of multiple vaccine components.
- Large surface area and surface functional groups allow conjugating of targeting moieties.

The main disadvantage of PLGA-based particulate vaccine delivery system: The negative charge of PLGA reduces the uptake by targeted cells.

➢ The PLGA particles are heat stable in some cases. The filtration of PLGA particles is not sterile, resulting in manufacturing complications.

The advantage of polymeric based nanoparticles

→They simulate stronger cellular immunity by increasing the antibody levels and secretion of cytokines [25][9].

→It provides the administration of vaccines in different routes [9] [29] [59]

→The circulation of antigen in the host body lasts for several months or years [36].

→They activate the immune responses such as Th1 or Th2 [26] [15]

→It has advanced adjuvant properties [25]

→The administration of vaccines can be needle free [9] [29] [59]

2.1.1.2 Lipid based nano vaccine.

In vaccine development; lipid nanocarriers have emerged as a practical approach. A liposome nanoparticle is constructed of spherical, self-assembled bilayer of phospholipids. These phospholipids have two distinct parts:

- A hydrophilic (water attracting) head.
- A hydrophobic (water repelling) tail

While hydrophobic tails face inward toward one another, hydrophilic heads face outward and interact with the surrounding water. This allows liposomes to carry several types of substances such as water-soluble molecules can be encapsulated in the aqueous core, while fat soluble molecules can be embedded within the lipid bilayer. [42]

The most crucial properties of lipid nanocarriers are the possibility of incorporating different antigens such as proteins, nucleic acids, peptides, carbohydrates and haptens. liposome formulations for vaccine delivery are commercialised now. As a vaccine delivery system, it has a number of benefits, such as carrying hydro pods, preventing antigen degradation before it reaches the target cell or site, providing the ideal antigen release profile, boosting cellular uptake, and enhancing antigen-specific immune responses.[42]

In vaccine development, another type of lipid-based nanoparticles is the micellar that are designed by synthetic copolymers. Thus, the lipolytic cavity or hydrophilic cavity (reverse micellar) facilitates the entrapment and delivery of antigens into the target cells.

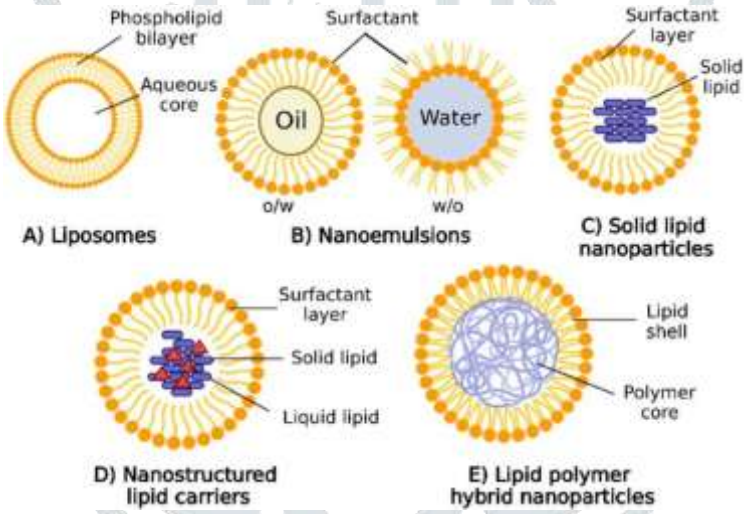

Figure 3: Different forms of lipid nanoparticles [42]

Lipid nanoparticles—the virosome—emerged as a bioinspiration tool to fight viral infections in place of virosomes. Solid lipid nanoparticles and nano emulsions are two more types of lipid nanoparticles. Spherical biphasic liquid droplets between 50 and 500 nm in size make up nano emulsions. They are composed of an internally dispersed oil phase covered by an externally continuous phase to transport either hydrophilic or hydrophobic active compounds. It is possible to prepare nano emulsions as either water in oil (w/o) or oil in water (o/w). Nano emulsions' colloidal structure makes it possible to solubilise and encapsulate hydrophilic medications, which lessens side effects. Nano emulsions are used in the food industry as preservatives, flavorings, colorings, and nutraceuticals. The cost-effectiveness, enhanced drug bioavailability, physical stability, and non-toxicity of nano emulsions make them advantageous for use as food additives, skin care products, or drug delivery systems. [42]

At room temperature, solid lipid nanoparticles (SLNS) were initially created as tiny, spherical particles with a solid lipid core. Following these developments, flat ellipsoidal or disc-like shapes with sizes ranging from 50 to 100 nm have emerged. In contrast to liposomes, two lipid cores or lipid shells can be added to SLNs with active ingredients that are distributed throughout the entire lipid matrix; this is a novel way to increase particle stability.When compared to other kinds of drug carriers, SLNs have both pharmaceutical benefits and drawbacks. The ability to co-deliver two antigens, enhanced physical stability, ease of sterilisation, and drug protection from chemical and enzymatic degradation are the technical benefits of SLNs. The main disadvantages are limited drug loading efficiency, short self-life due to cold storage requirements, poor long

term drug retention, low drug loading capacity, polydispersity, and high operative temperature [42].

2.1.1.3 **Virosomes**

Virosomes are spherical uni-lamellar vesicles 60 to 200 nm which are constructed with the viral envelopes of phospholipids with removed nuclear capsid. Virosomes are superior to VLPs in vaccine therapy because their fluidic phospholipid substrate surface contains protein antigens that improve interaction with host cell receptors, while VLPs' protein structure limits their mobility, especially when antigens are close to one another [1]. virosomes that, in terms of size and shape, are very similar to the virus. Three primary steps are involved in the presentation:

- Solubilizing the virus with detergent to remove nucleocapsid.
- Eliminating detergent and extracting viral protein.
- Reassembling the envelope with viral antigens and liquid into hollow membrane vesicles [1].

Some viruses are inactivated using UV before formulation like the Sendai virus. Virosome performance is impacted by the selection and removal of detergents (such as OG, triton, and X-100). Dialysis and biobeats are two methods that aid in eliminating leftover detergents.

Mechanism of action: Placing surface antigens is the most important step in getting Band T cells to respond.

- B cells make antibodies when they recognize surface antigens.
- APCs present antigen via MHC 1 and MHC 2 to activate CD8 + and CD 4 + t cells. ● Leads to long lasting IgG and memory cell production. Example influenza virosomes (IRIVs) containing HA and NA target APC sialic acid, initiating endocytosis and immune activation.[3][38].

2.1.1.4 Virus-like particles

Viral-like particles on nanoscale structure that are made of self-assembled viral proteins that lack viral genetic material that are non-infectious [4]. A variety of systems such as mammals, plants, insects, and bacteria are used to produce VLPs that are dispersed nanomaterial. For the delivery of bio and nanomaterials such as drugs, vaccines, Quantum dots, and imaging substance VLPs can be used by virtue of cavity inside their structure [13][64]. In the laboratory using recombinant viral proteins, VLPs can be experimentally generated. They are expressed in a range of expression systems such as prokaryotic cell, yeast, insects cell lines, plants, and mammalian cell lines [37][57][31][70][61]. While VLPs are commonly produced using proteins from a single virus type, chimeric VLPs can also be created by the assembly of structural proteins of various viruses [37].

VLPs are tiny structures made from the fragment of viral proteins, specifically the capsid protein but without the genetic material inside. To produce VLPs structural proteins from viruses such as Human Immunodeficiency virus (HIV), Adeno associated virus, Hepatitis B virus (HBV), Hepatitis C virus (HCV), Bacteriophage are used [31,70,61]. These particles are of assorted sizes ranging from 20 to 200 nm. VLPs are highly organized structures, because of their underlying geometry, they resemble pathogen associated structural proteins (PASP) that can be recognized efficiently by the immune cells [46][62]. Based on the presence of lipid envelopes VLPs have been classified into two types such as enveloped VLPs and non-enveloped VLPs and the presence of proteins organized into single layered or multilayered [30].

Since they contain an internal cavity, VLPs can be used for different purposes such as they can be used as an efficient delivery vehicle, and they have been used to deliver various biological materials such as genes, peptides, proteins, and small drugs. They can be used for targeted drug delivery, and their property of enhanced permeability and retention make these carriers an attractive means of drug delivery to tumour tissues for delivering treatment and for tumour imaging [21][34][53].

In conventional vaccine approaches, VLPs play an important potential application [22]. VLPs because of their size and shape that resembles the actual size of the native viruses, these structures effectively trigger the immune responses. The lack of viral genome in the VLPs inhibits the replication process, thus resulting in non-pathogenic state in the host body especially for immunocompromised patients and/or elderly vaccines [5]. To provoke more effective immune responses in innate immune system stimuli VLPs can be loaded with immune modulators while VLPs can stimulate both humoral and cellular immune responses [40][67].

2.1.1.5 Protein/Peptide based nano vaccine.

The protein that has a second and the third structure or a short fragment of the peptides is the main

composition of the peptide vaccine. A key feature in forming these peptide nano particles is self-assembly. These self-assemble peptides naturally organised into stable structures. This smart design allows the target and Nano vaccines to trigger strong immune responses all while avoiding sequences that might cause allergic or reactive side effects [33]. One of the most impressive examples of self-assembling nanoparticles is ferritin. A protein normally used by cells to store iron safely.

When modified, ferritin becomes a powerful Nano carrier. In fact, scientists have developed ferritin based in nano vaccine where the influence of matrix protein 2(M2C) is displayed on a recommended human heavy chain ferritin γHF . This intranasal Nano vaccine shows promise and offers protection against both similar and different sub types of influenza viruses, and it is currently being explored in preclinical studies. Notably this vaccine is thermostable and chemically robust making it even more attractive for real word use [47].

2.1.1.6 Self -adjuvating based nano vaccines.

These are the new generation of vaccines that use nano particles not only as careers for antigens but also as agents with intrinsic immune stimulatory activity which mean they can function as adjuvants themselves or at least minimise the need for additional adjuvants Numerous factors contribute to the superior efficiency of nanoparticles as a vaccine delivery system [66].

1.APC activation and antigen release [27, 68]
2.Improve antigen delivery [72]
3.Targeting pattern recognition receptors [41]
4.Encouraging APC development
5.Enhancing cross presentation [66]
6.Targeting signal in pathways
7.Mimicry of pathogen invasion by Bhaiyon

The primary purpose of using self-adjuvating nanoparticles and nano vaccine is to

- Enhance or improve antigen delivery and presentation.
- Increasing immune responses.
- Minimising the need of external adjuvants.
- Providing sustained and prolonged antigen.
- Targeting specific immune pathways [66][32].

3. NON BIODEGRADABLE NANOVACCINES

It is a type of nano vaccine which utilizes nanoparticles that are not easily broken down by the body's natural processes. They are designed to deliver antigens (parts of a pathogen that trigger an immune response). It persists in the body for a long duration.

3.1 Inorganic nano vaccine

Preclinical vaccine development has shown interest in inorganic nanoparticles. The majority of inorganic nanomaterials are perfect for delivering antigens as vaccines because they have smaller particle sizes, better stability, controlled tunability, increased permeability, high drug loading, and a triggered release profile.

These metallic hunks are double-edged weapons because they possess interstinic antimicrobial properties, unlike organic or polymer-based systems. They use their own strength to combat infections in addition to carrying viral antigen**.**

3.1.1 Gold nanoparticle

Applications for gold nanoparticles are numerous and include drug delivery, sensing probes, competing devices, and catalysis [35]. Because of their low toxicity and chemical diversity, which allows for the preparation of nanoparticles with various compositions, shapes, and surface functionalizations, AuNPs are promising candidates for vaccine development [17]. However, different AuNPS shapes and sizes affect the uptake, which lowers the immune response in the host cells [50].

Niltura et al used spherical, rod and cubic shaped AuNPs that are diameter of length, which are around form coated with west nile virus envelope protein and investigated the immune response [50].

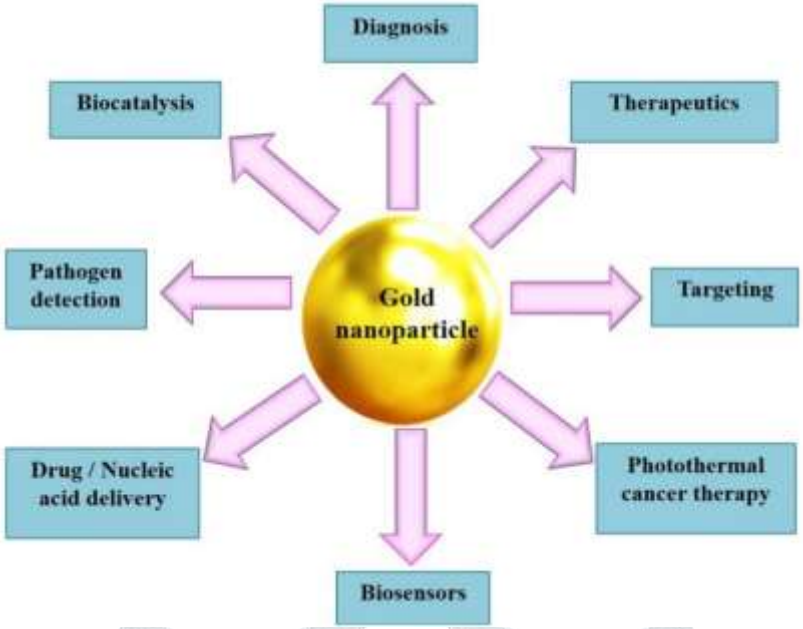

Figure 4: Applications of Gold Nanoparticles

Compared to cubic or spherical AuNPs, rod-shaped AuNPs are more efficiently absorbed by macrophages and dendritic cells. However, while rod-shaped AuNPs induce elevated levels of inflammatory- related cytokines like IL-1β and IL-18, spherical and cubic AuNPs induce higher levels of inflammatory cytokines like TNF-X, IL-6, IL-12, and GM-CSF [70].

3.1.2 Iron oxide nanoparticles

Iron oxide nanoparticles (IONPs) are small, magnetic particles that are mostly used as contrast agents in MRIs to help doctors see different diseases. Scientists have recently begun using them as adjuvants in vaccines to make the immune system work better. Studies have shown that IONPs can control immune cells and lower T cell-mediated reactions. This makes them a good base for making new vaccines, especially for infectious diseases. Most of the time, these nanoparticles are made of gamma iron oxide (γFe_2O_3) and magnetite (Fe_$3O_4$). They are made using methods like oxidative coprecipitation or thermal decomposition. Scientists have been using nanoparticles to improve vaccines. For example, nanoparticles that carried a tuberculosis protein caused different immune responses depending on whether they were given through the nose or the skin. [49]. Another study found that tiny iron oxide nanoparticles with a malaria vaccine antigen worked very well when injected because immune cells could easily take them up, which made the immune response stronger [56].

Researchers have made these nano vaccines even better by adding carbohydrate coatings to them. These coatings are meant to affect specific immune cells, such as dendritic cells. Nanoparticles coated with mannose effectively targeted these cells, significantly increasing the production of immune-system components.

3.1.3 Mesoporous silica nanoparticles

Researchers have attached vaccine antigens to MSNPs using a variety of techniques [53]. In one study, using MSNPs to administer bovine serum albumin (BSA) proved to be more effective than a standard aluminium-based vaccine adjuvant, particularly in mice with weakened immune responses [10]. A more comprehensive immune response resulted from the MSNPs activating both the Th1 and Th2 pathways, whereas the aluminium adjuvant only activated the Th2 pathway. According to a different study, MSNPs containing bacterial and snake venom proteins produced an immune response that was twice as potent as when the same proteins were administered with an aluminium adjuvant [43].

Additional studies have demonstrated the efficacy of MSNPs. They made the vaccine 38% more effective and offered longer-lasting protection than an aluminium-based vaccine when used to deliver an antigen for a parasite [52]. MSNPs with a pig virus protein were more successful in a different study. They possessed a mechanism that allowed the mice's immune response to grow stronger and last longer [23].

4. CONCLUSION:

Biodegradable and non-biodegradable nano vaccines offer two unique yet complementary strategies in the evolving landscape of vaccine development. Biodegradable nanocarriers are valued for their safety, compatibility with the body, and ability to release antigens in a controlled manner making them ideal for long-term, personalized vaccination. On the other hand, non-biodegradable nanocarriers stand out for their remarkable stability and sustained immune activation, which is especially important when long-lasting immunity is required. Together, these nano vaccine platforms are paving the way toward more precise, powerful, and adaptable immunization solutions. Moving forward, a deeper understanding of their mechanisms and careful assessment of their long-term impacts will be crucial in maximizing their potential against both existing and emerging health threats.

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