Chapter 15
Nanoparticle as an Effective Tool for the Diagnosis of Diseases and Vaccinology

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15.1 Introduction

The chemistry of nanoparticles and their various applications is quite intriguing. Although the advent and application of the smallest invisible particles are not new, the notion of nanotechnology has recently been at the forefront of scientific inquiry (El Sayed et al., 2022). Particles of minimum one dimension smaller than 100 nm in size, known as nanoparticles, were fruitfully employed in the assortment of biomedical science fields, ranging from diagnostics to prevention and therapeutics (Aljabali et al., 2020). Nanotechnology is becoming increasingly important in the life sciences, particularly because many elements of operational genetic units, like deoxyribonucleic acid (DNA), ribosomes, and ribonucleic acids in living cells, are primarily of nanoscale sizes (Yaqoob et al., 2020). This offers a chance for the use of nanotechnology to screen, discover any flaws, improve, combine, or even copy these cell components. The need for efficient vaccinations is becoming a major worldwide health-care issue due to the seasonal epidemics of pantropic infectious diseases (Oun et al., 2020). Inorganic and biological polymeric nanomaterials, such as exosomes and bacteriophages, and synthetic polymeric nanoparticles like nanobeads and immunostimulating complexes (ISCOMs) have been used as nanocarriers and adjuvants to treat both infectious and noninfectious disorders. Using dead organisms (Ni et al., 2020), live attenuated organisms, or inactivated toxin, vaccines have been created. Recently, new vaccination modalities have been investigated,

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including DNA vaccines that encode antigenic pathogenic proteins and subunit vaccines (Renu et al., 2020). Antigen or adjuvant targeting and/or sustained release to antigen-presenting cells can be facilitated by nanocarriers (Youssef et al., 2019). The functionality of vaccine formulations based on nanotechnology supports the use of nanocarriers in the vaccination industry. The improved antigen storage, minimal immunotoxicity, continued release, higher immunogenicity, and suppleness of physical features may all contribute to the effectiveness of nano-assembled vaccines (Feng et al., 2022a). Nanovaccines offer a great deal of potential and are relatively simple to engineer. Utilizing the potential of nanovaccines also makes it possible to create custom, individualized immune therapies. Understanding the precise biodistribution processes and potential commercialization of nanovaccines is a challenging issue that needs to be thoroughly researched and assigned. It is important to note that the efficacious use of the Pfizer/BioNTech and Moderna mRNA coronavirus disease (COVID-19) vaccines has underlined the importance of nanotechnology in the creation of new vaccines. Nanodiagnostics employs nanotechnology to recognize biomolecules and analytes relevant to medical analysis using specially designed instruments. It provides novel approaches for sample evaluation and early detection of disease biomarkers, with increased sensitivity and specificity. As a result of the development and optimization of nanoparticle platforms, complex procedures are now integrated onto a single, simple device that can be used for immediate diagnosis. The primary goal is to identify the medical problem being managed, treated, or endured. Identifying the root cause of a disease is regarded as the first step in any attempt to cure or manage a medical problem. Medical diagnosis has a long history, beginning with a rudimentary organoleptic evaluation of body samples and progressing through the age of microscopy to the current use of biosensors and body imaging. As a result, incorporating nanotechnology to improve diagnosis is not only prudent but also encouraging (Karch & Matyas, 2021). The demand for novel approaches, medications, and tools for the precise, rapid, and effective diagnosis and treatment of diseases is intensifying. More recently, nanotechnology has been used to deliver medications to a precise area and release them at a controlled rate, to improve immune responses against antigens for effective immunization, and to detect and identify diseases precisely and affordably. This chapter provides an overview of how advancements in nanotechnology have aided in disease prevention, diagnosis, and treatment.

15.2 Types of Nanoparticles Used in Diagnosis and Vaccinology

Nanomaterials are biomaterials that are widely used in the medical field. Surface modifications or coatings may improve biocompatibility by encouraging the interaction of living cells with the biomaterials (Lyons et al., 2020). These substances can be further regarded as nanocrystalline and nanostructured. Nanocrystalline

materials are easily produced and can be employed to substitute bulk materials that perform poorly. Drug encapsulation, bone replacement, prostheses, and implants can all be made with raw nanomaterials. Nanostructured materials, such as carbon nanotubes (CNTs), quantum dots, dendrimers, and fullerenes, are modified versions of basic nanomaterials that offer unique shapes or functions (Keshari et al., 2019). Nanodevices are tiny gadgets that operate at the nanoscale such as microfluidics, microarrays, and nano- and microelectromechanical systems. Nanoparticles (NPs) have been used in vaccine formulations more than any other type of nanomaterials. NPs with unique physical and chemical properties aid in the creation of innovative biochemical detection systems (Hasan et al., 2018). In fact, during the past few years, a wide range of incredibly creative methods such as assaying metal ions, small molecules, protein, and nucleic acid biomarkers have been used at nanoscale (noble metal nanoparticles, quantum dots, and magnetic nanoparticles) (Jeevanandam et al., 2018). Nanoparticles have a high surface-to-volume ratio that encourages downsizing, depending on the materials that make up their core, distinct optical, electrical, and magnetic capabilities. Furthermore, the properties of nanomaterials change depending on their chemical environment and their size and shape. Moreover, nanoparticles with a variety of tiny biochemical ligands and large biological polymers can be created using surface modification tools and methods. Because of these characteristics, researchers have been able to develop unique diagnostic systems with significant advantages in terms of sensitivity, selectivity, reliability, and usability. The composition of the NP material influences its biodegradability and biocompatibility, as well as its transport, cellular uptake, and intracellular trafficking (Han et al., 2022). Many materials, including lipids, viruses, natural and synthetic polymers, and inorganic compounds, immunogen or immunomodulatory agents, and targeting and immune stimulatory ligands, can be used to create nanoparticles for vaccine administration (Han et al., 2018). The following sections discuss various nanocarrier delivery methods used for vaccinations.

15.2.1 Polymeric Nanoparticles

Polymeric NPs have recently received much attention due to their use in the delivery of several vaccinations. This is primarily due to their ease of preparation, biodegradability, biocompatibility, low cytotoxicity, and the ability to modify surface properties as needed (Tao & Gill, 2015). Furthermore, the rate of vaccine release can be easily controlled by adjusting the composition or ratio of copolymers when the NP is prepared. Poly(lactic-co-glycolic acid) (PLGA) and poly(ethylene glycol) are the frequently used polymeric NPs for vaccine administration (Ys et al., 2010) due to their superior tissue compatibility and biodegradability (Wang et al., 2007). The use of PLGA-conjugated antigens demonstrated robust immunostimulatory properties by increasing cytokine and nitric oxide production in response to mycobacteria infection (Zhao et al., 2014). Natural biopolymers such as alginate, pullulans, inulins, and chitosan have been used as adjuvants along with synthetic polymers.

PLGA NPs have been used to deliver a variety of antigens, including hydrophobic antigens, hepatitis-B virus (HBV) antigens, *Bacillus anthracis*, tetanus toxoid, and ovalbumin (Shen et al., 2006; Demento et al., 2012), (Pusic et al., 2013; Zhao et al., 2014). Chitosan-based nanoparticles have been used in the development of a number of vaccines, including those against HBV and Newcastle disease, due to their biocompatibility, biodegradability, benign nature, and ease of modification (Ys et al., 2010; Tao & Gill, 2015). Furthermore, natural polymers were used to make hydrogel nanoparticles, which are a type of nanoscale, hydrophilic, three-dimensional polymer network. Nanogels have a variable mesh size, a large surface area for multivalent conjugation, a high water content, and a high antigen-loading capacity. Chitosan nanogels have been widely used in the delivery of antigens, such as recombinant *Neospora caninum* protein disulphide isomerase (NcPDI) antigen for *Neospora caninum* vaccination and *Clostridium botulinum* type-A neurotoxin subunit antigen Hc for an adjuvant-free intranasal vaccine (Ball et al., 1998). Chitosan nanoparticles have also been shown to act as nanovehicle molecules for HBV antigens, DNA vaccines, and Newcastle disease vaccines. A well-known alternate pathway complement activator, inulin, is a powerful adjuvant. Inulin improved defense against hepatitis B and influenza viruses (Nicol & Lachmann, 1973). AdvaxTM, an inulin-derived nanoparticle adjuvant, has demonstrated improved defense reactivity in vaccinations against a variety of viruses, including influenza Das et al., 2017) and hepatitis B.

15.2.2 Inorganic Nanoparticles

For application in vaccinations, many inorganic nanoparticles have been investigated. The advantage of these nanoparticles lies in their hard structure and regulated manufacture, despite the fact that they are largely nonbiodegradable. Because of the ease with which they shall be formed into an assortment of shapes and have a dimensional scale of 2–150 nm and can have their exterior changed with carbohydrates, gold nanoparticles (AuNPs) are employed in vaccine administration (Mitchell et al., 2021). By affixing the antigen to the surface of the gold nanorods, an antigen generated from the respiratory syncytial virus has been employed as a carrier. Gold nanoparticles have also been used as adjuvants for the human immunodeficiency virus (HIV) or as vehicles for immune triggers derived from other viruses like influenza and foot-and-mouth disease (Dykman, 2020). Another often-researched substances for the delivery of drugs and vaccines are carbon nanoparticles, manufactured into multiple kinds of nanotubes and mesoporous spheres, and are renowned for their good biocompatibility (Patra et al., 2018). Mesoporous carbon spheres are roughly 500 nm in size, whereas the diameter of carbon nanotubes (CNTs) used as carriers is typically 0.8–2 nm with a length of 100–1000 nm. CNTs can transmit numerous models of protein and peptide antigens and has increased the amount of immunoglobulin G (IgG) reaction. Mesoporous carbon nanoparticles were investigated for potential use as an adjuvant in oral vaccines (Zhu et al., 2014).

Silica is a promising substance for the construction of nanovaccination and delivery systems. In addition to being biocompatible, silica-based nanoparticles (SiNPs) are effective nanocarriers for the administration of vaccines and the selective targeting of tumors (Zhang et al., 2022). Mesoporous silica nanoparticles (MSNs) were investigated as nanocarriers and adjuvants for the carriage of potent antigens including those produced from the pig circovirus and HIV (Mody et al., 2013). These MSNs were about 50 to 200 nm in size and can be used to regulate the surface functionalization, pore size, and shape to regulate the release of antigens. Owing to their greater specific surface area and tunable hollow and mesoporous structure, MSNs perform better in delivery and controlled release as compared to solid SiNPs and can be broken down and eliminated in the urine (Afarid et al., 2022). These characteristics suggest that MSNs could develop into high-efficiency, measured-discharge nanocarriers in the next vaccine preparations. When sodium citrate, calcium chloride, and dibasic sodium phosphate are combined under particular circumstances, calcium phosphate nanoparticles can be produced (Sreeharsha et al., 2022). They may be shaped into sizes between 50 and 100 nm and are nontoxic. These nanoparticles exhibit high biocompatibility and are helpful adjuvants for DNA vaccines and mucosal immunity.

15.2.3 Liposomes

Liposomes are the second most researched vaccine and drug delivery method in the field of nanomedicine, after polymeric NPs. The hydration of lipids during the spontaneous production of liposomes allows for the formation of a lipid bilayer surrounding an aqueous core. So far, several types of liposomes have been used in vaccine trials, including unilamellar or multilamellar vesicles made of biodegradable phospholipids (such as phosphatidylserine, phosphatidylcholine, and cholesterol). Liposomes deliver vaccinations by fusing with the target cell membrane (Bozzuto & Molinari, 2015). Liposomes can combine viral envelope glycoproteins to form virosomes, including influenza virosomes, and they can encapsulate antigens within the core for delivery (Asadi & Gholami, 2021). In DNA vaccination research, liposome–polycation–DNA nanoparticles (LPD) are a popular adjuvant delivery method. They are made up of a cationic liposome that has been modified with 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) and a cationic polymer-condensed DNA (often protamine). Condensed DNA is housed within a liposome, in which the parts of LPD spontaneously reorganize into a 150-nm nanostructure (Schwendener, 2014). Maleimide-modified liposomes can be converted into interbilayer-crosslinked multilamellar vesicles (ICMVs) via cation-driven fusion and crosslinking, allowing for slower antigen release. Inflexal®V and Epaxal® that have been reviewed in earlier studies are two liposome systems that have been developed and given approval for human use (Gao et al., 2013). According to a previous research, antigenic proteins delivered in multilamellar lipid vesicles induce potent T- and B-cell responses. Clinical trials using liposome-based vaccination

nanoformulations against intracellular pathogens such as viruses and *Mycobacterium tuberculosis* have received approval (Kulkarni & Vaidya, 2010). Liposomal aerosol carriers' efficacy in the development of protective immunity against *M. tuberculosis* infection has already been demonstrated in one such study. Surface-modified liposomes that target immune cells, codeliver immunostimulatory chemicals, and simultaneously boost humoral and cell-mediated immune responses have been developed to improve the efficacy of liposomal vaccines. The protein surface antigen-to-lipid ratio can affect both liposome aggregation behavior and vaccine's overall stability during storage (Wang et al., 2019).

15.2.4 Immunostimulating Complex (ISCOM)

ISCOMs are 40-nm cage-like particles made of cholesterol, phospholipids, protein antigen, and saponin adjuvant. These spherical particles can capture the antigen via polar interactions. ISCOMATRIX™ includes ISCOMs that lack an antigen (Sun et al., 2009). It can be blended with antigen after removing the limiting hydrophobic antigens, allowing for a more versatile use than ISCOMs. ISCOMs have been developed using a variety of antigens, including flu, herpes simplex virus, HIV, and Newcastle disease antigens (Shen et al., 2018)

15.2.5 Virus-Like Particles (VLPs)

Virus-like particles (VLPs) are self-assembling nanoparticles that lack infectious nucleic acids and are formed by the self-assembly of biocompatible capsid proteins. VLPs are the ideal nanovaccine technology because they adopt the positive characteristics of viruses, namely the ability of the evolved viral structure to naturally engage with the immune system while avoiding the negative ones, namely the infectious components (Gao et al., 2018). Because of the spontaneously tuned nanoparticle size and the repeated structural order, VLPs elicit strong immune responses even in the absence of an adjuvant. In 1986, the first VLP vaccine against the hepatitis B virus was introduced, and VLP-based vaccinations were the first nanoparticle class to enter the market (Nooraei et al., 2021). These vaccines are now routinely administered to healthy populations. In nanovaccinology, VLPs provide the best evidence for safe use in healthy people. More recent VLP vaccinations for hepatitis E and the human papillomavirus were approved for human use in 2006 and 2011, respectively. VLPs can be manufactured using a variety of technologies, range in size from 20 nm to 800 nm, and can be obtained from a variety of viruses (Gregory et al., 2013). Numerous studies have sufficiently demonstrated the use of VLPs as vaccine carriers and their ability to activate their hosts' immune systems. In addition to viruses, VLPs can provide defense against heterologous antigens (Kushnir et al., 2012).

15.2.6 Self-Assembled Proteins

Self-assembling methods that aim to induce higher levels of protein quaternary structure have emerged for the creation of nanoparticle-based vaccinations in recognition of the effectiveness of the VLP method. Ferritin, a protein, has the ability to self-assemble into a nearly spherical 10-nm shape (López-Sagaseta et al., 2015). The main vault protein (MVP) is another self-assembling protein. The self-assembly of 96 units of MVP can produce a barrel-shaped vault nanoparticle with a diameter of about 40 nm and a length of about 70 nm. Genetically fused antigens with a limited interaction domain can self-assemble into vault nanoparticles when combined with MVPs (Zhao et al., 2014). Vault nanoparticles were used to encapsulate the main outer membrane protein of *Chlamydia muridarum* for research on mucosal immunity.

15.2.7 Emulsions

Nano-sized emulsions are another type of nanoparticle used as an adjuvant in the administration of vaccinations. Emulsions have lately been investigated as vaccine delivery systems after years of study as adjuvant formulations. Emulsions are dispersions of two or more immiscible liquids (Tayeb et al., 2021). The two main types are water-in-oil emulsions and oil-in-water emulsions. Emulsions can be combined with antigens or contain antigens within their core for efficient vaccination delivery. One of the most commonly used emulsions is MF59TM, an oil-in-water emulsion that has been approved as a safe and effective vaccination adjuvant in over 20 countries. It has undergone extensive research for use in influenza vaccines. Montanide™ is another name for a large family of emulsions that includes the ISA50V, ISA51, ISA201, ISA206, and ISA720. (Banzhoff et al., 2008). Malaria vaccines have used montanide ISA51 and ISA720, while foot-and-mouth disease vaccines have used montanide ISA 201 and 206. Recently, noncovalent click self-assembly was used to develop a tailorable nano-sized emulsion (TNE) platform technology for antigen and medication delivery (Dar et al., 2013). An oil-in-water nano-sized emulsion is created using specially formulated biosurfactant peptides and proteins. Using a self-assembling peptide–protein system, immune-evading polyethylene glycol (PEG) and a receptor-specific antibody can be strategically arranged on the aqueous interface of a nano-sized oil-in-water emulsion.

15.2.8 Dendrimers

Dendrimers are three-dimensional, mono-dispersed, hyperbranched amine- and amide-based nanostructures. According to the studies, dendrimers have only been used infrequently to transport various antigenic compounds. Dendrimers made of polypropyleneimine (PPI) and polyamidoamine (PAMAM) are the two most commonly used dendrimers for vaccine administration (Madaan et al., 2014). Dendrimers are thus promising candidates for the development of new generation vaccines with improved immunogenic properties because they can be tailored to achieve specific biological and physicochemical properties and because multiple ligands can be conjugated to a single molecule (Crampton & Simanek, 2007).

15.2.9 Nanotubes

Nanotubes are distinct cylindrical carbon molecules with numerous potential applications in nanotechnology, electronics, and material sciences. Because the chemical bonding is consistent with sp2 orbital hybridization, they have remarkable strength, distinct electrical properties, and good thermal conductivity. Fullerene, a carbon allotrope, is one example (Cui et al., 2011). Carbon nanomaterial delivery systems are insoluble and inert and resemble bacteria in both size and shape. Carbon nanotubes can transport many antigens, have low toxicity, are quickly absorbed by antigen-presenting cells, and are not naturally immunogenic. Such properties support the viability of using carbon nanotubes as antigen carriers (Scheinberg et al., 2013). According to reports, researchers at the University of Connecticut have developed a sensor that uses tightly packed carbon nanotubes coated with gold nanoparticles to detect oral cancer in samples. Carbon nanotubes and silicon nanowires (SiNWs) were used to identify different volatile organic chemicals in breath samples from patients with stomach and lung cancers, respectively (Mondal et al., 2022). Single-walled carbon nanotubes (SWCNTs) have been studied for their potential use in vaccine delivery systems by Zeinali et al. (2009). It has been noted that the antigen presentation process is impacted by the nanostructures of carbon-based materials. A number of studies have demonstrated the viability of carbon-based systems for systemic or oral antigen administration (Aqel et al., 2012).

15.2.10 Nanocrystals

Nanocrystals are crystalline materials with a minimum dimension of 1 μm, and their electrical and thermodynamic properties are size-dependent. One source of these crystals is Elan Pharma International Limited, an Irish company that specializes in the manufacturing of drugs for nanoparticles (Junghanns & Müller, 2008).

Nanocrystals in the 10-nm range have good semi-conductivity and a loose morphology with nanopores positioned between the crystals. The presence of silica molecules alters the surface of the pores, allowing them to adsorb protein. These hydroxyapatite nanoparticles are useful in the treatment of bone defects. An international cofunding framework for nano-drug development will also help to expand treatment (Shadjou & Hasanzadeh, 2015).

15.2.11 Nanorobots

Nanorobotics refers to robots of the nanometer (10^{-9} m) scale that have been used in medicine for early diagnosis and targeted medication administration for cancer therapy, pharmacokinetic monitoring of diabetes, and healthcare (Saadeh & Vyas, 2014). When used as toothpaste or mouthwash, nanobots dentifrices (dentifrobots) can cover all subgingival surfaces, metabolizing any trapped organic matter into harmless and odorless fumes. Dentifrobots are used to detect and eliminate pathogenic bacteria found in tooth plaque (Shetty et al., 2013). In fact, it is expected that patients will be given nanobot injections to perform tasks at the cellular level. Biochips and nubots are two examples of nanobots.

15.2.12 Nanowires (NWs)

A nanowire (NW) is a channel made of silicon, carbon nanotubes, or metal oxides that allows very low-amplitude electrical current to flow through it. They are extremely sensitive to even the smallest changes in their electrical properties, such as when a new molecule is bound to them, due to their tiny size and diameter of about 10 nm (Zhu et al., 2021). Antibodies are attached to the surface of nanowires to serve as detectors. When the antibodies interact with the target's biomolecules, a conformational change occurs, which is detected as an electrical signal on the nanowire. They can therefore be used as detectors for diseases like cancer when numerous nanowires with various antibodies attached are combined into a single device. Field effect transistors (FETs) made of silicon nanowire (SiNW) are used in sensors as an example (Vu & Chen, 2019). According to reports, FET-SiNWs have been used to monitor prostate cancer and predict the likelihood of biochemical relapse early, before full manifestation, for a number of prostate cancer biomarkers, including prostate-specific antigen (PSA) (Manceau et al., 2021).

15.2.13 Quantum Dots

Quantum dots (QDs) are 2- to 10-nm cadmium selenide nanocrystals coated with a shell (such as zinc sulfide) to improve optical properties and a cap (such as silica) to improve solubility. QDs may be used in a variety of biological applications because they are unique structures designed for targeted imaging by labeling molecules with a fluorescent probe. QDs have only positive properties as probes due to their quantum effect capability, such as stable and high quantum yield fluorescence that is not photobleached (Tandale et al., 2021). They are very useful in genotype determination, image-guided surgery, and molecular diagnostics because they require only such a basic excitation, resulting in high sensitivity and a broad excitation spectrum. Through the use of quantum dots in conjunction with other diagnostic methods, diagnostics and therapies have been combined. Quantum dots with detectable luminescence enclosed in carbohydrates, for example, are useful in cancer imaging (Fang et al., 2012).

15.3 Nanomaterial Vaccines for the Treatment and Prevention of Infectious Diseases

NPs are a favored tool among virologists and have been used extensively in the development of antiviral vaccination techniques. Its application in the treatment and prevention of infectious diseases such as influenza, hepatitis, dengue, and HIV has been studied. Nanovaccines have frequently been shown to improve immunogenicity over recombinant antigens alone. They can also transport and present antigens in native-like conformations. Gill et al. developed an influenza vaccine using gold NPs (AuNPs). They combined a 12-nm AuNP with an influenza virus matrix protein 2 (M2e) peptide's highly conserved N-terminal extracellular domain. The conjugates were given to BALB/c mice twice, which resulted in increased IgG1 and IgG2 production as well as improved defense against a lethal dose of PR8-H1N1 infection (Tao et al., 2014). As a potential therapeutic strategy, Negahdari et al. used a gene gun to deliver AuNP-coated hepatitis B virus surface antigen (HBsAg) DNA into epidermal cells. AuNPs were also given to mice along with plasmid DNA encoding HBsAg DNA as adjuvants. AuNPs cause rapid antibody synthesis, which expedites the animals' attainment of the peak antibody titer (Negahdari et al., 2019; Sengupta et al., 2022).

HIV-1 has remained a global epidemic since the 1980s. Despite the achievements of antiretroviral medications, it is unlikely that the virus will be eradicated from the population without a reliable vaccine. A variety of nanovaccine technologies were used in preclinical animal studies to create an effective HIV-1 vaccine, and these were capable of producing neutralizing antibodies (NAbs), non-neutralizing antibodies, and CD4+ and CD8+ reactivity. They have also been used as standalone immunogens and as components of prime-boost vaccination

programs. The potential and role of nanovaccines in the development of an effective HIV-1 vaccine are still being thoroughly researched, and a future research may reveal even greater significance (Lin, 2015).

15.4 Nanomaterial Vaccines for the Treatment and Prevention of Cancer

Cancer is still the leading cause of death in humans. The development of an anticancer vaccination enhanced by novel opportunities provided by nanomaterials is a critical first step toward individualized medicine for this widespread disease. STING, an endoplasmic reticulum-associated signaling molecule, regulates the transcription of a number of host defense genes. STING detects cyclic dinucleotides (CDNs) or abnormal DNA species, which induce the expression of type I interferons (IFNs) and proinflammatory cytokines. Through this fundamental mechanism, STING has been found to be involved in a wide range of pathological and biological processes. In response to DNA vaccines, STING-dependent signaling was supposed to induce adaptive immunity. Furthermore, it has been demonstrated that STING can detect viral entry-related membrane-fusion events independently of nucleic acid detection (Holm et al., 2012). STING can mediate type I interferon production by CD8+ dendritic cells (DCs), which can activate CD8+ T cells, for cancer treatment. Furthermore, leukocytes that can recognize tumor-derived STING-activating components, such as CD11b + and B cells, can activate STING, causing type I IFN production by leukocytes and preparing natural killer (NK) cells for cytotoxic death of tumor cells (Sundararaman & Barbie, 2018). These findings strongly suggest that STING plays an important role in a variety of innate and adaptive immune responses that can be used to treat cancer (Jing et al., 2022).

15.5 Dosage and Antigenicity of Nanoparticles

The immune system protects the body from harmful self-antigens, invasive infections, and their effects by recognizing and eliminating them as soon as they appear. The physicochemical properties of nanoparticles govern their interaction with the immune system. Plasma proteins bind to the surfaces of nanoparticles that do not contain polyethylene glycol (PEG) or other polymers, preparing them for a rapid uptake by phagocytic cells. Furthermore, it is well established that some nanoparticles can act as adjuvants, increasing the immunogenicity of weak antigens and facilitating vaccine production. Furthermore, modifying their size, surface charge, and administration method allows for effective antigen presentation to dendritic cells and lymphatic transport. The successful production of antibodies utilizing a protein-free formulation of particulate antigens was explained in support of

liposomes, which exhibit the hapten behavior of some nanoparticles; that is, they are not capable of eliciting an immunogenic response unless they are coupled to a protein carrier. Liposomes' antigenicity is entirely distinct from that of other nanomaterials that have been researched up to this point. For instance, antibodies are produced in reaction to substances other than lipids. A liposome interacted with the membrane phospholipids of cultured macrophages in addition to binding to phosphatidylcholine and cholesterol (Dobrovolskaia & McNeil, 2016).

15.6 Biodegradation and Elimination of Nanoparticles

The destiny of nanoparticles is critical for regulatory purposes due to concerns about long-term accumulation and patient safety. To successfully translate nanoparticles into the clinical side, it is necessary to first understand how the body eliminates them. Nanoparticles injected intravenously circulate in the blood until they are removed from circulation and eliminated via two primary routes, namely renal elimination and hepatobiliary elimination. Many prospective nanoparticle formulations for in vivo medicinal applications cannot be removed renally because they have sizes of 5.5 nm or greater and are nonbiodegradable. Although hepatobiliary elimination has been proposed as a route for these nanoparticles, limited information is known about their excursion through the body. Poon et al. investigated nanoparticle elimination via the hepatobiliary route and discovered that the fate of elimination is determined by the interaction of nanoparticles with liver nonparenchymal cells, such as Kupffer cells and liver sinusoidal endothelial cells. Cells at each stage of the pathway can entrap and chemically or physically change the nanoparticles, affecting how well they are eliminated through feces (Poon et al., 2019).

15.7 Contraindications and Adverse Effects

In the last decennium, an abundance of nanoparticles has been developed and researched for their potential application in disease diagnosis and treatment. Despite enormous progress in research, there are numerous factors that hinder the translation of nanotherapeutic, nanodiagnostic, and nanovaccine particles into the clinical domain. The concerns that exist include the impertinent physiochemical properties, difficulty in achieving repeatability in synthesis, its atypical biodegradation, elimination, and toxicity. A research showed that the intrinsic and extrinsic properties of the nanoparticles do not adequately coincide with the pharmacodynamic and pharmacokinetic requirements (Baetke et al., 2015).

Nanodiagnostic particles exhibit a short-lived circulation time, high specificity and sensitivity, and fast biodegradation and elimination without bringing about any pathophysiological effects. The nano-dimensions of the nanoparticles restrict the

biodistribution by affecting the uptake by the mononuclear phagocytic system, which is essential for the diagnostic agents that target the extravascular structures. They can easily extravasate out of the blood vessels and disseminate within the interstitial space (Nandedkar, 2012). Nanotherapeutic particles, on the other hand, should have a pharmacological activity and a longer circulation time to improve retention and permeability. When the nanoparticles reach the interstitial space, they should cause drug release (Kiessling et al., 2014). Because of their small size, nanoparticles can easily enter tissues and organs, which is a good thing overall. They can ingress the brain either by crossing the blood–brain barrier or via the olfactory epithelium on inhalation. Neutral and low-concentration anionic nanoparticles were not found to influence the integrity of blood–brain barrier, whereas high-concentration anionic and cationic nanoparticles were found to be toxic for the blood–brain barrier. Nanoparticles also bring about the formation of reactive oxygen species (ROS) and oxidative stress (Nel et al., 2006), which has been involved in the pathogenesis of neurodegenerative diseases such as Alzheimer's diseases and Parkinson's disease (CALDERon-GARCIDUEnas et al., 2004), (Cruz et al., 2014).

Toxicological evaluation of nanoparticle formulations is essential as they can result in varied distribution through the body, pass through the blood–brain barrier, and alter the pathways of coagulation. It has also been discovered that the toxicological profile of the bulk nanoformulation cannot be relied on, and thus, each case requires a safety evaluation. Nanoparticles may also cause mitochondrial damage, uptake through the olfactory epithelium, and platelet aggregation defects, particularly in immunocompromised subjects (De Jong & Borm, 2008). Cationic nanoparticles are found to cause hemolysis, vascular thrombosis, and blood clotting, while anionic particles are found to be comparatively less toxic (Gupta et al., 2007), (Nandedkar, 2012; Cruz et al., 2014). Studies on the biological behavior and toxicity of nanoparticles inhaled during drug delivery reveal concerning results due to the unintended release of ultrafine nanoparticles, which can cause a variety of adverse effects on the respiratory system, such as pulmonary inflammation, coagulation (thrombosis and platelet aggregation), cardiovascular system (like altered heart rate), and immune system defects (Oberdörster et al., 2005; De Jong & Borm, 2008). It is impossible to distinguish between the toxicity caused by the drug and the toxicity caused by the nanoparticle. As a result, the toxicity of non-drug-loaded particles should be highlighted, because drug delivery using slow or nondegradable particles may exhibit persistence and accumulation, resulting in chronic inflammatory reactions (De Jong & Borm, 2008).

Nanovaccines face challenges such as stability during production and storage, biocompatibility, toxicity, nonthermal sterilization, and toxicity with changing size and shape of the nanoparticle. One of the major barriers to the application of nanovaccines is the difficulty in the reproducibility of the formulation during synthesis, as they exhibit size-dependent immunogenicity (Sharma et al., 2009). Although small nanoparticles are quickly cleared, large counterparts accumulate in vital organs and are cleared slowly over time, causing toxic effects ((Nandedkar, 2009). The majority of the adverse effects are the result of long-term low-level exposure,

which can only be determined by long clinical trials (Nasir, 2009; Gonzalez-Aramundiz et al., 2012).

Orally administered nanovaccine must be administered at a higher concentration to overcome dilution during transit through the gastrointestinal tract, which can cause gastrointestinal disturbances (Bhavsar & Amiji, 2007). Nanovaccine intradermal injections have been linked to dermatological issues such as inflammation (Nandedkar, 2012). Nanovaccines administered orally are generally well tolerated and safe. The difficulties include dispensing minute amounts of vaccine and ensuring equal distribution to every extent of the nasal mucosa while ensuring minimal accumulation in the lungs (Sharma et al., 2009). Nasal drugs can also cause mucosal irritation, respiratory syndromes, poor particle distribution, and an unpleasant taste. Another barrier is that the free antigens delivered in the vaccine are easily removed from the nasal cavity, are only sparingly taken up by the nasal epithelium, and generate a low immune response, necessitating antigen encapsulation to prevent degradation (Nandedkar, 2009).

Nanomaterials are found to incite inflammation and the release of cytokines and inflammatory mediators like IF-1, tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-1β, and IFN-α causing an inflow of leukocyte, fever, swelling, vasodilation, and tissue damage. This effect has been shown by nanovaccines according to the reports by Gao et al. (2015) and Dinarello (2018). The immunogenicity of cytosine-phosphorothioate-guanine (CpG) oligonucleotides carried by polystyrene particles was found to be size-dependent, with differential expression of IL-6 and IFN-α (Azharuddin et al., 2022). The in vivo studies to test the toxicology of nanoparticles are expensive. Cell-based assays can be used to find the sequence of activity and the range of responses (Shaw et al., 2008). Although multiple tests are required to predict the toxicity of nanomaterials, these assays will benefit by accelerating toxicological testing of nanomaterials in order to increase their clinical applications to efficiently diagnose, prevent, and cure diseases (Nandedkar, 2009).

15.8 Future Scope of Nanoparticles in Diagnosis and Vaccinology

The small particle size, adulatory sensitivity, ability to detect diseases and genetic disorders in the initial stages, and accuracy in imaging methods have contributed in making nanomedicine a research hub (Jackson et al., 2017). Biological information can be obtained quickly and cheaply using nanotechnology and then analyzed using DNA sequence analysis (Jain, 2003), greatly expanding the scope of preventive medicine. Such advancements in diagnosis transform therapy into a more personalized approach. In fact, therapy and diagnostics are now combined into a new field known as theranostics, in which nanotechnology methods and medicines perform both diagnostic and therapeutic functions. This is applicable in nanoformulations such as contrast medium, which can function as a functional unit in the event of a

pathological change in tissue or circulate preventively when the organism is capable of secreting active substance in response to endogenous signals (Jackson et al., 2017). These also have the ability to recognize and repair individual genes, cells, or cell constituents that are damaged. This allows for the early detection and treatment of conditions with a genetic predisposition (Lu et al., 2007). Nanotechnology is not only improving the efficacy of conventional vaccines, but also assisting researchers in developing vaccines for diseases that were previously thought to be incurable. Nanovaccines are mostly delivered noninvasively, via the oral route, nasal route, patches, or microneedles, allowing for pain-free delivery with negligible tissue damage and thus increasing their efficacy over conventional vaccines. The physiochemical properties that influence potency can be easily controlled by using the appropriate polymer mixture, resulting in successful nanovaccine formulation (Kendall, 2006).

The latest developments in the field of immunology upraise an invigorative panorama in the formulation of nanovaccines. The populations of regulatory T cells and X lymphocytes have broadened the cognizance of the mechanism of protection against diseases (MacDonald et al., 2019; Ahmed et al., 2019). Nanovaccines may develop on their own, leading to pioneering treatment modalities with an increased efficacy. Liposomes and lipid nanoparticles used in nanovaccine formulations have demonstrated excellent biocompatibility and biosafety, with future research and development opportunities. A nanovaccine based on mRNA is expected to show a great promise in the treatment of cancer and the prevention of infectious diseases. Multidisciplinary research addressing physicochemical properties, biointerfacing, and quality control will improve disease and cancer management (Feng et al., 2022b). Cancer immunotherapies are a prominent field of nanoresearch that focuses on overcoming tolerance to self-antigens in order to defeat the tumor. Organic compounds serve as the reductant in the biofabrication method, which uses plants, algae, and microorganisms to create nanoparticles that could be used in vaccine design (Ahmed et al., 2017). Exploring the use of nanocomposites in vaccinology can lead to multifunctional nanomaterials with optimal immunogenicity (Korupalli et al., 2019) and pave the way for advanced versions of nanovaccines with improved safety and immunogenicity (Rosales-Mendoza & González-Ortega, 2019).

The use of nanotechnology in medicine suggests virtually limitless possibilities for advancement in the areas of early diagnosis, prevention, and minimally invasive treatments for cardiac disorders, cancer, diabetes, and other diseases (Yezdani et al., 2018). However, nanomedicine raises ethical and legal concerns about the custody of individuals' genetic sequence records (Jackson et al., 2017). Furthermore, because of their high surface area-to-volume ratio, nanoparticles interact negatively with biological systems and the environment, resulting in toxicity (Rezaei et al., 2019). Global collaboration is required to establish and maintain international standards for safety, nomenclature, risk assessment, toxicity testing, and mitigation, which will enable internationally accepted and standardized characterization protocols. With proper attention to ethical concerns and global participation, the possibilities in the field of health-care delivery will expand due to its great potential for disease diagnosis, prevention, and treatment with efficacy and safety (Jackson et al.,

2017). Because we are still in the early stages of research into the applications of nanotherapeutic, nanodiagnostic, and nanovaccine production, only a few nanoparticle formulations are in the early clinical stages (Gheibi Hayat & Darroudi, 2019). Nanotechnology will play an important role in the future by enabling early disease detection and therapeutic procedures that improve health and enable effective patient-tailored therapy (Yezdani et al., 2018).

15.9 Conclusion

The last decade has seen unprecedented growth in nanoscience and nanotechnology research. The nanoparticles' small size allows for easy diffusion into cells, adding to their enormous potential in the development of diagnostic devices, analytical tools, contrast agents, gene delivery, and drug delivery vehicles. There is growing optimism that nanomedicine will lead to significant advances not only in disease diagnosis and treatment, but also in vaccine development. Nanoparticles have properties that can be used in a variety of imaging applications. Theranostic nanoparticles can be used to visualize and quantify nanoparticle biodistribution and accumulation and to monitor drug release and predict treatment response. Nanoparticles have been found to deposit the active agent at the desired location, significantly reducing drug consumption and, as a result, its adverse effects. Nanovaccines are one of the most enthralling advances in the last decade. Their goal is to create an ideal vaccination system that does not require refrigeration, uses a single dose, and is painless to administer. Nanovaccines are thought to improve antigen stability, targeted delivery, and antigen release over time. A wide range of nanovaccines are currently being tested in clinical trials, with more on the way. The antigenicity and inflammatory response are affected by the shape, size, surface characteristics, and level of hydrophobicity. Vaccines for the treatment of cancer, Alzheimer's disease, and other emerging infectious diseases are being developed. Although nanovaccination is changing the vaccinology field, there is still no complete validation of their toxicity in mucosal immunization schemes. Nanotechnology will enable the development of vaccines that are stable enough to be distributed in remote areas with a limited access to medical facilities without refrigeration. Many lives could be saved by early detection, treatment, and slowing the spread of major infectious diseases. As a result, in order to promote the clinical translation of nanoparticles for diagnostic, therapeutic, and vaccine purposes, an interdisciplinary collaboration is required, which will make it a reality for society, with subsequent benefits for global health, quality of life, and life expectancy.

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