Kaushik Pal *Editor*

Nanovaccinology

Clinical Application of Nanostructured Materials Research to Translational Medicine

Springer

Nanovaccinology

Kaushik Pal
Editor

Nanovaccinology

Clinical Application of Nanostructured Materials Research to Translational Medicine

Springer

Editor
Kaushik Pal
University Centre for Research and Development (UCRD)
Chandigarh University
Mohali, Punjab, India

ISBN 978-3-031-35394-9 ISBN 978-3-031-35395-6 (eBook)
https://doi.org/10.1007/978-3-031-35395-6

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Vaccination is so far the most effective way of eradicating infections. Nanoparticle (NP)-based delivery vehicles like microemulsions, liposomes, virosomes, nanogels, micelles and dendrimers offer promising strategies to overcome limitations of traditional vaccine adjuvants. Flexibility in the design of nanomedicine allows for the programming of immune responses, thereby addressing the many challenges encountered in vaccine development. The conjugation of antigens to nanoparticles by covalent bonds ensures co-delivery of these components to the same subset of immune cells in order to trigger the desired immune responses. Whereas, nanovaccines can improve targeted delivery, antigen presentation, stimulation of body's innate immunity, strong 'T'-cell response combined with safety to combat infectious diseases and cancers. Further, nanovaccines can be highly beneficial to generate effective immutherapeutic formulations against cancer, malaria, HIV and serious microbial infections as well as control SARS-CoV2 or COVID-19 viral pandemic worldwide. Hence, this book emphasizes conjugation strategies for the development of protein-based subunit nanovaccines and the advantages and disadvantages of each strategy. Indeed, the most significant research trends emphasizes in this Springer book series Nanovaccinology subtitled *Clinical Application of Nanostructured Materials Research to Translational Medicine* exploring recent breakthroughs of exciting novelties finding inter-and cross multidisciplinary biosciences belongs to micro- to nanofabrication of bio-engineered nanomaterial for spectroscopic characterization and promising avenues of eco-friendly products as well as sustainable biomedical applications industrial potential scale. This book comprises 19 significant chapters written in diverse fields of studies on Green Chemistry, Nanotechnology, Advanced Materials processing, Nano-Biotechnology as well as Next-Generation Biomedical Technology. Throughout innovative performance explores the 'vaccine' candidate capable of eliciting robust immune responses against different diseases.

The book is informed by evidence from academicians, scientists, scholars, doctors, engineers and medical industry. Overall investigation illustrates wide variety of research interests in these areas and provides a background to the later chapters, which address importantly, some antigen-conjugated nanoparticles, can induce

robust immune responses without the use of additional immunopotentiators, such as TLR agonists. However, many of them still require the use of external adjuvant for optimal efficacy. Still, there are clear advantages of these delivery systems compared to non-conjugated strategies. As the use of NP-based vaccine delivery systems becomes increasingly popular, we can expect further development in protein–NP conjugation techniques, as well as greater application in vaccine designs. Hence, brand new strategies are leading in various implications of Green Nanotechnology based multifunctional nanomaterials potential applications. Liable appropriate regulation alongside the topics indicated that commercial production of manufactured novel composite materials can be realized. Furthermore, the many brilliant discoveries and explorations highlighted in this book can modulate spectroscopic performances with technical excellence in the nanovaccinology research with high competence.

Lastly, I would like to express my overwhelming gratitude to all the authors/co-authors for their excellence of research contributions throughout this significant book. I would like to thank the entire team of the Springer members for their efficient handling of this book at all harder stages of its publication. As an editor am very much confident and also pitty sure is that, the Springer book series will be more popular world-wide readers from academic Institutions, Universities and libraries and within short interval hopefully will achieve the highest citation.

Mohali, Punjab, India Kaushik Pal

Contents

Chapter 1
Diversities of Various Nanomaterials-Based Vaccines for Healthcare Applications

Amjad Islam Aqib, Mahreen Fatima, Kaushik Pal, Sana Zia, Muhammad Arslan, Asyia Shafiq, Junaid Sattar, Tean Zaheer, and Tasleem Kausar

1.1 Introduction

Few preventive health initiatives are as successful as vaccine development. Active acquired immunity to a specific disease is provided by vaccines, which are biological preparations. Vaccines come in a wide range of forms, such as attenuated,

A. I. Aqib (✉)
Department of Medicine Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan
e-mail: amjadislamaqib@cuvas.edu.pk

M. Fatima
Faculty of Biosciences, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan

K. Pal
University Centre for Research and Development (UCRD), Chandigarh University, Mohali, Punjab, India

S. Zia · T. Kausar
Department of Zoology, Govt Sadiq College Women University, Bahawalpur, Punjab, Pakistan

M. Arslan
Department of Poultry Science, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan

A. Shafiq
Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Punjab, Pakistan

J. Sattar
Faculty of Veterinary, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan

T. Zaheer
Department of Parasitology, University of Agriculture, Faisalabad, Punjab, Pakistan

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_1

inactivated, DNA, vector, live, and toxoid vaccines (Li et al., 2016). In the past two decades, vaccines based on nanomaterials have emerged as an innovative vaccination strategy (Butkovich et al., 2021). It has been shown that nanomaterials as vaccine carriers or delivery vehicles have significant adjuvant effects than solid particles with diameters ranging from 1 to 1000 nanometers (Sun & Xia, 2016). Infectious diseases, including viral, bacterial, parasitic, and cancer, have been controlled by effective vaccines. Developing next-generation vaccines requires the development of adjuvants and delivery systems that can be applied to next-generation molecules (O'Hagan & Valiante, 2003). As vaccine use enters the fourth century, it faces numerous challenges in protecting against many complex infectious diseases (Harandi et al., 2010). Due to the problems associated with conventional vaccines, new delivery systems and adjuvants have been developed to address those issues (Rice-Ficht et al., 2010). It has been reported that polymer particles have elicited the most protective immune response among all the delivery systems developed so far (Look M et al., 2010).

Polymer particle-based delivery systems have primarily been investigated for developing single-dose vaccines for various diseases. However, as nanotechnology has advanced, the scope and application of vaccine development have expanded significantly. One of the major applications of nanomaterials is drug, vaccine, and therapeutic delivery, where systems such as nanoemulsion, liposomes, nanoparticles, polymer micelles, dendrimers, and others provide higher therapeutic indices. Using a nanomaterial-based particulate system to entrap a candidate vaccine, an adjuvant/immunomodulator and a dendritic cell activator provide an enormous opportunity for vaccine development (Draper & Heeney, 2010). The development of a universal vaccine is challenging since it does not utilize live pathogens to induce sustained immune responses. A biodegradable nanoparticle that mimics a virus' structure and function but is not contagious and does not reproduce might serve this need (Basu et al., 2018).

1.2 Role of Nanoparticles as Adjuvants in Vaccines

1.2.1 Nanoparticles in Vaccine

Vaccine development is increasingly relying on nanotechnology. Formulations that increase antigen effectiveness are required more and more as vaccine development shifts toward "minimalist" compositions that are less immunogenic. Improved immunogenicity and antigen stability are made possible by the use of nanoparticles in vaccine formulations, along with targeted delivery and slow release. There are many different types of nanoparticle vaccines that have been approved for use in humans, and the number of candidates is growing. It is fundamentally important to understand how nanoparticles behave in vivo, whether they are used as delivery systems for antigens or as immunostimulant adjuvants to enhance immunity. In-depth summaries of recent advances in preventative nanovaccinology are

provided in this review. Various types of nanoparticles that are used are described, along with how they interact with immune cells and the biosystem. Developing rational vaccines containing nanoparticles will be more effective when we know more about nanoparticle mechanisms of action both in immunostimulatory and delivery modes, and also how nanoparticles distribute and behave in vivo (Zhao et al., 2014).

1.2.1.1 Nanoparticles as Adjuvants in Vaccines

An excellent foundation for creating a new generation of vaccines is provided by nanotechnology, which are based on nucleic acids, proteins, and synthetic peptides made from recombinant DNA, polysaccharides, or purified subunit proteins. These vaccines might not be sufficiently immunogenic, so adjuvants that boost their immunogenicity are necessary. An adjuvant (NA) is a nanoparticle (NP) that can act as an adjuvant for a vaccine (NA). In a suitable formulation, vaccine antigen or DNA can be encapsulated or adsorbed by nanoparticles, enhancing stability, cellular uptake, and immunogenicity. Different NA formulations can also be used to regulate a vaccine's biodistribution and systemic release (Garg & Dewangan, 2020).

1.2.2 Role of Metallic Nanoparticle

For subunit vaccines, the adjuvant choice is crucial for boosting immunogenicity, directing innate immunity stimulation, and creating the proper protective response to fight the target microorganism. Adjuvants can be immunomodulatory molecules, particulate formulations, or a combination of the two. A number of ways in which they may function include reducing the vaccine dose, enhancing the immune response, or extending the immune response, as well as providing a variety of immune responses, including humoral and cellular responses. Of the seven vaccine adjuvants that have been approved for use in humans, three are categorized as particulate formulations: aluminum salts (alum), extended immune response emulsions (like MF59), and virosomes. Alum increases antibody production as well as T-helper 1 (Th1) and Th2 responses, while the other two can also stimulate these responses. A TLR4 agonist, monophosphoryl lipid A, was combined with immunomodulatory molecules in AS 01 and 04, along with a particulate formulation of this molecule. As01 combined saponin (QS-21) with liposomes to favor Th1 responses, whereas ASo4 contained alum to enhance humoral responses. As agonists of TLR7 and TLR8, imidazoquinolines, as well as lipid A analogs, can trigger a Th1 response through their immunomodulatory effects (Marques et al., 2017).

1. Vaccines serve as essential triggers for activating both the innate and adaptive defenses of the body, enabling a robust response against infections. As a result, vaccines emerge as highly valuable and effective tools in promoting public

health. By harnessing the power of vaccination, we can enhance the body's natural ability to combat diseases and significantly reduce their impact on communities, thereby safeguarding the well-being of the population. With increased knowledge about cancer and how it affects the immune system, the goal of activating host immune defenses to provide a targeted and comprehensive antitumor response is becoming more important. It is promising to use nanoparticle systems as vaccines to deliver antigens efficiently and as adjuvants to enhance immune responses (Wen et al., 2019).

2. Mucosal tract pathogenic infiltrations cause most infectious diseases. There is extensive research underway on vaccinations to prevent a number of diseases, including infectious diseases, infertility, immune disorders, cancer, and allergies. It has been investigated how to stimulate the immune system more effectively against particular antigens using broad-spectrum adjuvant substances. Adjuvants have been developed that are inorganic, emulsion-based, oil-based, and bacterial-derived, both with cytosine-guanine dinucleotide motifs and with cytokines. Delivery of vaccines through the mucosa is an alternative to trigger cellular and humoral immune reactions. Antigen stability and immunogenicity can be increased, and targeted delivery and concentration of antigens are enhanced when nanoparticles are incorporated into vaccine formulations. Chitosan nanoparticles have mucoadhesive and immunologically active characteristics. Many antigens have served as a mucosal vaccine delivery system (Mehrabi et al., 2018).
3. There is a need for improvements in traditional vaccines due to their low immunogenicity, toxicity, instability, and multiple vaccination administration requirements. Recently, nanotechnology has been incorporated into vaccine development to address the aforementioned issues. The development of vaccines using nanocarrier-based delivery systems, which provide a chance to boost cellular and humoral immune responses, is becoming increasingly dependent on nanotechnology. Nanoparticles enable targeted delivery, slow release, and improved immunogenicity and antigen stability in vaccine formulations. Polymeric, inorganic nanoparticles, ISCOMs, virus-like particles, liposomes, and emulsions are examples of nanoscale-size materials that have drawn attention as potential vaccine antigen delivery systems because they can both stabilize vaccine antigens and function as adjuvants. This benefit is due to the nanoscale particle size, which makes it easier for antigen-presenting cells (APCs) to absorb the material, resulting in effective antigen recognition and presentation. By adding various targeting moieties to the surfaces of nanoparticles, it is possible to deliver antigens to particular cell surface receptors, inducing specialized immune responses (Kheirollahpour et al., 2020).

1.2.3 Carbohydrates Containing Nanoparticles as Vaccines

Vaccine adjuvants are necessary for immunopotentiation, which induces the production of protective immunity. Unfortunately, traditional aluminum-based adjuvants are only capable of stimulating limited cellular responses. Developing vaccines against emerging pathogens requires adjuvants with better profiles in order to be effective. A greater balance of humoral and cellular immune responses can be stimulated by carbohydrate-containing nanoparticles (NPs) with immunomodulatory activity. We looked at a number of carbohydrates that have immunomodulatory effects. They include mannan, saponins, glucan, chitosan, and others that have been incorporated into vaccine formulations. These carbohydrate-containing NPs' mode of action, preparation techniques, characterization, and associated vaccines are presented. Numerous NPs that contain carbohydrates have either reached the clinical stage or have been included in approved human vaccines. A vaccine against the pathogen responsible for the global pandemic, SARS-CoV-2, is being tested that contains saponin. Preclinical research and late-stage clinical trials are both stages of the development process for vaccines containing NPs that contain carbohydrates. The development of next-generation vaccines against cancer and infectious diseases may benefit from understanding the mechanism of action of carbohydrate-containing nanoparticles as carriers and immunostimulants (Smith et al., 2015).

1.2.4 Polymer-Based Nanoparticles

Polymer-based nanoparticles are solubilized, stable, safe, and have a sustained release, they are able to enhance drug absorption, protect them from deterioration, so that they can travel through the body for a longer period of time, and can increase targeted drug delivery. Vaccination is the best method of preventing and managing infectious diseases. There are several drawbacks to vaccines, including immunity tolerance, low immunogenicity, low expression levels, and the induction of pathological changes in respiration, including live attenuated vaccines, inactivated vaccines, protein subunit vaccines, recombinant subunit vaccines, and synthetic peptide vaccines. The antigens of vaccines are released more slowly through the use of biodegradable natural and synthetic polymers. Furthermore, these polymers enhance vaccine immunogenicity as well as act as adjuvants (Guo et al., 2019).

1.2.5 Nanomaterial Vaccines

As engineering devices have been modified to deliver drugs to and interact with cell environments, nanomedicines have gained increasing interest from the medical community in recent years (Shah et al., 2015). A wide variety of biodegradable

materials, including cholesterol, phosphatidylserine, and other lipids, have been used to manufacture nanoparticles (NP) in addition to natural and synthetic polymers (PLGA) and metals (gold, copper oxide, silver, zinc oxide, and aluminum oxide) (Pati et al., 2018).

It is widely accepted that nanoparticles (NPs) and microparticles (MPs) are useful methods for delivering drugs, especially cytotoxic drugs or immunosuppressing treatments for organ transplants. Drug delivery is controlled using these NPs as they target specific organs to distribute medications. In transporting drugs to cancerous sites or other diseased organs, these carriers protect drugs from degradation (He et al., 2011). Metal nanoparticles themselves may work as carriers and targets for the delivery of tumor-associated markers since they are incorporated into the membranes of cancer cells. The outer layer of PLGA nanoparticles was coated with membranes derived from mouse melanoma cancer cells, according to Fang et al. 2014. In response to these molecules, cellular endocytosis caused dendritic cells to mature, presenting a special presentation to T cells with TCRs that bind to gp100 and produce IFN-γ.

A second finding was that PLGA-covered membranes were equipped with receptors for interacting with cancer cells and allowing drugs to be delivered (Fan et al., 2019). To prevent the rejection of organs, drugs were delivered through the NPs to the transplant site. The use of immunosuppressive agents in high doses is harmful to organ transplant recipients, as it can lead to infection and even death. Researchers have previously established that rapamycin MPs prevent islet rejection in vivo in diabetic mice with islets transplanted into the eye. Islets transplanted into immunosuppressive drug MPs survived for more than a month, while islets transplanted into a control group (empty MPs) were rejected after the second week (Sridhar et al., 2015). We have gained a greater understanding of infectious diseases and immune evasion mechanisms in the past few decades. Designing new vaccines and adjuvants to combat antibiotic-resistant pathogenic microorganisms is becoming increasingly challenging. It is currently possible to develop vaccines from live-attenuated microorganisms or killed pathogens (first-generation vaccines) (Lugade et al., 2013). In the third generation, there are DNA vaccines (Levine & Sztein, 2004), subunit vaccines (Levine & Sztein, 2004), and synthetic peptides (second-generation vaccines). In addition to these three vaccine types, an adequate adjuvant or delivery system is required to eliminate the risk of developing the disease (Eidi et al., 2010).

There should be no strict storage requirements for the vaccine and adjuvant combined, and it should induce long-lasting memory B and T cell responses (Levine & Sztein, 2004). The vaccine should be safe, stable, and able to induce memory B and T cell responses. The last three vaccine types must be combined with an adequate adjuvant or delivery system to eliminate the risk of developing the disease (Eidi et al., 2010). Recombinant proteins or DNA from another vector are needed to boost DNA and RNA vaccines. Some studies have demonstrated that nanoparticles improve vaccine efficacy by slowing antigen release, allowing for easy antigen uptake, and inducing humoral and cellular responses (Lugade et al., 2013).

1.2.5.1 Concept of Nanomaterial-Based Vaccines

One of the best methods for triggering immune responses that protect against infectious diseases is vaccination (Whitney et al., 2001; Pulendran 2006). Vaccination has prevented millions of deaths since Edward Jenner used cowpox materials to treat smallpox in 1796. It has virtually eradicated poliomyelitis and smallpox and significantly reduced the prevalence of diseases like hepatitis A, hepatitis B, diphtheria, tetanus, pertussis, measles, and others (Rappuoli et al., 2011). Additionally, vaccinations can ward off conditions that can result in cancer. In terms of preventing infections, chronic disease, and liver cancer, the hepatitis B vaccine has a 95% success rate. A vaccine for human papillomavirus (HPV) that prevents HPV-related vulvar cancer, vaginal cancer, and cervical cancer can prevent 50% of these diseases. Gardasil is effective against two varieties of HPV. To achieve optimum and long-lasting immunogenicity, vaccines need protective antigens and adjuvants (Wegmann et al., 2012). Vaxjo, a website that houses vaccine adjuvants and their uses in vaccine development, reports the use of 93 vaccine adjuvants in 379 vaccines designed to combat 78 pathogens, cancers, and allergies (Sayers et al., 2012). Adjuvants for vaccines are only approved for use in humans in a limited number of cases. A total of four adjuvants have been approved by the U.S. Food and Drug Administration (FDA), including aluminum salts, AS03, AS04, and MF59. Whether they enhance innate or adaptive immune responses or improve antigen delivery to the immune system, nanomaterials act as adjuvants (Smith et al., 2013). To elicit robust immune responses, researchers have developed different ENM-based adjuvants. Alum-based vaccine adjuvants have been used for many years in vaccinations for hepatitis A and B, human papillomavirus, pneumococcal disease, and DTaP (Diphtheria, Tetanus, and Acellular Pertussis) (Baylor et al., 2002). In addition to aluminum hydroxide (Alhydrogel), aluminum phosphate (Adju-Phos), aluminum potassium sulfate (Alum), and aluminum hydroxy phosphate sulfate (AHSA), some commercial sources offer aluminum and magnesium hydroxide mixtures (Inject Alum) (Baylor et al., 2002).

These aluminum salts that are offered for sale have various physicochemical characteristics. In-depth research is being done on gold nanoparticles (AuNPs) with controlled physicochemical properties for use in biology and medicine (H. Sawa et al., 2013). Silver nanoparticles are widely used in commercial products due to their special antimicrobial properties, and more than 30% of consumer products based on nanomaterials contain nano-silver (Niikura et al., 2013). It is important to get vaccinated against infectious diseases and conditions that may lead to cancer in order to prevent these diseases and conditions. It is common for vaccine formulations to include adjuvants because many recombinant and synthetic antigens are not immunogenic, improve immune response, and reduce antigen requirement for protective immunity. More than 400 vaccines are either in development or commercially available, employing over 100 different types of adjuvants. In addition to nanomaterial-based vaccines that enhance immunity caused by different antigens, there are many vaccines under development or on the market today. A better understanding of the molecular mechanisms is required, however, in order to ensure safety (Sun et al., 2016).

1.2.5.2 Current Market Scenario of Nanomaterial-Based Vaccines

A potential strategy for creating efficient and secure vaccinations against a variety of infectious illnesses is the use of nanomaterials. Due to the rising incidence of infectious diseases, the demand for targeted drug delivery systems, and the expansion of research and development efforts in the field of nanotechnology, it is anticipated that the global market for nanomaterial-based vaccines will experience significant growth in the upcoming years. The COVID-19 pandemic has also sped up the development and usage of vaccines based on nanomaterials, with a number of candidates now in clinical studies or authorized for use in an emergency. Nevertheless, in the near future, market expansion can be hampered by the high cost of production, regulatory obstacles, and the lack of knowledge and infrastructure in developing nations (Datta & Mandal, 2020).

Nanotechnology is used in vaccinations made of nanomaterials to increase their effectiveness. There are currently just a few vaccines in clinical trials, and the market for vaccines based on nanomaterials is still in its infancy. Yet, given the potential benefits of these vaccines, including enhanced stability, tailored distribution, and greater immunogenicity, there is a rising interest in this subject. As the need for safe and efficient immunizations increases, it is projected that the market for vaccines based on nanomaterials would expand fast in the future years (Bachmann & Jennings, 2019).

There were a number of nano material-based vaccines being developed, some of which were for COVID-19 and some of which had previously received emergency use authorization, but their market performance and effect were still in the early stages and were still being studied (Pardi et al., 2018).

The pharmaceutical industry's field of nanomaterial-based vaccine research was one that was expanding quickly. These vaccines include nanotechnology to increase their potency and the antigens' ability to reach the immune system, potentially enhancing vaccination effectiveness and lowering adverse effects. There were several nanomaterial-based vaccinations for infectious illnesses such as COVID-19, influenza, and cancer that were in various phases of clinical studies. Yet, regulatory clearance and consumer and healthcare professional acceptability might be determining factors in their commercial availability and market penetration (Dadu et al., 2021).

1.2.6 Inorganic Nanoparticles

There have been studies on the delivery of vaccines using silica, carbon, and gold, and these are biocompatible inorganic NPs (Wang et al., 2011). There are various forms, sizes, and surface modifications that can be used to synthesize these nanoparticles. It has been demonstrated that inorganic nanoparticles can successfully deliver viral antigens. Proteolytic enzymes couldn't prematurely digest antigens this way. When influenza, immunodeficiency virus, foot and mouth disease, and TB antigens

were delivered with gold nanoparticles, a robust immune response was induced in mice (Villa et al., 2011). In mice infected with Mycobacterium tuberculosis, the causative agent of human tuberculosis, gold nanoparticles encapsulating plasmid DNA encoding the hsp65 antigen reduced the burden of the bacterium by a significant amount (Silva et al., 2005). As adjuvants for enhancing immunogenicity and antigen delivery, hollow mesoporous silica, nanotubes, and spherical carbon nanoparticles have been used in many studies (Kawano et al., 2013). A significant amount of silanol groups can be introduced on the surfaces of silica-derived nanoparticles to provide access to the target cells for vaccine molecules (He et al., 2003). Inorganic nanoparticles have the advantages of being low-cost, reproducible, and safe.

1.2.6.1 Liposomes

Among the nanomedicine delivery vehicles, liposomes are the second most widely tested after polymeric nanoparticles. It is through liposome fusion that vaccines are delivered to target cells. Both hydrophilic and hydrophobic substances can be encapsulated by structurally flexible liposomes. An aqueous core can accommodate hydrophilic molecules, while phospholipid bilayers enclose hydrophobic molecules. Multilamellar lipid vesicles have previously been shown to elicit strong T- and B-cell responses when they transport antigenic proteins (Moon et al., 2011). APCs readily internalized phosphatidylserine liposomes containing antigenic peptides to potentiate T-helper cell-mediated immunity (Ichihashi et al., 2013). Vaccine DNA encoding heat shock proteins delivered using liposomes induced strong protection against fungal infections (Ribeiro et al., 2013). Clinical trials of liposome-based vaccine nano-formulations against intracellular pathogens have been approved as a result of their expected applications, including viruses and M. tuberculosis (Watson et al., 2012). The effectiveness of liposomal aerosol carriers has already been demonstrated for the generation of protective immunity against Mycobacterium tuberculosis infection (Vyas et al., 2005). DDA-lipid liposomes and various immunomodulators have also been used in other studies to boost immunity against influenza and chlamydia (Alving et al., 2016). The delivery of lipid-DNA complexes to monkey lungs has been successful in the context of DNA vaccines.

1.2.6.2 VLPs (Virus-Like Particles)

VLPs have been demonstrated to be capable of serving as vaccine carriers and stimulating host immune responses in several studies. An immunologically active VLP consists of an epitope-rich monomeric complex viral membrane that self-assembles (Grgacic & Anderson, 2006). Fusing proteins with the particles can also enable VLPs to endogenously express multiple antigens, as well as express additional proteins. The surface of VLPs can also be chemically conjugated to produce bioconjugates with nonprotein antigens (Patel & Swartz, 2011). This distinguishing feature

of VLPs enables them to provide both virus and antigen protection (Grgacic & Anderson, 2006). When SV40 virus capsid protein is delivered to mammalian cells, a specific immune response is induced (Kawano et al., 2013). The immunogenicity of weak antigens was also increased by VLPs. *Salmonella typhi* membrane antigens, the influenza A M2 protein, and GnRH-assembled VLPs from H1N1 Nef all evoked antigen-specific humoral and cellular immune responses (Gao et al., 2018). According to some theories, VLP-based nanoformulations can help antigens to produce conformations that are similar to those of native antigens, thus stimulating the immune system more effectively (Gao et al., 2018).

1.2.6.3 Dendrimers

Benzene amines and amides are the essential ingredients in dendrimers, which are three-dimensional, monodisperse, and hyperbranched nanostructures. Dendrimers have been used to deliver antigenic molecules in a few studies. PPI and PAMAM dendrimers are two of the most widely used dendrimers for vaccine delivery. It was found that dendrimers encapsulating multiple antigens produced well-trained antibodies and T cells against the Ebola virus, H1N1 influenza, and *Toxoplasma gondii* (Chahal et al., 2016). Dendrimers are efficiently absorbed by the host cells, generating robust immune responses. In a similar study, increased cellular uptake of PMAM dendrimers led to significant improvements in HIV transactivator of transcription (TAT)-based DNA vaccine efficacy (Bahadoran et al., 2016). Dendrimers have proven to be a promising candidate for developing new-generation vaccines with enhanced immunogenic properties because they can be tailored to achieve specific biological and physicochemical properties and are capable of conjugating multiple ligands into one molecule.

1.3 Applications of Nanomaterials-Based Vaccines in Healthcare

(a) *Viral Diseases*

Before a vaccine is approved for production and marketing, it must undergo several preclinical and clinical testing stages. An infectious agent is screened for immunogenic antigens at the beginning of the vaccine development process, which stimulates an immune response in the host. Once clinical studies have been reviewed by the regulator, the developer will be allowed to begin clinical trials if the potential benefits of the new vaccine outweigh the risks of toxic or unpleasant side effects (Kim et al., 2020). Three phases of clinical trials are used to evaluate the effects of vaccines currently being developed on human subjects. The effects of vaccines currently being developed on human subjects are examined in three phases throughout clinical trials.

Phase 1 of vaccine development involves testing the vaccine on a small group of healthy adult volunteers to evaluate the induced immune response and address any safety concerns. The phase 2 trials begin with a pilot efficacy study, which confirms safety. Potential vaccines will be enrolled in phase 3 clinical trials if they demonstrate efficacy and low toxicity risks. As part of phase 3 trials, a wider number of people from a wide range of populations are involved to confirm the safety, efficacy, and dose levels of the vaccine in these groups. Tens of thousands of volunteers are often recruited from regions where viral transmission is prevalent. The higher the rate, the older the age. This includes individuals over the age of 18, as well as those suffering from underlying health issues. Phase 3 vaccines that are effective can apply to regulatory agencies for marketing approval before being produced in large quantities and marketed. Phase 4 of pharmacovigilance, during which the vaccine product is continuously and carefully monitored for safety and efficacy, will begin after marketing authorization is granted (Shin et al., 2020). It is possible to develop a vaccine under an emergency use authorization (EUA) to make it available for mass vaccination in emergencies and pandemics like the current COVID-19, even if a clinical trial is still ongoing (Kuwentrai et al., 2020). Almost 320 SARS-CoV-2 vaccine products are currently in development, with about 126 undergoing clinical trials and 194 undergoing preclinical testing (Chen et al., 2019). Only eight candidates, though, have entered phase 4 clinical trials. Because more candidates must be developed and marketed before being evaluated in real-world situations and for population-wide effects (Boopathy et al., 2019), out of these eight, only Pfizer-BNT162b2 BioNTech's and Moderna's mRNA-1273 are nonviral vector nanocarriers that rely on LNPs.

Despite the drawbacks of traditional vaccines, such as time-consuming manufacturing, very few fully approved vaccines against viruses are developed and delivered using advanced biomaterials or nonviral nanocarriers. Process, toxicity, and over infection are ongoing concerns (Tebas et al., 2021), especially the prophylactic HPV vaccines, virosomes, and VLP systems, as well as the HAV, HBV, and influenza vaccines in the past (Petkar et al., 2021). As an alternative, Shingrix®, which contains a liposomal carrier that delivers the viral antigen cargo, is authorized for clinical use by GlaxoSmithKline, a manufacturer of nano vaccines (Petkar et al., 2021). Therefore, developing clinically approved nano vaccines that meet regulators' strict quality, safety, and efficacy requirements still requires much work. SARS-CoV-2 vaccines based on LNPs, which are undergoing phase 4 clinical evaluation, have made it possible to develop additional nano vaccines using cutting-edge nanotechnological methods (Gutjahr et al., 2016). BNT162b2 and mRNA-1273 contain nucleoside-modified mRNA encoding SARS-CoV-2 spike (S) glycoproteins. As a result of these vaccines, SARS-CoV-2 was protected from infection by both humoral and cell-mediated responses. It is the stability of Pfizer-BioNTech and Moderna vaccines that makes them the most difficult to store at temperatures below 80 to 60 °C or 50 to 15 °C. It is only possible to freeze the Pfizer-BioNTech vaccine

for two weeks at 25 to 15 °C, which necessitates specialized transportation and storage equipment (Sulczewski et al., 2018).

(b) *Bacterial Diseases*

As a result of the global pandemic caused by SARS-CoV-2, antiviral defenses are urgently needed. Public health measures such as vaccination are effective. There is evidence that these vaccines are effective against specific species of *Mycobacterium*, *Burkholderia*, *Listeria monocytogenes*, *Chlamydia trachomatis*, *Borrelia burgdorferi*, *Brucella abortus*, and *Shigella flexneri*, but not against other species of bacteria (Palmer et al., 2016). During their invasion and persistence inside host cells, intracellular bacterial pathogens create a blockage to the development of defense mechanisms, which explains their success. It is common for bacteria to take advantage of nutrients, environments, locomotion potential, or other properties of host cells by exploiting antigen-presenting cells (APCs) or epithelial stromal cells. Antigenic variation, the release of immunomodulators, or the suppression of immune responses are some of the tactics bacteria can employ to evade detection inside the host cell (Chai et al., 2020). Thus, bacterial invasion of the host cell creates a safe environment that enables some bacteria to develop long-lasting or persistent and hard-to-treat infections (Thakur et al., 2019). There are multiple strains of bacteria that are drug-resistant, which complicates treatment and contribute to ongoing transmission. Despite the fact that these bacterial infections can sometimes be treated with antibiotics, new classes of antibiotics are slow to reach the clinic.

Subunit vaccines and attenuated or inactivated organisms are used in conventional vaccination methods. Live attenuated vaccines, such as the oral typhoid vaccine and Mycobacterium bovis bacillus Calmette-Guarin (BCG), may be dangerous in susceptible populations (such as the immunocompromised) (Acosta et al., 2005; Hanley, 2011). However, inactivated organisms are usually at low risk of losing their immunogenicity due to the inactivation process. Subunit vaccines are significantly less dangerous than live attenuated vaccines, but they must also be administered with adjuvants because of their low immunogenicity. In developing vaccines, the challenge is to maintain safety while delivering potent cell-mediated and humoral responses (González-Miró et al., 2018).

(c) *Parasitic Diseases*

TBV targets the sexual stages of malaria transmission and is the ideal intervention to lessen the disease's impact by preventing the vector-mediated spread and eventually eradicating the parasite from the population in endemic areas (Ariey et al., 2014). The parasite development inside the mosquitoes is hampered by immune reactions to sexual-stage antigens, which reduces transmission. Pfs230, Pfs48/45, and Pfs25 are three of the primary target antigens for TBVs, and their orthologs exist in *P. vivax*. Pfs25, which is expressed on the surface of zygotes and ookinetes, is one of the most promising targets for TBV development. Clinical trials in phase 1 and preclinical models have been conducted on the drug (Nunes et al., 2014). Recombinant Pfs25 expression in yeast and cell-free translation using wheat germ, plants, and algae has all been reported in some studies with varying degrees of transmission-blocking efficacy in preclinical

studies and phase 1 clinical trials has been reported (Kumar et al., 2014). Generating Pfs25 in its native form in any heterologous expression system has been difficult due to its complex tertiary structure, which is characterized by 22 conserved cysteine residues important for the structural integrity of the antigen (Kumar et al., 2013). An antigen formulation must trigger potent and, ideally, long-lasting antibody responses to be a successful vaccine. Effective adjuvants are incorporated, delivery methods are optimized, and particulate vaccine size is adjusted to modulate immune responses (Jones et al., 2013). Since there are no reliable adjuvants or delivery mechanisms, vaccine development has been generally difficult. Nanoparticles can be used to deliver antigens faster, resulting in a better immune response than soluble antigens (Reed et al., 2013). This is because gold nano (GN)-particles can be molded into various particle shapes and sizes, are biocompatible, and have unique physicochemical properties (Rana et al., 2012). Dendritic cells and other antigen-presenting cells can easily take up GN particles because they are nontoxic and can enhance vaccine antigen delivery. Few studies have reported GN particles' ability to deliver vaccine antigens, despite their enormous potential in biomedical imaging and diagnostics (Arnáiz et al., 2012).

(d) *Cancer Diseases*

Cancer, a major public health concern, is now seriously threatened by people's safety and health. In addition to vaccination, vaccines have also been found effective in treating cancer to reduce the risk of contracting infectious diseases. Vaccines that are known as cancer vaccines prevent both infections with cancer-causing viruses and prevent cancer from developing known as prophylactic vaccines) and treat cancer that has already developed known as therapeutic cancer vaccines (Bolhassani et al., 2011). In 2010, sipuleucel-T (Provenge), the first therapeutic cancer vaccine, received US approval to treat prostate cancer (Melief et al., 2015). Recent clinical findings suggest that cancer vaccines can improve overall survival and reduce cancer recurrence. Therefore, the clinical value of therapeutic cancer vaccines has been proven. Various methods are used in cancer vaccination to produce, enhance, or skew antitumor immunity (or a combination of these) (Jia et al., 2017). Creating more effective cancer vaccines requires a thorough understanding of how they work. Cancer vaccines are exposed to APCs when administered intradermally, subcutaneously, or intramuscularly. Vaccine antigens are presented on MHC class I or II by APCs after they have ingested the vaccine.

APCs enter the lymph nodes via the afferent lymph, prime, and activate T cells. The activated effector T cells recognize the cancer cells in the tumor bed as they move through the efferent lymph and into the blood (Chen & Mellman, 2013). Among the various cell types that can serve as APCs, dendritic cells (DCs), B cells, and macrophages are the most effective (Schlom, 2012). Two main pathways are thought to be involved in DCs' mediation of antigen presentation. In the first pathway, proteasomes in the cytosol of DCs break down endogenous antigenic proteins. CD8+ cytotoxic T lymphocytes (CTLs), the primary immunosuppressive cells that kill tumors, are stimulated by MHC-I molecules and

their peptide fragments (Gordon et al., 2014). On the other hand, exogenous antigenic proteins are taken up by DCs via endocytosis and degraded by lysosomes after they've been tinged with endosomes. The peptide fragments present in MHC-II molecules activate CD4 T lymphocytes, allowing lymphocytes to become activated, function, and survive (Alloatti et al., 2016). As such, CD4+ T cells and CTLs must be activated in order to effectively administer cancer vaccines for antitumor immunotherapy.

1.4 Challenges in the Development of Nano-Based Vaccines

Vaccination is an effective solution in fighting infectious diseases by activating immune responses for more than 200 years; however, the advancement in immunology and molecular biology demands improvement regarding the adjuvant and immunostimulant properties of vaccines. The use of nanotechnology in vaccine development provides numerous benefits over conventional vaccines (Panda, 2012a). Nano-based vaccines are the modern generation of vaccines developed by using nanoparticles as a carrier or adjuvants which have multiple benefits including improved immunogenicity, lower dosing frequency, optimum size, stability of vaccine, excellent presentation of antigen with the loading of antigen in an optimum way (Yin et al., 2022). Undoubtedly, the use of nanocarrier-based vaccines is a quite effective tool to combat infectious diseases.

This safety risk is probably the main public concern regarding nano-based vaccines. The application history of the nanoparticles with short history produces some serious concerns about long-lasting safety profiles. The prolonged exposure of inorganic nanoparticles might cause inherent toxicity while it can also cause cytotoxicity in dose-dependent manner. The biocompatibility and biodegradability profiles of nanoparticles are also important for the development of future vaccines. The scaling-up process after manufacturing is another barrier to nanovaccine development. The nanoparticles can cause unexpected immune responses after biodegradation due to varying physicochemical properties of these particles after manufacturing. Lack of guidelines regarding nanoparticle production can cause certain degrees of ambiguity for developers. The elevated production cost of nanoparticles, such as liposomes, along with various other drawbacks associated with these particles, may hinder the progress of vaccine development (Vincken et al., 2007).

The unforeseen safety risks can be compensated through the use of nanotechnology that requires good considerate of the nanoparticle's effects on immune response (Azharuddin et al., 2022). Vaccines could potentially face various types of vaccine-dependent problems and challenges including storage, administration, manufacturing, administration, acceptability, ethical considerations, and adverse reactions (Helmy et al., 2022).

The commercialization of the nano-based vaccine, cost of production, and increased complexity are also significant challenges in vaccine development. While the toxicity, lack of regulatory framework, and scaling-up process are even more important limiting factors for nanovaccine development (Bhardwaj et al., 2020).

Furthermore, lack of safety testing and analytical procedures for nano-based vaccine development regarding various compositions are to be focused for future considerations (Azharuddin et al., 2022).

1.5 Future Perspectives

Nano-based vaccines have many advantages over traditional vaccines including adjuvancity, controlled release of antigen, antigenicity, efficiency, dose regimes, nanoparticles variety, biodegradability, and administration routes. These vaccines are developed against highly infectious diseases including COVID-19, HIV, and influenza. While traditional vaccines typically necessitate frequent booster doses, nano-based vaccines have the potential to induce memory effector responses, along with humoral and cellular immune responses. Consequently, frequent boosters are not required. Various nano-based vaccines are being studied in pre-clinical trials to combat the future pandemics but the adaptation of these vaccines is not reasonable. The slow adaptation is mainly ascribed to certain issues including regulatory framework, manufacturing, and safety risks. The application of the nanoparticles in terms of their safety profiles is not well studied and, therefore, detailed studies and clinical trials are necessary to ensure the safety of nanoparticles for vaccine delivery. The provision of good regulatory framework for vaccine developers can be helpful in the development of good standard vaccines in future. Nevertheless, the successful utilization of nano-based vaccines such as the Pfizer/BioNTech and Moderna mRNA COVID-19 vaccines has provided valuable experience and highlights the potential future applications of nanobased vaccines in combating infectious diseases (Vincken et al., 2007).

Nanotools are helpful in developing seasonal or personalized vaccines to generate better immunity with broader spectrum. However, certain issues necessitate the development of an optimal solution, which should be determined through a comprehensive risk-benefit analysis. The safety testing of nano-based vaccines against various risk factors and development of analytical procedures for development of the vaccines will be required to be prioritized (Azharuddin et al., 2022). However, the advancement in nanoparticle design and upgraded knowledge of virus entry will provide further improvement in future to develop nano-based vaccines with advanced features (Ftouh et al., 2021). This advanced nanotechnology will provide innovative vaccine formulations against infectious, metabolic, and complex diseases as well as cancer (Panda, 2012b). Future vaccines are expected to be safe and more stable with strong antitumor responses (Yin et al., 2022).

1.6 Conclusions

Vaccines activate the immune system and produce adaptive immunity against a pathogen; a person is vaccinated by injecting an antigenic substance into their body.

It will be advantageous to use NPs to deliver vaccine components because they can easily encapsulate target antigens, proteins, bacteria, and parasites. NPs can also deliver a sustained release of vaccine payload into immune cells and produce long-lasting immunological effects after crossing biological barriers. Although these NP vehicles might offer promising possibilities for upcoming vaccination strategies, it's important to be aware of any potential risks, particularly those related to cytotoxicity. The recent medical history of NPs makes them unfit for long-term human use. However, the medical community has become more confident about nanovaccines with high efficacy and even a good safety profile as a result of the recent success of LNP-based COVID-19 vaccines.

References

Acosta, C. J., Galindo, C. M., Deen, J. L., Ochiai, R. L., Lee, H. J., von Seidlein, L., et al. (2005). Vaccines against cholera, typhoid fever and shigellosis for developing countries. *Expert Opinion on Biological Therapy, 4*, 1939–1951. https://doi.org/10.1517/14712598.4.12.1939

Alloatti, A., Kotsias, F., Magalhaes, J. G., & Amigorena, S. (2016). Dendritic cell maturation and cross-presentation: Timing matters! *Immunological Reviews, 272*(1), 97–108.

Alving, C. R., Beck, Z., Matyas, G. R., & Rao, M. (2016). Liposomal adjuvants for human vaccines. *Expert Opinion on Drug Delivery, 13*, 807–816. https://doi.org/10.1517/17425247.2016.1151871

Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A., Khim, N., et al. (2014). A molecular marker of artemisinin-resistant plasmodium falciparum malaria. *Nature, 505*, 50–55.

Arnáiz, B., Martínez-Ávila, O., Falcon-Perez, J. M., & Penadés, S. (2012). Cellular uptake of gold nanoparticles bearing HIV gp120 Oligomannosides. *Bioconjugate Chemistry, 23*(4), 814–825.

Azharuddin, M., Zhu, G. H., Sengupta, A., Hinkula, J., Slater, N. K., & Patra, H. K. (2022). Nano toolbox in immune modulation and nanovaccines. *Trends in Biotechnology*.

Bachmann, M. F., & Jennings, G. T. (2019). Vaccine delivery: A matter of size, geometry, kinetics and molecular patterns. *Nature Reviews Immunology, 10*, 1–15. https://doi.org/10.1038/s41577-019-0144-4

Bahadoran, A., Moeini, H., Bejo, M. H., Hussein, M. Z., & Omar, A. R. (2016). Development of Tat-conjugated dendrimer for transdermal DNA vaccine delivery. *Journal of Pharmacy & Pharmaceutical Sciences, 19*, 325–338. https://doi.org/10.18433/J3G31Q

Basu, R., Zhai, L., Contreras, A., & Tumban, E. (2018). Immunization with phage virus-like particles displaying Zika virus potential B-cell epitopes neutralizes Zika virus infection of monkey kidney cells. *Vaccine, 36*, 1256–1264. https://doi.org/10.1016/j.vaccine.2018.01.056

Baylor, N. W., Egan, W., & Richman, P. (2002). Aluminum salts in vaccines—US perspective. *Vaccine, 20*, S18–S23.

Bhardwaj, P., Tripathi, P., Gupta, R., & Pandey, S. (2020). Niosomes: A review on niosomal research in the last decade. *Journal of Drug Delivery Science and Technology, 56*, 101581.

Bolhassani, A., Safaiyan, S., & Rafati, S. (2011). Improvement of different vaccine delivery systems for cancer therapy. *Molecular Cancer, 10*, 3.

Boopathy, A. V., Mandal, A., Kulp, D. W., Menis, S., Bennett, N. R., Watkins, H. C., Wang, W., Martin, J., Thai, N. T., He, Y., et al. (2019). Enhancing humoral immunity via sustained-release implantable microneedle patch vaccination. *Proceedings of the National Academy of Sciences, 116*, 16473–16478.

Butkovich, N., Li, E., Ramirez, A., Burkhardt, A. M., & Wang, S. W. (2021). Advancements in protein nanoparticle vaccine platforms to combat infectious disease. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 13*(3), e1681. https://doi.org/10.1002/wnan.1681

Chahal, J. S., Khan OF, Cooper, C. L., McPartlan, J. S., Tsosie, J. K., Tilley, L. D., et al. (2016). Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose. *Proceedings of the National Academy of Sciences USA, 113*, E4133–E4142. https://doi.org/10.1073/pnas.1600299113

Chai, Q., Wang, L., Liu, C. H., & Ge, B. (2020). New insights into the evasion of host innate immunity by mycobacterium tuberculosis. *Cellular & Molecular Immunology, 17*, 901–913. https://doi.org/10.1038/s41423-020-0502-z

Chen, D. S., & Mellman, I. (2013). Oncology meets immunology: The cancer-immunity cycle. *Immunity, 39*(1), 1–10.

Chen, Y.-H., Lai, K.-Y., Chiu, Y.-H., Wu, Y.-W., Shiau, A.-L., & Chen, M.-C. (2019). Implantable microneedles with an immune-boosting function for effective intradermal influenza vaccination. *Acta Biomaterialia, 97*, 230–238.

Dadu, A., Kumar, P., & Kumar, A. (2021). Nanoparticle-based vaccines for infectious diseases: A review. *Journal of Biomedical Nanotechnology, 17*(8), 1425–1450. https://doi.org/10.1166/jbn.2021.3089

Datta, P., & Mandal, A. K. (2020). Nanotechnology-based approaches in anticancer research. *International Journal of Pharmaceutical Sciences and Research, 11*(7), 3072–3087. https://doi.org/10.13040/IJPSR.0975-8232.11(7).3072-87

Draper, S. J., & Heeney, J. L. (2010). Viruses as vaccine vectors for infectious diseases and cancer. *Nature Reviews. Microbiology, 8*, 62–73.

Eidi, H., Joubert, O., Attik, G., Duval, R. E., Bottin, M. C., Hamouia, A., et al. (2010). Cytotoxicity assessment of heparin nanoparticles in NR8383 macrophages. *International Journal of Pharmaceutics, 396*(1–2), 156–165.

Fan, Y., Zheng, X., Ali, Y., Berggren, P. O., & Loo, S. C. J. (2019). Local release of rapamycin by microparticles delays islet rejection within the anterior chamber of the eye. *Scientific Reports, 9*(1), 1–9.

Fang, R. H., Hu, C. M. J., Luk, B. T., Gao, W., Copp, J. A., Tai, Y., et al. (2014). Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery. *Nano Letters, 14*(4), 2181–2188.

Ftouh, M., Kalboussi, N., Abid, N., Sfar, S., Mignet, N., & Bahloul, B. (2021). Contribution of nanotechnologies to vaccine development and drug delivery against respiratory viruses. *PPAR Research*, 2021.

Gao, Y., Wijewardhana, C., & Mann, J. F. S. (2018). Virus-like particle, liposome, and polymeric particle-based vaccines against HIV-1. *Frontiers in Immunology, 9*, 345. https://doi.org/10.3389/fimmu.2018.00345

Garg, A., & Dewangan, H. K. (2020). Nanoparticles as adjuvants in vaccine delivery. *Critical Reviews™ in Therapeutic Drug Carrier Systems, 37*(2).

González-Miró, M., Rodríguez-Noda, L., Fariñas-Medina, M., Cedré-Marrero, B., Madariaga-Zarza, S., Zayas-Vignier, C., et al. (2018). Bioengineered polyester beads co-displaying protein and carbohydrate-based antigens induce protective immunity against bacterial infection. *Scientific Reports, 8*, 1–15. https://doi.org/10.1038/s41598-018-20205-7

Gordon, J. R., Ma, Y., Churchman, L., Gordon, S. A., & Dawicki, W. (2014). Regulatory dendritic cells for immunotherapy in immunologic diseases. *Frontiers in Immunology, 5*, 7.

Grgacic, E. V. L., & Anderson, D. A. (2006). Virus-like particles: passport to immune recognition. *Methods, 40*, 60–65. https://doi.org/10.1016/j.ymeth.2006.07.018

Guo, S., Fu, D., Utupova, A., Sun, D., Zhou, M., Jin, Z., & Zhao, K. (2019). Applications of polymer-based nanoparticles in vaccine field. *Nanotechnology Reviews, 8*(1), 143–155.

Gutjahr, A., Phelip, C., Coolen, A.-L., Monge, C., Boisgard, A.-S., Paul, S., & Verrier, B. (2016). Biodegradable polymeric nanoparticles-based vaccine adjuvants for lymph nodes targeting. *Vaccine, 4*, 34.

Hanley, K. A. (2011). The double-edged sword: How evolution can make or break a live-attenuated virus vaccine. *Evolution: Education and Outreach, 4*, 635. https://doi.org/10.1007/S12052-011-0365-Y

Harandi, A. M., Medaglini, D., & Shattock, R. J. (2010). Vaccine adjuvants: A priority for vaccine research. *Vaccine, 28*, 2363–2366.

He, X., Wang, K., Tan, W., Liu, B., Lin, X., et al. (2003). Bioconjugated nanoparticles for DNA protection from cleavage. *Journal of the American Chemical Society, 125*, 7168–7169. https://doi.org/10.1021/ja034450d

He, Q., Gao, Y., Zhang, L., Zhang, Z., Gao, F., Ji, X., et al. (2011). A pH-responsive mesoporous silica nanoparticles-based multi-drug delivery system for overcoming multi-drug resistance. *Biomaterials, 32*(30), 7711–7720.

Helmy, M. T., Sroor, F. M., Mahrous, K. F., Mahmoud, K., Hassaneen, H. M., Saleh, F. M., ... & Mohamed Teleb, M. A. (2022). Anticancer activity of novel 3-(furan-2-yl) pyrazolyl and 3-(thiophen-2-yl) pyrazolyl hybrid chalcones: Synthesis and in vitro studies. *Archiv der Pharmazie, 355*(3), 2100381

Ichihashi, T., Satoh, T., Sugimoto, C., & Kajino, K. (2013). Emulsified phosphatidylserine, simple and effective peptide carrier for induction of potent epitope-specific T cell responses. *PLoS One, 8*, e60068. https://doi.org/10.1371/journal.pone.0060068

Jia, Y., Omri, A., Krishnan, L., & McCluskie, M. J. (2017). Potential applications of nanoparticles in cancer immunotherapy. *Human Vaccines & Immunotherapeutics, 13*(1), 63–74.

Jones, R. M., Chichester, J. A., Mett, V., Jaje, J., Tottey, S., Manceva, S., et al. (2013). A plant-produced Pfs25 VLP malaria vaccine candidate induces persistent transmission blocking antibodies against Plasmodium falciparum in immunized mice. *PLoS One, 8*(11), e79538.

Kawano, M., Matsui, M., & Handa, H. (2013). SV40 virus-like particles as an effective delivery system and its application to a vaccine carrier. *Expert Review of Vaccines, 12*, 199–210. https://doi.org/10.1586/erv.12.149

Kheirollahpour, M., Mehrabi, M., Dounighi, N. M., Mohammadi, M., & Masoudi, A. (2020). Nanoparticles and vaccine development. *Pharmaceutical Nanotechnology, 8*(1), 6–21.

Kim, J. S., Choi, J., Kim, J. C., Park, H., Yang, E., Park, J. S., Song, M., & Park, J. (2020). Microneedles with dual release pattern for improved immunological efficacy of hepatitis B vaccine. *International Journal of Pharmaceutics, 591*, 119928.

Kumar, R., Nyakundi, R., Kariuki, T., Ozwara, H., Nyamongo, O., Mlambo, G., et al. (2013). Functional evaluation of malaria Pfs25 DNA vaccine by in vivo electroporation in olive baboons. *Vaccine, 31*(31), 3140–3147.

Kumar, R., Angov, E., & Kumar, N. (2014). Potent malaria transmission-blocking antibody responses elicited by plasmodium falciparum Pfs25 expressed in Escherichia coli after successful protein refolding. *Infection and Immunity, 82*(4), 1453–1459.

Kuwentrai, C., Yu, J., Rong, L., Zhang, B., Hu, Y., Gong, H., Dou, Y., Deng, J., Huang, J., & Xu, C. (2020). Intradermal delivery of receptor-binding domain of SARS-CoV-2 spike protein with dissolvable microneedles to induce humoral and cellular responses in mice. *Bioengineering & Translational Medicine, 6*, e10202.

Levine, M. M., & Sztein, M. B. (2004). Vaccine development strategies for improving immunization: The role of modern immunology. *Nature Immunology, 5*(5), 460–464.

Li, J., Yu, J., Xu, S., Shi, J., Xu, S., Wu, X., Fu, F., Peng, Z., Zhang, L., & Zheng, S. (2016). Immunogenicity of porcine circovirus type 2 nucleic acid vaccine containing CpG motif for mice. *Virology Journal, 13*, 1–7.

Look, M., Bandyopadhyay, A., Blum, J. S., & Fahmy, T. M. (2010). Application of nanotechnologies for improved immune response against infectious diseases in the developing world. *Advanced Drug Delivery Reviews, 62*, 378–393.

Lugade, A. A., Bharali, D. J., Pradhan, V., Elkin, G., Mousa, S. A., & Thanavala, Y. (2013). Single low-dose un-adjuvanted HBsAg nanoparticle vaccine elicits robust, durable immunity. *Nanomedicine: Nanotechnology, Biology and Medicine, 9*(7), 923–934.

Marques Neto, L. M., Kipnis, A., & Junqueira-Kipnis, A. P. (2017). Role of metallic nanoparticles in vaccinology: Implications for infectious disease vaccine development. *Frontiers in Immunology, 8*, 239.

Mehrabi, M., Montazeri, H., Mohamadpour Dounighi, N., Rashti, A., & Vakili-Ghartavol, R. (2018). Chitosan-based nanoparticles in mucosal vaccine delivery. *Archives of Razi Institute, 73*(3), 165–176.

Melief, C. J., van Hall, T., Arens, R., Ossendorp, F., & van der Burg, S. H. (2015). Therapeutic cancer vaccines. *Journal of Clinical Investigation, 125*(9), 3401–3412.

Moon, J. J., Suh, H., Bershteyn, A., Stephan, M. T., Liu, H., Huang, B., et al. (2011). Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. *Nature Materials, 10*, 243–251. https://doi.org/10.1038/nmat2960

Niikura, K., Matsunaga, T., Suzuki, T., Kobayashi, S., Yamaguchi, H., Orba, Y., ... & Sawa, H. (2013). Gold nanoparticles as a vaccine platform: influence of size and shape on immunological responses in vitro and in vivo. *ACS Nano, 7*(5), 3926–3938.

Nunes, J. K., Woods, C., Carter, T., Raphael, T., Morin, M. J., Diallo, D., et al. (2014). Development of a transmission-blocking malaria vaccine: Progress, challenges, and the path forward. *Vaccine, 32*(43), 5531–5539.

O'Hagan, D. T., & Valiante, N. M. (2003). Recent advances in the discovery and delivery of vaccine adjuvants. *Nature Reviews. Drug Discovery, 2*, 727–735.

Palmer, G. H., Bankhead, T., & Seifert, H. S. (2016). Antigenic variation in bacterial pathogens. *Microbiology Spectrum, 4*, 4.1.03. https://doi.org/10.1128/microbiolspec.VMBF-0005-2015

Panda, M., Abraham, A., & Patra, M. R. (2012a). A hybrid intelligent approach for network intrusion detection. *Procedia Engineering, 30*, 1–9.

Panda, M. K., Ladomenou, K., & Coutsolelos, A. G. (2012b). Porphyrins in bio-inspired transformations: Lightharvesting to solar cell. *Coordination Chemistry Reviews, 256*(21–22), 2601–2627.

Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines — a new era in vaccinology. *Nature Reviews Drug Discovery, 17*(4), 261–279.

Patel, K. G., & Swartz, J. R. (2011). Surface functionalization of virus-like particles by direct conjugation using azide–alkyne click chemistry. *Bioconjugate Chemistry, 22*, 376–387. https://doi.org/10.1021/bc100367u

Pati, R., Shevtsov, M., & Sonawane, A. (2018). Nanoparticle vaccines against infectious diseases. *Frontiers in Immunology, 9*, 2224.

Petkar, K., Patil, S., Chavhan, S., Kaneko, K., Sawant, K., Kunda, N., & Saleem, I. (2021). An overview of nanocarrier-based adjuvants for vaccine delivery. *Pharmaceutics, 13*, 455.

Pulendran, B. (2006). Division of labor and cooperation between dendritic cells. *Nature Immunology, 7*(7), 699–700.

Rana, S., Bajaj, A., Mout, R., & Rotello, V. M. (2012). Monolayer coated gold nanoparticles for delivery applications. *Advanced Drug Delivery Reviews, 64*(2), 200–216.

Rappuoli, R., Mandl, C. W., Black, S., & De Gregorio, E. (2011). Vaccines for the twenty-first century society. *Nature Reviews Immunology, 11*(12), 865–872.

Reed, S. G., Orr, M. T., & Fox, C. B. (2013). Key roles of adjuvants in modern vaccines. *Nature Medicine, 19*(12), 1597–1608.

Ribeiro, A. M., Souza, A. C. O., Amaral, A. C., Vasconcelos, N. M., Jeronimo, M. S., Carneiro, F. P., et al. (2013). Nanobiotechnological approaches to delivery of DNA vaccine against fungal infection. *Journal of Biomedical Nanotechnology, 9*, 221–230. https://doi.org/10.1166/jbn.2013.1491

Rice-Ficht, A. C., Arenas-Gamboa, A. M., Kahl-McDonagh, M. M., & Ficht, T. A. (2010). Polymeric particles in vaccine delivery. *Current Opinion in Microbiology, 13*, 106–112.

Sayers, E. W., Barrett, T., Benson, D. A., Bolton, E., Bryant, S. H., Canese, K., ... & Ye, J. (2012). Database resources of the national center for biotechnology information. *Nucleic Acids Research, 40*(D1), D13–D25.

Sawa, T., Ihara, H., Ida, T., Fujii, S., Nishida, M., & Akaike, T. (2013). Formation, signaling functions, and metabolisms of nitrated cyclic nucleotide. *Nitric Oxide, 34*, 10–18.

Schlom, J. (2012). Therapeutic cancer vaccines: Current status and moving forward. *Journal of the National Cancer Institute, 104*(8), 599–613.

Shah, M. A. A., Ali, Z., Ahmad, R., Qadri, I., Fatima, K., & He, N. (2015). DNA-mediated vaccines delivery through nanoparticles. *Journal of Nanoscience and Nanotechnology, 15*(1), 41–53.

Shin, M. D., Shukla, S., Chung, Y. H., Beiss, V., Chan, S. K., Ortega-Rivera, O. A., Wirth, D. M., Chen, A., Sack, M., Pokorski, J. K., et al. (2020). COVID-19 vaccine development and a potential nanomaterial path forward. *Nature Nanotechnology, 15*, 646–655.

Silva, C. L., Bonato, V. L. D., Coelho-Castelo, A. A. M., De Souza, A. O., Santos, S. A., Lima, K. M., et al. (2005). Immunotherapy with plasmid DNA encoding mycobacterial hsp65 in association with chemotherapy is a more rapid and efficient form of treatment for tuberculosis in mice. *Gene Therapy, 12*, 281–287. https://doi.org/10.1038/sj.gt.3302418

Smith, P., Haberl, H., Popp, A., Erb, K. H., Lauk, C., Harper, R., ... & Rose, S. (2013). How much land-based greenhouse gas mitigation can be achieved without compromising food security and environmental goals?. *Global Change Biology, 19*(8), 2285–2302.

Smith, J. D., Morton, L. D., & Ulery, B. D. (2015). Nanoparticles as synthetic vaccines. *Current Opinion in Biotechnology, 34*, 217–224.

Sridhar, S., Brokstad, K. A., & Cox, R. J. (2015). Influenza vaccination strategies: Comparing inactivated and live attenuated influenza vaccines. *Vaccine, 3*(2), 373–389.

Sulczewski, F., Liszbinski, R. B., Romão, P. R. T., & Junior, L. C. R. (2018). Nanoparticle vaccines against viral infections. *Archives of Virology, 163*, 2313–2325.

Sun, L., Campbell, M. G., & Dincă, M. (2016). Electrically conductive porous metal–organic frameworks. *Angewandte Chemie International Edition, 55*(11), 3566–3579.

Sun, B., & Xia, T. (2016). Nanomaterial-based vaccine adjuvants. *Journal of Materials Chemistry B, 4*(33), 5496–5509. https://doi.org/10.1039/c6tb01131d

Tebas, P., Yang, S., Boyer, J. D., Reuschel, E. L., Patel, A., Christensen-Quick, A., Andrade, V. M., Morrow, M. P., Kraynyak, K., Agnes, J., et al. (2021). Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: A preliminary report of an open-label, phase 1 clinical trial. *EClinicalMedicine, 31*, 100689.

Thakur, S., Singh, A. K., Ghrera, S. P., & Elhoseny, M. (2019). Multi-layer security of medical data through watermarking and chaotic encryption for tele-health applications. *Multimedia Tools and Applications, 78*, 3457–3470.

Villa, C. H., Dao, T., Ahearn, I., Fehrenbacher, N., Casey, E., Rey, D. A., et al. (2011). Single-walled carbon nanotubes deliver peptide antigen into dendritic cells and enhance IgG responses to tumor-associated antigens. *ACS Nano, 5*, 5300–5311. https://doi.org/10.1021/nn200182x

Vincken, P. W., ter Braak, A. P., van Erkel, A. R., Coerkamp, E. G., de Rooy, T. P., de Lange, S., ... & Bloem, J. L. (2007). MR imaging: effectiveness and costs at triage of patients with nonacute knee symptoms. *Radiology, 242*(1), 85–93.

Vyas, S., Quraishi, S., Gupta, S., & Jaganathan, K. (2005). Aerosolized liposome-based delivery of amphotericin B to alveolar macrophages. *International Journal of Pharmaceutics, 296*, 12–25. https://doi.org/10.1016/j.ijpharm.2005.02.003

Wang, T., Zou, M., Jiang, H., Ji, Z., Gao, P., & Cheng, G. (2011). Synthesis of a novel kind of carbon nanoparticle with large mesopores and macropores and its application as an oral vaccine adjuvant. *European Journal of Pharmaceutical Sciences, 44*, 653–659. https://doi.org/10.1016/j.ejps.2011.10.012

Watson, D. S., Endsley, A. N., & Huang, L. (2012). Design considerations for liposomal vaccines: Influence of formulation parameters on antibody and cell-mediated immune responses to liposome associated antigens. *Vaccine, 30*, 2256–2272. https://doi.org/10.1016/j.vaccine.2012.01.070

Wegmann, F., Gartlan, K. H., Harandi, A. M., Brinckmann, S. A., Coccia, M., Hillson, W. R., ... & Sattentau, Q. J. (2012). Polyethyleneimine is a potent mucosal adjuvant for viral glycoprotein antigens. *Nature Biotechnology, 30*(9), 883–888.

Wen, R., Umeano, A. C., Kou, Y., Xu, J., & Farooqi, A. A. (2019). Nanoparticle systems for cancer vaccine. *Nanomedicine, 14*(5), 627–648.

Whitney, S. M., Baldet, P., Hudson, G. S., & Andrews, T. J. (2001). Form I Rubiscos from non-green algae are expressed abundantly but not assembled in tobacco chloroplasts. *The Plant Journal, 26*(5), 535–547.

Yin, W., Xu, Y., Xu, P., Cao, X., Wu, C., Gu, C., ... & Xu, H. E. (2022). Structures of the Omicron spike trimer with ACE2 and an anti-Omicron antibody. *Science, 375*(6584), 1048–1053.

Zhao, L., Seth, A., Wibowo, N., Zhao, C. X., Mitter, N., Yu, C., & Middelberg, A. P. (2014). Nanoparticle vaccines. *Vaccine, 32*(3), 327–337.

Chapter 2
Nanomedicine: Insight Analysis of Emerging Biomedical Research and Developments

Suma Sarojini, Sreeja Puthenveetil Balakrishnan, Kaviya Parambath Kootery, Soma Biswas, Indhu Philip, Anushka Shitut, Anjana Baby, and Saranya Jayaram

2.1 Scope of Nanomedicine

The concept of "nanomedicine," first put forth by Freitas in 1993, was used to describe the observations, control, and treatment of biological systems of human physiology at molecular level using nanodevices and nanostructures (Freitas, 2000). With advances in research, nanomedicine (NM) has evolved to enable the diagnostics, therapeutics, and prevention of various disorders and ailments. The wide-ranging applications of nanotechnology (NT) in different industrial advancements are attributed to the novel physicochemical properties of nanoparticles (NP) like smaller size and larger size: charge ratio enhancing their efficiency when compared to other larger compounds. Numerous examples of NT can be enlisted in the field of drug design and delivery, owing to the superior specificity toward target molecules, larger size: charge ratio, reduced toxicity toward nonspecific tissues, use of "green synthesis" techniques, and so on. But on the downside, the physicochemical properties of nanoformulations have certain drawbacks like alterations to pharmacokinetics of absorption, distribution, metabolism, and elimination, the potential to cross biological barriers and persistence in human body and environment. NPs are predominantly utilized in medical applications in three areas, i.e., nanodiagnosis, nanotherapy (drug delivery), and regenerative medicine in addition to theranostics. Nanoformulations have recently been applied in treating neuropathic pain and are being tested in clinical trials, in addition to few of these formulations that have obtained FDA approval for commercialization (Ventola, 2017). Novel properties of NM include integration of effective molecules that otherwise

S. Sarojini (✉) · K. P. Kootery · S. Biswas · I. Philip · A. Shitut · S. Jayaram
Department of Life Sciences, CHRIST (Deemed to be University), Bangalore, India
e-mail: suma@christuniversity.in

S. P. Balakrishnan · A. Baby
Department of Chemistry, CHRIST (Deemed to be University), Bangalore, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_2

possess high toxicity, utilization of multiple mechanisms of action, maximization of efficiency, reduction in dosage and toxicity, and enabling controlled and targeted drug delivery combined with site-specific release. NP possesses high surface area: volume and consequently a high particle surface energy. This renders them more reactive and enables them to readily absorb biomolecules like proteins, lipids, and so on (Soares et al., 2018). Organic NPs have been known to be easily degradable into their elemental forms, thus eliminating any cytotoxic effects, and in animal trials they have been proven to be tolerable (Kuthati et al., 2020). Hence, NM encompassing a thorough understanding of pathology and diagnostics will provide efficient and effective ways to alleviate problems associated with diagnostic and therapeutic techniques.

2.1.1 Nanomedicine: R&D in Prophylaxis

Nanovaccines (NVs) are made up of polymers, macromolecules, and metals and they can be designed to interact with immune cells like B cells, phagocytic cells, etc. (Zaheer et al., 2021). Some NPs can carry antigens or immunostimulatory molecules encapsulated or as a structural element of the particle itself; however, the majority of NVs have antigens of interest coated on their surface, which allows for direct interaction with B-cell receptors (Facciola et al., 2019). In recent times, the rate of virus mutations is so high, and thus rapid development of vaccines is necessary against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Compared to nucleic acid vaccines, subunit vaccines have less safety concerns and they also showed good results in clinical trials (Chen et al., 2020). In a recent study, biodegradable silica NP encapsulated with CpG (cytosine-phosphate-guanine oligodeoxynucleotide) and coated with genetically engineered cell membrane, containing SARS-CoV-2 receptor binding domain (RBD), was used as a vaccine. After extensive studies, the specific vaccine was administered to mice to initiate immunization. After receiving this NV, they exhibited strong immune defenses and high titers of SARS-CoV-2 neutralizing antibodies. Also, using a flash nanocomplexation technique, the mass production of this vaccine was possible (Chen et al., 2022).

Another study was done by designing a nasal NV which can be inhaled against COVID-19. This vaccine to a greater extent mimics the structure of SARS-CoV-2 and promotes better mucosal immunity (Zheng et al., 2021). The vaccines which brought us hope in the midst of COVID-19 pandemic were BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna). Lipid NPs were used in these two vaccines as nanocarriers. These nanocarrier-based vaccinations demonstrate the significance of NT and the power of nanoscale delivery systems to shield payloads from deterioration and to deliver drugs directly to cells (Khurana et al., 2021). In another study, gold NPs were used as an antigen carrier and also as an adjuvant of recombinant S protein (spike protein) present in the spikes of SARS-CoV virus. Gold NP adjuvanted protein showed a very high immune response (IgG) (Sekimukai et al., 2020).

Even after the century-old BCG vaccination routine, TB remains as one of the topmost infectious diseases. In a study, BCG-primed mice were immunized with Ag85B CD4+ T cells, coated in peptide nanofibers (PNFs). After the immunization there was a steep increase in antigen-specific T cells along with resident memory (Trm) and effector memory (Tem) cells and also cytokines (Megan et al., 2022). An intranasal vaccine was developed against *Mycobacterium* based on a goal to create mucosal protection and thus prevent the infection at the site of entrance. In this study polymeric nanocapsules (NCs) were used with a chitosan polymer shell and an oily core. A Toll-like receptor-7 (TLR-7) Imiquimod, which is an immunostimulant, was encased in the oily core, and a fusion protein made with two antigens was expressed in the NC surface. This study was successful after trials in mice but prior immunization with BCG showed heightened immune response was observed (Diego et al., 2022). Johne's disease caused by *M. avium* subsp. *paratuberculosis* affects ruminants and is a major reason for economic losses in the dairy industry. A novel polyanhydride NP-based vaccine was designed against this bacterium with a core composed of cell lysate and culture filtrate. Immunization in mice was successful with no inflammatory lesions and sizable increase in T cell responses, cytokines, etc. (Thukral et al., 2020).

Infections caused by *Pseudomonas aeruginosa* are challenging because of the drug resistance these strains exhibit. Engineered mesoporous silica nanospheres (MSNs) containing cytosolic and membrane antigens from PAO1, which is a drug-resistant *P. aeruginosa* strain, were used as vaccines without any additional adjuvants. The vaccine showed a notable increase in humoral and cellular immune response in mice and protected them from drug-resistant *Pseudomonas* (Guo et al., 2022). Influenza is a persistent health hazard that causes high morbidity and mortality rate. Due to frequent changes and drifts in antigens, the efficacy of vaccines is limited to only 10–60%. A biepitope NV was discovered recently, which showed full protection against H3N2 and H1N1 viruses. The vaccine contained two conserved epitopes (M2e and CDhelix) expressed on the ferritin surface. Subcutaneous injection in mice with this vaccine showed a rise in humoral and cellular response (Qiao et al., 2022).

The Zika virus spreads through the bite of infected *Aedes* mosquitoes and this disease is creating a threat to humans, leading to epidemics. Therefore, the efficient development of vaccines is a challenge. In a recent study, a self-assembling NV was constructed with complete protection against Zika virus infection. zEDIII, a Zika virus envelope protein domain III, was expressed on recombinant human heavy chain ferritin NPs (rHF). Without any adjuvants, this zEDIII-rHF vaccine showed a remarkable upshoot in both humoral and cellular immune responses in mice. Another advantage of this vaccine is that it does not counteract with the dengue virus-2, thus overcoming the antibody-dependent enhancement (ADE) problem, which was a safety issue in Zika virus development (Rong et al., 2022). To better tackle infectious diseases and malignancies, NVs can enhance targeted delivery, antigen presentation, stimulation of the body's innate immunity, strong T cell response, and safety. Various modes of NV strategies against superbugs have been explored (Santhosh et al., 2022). Future objectives of ideal NVs include more logical design, enhanced antigen loading, extended functionalization, and targeted administration (Das & Ali, 2021).

2.1.2 Nanomedicine: R&D in Diagnostics

The use of NPs help address the quantum confinement effect. This is demonstrated with the blue shift with widerenergy gaps and more discrete energy levels, increasing the distance between the ground state and the excited state (Malhotra & Ali, 2018). They can be visualized by acting as probes or being labeled as probes while frequently acting as biosensors due to antigen-antibody reactions, allowing the diseases to be identified in the primitive stages on the basis of their biochemical markers. Based on sizes, NPs can be classified as zero-, one-, two-, and three–dimensional particles. A zero-dimensional particle includes quantum dots and metal NPs; one-dimensional particle includes nanomaterials made of metals like gold, palladium, etc.; A zero-dimensional particle includes quantum dots and metal NPs; one-dimensional particle includesnanomaterials made of metals like gold, palladium, etc.; two-dimensional particles which are bifacial, includes oneside on the nanoscale and the other in macro scale and three-dimensional particle includes bulk NPs composed of individual nanoblocks. As mentioned earlier, several modifications can be made to the NPs for them to be visualized or for them to act as biosensors. The fact that they have a large surface area makes them ideal candidates for the immobilization of larger biomolecules and as efficient biosensors. Microfluidic analyses have revealed that, by virtue of the association with groups complementary to the target biomolecules, NPs can be quite efficient. For the synthesis of these bioparticles, a signal transducer, bioreceptor, a signal processor, a site to display, and biocompatibility of the parts are essential. Precisely designed NPs can detect a multitude of diseases at initial stages itself, even with only a miniscule amount of the target binding biomolecule.

Meanwhile, NPs with high contrast can be efficient when it comes to functional and molecular imaging. NPs of sizes ranging from 5 to 100 nm are highly efficient when it comes to visualization for diagnostic purposes (Kiessling et al., 2014). Being of smaller size and capable of bonding to functional groups, they are more specific and have less noise during visualization. They have a long half-life and can be efficiently transported through the reticuloendothelial system, which helps in locating the disease or tumor site. Liposomes, iron oxide NPs, and gold NPs are examples of efficient contrast agents, supporting visualization at a nanoscale. Quantum dots are semiconductors which upon exposure to UV light shift to a higher energy level, demonstrating optical and electronic properties which can be visualized, aiding in diagnosis. They help to redimension the magnitude of the scale, allowing for ease in diagnosis at a molecular level. Iron oxide and other metal NPs can be put into resonance with the magnetic NPs. Interesting properties of iron oxide NPs however are superparamagnetic characteristic and low toxicity, making them ideal for diagnosis as well as treatment. This indicates that they display magnetic properties upon exposure to an external magnetic field allowing for MR-based imaging (Thorek et al., 2006). By increasing the specificity and precise synthesis of NPs, multimodal imaging using various techniques such as optical, MRI, CT, and PET is possible, some of which are listed in Table 2.1.

Table 2.1 Applications of nanoparticles in the molecular diagnostics of diseases

Sl no	Disease name	Disease biomarker detected	Type of nanoparticle	Method of visualization	Reference
1.	HIV	CD4+ cells produced during the infection	Nanobiosensor like NPs and QDs	Biosensor that allows for real-time ligand-receptor interaction	Farzin et al. (2020)
2.	Sepsis	C reactive protein and procalcitonin	Metal, magnetic, or lipid-based NPs	Colorimetric & electrochemical detection and magnetic separation respectively	Lim et al. (2021)
3.	Coronary artery disease	Fibrin deposition accompanied with tissue factor	Metallic-like iron oxide NPs	In vivo by ultrasound and in vitro by MRI	Lanza and Wicklin (2003)
4.	SARS-CoV-2 2	Presence of the N gene in the virus	Gold NPs with oligonucleotide dual-function biosensor	Plasmonic photothermal effect and localized surface plasmon resonance (LSPR) sensing transduction	Talebian et al. (2020)
5.	Traumatic brain injury	Plasma levels of ubiquitin-C-terminal hydrolase-L1 (UCH-L1)	Gold NPs	By high-resolution microscope or by fluorescence microscope or Raman spectroscopy	Liao et al. (2012)
6.	Alzheimer's	Aβ peptides τ (Thr181 and Thr231) proteins in varied levels between CS fluid	Iron oxide NPs	PET to view amyloid plaque, MRI for visualization of alpha beta aggregation along with morphological and structural modification	Luo et al. (2020)
7.	Brain tumor	Uptake of particle CD11b + cells and primary tumor cells	Multimodal NP CLIO-Cy5.5	Fluorescence and MRI	Kumar and Das (2017)
8	Cardiovascular disease	Myoglobin and cardiac tropin 1	NDs in HsGDY	Real-time detection through label-free visualization	Wang et al. (2021)

2.1.3 Nanomedicine in Therapeutics

Physicochemical characteristics such as particle size (>100 nm), shape (rod, sphere, wire, sheets), and chemical composition enable these nano-sized compounds to attain their function (Yetisgin et al., 2020). Since nanodrugs can move through the blood vessels and lymphatic vessels throughout the body, they can easily bind to the target site. Major goals in nanomedical research are (i) targeted drug delivery, (ii)

site-specific drug delivery, and (iii) designing of biocompatible nanocompounds (Bawarski et al., 2008).

2.1.3.1 Nanomedicine in Drug Delivery

NM can be delivered intracellularly through oral, buccal, intravenous, and subcutaneous routes (Sultana et al., 2022). Intracellular transport of NM facilitates its absorption, diffusion, and excretion. NM coated with surfactants and polymers degrades after the NM is absorbed by the cell surface (Patra et al., 2018). NPs coupled with proteins or antibodies open at the specific tight junctions where the permeability of the drug is easier. Anticancer drugs capped with NPs are also studied to bind with the target sites making the drug release and treatment easier (Liu et al., 2021). The enhanced permeability and retention property of the NP help them to transmit the NM directly at the target tissues by combining with polysaccharides, antigens, enzymes, or peptides. Internal and external stimuli also trigger some of the NP which helps in releasing the drug at the target site. Magnetic and electric fields developed by the NP also release the drugs to gather at the target site for target-specific action (Mullner, 2022).

2.1.3.1.1 Polymer-Based Nanoparticles

Biodegradable polymers include natural polymers like chitosan, alginate, gelatin, and albumin and synthetic polymers like poly D,l-lactide (PLA), poly D,l glycolide (PLG), polylactide-co-glycolide (PLGA), etc. The wide availability and the stability of the biopolymeric NP together with the body fluids is a characteristic of polymeric NP. The therapeutic agents will be coated at the surface of the polymer, and on entering the body fluids, the drugs will be released and the polymer degrades to monomers and polymers further leading to adsorption (Naki & Aderibigbe, 2022). NP coated with polyethylene glycol (PEG) is widely studied to increase the circulation of NP through blood. Polymer-based NP includes dendrimers, polymeric NP, micelles, drug conjugates, protein NP, and nanogels. The main disadvantages of polymeric NP include its high cost, complex preparation method, and agglomeration in blood streams (Ebhodaghe, 2022).

(a) Dendrimers—Dendrimers are nano-sized artificial macro molecules designed by a combination of a large number of functional groups with a dispersed structure (Marwah et al., 2022). Biological properties like nontoxicity, stability, self-assembling, electrostatic interaction, solubility, etc., make them compatible in the field of NM. Dendrimers also have the property of controlled drug delivery, making them a potential platform for drug delivery. Dendrimers are used in transdermal drug delivery and gene delivery. Dendrimers functionalized with vitamins, proteins, and antibodies improve biocompatibility further increasing site-specific drug delivery (Seidu et al., 2022).

(b) Micelles—Polymeric micelles are spherical shell-shaped NPs formed by self-assembly of polymers in aqueous solution (Jhaveri & Torchilin, 2014). The small size (30–100 nm), shapes (cylindrical, spherical, bilayer), and solubility property make the polymeric micelles a good candidate for drug administration. Polymeric micelles act as an efficient drug carrier for poorly water-soluble drugs. Advantages of micelles include reduced side effects of the drug encapsulated, easy to scale up the preparation, longer circulation time due to hydrophobic moieties, etc. A drawback of polymeric micelles is the sudden rupture of the micelles due to low molecular weight, where the drugs are released before reaching the target site. Thermosensitive micelles are used in drug delivery where structural changes occur due to thermal stimuli leading to the release of drug (Khot et al., 2022).

(c) Protein NP—Natural bio-molecules such as proteins are used as an alternative for polymers in developing nanodrug formulation (Habibi et al., 2022). The biocompatible, biodegradable, availability in natural environment, easy synthesis process, and cost-effectiveness of the proteins gives the advantage of using them in drug delivery. Protein NPs are delivered intracellularly through endocytosis. Different proteins including silk protein fibroin, human serum albumin, gelatin, legumin, lipoprotein, ferritin, etc., are used as nanocarriers (Li et al., 2022). Protein NPs are used to deliver genetic materials, anti-cancer drugs, growth factors, etc. Compared with other NPs, protein NPs are nontoxic and enable targeted drug delivery to tissues and organs. NMs from natural compounds are also studied for the treatment against cancer, diabetes mellitus, neurodegenerative diseases, etc., which has to studied further. Proteins and peptides of therapeutic importance are clustered to form nanoclusters for drug delivery (Danielsen et al., 2022).

(d) Nanogel—NGs are cross-linked polymeric NP or hydrogels with 3-D property, which are used as drug carriers (Shah et al., 2020). NGs are highly stable, permeable, and environment friendly. NGs encapsulated with hydrophilic or hydrophobic drugs can produce responses to temperature pressure and to stimulus. NGs encapsulation includes conjugation, self-assembly, physical entrapment, etc. NGs doped with ligands are developed for achieving targeted drug delivery where the ligands directly bind to the protein at the target site. Drug release from the NGs includes diffusion, ion movements, pH, and thermosensitive techniques (Sindhu et al., 2022).

(e) Nanofibers—Nanofibers are developed using electrospinning techniques and are studied for its therapeutic application (Odularu, 2022). The fundamental principle of NPs is that higher the surface area of the drug carrier, faster is the drug dispersal. Polymers coated with therapeutics are woven as fibers and are used in wound healing as the drug is having direct contact with the wound and healing occurs. Antifungal nanofibers are also used in wound healing function. Table 2.2 shows the use of different types of NP in therapeutics.

Table 2.2 Application of polymeric nanoparticles in therapeutics

Types of Nanoparticles	Size	Compounds used for therapeutics	Application	References
Dendrimers	1–5 nm	Poly(amidoamine) dendrimer Tryptophan-rich peptide dendrimer	Antibacterial activity Tumor therapy	Chauhan et al. (2020)
Micelles	>100 nm	Micelles with polyesteramide and poly(urea-urethane) PEGylated uricase micelles	Anticancer therapeutics Treatment of gout	Majumder et al. (2020)
Drug conjugates	>100 nm	Polyethylene glycol with cisplatin, carboplatin PEG-Embelin conjugate	Cancer-targeted drug delivery Antidiabetic, anti-inflammatory	Manandhar et al. (2021)
Protein nanoparticles	>100 nm	Gelatin nanoparticles Gliadin nanoparticles	Anti-cancer drugs, anti-AIDS drugs, antimalarial drugs Gastric diseases	Kianfar (2021)
Nanogels	>100 nm	Alginate (ALG) nanogel Paclitaxel nanogel	Antitumor activity Hepatocellular carcinoma Chemo immunotherapy	Yin et al. (2020)
Nanosponges	>1 μm	Curcumin nanosponges	Tumor treatment	Tiwari and Bhattacharya (2022)

2.1.3.1.2 Lipid-Based NPs

Lipid-coated NPs are considered more efficient than polymeric NP. Physiological and biodegradable lipids carriers are used as nano-safe carriers of drugs. Surface modification of the lipid NP using carbohydrates is also used in drug delivery technology (Gagliardi et al., 2021). Lipid-based NPs are used for the delivery of various biomolecules like enzymes, hormones, ribozymes, nucleic acids, and mRNAs.

(a) Solid-lipid NPs—Solid-lipid NPs have solid matrices ranging in size from 1 to 1000 nm. They are spherical, pellet, rod, or anisotropic in shape (Hamid & Manzoor, 2020). Lipid NPs are studied extensively in gene delivery, cancer therapy, protein, peptide therapy, etc. They are studied as oral drug delivery systems where rifampicin, isoniazid, and pyrazinamide-loaded nanodrugs for tuberculosis and camptothecin and tamoxifen for cancer are examples (Shirodkar et al., 2019). Lipid NPs are also studied in brain drug delivery where the particles easily pass through the blood-brain barrier. Solid-lipid NPs containing cationic lipids serve as a reservoir for hydrophobic drugs and the positively charged lipids enable cellular intake of drugs, which helps in blood-

brain penetration. Drug leakage, drug storage, and crystallization of the NP are the major drawbacks.

(b) Liposomes—These are vesicles made of one or more phospholipid bilayer with an aqueous pore at the center. Liposomes are nontoxic and biodegradable with a size of 100 nm to 5 μm (Lamichhane et al., 2018). The phosphor lipid layers of the liposomes are arranged in an order that both hydrophobic and hydrophilic molecules can bind to the liposomes. The most important advantage of liposomes is to bind itself with the cell membrane and release the drug into the cytoplasm which can be used for targeted drug delivery. Another advantage of liposomes is that more than one type of drug can be attached with the different layers of liposomes. Liposomes coated with drugs are studied to travel through the blood-brain barrier and release the drug at the target site (van der Koog et al., 2022). Liposomes are studied extensively for their therapeutic application for brain-targeted drug delivery, as vaccines, and in cancer diagnostics.

(c) Exosomes—These are extracellular vesicles of 30–150 nm size and are found in breast milk, saliva, urine, and blood (Pegtel & Gould, 2019). The vesicles isolated from the body fluids are studied to improve targeted drug delivery since the cells have an advantage over immune systems. Exosomes are studied to deliver drugs for autoimmune disease, cancer, and tissue regeneration.

(d) Virus-like particles—VLPs are nonreplicative, noninfectious proteins originated from viruses (Tornesello et al., 2022). Virus-like particles encapsulate molecules like nucleic acid and other drug compounds for drug delivery application. Hepatitis B virus cores are highly efficient NPs ranging from 30 to 34 nm in diameter for drug delivery. VLP vaccines were studied against Epstein-Barr virus-related cancer, cancer caused by T-lymphotropic virus, and cancer caused by human HPV.

2.1.3.1.3 Nonpolymeric Nanoparticles

(a) Carbon Nanotubes—CNTs are made of carbon compounds having tubular structure with a particle size of 1 nm in diameter and 100 nm in length. CNT is formed as multiwalled, single-walled nanotubes and C60 fullerenes (Kazemzadeh & Mozafari, 2019). The nanotubes enter cells by endocytosis or through insertions in cell membranes. C60 fullerenes are studied for their ability to protect mitochondria as they release free radicals, which is considered a positive property to use them as an agent for targeted drug delivery (Kaur et al., 2019). CNTs are studied for their ability to deliver antibiotics, antiviral drugs, and cancer drugs. Surface-functionalized nanotubes are used to deliver therapeutic materials at specialized target sites.

(b) Metallic NPs—Metallic NPs (1–100 nm in size) made of gold, iron, nickel, cobalt, and its oxides are synthesized with an outer functional group so that the therapeutic agent can be bound to the functional group (Kovács et al., 2022). Since these compounds have magnetic properties, they are used for diagnostic purposes. Many such NPs have been explored as having multiple modes of

antibacterial activity too (Sarojini & Jayaram, 2021). Gold NPs are widely used in cancer therapy and diagnostics as they are relatively less cytotoxic and targeted to tumor tissues to kill cancer cells using its photothermal therapeutical characteristics which in response to light or magnetic stimuli were studied for controlled drug release (Clasky et al., 2021).

(c) Quantum dots—These are NPs with 2–10 nm in diameters and are made with a semiconducting material (Campbell et al., 2021). They are made of inorganic core and aqueous outer shell. The outer shell is used to conjugate biomolecules. Quantum dots are highly toxic on exposure to UV light or aqueous regions which is considered as a major drawback in therapeutic research.

(d) Silica-based NPs—Silica NPs have high surface area, have larger pore size ranging from 2 to 50 nm, are biodegradable, and exhibit aqueous dispersal property, which make them a good drug carrier (Trzeciak et al., 2021). Poorly water-soluble drug camptothecin (cancer drug) incorporated with silica mesoporous NPs was used for targeted drug delivery resulting in apoptosis of cancerous cells. Mesoporous silica NPs are biocompatible with high loading capacity with targeted drug delivery capacity.

2.2 Nanoparticles in Cancer Diagnosis

Early-stage detection is of paramount significance for successful cancer therapy, which can lead to a drastic reduction in cancer-related mortality (Zhang et al., 2019). The large surface area and volume ratio make NPs an excellent material for cancer detection compared to bulk material. Due to their adaptability and affordability, NT-based solutions are presently being created and developed as a viable tool for real-time cancer diagnosis and detection (Chen et al., 2018). NPs can be easily surface functionalized with functional groups, making cancer diagnosis more efficient (Chaturvedi et al., 2018). Figure 2.1 shows some of the applications of biosensors in the field of medicine.

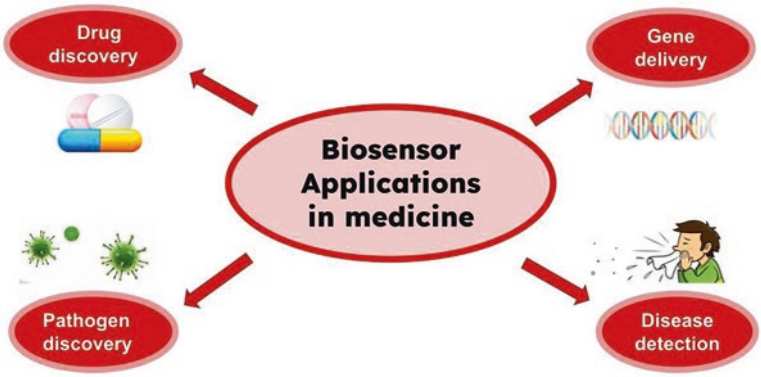


Fig. 2.1 Applications of biosensors in the field of medicine

2.2.1 Nanoparticles in Bioimaging/Biosensing

Early disease identification and development of novel treatments are made possible by biosensing and bioimaging techniques, which enable researchers to visualize the internal structures and investigate the physiological processes of living systems at molecular level (Pu, 2019). They are particularly useful for examining the dynamics of tumor progression by locating tumor sites, characterizing cell phenotype, and visualizing key biological processes using cell composition and other indicators inside the tumor microenvironment (Chung et al., 2021). An analyte-binding input modality and a detectable output modality are both included in a biosensor (Yur et al., 2021). Optical imaging (fluorescence (FL), two-photon FL, and Raman imaging) (Berckman & Chen, 2020), PET/SPECT (positron emission tomography/single-photon emission computed tomography) (Cui et al., 2018), MRI (magnetic resonance imaging) (Wu & Huang, 2017), PAI (photoacoustic imaging) (Lemaster & Jokerst, 2017), and multimodal imaging CT (computed tomography) represent some of the molecular imaging techniques currently adopted using NPs (Lin et al., 2016). Each bio-imaging strategy is distinct and has its own set of benefits and drawbacks. As a result, single imaging will not meet the requirements. To overcome the issue a series of modulation imaging for visualization is being researched (Rostami et al., 2019). Biomedical imaging probes based on inorganic NPs have been intensively researched as a potential alternative to conventional molecular imaging probes. They have better adaptability in terms of enhanced multimodal, stimuli-responsive, and targeted imaging performance. Revolutionary findings in the field of imaging probes have led to excellent molecular and subcellular bioimaging (Kim et al., 2018).

2.2.2 Nanoparticles in Drug Delivery

One important area of NT with many potential applications in the field of cancer research is the delivery of drugs and the targeting of malignant cells using a variety of nano-based biomaterials (Limeres et al., 2019). The drug nanocarrier system is versatile as it enhances cancer treatment efficiency, selectively raises the number of drugs accumulated within target tumor cells, and reduces therapeutic agent toxicity in neighboring cells. Nanocarriers are highly soluble, capable of transporting a wide range of medications, have an expanded surface area, and can be used for a variety of delivery methods (Khodabandehloo et al., 2016). The difficulty for the drug delivery system is to deliver drug molecules exactly at the target region without harming healthy cells (Yaqoob et al., 2020).

Two targeting techniques—active targeting and passive targeting—are used to deliver NPs to the tumor tissue. Enhanced permeation and retention effect (EPR) is the phenomenon in passive targeting. The most promising and efficient method for increasing the specificity of the NPs is termed “active targeting” (Ingle et al., 2017).

Due to their optical, mechanical, electrical, and thermal properties, several carbon-based nanomaterials (CNMs) have recently attracted significant research interest in the development of cancer treatments. They are more biocompatible and safer than metal-based nanomaterials (Kumari et al., 2016). These CNMs can be functionalized, either covalently (chemical bond formation) or noncovalently (physio adsorption), as per the requirement, to make them biocompatible (Clemons et al., 2018).

Photodynamic therapy (PDT) is used to treat abnormal cells in the presence of light. Earlier, Eosin was used as a photosensitizer for tumors which developed cytotoxicity and skin damage. The issue was addressed by introducing hematoporphyrin as a photosensitizing agent, which acts as a carrier too. The superior high mechanical strength, optical properties, low toxicity, and biocompatibility have made carbon-based nanomaterials a potential material in PDT (Bhattacharya et al., 2016). NPs are an effective material in PDT because of their distinctive properties as carriers, sensitizers, energy transducers, or enhancers for reactive oxygen species (ROS) formation. Compared to other therapeutic techniques, PDT is considered less harmful to healthy cells. Metal-organic frameworks are yet another class of nanomaterials used in PDT. These highly flexible hybrid structures are designed from metal ions or clusters using organic linkers. In order to increase blood flow time and improve their ability to target tumors, MOF NPs are loaded and altered (van Straten et al., 2017).

Another technique used for the cancer treatment is photothermal therapy (PTT), in which near-infrared radiations are used. In this therapeutic technique, radiations are used to inflict heat for damaging the cancer cells (Fig. 2.2). PTT uses photothermal

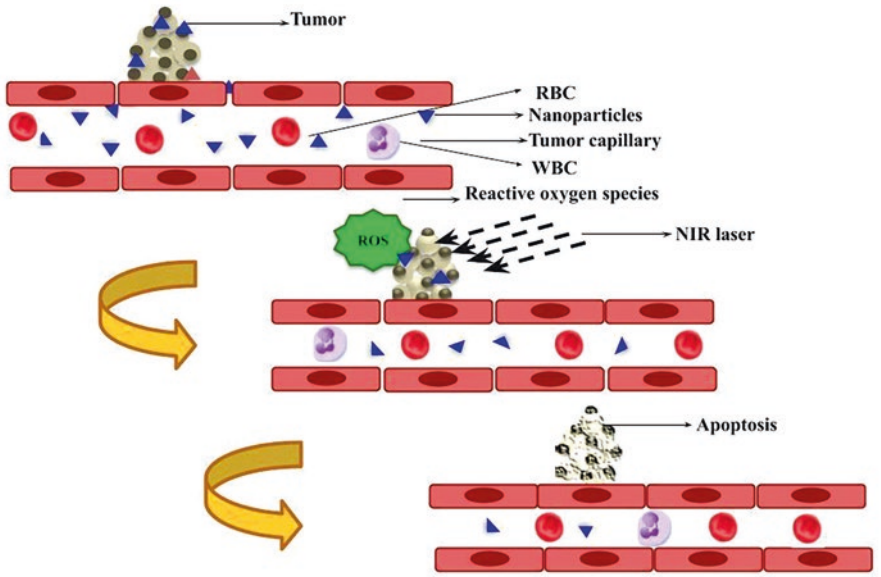


Fig. 2.2 Schematic illustration of PTT mechanism

agents (PTAs) with high PT conversion efficacy to convert light to heat using NIR (Lucky et al., 2015). The anticancer effects of FA-GO/SF (folic acid (FA)-conjugated polyvinyl pyrrolidone functionalized graphene oxides) were improved by the photothermal effect while exposing GO to near-infrared irradiation (Babu et al., 2022). FA-GO transforms it into an anticancer drug carrier capable of pharmacological and photothermal effects. Gold NPs and CNTs have attracted interest recently for potential PTT applications. A two-step reduction technique was used to create the hybrid of gold nanostars and MWCNTs. The biocompatibility of these hybrids exhibited high photothermal efficiency compared to the gold nanostars (Zou et al., 2016).

2.3 Challenges in Nanomedicine R & D

NM has potential advantages such as the delivery of NP-based drugs, vaccine delivery, and cell-based diagnosis. Research and development in this field has greatly enhanced NP-based screening, diagnosis, and treatment of diseases (Kargozar & Mozafari, 2018). However, very few NP-based drugs get clinical approval to use because of challenges at different stages in drug development.

The safety challenges arise when nanotherapeutics interact with the body once it enters the bloodstream, extracellular matrix, cell organelles and cytoplasm. Different NMs require different pharmacokinetic parameters that are very different from the commercially available drugs. Thus, there remains a lack of proper methods and standards to evaluate the safety of nanodrugs. In recent years, researchers are addressing toxicity issues of nanodrugs for human health. These NMs upon interacting with intracellular organelles, proteins, polysaccharides, and enzymes may alter their signaling pathways, complicating the safety issues. Thus, the development of standard tests for toxicity evaluation of NM becomes the need of the hour (Wu et al., 2020; Zhang et al., 2020).

Scale-up issues—For clinical and pre-clinical studies of NM, only a small amount of product is needed; thus, robustness and effectiveness can be maintained in small-scale production of the nanodrugs. On the other hand, large-scale production of nanodrugs shows polydispersity and changes in physical and chemical properties such as drug composition, size, surface charge, crystallinity, and therapeutic outcomes of nanodrugs, thus compromising the stability in manufacturing process. Methods for synthesis and characterization of nanoformulations and upscaling their production remains a time-consuming and costly affair (Metselaar & Lammers 2020; Zhang et al., 2020).

Biological Challenges—First challenge is the increased biodistribution of nanodrugs to target sites such as cells and tissues and reduced accumulation in healthy tissues. Secondly, the interaction between nanodrugs and biological barriers such as cells, skin, organs, etc., may interfere with the drug delivery system thereby, affecting the efficiency and stability of nanotherapeutics. The understanding of nano-bio interactions with the goal of overcoming and transporting the nanodrugs to target sites is the need of the hour. Another problem is the storage of NM leads to loss of its stability. The nanomaterials used in the preparation of NM are usually

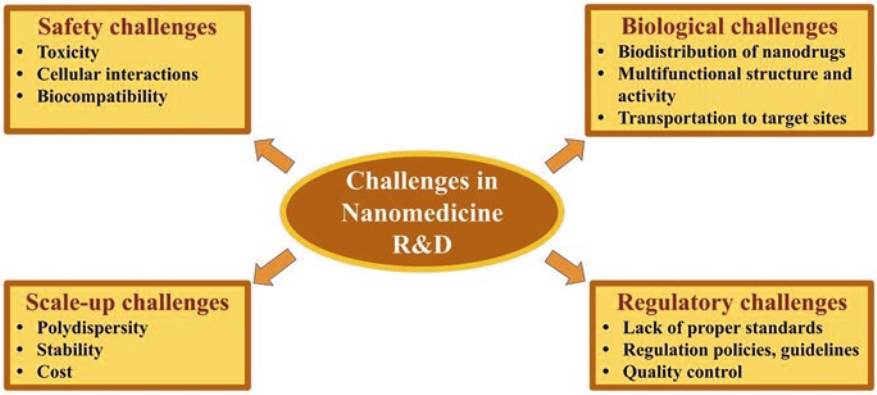


Fig. 2.3 Schematic representation of challenges faced in nanomedicine R&D

biodegradable, so while storing, its property could alter (Wu et al., 2020; Abdel-Mageed et al., 2021).

Regulatory Challenges—Regulations laid down by FDA and EMA (European Medicines Agency) are very different and change from time to time which may affect the approval of nanodrugs, which are in the clinical trial stage. Another obstacle is nanotherapeutics approved in one country do not get approval from other countries. Thus, due to the lack of proper standards and regulation policy, detailed guidelines for proper characterization and quality control for nanodrugs are the major barriers to develop nanotherapeutics (Farjadian et al., 2019). Major challenge in developing cancer-targeted anti-angiogenic NM demands intensive understanding of cancer-related metabolic processes, their interaction with immune cells and body, optimizing pharmacokinetic parameters, and grasping their biodegradative property (Mukherjee et al., 2020). A few of the challenges faced in nanomedicine R&D is depicted in Fig. 2.3.

2.4 Future Perspectives

Recent times have witnessed the emergence of different diseases, many of which have their underlying pathophysiology in the disturbances at the nanoscale levels of genes and molecules (like gene mutations and protein misfolding). The correlation between nanoscale understanding of disease mechanisms, progression, and therapy, with the need for comprehensive knowledge about physicochemical properties of NP, forms the basis of NM for targeted and effective approaches. There exists an additional concern that needs to be resolved to eliminate nonspecific toxicity, i.e., regulating the interaction between NP and the biological environment. Here, considerable importance needs to be given to understand the interactions between the surface of NPs and surrounding biological components and fluids (phospholipids,

vesicles, etc.) that could subsequently affect the pathophysiological responses. This interaction is affected by the composition of NP and nature of the suspending media (solvents, water molecules, salts, etc.) used. Together, there arises variations in the physicochemical properties of NP size, surface area, shape, surface charge, roughness, porosity, conduction states, hydrophobic/hydrophilic nature, and so on. In tandem, these factors will affect the physiological responses brought about by the cells being targeted with these NP (Nel et al., 2009). Small-scale setups enable easy regulation of nanoformulations, but at the larger scale, even slight errors could result in significant changes in physicochemical properties of NP that will impact their efficiency, quality, reproducibility, and safety. Subsequently, these erroneous particles when administered in diagnostics or therapy would elicit inappropriate physiological responses. Hence, in accordance with specific disease pathophysiology, we need to augment the physicochemical properties of NP and tailor them to resolve the disease or diagnostics being targeted. A global consensus aimed toward harmonizing and integrating the regulatory, safety, and scientific protocols of NM will further catalyze the standardization of the usage of NP with maximum reproducibility, safety, efficacy, and specificity.

Acknowledgments The authors thank the Department of Life Sciences and the Department of Chemistry, CHRIST (Deemed to be University), Bangalore, India, for the support and facilities provided for the study.

References

Abdel-Mageed, H. M., AbuelEzz, N. Z., Radwan, R. A., & Mohamed, S. A. (2021). Nanoparticles in nanomedicine: A comprehensive updated review on current status, challenges and emerging opportunities. *Journal of Microencapsulation, 38*(6), 414–436. https://doi.org/10.1080/02652048.2021.1942275

Babu, N., Rahaman, S. A., John, A. M., & Balakrishnan, S. P. (2022). Photosensitizer anchored nanoparticles: A potential material for photodynamic therapy. *Chemistry Select, 7*(17). https://doi.org/10.1002/slct.202200850

Bawarski, W. E., Chidlowsky, E., Bharali, D. J., & Mousa, S. A. (2008). Emerging nanopharmaceuticals. *Nanomedicine: Nanotechnology, Biology and Medicine, 4*(4), 273. https://doi.org/10.1016/j.nano.2008.06.002

Berckman, E. A., & Chen, W. (2020). A modular approach for dCas9-mediated enzyme cascadingviaorthogonal bioconjugation. *Chemical Communications, 56*(77), 11426–11428. https://doi.org/10.1039/d0cc04196c

Bhattacharya, K., Mukherjee, S. P., Gallud, A., Burkert, S. C., Bistarelli, S., Bellucci, S., et al. (2016). Biological interactions of carbon-based nanomaterials: From coronation to degradation. *Nanomedicine: Nanotechnology, Biology, and Medicine, 12*(2), 333–351. https://doi.org/10.1016/j.nano.2015.11.011

Campbell, E., Hasan, M. T., Gonzalez-Rodriguez, R., Truly, T., Lee, B. H., Green, K. N., et al. (2021). Graphene quantum dot formulation for cancer imaging and redox-based drug delivery. *Nanomedicine, 37*, 102408. https://doi.org/10.1016/j.nano.2021.102408

Chaturvedi, V. K., Singh, A., Singh, V. K., & Singh, M. P. (2018). Cancer nanotechnology: A new revolution for cancer diagnosis and therapy. *Current Drug Metabolism, 20*(6), 416–429. https://doi.org/10.2174/1389200219666180918111528

Chauhan, A., Krynicka, D., & Singh, M. K. (2020). Therapeutic dendrimers. In *Pharmaceutical applications of dendrimers* (Micro and Nano Technologies) (pp. 275–287). Elsevier. https://doi.org/10.1016/B978-0-12-814527-2.00012-3

Chen, X. J., Zhang, X. Q., Liu, Q., Zhang, J., & Zhou, G. (2018). Nanotechnology: A promising method for oral cancer detection and diagnosis. *Journal of Nanobiotechnology, 16*, 52. https://doi.org/10.1186/s12951-018-0378-6

Chen, W.H., Strych, U., Hotez, P. J., Bottazzi , M.E. (2020). The SARS-CoV-2 vaccine pipeline: an overview.*Current tropical medicine reports, 7*(2), 1-4.

Chen, X., Shi, T., Yang, C., Chen, F., He, X., Zhang, K., et al. (2022). Scalable biomimetic SARS-CoV-2 nanovaccines with robust protective immune responses. *Signal Transduction and Targeted Therapy, 96*(7), 1–3. https://doi.org/10.1038/s41392-022-00942-y

Chung, S., Revia, R. A., & Zhang, M. (2021). Graphene quantum dots and their applications in bioimaging, biosensing, and therapy. *Advanced Materials, 33*(22), 1–26. https://doi.org/10.1002/adma.201904362

Clasky, A. J., Watchorn, J. D., Chen, P. Z., & Gu, F. X. (2021). From prevention to diagnosis and treatment: Biomedical applications of metal nanoparticle-hydrogel composites. *Acta Biomaterialia, 122*, 1. https://doi.org/10.1016/j.actbio.2020.12.030

Clemons, T. D., Singh, R., Sorolla, A., Chaudhari, N., Hubbard, A., & Iyer, K. S. (2018). Distinction between active and passive targeting of nanoparticles dictate their overall therapeutic efficacy. *Langmuir, 34*(50), 15343–15349. https://doi.org/10.1021/acs.langmuir.8b02946

Cui, S., Zhang, S., & Yue, S. (2018). Raman spectroscopy and imaging for cancer diagnosis. *Journal of Healthcare Engineering, 2018*. https://doi.org/10.1155/2018/8619342

Danielsen, M., Hempel, C., Andresen, T. L., & Urquhart, A. J. (2022). Biopharmaceutical nanoclusters: Towards the self-delivery of protein and peptide therapeutics. *Journal of Controlled Release, 347*, 282. https://doi.org/10.1016/j.jconrel.2022.04.050

Das, A., & Ali, N. (2021). Nanovaccine: An emerging strategy. *Expert Review of Vaccines, 20*(9), 1–8. https://doi.org/10.1080/14760584.2021.1984890

Diego, L., Campo, J., Paul, J., Singh, M., Reljic, R., Alonso, J., et al. (2022). Design of polymeric nanocapsules for intranasal vaccination against mycobacterium tuberculosis: Influence of the polymeric shell and antigen positioning. *Pharmaceutics, 12*(6), 1–22. https://doi.org/10.3390/pharmaceutics12060489

Ebhodaghe, S. O. (2022). A scoping review on the biomedical applications of polymeric particles. *International Journal of Polymeric Materials and Polymeric Biomaterials, 1*. https://doi.org/10.1080/00914037.2022.2032708

Facciola, A., Visalli, G., Lagana, P., La Fauci, V., Squeri, R., Pellicano, G. F., et al. (2019). The new era of vaccines: the "nanovaccinology". *European Review for Medical and Pharmacological Sciences, 23*(16), 7163–7182. https://doi.org/10.26355/eurrev_201908_18763

Farjadian, F., Ghasemi, A., Gohari, O., Roointan, A., Karimi, M., & Hamblin, M. R. (2019). Nanopharmaceuticals and nanomedicines currently on the market: Challenges and opportunities. *Nanomedicine, 14*(1), 93–126. https://doi.org/10.2217/nnm-2018-0120

Farzin, L., Shamsipur, M., Samandari, L., & Sheibani, S. (2020). HIV biosensors for early diagnosis of infection: The intertwine of nanotechnology with sensing strategies. *Talanta, 206*, 120201.

Ferrari, M. (2005). Cancer nanotechnology: Opportunities and challenges. *Nature Reviews Cancer, 5*(3), 161–171.

Freitas, R. A., Jr. (2000). Nanodentistry. *Journal of the American Dental Association (1939), 131*, 1559.

Gagliardi, A., Giuliano, E., Venkateswararao, E., Fresta, M., Bulotta, S., Awasthi, V., & Cosco, D. (2021). Biodegradable polymeric nanoparticles for drug delivery to solid tumors. *Frontiers in Pharmacology, 12*, 601626. https://doi.org/10.3389/fphar.2021.601626

Guo, Z., Zhu, Y., Du, Z., Qin, M., He, C., He, P., et al. (2022). Rapid development of a subunit nano-vaccine against drug-resistant Pseudomonas aeruginosa with effective cross-protection. *Nano Today, 43*(1), 1–10. https://doi.org/10.1016/j.nantod.2022.101398

Habibi, N., Mauser, A., Ko, Y., & Lahann, J. (2022). Protein nanoparticles: Uniting the power of proteins with engineering design approaches. *Advanced Science, 9*(8), 2104012. https://doi.org/10.1002/advs.202104012

Hamid, R., & Manzoor, I. (2020). Nanomedicines: Nano based drug delivery systems challenges and opportunities. *Alternative Medicine-Update.* https://doi.org/10.5772/intechopen.94353

Ingle, A. P., Paralikar, P., Grupenmacher, A., Padovani, F. H., Ferrer, M. T., Rai, M., & Alves, M. (2017). Nanotechnological interventions for drug delivery in eye diseases. In *Nanotechnology applied to pharmaceutical technology* (pp. 279–306). https://doi.org/10.1007/978-3-319-70299-5_12

Jhaveri, A. M., & Torchilin, V. P. (2014). Multifunctional polymeric micelles for delivery of drugs and siRNA. *Frontiers in Pharmacology, 5*, 77. https://doi.org/10.3389/fphar.2014.00077

Kargozar, S., & Mozafari, M. (2018). Nanotechnology and nanomedicine: Start small, think big. *Materials Today: Proceedings, 5*(7), 15492–15500. https://doi.org/10.1016/j.matpr.2018.04.155

Kaur, J., Gill, G. S., & Jeet, K. (2019). Applications of carbon nanotubes in drug delivery: A comprehensive review. In *Characterization and biology of nanomaterials for drug delivery* (Vol. 113). Elsevier. https://doi.org/10.1016/j.cbpa.2005.10.005

Kazemzadeh, H., & Mozafari, M. (2019). Fullerene-based delivery systems. *Drug Discovery Today, 24*(3), 898. https://doi.org/10.1016/j.drudis.2019.01.013

Khodabandehloo, H., Zahednasab, H., & Hafez, A. A. (2016). Nanocarriers usage for drug delivery in cancer therapy. *International Journal of Cancer Management, 9*(2). https://doi.org/10.17795/ijcp-3966

Khot, P., Nangare, K., Payghan, V., Kamble, T., & Payghan, S. (2022). Drug delivery systems based on polymeric micelles. *Asian Journal of Research in Pharmaceutical Science, 12*(1). https://doi.org/10.52711/2231-5659.2022.00007

Khurana, A., Allawadhi, P., Khurana, I., Allwadhi, S., Weiskirchen, R., Banothu, A., et al. (2021). Role of nanotechnology behind the success of mRNA vaccines for COVID-19. *Nano Today, 38*(1), 1–6. https://doi.org/10.1016/j.nantod.2021.101142

Kianfar, E. (2021). Protein nanoparticles in drug delivery: Animal protein, plant proteins and protein cages, albumin nanoparticles. *Journal of Nanobiotechnology, 19*(1), 1–32. https://doi.org/10.1186/s12951-021-00896-3

Kiessling, F., Mertens, M. E., Grimm, J., & Lammers, T. (2014). Nanoparticles for imaging: Top or flop. *Radiology, 273*(1), 10.

Kim, D., Kim, J., Park, Y. I., Lee, N., & Hyeon, T. (2018). Recent development of inorganic nanoparticles for biomedical imaging. *ACS Central Science, 4*(3), 324–336. https://doi.org/10.1021/acscentsci.7b00574

Kovács, D., Igaz, N., Gopisetty, M. K., & Kiricsi, M. (2022). Cancer therapy by silver nanoparticles: Fiction or reality. *International Journal of Molecular Sciences, 23*(2), 839. https://doi.org/10.3390/ijms23020839

Kumar, M. S., & Das, A. P. (2017). Emerging nanotechnology-based strategies for diagnosis and therapeutics of urinary tract infections: A review. *Advances in Colloid and Interface Science, 249*, 53–65.

Kumar, S., Bajpai, R. P., & Bharadwaj, L. M. (2004). Diagnosis of tuberculosis based on BioMEMS. In *International conference on intelligent sensing and information processing, Proceedings of IEEE* (Vol. 237). https://doi.org/10.1109/ICISIP.2004.1287659

Kumari, P., Ghosh, B., & Biswas, S. (2016). Nanocarriers for cancer-targeted drug delivery. *Journal of Drug Targeting, 24*(3), 179–191. https://doi.org/10.3109/1061186X.2015.1051049

Kuthati, Y., Navakanth Rao, V., Busa, P., Tummala, S., Davuluri Venkata Naga, G., & Wong, C. S. (2020). Scope and applications of nanomedicines for the management of neuropathic pain. *Molecular Pharmaceutics, 17*(4), 1015.

Lamichhane, N., Udayakumar, T. S., D'Souza, W. D., Simone, C. B., Raghavan, S. R., Polf, J., & Mahmood, J. (2018). Liposomes: Clinical applications and potential for image-guided drug delivery. *Molecules, 23*(2), 288. https://doi.org/10.3390/molecules23020288

Lanza, G. M., & Wickline, S. A. (2003). Targeted ultrasonic contrast agents for molecular imaging and therapy. *Current Problems in Cardiology, 28*(12), 625–653.

Lemaster, J. E., & Jokerst, J. V. (2017). What is new in nanoparticle-based photoacoustic imaging? *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 9*(1). https://doi.org/10.1002/wnan.1404

Li, Z., Zhao, T., Li, J., Yu, Q., Feng, Y., Xie, Y., & Sun, P. (2022). Nanomedicine based on natural products: Improving clinical application potential. *Journal of Nanomaterials*. https://doi.org/10.1155/2022/3066613

Liao, Y. H., Chang, Y. J., Yoshiike, Y., Chang, Y. C., & Chen, Y. R. (2012). Negatively charged gold nanoparticles inhibit Alzheimer's amyloid-β fibrillization, induce fibril dissociation, and mitigate neurotoxicity. *Small, 8*(23), 3631–3639.

Lim, J., Lee, Y. Y., Choy, Y. B., Park, W., & Park, C. G. (2021). Sepsis diagnosis and treatment using nanomaterials. *Biomedical Engineering Letters, 11*(3), 1–14. https://doi.org/10.1007/s13534-021-00200-0

Limeres, M. J., Moretton, M. A., Bernabeu, E., Chiappetta, D. A., & Cuestas, M. L. (2019). Thinking small, doing big: Current success and future trends in drug delivery systems for improving cancer therapy with special focus on liver cancer. *Materials Science and Engineering C, 95*, 328–341. https://doi.org/10.1016/j.msec.2018.11.001

Lin, J., Chen, X., & Huang, P. (2016). Graphene-based nanomaterials for bioimaging. *Advanced Drug Delivery Reviews, 105*, 242–254. https://doi.org/10.1016/j.addr.2016.05.013

Liu, G., Yang, L., Chen, G., Xu, F., Yang, F., Yu, H., Li, L., Dong, X., Han, J., Li, B., et al. (2021). A review on drug delivery system for tumor therapy. *Frontiers in Pharmacology, 12*, 735446. https://doi.org/10.3389/fphar.2021.735446

Lucky, S. S., Soo, K. C., & Zhang, Y. (2015). Nanoparticles in photodynamic therapy. *Chemical Reviews, 115*(4), 1990–2042. https://doi.org/10.1021/cr5004198

Luo, S., Ma, C., Zhu, M. Q., Ju, W. N., Yang, Y., & Wang, X. (2020). Application of iron oxide nanoparticles in the diagnosis and treatment of neurodegenerative diseases with emphasis on Alzheimer's disease. *Frontiers in Cellular Neuroscience, 14*, 21.

Majumder, N., Das, N. G., & Das, S. K. (2020). Polymeric micelles for anticancer drug delivery. *Future Sciences, 11*(10), 613–635. https://doi.org/10.4155/tde-2020-0008

Malhotra, B. D., & Ali, M. A. (2018). Nanomaterials in biosensors: Fundamentals and applications. Nanomaterials for Biosensors. 1–74. PMCID: PMC7152100. https://doi.org/10.1016/B978-0-323-44923-6.00001-7

Manandhar, S., Sjöholm, E., Bobacka, J., Rosenholm, J. M., & Bansal, K. K. (2021). Polymer-drug conjugates as nanotheranostic agents. *Journal of Nanotheranostics, 2*(1), 63–81. https://doi.org/10.3390/jnt2010005

Marwah, H., Singh, S., Kesharwani, P., & Arora, S. (2022). Dendrimer nanohybrid systems for drug delivery. In *Hybrid nanomaterials for drug delivery* (p. 245). Woodhead Publishing. https://doi.org/10.1016/b978-0-323-85754-3.00009-5

Megan, A., Naqvi, F., & Saito, B. (2022). Self-adjuvanting nanovaccines boost lung-resident $CD4^+$ T cell immune responses in BCG-primed mice. *npj Vaccines, 7*(48), 1–14. https://doi.org/10.1038/s41541-022-00466-0

Metselaar, J. M., & Lammers, T. (2020). Challenges in nanomedicine clinical translation. *Drug Delivery and Translational Research, 10*(3), 721–725. https://doi.org/10.1007/s13346-020-00740-5

Mukherjee, A., Madamsetty, V. S., Paul, M. K., & Mukherjee, S. (2020). Recent advancements of Nanomedicine towards antiangiogenic therapy in cancer. *International Journal of Molecular Sciences, 21*(2), 455. https://doi.org/10.3390/ijms21020455

Müllner, M. (2022). Molecular polymer bottlebrushes in nanomedicine: Therapeutic and diagnostic applications. *Chemical Communications, 58*(38), 5683. https://doi.org/10.1039/D2CC01601J

Naki, T., & Aderibigbe, B. A. (2022). Efficacy of polymer-based nanomedicine for the treatment of brain cancer. *Pharmaceutics, 14*(5), 1048.

Nel, A. E., Mädler, L., Velegol, D., Xia, T., Hoek, E. M., Somasundaran, P., et al. (2009). Understanding biophysiochemical interactions at the nano-bio interface. *Nature Materials, 7*, 543. https://doi.org/10.1038/nmat2442

Odularu, A. T. (2022). Basic principles of electrospinning, mechanisms, nanofibre production, and anticancer drug delivery. *Journal of Chemistry*. https://doi.org/10.1155/2022/9283325

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta-Torres, L. S., et al. (2018). Nano based drug delivery systems: Recent developments and future prospects. *Journal of Nanobiotechnology, 16*(1), 1. https://doi.org/10.1186/s12951-018-0392-8

Pegtel, D. M., & Gould, S. J. (2019). Exosomes. *Annual Review of Biochemistry, 88*, 487. https://doi.org/10.1146/annurev-biochem-013118-111902

Pu, K. (2019). Biosenors and bioimaging. *Chembiochem, 20*(4), 420–421. https://doi.org/10.1002/cbic.201900021

Qiao, Y., Zhang, Y., & Chen, J. (2022). A biepitope, adjuvant-free, self-assembled influenza nanovaccine provides cross-protection against H3N2 and H1N1 viruses in mice. *Nano Research, 20*(1), 1–11. https://doi.org/10.1007/s12274-022-4482-4

Rong, H., Qi, M., Pan, J., Sun, Y., Gao, J., Zhang, X., Li, W., Zhang, B., Zhang, X. E., & Cui, Z. (2022). Self-assembling nanovaccine confers complete protection against Zika virus without causing antibody-dependent enhancement. *Frontiers in Immunology, 13*(1), 1–13. https://doi.org/10.3389/fimmu.2022.905431

Rostami, I., Alanagh, H. R., Hu, Z., & Shahmoradian, S. H. (2019). Breakthroughs in medicine and bioimaging with up-conversion nanoparticles. *International Journal of Nanomedicine, 14*, 7759–7780. https://doi.org/10.2147/IJN.S221433

Santhosh, S. K., Kootery, K. P., Umesh, M., Alex, P. M., Mani, M., Roy, A., & Sarojini, S. (2022). Nanovaccinology and superbugs. In *Nanovaccinology as Targeted Therapeutics* (p. 53). John Wiley & Sons.

Sarojini, S., & Jayaram, S. (2021). An impact of antibacterial efficacy of metal oxide nanoparticles: A promise for future. *Bio-manufactured Nanomaterials, 393*.

Seidu, T. A., Kutoka, P. T., Asante, D. O., Farooq, M. A., Alolga, R. N., & Bo, W. (2022). Functionalization of nanoparticulate drug delivery systems and its influence in cancer therapy. *Pharmaceutics, 14*, 1113. https://doi.org/10.3390/pharmaceutics14051113

Sekimukai, H., Yoshikawa, N., Fukushi, S., Tani, H., Kataoka, M., Suzuki, T., et al. (2020). Gold nanoparticle-adjuvanted S protein induces a strong antigen-specific IgG response against severe acute respiratory syndrome-related coronavirus infection but fails to induce protective antibodies and limit eosinophilic infiltration in lungs. *Microbiology and Immunology, 64*(1), 33–51. https://doi.org/10.1111/1348-0421.1275

Shah, S., Rangaraj, N., Laxmikeshav, K., & Sampathi, S. (2020). Nanogels as drug carriers–introduction, chemical aspects, release mechanisms and potential applications. *International Journal of Pharmaceutics, 581*, 119268. https://doi.org/10.1016/j.ijpharm.2020.119268

Shirodkar, R. K., Kumar, L., Mutalik, S., & Lewis, S. (2019). Solid lipid nanoparticles and nanostructured lipid carriers: Emerging lipid-based drug delivery systems. *Pharmaceutical Chemistry Journal, 53*(5), 440. https://doi.org/10.1007/s11094-019-02017-9

Sindhu, R. K., Gupta, R., Wadhera, G., & Kumar, P. (2022). Modern herbal nanogels: Formulation, delivery methods, and applications. *Gels, 8*(2), 97. https://doi.org/10.3390/gels8020097

Soares, S., Sousa, J., Pais, A., & Vitorino, C. (2018). Nanomedicine: Principles, properties, and regulatory issues. *Frontiers in Chemistry, 6*, 360. https://doi.org/10.3389/fchem.2018.00360

Sultana, A., Zare, M., Thomas, V., Kumar, T. S., & Ramakrishna, S. (2022). Nano-based drug delivery systems: Conventional drug delivery routes, recent developments, and future prospects. *Medicine in Drug Discovery, 100134*. https://doi.org/10.1016/j.medidd.2022.100134

Talebian, S., Wallace, G. G., Schroeder, A., et al. (2020). Nanotechnology-based disinfectants and sensors for SARS-CoV-2. *Nature Nanotechnology, 15*, 618–621.

Thorek, D. L., Chen, A. K., Czupryna, J., & Tsourkas, A. (2006). Superparamagnetic iron oxide nanoparticle probes for molecular imaging. *Annals of Biomedical Engineering, 34*(1), 23–38.

Thukral, A., Ross, K., & Hansen, C. (2020). A single dose polyanhydride-based nanovaccine against paratuberculosis infection. *npj Vaccines, 5*(15), 1–10. https://doi.org/10.1038/s41541-020-0164-y

Tiwari, K., & Bhattacharya, S. (2022). The Ascension of nanosponges as a drug delivery carrier: Preparation, characterization, and applications. *Journal of Materials Science: Materials in Medicine, 33*(3), 1–21. https://doi.org/10.1007/s10856-022-06652-9

Tornesello, A. L., Tagliamonte, M., Buonaguro, F. M., Tornesello, M. L., & Buonaguro, L. (2022). Virus-like particles as preventive and therapeutic cancer vaccines. *Vaccine, 10*(2), 227. https://doi.org/10.3390/vaccines10020227

Trzeciak, K., Chotera-Ouda, A., Bak-Sypien, I. I., & Potrzebowski, M. J. (2021). Mesoporous silica particles as drug delivery systems-the state of the art in loading methods and the recent progress in analytical techniques for monitoring these processes. *Pharmaceutics, 13*(7), 950. https://doi.org/10.3390/pharmaceutics13070950

van der Koog, L., Gandek, T. B., & Nagelkerke, A. (2022). Liposomes and extracellular vesicles as drug delivery systems: A comparison of composition, pharmacokinetics, and functionalization. *Advanced Healthcare Materials, 11*(5), 2100639. https://doi.org/10.1002/adhm.202100639

van Straten, D., Mashayekhi, V., de Bruijn, H. S., Oliveira, S., & Robinson, D. J. (2017). Oncologic photodynamic therapy: Basic principles, current clinical status and future directions. *Cancers, 9*(2), 1–54. https://doi.org/10.3390/cancers9020019

Ventola, C. L. (2017). Progress in nanomedicine: Approved and investigational nanodrugs. *Pharmacy and Therapeutics, 42*(12), 742.

Wang, C., Li, J., Kang, M., Huang, X., Liu, Y., Zhou, N., & Zhang, Z. (2021). Nanodiamonds and hydrogen-substituted graphdiyne heteronanostructure for the sensitive impedimetric aptasensing of myocardial infarction and cardiac troponin I. *Analytica Chimica Acta, 1141*, 110–119. https://doi.org/10.1016/j.aca.2020.10.044

Wu, M., & Huang, S. (2017). Magnetic nanoparticles in cancer diagnosis, drug delivery and treatment (review). *Molecular and Clinical Oncology, 7*, 738–746. https://doi.org/10.3892/mco.2017.1399

Wu, L. P., Wang, D., & Li, Z. (2020). Grand challenges in nanomedicine. *Materials Science & Engineering C, 106*, 110302. https://doi.org/10.1016/j.msec.2019.110302

Yaqoob, S. B., Adnan, R., Rameez Khan, R. M., & Rashid, M. (2020). Gold, silver, and palladium nanoparticles: A chemical tool for biomedical applications. *Frontiers in Chemistry, 8*(June), 1–15. https://doi.org/10.3389/fchem.2020.00376

Yetisgin, A. A., Cetinel, S., Zuvin, M., Kosar, A., & Kutlu, O. (2020). Therapeutic nanoparticles and their targeted delivery applications. *Molecules, 25*(9), 2193. https://doi.org/10.3390/molecules25092193

Yin, Y., Hu, B., Yuan, X., Cai, L., Gao, H., & Yang, Q. (2020). Nanogel: A versatile nano-delivery system for biomedical applications. *Pharmaceutics, 12*(3), 290. https://doi.org/10.3390/pharmaceutics12030290

Yur, D., Lieser, R. M., Sullivan, M. O., & Chen, W. (2021). Engineering bionanoparticles for improved biosensing and bioimaging. *Current Opinion in Biotechnology, 71*, 41–48. https://doi.org/10.1016/j.copbio.2021.06.002

Zaheer, T., Pal, K., & Zaheer, I. (2021). Topical review on nano-vaccinology: Biochemical promises and key challenges. *Process Biochemistry, 100*, 237–244. https://doi.org/10.1016/j.procbio.2020.09.028

Zhang, Y., Li, M., Gao, X., & Chen, Y. (2019). Nanotechnology in cancer diagnosis: Progress, challenges and opportunities. *Journal of Hematology & Oncology, 3*, 1–13.

Zhang, C., Yan, L., Wang, X., Zhu, S., Chen, C., Gu, Z., & Zhao, Y. (2020). Progress, challenges, and future of nanomedicine. *Nano Today, 35*, 101008. https://doi.org/10.1016/j.nantod.2020.101008

Zheng, B., Peng, W., Guo, M., Huang, M., Gu, Y., Wang, T., Ni, G., & Ming, D. (2021). Inhalable nanovaccine with biomimetic coronavirus structure to trigger mucosal immunity of respiratory tract against COVID-19. *Chemical Engineering Journal, 418*(129392), 1–13. https://doi.org/10.1016/j.cej.2021.129392

Zou, L., Wang, H., He, B., Zeng, L., Tan, T., Cao, H., He, X., Zhang, Z., Guo, S., & Li, Y. (2016). Current approaches of photothermal therapy in treating cancer metastasis with nanotherapeutics. *Theranostics, 6*(6), 762–772. https://doi.org/10.7150/thno.14988

Chapter 3
Nanomedicine: Therapeutic Approach of Vaccinology to Fight Against SARS/COVID-19

Anjali Yadav, Manpreet Kaur Aulakh, Kirtanjot Kaur, Kaushik Pal, and Vishal Mutreja

3.1 Detection of SARS/COVID-19

In December 2019, in Wuhan, the capital of China's Hubei territory, the unrivaled COVID-19 came out of nowhere, which brought the world down to its verge. It caused adverse break-outs on human health (such as diseases related to lungs), life, and economies worldwide. The well-established fact is that in people it originates through respiratory tract syndrome coronavirus-2 (SARS-CoV-2). Coronavirus is well recognized for its fleeting spread and high infectivity which led to threats across the globe and was manifested by the great mortality in the past 27 months (Abd Elkodous et al., 2021). The ailment is usually set about with cough, fever, and difficulty in breathing, coming after with different acute lung disorders such as chronic obstructive pulmonary diseases (COPDs), which may result in death. But somehow, there are asymptomatic cases that have been reported. This has created an urgency for the development of sensors for SARS-CoV-2 to promptly and accurately confirm the presence of coronavirus.

To this time, the development of these tests has verified that spike (S), envelope (E), membrane (M), and nucleocapsid (N) are the only notable structural and basic

A. Yadav · V. Mutreja (✉)
Department of Chemistry, University Institute of Science, Chandigarh University, Mohali, Punjab, India

M. K. Aulakh
University Center for Research and Development (UCRD), Chandigarh University, Gharuan, Mohali, Punjab, India

K. Kaur
PG Department of Chemistry, Sri Guru Gobind Singh College, Chandigarh, India

K. Pal
University Centre for Research and Development (UCRD), Chandigarh University, Mohali, Punjab, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_3

proteins for the COVID-19 virus. It has been revealed through various studies that the spike (S) protein holds the highest importance in the entry of viruses and binding with the human renin-angiotensin system's (RAS's) enzyme, i.e., angiotensin-converting enzyme (ACE-2) which is also responsible for blood pressure controls. The spike binds to the ACE-2 receptor over the cell surface; hence, the spike (S) protein could be the most favorable noble target site for sensors/detectors (Mushtaq, 2022).

Several methods were summoned for the identification of coronavirus like nucleic acid, reverse transcription-polymerase chain reaction test (RT-PCR), CT-imaging, enzyme-linked immunosorbent assay (i.e., ELISA), point-of-care tests, and tests for some hematological parameters. Even though such methods hold appreciable comfort in spotting the SARS-CoV-2, diagnosing COVID-19 many have limitations. For instance, the most commonly employed, however, questionable, reverse transcription real-time-PCR (RT-PCR), which requires viral genetic substance (i.e., RNA) in samples collected from nasopharyngeal swabs, demands high expertise. It is also a time-consuming process and is prone to give false negatives (Bidram et al., 2021). This fastens the need for a more sensitive, simpler, accurate, and reliable method for the timely treatment of patients and preventing it from spreading (Krishnan et al., 2021).

Numerous researchers have been working to create cutting-edge nanoparticles (NPs)-based viral sensors for effectively managing and preventing the pandemic ever since the uncovering of COVID-19 and its mechanism of influence. The term "nanoparticles" refers to particles which are having a size of less than 100 nm in at least one dimension. NPs are distinctive because of their tiny particle size, greater surface area, shape and size-dependent activity, unique bonding and conjugation characteristics (such as interactions between gold and thiol), encapsulation of unstable biomolecules (such as mRNA), and surface plasmonic nature. Therefore, efforts are being made to fabricate ultrasensitive biosensors by using NPs for obtaining different sensors such as colorimetric, scientometric, electrochemical, and fluorometric, etc., to detect well-known human viruses (Bidram et al., 2021; Liu et al., 2022).

3.2 Diagnosis of SARS-CoV-2

The recent developments in nanobiotechnology have opened up new avenues for diagnostic and therapeutic science. Nanomaterials' distinctive characteristics, as opposed to those of their bulk counterpart, are advantageous in several biomedical applications. For instance, the size and morphology of the NPs could influence their efficiency in diagnostic applications, and their surface can be modified with various types of ligands covalently or non-covalently bonded to improve detecting limits and selectivity or sensitivity (Mahmud et al., 2022).

The main obstacle in the detection of biological materials like DNA (deoxyribonucleic acid), RNA (ribonucleic acid), and spike proteins is their very low

concentrations, which produce a weak signal. However, unique properties like the plasmonic characteristics of gold nanoparticles (AuNPs), the magnetic characteristics of some metal NPs, the distinctive electrochemical characteristics of carbon-based NPs, the fluorescence properties of quantum dots, and a range of polymeric NPs provide a stage for quick and inexpensive diagnostic platforms with the lowest detection limit (LOD). Selectivity is a significant obstacle to biological material detection that can be removed by coating the surface of the nanoparticles with a particular ligand (such as antibodies, proteins, or thiolated molecules).

3.2.1 Colorimetric

The colorimetric analysis is a straightforward method that is uncalled for any complex instrumentation. Specifically, metal NPs are usually employed in the colorimetric assay to detect human aliment, and the establishment of such biosensors has validated the easiness of a quick detection test that doesn't require highly skilled manpower. Particularly, AuNPs are repeatedly used in colorimetric analysis owing to their easy synthesis, comprehensibility, viability, distinctive optical properties, and effective surface areas. Colorimetric assays equipped with gold nanoparticles grasp merit in the change of color from red to purple, into colloidal suspension through antigen-antibody (Ag-Ab) interaction. This approach improves the detection of coronavirus spike protein with greater specificity and sensitivity (Jeevika et al., 2021).

The LSPR (Localized Surface Plasmon Resonance) results in cohesive and non-moving oscillations of self-directing electrons in metal NPs when they fraternize with an electromagnetic (EM) wave whose frequency is in resonance with a plasmonic one, is the physical mechanism underlying this category of biosensors. For instance, a colloidal suspension of AuNPs could show color changes from red to blue due to LSPR coupling in a colorimetric-based diagnostic method (Liu et al., 2022; Jeevika et al., 2021). AuNPs clustering can be synchronized by biological mechanisms namely antigen-antibody (Ag-Ab) interaction. Though different techniques can be inclined to inactivate antigen-antibody (Ag-Ab) when rightly encountered with the facet of the Au nanoparticles, the sophistication of such standard methods leaves them impractical for commercial processes on a huge scale.

High-density functionalized surface can be created in a matter of minutes via the Photochemical Immobilization Technique (PIT) with UV (ultraviolet) activation of the antigen-antibody system. PIT was shown to be successful for holding antibodies (Abs) upright on surfaces other than flat surfaces, such as AuNPs, which were either used to counterweight small antigens or to create a colorimetric biosensor for recognizing estradiol and IgGs[28]. In the later instances, antigen presence showed a shift in absorbance that was simple to measure with a spectrophotometer or even with the human eye. The influenza A virus was also detected using a method that relied on NPs aggregation by the occupancy of the antigen Although no clinical trials were reported to show the procedure's efficacy in actual clinical situations (Syed, 2020).

A study by Sergio et al. (Christian, 2011; Ventura et al., 2020) revealed that the development of a colorimetric biosensor that was applied to SAR-CoV-2 mass testing with both susceptivity and explicitness of greater than 95%, as shown by a comparative evaluation performed over 94 samples (45 positives and 49 negatives), tested by conventional RT-PCR in the Virology unit of A.O.U. Federico II/ Department of Translational Medicine of the University of Naples. The detection method depicted in Fig. 3.1 involves the use of the colloidal solution of PIT-functionalized Au nanoparticles (f-AuNPs) against the membrane, spike, and envelope surface proteins of coronavirus (M, S, and E respectively, in Fig. 3.1a). In this method, the specimens were dipped into a solution of Universal Transport Medium (UTM, Copan Brescia, Italy) after being taken from the patient and without any further processing. For analysis, 100**μ**L of the sample, 50**μ**L of f-AuNP colloidal solution, and 100**μ**L of ultrapure water were combined to conduct the test. The presence of virions (i.e., the viral protein particles) caused a coating of AuNPs to develop on their surface (Fig. 3.1b), which caused a redshift in the optical density (OD) (Fig. 3.1c). When the viral levels were considerably large, the change in color from red to purple was visible even through naked eyes (Fig. 3.1a, b) (Ventura et al., 2020).

The COVID-19 proteins (S, M, and E) are highlighted in red, dark green, and violet, respectively, and are associated with respective antibodies. The purple colloidal solution with anti-SARS-CoV-2 synthesized functionalized AuNPs (f-AuNP) is depicted in the inset of Fig. 3.1b. The f-AuNPs encircle the virion and create a coating of nanoparticles on its upper surface. Due to their interaction, there is a shift in the extinction spectrum of the resonance peak, which results in the change of color as shown in Fig. 3.1c. Extinction spectra showing the optical density of f-AuNPs colloidal mixture combined with patient samples having various virus loads. The extinction spectra of virions cannot be distinguished from the spectrum of f-AuNPs at very low virion concentrations (curve C_{t32}) (black continuous line). The extinction spectrum is slightly redshifted at intermediate virion concentrations (curve C_{t15}), and this difference from the control (f-AuNPs) results in the curve C_{t15}-(f-AuNPs), which shows the virion's involvement. The extinction spectra for C_{t7} (high virion concentration) peak at 560 nm (f-AuNPs) are shown in Fig. 3.1c (Ventura et al., 2020).

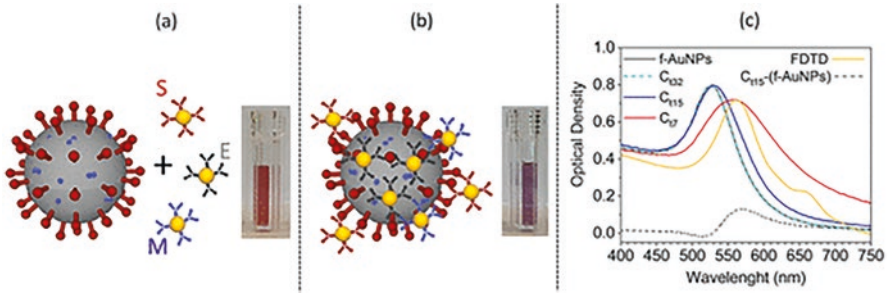


Fig. 3.1 A layout of the SARS-CoV-2 detection method using nanostructured AuNPs (Reprinted with permission from Ventura et al., 2020)

3.2.2 Biosensor

Biosensor is a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element that is in direct spatial contact with a transducer element. Various biological elements such as antibodies and enzymes have been employed as receptors. AuNPs-based biosensors have been tailored in such a manner that they owe rare optical and plasmonic properties. They are a great option for sensitive biosensors for numerous pathogens and biomarkers due to their customizable size, simplicity of production, stability, and biocompatibility. Because of their compelling interactions with sulfur (S), AuNPs are frequently used in colorimetric viral detections. The fundamental idea behind AuNPs-based biosensor assays is to target RNA/DNA with thiol-altered nucleic acid surfaces in the vicinity of an electrolyte that is positively charged, which then interfaces with AuNPs and alters its color due change in the charge. Recently, Pramanik et al. (Mahmud et al., 2022; Chauhan et al., 2020; Christian, 2011) disclosed a methodology for the rapid identification of SAR-CoV-2 via surface-enhanced Raman scattering (SERS)-based detection using 4-amino thiophenol (4-ATP) nanostructured AuNPs. To cause aggregation inside the AuNPs colloidal system, 4-ATP functionalized AuNPs are further modified with an anti-spike antibody, which subsequently reacts with the antigen present in the coronavirus. Because of the shift in absorbance occurring in the visible band of the light spectrum, this aggregation behavior can be seen by the naked eye and can be measured using a UV-absorbance spectrometer (Liu et al., 2022). SERS and the AuNPs sulfur bond open the door for detecting at the POC (point of care). Viral membrane protein can interact with AuNPs and organic substructure in paper-like strips, breaking the connections between the metal and the organic scaffolding. This property can be utilized in making masks having high coronavirus sensitivity. The intensity and color of SERS's AuNPs would appear to alter as a result of the protein-AuNPs interaction, which is effectively thought of as an electron-hole effect. One clear example is that when the bare AuNPs are encountered with SERS the AuNPs due change in the conjugation site, leaves a hole in the previous site, hence, creating an electron-hole effect. Therefore, rapid detection using AuNP-based point of care is feasible. Such a technique can be adapted for common use by making a mask with NPs having different properties when interacting with the foreign particles specifically SARs-CoV-2 by binding and locking system, depicted through color change as shown in Fig. 3.2 (Mahmud et al., 2022).

3.3 Types of Nanocarrier for Delivery of Drugs

There are several approaches made for nanomaterial-based drug delivery systems. These include the use of metal nanoparticles such as gold, silver, and organic nanoparticles in drug delivery systems without any adverse effect over vaccinology.

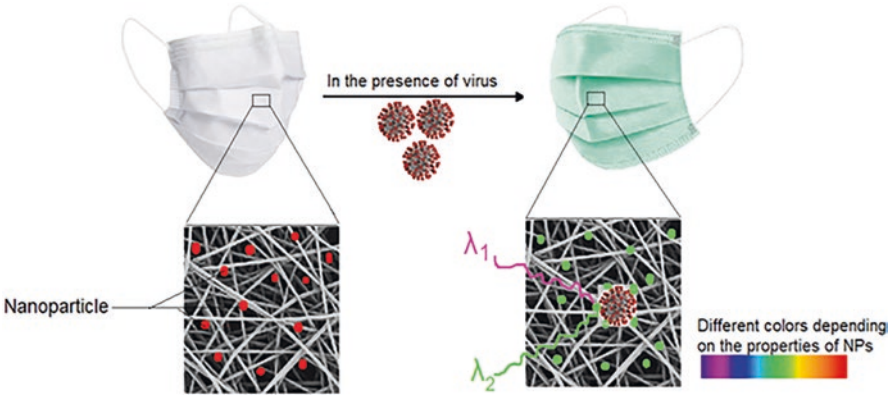


Fig. 3.2 Mask-embedded AuNPs undergo SERS intensity and color variations in the environment of SARS-CoV-2 (Mahmud et al., 2022)

There are abundant organic materials-based drug delivery systems such as liposomes, dendrimers, nanoemulsions, nanosuspensions, hydrogel, etc. Some widely acknowledged benefits of nanocarriers that make them excellent choices for the delivery of antiviral drugs are as follows: (1) Under the right circumstances, medications with low water solubility and hazardous substances could host themselves inside the nanocarrier to gain increased stability and solubility. (2) Phagocytotic cells, that rapidly absorb nanocarriers are the main viral reservoirs. (3) Nanocarriers increase the bioavailability of capsulated active ingredients. (4) Nanocarriers give adhesiveness to microbicidal gels. (5) The medicine must be delivered intracellularly using nanocarriers (Jagessar, 2020). (6) Nanocarriers have the potential to lower the toxicity of antiviral medications, improve yield, and postpone the onset of resistance. (7) Nanocarriers are purported to be able to traverse biological barriers, for example, the blood-brain barrier which is a semipermeable membrane that separates the blood from cerebrospinal fluid, due to their nano-size. (8) Nanocarriers may improve tissue tolerance, cellular uptake, and transportation, enabling efficient administration to the intended destination. (9) Nanocarriers can always be released sustainably. Nanoparticle-based drug delivery systems can be of various types as shown in Fig. 3.3, depending on (1) the type of material, (2) the size, (3) the type of shape or geometry chosen, and (4) the type of surface epitome used in the fabrication of nanoparticles.

3.3.1 Liposomes

Liposomes are lipid vesicles that resemble cells and have a sequenced phospholipid bilayer. They have many benefits, including non-toxicity or immunogenic nature, altering the distribution of drugs in vivo, enabling sustained drug release,

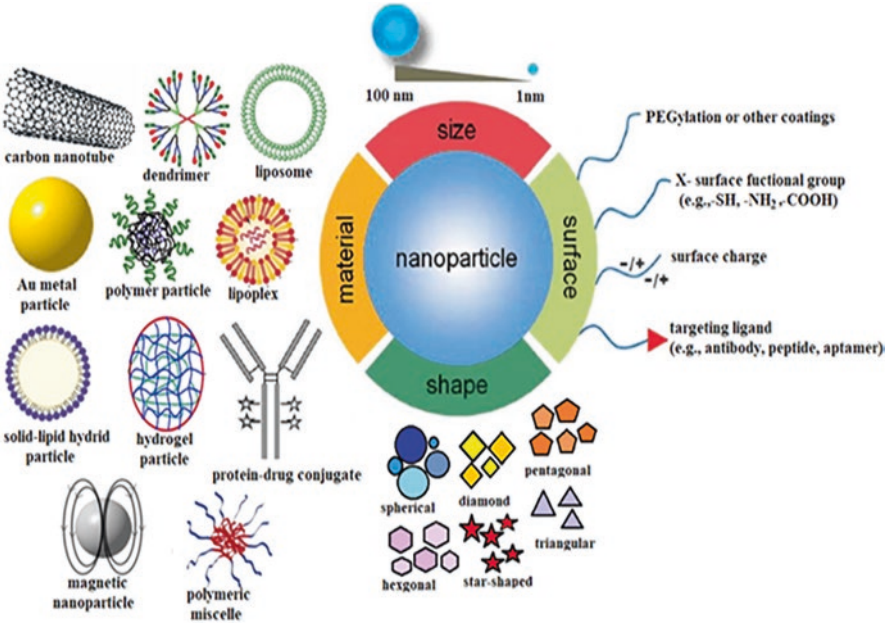


Fig. 3.3 Schematic representation of shape, size, material, and a surface group of nanomaterial-based drug delivery system

lengthening drug action time, uplifting drug treatment index, and lowering side effects specifically for entrapping hydrophilic, ionic molecules, and hydrophobic drugs. Interestingly, reticuloendothelial (RES) phagocytic cells are specialized for their removal from regular circulation.

The liposome-based nanoparticles are quintessential for endovenous administration. Such nanomaterials could be identified by lipoproteins and plasma opsonins (antibodies or other substances which bind to foreign microorganisms), proceeding with their speedy phagocytosis and discardation from circulation (Christian, 2011). In a liposomes-based drug delivery system, as illustrated in Fig. 3.4, drug molecules can be loaded into the aqueous core of the liposomes which are shielded from the body's aqueous environment by a lipid bilayer to protect the drug from the autoimmune system. The surface is an epitome with a specific surface protein for binding to the targeted site. Over time, the bilayer deteriorates and the liposome releases its internal drug essence. Further, a study depicted that liposomes usually are positively charged and carry the raised potential for mucosal vaccinations because nasal cavity retention carries a negative charge resulting in a vigorous immune effect, giving rise to the generation of enormous levels of immunoglobins to fight COVID-19 (Jagessar, 2020; Christian, 2011).

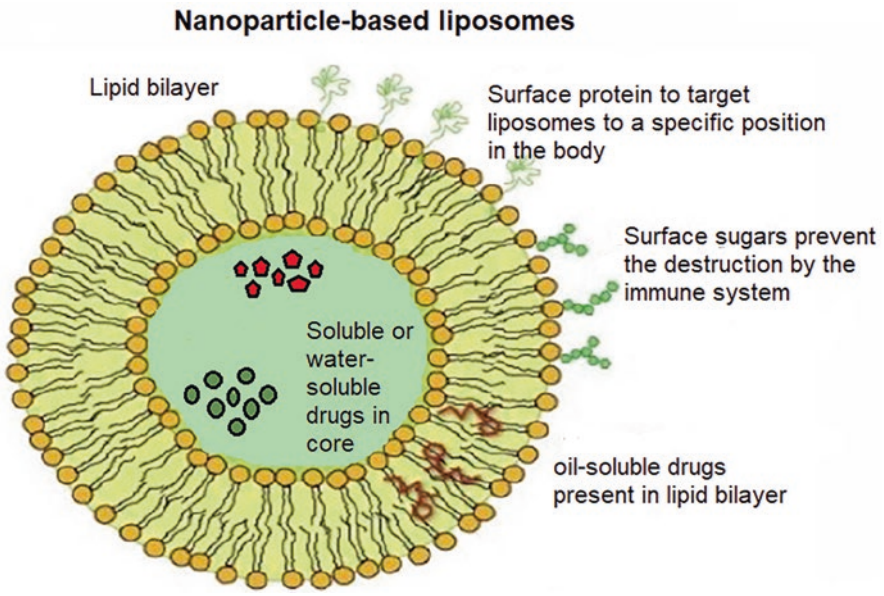


Fig. 3.4 Schematic representation of liposome-based nanoparticles

3.3.2 Dendrimer

Dendrimers are artificial, branched drug delivery structures that have diversity in configuration and are frequently engrossed to infuse insoluble medicines. Moreover, these can be conjugated with different surface functional groups, leading to an eccentric drug delivery product, and their molecular weight is tuneable.

Furthermore, these are frequently employed in biomedical and pharmaceutical applications because of their excellent biological properties. Additionally, dendrimers showed enhanced antiviral potential due to their strong interactions with some viruses, which helps them to control the host's illness. Thus, they emerged as leading contenders to treat viral diseases like HIV, COVID-19, and influenza virus. It was discovered that dendrimer nanoparticles had a replicon mRNA that expresses an antigen, enabling critical CD8+ T-cells as well as antibody responses to protect against deadly viruses including Ebola and H1N1. Dendrimers naturally incorporate conjugation with the chemical species to their surface which functions as a detecting agent (for instance dye molecules). The main conclusions can be screened against SARS-CoV-2 which can lessen COVID-19's onslaught (Chelliah et al., 2021). Dendrimers have promising potential for such applications as their structure leads to a multivalent system. Moreover, one dendrimer has hundreds of feasible localities to couple an active species as shown in Fig. 3.5.

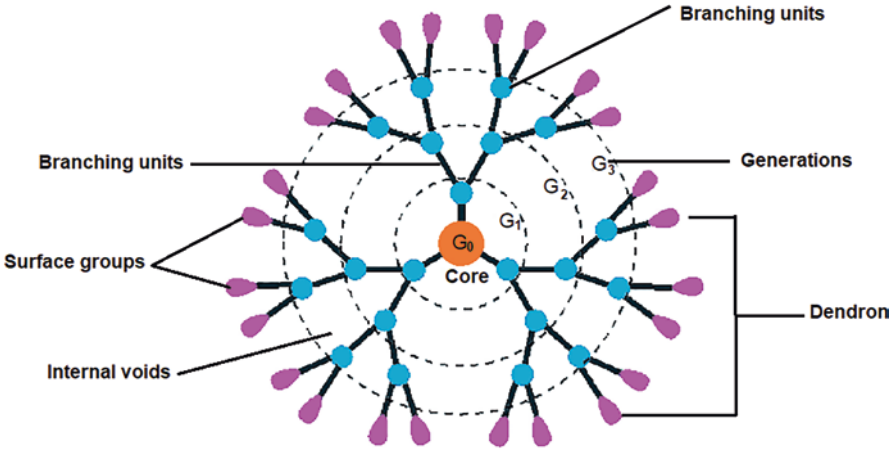


Fig. 3.5 Schematic representation of dendrimer-based nanoparticles

3.3.3 Polymer-Based Nanoparticles

Polymer-based nanoparticles are usually summoned as nanospheres and nanocapsules. Such kinds of nanostructures are hollow and contain polymer membranes and can be loaded with drug molecules. Polymers such as synthetic homopolymers, natural polymers, and co-polymers can be employed in the preparation of NPs**.** The drug delivery systems could work by (a) phagocytosis, (b) pinocytosis in the fluid process, (c) endocytosis mediated by receptors, and (d) pit-mediated endocytosis coated with clathrin. To return to the cell, these systems travel from primary endosomes to secondary endosomes, which eventually combine with lysosomes. There is a time constraint for engagement with the target cells since they are rapidly consumed by the RES or in an opsonized state. According to studies, a disproportionately high proportion of monocyte-macrophage cells take up these components, acting as carriers for the virions to spread and multiply in other tissues throughout the body. The polymeric NPs can be made compatible with both hydrophilic and hydrophobic medicines which is more advantageous than other drug delivery systems. Thus polymeric drug delivery systems could be employed as a formulation or a tool enabling the introduction of therapeutics into the body. It improves the safety and efficacy of the drug because of its unique geometry by controlling the rate, time, and place of release of drugs into the body as shown in Fig. 3.6.

Such NPs can also be employed in diagnosing sensors for identifying viral pathogens, physical barriers like masks and shields to ward off viruses, and vaccinations.

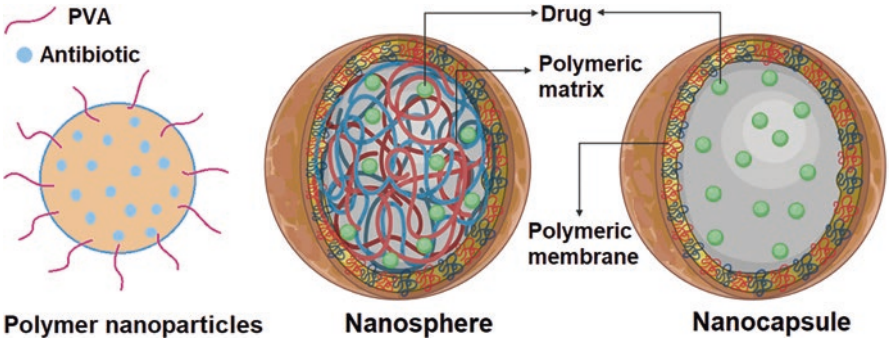


Fig. 3.6 Schematic representation of polymer-based NPs, nanosphere, and nanocapsules

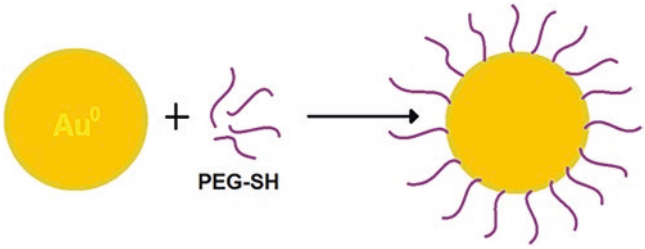


Fig. 3.7 Schematic representation of gold nanoparticles tailored with PEG-SH

3.3.4 Coating with PEG

Coating the NPs with PEG (polyethylene glycol) makes them more liable as therapeutic agents. PEG is being exhaustively used in the formulation of nanomaterials or nanoparticles due to its strength of having specialized structure and performance. PEG coating provides nanomaterial a significant modification such as dispersion, structure-directing nature, functionalization, and protection of bio-molecules including proteins, peptides, and oligonucleotides from degradation. Owing to their unique polymeric structure, it has been seen that coating is feasible for both types of the drug, i.e., hydrophobic as well as hydrophilic. These properties are useful to extemporize the dissolving ability of the drug and can minimize coupled issues like environmental precipitation, perniciousness, etc. Eventually, these properties hinder the fast production and destabilization, which successively enable drug regulation in the slightest dosage. The miniature size of NPs permits the chemical or drug to probe inside the cells without any extracellular deterioration, thus, enabling intracellular drug delivery. Consequently, being small-sized particles, these can overcome the mucosal barriers namely mucus, altogether with epithelial and subepithelial penetration (Liu et al., 2013). Coatings of PEG on nanoparticles shield their surface from aggregation, and phagocytosis, and enhance the prolonged systematic circulation period of Au nanoparticles as shown in Fig. 3.7.

A study revealed that tailoring the NPs surfaces with PLGA{i.e., poly(lactic-co-glycolic acid)} has boosted the specific surface area of polymer NPs by adding them with magnificent penetrating and transporting properties. Moreover, such NPs have a lesser molecular weight in contrast to pure PLGA NPs and better grafting capacity. These formulations have led to an effective fight against COVID-19 without side effects (Bidram et al., 2021; Liu et al., 2022).

3.3.5 Nanosuspension

Nanosuspension-based drug delivery system is a submicron colloidal dispersion containing therapeutic particles and/or oil droplets that are nanosized and stabilized by surfactants. A biphasic solid-in-liquid or solid-in-semisolid system known as a nanosuspension comprising dispersed components such as a pure active ingredient or a mixture of pure active compounds. However, active components can be lyophilized, spray-dried, or combined with NPs in a nanosuspension to form a solid carrier matrix. The transporting challenges of drugs that are lipid as well as water-soluble can also be handled by nanosuspension compositions. Nanosuspensions are also suitable to incorporate drugs that are poorly soluble in water as well as lipid media. As a result of enhanced solubility, the rate of emerging the active compounds increases and the maximum plasma level is achieved faster. Hence, such an approach is a boon for molecules with poor solubility, permeability, and both as illustrated in Fig. 3.8.

These systems can quickly enter the cell due to their nanosized shape, which allows them to pass the cellular barrier and demonstrate a variety of uses in the case of oral, intravenous, and pulmonary delivery systems. Additionally, these are utilized for a variety of medication methods including ophthalmic, parenteral, pulmonary, and nasal and their antiviral actions have been the subject of extensive research (Wilson & Mukundan, 2022).

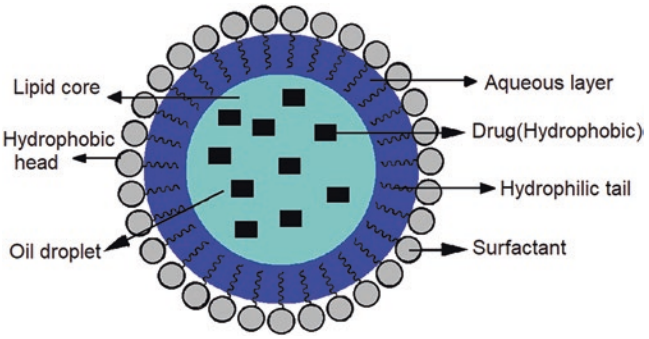


Fig. 3.8 Schematic representation of drug incorporation in nanosuspensions

3.3.6 Nanoemulsions

The terms "oil and water dispersions" are frequently employed to refer to emulsions, finely dispersed emulsions, or submicron emulsions, which are surface-active films made up of both surfactant and co-surfactant that disperse and stabilize nanosized liquid droplets (20–200 nm). Nanoemulsions are transparent or translucent, optically isotropic, and composed of a kinetically stable heterogeneous system of two immiscible liquids, i.e., water and oil stabilized via amphiphilic surfactant for enhancement of drug solubility depicted by Fig. 3.9.

These can be differentiated from suspension by a high degree of steadiness, which is primarily the result of strong steric stabilization among droplets. They offer several benefits that enhance their medication delivery platforms and nanocarriers.

Due to the minute droplet size, the force due to gravity is significantly reduced owing to Brownian motion which is strong enough to counteract gravity effects and shows zero creaming (i.e., the forced migration of the dispersed phase of an emulsion under the influence of buoyancy) or sedimentation during storage of nanoemulsions. Besides having various benefits, these systems nowadays have some flaws that prevent wider adoption. The main barrier to the evolution of nanoemulsions is stability. Prolonged shelf times during the chilling process may cause hydrophilic medicines to recrystallize in the aqueous phase in nanoemulsion systems (Shen et al., 2017).

3.4 Conclusion

To cure viral illnesses, therapeutics have been investigated by employing a multitude of conventional carriers along with sophisticated nanocarriers such as dendrimers and polymeric nanoparticles. However, there are limited reports on carbon-based nanomaterials such as carbon dots, nanotubes, and chitosan NPs which could be potential alternatives as drug carriers. It is necessary to put more

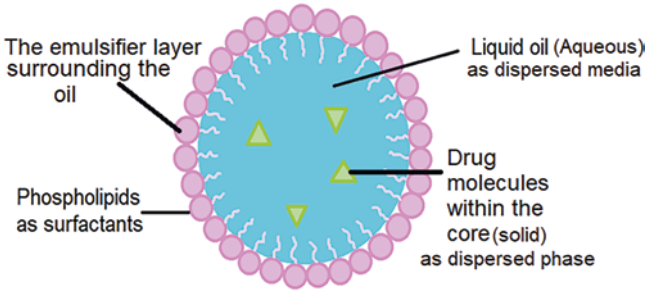


Fig. 3.9 Schematic representation of nanoemulsions

emphasis on researching antiviral delivery nanocarriers which could concentrate especially on viral entrance routes. The methods through which nanocarriers are employed for viral suppression is the thrust area that demands further research. Eventually, these nanocarriers will boost medicinal compliance and eliminate the possibility of partial or total resistance.

Apart from therapeutics, employing nanomaterials in the fabrication of sensors for the detection of COVID-19 has also shown promising results. Specifically, modifying the surface of nanomaterials with functional groups or proteins could improve the selectivity and decrease the response time of sensors. The efforts in this approach might lead to the development of rapid detection kits for viruses with high selectivity.

References

Abd Elkodous, M., Olojede, S. O., Morsi, M., & El-Sayyad, G. S. (2021). Nanomaterial-based drug delivery systems as promising carriers for patients with COVID-19. *RSC Advances, 11*(43), 26463–26480. https://doi.org/10.1039/D1RA04835J

Bidram, E., Esmaeili, Y., Amini, A., Sartorius, R., Tay, F. R., Shariati, L., & Makvandi, P. (2021). Nano-based platforms for diagnosis and treatment of COVID-19: From benchtop to bedside. *ACS Biomaterials Science and Engineering, 7*(6), 2150–2176. https://doi.org/10.1021/acsbiomaterials.1c00318

Chauhan, G., Madou, M. J., Kalra, S., Chopra, V., Ghosh, D., & Martinez-Chapa, S. O. (2020). Nanotechnology for COVID-19: Therapeutics and vaccine research. *ACS Nano, 14*(7), 7760–7782. https://doi.org/10.1021/acsnano.0c04006

Chelliah, R., Daliri, E. B.-M., Elahi, F., Yeon, S.-J., Tyagi, A., Park, C. R., Kim, E. J., kyoung Hee, J., & Oh, D.-H. (2021). Role of peptides in antiviral (COVID-19) therapy. *Journal of the Food Hygiene and Safety, 36*(5), 363–375. https://doi.org/10.13103/jfhs.2021.36.5.363

Christian, P. (2011). Polymer chemistry. *Electrospinning for Tissue Regeneration, 207890*, 34–50. https://doi.org/10.1016/B978-1-84569-741-9.50002-1

Jagessar, R. C. (2020). Plant extracts based nanoparticles, potential nanomedicine in fight against COVID-19. *Journal of Nanosciences Research & Reports, 2*(4), 1–4. https://doi.org/10.47363/JNSRR/2020(2)116

Jeevika, K., Sobiya, R. A., Indhumathi, T., & Kathiravan, S. (2021). Feasibility of nano-scaffold for Covid-19 infected lung tissue regeneration: A review. *Asian Journal of Pharmacy and Pharmacology, 7*(3), 118–125. https://doi.org/10.31024/ajpp.2021.7.3.2

Krishnan, S., Dusane, A., Morajkar, R., Venkat, A., & Vernekar, A. A. (2021). Deciphering the role of nanostructured materials in the point-of-care diagnostics for COVID-19: A comprehensive review. *Journal of Materials Chemistry B, 9*(30), 5967–5981. https://doi.org/10.1039/d1tb01182k

Liu, G., Liu, W., & Dong, C. M. (2013). UV- and NIR-responsive polymeric nanomedicines for on-demand drug delivery. *Polymer Chemistry, 4*(12), 3431–3443. https://doi.org/10.1039/c3py21121e

Liu, K., Salvati, A., & Sabirsh, A. (2022). Physiology, pathology and the biomolecular corona: The confounding factors in nanomedicine design. *Nanoscale, 14*(6), 2136–2154. https://doi.org/10.1039/d1nr08101b

Mahmud, N., Anik, M. I., Hossain, M. K., Khan, M. I., Uddin, S., Ashrafuzzaman, M., & Rahaman, M. M. (2022). Advances in nanomaterial-based platforms to combat COVID-19: Diagnostics, preventions, therapeutics, and vaccine developments. *ACS Applied Bio Materials, 5*(6), 2431–2460. https://doi.org/10.1021/acsabm.2c00123

Mushtaq, A. (2022). Antiviral effects of coinage metal-based nanomaterials to combat COVID-19 and its variants. *Journal of Materials Chemistry B, 10*(28), 5323–5343. https://doi.org/10.1039/d2tb00849a

Shen, S., Wu, Y., Liu, Y., & Wu, D. (2017). High drug-loading nanomedicines: Progress, current status, and prospects. *International Journal of Nanomedicine, 12*, 4085–4109. https://doi.org/10.2147/IJN.S132780

Syed, T. R. (2020, June). *Nanomedicine: An essential resource in treatment and diagnosis of viral accepted manuscript accepted manuscript (uncorrected proof)*.

Ventura, B. D., Cennamo, M., Minopoli, A., Campanile, R., Censi, S. B., Terracciano, D., Portella, G., & Velotta, R. (2020). Colorimetric test for fast detection of SARS-COV-2 in nasal and throat swabs. *ACS Sensors, 5*(10), 3043–3048. https://doi.org/10.1021/acssensors.0c01742

Wilson, B., & Mukundan, K. (2022). Lipid nanoparticles in the development of mRNA vaccines for COVID-19. *Journal of Drug Delivery Science and Technology, 74*(May), 103553. https://doi.org/10.1016/j.jddst.2022.103553

Chapter 4
Drug Delivery and Therapeutics for the Treatment of Infectious Diseases

Dominic Augustine, K. V. Sandhya, Jithu Jerin James, S. V. Sowmya, C. Pushpalatha, G. R. Saraswathy, Arshiya Shakir, and Reshma Dhodwad

4.1 Introduction

Infectious diseases are a leading cause of death worldwide, mainly in low- and middle-income nations, especially in infants. Infectious diseases, whether intracellular, extracellular, biofilm-mediated, or medical device-associated, have always been a global problem in public health causing millions of deaths each year (Michaud, 2009). Tuberculosis, Brucellosis (*Brucella spp*), Salmonellosis (genus *Salmonella*), and Lysteriosis (*Lysteria monocytogenes*) are some of the common infections affecting most of the population. The discovery of antibiotics at the turn of the twentieth century greatly reduced morbidity and mortality due to infectious diseases (Rahman et al., 2020). Antibiotic resistance and the emergence of new diseases are caused by alterations acquired over the course of a person's lifetime from exposure to the environment combined with the constantly changing

D. Augustine · S. V. Sowmya
Department of Oral Pathology and Microbiology, Faculty of Dental Sciences, M.S. Ramaiah University of Applied Sciences, Bengaluru, India

K. V. Sandhya · J. J. James
Department of Pharmaceutics, Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bengaluru, India

C. Pushpalatha (✉) · A. Shakir
Department of Pedodontics and Preventive Dentistry, Faculty of Dental Sciences, M. S. Ramaiah University of Applied Sciences, Bengaluru, India

G. R. Saraswathy
Department of Pharmacy Practice, Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bengaluru, India

R. Dhodwad
Department of Pedodontics and Preventive Dentistry, Krishnadevaraya College of Dental Sciences, Bengaluru, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_4

characteristics of bacteria (Michael et al., 2014). Existing antibiotics can be pharmacologically modified to overcome microbial resistance. However, even these modifications and new antimicrobial drugs fall short of ensuring that there will be no development of resistance in the future. There exists a dire need to curb the phenomena of antibiotic resistance (Fair & Tor, 2014). The key factor in antibiotic resistance is patients' sublethal antibiotic doses, which allows organisms to resist the antibiotic's effects. The effects can be witnessed by rising nosocomial infections (Zhang et al., 2020). Researchers have hypothesized that viral infections could be transmitted at a very rapid rate even in a technologically advanced world. The Johns Hopkins Centre for Health Security has ranked aerosol-transmitted viral infections as a primary reason for any wide-spreading pandemic (Triggle et al., 2021). Development of effective anti-infectious drug delivery strategies to overcome resistance is the need of the hour.

Current research should enable us to effectively combat new emerging infections and maintain a high level of preparedness to future infections that could challenge healthcare systems globally. Climate change and shift in geographical distribution also play pivotal roles in disease transmission leading to increased incidence of infectious disease: the Zika and dengue viruses transmitted by mosquitoes or the spread of *Borrelia burgdorferi* by ticks are a few well-known examples (Rocklöv & Dubrow, 2020). Lower respiratory tract infections (LRTIs) continue to be one of the top-ten fatal diseases worldwide, which are more prevalent in low-income countries. Infectious diseases such as malaria, tuberculosis, and HIV/AIDS closely follow LRTI (Saleri & Ryan, 2019). Challenges always present various opportunities. The current awareness of the threats of infectious disease and their impact on modern life further emphasize the need for effective drug delivery mechanisms that will play an imperative role in these intercessions (Feddema et al., 2021). There are many possibilities for drug delivery technologies. More research is required to develop and evaluate alternative approaches, particularly based on human cells and tissues, and to use (patho)physiology-based pharmacokinetics to examine interactions between the patient, the microorganism, and the treatment either In vitro or In silico. Establishment of these novel drug delivery systems will change the treatment protocol of infectious diseases. This chapter focuses on drug delivery modalities and therapeutic strategies for infectious diseases. It also discusses the limitations of conventional drug delivery, and the advantages of various novel drug delivery systems as effective therapeutics for infectious diseases.

4.2 Conventional Drug Delivery and Therapeutics for Infectious Diseases

Antimicrobial treatment is the treatment of infectious diseases using chemotherapeutic agents, antibiotics, anti-virals, and anti-parasitic drugs by either killing the microbes or interfering with their growth leading to death (Dhingra et al., 2020).

Conventional Drug Delivery Systems (CDDS) encompass the oral and parenteral route of administration. These routes have been proven to eliminate infections, however, not without its own adverse effects and drawback as well. The conventional DDS have several limitations, and a higher dosage cannot be used to ensure there is no systemic toxicity. Plasma concentration of the drug is not a constant; it varies and so multiple doses must be administered. A significant reduction in concentration occurs through first-pass metabolism and intestinal barriers further reduce the final desired concentration resulting in poor bioavailability (Adepu & Ramakrishna, 2021). Low therapeutic indices and poor water solubility are some of the other disadvantages of the conventional DDS as well. Without an effective delivery system, the active drug is most often rendered hopeless.

In terms of infectious diseases, the limitations of CDDS can be catastrophic if the drug is not adequately delivered to the site of infection or if the desired bioavailability is not reached. Apart from the development of resistance, the infection could spread leading to sepsis if the management is compromised (Philip & Philip, 2010). To overcome the above-mentioned constraints, researchers are in quest to develop polymeric and novel drug-based delivery systems. These materials act as excellent carriers to ensure targeted delivery and high local concentrations.

4.3 Drug Delivery Systems (DDS) to Combat Infectious Diseases

In addition to the conventional dosage forms, there are many novel drug delivery systems which could be effectively used for the delivery of drug. One of the most popular novel drug delivery systems is nanoparticulate DDS (Devi et al., 2010). Nanotechnology is used to develop and fabricate nanostructures for medicine delivery systems. It is possible to see perforated vasculature and an overexpression of certain epitopes or receptors in the localized diseases like infection and inflammation. Nanomedicines can target these areas (Brusini et al., 2020). Infectious disease therapy is hampered by pathogen resistance. In addition to forming biofilms, adopting an intracellular life cycle, secreting drug-inactivating enzymes, or becoming obligatory intracellular pathogens, pathogens can also suppress metabolic function (Kirtane et al., 2021). Drug effectiveness, pharmacokinetics, toxicity, stability, and regulatory control are some technology challenges.

Fast, accurate, and sensitive disease diagnostics, the formulation of antimicrobial drugs from metals, metal oxides, and biological particles to combat pathogens resistant to antibiotics, and targeted drug delivery that enhances biodistribution and drug accumulation in resistant body sites are all potential applications of NPs in the control of infection (Sánchez-López et al., 2020). Drug delivery strategies include biodegradable, polymeric, lipid, and metal NPs. Systemic, oral, pulmonary, transdermal, and other administration methods employ NPs to explore medication targeting, bioavailability, bioactivity, and stability (Begines et al., 2020).

NPs can transport greater doses of antimicrobials to the site of infection, bypassing resistance mechanisms with fewer side effects. NPs target infections passively or actively. Passively targeted NPs extravasate in infection locations. Actively targeted NPs include ligands that activate infection-site receptors. Controlling NP size, surface properties, and drug release produces site-specific action at the appropriate price and dose (Yoo et al., 2019).

NPs of metals, metal oxides, and many biologically derived compounds are efficient antimicrobials owing to their nanosize and unique chemical and physical features, such as high surface-to-volume ratio and reactivity. Silver, gold, copper, oxides of zinc, magnesium, titanium, aluminum, copper, iron, and nitric oxide NPs are antimicrobial against enterotoxigenic *E. coli* (Wang et al., 2017). The popular drug delivery systems include oral route, local delivery, pulmonary delivery, topical delivery, ocular delivery, brain delivery, etc. (Nikolova & Chavali, 2020). Oral delivery is the most preferred route despite various limitations. Local distribution to the infection site may increase on-target medication exposure compared to systemic administration, whereas pulmonary delivery may increase on-target medication exposure for respiratory infections and is non-invasive (Date et al., 2016). Chronic infections caused by opportunistic bacteria may be treated topically. Chronic wounds are caused by malfunctions in the wound-healing pathway. In mouse skin infection models including methicillin-resistant *S. aureus* biofilms and *S. aureus* infections, nitric oxide-releasing NPs decreased wound bacterial load (Negut et al., 2018).

Ocular delivery may deliver medications insoluble in lachrymal fluids. SLN delivered tobramycin to rabbit eyes dramatically increased medication bioavailability within 6 hours (Gaudana et al., 2010). The blood-brain barrier (BBB) is a challenging barrier. Polyamidoamine dendrimers loaded with lamivudine, a standard HIV therapy, were tested in HIV-1-infected MT2 cells. Figure 4.1 shows the different types of nanoparticulate systems for delivering various therapeutics to treat infectious diseases.

4.3.1 Polymer-Based Nanoparticulate Drug Delivery System

Polymer-based NPs are submicron-sized polymeric colloidal particles in which a medicinal substance may be embedded, encapsulated, adsorbed, or conjugated. Polymer–drug conjugates (PDCs), polymer–protein conjugates (PPCs), and dendrimers are prominent polymeric therapies (Larson & Ghandehari, 2012). PDCs consist of a polymeric carrier, bioactive drug molecule, linking agent, solubilizing group, and targeting moiety. Nicoletti et al. (2009) demonstrated that N-(2-hydroxypropyl) methacrylamide–drug conjugates have increased antileishmanial effectiveness. PPCs overcome limitations associated with protein delivery by conjugating proteins onto polymers resulting in physicochemically better therapies. Polymeric NPs protect bioactive compounds from enzymatic and hydrolytic breakdown (Bailly et al., 2012). Therapeutic polymeric NPs are made of biodegradable or

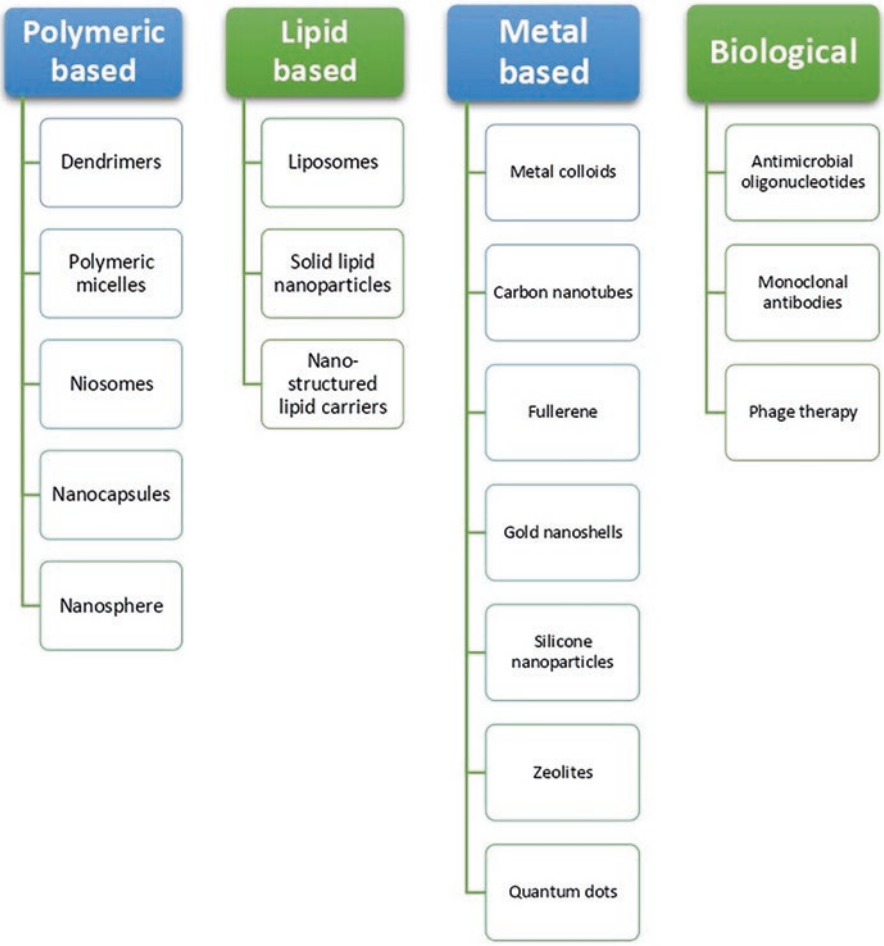


Fig. 4.1 Types of nanoparticulate drug delivery system

biocompatible materials, such as poly (ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), alginic acid, gelatin, and chitosan. In a study by Fattal et al., oral treatment of *S. typhimurium* phosphorylcholine antigen encapsulated in PLGA particles protected mice against *S. typhimurium* (Fattal et al., 2002).

Polymeric therapeutics also include polymeric micelles, niosomes, nanocapsules, and nanospheres. Their benefits make them potential drug-resistance systems. Some benefits include decreased toxicity and immunogenicity, increased specificity that protects healthy cells, tissues, and organs from toxic side effects, better bioavailability, greater water solubility, prolonged half-life resulting in lower renal clearance, and protection of incorporated drugs against premature degradation by enzymatic reactions and other scavenging mechanisms (Yetisgin et al., 2020).

4.3.1.1 Dendrimers

Dendrimers are highly branched, nanosized 3D macromolecules with great surface functionality, stability, and versatility. They are highly structured, globular, layers of branched repeat units, and functional end groups on the outside layer (Khandare et al., 2010). Dendrimers are manufactured from biocompatible materials including polyamidoamine (PAMAM), polyethylene oxide (PEO), polypropylene imine (PPI), polyethyleneimine (PEI), polyethylene glycol (PEG), etc. Hyperbranched structures can be functionalized to improve physicochemical qualities. They are used to administer therapeutically effective drugs. The results exhibited excellent therapeutic benefits. Amphotericin B was conjugated to poly(propyleneimine) dendrimers for Leishmaniasis by Jain et al. (2015).

4.3.1.2 Polymeric Micelles

Micelles are submicroscopic surfactant molecule aggregates that self-assemble in aqueous conditions to create structures with a hydrophobic core out of amphiphilic block copolymers, polymer-lipid conjugates, or other surface-active molecules (Hanafy et al., 2018). Small size, excellent stability, biocompatibility, safety profile, etc., affect micelle functionalization. By altering the co-polymer composition, these micelles' disintegration behavior can be altered, making them ideal drug carriers. For pediatric HIV medication, efavirenz was encapsulated in polymeric micelles to increase oral bioavailability of the drug (Chiappetta et al., 2010).

4.3.1.3 Niosomes

Niosomes are vesicles composed of non-ionic surfactants, such as creatinine, creatinine derivatives, a-tocopherol, and other renewable resources which are biodegradable, biocompatible, relatively nontoxic, more stable, and inexpensive, a substitute for liposomes because phospholipids in the GIT are susceptible to oxidative breakdown (Kazi et al., 2010).

4.3.1.4 Nanocapsules

In nanocapsules, the pharmaceutical is contained inside an aqueous or oily chamber that is encircled by a polymeric barrier which provides protection from rapid degradation. Drugs diffuse out of the core under appropriate conditions, by responding to environmental, chemical, thermal, or biological triggers. Nanocapsules range in size from 5 to 1000 nm, although most are between 100 and 500 nm. Nanocapsules are colloidal in nature and are synthesized by interfacial deposition of preformed polymers (PLA, PLGA, PCL, and PEG). It could be produced using several techniques, including layer-by-layer synthesis, double emulsification polymer coating,

emulsion diffusion, and nanoprecipitation (Mora-Huertas et al., 2010). These hollow nanocapsules could encapsulate chemicals, including pigments, medications, catalysts, and biopolymers including proteins and nucleic acids. Additionally, they display stability, delayed and prolonged release of loaded medicines, and site specificity. These hollow nanocapsules could encapsulate chemicals, including pigments, medications, catalysts, and biopolymers including proteins and nucleic acids. Additionally, they display stability, delayed and prolonged release of loaded medicines, and site specificity (Patra et al., 2018). Incorporation of pH-sensitive, temperature-sensitive, or redox-sensitive polymers could alter the permeability of capsule.

4.3.1.5 Nanosphere

Nanospheres are matrix systems in which the pharmaceutical is equally spread in the polymeric core and released through diffusion. Nanospheres can absorb pharmaceuticals on its surface. These nanostructures offer a high loading capacity for weakly water-soluble medicines because they can integrate hydrophobic pharmaceuticals at concentrations above their inherent water solubility (Rizvi & Saleh, 2018). They possess sustained and controllable drug-release profiles. Emulsification-evaporation and nanoprecipitation can produce nanospheres.

4.3.2 Lipid-Based NPs (LBNP)

LBNPs are potential colloidal carriers for bioactive compounds. LBNPs have great temporal and thermal stability, high loading capacity, easy preparation, cheap manufacturing costs, and large-scale industrial production from natural sources. LBNPs include liposomes, solid lipid NPs (SLN), and nanostructured lipid carriers (NLC) (Martins et al., 2007). These NPs can carry hydrophobic and hydrophilic molecules, have minimal or no toxicity, and extend pharmacological activity by prolonged half-life and controlling drug release. Lipid nanosystems may be chemically modified to prevent immune system detection or increase medication solubility (Tenchov et al., 2021). They could also be manufactured in pH-sensitive formulations to increase drug release in an acidic environment and linked with antibodies that identify their receptors.

4.3.2.1 Liposomes

Liposomes are phospholipid bilayers with an entrapped aqueous volume. Liposomes are small spherical vesicles in which one or more aqueous parts are surrounded by molecules that have hydrophilic and hydrophobic functionality (Akbarzadeh et al., 2013). Drugs are distributed in the hydrophilic or hydrophobic compartments of

liposomes according to their lipophilicity (Bozzuto & Molinari, 2015). Liposomes offer potential qualities for drug delivery, including high biocompatibility, entrapment efficiency, regulated drug release, a favorable safety profile, easy drug loading and surface modification, and payload protection. Composition, size, surface charge, and production process affect liposomes. Single or multiple bilayers of liposomes are possible. They are classified into multilamellar (diameter more than 200 nm), unilamellar (large unilamellar vesicles (diameter between 100 to 400 nm), and small unilamellar vesicles (Liu et al., 2022).

4.3.2.2 Solid Lipid NPs (SLN)

SLNs are colloidal drug carriers or lipospheres. SLNs are a controlled drug delivery alternative to emulsions, liposomes, and polymeric NPs. 50–100 nm submicron-sized particles consist of biocompatible lipids that stay solid at body and room temperature and distributed in aqueous solution. SLNs are emulsified from lipids, waxes, and surfactants. (Naseri et al., 2015).

4.3.2.3 Nanostructured Lipid Carriers (NLCs)

NLCs comprise lipids, surfactants, and co-surfactants that are biocompatible and physiological. NLCs are made using biodegradable solid and liquid lipids and emulsifiers. NLC has replaced SLNs, polymeric NPs, emulsions, microparticles, and liposomes. Incorporating liquid lipids produces structural defects in solid lipids, resulting in a less ordered crystalline arrangement that prevents drug leakage and increases drug load. Nanocarriers can transport hydrophilic and hydrophobic medicines (Elmowafy & Al-Sanea, 2021). NLCs are a potential carrier for oral, parenteral, ophthalmic, pulmonary, topical, and transdermal drug administration. NLCs improve skin moisture, occlusion, bioavailability, and targeting. NLC's easy preparation, biocompatibility, scalability, non-toxicity, increased drug loading, and stability make them a potential drug delivery system (Czajkowska-Kośnik et al., 2019).

4.3.3 Metal-Based Nanoparticulate Drug Delivery System

Diverse shaped and sized metal-based NPs (between 10 and 100 nm) have been studied as diagnostic and medication delivery systems. Gold, nickel, silver, iron oxide, zinc oxide, gadolinium, and titanium dioxide particles comprise most metallic NPs (Păduraru et al., 2022).

4.3.3.1 Metal Colloids

Linear polymers and metal NPs comprise colloidal metal NPs. The linear polymers can produce a first protective shell as a ligand and a second protective shell as a steric hindrance, which stabilize NPs by interacting with the particle surface and heteroatoms (Clarkson et al., 2017). Poly(N-vinyl-2-pyrrolidone) (PVP) is a linear polymer used to produce metal NPs because of its nontoxicity, solubility in numerous solvents, and excellent metal stabilization efficiency (Franco & De Marco, 2020).

4.3.3.2 Carbon Nanotubes

Carbon nanotubes (CNTs) are cylinder-shaped allotropic carbon structures generated by chemical vapor deposition. They have remarkable chemical, electrical, mechanical, and optical characteristics (Eatemadi et al., 2014). Multiwalled and single-walled carbon nanotubes are common. After functionalization, carbon nanotubes could be used for biosensing and medication delivery. It is indeed chemically and mechanically stable and non-cytotoxic. Carbon nanotubes act as a good medication delivery mechanism since it is chemically modifiable (Dresselhaus et al., 2000).

4.3.3.3 Fullerene

A brand-new kind of carbon called a fullerene comes in three shapes: a hollow sphere, an ellipsoid, or a tube. They provide therapeutic potential due to their tiny size, spherical form, and hollow interior. The Buckminsterfullerene (C60), which has 60 carbon atoms organized in a spherical configuration, is the most prevalent kind of fullerene (Bhakta & Barthunia, 2020).

4.3.3.4 Gold Nanoshells

Silica serves as the dielectric core of gold nanoshells, which are then covered in a thin coating of gold. Typically, gold nanoparticles (gold NPs) are attached to the dielectric core initially, and then grow under certain conditions to create a shell. This process of fabricating gold nanoshells is known as seed-mediated growth. Gold nanoshells are highly biocompatible, water-soluble, and commercially available (Wang et al., 2018).

4.3.3.5 Silicone NPs

Silica materials are appropriate for several crucial biological applications, including oxygen transport, imaging, drug administration, and controlled release (Colilla & Vallet-Regí, 2020). Silica materials have been shown to be effective antibiotic delivery systems, which may be relevant in the context of biofilm-associated infections, and which provide a significant issue for contemporary medicine (Selvarajan et al., 2020).

Zeolites are solid, hydrated, crystalline minerals having regular-sized nanochannels and cages and silicon, aluminum, and oxygen-based frameworks. Oxygen balances the silicon ion's positive charge. The ratio of silica to alumina in the structure affects the capacity of cation exchange (Liguori et al., 2019).

4.3.3.6 Quantum Dots

With their appealing photophysical features, high quantum yield, photobleach resistance, and harmonic photoluminescence, quantum dots which are nanocrystals generated by semiconductor materials have the potential to be effective in a variety of biomedical applications (Wagner et al., 2019).

4.3.4 Biologicals

Antimicrobial oligonucleotides: To inhibit the production of harmful genes, short synthetic nucleic acid sequences called oligonucleotides may be utilized therapeutically. To inhibit the production of harmful genes, short synthetic nucleic acid sequences called oligonucleotides may be utilized therapeutically. Antisense oligonucleotides (ASOs) and short interfering RNAs are two oligonucleotide-based platforms for regulating gene expression (Dhuri et al., 2020). Both ASOs and siRNAs possess complementary sequences to their target mRNA.

Monoclonal antibodies (mAbs): Before the antibiotic revolution, serum treatment was routinely employed. In the early 1900s, animal sera were used to treat diphtheria, tetanus, scarlet fever, and pneumococcal pneumonia. Humanized and completely human mAbs provide great specificity and lower toxicity in today's antibody treatments (Stray-Pedersen et al., 2005).

Phage therapy: Phages are viruses that infect bacteria alone. Exploiting these viruses' bactericidal effect enables a very targeted therapy. Some phages may infect a variety of bacterial groups, although most are strain-specific. Receptor type determines host range. Phages inject their genetic material after attaching to a receptor. Lytic phages hijack bacterial replication machinery to make offspring in the bacterial cytoplasm (Lin et al., 2017).

Each alternative therapeutic agent faces several challenges before broad use. Since these medicines are extremely specific to the pathogen being treated,

high-specificity diagnostic testing is needed. Minimum inhibitory concentrations can only be used to estimate the in vivo effectiveness of directly bactericidal or static drugs. Parallel diagnostic and therapeutic panel design is an intriguing idea (Streicher, 2021). A fast multiplex nucleic acid test might identify genes targeted by antisense or CRISPR-Cas treatments. Such a technique would be particularly useful for diagnosing and treating infections in sterile bodily areas like blood or spinal fluid, where most infections are caused by a well-defined set of microorganisms (Jolany Vangah et al., 2020). Rare polymicrobial illnesses need prompt treatment.

4.4 Biomimetic/Bioinspired Nano Therapies to Combat Infectious Diseases

Nanotechnology and biomimetics have been combined to generate bioinspired NPs for medication delivery and vaccine development. These biomimetic NPs combine synthetic nanomaterial diversification, tolerability, and repeatability with biological functioning, complexity, and biocompatibility (Zhou et al., 2020). They may also function as site-specific nanocarriers for medicines or vaccinations. NPs produced from viruses, bacteria, and mammalian cells have been generated for infectious illness management. These biomimetic nanovehicles can carry cargo molecules to diseased areas without triggering immune reactions (Naskar et al., 2021). Here, we explore biomimetic and bioinspired NPs for infectious disease treatment.

4.4.1 Biomimetic NPs for Treatment

Biomimetic techniques have developed virus-mimetic NPs. Hydrophilic and hydrophobic pharmaceuticals may be encapsulated in virus-mimetic nanocapsules comprised of iron oxide NPs and lactoferrin. Albumin NPs packed with antiviral medication efavirenz were developed for HIV therapy, resulting in better drug delivery to organs. Similarly different membrane components of bacteria/fungi have been used to generate NPs that partly reproduce their natural features or treat bacterial or fungal diseases (Yang et al., 2019). Chitosan, an essential component of *Cryptococcus neoformans* vegetative cell wall, was used to manufacture NPs, resulting in a tailored drug delivery method. Also NPs mimicking mammalian cells like the actions of red blood cells (RBCs), platelets, leukocytes, dendritic cells, and stem cells have been produced. PLGA NPs were applied to erythrocyte membranes to increase in vivo circulation. Other biomimetic nanoplatforms, such as hepatitis B virus envelope L particles and lipid NPs, have been studied as drug delivery vehicles. High-density lipoproteins are the most investigated drug delivery nanovehicles (Chen et al., 2021).

4.4.2 Biomimetic/Bioinspired NPs as Vaccines

Virosome-based nanovaccines contain antigens and adjuvants which activate humoral and cellular immune responses. Lederhofer et al. (2018) produced a virosomal respiratory syncytial virus (RSV) vaccine by incorporating 3-deacyl-phosphorylated hexa-acyl disaccharide, a lipid adjuvant into viral membranes containing G and F glycoproteins. Mice immunized in vivo produced RSV-neutralizing antibodies.

Vaccines from virus-like particles (VLPs) generated from capsid or envelope proteins are appealing preventive and therapeutic vaccination candidates because of their unique structural properties, safe manufacture, fast development time, and various expression platforms (Nooraei et al., 2021). Some VLP-based vaccinations, such as hepatitis B and HPV-based VLPs, are authorized for clinical use, while others are under clinical testing. VLPs may be utilized to deliver exogenous antigens in immunogenic and multivalent forms (Tariq et al., 2022).

Self-assembling NPs containing antigens have been studied as viral vaccinations. It has been found that ferritin-viral hemagglutinin (HA) NPs induce wider and more powerful protection than standard influenza vaccinations (Kanekiyo et al., 2013).

In addition to the virus-mimicking vaccination techniques, additional ways have been explored. Recombinant influenza virus HA was incorporated into HDL-like nanodisc particles made of lipid bilayers and membrane scaffold proteins. Intranasal immunization with HA-loaded nanodisc induced a robust anti-HA IgA response, protecting against influenza (H1N1) virus challenge with effectiveness equivalent to Fluzone and FluMist (marketed vaccines) (Bhattacharya et al., 2010). Bacterial extracellular vesicles have gained interest in vaccine research due to their immunogenic features, self-adjuvant capacity, modification potential, antigen-presenting cell uptake ability, and ability to deliver exogenous antigens. Nanotechnology and bacterial mimic methods have been used to build vaccination platforms. By cavitating nitrogen, double-layered membrane vesicles (DMVs) can be easily made (Pati et al., 2018).

Biomimetic nanotherapy has advanced considerably in the recent decade. Bioinspired NPs have varied activities, such as extended circulation, greater accumulation at infected areas, and decreased off-target effects in healthy tissues. Despite virosomes and VLPs being approved as new vaccines, most biomimetic nanotherapies face translation difficulties.

4.5 Oral Nanobiotics: Delivery and Therapy for Infectious Diseases

Resistance to antimicrobial treatments threatens hospitals and communities, leading to fewer effective medications to address "old" well-known bacterium diseases. Nanotechnology with antibiotics is a potential antibacterial treatment. Different antibiotics have been incorporated into NPs with diverse bio-physicochemical characteristics to enable site-specific or intracellular delivery of medicines, especially for treating multidrug-resistant bacteria (Alabresm et al., 2021). Antibiotics of different forms may be physically absorbed on, entrapped in, or covalently coupled with metal, inorganic, and polymer NPs, nanogels, nanoemulsions, liposomes, and hybrid nanovehicles. Synergistic effects may be achieved by co-delivering antibacterial agents using nanocarriers. In addition to diffusion, antimicrobial payloads may be released by pH, reactive oxygen species, bacterial toxins, or lipases. Drug-loaded antibiotic NPs showed improved drug stability, targeted release, longer retention, sustained or responsive release, and penetrating ability. Antibacterial nanotherapies may overcome lower drug absorption and enhanced efflux, inhibit biofilm development, and target intracellular bacteria (Baptista et al., 2018).

4.5.1 Benefits of Nanoantibiotics

Drug-loaded surface-modified NPs can bypass immune systems and target specific tissues. Nanocarriers may reduce drug-induced adverse effects and improve drug solubility or stability. Nanotechnology may allow co-delivery of two or more medications for combination treatment. NP-based antimicrobial medication delivery can help overcome pathogens' antibiotic resistance. NPs can improve therapeutic index, lengthen drug circulation, and accomplish controlled drug release, enhancing pharmacokinetics. It can be utilized for oral, nasal, parenteral, and intraocular administration. Thus, antimicrobial NPs are of tremendous interest because they offer several advantages over free antimicrobial drugs (Garg et al., 2019).

4.5.2 Drawbacks of Nanoantibiotics

This intriguing technology's clinical use faces several hurdles. The harmful effects of antimicrobial NPs on central nervous system (CNS) cells and tissues remain unclear. NPs' size-specific features restrict the broad application of in vitro research, and there is no defined definition for NP dose in mass, number, surface area, and biological samples (e.g., blood, urine, and inside organs) (Kashizadeh et al., 2022). This indicates that novel characterization approaches not impacted by NP characteristics and biological media are needed. NPs have a limited half-life owing to the

mononuclear phagocytic system's innate defensive mechanism for removing them. To avoid opsonization, particle surfaces must be modified.

4.6 Future Scope, Emerging Trends, and Road Ahead

The unveiling of the human genome presents enormous data to the researchers to adopt various rational approaches to design strategies to block infections. Targeting the viral genome would be a realistic strategy for designing therapeutic agents with highly selective toward virus. To enhance selective toxicity, application of drug delivery systems (DDS) is being seriously considered. The control of various viral infections by artificial oligonucleotides has been tried since it was assumed that artificial oligonucleotides including antisense DNA were effective against communicable diseases. In the summer of 1998, an antisense drug product (Fomivirsen) for cytomegalovirus received FDA approval as the first antisense DNA (Hui, 2006). The most severe complication is lead blindness. In advanced-stage AIDS patients, blindness from cytomegaloviral infection becomes an important issue when other antiviral medication is contraindicated and is serious from the point of view of quality of life. Fomivirsen, the first antisense DNA, is administered directly into the vitreous. Cationic carriers utilize a carrier in order to avoid complexity involving chemical modification of oligonucleotide. GS2888 cytofectin has been reported recently, and is assumed to promote cytoplasmic delivery (Juliano et al., 2008). Cytofectin is a complex of a new cationic lipid, GS2888, and dioleophosphatidylethanolamine. Transfection efficacy is enhanced when polycation such as poly-L-lysine or protamine is added to cationic liposomes. An oligonucleotide that specifically binds to protein is called an aptamer (Nchinda et al., 2002). Selection and isolation of aptamers have been enthusiastically carried out.

Below the human body's temperature, pluronic gel is a liquid, but when it reaches body temperature, it transforms into a gel. Pluronic gel is liquid below the body temperature while it starts gel form at the body temperature. This characteristic has advantage in adjusting appropriate formulation and easiness for administration. Antisense DNA to c-myc and c-myb was administered to the perivascular aspect of injured rat carotid arteries via polymer-based delivery system (Al Khateb et al., 2016). Gene-gun is a unique physical method to increase the delivery efficiency of gene. The DNA- or RNA-coated gold microcarriers are directly transferred in vivo. Because special apparatus is necessary, it is not widely used. However, transfection efficiency is extraordinarily superior. Further, as companies like Incyte company are offering inexpensive genome data to individual researchers, it could be suggested that the future of gene medicine looks bright despite the major challenges.

IDs are a major driver of morbidity and mortality globally, and their impact on low SDI countries is particularly grave. Simplifying the use of medicines and making drugs safer and more efficacious can improve patients' quality of life and reduce disease burden. In this sense, drug delivery scientists are searching for the ideal vehicle for delivering drugs (Tsoumpra et al., 2019). One system that would

dramatically reduce drug dosage, improve in the drug absorption so that the patient can take a smaller dose, and yet have the same benefit, deliver the drug to the right place in the living system, increase the local concentration of the drug at the favorite site, and limit or eliminate side effects.

This chapter highlights many promising therapeutic strategies for developing medicines like the nanotechnology-based approach, and biomimetic delivery to treat and prevent IDs. However, to have the maximum impact, all these formulations will need to overcome several financial, manufacturing, and regulatory challenges.

References

Adepu, S., & Ramakrishna, S. (2021). Controlled drug delivery systems: Current status and future directions. *Molecules (Basel, Switzerland), 26*(19), 5905. https://doi.org/10.3390/molecules26195905

Akbarzadeh, A., Rezaei-Sadabady, R., Davaran, S., Joo, S. W., Zarghami, N., Hanifehpour, Y., Samiei, M., Kouhi, M., & Nejati-Koshki, K. (2013). Liposome: Classification, preparation, and applications. *Nanoscale Research Letters, 8*(1), 102. https://doi.org/10.1186/1556-276X-8-102

Al Khateb, K., Ozhmukhametova, E. K., Mussin, M. N., Seilkhanov, S. K., Rakhypbekov, T. K., Lau, W. M., & Khutoryanskiy, V. V. (2016). In situ gelling systems based on Pluronic F127/Pluronic F68 formulations for ocular drug delivery. *International Journal of Pharmaceutics, 502*(1–2), 70–79. https://doi.org/10.1016/j.ijpharm.2016.02.027

Alabresm, A., Chandler, S. L., Benicewicz, B. C., & Decho, A. W. (2021). Nanotargeting of resistant infections with a special emphasis on the biofilm landscape. *Bioconjugate Chemistry, 32*(8), 1411–1430. https://doi.org/10.1021/acs.bioconjchem.1c00116

Bailly, N., Thomas, M., & Klumperman, B. (2012). Poly (N-vinylpyrrolidone)-block-poly (vinyl acetate) as a drug delivery vehicle for hydrophobic drugs. *Biomacromolecules, 13*(12), 4109–4117.

Baptista, P. V., McCusker, M. P., Carvalho, A., Ferreira, D. A., Mohan, N. M., Martins, M., & Fernandes, A. R. (2018). Nano-strategies to fight multidrug resistant bacteria—"A Battle of the Titans". *Frontiers in Microbiology, 9*, 1441.

Begines, B., Ortiz, T., Pérez-Aranda, M., Martínez, G., Merinero, M., Argüelles-Arias, F., & Alcudia, A. (2020). Polymeric nanoparticles for drug delivery: Recent developments and future prospects. *Nanomaterials (Basel, Switzerland), 10*(7), 1403. https://doi.org/10.3390/nano10071403

Bhakta, P., & Barthunia, B. (2020). Fullerene and its applications: A review. *Journal of Indian Academy of Oral Medicine and Radiology, 32*(2), 159.

Bhattacharya, P., Grimme, S., Ganesh, B., Gopisetty, A., Sheng, J. R., Martinez, O., Jayarama, S., Artinger, M., Meriggioli, M., & Prabhakar, B. S. (2010). Nanodisc-incorporated hemagglutinin provides protective immunity against influenza virus infection. *Journal of Virology, 84*(1), 361–371. https://doi.org/10.1128/JVI.01355-09

Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. *International Journal of Nanomedicine, 10*, 975–999. https://doi.org/10.2147/IJN.S68861

Brusini, R., Varna, M., & Couvreur, P. (2020). Advanced nanomedicines for the treatment of inflammatory diseases. *Advanced Drug Delivery Reviews, 157*, 161–178. https://doi.org/10.1016/j.addr.2020.07.010

Chen, L., Hong, W., Ren, W., Xu, T., Qian, Z., & He, Z. (2021). Recent progress in targeted delivery vectors based on biomimetic nanoparticles. *Signal Transduction and Targeted Therapy, 6*(1), 1–25.

Chiappetta, D. A., Hocht, C., Taira, C., & Sosnik, A. (2010). Efavirenz-loaded polymeric micelles for pediatric anti-HIV pharmacotherapy with significantly higher oral bioavailability [corrected]. *Nanomedicine (London, England), 5*(1), 11–23. https://doi.org/10.2217/nnm.09.90

Clarkson, J. J., Dodsworth, S., & Chase, M. W. (2017). Time-calibrated phylogenetic trees establish a lag between polyploidisation and diversification in Nicotiana (Solanaceae). *Plant Systematics and Evolution, 303*(8), 1001–1012.

Colilla, M., & Vallet-Regí, M. (2020). Targeted stimuli-responsive mesoporous silica nanoparticles for bacterial infection treatment. *International Journal of Molecular Sciences, 21*(22), 8605. https://doi.org/10.3390/ijms21228605

Czajkowska-Kośnik, A., Szekalska, M., & Winnicka, K. (2019). Nanostructured lipid carriers: A potential use for skin drug delivery systems. *Pharmacological Reports, 71*(1), 156–166.

Date, A. A., Hanes, J., & Ensign, L. M. (2016). Nanoparticles for oral delivery: Design, evaluation and state-of-the-art. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 240*, 504–526. https://doi.org/10.1016/j.jconrel.2016.06.016

Devi, V. K., Jain, N., & Valli, K. S. (2010). Importance of novel drug delivery systems in herbal medicines. *Pharmacognosy Reviews, 4*(7), 27–31. https://doi.org/10.4103/0973-7847.65322

Dhingra, S., Rahman, N. A. A., Peile, E., Rahman, M., Sartelli, M., Hassali, M. A., et al. (2020). Microbial resistance movements: An overview of global public health threats posed by antimicrobial resistance, and how best to counter. *Frontiers in Public Health, 8*, 535668.

Dhuri, K., Bechtold, C., Quijano, E., Pham, H., Gupta, A., Vikram, A., & Bahal, R. (2020). Antisense oligonucleotides: An emerging area in drug discovery and development. *Journal of Clinical Medicine, 9*(6), 2004. https://doi.org/10.3390/jcm9062004

Dresselhaus, M. S., Dresselhaus, G., Eklund, P. C., & Rao, A. M. (2000). Carbon nanotubes. In *The physics of fullerene-based and fullerene-related materials* (pp. 331–379). Springer.

Eatemadi, A., Daraee, H., Karimkhanloo, H., Kouhi, M., Zarghami, N., Akbarzadeh, A., et al. (2014). Carbon nanotubes: Properties, synthesis, purification, and medical applications. *Nanoscale Research Letters, 9*(1), 1–13.

Elmowafy, M., & Al-Sanea, M. M. (2021). Nanostructured lipid carriers (NLCs) as drug delivery platform: Advances in formulation and delivery strategies. *Saudi Pharmaceutical Journal, 29*(9), 999–1012.

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in Medicinal Chemistry, 6*, 25–64. https://doi.org/10.4137/PMC.S14459

Fattal, E., Pecquet, S., Couvreur, P., & Andremont, A. (2002). Biodegradable microparticles for the mucosal delivery of antibacterial and dietary antigens. *International Journal of Pharmaceutics, 242*(1–2), 15–24.

Feddema, J. J., van der Geest, A. M., Claassen, E., & van de Burgwal, L. (2021). Lower respiratory tract infection trends in East and South-East Asia: In the light of economic and health care development. *Global Pediatric Health, 8*, 2333794X21989530. https://doi.org/10.1177/2333794X21989530

Franco, P., & De Marco, I. (2020). The use of poly(*N*-vinyl pyrrolidone) in the delivery of drugs: A review. *Polymers, 12*(5), 1114. https://doi.org/10.3390/polym12051114

Garg, U., Chauhan, S., Nagaich, U., & Jain, N. (2019). Current advances in chitosan nanoparticles based drug delivery and targeting. *Advanced Pharmaceutical Bulletin, 9*(2), 195–204. https://doi.org/10.15171/apb.2019.023

Gaudana, R., Ananthula, H. K., Parenky, A., & Mitra, A. K. (2010). Ocular drug delivery. *The AAPS Journal, 12*(3), 348–360. https://doi.org/10.1208/s12248-010-9183-3

Hanafy, N., El-Kemary, M., & Leporatti, S. (2018). Micelles structure development as a strategy to improve smart cancer therapy. *Cancers, 10*(7), 238. https://doi.org/10.3390/cancers10070238

Hui, E. K. (2006). Reasons for the increase in emerging and re-emerging viral infectious diseases. *Microbes and Infection, 8*(3), 905–916. https://doi.org/10.1016/j.micinf.2005.06.032

Jain, K., Verma, A. K., Mishra, P. R., & Jain, N. K. (2015). Characterization and evaluation of amphotericin B loaded MDP conjugated poly (propylene imine) dendrimers. *Nanomedicine:*

Nanotechnology, Biology, and Medicine, 11(3), 705–713. https://doi.org/10.1016/j.nano.2014.11.008

JolanyVangah, S., Katalani, C., Booneh, H. A., Hajizade, A., Sijercic, A., & Ahmadian, G. (2020). CRISPR-based diagnosis of infectious and noninfectious diseases. *Biological Procedures Online, 22*, 22. https://doi.org/10.1186/s12575-020-00135-3

Juliano, R., Alam, M. R., Dixit, V., & Kang, H. (2008). Mechanisms and strategies for effective delivery of antisense and siRNA oligonucleotides. *Nucleic Acids Research, 36*(12), 4158–4171. https://doi.org/10.1093/nar/gkn342

Kanekiyo, M., Wei, C. J., Yassine, H. M., McTamney, P. M., Boyington, J. C., Whittle, J. R., Rao, S. S., Kong, W. P., Wang, L., & Nabel, G. J. (2013). Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. *Nature, 499*(7456), 102–106. https://doi.org/10.1038/nature12202

Kashizadeh, A., Pastras, C., Rabiee, N., Mohseni-Dargah, M., Mukherjee, P., & Asadnia, M. (2022). Potential nanotechnology-based diagnostic and therapeutic approaches for Meniere's disease. *Nanomedicine: Nanotechnology, Biology and Medicine, 46*, 102599.

Kazi, K. M., Mandal, A. S., Biswas, N., Guha, A., Chatterjee, S., Behera, M., & Kuotsu, K. (2010). Niosome: A future of targeted drug delivery systems. *Journal of Advanced Pharmaceutical Technology & Research, 1*(4), 374–380. https://doi.org/10.4103/0110-5558.76435

Khandare, J., Mohr, A., Calderón, M., Welker, P., Licha, K., & Haag, R. (2010). Structure-biocompatibility relationship of dendritic polyglycerol derivatives. *Biomaterials, 31*(15), 4268–4277.

Kirtane, A. R., Verma, M., Karandikar, P., Furin, J., Langer, R., & Traverso, G. (2021). Nanotechnology approaches for global infectious diseases. *Nature Nanotechnology, 16*(4), 369–384.

Larson, N., & Ghandehari, H. (2012). Polymeric conjugates for drug delivery. *Chemistry of Materials: A Publication of the American Chemical Society, 24*(5), 840–853. https://doi.org/10.1021/cm2031569

Lederhofer, J., van Lent, J., Bhoelan, F., Karneva, Z., de Haan, A., Wilschut, J. C., & Stegmann, T. (2018). Development of a Virosomal RSV vaccine containing 3D-PHAD® adjuvant: Formulation, composition, and long-term stability. *Pharmaceutical Research, 35*(9), 172. https://doi.org/10.1007/s11095-018-2453-y

Liguori, B., Aprea, P., Gennaro, B., Iucolano, F., Colella, A., & Caputo, D. (2019). Pozzolanic activity of zeolites: The role of Si/Al ratio. *Materials (Basel, Switzerland), 12*(24), 4231. https://doi.org/10.3390/ma12244231

Lin, D. M., Koskella, B., & Lin, H. C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World Journal of Gastrointestinal Pharmacology and Therapeutics, 8*(3), 162–173.

Liu, P., Chen, G., & Zhang, J. (2022). A review of liposomes as a drug delivery system: Current status of approved products, regulatory environments, and future perspectives. *Molecules (Basel, Switzerland), 27*(4), 1372. https://doi.org/10.3390/molecules27041372

Martins, S., Sarmento, B., Ferreira, D. C., & Souto, E. B. (2007). Lipid-based colloidal carriers for peptide and protein delivery--liposomes versus lipid nanoparticles. *International Journal of Nanomedicine, 2*(4), 595–607.

Michael, C. A., Dominey-Howes, D., & Labbate, M. (2014). The antimicrobial resistance crisis: Causes, consequences, and management. *Frontiers in Public Health, 2*, 145.

Michaud, C. M. (2009). Global burden of infectious diseases. *Encyclopedia of Microbiology*, 444–454. https://doi.org/10.1016/B978-012373944-5.00185-1

Mora-Huertas, C. E., Fessi, H., & Elaissari, A. (2010). Polymer-based nanocapsules for drug delivery. *International Journal of Pharmaceutics, 385*(1–2), 113–142.

Naseri, N., Valizadeh, H., & Zakeri-Milani, P. (2015). Solid lipid nanoparticles and nanostructured lipid carriers: Structure, preparation and application. *Advanced Pharmaceutical Bulletin, 5*(3), 305–313. https://doi.org/10.15171/apb.2015.043

Naskar, A., Cho, H., Lee, S., & Kim, K. S. (2021). Biomimetic nanoparticles coated with bacterial outer membrane vesicles as a new-generation platform for biomedical applications. *Pharmaceutics, 13*(11), 1887. https://doi.org/10.3390/pharmaceutics13111887

Nchinda, G., Überla, K., & Zschörnig, O. (2002). Characterization of cationic lipid DNA transfection complexes differing in susceptibility to serum inhibition. *BMC Biotechnology, 2*(1), 1–10.

Negut, I., Grumezescu, V., & Grumezescu, A. M. (2018). Treatment strategies for infected wounds. *Molecules (Basel, Switzerland), 23*(9), 2392. https://doi.org/10.3390/molecules23092392

Nicoletti, S., Seifert, K., & Gilbert, I. H. (2009). N-(2-hydroxypropyl)methacrylamide-amphotericin B (HPMA-AmB) copolymer conjugates as antileishmanial agents. *International Journal of Antimicrobial Agents, 33*(5), 441–448. https://doi.org/10.1016/j.ijantimicag.2008.10.013

Nikolova, M. P., & Chavali, M. S. (2020). Metal oxide nanoparticles as biomedical materials. *Biomimetics (Basel, Switzerland), 5*(2), 27. https://doi.org/10.3390/biomimetics5020027

Nooraei, S., Bahrulolum, H., Hoseini, Z. S., Katalani, C., Hajizade, A., Easton, A. J., & Ahmadian, G. (2021). Virus-like particles: Preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. *Journal of Nanobiotechnology, 19*(1), 1–27.

Păduraru, D. N., Ion, D., Niculescu, A. G., Mușat, F., Andronic, O., Grumezescu, A. M., & Bolocan, A. (2022). Recent developments in metallic nanomaterials for cancer therapy, diagnosing and imaging applications. *Pharmaceutics, 14*(2), 435. https://doi.org/10.3390/pharmaceutics14020435

Pati, R., Shevtsov, M., & Sonawane, A. (2018). Nanoparticle vaccines against infectious diseases. *Frontiers in Immunology, 9*, 2224. https://doi.org/10.3389/fimmu.2018.02224

Patra, J. K., Das, G., Fraceto, L. F., Campos, E., Rodriguez-Torres, M., Acosta-Torres, L. S., Diaz-Torres, L. A., Grillo, R., Swamy, M. K., Sharma, S., Habtemariam, S., & Shin, H. S. (2018). Nano based drug delivery systems: Recent developments and future prospects. *Journal of Nanobiotechnology, 16*(1), 71. https://doi.org/10.1186/s12951-018-0392-8

Philip, A. K., & Philip, B. (2010). Colon targeted drug delivery systems: A review on primary and novel approaches. *Oman Medical Journal, 25*(2), 79.

Rahman, M. T., Sobur, M. A., Islam, M. S., Ievy, S., Hossain, M. J., El Zowalaty, M. E., Rahman, A. T., & Ashour, H. M. (2020). Zoonotic diseases: Etiology, impact, and control. *Microorganisms, 8*(9), 1405. https://doi.org/10.3390/microorganisms

Rizvi, S., & Saleh, A. M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal :SPJ: The Official Publication of the Saudi Pharmaceutical Society, 26*(1), 64–70. https://doi.org/10.1016/j.jsps.2017.10.012

Rocklöv, J., & Dubrow, R. (2020). Climate change: An enduring challenge for vector-borne disease prevention and control. *Nature Immunology, 21*, 479–483. https://doi.org/10.1038/s41590-020-0648-y

Saleri, N., & Ryan, E. T. (2019). Respiratory infections. *Travel Medicine*, 527–537. https://doi.org/10.1016/B978-0-323-54696-6.00059-8

Sánchez-López, E., Gomes, D., Esteruelas, G., Bonilla, L., Lopez-Machado, A. L., Galindo, R., Cano, A., Espina, M., Ettcheto, M., Camins, A., Silva, A. M., Durazzo, A., Santini, A., Garcia, M. L., & Souto, E. B. (2020). Metal-based nanoparticles as antimicrobial agents: An overview. *Nanomaterials (Basel, Switzerland), 10*(2), 292. https://doi.org/10.3390/nano10020292

Selvarajan, V., Obuobi, S., & Ee, P. L. R. (2020). Silica nanoparticles—A versatile tool for the treatment of bacterial infections. *Frontiers in Chemistry, 8*, 602.

Stray-Pedersen, A., Aaberge, I. S., Früh, A., & Abrahamsen, T. G. (2005). Pneumococcal conjugate vaccine followed by pneumococcal polysaccharide vaccine; immunogenicity in patients with ataxia-telangiectasia. *Clinical and Experimental Immunology, 140*(3), 507–516. https://doi.org/10.1111/j.1365-2249.2005.02791.x

Streicher, L. M. (2021). Exploring the future of infectious disease treatment in a post-antibiotic era: A comparative review of alternative therapeutics. *Journal of Global Antimicrobial Resistance, 24*, 285–295.

Tariq, H., Batool, S., Asif, S., Ali, M., & Abbasi, B. H. (2022). Virus-like particles: Revolutionary platforms for developing vaccines against emerging infectious diseases. *Frontiers in Microbiology, 12*, 790121.

Tenchov, R., Bird, R., Curtze, A. E., & Zhou, Q. (2021). Lipid nanoparticles─ from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. *ACS Nano, 15*(11), 16982–17015.

Triggle, C. R., Bansal, D., Ding, H., Islam, M. M., Farag, E. A. B. A., Hadi, H. A., & Sultan, A. A. (2021). A comprehensive review of viral characteristics, transmission, pathophysiology, immune response, and management of SARS-CoV-2 and COVID-19 as a basis for controlling the pandemic. *Frontiers in Immunology, 12*, 631139.

Tsoumpra, M. K., Fukumoto, S., Matsumoto, T., Takeda, S., Wood, M., & Aoki, Y. (2019). Peptide-conjugate antisense based splice-correction for Duchenne muscular dystrophy and other neuromuscular diseases. *eBioMedicine, 45*, 630–645. https://doi.org/10.1016/j.ebiom.2019.06.036

Wagner, A. M., Knipe, J. M., Orive, G., & Peppas, N. A. (2019). Quantum dots in biomedical applications. *Acta Biomaterialia, 94*, 44–63. https://doi.org/10.1016/j.actbio.2019.05.022

Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *International Journal of Nanomedicine, 12*, 1227–1249. https://doi.org/10.2147/IJN.S121956

Wang, Y. C., Rhéaume, É., Lesage, F., & Kakkar, A. (2018). Synthetic methodologies to gold Nanoshells: An overview. *Molecules (Basel, Switzerland), 23*(11), 2851. https://doi.org/10.3390/molecules23112851

Yang, G., Chen, S., & Zhang, J. (2019). Bioinspired and biomimetic nanotherapies for the treatment of infectious diseases. *Frontiers in Pharmacology, 10*, 751. https://doi.org/10.3389/fphar.2019.00751

Yetisgin, A. A., Cetinel, S., Zuvin, M., Kosar, A., & Kutlu, O. (2020). Therapeutic nanoparticles and their targeted delivery applications. *Molecules (Basel, Switzerland), 25*(9), 2193. https://doi.org/10.3390/molecules25092193

Yoo, J., Park, C., Yi, G., Lee, D., & Koo, H. (2019). Active targeting strategies using biological ligands for nanoparticle drug delivery systems. *Cancers, 11*(5), 640. https://doi.org/10.3390/cancers11050640

Zhang, K., Li, X., Yu, C., & Wang, Y. (2020). Promising therapeutic strategies against microbial biofilm challenges. *Frontiers in Cellular and Infection Microbiology, 10*, 359.

Zhou, J., Kroll, A. V., Holay, M., Fang, R. H., & Zhang, L. (2020). Biomimetic nanotechnology toward personalized vaccines. *Advanced Materials (Deerfield Beach, Fla.), 32*(13), e1901255. https://doi.org/10.1002/adma.201901255

Chapter 5
Emerging Vaccine for the Treatment of Cancer Via Nanotechnology

K. Jagadeesh Chandra Bose and Jyoti Sarwan

5.1 Introduction

The immune system of homo sapiens is organized as a complex network comprising of various cells, organs, proteins, and chemical modulators along with anatomical and physical barriers which work collectively to resist many pathogens and antigen-causing infections. It can be also explained as the capability of the body to vanish foreign molecules in the body reduce risks for severe infections, but it has observed if it has not strength enough can lead to numerous diseases. Sometimes even an extremely active immune system can lead to autoimmunity and some inflammatory responses to the production of healthy tissues (Rosenblum et al., 2015; Wang et al., 2015). The immune system not only functions against external antigens with which our body encounters the clinical illness in the form of exo-antigens, but also can respond to numerous endo-antigens such as intracellular pathogenic viruses as well as certain tumour antigens. The natural immune system always tries to eliminate foreign pathogens, including cancer cells. The proper functioning of the immune system is a very important aspect of the treatment of cancer patients as either cancer itself can weaken the individual immune system or various cancer therapies might also weaken the immune system instead of fighting against the cancer.

K. Jagadeesh Chandra Bose (✉) · J. Sarwan
University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India
e-mail: jagadeesh.e8748@cumail.ins

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_5

5.2 Cancer and Treatments May Weaken Immunity

Cancer generally targets human immunity and generally undermines the functions of the immune system, especially when it causes the metastasis of bone marrow which makes diverse blood cells that will be the sole part of resistance to fight against infection. This will be most often observed in the case of certain types of cancers such as lymphoma or leukaemia in addition to other cancers. Cancer therapy inhibits bone marrow from being effective hemopoiesis that can make so many blood cells. Certain cancer treatments, such as radiation and chemotherapy, will target cell division and cannot have selective toxicity by which only the cancer cells will be destroyed and equally toxic to cancer cells as well as normal dividing cells which will temporarily weaken the immune system. This is because of the drop in the number of blood leukocytes which are generated in the bone marrow. Among the cancer treatments that are most likely to weaken the immune system are chemotherapy, radiotherapy, and high doses of steroids.

5.3 Cancer Treatments That Use the Immune System

Some of the cells of our immune system can recognise a wide range of different cancer cells as abnormal cells and effectively kill them through the identification of tumour markers on their surfaces. But this mechanism may not be sufficient to dispose of a cancer altogether. Hence some treatment strategies will aim to induce the individual immune system to fight against cancer, either with the inbuilt immune protection or with the protection methods developed after exposure to certain disease-causing agents by acquired immunity. Some advanced types of cancer treatments are currently used to boost the immune system to fight effectively against cancer, such as immunotherapy. This is one of the best treatment options for some types of cancer. It induces the immune system to precisely find the cancer cells, target them and kill cancer cells. They are much more helpful in cancer treatment since the cancer cells are metabolically and immunologically different from the normal cells of our body. Hence the targeted immune therapy helps the immune system to differentiate and kill the abnormal cancer cells. There are different types of chemicals that are part of the immune response with immunotherapy, such as monoclonal antibodies (MABs), which exclusively recognise and attack certain protein markers generally known as targeted antigens on the surface of cancer cells.

But if we can block the cellular transformation

- Vaccines to help the immune system to recognise and attack cancer,
- Cytokines to help to boost the immune system,
- CAR T-cell therapy (also called adoptive cell transfer) to change the genes in a person's white blood cells.

5.4 Cellular Genomic Changes and Cancer

All cancers begin within a single cell. Our body are made up of more than a hundred million million cells. Generally, the cancer starts with genomic changes within one cell or a small group of cells. The cells will have cellular machinery to create signals to control the cellular growth and cell division. If any of these regulatory signals are faulty or missing, these cells attain the capacity to start to grow and multiply excessively, thereby leading to uncontrollable proliferation to form a lump called a primary tumour. A primary tumour of the body is generally brought about by genomic instability and escape from the natural defence of our immune system, making it the site where the cancer starts. Some types of cancers, such as like leukaemia, are associated with blood cells; in some circumstances, abnormal immature blood cells will be accumulated, and routine functions will not be held. They will not form solid tumours. Instead, the cancer cells build up either in the blood or in the bone marrow. For a cancer to start, certain cellular changes take place within the genes of a cell or a group of cells. Diverse types of cells in our body perform different functions, even though they are basically similar. But each of the cells have a unique genetic makeup to engage typical functionalities by expressing diverse proteins via RNA (ribonucleic acid). Genes will make sure that cells grow and reproduce in an orderly and controlled way to keep the body normal and healthy. Sometimes, a sudden change may happen because of either intrinsic or extrinsic factors in the genes when a cell divide is known as mutation. These mutations are also time to time restored by DNA damage repair, a natural phenomenon that occurs in all cells. If these mutations accumulated more and irreversibly damages the DNA, then this may lead to the genomic instability, which is a hallmark of cancer. These mutations can happen by chance when a cell is dividing. Some mutations insensitive to the cell no longer recognises cellular instructions. It can twitch to grow out of control. There must be about 6 diverse mutations before a normal cell transform into a cancer cell. Mutations results in a cell cause the expression of many proteins that trigger cell division; with mutation, a cell may stop protein expression that normally causes a cell to stop dividing. Alternatively, an abnormal proteins expression may produce a cell that works in an abnormal manner.

5.5 How Mutations Will Happen in the Cells

Mutations can happen by chance when a cell is in the process of division at the DNA level. This may occur either by the improper processes of metabolism inside the cell such as oxidative stress or by carcinogens and mutagens from outside the body, such as the chemicals in processed food, tobacco smoke, chronic alcoholism etc. In some cases, people will inherit activated oncogenes, in particular, the genes that make them more likely to prone to varieties of cancer. Some genes in the cells get damaged

every day and cells are very good at repairing them. Over time, however, the damages that may accumulate may lead to the cells to start growing too fast; as a process, they are much prone to pick up additional mutations and less likely to be able to repair of these damaged genes.

5.6 What Are Cancer and Cancer Immunotherapy?

Cancer is usually defined as uncontrolled and mutated cells that aggregate at a point and are responsible for relentless cell proliferation. Tumours of cancers grows continuously and over time to escape from the recognise and in always make themselves to get a relief in continues battle with immune system (Kim et al., 2007). The biggest challenge to the modern technology is that the random behaviour of mutations in cells of cancers can even lead to high malignancy within the cells of the tumours. Due to this higher malignancy and the unstable mechanisms of immune response and against mutant cells still unknown and undetected (de Titta et al., 2013; Dagogo-Jack & Shaw, 2018). Therefore, a mechanism to target different cancer mutants and escape the behaviour of the immune system are as yet undefined Law, 1991). Therefore, cancer cells belong to the normal cells and normal cells releases a number of waste and unwanted products. These waste products also carry tumour-specific antigens and resist the parent tumour cells in recognition of cytotoxic T cells (Fig. 5.1).

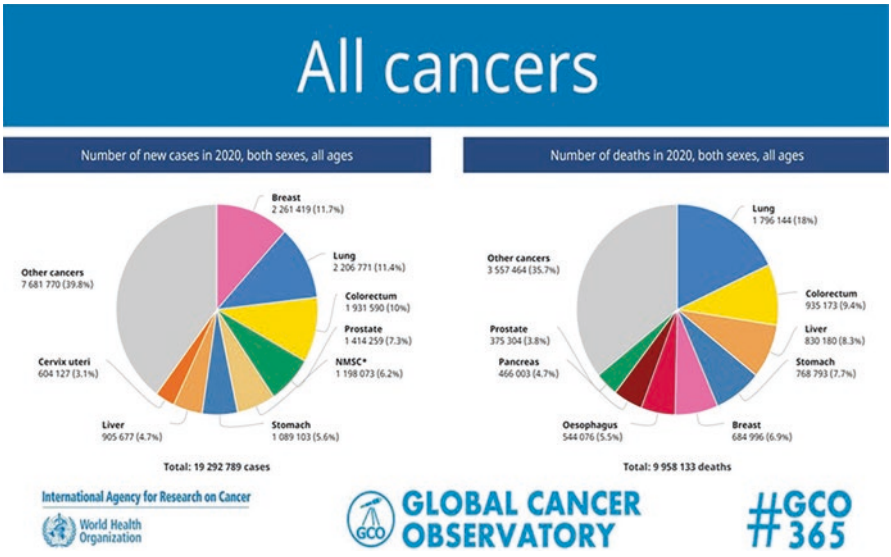


Fig. 5.1 Figure shown Different types of Cancers among both males and females and their percentages in cases

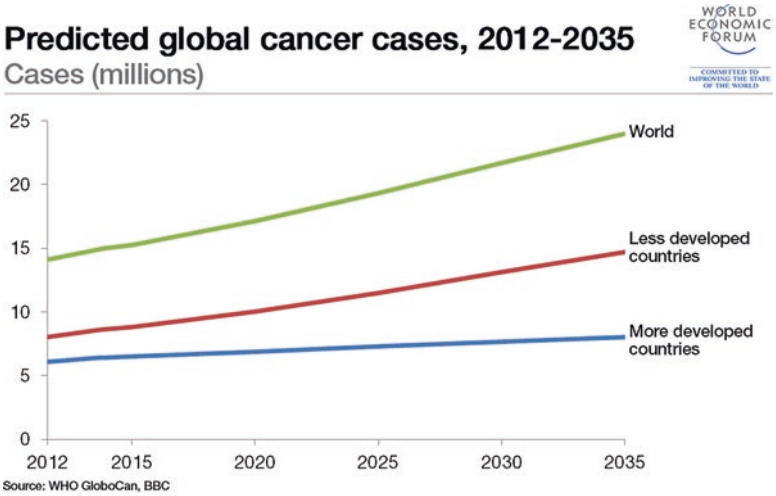


Fig. 5.2 Figure shown estimated cases of cancers worldwide in developed and less developed countries by 2050

Figure has been taken from the World Health Organization observatory of year 2020, collective research of worldwide cancer cases (Fig. 5.2).

Anticancer vaccines have been developed which allow the immune system to identify the tumours and enhance the immune response for a particular antigen such as tumours. This therapy is also known as immune therapy which has its main focus of training the immune system with releasing many components at a time (Rosenberg et al., 2004). These immunotherapies are antigen specific in their characteristics (Fig. 5.3).

Vaccines were made to exercise control over the spreading of diseases all over the world. These vaccines work upon specific infections to accelerate immunity in that person. To date, vaccines have achieved benchmarks in health sciences, leading even in many cases to the complete eradications of some communicated diseases (Rosenberg et al., 2004; Guo et al., 2013) (Fig. 5.4).

To date, vaccine research has achieved high degree of success in terms of both antiviral and antibacterial vaccines. Researchers have been continuously trying to make anticancer vaccines, but the biggest challenge for researchers has been the low immunogenicity of tumours. The main problem with tumours is that they arise from the healthy cells, and they are unable to accelerate immune responses. By 2010, the USA has already approved vaccines for cancer therapy with success rates of cancer therapies. Nanoparticles are highly advanced tools to cure diseases such as cancer. As traditional methods are not much enough effective upon tumours because of their low immunogenicity (Fang et al., 2015) (Figs. 5.5 and 5.6).

Therefore, the mechanism to treat tumours by immune response is non-specific. To attain specific target therapies, genetic engineering is the most favourable approach in cancer treatment therapies (Fesnak et al., 2016; Brudno & Kochenderfer,

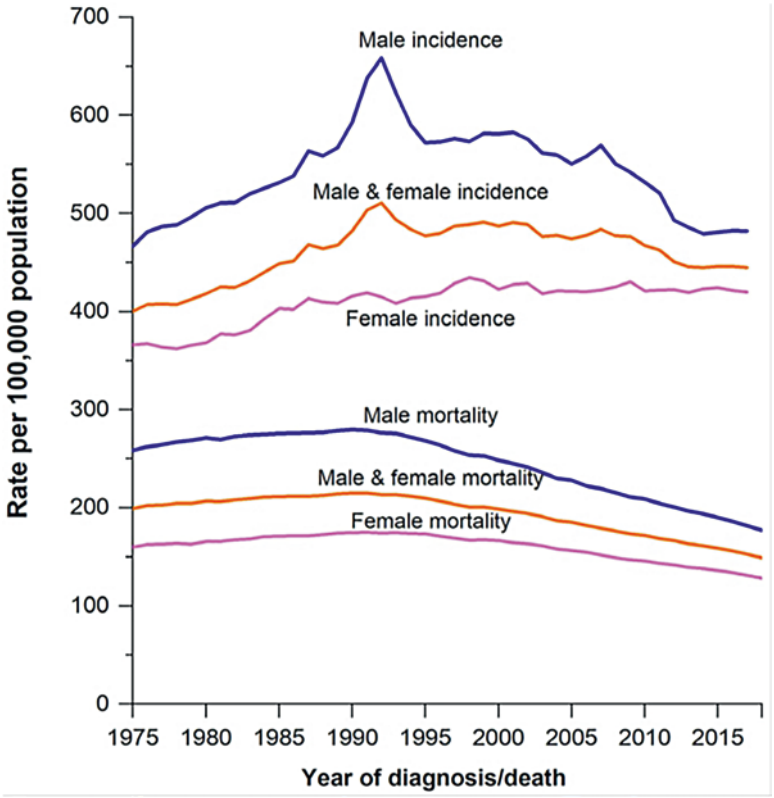


Fig. 5.3 Figure shown number of deaths by cancer in both females and males over the time/year (Siegel et al., 2021)

2018). Chimeric antigen receptors isolated from donor or patient of leukapheresis (Rosenberg et al., 2008). In these techniques, the modifications can be done in the cells to identify tumour-associated antigens. These tumour-associated antigens are capable of recognition and deletion of cancerous cells.

5.6.1 The Current Status of Vaccines and Cancers

Cancer vaccines are made to reduce the tumour formation and their negative impacts over the immune system. If we are comparing traditional therapies with modern technology such as nanovaccines, they are specialized to work upon a specific target, unlike traditional therapies. The Food and Drug Administration of United States has already given approval to the anticancer vaccines in therapies in April 2010. These approvals were involved in the treatment of prostate cancer with sipuleucel-T (Cheever & Higano, 2011) (Fig. 5.7).

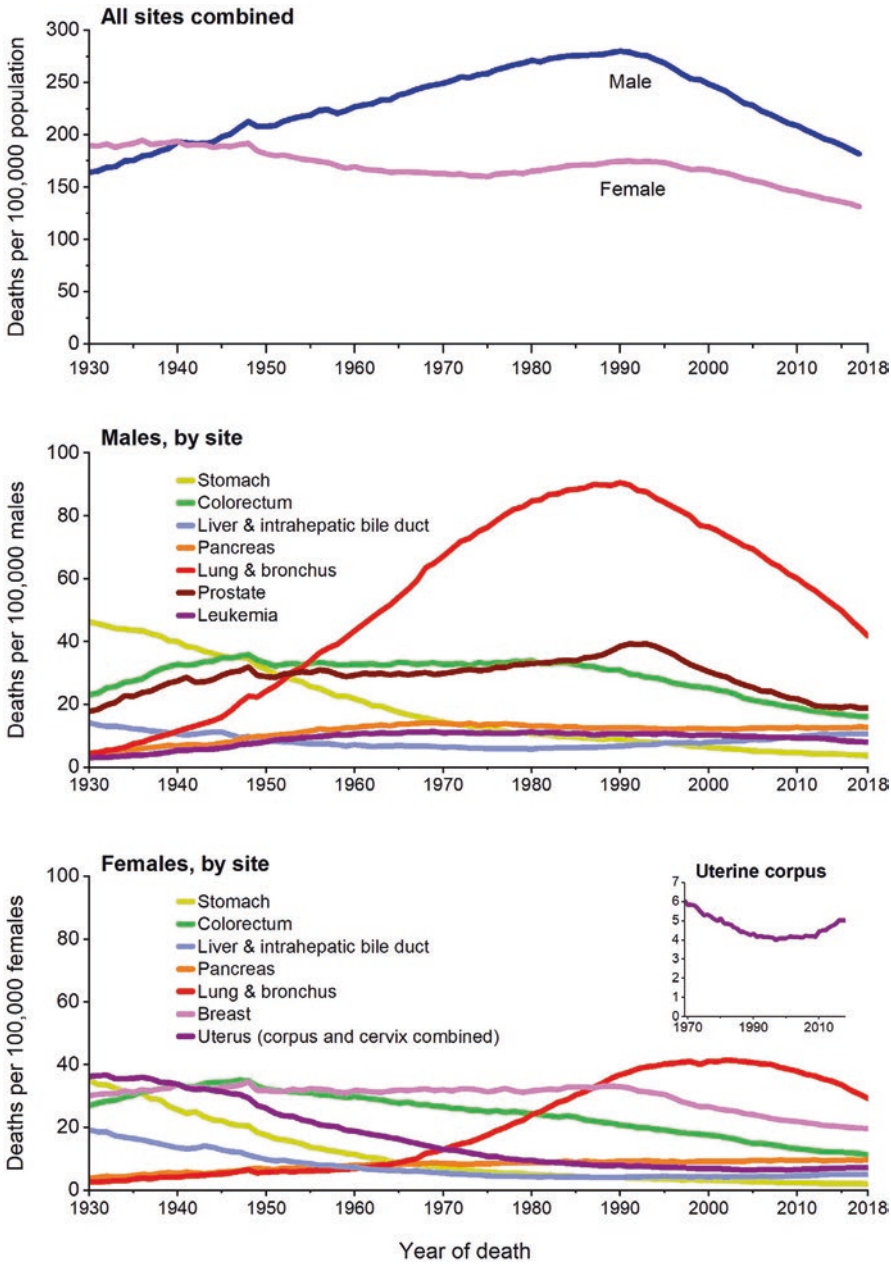


Fig. 5.4 Figure showing the number of deaths increasing in males and females up to the year 2018 (Siegel et al., 2021)

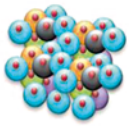
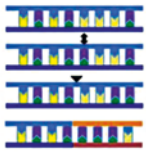
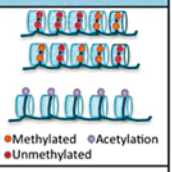
Types of heterogeneity				
Patient	**Tumour**	**Cellular**	**Genomic**	**Epigenetic**
				
Different primary tumours in different patients. Different metastatic tumours in different locations.	Different tumours in the same prostate of a single patient (multi-focal disease). Recurrent tumours that have been altered or selected for by treatment.	Different cell types within each tumour mass. These range from stem cells to terminally differentiated cells.	Different mutations (small nucleotide polymorphisms, insertions, deletions or genome rearrangements).	Different methylation and acetylation patterns between the same genes in the same cells, between normal and cancer cells and between different cancer cells.

Fig. 5.5 Figure describing heterogeneity in cancer in different patients (Kemp & Kwon, 2021)

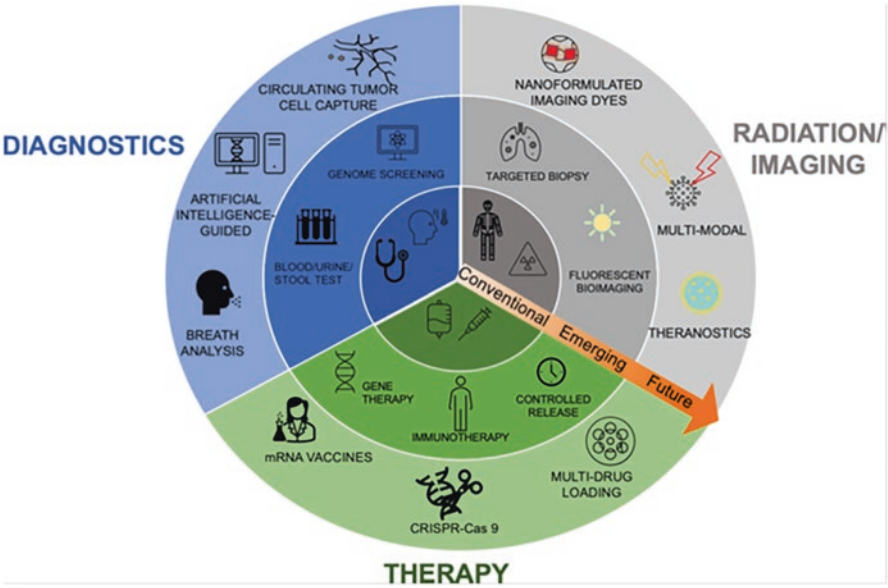


Fig. 5.6 Figure shown detection methods of cancers (Kemp & Kwon, 2021)

These kind of therapies involved prostate cancers as described earlier, involving the treatment of dendritic cells were isolated and exposed with the prosthetic acid phosphatase. This acid phosphatase was significantly observed in a number of patients of prostate cancer (Graddis et al., 2011).

The isolated cells from prostate cancer patients were injected again to the patients after exposing with granulocytes with macrophages. It was observed in the patient that

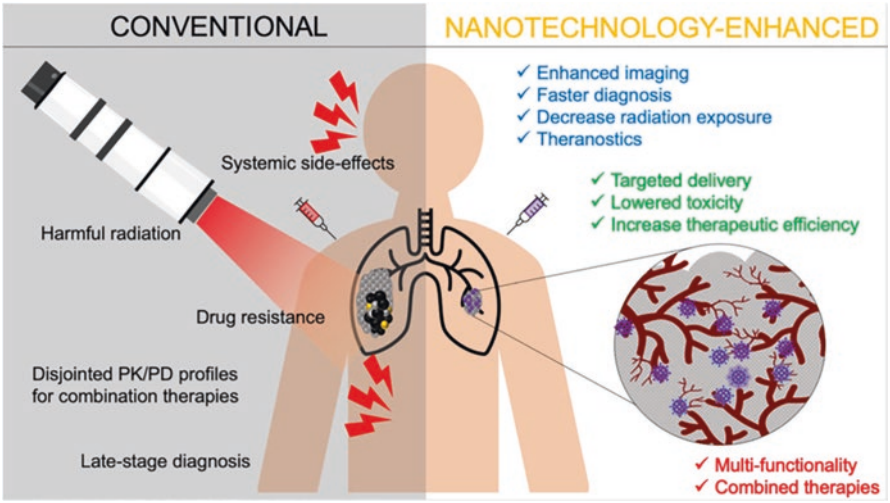


Fig. 5.7 Figure showing how nanovaccines differ from traditional methods (Kemp & Kwon, 2021)

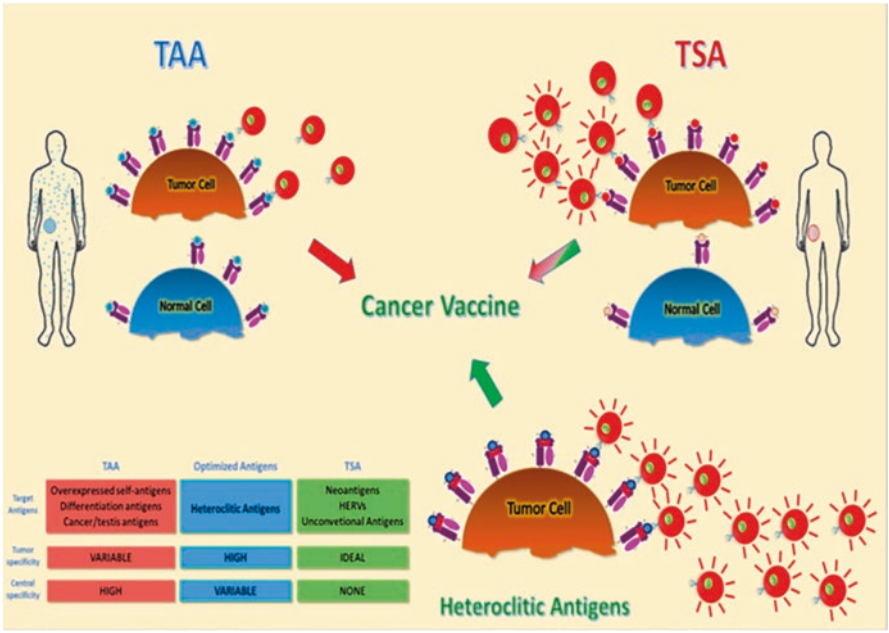


Fig. 5.8 Figure illustrating the basic mechanism behind designing cancer vaccines

the survival time was up to 4.1 months than other untreated cases. Therefore, the government had to approve this method of treatment (Cheever & Higano, 2011) (Fig. 5.8).

Even now, there are more than 200 active trials for cancer therapies. One of the most recent examples of these nanovaccines is glioblastoma or oncolytic ovarian cancer and also the recurrence of breast cancers with dendritic cell therapies.

5.6.2 *Nanovaccines and Their Advantages*

Nanotechnology has various applications in different fields such as food, waste treatment and environment etc., but medical sciences also need these advanced and emerging techniques to interlink different pathways that are unknown. Nanotechnologies are opening doors for many opportunities in cancer research. Unfortunately, the very low efficacy rate of traditional technologies in cancer therapies, nanotechnology can boost various research platforms (Fischer et al., 2013) (Fig. 5.9).

The presence of flexibilities in nanotechnology can stimulate various components in cells such as proteins, polysaccharides, hormones, antibodies, adjuvants and many more. These nanoparticles are encapsulated particularly in biological components such as membranes, cells and proteins for targeting the specific site in the cell. There are some special chemicals that are involved in coating nanoparticles such as calcium phosphate etc. (Tam et al., 2016).

Therefore, nanocarriers are available to carry both antigen and adjuvants at same time (Tam et al., 2016). Nanoparticle-based vaccines are extremely nanosized in nature, which is why they can better design to enhance immune response, decrease the level of immunosuppression and target specificity. As they have narrow-sized characteristics so they can flow in lymph nodes and can achieve drug delivery in immune cells (Bachmann & Jennings, 2010; Shannahan et al., 2015; Reddy et al., 2007).

Nanoformulation can be undertaken in order to maximise biological activity to the payloads and increase direct penetration of cell membranes, Toll -ike receptors and surface molecule by coated with nanoparticles (Fig. 5.10).

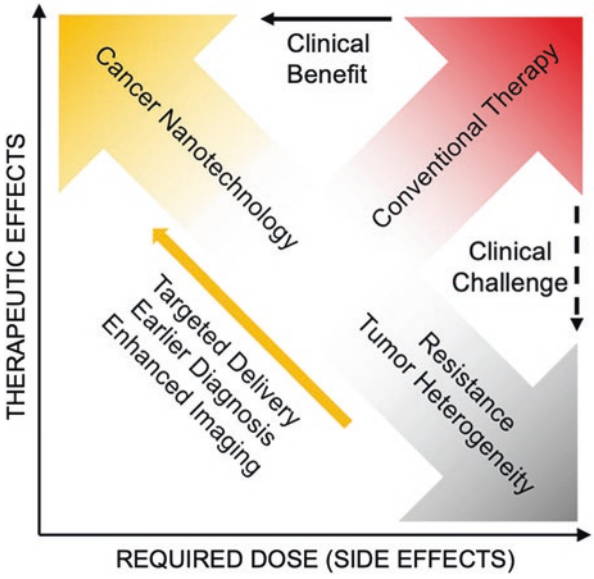


Fig. 5.9 Figure showing conventional therapies, their side effects and the urgency of cancer nanovaccines (Kemp & Kwon, 2021)

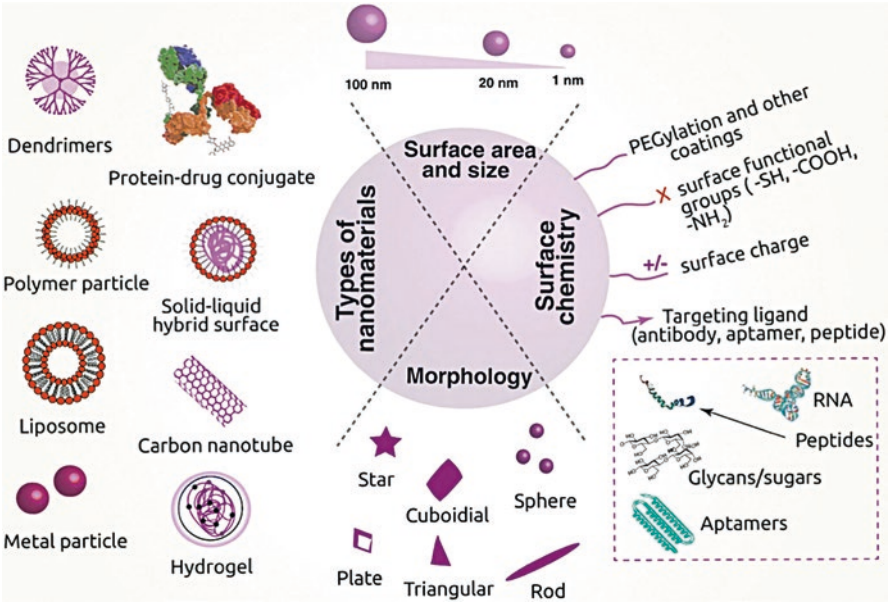


Fig. 5.10 Selection criteria based upon surface chemistry for formulations of nanovaccines in cancer therapies (Kemp & Kwon, 2021)

5.6.3 Nanoparticles Incorporated with Cancer Vaccines

5.6.3.1 Lack of Specificity

There are many immunomodulators based upon nanoparticles; these are the so-called non-specific because they can boost the immune system but are not target-specific in nature. These vaccines are not considered as vaccines due to their non-specificity and only rely upon the immune system of the patient, including tumour recognition and antigen presentation. Thus, the process can be attained by manipulations in immune response through a reduction in immunosuppression and an enhancement of the potential against cancer cells (Wei et al., 2018).

5.6.3.2 Enhancement of Physical Proximity of Immune Cells

The main motive in enhancing the immune system of the patient and the elimination of the tumour cells from his or her body through specific targeting . In this technique the nanoparticles are used to design and in association of two antibodies take place. These two antibodies are being directed; one is to target the immune response to accelerate and other is to target tumour cells. These phenomena not only enhance immune system but are also helpful in releasing tumour antigens and antigen presentation. For example, nanoparticles coated with biodegradable components such

as poly lactic to load antibodies which works against CD40+ markers that are co-stimulators of dendritic cells markers and HER2. HER2 are human epidermal growth factors that are commonly present in its overexpression in breast cancer patients (Dominguez & Lustgarten, 2010; Ashwini & Kumar, 2011).

5.6.3.3 Immunosuppression Reduction

We discussed earlier that immunosuppression is quite necessary in cases like autoimmune diseases. In some cases, it can be defined as being like the early stages of melanoma cancer vaccines are extremely effective in melanoma-specific antibodies. The specificity does not lead to effective treatment in the later stages of melanoma. This specificity is not retained in the later stages because of the increased level of immunosuppression of cytokines such as tumour factors TGF (Xu et al., 2013).

To overcome this condition, nanoparticles are coated with lysosome protamine-hyaluronic acid. In such cases, drug delivery are made to reduce TFGβ tumour effects with siRNA (Xu et al., 2013). But these nano-based drug deliveries are not so simple; it has many side effects to reduce immunosuppression such as inflammation and the blockages of different pathways such as PD1 (Mullard, 2013). One study suggests that these nano-based drug deliveries are also effective in antibodies that work against death ligand known as PDL1 (Zhu et al., 2014).

5.6.3.4 Activation of Immune System

Pathogen-associated molecular patterns are those molecules which are responsible for boosting the immune system of the patient. There are some other factors, such as co-stimulatory markers, signalling proteins and cytokines which are used to enhance the mechanisms in immune responses. It has been found that extremely nonspecific and strong modulator called adjuvants are used in the treatment of cancer. PAMs are mainly involved lipopolysaccharides, double-stranded RNA and single-stranded DNA and helpful in the activation of immune responses in cancer. Polysaccharides are involved in the mechanism of suppressing the effect of inflammation by the immune system and are recognised by toll-like receptors. These PAMs are recognised by TLR9 and patterns that are being recognized are CpG oligonucleotides by endosomal TLR9 (de Titta et al., 2013; Chinnathambi et al., 2012).

5.6.3.5 Combination of Immunosuppressive Intervention and Immune Activation

There are a number of different ways to enhance the immune system and its activation but combining immunosuppressive intervention with it can yield highly favourable results. It can be explained with an example for visualizing the efficacy of the

immune response in a balanced for with Th1 and Th2 cytokines by mimicking the pathogenic behaviour of nanoparticles when combined with CpG ODN and si RNA for IL10 (Pradhan et al., 2014). There is an another example to explain it encapsulation of T cells in nanoparticles for targeting PDI (T cell expressing cells) for activation of R848 (Schmid et al., 2017). This technique can further explain the enhancement of the immune system as well as immunosuppressive intervention by achieving dual targeting nanoparticles which carry both antagonistic and agonistic antibodies on a same surface (Kosmides et al., 2017).

5.6.3.6 Nanoparticles Combining with Traditional Methods of Cancer Treatment

Some studies suggest that traditional therapies rely completely upon the tumour activation of the immune system in such cases as combine traditional methods with nanoparticles and relying of them onto tumour cells can give an accelerated response to new technology. In terms of decreasing the immune activation, nanoparticles have advantages over tumours. For example, potato virus X clone can be used to deactivate the growth of B16-F10 cancer cells (Lee et al., 2017). So-called sticky nanoparticles are being designed to recognise and capture antigens that are available for phagocytosis for immune cells (Min et al., 2017) (Fig. 5.11).

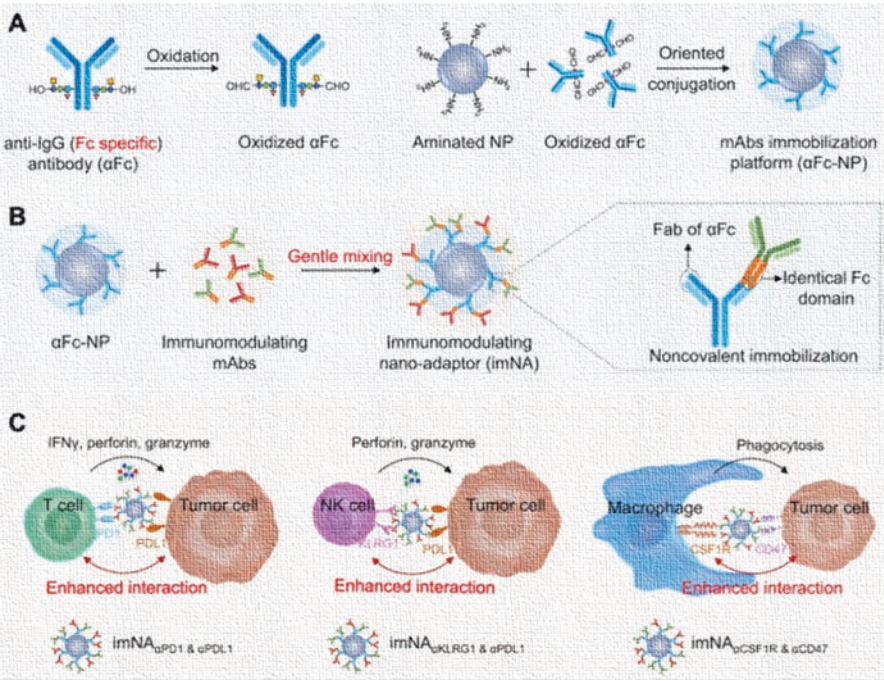


Fig. 5.11 Figure shown mechanism of cancer nanovaccines as immunomodulators and targeting tumours (Kemp & Kwon, 2021)

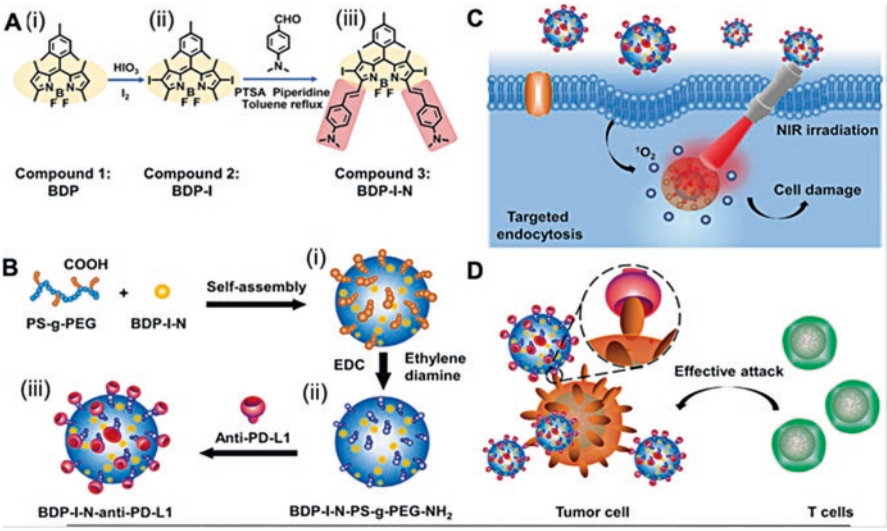


Fig. 5.12 (**a**) and (**b**) representing boron dipyrromethene nanoparticles (**c**) and (**d**) representing phytotoxicity and immune efficacy of tumour cells (Kemp & Kwon, 2021)

5.6.4 Need for Vaccines with Specificity

The main purpose in combining nanoparticles with cancer therapies is to achieve the target of designing and applicable cancer vaccines with specificity. The already present vaccines are not so sufficient to downstream the immunosuppression and low impact on tumours. The ultimate goal of the nanoparticle-based vaccines for cancer must be specific for the tumours, and contains targeted antibodies and adjuvants. These cancer vaccines are found to be more effective; they have cross-representation and their dual targeting nature has an enhanced ability in terms of immune response. The characteristics of nano-based vaccines in cancer treatment are detailed below:

I. Entry in cytosol.
II. Targeting immune cells.
III. Dual deliver/co-delivery of adjuvants and antibodies.
IV. Adjuvancy of inherited nanoparticles (Figs. 5.12 and 5.13).

5.6.5 Types of Nanovaccines for Cancer Therapy (Fig. 5.14)

5.6.5.1 The Background of Nanovaccines for Anticancer Therapies

The treatment of diseases such as cancer offer never-ending challenges to medical researchers. New technologies are always being developed, but they are often slow to establish a secure footing. Nanovaccines are an advanced technology that can

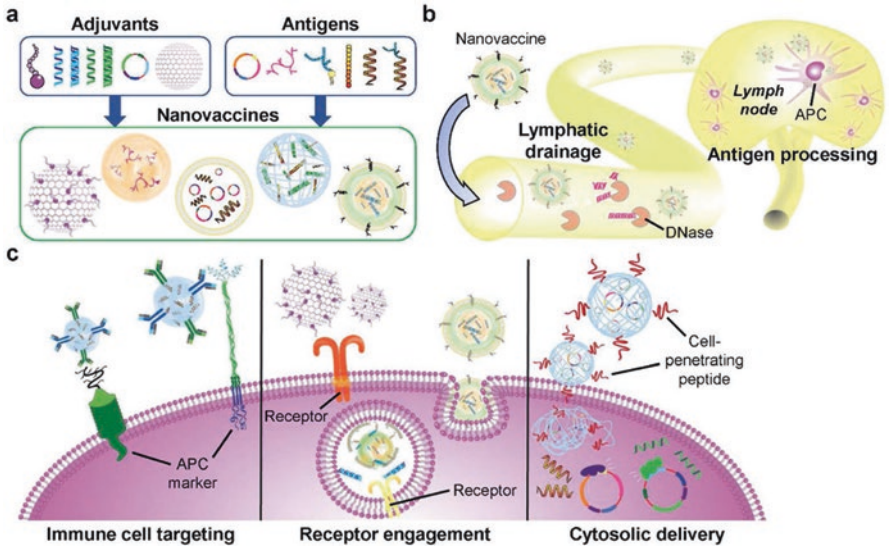


Fig. 5.13 (**a**) Various types of adjuvants, (**b**) nanovaccines for representing APCs. (**c**) drug delivery by nanovaccines (**d**) entry of nanovaccines in cystosol (Wei et al., 2018)

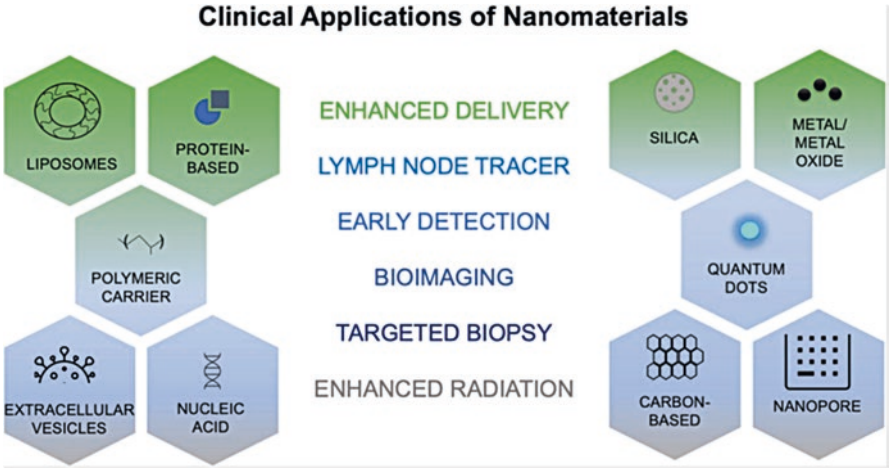


Fig. 5.14 Types of nanoparticles used in nanovaccines for cancer therapies (Kemp & Kwon, 2021)

have offer many more applications in the field of cancer treatment if they are combined with an appropriate target molecule. Some of the previous studies have shown that both non-specificity and immunosuppression can play a huge part in cancer therapies. Upgrading treatment from specific to non-specific and reduction of immunosuppression has indeed demand of the immune system. Enhancing the immune response with target-based cell membranes of nanoparticles offer an

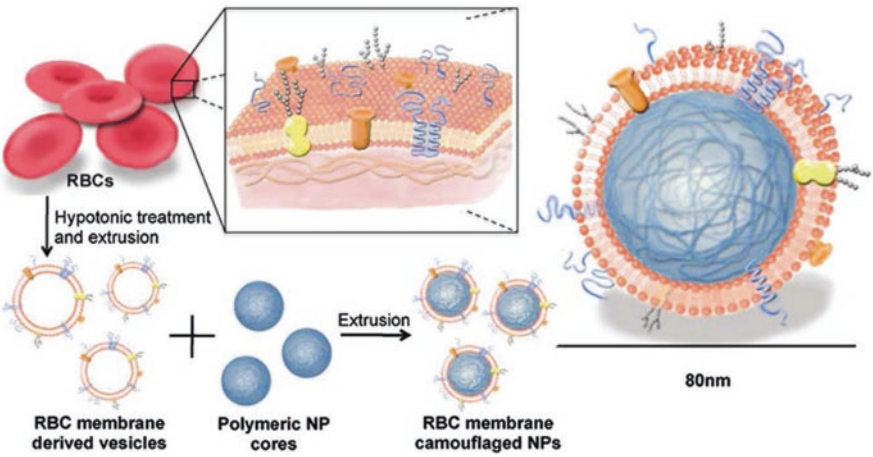


Fig. 5.15 Usage of RBCs for antibacterial nanovaccines (Wei et al., 2018)

advance in technology and highly acceptable in future perspectives. For example, RBC-coated cells are used with nanoparticles for an enhancement of immune clearance (Hu et al., 2011) (Fig. 5.15).

The cell-mimicking abilities of nanoparticles are used to transfer proteins of cell membranes onto the other membranes on the same surface (Hu et al., 2013; Angsantikul et al., 2018). Another example is to be found in the cases of nanoparticles coated with platelets to reduce bacterial vasculatures (Wei et al., 2018; Angsantikul et al., 2018; Plotkin, 2014). In another example WBCs are coated with nanoparticles to release toxins and remove infections such as sepsis (Gao et al., 2015; Thamphiwatana et al., 2017; Angsantikul et al., 2015).

5.6.5.1.1 Nanoparticles Coated with Cell Membrane as Antibacterial Vaccine

Vaccines can be defined as the world's most effective and efficient way to resist infection and enhance the capabilities of the immune system. With the help of vaccines it has now become a simple task to combat and overcome many infectious diseases (Angsantikul et al., 2018). There is an effective form of vaccines which is known as toxoids vaccines. These vaccines are available in the health sector to kill infections such as tetanus and diphtheria (Mendoza et al., 2009). In the strategies of making antibacterial vaccines are meant for reduction of virulent protein present in the bacteria that is not capable of causing infection but able to generate immune system. These vaccines are made by reducing harmful virulent proteins, either by treating with harsh chemicals or through the heat treatment of proteins. When nanoparticles are mixed with bacterial proteins of RBC, which have the ability to neutralise bacterial toxins called nanosponges (Angsantikul et al., 2018; Gao et al., 2015).

Staphylococcus aureus is also used in making vaccines against bacteria MRSA infection. In this strategy, bacterial toxins are reduced through the use of heat treatment to denature the toxins of the proteins and make them nanotoxoids (Rosenberg et al., 2008; Plotkin, 2014). Outer membrane vesicles (OMVs) are also used in antibacterial vaccines, for example, sometimes E. coli is coated with nanoparticles of gold, which makes it appropriate for further use (Wei et al., 2018).

5.6.5.2 Anticancer Vaccines with Nanoparticles Coated by Cell Membrane

As we have seen, antibacterial vaccines has achieved a great success all across the world. In the case of cancer, the same scenario hadn't predicted and not even more successful targets attained. IN recent studies, RBC have become a source of anticancer vaccines and came to the attention of researchers. Accordingly, anticancer vaccines loaded with nanoparticles are becoming the major area of research nowadays (Wang et al., 2015; Xu et al., 2013; Angsantikul et al., 2018). This platform of nanoparticles can be further modified with mannose to allow the surface targeting of dendritic cells. This surface target can enhance the immune response as well as the draining of lymph nodes. In the case of cancer cell membranes of autologous tumours contain plethora, which can be used as the nanoparticles coated cell membrane in anticancer activity. Initially, it was observed in B16-F10 melanoma. These melanoma membranes coated with nanoparticles are significant in enhancing the stimulation of T cells for antigen presentation and are also helpful in maturing dendritic cells from bone marrow (Gao et al., 2015) (Fig. 5.16).

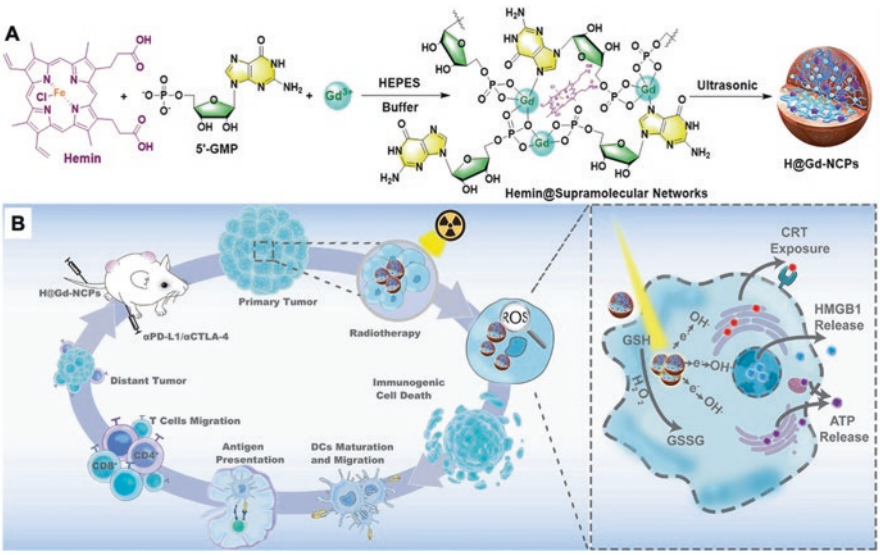


Fig. 5.16 Caption

It can also be defined as both an anticancer response, as in the case of antibacterial vaccines and targeting ligand, may work together and led to the artificial stimulation of the immune response. These cell membrane-coated nanoparticles work in a manner like the artificial generation of the immune response with target specificity (Zhu et al., 2014; Kang et al., 2018; Rosenberg, 2014; Rabinovich et al., 2007).

5.7 Conclusion

In this review the authors tried to explain different applications of the nanoparticles in treatment of diseases like cancer. Also, in this review authors tried to summarise all the factors and challenges that can affect the pathways and delivery system of cancer therapies. Therefore, it is extremely difficult to achieve a target drug delivery system and lower the immunogenicity with respect to time. Tumours can generate an immune response but can also lead to immunosuppression because tumours are arising from the same cells of the body. Nanovaccines can lead to a revolution in the field of cancer research with highly specific target attacking and the generation of an immune response. Nanovaccines have already achieved huge success in antibacterial vaccines in a similar manner, nanoparticle-coated cell membranes are principally effective with regard to tumours and T cells.

Furthermore, nanoparticles are becoming a boon to the medical science through their specific ability to recognise tumours and dual target abilities and antibodies-adjuvants loading characteristics. In this review the authors tried to explain the need for nanovaccines to be used in cancer treatment. By comparing nanovaccines with traditional medicines, nanoparticles are not non-specific, highly effective, and mainly work at dual targeting at a time. It is therefore clear that future studies will be required to developed the use of these nanovaccines as anti-cancer vaccines in an innovative fashion.

References

Angsantikul, P., Thamphiwatana, S., Gao, W., & Zhang, L. (2015). Cell membrane-coated nanoparticles as an emerging antibacterial vaccine platform. *Vaccine, 3*(4), 814–828. https://doi.org/10.3390/vaccines3040814

Angsantikul, P., Fang, R. H., & Zhang, L. (2018). Toxoid vaccination against bacterial infection using cell membrane-coated nanoparticles. *Bioconjugate Chemistry, 29*(3), 604–612. https://doi.org/10.1021/acs.bioconjchem.7b00692

Ashwini, K., Kumar, G., & Bhaskara Rao, K. V. (2011). Optimization, production and partial purification of extracellular α-amylase from Bacillus sp. marini. *Archives of Applied Science Research, 3*(1), 33–42. [Online]. Available: www.scholarsresearchlibrary.com

Bachmann, M. F., & Jennings, G. T. (2010). Vaccine delivery: A matter of size, geometry, kinetics and molecular patterns. *Nature Reviews. Immunology, 10*(11), 787–796. https://doi.org/10.1038/nri2868

Brudno, J. N., & Kochenderfer, J. N. (2018). Chimeric antigen receptor T-cell therapies for lymphoma. *Nature Reviews. Clinical Oncology, 15*(1), 31–46. https://doi.org/10.1038/nrclinonc.2017.128

Cheever, M. A., & Higano, C. S. (2011). PROVENGE (Sipuleucel-T) in prostate cancer: The first FDA-approved therapeutic cancer vaccine. *Clinical Cancer Research, 17*(11), 3520–3526. https://doi.org/10.1158/1078-0432.CCR-10-3126

Chinnathambi, S., Chen, S., Ganesan, S., & Hanagata, N. (2012). Binding mode of CpG oligodeoxynucleotides to nanoparticles regulates bifurcated cytokine induction via toll-like receptor 9. *Scientific Reports, 2*, 534. https://doi.org/10.1038/srep00534

Dagogo-Jack, I., & Shaw, A. T. (2018). Tumour heterogeneity and resistance to cancer therapies. *Nature Reviews. Clinical Oncology, 15*(2), 81–94. https://doi.org/10.1038/nrclinonc.2017.166

de Titta, A., et al. (2013). Nanoparticle conjugation of CpG enhances adjuvancy for cellular immunity and memory recall at low dose. *Proceedings of the National Academy of Sciences of the United States of America, 110*(49), 19902–19907. https://doi.org/10.1073/pnas.1313152110

Dominguez, A. L., & Lustgarten, J. (2010). Targeting the tumor microenvironment with anti-neu/anti-CD40 conjugated nanoparticles for the induction of antitumor immune responses. *Vaccine, 28*(5), 1383–1390. https://doi.org/10.1016/j.vaccine.2009.10.153

Fang, R. H., Kroll, A. V., & Zhang, L. (2015). Nanoparticle-based manipulation of antigen-presenting cells for cancer immunotherapy. *Small, 11*(41), 5483–5496. https://doi.org/10.1002/smll.201501284

Fesnak, A. D., June, C. H., & Levine, B. L. (2016). Engineered T cells: The promise and challenges of cancer immunotherapy. *Nature Reviews. Cancer, 16*(9), 566–581. https://doi.org/10.1038/nrc.2016.97

Fischer, N. O., Rasley, A., Corzett, M., Hwang, M. H., Hoeprich, P. D., & Blanchette, C. D. (2013). Colocalized delivery of adjuvant and antigen using nanolipoprotein particles enhances the immune response to recombinant antigens. *Journal of the American Chemical Society, 135*(6), 2044–2047. https://doi.org/10.1021/ja3063293

Gao, W., et al. (2015). Modulating antibacterial immunity via bacterial membrane-coated nanoparticles. *Nano Letters, 15*(2), 1403–1409. https://doi.org/10.1021/nl504798g

Graddis, T. J., McMahan, C. J., Tamman, J., Page, K. J., & Trager, J. B. (2011). Prostatic acid phosphatase expression in human tissues. *International Journal of Clinical and Experimental Pathology, 4*(3), 295–306.

Guo, C., Manjili, M. H., Subjeck, J. R., Sarkar, D., Fisher, P. B., & Wang, X.-Y. (2013). Therapeutic cancer vaccines: Past, present, and future. *Advances in Cancer Research, 119*, 421–475. https://doi.org/10.1016/B978-0-12-407190-2.00007-1

Hu, C.-M. J., Zhang, L., Aryal, S., Cheung, C., Fang, R. H., & Zhang, L. (2011). Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *Proceedings of the National Academy of Sciences of the United States of America, 108*(27), 10980–10985. https://doi.org/10.1073/pnas.1106634108

Hu, C.-M. J., Fang, R. H., Luk, B. T., & Zhang, L. (2013). Nanoparticle-detained toxins for safe and effective vaccination. *Nature Nanotechnology, 8*(12), 933–938. https://doi.org/10.1038/nnano.2013.254

Kang, T., et al. (2018). Necroptotic cancer cells-mimicry nanovaccine boosts anti-tumor immunity with tailored immune-stimulatory modality. *Biomaterials, 164*, 80–97. https://doi.org/10.1016/j.biomaterials.2018.02.033

Kemp, J. A., & Kwon, Y. J. (2021). Cancer nanotechnology: Current status and perspectives. *Nano Convergence, 8*(1), 34. https://doi.org/10.1186/s40580-021-00282-7

Kim, R., Emi, M., & Tanabe, K. (2007). Cancer immunoediting from immune surveillance to immune escape. *Immunology, 121*(1), 1–14. https://doi.org/10.1111/j.1365-2567.2007.02587.x

Kosmides, A. K., Sidhom, J.-W., Fraser, A., Bessell, C. A., & Schneck, J. P. (2017). Dual targeting nanoparticle stimulates the immune system to inhibit tumor growth. *ACS Nano, 11*(6), 5417–5429. https://doi.org/10.1021/acsnano.6b08152

Law, S. K. (1991). Antigen shedding and metastasis of tumour cells. *Clinical and Experimental Immunology, 85*(1), 1–2. https://doi.org/10.1111/j.1365-2249.1991.tb05672.x

Lee, K. L., et al. (2017). Combination of plant virus nanoparticle-based in situ vaccination with chemotherapy potentiates antitumor response. *Nano Letters, 17*(7), 4019–4028. https://doi.org/10.1021/acs.nanolett.7b00107

Mendoza, N., Ravanfar, P., Satyaprakash, A., Pillai, S., & Creed, R. (2009). Existing antibacterial vaccines. *Dermatologic Therapy, 22*(2), 129–142. https://doi.org/10.1111/j.1529-8019.2009.01225.x

Min, Y., et al. (2017). Antigen-capturing nanoparticles improve the abscopal effect and cancer immunotherapy. *Nature Nanotechnology, 12*(9), 877–882. https://doi.org/10.1038/nnano.2017.113

Mullard, A. (2013). New checkpoint inhibitors ride the immunotherapy tsunami. *Nature Reviews Drug discovery, 12*(7), 489–492. https://doi.org/10.1038/nrd4066

Plotkin, S. (2014). History of vaccination. *Proceedings of the National Academy of Sciences of the United States of America, 111*(34), 12283–12287. https://doi.org/10.1073/pnas.1400472111

Pradhan, P., et al. (2014). The effect of combined IL10 siRNA and CpG ODN as pathogen-mimicking microparticles on Th1/Th2 cytokine balance in dendritic cells and protective immunity against B cell lymphoma. *Biomaterials, 35*(21), 5491–5504. https://doi.org/10.1016/j.biomaterials.2014.03.039

Rabinovich, G. A., Gabrilovich, D., & Sotomayor, E. M. (2007). Immunosuppressive strategies that are mediated by tumor cells. *Annual Review of Immunology, 25*, 267–296. https://doi.org/10.1146/annurev.immunol.25.022106.141609

Reddy, S. T., et al. (2007). Exploiting lymphatic transport and complement activation in nanoparticle vaccines. *Nature Biotechnology, 25*(10), 1159–1164. https://doi.org/10.1038/nbt1332

Rosenberg, S. A. (2014). IL-2: The first effective immunotherapy for human cancer. *Journal of Immunology, 192*(12), 5451–5458. https://doi.org/10.4049/jimmunol.1490019

Rosenberg, S. A., Yang, J. C., & Restifo, N. P. (2004). Cancer immunotherapy: Moving beyond current vaccines. *Nature Medicine, 10*(9), 909–915. https://doi.org/10.1038/nm1100

Rosenberg, S. A., Restifo, N. P., Yang, J. C., Morgan, R. A., & Dudley, M. E. (2008). Adoptive cell transfer: A clinical path to effective cancer immunotherapy. *Nature Reviews. Cancer, 8*(4), 299–308. https://doi.org/10.1038/nrc2355

Rosenblum, M. D., Remedios, K. A., & Abbas, A. K. (2015). Mechanisms of human autoimmunity. *The Journal of Clinical Investigation, 125*(6), 2228–2233. https://doi.org/10.1172/JCI78088

Schmid, D., et al. (2017). T cell-targeting nanoparticles focus delivery of immunotherapy to improve antitumor immunity. *Nature Communications, 8*(1), 1747. https://doi.org/10.1038/s41467-017-01830-8

Shannahan, J. H., Bai, W., & Brown, J. M. (2015). Implications of scavenger receptors in the safe development of nanotherapeutics. *Receptors & Clinical Investigation, 2*(3), e811. https://doi.org/10.14800/rci.811

Siegel, R. L., Miller, K. D., Fuchs, H. E., & Jemal, A. (2021). Cancer statistics, 2021. *CA: a Cancer Journal for Clinicians, 71*(1), 7–33. https://doi.org/10.3322/caac.21654

Tam, H. H., et al. (2016). Sustained antigen availability during germinal center initiation enhances antibody responses to vaccination. *Proceedings of the National Academy of Sciences of the United States of America, 113*(43), E6639–E6648. https://doi.org/10.1073/pnas.1606050113

Thamphiwatana, S., et al. (2017). Macrophage-like nanoparticles concurrently absorbing endotoxins and proinflammatory cytokines for sepsis management. *Proceedings of the National Academy of Sciences of the United States of America, 114*(43), 11488–11493. https://doi.org/10.1073/pnas.1714267114

Wang, L., Wang, F.-S., & Gershwin, M. E. (2015). Human autoimmune diseases: a comprehensive update. *Journal of Internal Medicine, 278*(4), 369–395. https://doi.org/10.1111/joim.12395

Wei, X., et al. (2018). Nanoparticle functionalization with platelet membrane enables multifactored biological targeting and detection of atherosclerosis. *ACS Nano, 12*(1), 109–116. https://doi.org/10.1021/acsnano.7b07720

Xu, Z., Ramishetti, S., Tseng, Y.-C., Guo, S., Wang, Y., & Huang, L. (2013). Multifunctional nanoparticles co-delivering Trp2 peptide and CpG adjuvant induce potent cytotoxic T-lymphocyte response against melanoma and its lung metastasis. *Journal of Controlled Release, 172*(1), 259–265. https://doi.org/10.1016/j.jconrel.2013.08.021

Zhu, M., Wang, R., & Nie, G. (2014). Applications of nanomaterials as vaccine adjuvants. *Human Vaccines & Immunotherapeutics, 10*(9), 2761–2774. https://doi.org/10.4161/hv.29589

Weblink

https://www.weforum.org/agenda/2016/02/did-we-just-get-a-step-closer-to-curing-cancer/

Chapter 6
Benefits of Molecular Medicine from Self-Assembled Nanostructured Materials

S. V. Sowmya, C. Pushpalatha, Dominic Augustine, Ishitha Singh, Arshiya Shakir, and Reshma Dhodwad

6.1 Introduction to Self-Assembled Nanostructured Materials

Democritus, a Greek philosopher in 400 BC proposed that the earth and solar system have emerged from atomic organization. A French philosopher Descartes hypothesized that as per the natural laws of physics, the universe had emerged from self-structure, i.e., organization of small components into large assemblages. Later in 1935, Langmuir and Blodgett developed a method to form a closely packed monolayer of amphiphilic molecules on solid and liquid surfaces. Bigelow observed the assembly of a monolayer of long alkylamine chains on a solid surface in 1946. Self-assembled monolayers (SAMs) were the initial terminology for self-assembly since 1983 when Nuzzo et al. developed well-ordered monolayers of alkanethiolate molecules chemosorbed on gold surfaces. Currently, nanostructures are fabricated via self-assembly (Castillo-León et al., 2011).

Self-assembly is the characteristic assembly of biological structures such as atoms, molecules, or nanoscale building blocks from their component parts into structured, non-covalent aggregates through random molecular movement. The individual components get organized without any human involvement, for example, the phospholipid bilayer of the cell membrane and the formation of bacteriophage

S. V. Sowmya · D. Augustine · I. Singh
Department of Oral Pathology and Microbiology, Faculty of Dental Sciences, M.S. Ramaiah University of Applied Sciences, Bengaluru, India

C. Pushpalatha (✉) · A. Shakir
Department of Pedodontics and Preventive Dentistry, Faculty of Dental Sciences, M. S. Ramaiah University of Applied Sciences, Bengaluru, India

R. Dhodwad
Department of Pedodontics and Preventive Dentistry, Krishnadevaraya College of Dental Sciences, Bengaluru, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_6

and viral particles (Ariga et al., 2019). Molecular self-assembly occurs through weak noncovalent bonds like electrostatic interactions (ionic bonds), hydrogen bonds, hydrophobic and hydrophilic interactions, water-mediated hydrogen bonds, and van der Waals interactions resulting in structurally and chemically stable structures. These forces and their kinetic pathways are controlled by specific properties such as charge, binding affinity, and hydrophobicity. Best example of natural molecular self-assembly is a cell that is formed by biological molecules like proteins, peptides, nucleic acids, lipids, etc. with self-assembly of complementary properties (Wang et al., 2019). Molecular self-assembly is also a prominent feature of antigen–antibody recognition, amyloid fibril formation, and chromatin assembly. The orderly arrangement of the constructs in the self-assembly is dependent on the shape, size, and surface properties of the blocks (Zhang, 2002).

6.2 Description of Nanostructures Formed by Self-Assembly

Self-assembled peptide and lipid systems have been described extensively in the literature due to their wide bionanotechnology applications. The self-assembly of peptide and protein systems forms nanostructures such as nanofibers, nanotubes, vesicles, helical ribbons, and fibrous scaffolds. It is the most promising practical low-cost and high-throughput approach for nanofabrication (Delfi et al., 2021).

6.2.1 Peptide Nanostructures Formed by Self-Assembly

6.2.1.1 Nanofibers

Nanofibers as nanostructures have drawn great attention as building blocks for their application as the next-generation biosensors. They have been used along with different structures in a self-assembly configuration. Insoluble amyloid fibrils made of aggregates of beta sheets of amino acids are associated with rare pathologies such as amyloidosis. However, this insoluble property has been made use of in biosensor fabrication as this will ensure long-term stability of the sensor (Jeevanandam et al., 2018).

6.2.1.2 Nanotubes

Functionalization of peptide shell–coated metal nano-wires and drug-filled cavitated structures can be performed using nanotubes. They can be formed using various types of monomers like linear and cyclic peptides, disc-shaped motifs, etc. (Gao & Matsui, 2005).

6.2.1.3 Nanoparticles

They cover structures such as solid structures as well as nanospheres with hollow cores. These hollow nanospheres can be a possible drug delivery system while the non-hollow nanospheres can be used as the biological counterpart of nanobeads.

6.2.1.4 Nano-tapes

Peptide beta sheets are stacked together to form peptide nano-tapes. Hydrogels are formed when their concentration increases above a certain limit (Lee et al., 2019).

6.2.1.5 Hydrogels

Hydrogels are materials with the properties of a gel depending on variables such as ionic strength, pH, salinity, and temperature.

6.2.1.6 Hydrophobic Dipeptides

They can be self-assembled to form nanofibers or nanotubes based on their condition during the formation process. It includes dipeptides such as diphenylalanine. Spherical nanostructures can also be formed when a protective group is added to the amino group of the dipeptide structure. These structures have hydrophobic side chains attached to both the amino groups of the dipeptide because of which they are named "hydrophobic dipeptides". These interactions take place due to hydrophilic-hydrophobic reactions along with stabilization through hydrogen bonding in between the peptides (Ahmed, 2015).

6.2.2 Lipid-Based Self-Assembled Nanostructures

6.2.2.1 Liposomes

They are phospholipid vesicles that contain concentric bilayers of lipid which in turn contain aqueous spaces. Liposomes have been used as drug delivery agents since the mid-twentieth century in both pharmacy and medicine. The commonly used liposomes are cholesterol and phosphatidylcholine. They can encapsulate compounds like glucose, peptides, or proteins. Hydrophobic compounds are stored in the bilayers of the vesicle whereas the hydrophilic compounds are stored in the aqueous compartment. Liposomes require vital geometrical analysis for self-assembly and aggregation. Based on their size and the number of bilayers present, they can be classified into multi-lamellar liposomes (MLV) and large and small

unilamellar vesicles (LUV and SUV). The quantity of the drug loaded, the permeability of liposomes, and their half-life can be varied by modifying these parameters. Another classification of liposomes includes first, second, and third generation of liposomes. The first one consists of a liposome with no modifications, like cholesterol and phospholipids. But, one of their disadvantages includes accelerated elimination and hence hampers their bioavailability. In the second generation of liposomes, the liposome group is coated with unreactive polymeric molecules like glycoproteins, oligosaccharides, PEG, etc. This coating increases their half-life and bioavailability, due to their hydrophilic nature. In the third group, antibodies or peptides are attached to the liposome or PEG compounds, which in turn improves the efficacy of the drug. Various techniques to characterize the preparation of liposomes in the laboratory have been developed which include hydrating a previously dried lipid film with an aqueous solution, reverse phase evaporation method, solvent injection technique, sonication, and homogenization. Liposomes have made a huge impact on medical care, but regulatory and clinical limitations are still a challenge. Some of the regularly used liposomes include Ambisome (injectable liposomal formulation of amphotericin B), Doxil (liposomal doxycycline), Marqibo (liposomal formulation of vincristine), Visudyne (lyophilized formulation of liposomal verteporfin), etc. (Tan et al., 2019).

6.2.2.2 Solid Lipid Nanoparticles (SLN)

They are nanoparticles that are formed from a lipid matrix that stays in the solid state at room and body temperature. They provide protection of the incorporated drug from degradation, physical stability, and controlled release with low cytotoxicity, they do not require the use of organic solvents for their assembly, and the synthesis process is cost-effective and readily performed on a large scale. A few of their limitations include drug expulsion during storage and drug-loading capacity. Formulation techniques for encapsulation of drug solutions into SLN can be done using high-pressure microemulsion formation, homogenization, ultrasonication, solvent injection (or solvent displacement), phase inversion, emulsification/solvent evaporation (precipitation), multiple emulsion technique, and the membrane contractor technique. The readily used formulations of SLN includes Acyclovir-loaded, SLN as carriers of glibenclamide, SLN-based stearic acid, and injectable soya lecithin, etc. In general, SLNs act as carriers of poorly water-soluble drugs to improve their bioavailability and therapeutic potential (Scioli Montoto et al., 2020).

6.2.2.3 Lipid Nano-capsules (LNC)

Lipid nano-capsules have the advantageous properties of both lipid delivery systems and colloidal particles. They contain three principal constituents which include an oily phase, an aqueous phase, and a non-ionic surfactant. These constituents can

be varied which in turn changes the stability and formulations of LNC. They are a hybrid between liposomes and polymeric nano-capsules because their structure is similar to lipoproteins. LNCs have good stability in contrast to liposomes which are unstable and leaky in body fluids. The formulation technique needs soft energy process and is free of organic solvents. LNCs can be used for targeted drug delivery as a therapeutic and diagnostic cancer agent (Huynh et al., 2009).

6.2.2.4 Microemulsions

They contain solutions with self-assembly systems dispersed disorderly. The internal structure contains both hydrophilic and hydrophobic regions. These microemulsions are consisting of water, oil, and amphiphiles (surfactant, usually in combination with a cosurfactant) optically transparent and isotropic nanostructured solutions, thermodynamically stable systems. They can be classified, according to Winsor, into, Winsor I (oil-in-water) (o/w) ME; Winsor II (water-in-oil) (w/o) ME; Winsor III (bi-continuous or middle self-assembled nanomaterials 75 phases ME); and Winsor IV (simple phase ME or pure ME). Properties like oil-surfactant ratio, surfactant mixture, and temperature change the formation of each type. The use of ME as a drug delivery agent has been shown to improve the bioavailability at the site of action of various drugs (Suhail et al. 2021).

6.2.2.5 Self-Microemulsifying Drug Delivery Systems (SMEDDS)

SMEDDS are pre-concentrates of microemulsions which contain oil, surfactant, and drugs**.**

6.3 Types of Self Assembled Systems

6.3.1 Discrete System Versus Continuum System

Self-assembled systems can be classified based on the building blocks into two categories: Discrete and continuum.

A system that uses prefabricated building blocks with fixed sizes and shapes is a discrete system. A bond forms when two particles are close to each other with matching orientations. A self-assembled chain of alternating structures is formed due to selective bonding when there are two bonding sites. When there are multiple bonding sites they self-assemble into a network of alternating particle chains. Nanoparticles and nanorods can be assembled into various configurations by modifying the electric and magnetic fields which induces a dipole-type interaction or shear forces which utilize hydrodynamic interactions.

Continuum system makes use of the formation of nanoscale domains in the form of patterns such as binary monolayers, block polymers, and organic molecular adsorbates on metal surfaces. A binary monolayer is 1–10 nm in size, stable on annealing, and can be self-assembled into triangular lattices of dots, parallel stripes, or serpentine stripes patterns in an orderly fashion. Polymer systems develop by phase separation and consist of two immiscible polymer fragments joined by a covalent bond. The size ranges from 10 to 100 nm. The advantages of a continuum system are presynthesied building blocks that are not required as the domains and their patterns self-assemble simultaneously, it provides significant flexibility and control of the assembly process and this approach can be used for other different systems (Lu, 2012).

6.3.2 Static Self-Assembly Versus Dynamic Self-Assembly

The types of self-assembly categorized based on the nature of interactions are static and dynamic. The process involving the formation of static structures using energy minimization leads to static self-assembly. Microphase separation and colloidal crystals are classic examples of this process. When the self-assembly system dissipates energy, dynamic type is observed with the formation of structures away from the thermodynamic equilibrium. However, if the influx of energy stops, structural disintegration occurs. The most typical example of dynamic self-assembly is a living organism (Subramani & Ahmed, 2018).

6.4 Applications of Self-Assembled Nanostructures

There are various applications of self-assembled nanostructures. The films can be used as membranes, sensors, insulators, electronic, and magnetic optical devices such as the construction of Bragg diffraction devices for Raman spectrometers (Chen et al., 2018). The incorporation of nanoparticles into self-assembled structures has been employed in optoelectronic devices, plasmon waveguides, focusing lenses, light generators, optical switches, nanoscale thermometers, plasmon rulers, and pH meters (Nie et al., 2010). Pharmacological, biological, and medical fields demonstrate the use of polymersomes, liposomes, or polymer capsules incorporated self-assembled nanoparticles as carriers for delivering biologically active species (Puri et al., 2009). The self-assembled nanostructures have also been used to stimulate pre-osteoblast cell attachment and growth. They are incorporated in extracellular matrix for the growth of human fibroblasts. They possess therapeutic applications as wound dressing materials, detection of neurotoxins, antiviral agents, and as immunosensors (Habibi et al., 2016).

6.5 Techniques for Characterization of Self-Assembled Nanostructures

Self-assembled nanostructures play a vital role in various biologic processes and has led to a lot of research due to their potential use in diagnosis and biosensing or designing biocompatible products. The designing and development of artificial nanostructures through different techniques for self-assembly is a method of analysing the basis of different functional structures with superior and desirable properties. These factors have made the characterization of self-assembled nanostructures more challenging. Significant research is being done on polymer self-assembly for similar reasons.

Researchers consider nanostructures as dynamic systems with continuously evolving and improving properties and structures, as they interact with the environment. Hence, the characterization of these nanostructures becomes very important to utilize their properties in various applications (Kasotakis et al., 2009).

This section gives an overview of the different techniques for characterizing self-assembled nanostructures based on their properties.

6.5.1 Atomic Force Microscopy

Atomic force microscopy (AFM) is a method of characterization which creates 3-D images of the surface of a nanostructure at high magnification. This method was introduced by 'Gerard Binning and Heinrich Rohrer' in the year 1986. AFM has been a topic of interest because it is simple to perform and is very flexible. The main principle of AFM is measuring the force between a fine probe of the microscope and the sample. The apparatus contains a cantilever, which has a probe attached at the tip, made of silicon. As AFM scans the sample, the attraction or repulsion involving the tip and the surface of the sample may lead the cantilever to deflect. This deflection can be computed with the help of a laser beam. The final calculation is done by adding the values obtained through laser alteration and the previously established cantilever stiffness. AFM tips can also be used to bring about surface reactions by carrying different catalysts. AFM tips can also cause direct local oxidation on surfaces.

The three types of AFM-based nanofabrication techniques include nano-shaving, nano-grafting, and nano-pen reader and writer (NPRW). In nano-shaving, the AFM tip applies excessive localized pressure at the point of contact of the sample. This pressure leads to the production of high shear force while scanning, which in turn leads to the displacement of self-assembled monolayer adsorbates. In nano-grafting, the self-assembled monolayer and the AFM cantilever are dipped in a solution with a thiol group. These thiol groups adsorb to the exposed surface of the sample. In NPRW, the required molecules and groups are pre-coated to the exposed surface of

the sample under high force. Following this, the nanostructures are characterized by decreased force.

AFM can function with three types of modes based on the proximity between the probe and the surface of the sample, into 'contact', 'non-contact', and 'tapping'. Tapping is the most commonly used mode for characterization of nanostructures. Using AFM an 'edge resolution' of 1 nm can be achieved, high spatial resolution characterization of nanostructures can be done in situ, complicated patterns can be produced automatically, and various constituents can be produced by modifying the components of the tip or the solution. The fabricated patterns can be modified in situ without modifying the process of fabrication and the height of the nanostructure can be analysed and visualized. In addition, the resolution of images produced by AFM is comparable to SEM and TEM. It is cost-effective and requires minimum laboratory equipment and space. Nevertheless, AFM displays slower scanning times than any electron microscope.

6.5.2 Electron Microscopy

6.5.2.1 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is a dominant and among the most popular technique used for material characterization and imaging of the surfaces of various materials. SEM can provide a resolution of up to 1 nm, highly focused, and a fairly straightforward image interpretation, making the imaging of three-dimensional nano-structures feasible.

SEM works on a voltage range of 100 V–30,000 V with the electron probe with a diameter of 1–3 nm and a field emission source. As the electron beam and the sample interact, it produces secondary electrons. The efficiency of emission of these secondary electrons varies based on the surface topography and chemical properties of the sample.

Undesirable excitation of electrons is inevitably involved in this interaction which makes radiation damage one of the most important set back of SEM imaging low-voltage SEM has been used for imaging such beam radiation-sensitive specimens (Zuccheri & Samorì, 2011).

6.5.2.2 Transmission Electron Microscopy

Transmission electron microscopy (TEM) has been utilized in the characterization of nanostructures in the form of a tool that can be used to analyse the spatial arrangement at high resolution and their chemical nature. A modern transmission electron microscope makes imaging atoms in crystalline samples directly, possible. TEM utilizes the interaction between the electron beam with a homogenous current density and the sample to be investigated, to determine the physical properties of the

nanostructure. The extent of the interaction depends on various properties including the density, size, and elemental composition of the sample. The transmitted electrons, in turn, form an image by utilising the information assimilated from their interaction with the sample. They can be analysed at resolutions close to 0.1 nm, which is lower than inter-atomic distance.

One of the most valuable applications of TEM is to determine the shape of nanostructures. Electron diffusion along with high-resolution lattice imaging can be used to analyse the facets and structure of nanocrystals. The 3D structure of a nanocrystal can be identified and analysed by using a minimum of two TEM images which are captured from different orientations. Simple nanostructures can be determined directly from TEM images while some of the more complex nanocrystals may require the analysis of experimental and theoretically obtained images.

TEM has been used as a method of characterization of nanostructures for various bio-medical purposes such as:

(1) Diagnostics and bio-sensing, where the aggregation changes based on the presence or absence of the concerned biomarkers.
(2) Therapy, where the aggregation enhances the therapeutic effect of the nanostructures.
(3) Imaging, in which, the aggregation enhances the response received from the tissue being visualised, in the form of signals.

TEM also aids in the identification and differentiation between hollow or solid pathologies which makes it a vital tool in diagnosis. In TEM, high-energy electrons interact with the concerned tissue so that the intensity of this interaction can be recorded in the image. The centre of hollow structures appears lighter than its capsule or wall in the image recorded.

TEM is one of the most readily used techniques to analyse the shape and size of various nanostructures because it provides the accurate valuation of nanostructure homogeneity and direct images of the sample. A few limitations of this technique include inaccurate images due to flawed orientations and the inability to quantify more particles.

6.5.2.3 High-Resolution Transmission Electron Microscopy

High-resolution TEM (HRTEM) is a technique that uses both scattered and transmitted electrons to produce an image, known as ‘phase-contrast imaging’. Phase-contrast imaging produces images with the highest resolution among others which aids in the identification of various atoms in crystalline structures. While conventional electron microscopy can only aid in the statistical characterization of nanostructures, HRTEM can produce images of a single particle crystal structure. Therefore, HRTEM is constantly being utilized to characterize the internal structure of nanostructures. This ability of HRTEM can also be used to assess the structural defects of these nanocrystals which can explain some of their atypical properties.

The characterization of all the nanostructures is not always possible using HRTEM. The random and haphazard orientation of nanocrystals can result in the formation of complex images that may be inaccurate.

6.5.2.4 Liquid Transmission Electron Microscopy

A vacuum system is used in TEM which protects the filament, used in the apparatus, along with the reduction of electron beam scattering. Traditional TEM imaging is not used to assess liquids since it can compromise this vacuum system. Therefore, liquid systems characterization has not been explored as much as solid systems. Due to these limitations, the other imaging techniques are only able to produce data in a single time frame, most commonly after the completion of nanoparticle growth and not during the growth process. Liquid TEM gives the opportunity to track and analyse the nanoparticles. It permits the tracking of the course of nanoparticles while they are growing and hence provide a direct outlook on the evolution of nanoparticles. Liquid TEM also enables researchers to analyse the movement of nanoparticles with the liquid, which may be due to Brownian motion or some other parameters contributing to this movement.

6.5.2.5 Cryo-Electron Microscopy

Cryo-electron microscopy (Cryo-EM) is an effective method of characterization used to analyse the structure of polymer and protein self-assembly nanostructures.

The sample preparation in traditional TEM comprises drying and staining steps which might end up affecting their morphology and structure. In contrast, cryo-EM samples are processed and stored in a hydrated and frozen state, which is close to their inherent form, by vitrifying them and studying at cryogenic temperature using a TEM. Liquid nitrogen is commonly used to freeze-dry the nanostructure samples (Mourdikoudis et al., 2018).

Cryo-TEM can be used to view nanostructures at sub-nanomolar resolutions. It has been utilised to study the complicated mechanism of nanoparticle aggregation. Cryo-TEM imaging is vital to observe the atypical form of nanostructures because the rapid plunge freezing prevents the particles from rearranging during the preparation process of the sample and its characterization can be done.

6.5.3 Focused Ion Beam

Focused ion-beam (FIB) bombardment is a fascinating and alternative method used to prepare self-assembly nanostructures. This technique permits in-situ analysis of the sample using ion-beam or scanning electron beam imaging. FIB enables locating the desired spot for deposition or removal of materials using ‘high energy ion beam

sputtering' and 'ion-assisted chemical vapor deposition' (Kochovski et al., 2020). The merits of the FIB technique are high resolution, mask-less processing, provide prompt prototyping, and flexibility to adapt to different materials and structures. One of its major drawbacks is the time consumed to analyse nanostructures. A single ion beam is utilised to analyse various structures, making it inefficient for large-scale production characterization of nanostructures. The FIB is a vital tool in defect characterization, design modification, failure analysis, and process control for various applications.

6.5.4 Fourier Transformed Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) is the method that measures the absorption of electromagnetic(EM) radiation with wavelengths in the range of the infrared spectrum. FT-IR spectroscopy gives important information regarding the various functional groups which are present in a system through the frequency of vibrations of chemical bonds present in the nanocrystals. It recognizes the composition of different microorganisms by observing the difference in the functional groups in their structure. It can produce data about the kind of interaction that takes place between microorganisms and metal ions.

Biochemical bonds are identified depending on their molecular degree of rotation and the type of movement, like twisting, stretching, or bending.

The mechanism of FTIR includes an infrared source emitting radiation through an interferometer consisting of a moving and a fixed mirror along with a beam splitter. The interferometer gauges the wavelength of the emitted light. IR spectra are found by exposing infrared radiation to the nanostructure sample and quantifying the intensity of radiation at a particular wavenumber.

Some of the types of FTIR techniques include attenuated total reflectance (ATR–FTIR), transmittance FTIR, and micro-spectroscopy FTIR (Stephen et al., 2009).

The advantages of FTIR include time efficiency and both solid and liquid samples can be analysed using this technique. The quantity of samples required for analysis is also small and is a cost-efficient alternative for the identification of microorganisms.

However, there are a few demerits such as the need for numerous sample and background scans in order to avoid artefacts. Processing of the sample prior to analysis is generally needed to avoid peaks due to overlapping. Even after processing, the information and data require further analysis.

6.5.5 X-Ray Diffraction

X-ray diffraction (XRD) is one of the most commonly used characterization methods to determine the formation of crystalline self-assembly, as long as there is a sufficient quantity of the sample. Traditionally, XRD gives information about the crystalline structure, lattice parameters, phase, and crystalline grain size. The

spectrum of diffraction can directly be correlated to the atomic structure of the nanocrystals at high angle regions, while at low angle regions, the spectrum is linked to the organised arrangement of nanocrystals. This analysis is done assuming that every particle in the nanostructure is similar in shape, size, and orientation so that the principles of diffraction physics can be used for the analysis. But practically, this assumption cannot be made because there is evident variability in their shape, size, and orientation. XRD is one of the most popular method used to measure the average inter-particle distance, and it is one of the few techniques that can be used to study the 'in situ temperature as well as pressure induced phase transformation' in nanostructures (Zuccheri & Samorì, 2011). XRD can be used to study the powder form of samples, once their colloidal forms are dried. It leads to statistically averaged volume values. The intensity and position of the peaks correspond to the composition of particles, which can be compared with the already available data for reference. However, XRD peaks are extremely wide and broad for particles with a size less than 3 nm and hence is not suitable for amorphous nanostructures.

6.5.6 Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) is a technique used in thermal analysis that is readily used by researchers because it is simple to use, easily available, and gives rapid results. In this technique, a nanostructure sample and a control for reference are placed in the DSC apparatus. Heaters either gradually raise the temperature at a specific pace or hold the DSC at a previously determined temperature. This instrument evaluates the variation in the heat flow among the nanostructure sample and the control reference.

Modern DSC apparatuses use software that helps the user in characterizing different physical properties of nanostructures like melting points, heat capacity values, and glass transition temperatures (Koshy, 2017).

The quantitative applications of DSC include the assessment of fusion heat and the degree of crystallization for nanocrystals. DSC is used for quality control applications because the melting points of different materials correlate with their purity. One of the major applications of DSC is glass transition temperature ($T_{g)}$ determination. At T_g, the polymer undergoes changes in its physical characteristics like volume and expansion, heat capacity, and heat flow.

6.5.7 Thermogravimetric Analysis

Thermal gravimetric analysis (TGA) helps in the analysis of information about the composition and mass of nanostructures. In this method of characterization, a nanomaterial sample is heated which makes its constituents degrade at different temperatures and end up decomposing and vaporising, which at the end leads to

variation of mass. This can also help in determining the quantity and type of nanoparticle organic ligands present in the nanocrystal sample.

TGA is used for analysing a few thermal processes like adsorption, absorption, desorption, sublimation, vaporization, oxidation, decomposition, and reduction. TGA can also be used to study volatile or gaseous components which are lost during such thermal reactions for samples such as nanomaterials. TGA can also be utilised to analyse the kinetics of chemical reactions of nanostructures under various conditions.

The thermobalance is regarded as the functional unit of TGA. The subunits of TGA include a sample holder, electronic microbalance, temperature programmer, recorder, and a furnace. It consists of a clamp that is used to stabilize the electron microbalance.

Some of the advantages of TGA are its simplicity and no requirement for special sample preparation. A disadvantage of TGA is the requirement of few milligrams of a sample, which in turn leads to increased cost and lab production feasibility problems (Table 6.1).

Table 6.1 Characterization techniques corresponding to specific parameters to be analysed (Zuccheri & Samorì, 2011)

Parameter	Characterization techniques suitable for analysis
Size	Transmission electron microscopy, X-ray diffraction, high-resolution transmission electron microscopy, scanning electron microscopy, atomic force microscopy, differential scanning calorimetry
Shape	Transmission electron microscopy, high-resolution transmission electron microscopy, atomic force microscopy
Chemical and elemental composition	X-ray diffraction
Crystalline structure	X-ray diffraction, high-resolution transmission electron microscopy
Size distribution	Differential scanning calorimetry, scanning electron microscopy
Growth kinetics	Transmission electron microscopy, cryo-transmission electron microscopy, liquid-transmission electron microscopy
Composition/ligand binding/density/surface composition/arrangement/mass	Fourier transform infrared spectroscopy, thermal gravimetric analysis
Concentration	Differential scanning calorimetry
Agglomeration state	Differential scanning calorimetry, scanning electron microscopy, cryo-transmission electron microscopy, transmission electron microscopy
Density	Differential scanning calorimetry
Properties of Single particle properties	High-resolution transmission electron microscopy, liquid transmission electron microscopy
Three-Dimensional visualization	Atomic force microscopy, scanning electron microscopy
Dispersion of nanoparticles	Scanning electron microscopy, Atomic force microscopy, Transmission electron microscopy
Defects in the structure	High-resolution transmission electron microscopy
Detection of nanoparticles	Transmission electron microscopy, scanning electron microscopy

6.6 Advantages of Self-Assembled Nanostructured Materials

6.6.1 Synthesis

Self-assembled nanostructures are synthesised under non-harsh conditions, at room temperature without specialized equipment. This reduces the cost of these nanostructures.

They can be used to synthesize structures like nanotubes, nanoparticles, or nanofibers, by changing the initial building block used to fabricate these nanostructures. Hence, it can be used to obtain structures of varying shapes.

6.6.2 Functionalization

Self-assembled nanostructures can be used as agents used in contrast imaging or as biosensors. They can perform such specific functions because they are combined with suitable functional molecules which give them desired properties. Some of these functional groups include functional antibodies, enzymes, magnetic or metallic particles, fluorescent compounds, etc.

6.6.3 Biocompatibility

Studies have shown that these nanostructures can be used as a cell culture medium. They simplify the loading and handling of the culture medium. The matrix density of these cultures can be fully structured by tailoring it chemically. It has been seen that cell growth in these media is unhindered (Pignatello, 2011).

6.7 Mechanisms of Molecular Self-Assembly

Molecular self-assembly is the assembly and arrangement of molecules without manipulation from an external source. By definition, molecular self-assembly is, "the spontaneous organization of molecules under near-thermodynamic equilibrium conditions into structurally well-defined and stable arrangements through noncovalent interactions". This is largely regulated by weak noncovalent bonds like hydrogen bonds, electrostatic interactions (ionic bonds), water-mediated H-bonds, van der Waals forces, and hydrophilic and hydrophobic interactions. The collective interaction between these weak bonds produces chemically and structurally stable nanostructures (Li, 2017).

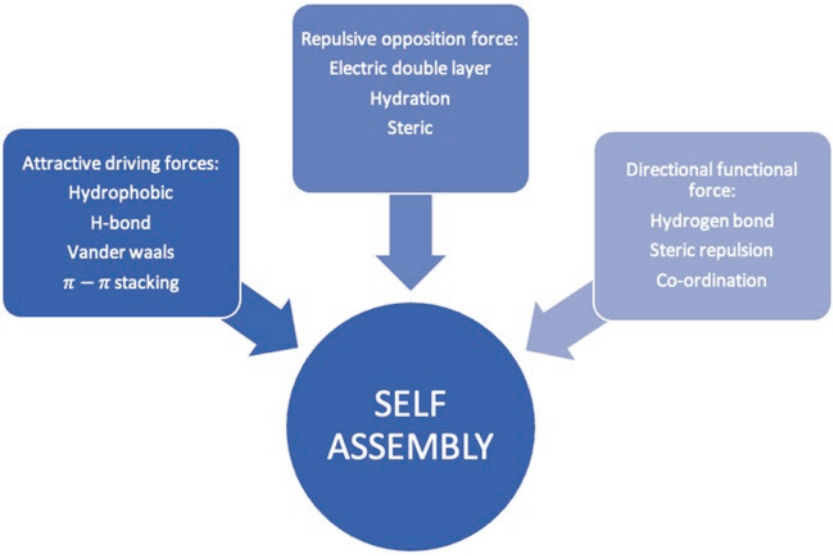


Fig. 6.1 The forces acting in the process of molecular self-assembly

Molecular self-assembly depends on two factors: structural compatibility and chemical complementarity. The molecular constituents require complementary properties like specific surface charge, surface characteristics, polarizability, surface functionalities, and mass, in order to self-assemble to give rise to various physiological forms. Biological components such as lipids, nucleic acids, and proteins, among other cellular components, also self-assemble to form a cell. Some of the examples of molecular self-assembly include antigen–antibody recognition, phospholipid membrane, chromatin assembly, and amyloid fibril formation.

Self-assembly involves maintaining a balance between three types of forces. They include 'driving force (attractive in nature)', 'opposition force (repulsive in nature)', and 'directional force'. A non-hierarchical self-assembly is observed in most of the nanostructures. The process becomes directional because of the supplementary l directional forces (Fig. 6.1).

6.8 Benefits of Molecular Medicine from Self-Assembled Nanostructured Materials

Studies done previously have shown that self-assembled nanostructures can be used to manufacture various molecular medicines which have properties which are superior to the ones made conventionally. The benefits of using these nanostructures for the assembly of molecular medicines for tissue engineering include:

1. Designing using active biological peptides.
2. Incorporation of different functional groups and their combinations with nanostructures can help to study cell behaviour.
3. Good biocompatibility since it degrades in the body by natural enzymes.
4. Molecules can be altered at the level of single amino acids based on the requirement in a cost effective way using conventional methods.
5. They can be used to analyse cell signalling processes and controlled gene expression.

Thus, these self-assembled nanostructures derived molecular medicines are being used readily for 'bionanotechnological applications'.

Nanoparticles can be used as potential vessels for drug targeting because their pharmacodynamics and pharmacokinetics in the body are controlled by their physicochemical properties, such as hydrophobicity, particle size, and surface charge. Colloidal particles can overcome the setback of bringing a hydrophobic substance into the aqueous blood medium. Another advantage of colloidal carrier systems is their protective action towards sensitive drugs, such as specific peptides and synthetic vaccines (Li, 2017).

Gene therapy is another therapeutic technique that can benefit from nanotechnology since it also has the problem of drug delivery given that it faces the same problems of delivering the gene to the target cell.

In the future, nanostructures can also be used to construct well-defined two-dimensional and three-dimensional structures. The fabrication of these nanostructures with different substrates can be explored.

6.9 Challenges When Using Self-Assembled Nanostructures in Molecular Medicine

Even though self-assembled nanostructures have many advantages, a few challenges regarding the manipulation, size control, stability in liquids, and immobilization have to be considered.

6.9.1 Size Control

Size control becomes a very important parameter when it comes to applications like drug-delivery systems and biosensors. Due to the process of self-assembly, size control during the synthesis process is very challenging.

6.9.2 *Stability of Nanostructures in Liquid Environment*

The biomedical applications of self-assembled peptide nanostructures, where they come in direct contact with a liquid environment are numerous, such as drug-delivery systems, contrast imaging agents, and biosensors. They have to be highly stable in the solution it is interacting with, in the body. It has been noted that nanostructures are unstable when dissolved in water, methanol, or phosphate buffer. This limits their application in various biomedical processes.

6.9.3 *Manipulation*

Manipulation of nanostructures is a very critical process because of their dimensions. It is a challenge to link nanostructures, such as nanofibers, nanotubes, and nanoparticles, with macroscopic applications.

It is also very vital to avoid damage to nanostructures when they interact with the object being manipulated and the instrument. Any alteration in the structure of the self-assembled nanostructure, during processing and manipulation, can cause a change in the behaviour required for particular medicine applications (Pignatello, 2011).

6.9.4 *Conductivity*

Another factor that makes the application of self-assembled nanostructures challenging is their low conductivity limits. Their use in the production of biosensors or diagnostic aids omitting the functionalization step incorporates molecules and compounds to improve conductivity.

6.10 Conclusion

Self-assembled nanostructures are biomaterials with various merits, which makes them an excellent candidate for biomedical applications. Nanostructures used in molecular medicine are developed under mild environments, in a rapid and cost-effective way. It can also be readily functionalized with various other functional groups like enzymes, fluorescent molecules, and antibodies. Nevertheless, it is notable to acknowledge the intricacies involved while studying self-assembled nanostructures. Multiple ways to overcome problems associated with manipulation, size control, stability, and low conductivity have been continuously explored. In spite of these limitations, nanostructures play a very important role

in biomedical applications. Until now, the size and shape of nanoparticles have been characterized. Some of the other parameters that can also be characterized and explored include 'surface area','degree of aggregation', 'surface charge', and 'size distribution', and also appraise their 'surface chemistry'. These parameters can affect the properties and applications of nanostructures. Reliable and accurate characterization techniques for nanostructures will significantly have an impact on the use of these materials, commercially. The next step in self-assembled nanostructures can be aimed at a comprehensive analysis of the immunogenicity and biocompatibility of these nanostructures with the purpose of understanding the outcome of their use.

References

Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research, 6*(2), 105–121.

Ariga, K., Nishikawa, M., Mori, T., Takeya, J., Shrestha, L. K., & Hill, J. P. (2019 Jan 31). Self-assembly as a key player for materials nanoarchitectonics. *Science and Technology of Advanced Materials, 20*(1), 51–95.

Castillo-León, J., Andersen, K. B., & Svendsen, W. E. (2011). Self–assembled peptide nanostructures for biomedical applications: Advantages and challenges. *Biomaterials Science and Engineering, 10*, 23322.

Chen, J., Guo, L., Qiu, B., Lin, Z., & Wang, T. (2018). Application of ordered nanoparticle self-assemblies in surface-enhanced spectroscopy. *Materials Chemistry Frontiers, 2*(5), 835–860.

Delfi, M., Sartorius, R., Ashrafizadeh, M., Sharifi, E., Zhang, Y., De Berardinis, P., Zarrabi, A., Varma, R. S., Tay, F. R., Smith, B. R., & Makvandi, P. (2021). Self-assembled peptide and protein nanostructures for anti-cancer therapy: Targeted delivery, stimuli-responsive devices and immunotherapy. *Nano Today, 38*, 101119.

Gao, X., & Matsui, H. (2005). Peptide-based nanotubes and their applications in bionanotechnology. *Advanced Materials, 17*(17), 2037–2050. https://doi.org/10.1002/adma.200401849

Habibi, N., Kamaly, N., Memic, A., & Shafiee, H. (2016). Self-assembled peptide-based nanostructures: Smart nanomaterials toward targeted drug delivery. *Nano Today, 11*(1), 41–60.

Huynh, N. T., Passirani, C., Saulnier, P., & Benoit, J. P. (2009). Lipid nanocapsules: a new platform for nanomedicine. *International Journal of Pharmaceutics, 379*(2), 201–209.

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology, 9*, 1050–1074.

Kasotakis, E., Mossou, E., Adler-Abramovich, L., Mitchell, E. P., Forsyth, V. T., Gazit, E., & Mitraki, A. (2009). Design of metal-binding sites onto self-assembled peptide fibrils. *Peptide Science: Original Research on Biomolecules, 92*(3), 164–172.

Kochovski, Z., Chen, G., Yuan, J., & Lu, Y. (2020). Cryo-Electron microscopy for the study of self-assembled poly(ionic liquid) nanoparticles and protein supramolecularstructures. *Colloid & Polymer Science, 298*, 707–717.

Koshy, O. (2017). Differential scanning calorimetry in nanoscience and nanotechnology. In *Thermal and rheological measurement techniques for nanomaterials characterization* (pp. 109–122). Elsevier.

Lee, S., Trinh, T. H. T., Yoo, M., Shin, J., Lee, H., Kim, J., Hwang, E., Lim, Y. B., & Ryou, C. (2019). Self-assembling peptides and their application in the treatment of diseases. *International Journal of Molecular Sciences, 20*(23), 5850.

Li, J. (2017). *Supramolecular chemistry of biomimetic systems. Advantages of self-assembled supramolecular polymers toward biological applications* (pp. 9–35). Springer. https://doi.org/10.1007/978-981-10-6059-5

Lu, W. (2012). Self-assembly of nanostructures. In B. Bhushan (Ed.), *Encyclopedia of nanotechnology*. Springer.

Mourdikoudis, S., Pallares, R. M., & Thanh, N. T. K. (2018). Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties. *Nanoscale, 10*, 12871–12934.

Nie, Z., Petukhova, A., & Kumacheva, E. (2010). Properties and emerging applications of self-assembled structures made from inorganic nanoparticles. *Nature Nanotechnology, 5*(1), 15–25.

Pignatello, R. (2011). Self-assembled peptide nanostructures for biomedical applications: Advantages and challenges. *Biomaterials Science and Engineering*. https://doi.org/10.5772/1956

Puri, A., Loomis, K., Smith, B., Lee, J. H., Yavlovich, A., Heldman, E., & Blumenthal, R. (2009). Lipid-based nanoparticles as pharmaceutical drug carriers: From concepts to clinic. *Critical Reviews in Therapeutic Drug Carrier Systems, 26*(6), 523–580.

Scioli Montoto, S., Muraca, G., & Ruiz, M. E. (2020). Solid lipid nanoparticles for drug delivery: pharmacological and biopharmaceutical aspects. *Frontiers in Molecular Biosciences, 7*, 587997.

Stephen, V. J., Andrew, M. R., Nigel, R. H., Hodson, W., Saiani, A., Gough, J. E., & Ulijin, V. (2009). Introducing chemical functionality in Fmoc-peptide gels for cell culture. *Acta Biomaterialia, 5*(3), 934–943.

Subramani, K., & Ahmed, W. (2018). Self-assembly of proteins and peptides and their applications in bionanotechnology and dentistry. In *Emerging nanotechnologies in dentistry* (pp. 231–249). William Andrew Publishing.

Suhail, N., Alzahrani, A. K., Basha, W. J., Kizilbash, N., Zaidi, A., Ambreen, J., & Khachfe, H. M. (2021). Microemulsions: Unique properties, pharmacological applications, and targeted drug delivery. *Frontiers in Nanotechnology, 69*.

Tan, A., Hong, L., Du, J. D., & Boyd, B. J. (2019). Self-assembled nanostructured lipid systems: is there a link between structure and cytotoxicity? *Advanced Science, 6*(3), 1801223.

Wang, L., Gong, C., Yuan, X., & Wei, G. (2019). Controlling the self-assembly of biomolecules into functional nanomaterials through internal interactions and external stimulations: A review. *Nanomaterials (Basel), 9*(2), 285.

Zhang, S. (2002). Emerging biological materials through molecular self-assembly. *Biotechnology Advances, 20*(5–6), 321–339.

Zuccheri, G., & Samorì, B. (2011). Methods in molecular biology; DNA nanotechnology. In *Synthesis and characterization of self-assembled DNA nanostructures (Chapter 1)* (Vol. 749, pp. 1–11). https://doi.org/10.1007/978-1-61779-142-0

Chapter 7
Functional Metal Oxide Nanoparticles Avenues for Nanomedicinal Research

Praseetha P. Nair and Kaushik Pal

7.1 Research Background and Overviews

The association of nanotechnology with material science, biology and medicine, combines the knowledge to explore the potential applications to many avenues of nanomedicinal research. Diverse nanomaterials or its nanoformulations are available and approved for the prevention, diagnosis, and therapy of physiological ailments (Soetaert et al., 2020). Very small size and the high specific surface area of nanoparticles define their reactivity and properties (Kaur et al., 2021). Nanomaterials exhibit astonishing features that are entirely different from their parent elements. The size range of engineered nanoparticles that are similar to biological entities make them compatible with the biomolecules and ease their interactions.

The major challenges faced by nanomedicinal research are the inconsistencies between the preclinical performance of the drug and its clinical application (Soetaert et al., 2020). The modest performance in in vivo application is due to many factors, particularly its instability in physiological fluids. This tends to the agglomeration and precipitation of particles in the body leading to toxicity and safety-related issues. Functionalization, coating, nanoencapsulation, etc., are the strategies that are proven and successful to overcome these drawbacks (Boyer et al., 2010; Safi et al., 2011). Figure 7.1 represents the various possible modifications of metal oxide nanoparticles for biomedical applications (Nikolova & Chavali, 2020).

P. P. Nair (✉)
Department of Chemical Engineering, Government Engineering College, Thrissur, Kerala, India
e-mail: praseetha@gectcr.ac.in

K. Pal
University Centre for Research and Development (UCRD), Chandigarh University, Mohali, Punjab, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_7

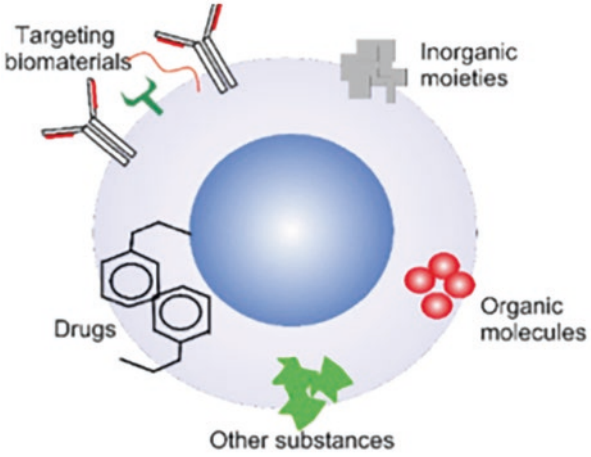


Fig. 7.1 Illustration of possible modifications of metal oxide nanoparticles for biomedical applications (Nikolova & Chavali, 2020). (Copyright at MDPI, 2020)

7.2 Metal Oxide Nanoparticles

7.2.1 Properties and Biomedical Applications

Nanoparticles are categorized based on the base matrix materials, into polymer, metal, ceramic, lipid, semiconductor, carbon-based materials, etc. Metal oxide-based nanoparticles are the next-generation materials that are extensively used for biomedical applications. The properties of nanomaterials can be tailored for suitable applications by optimizing the synthesis procedures. Oxides of metal nanoparticles such as nickel, silicon, copper, silver, gold, tin, magnesium, titanium, zinc, iron, etc., have broad applications in biomedical and pharmaceutical sectors. Among the various metal oxide nanomaterials, superparamagnetic nanoparticles, especially iron-oxide nanoparticles (IONPs), exhibit powerful and promising properties suitable for biomedical applications. Based on the size of IONPs, they are classified into super paramagnetic iron-oxide nanoparticles (SPION) and ultra-small superparamagnetic iron-oxide (USPIO) nanoparticles. The average size range of SPIONs is 50–100 nm and that of USPIONs is below 50 nm (Boyer et al., 2010).

The characteristic features of IONPs are summarized in Fig. 7.2. As IONPs exhibit core-shell characteristics, they possess the features of hydrous iron as well as metallic iron (Kaur et al., 2021). They are known as “nanozymes” as they exhibit the characteristics of enzymes. They are highly biocompatible, exhibit paramagnetism and antimicrobial property, are stable in liquid media, and are less toxic in nature. Superparamagnetism is explored for the transportation of MRI contrast agents and drug-delivery carriers to targeted sites by regulating the magnetic field externally (Hong et al., 2011; Valdiglesias et al., 2016). Another metal oxide nanoparticle having a potential biomedical applications is zinc oxide (Rasmussen

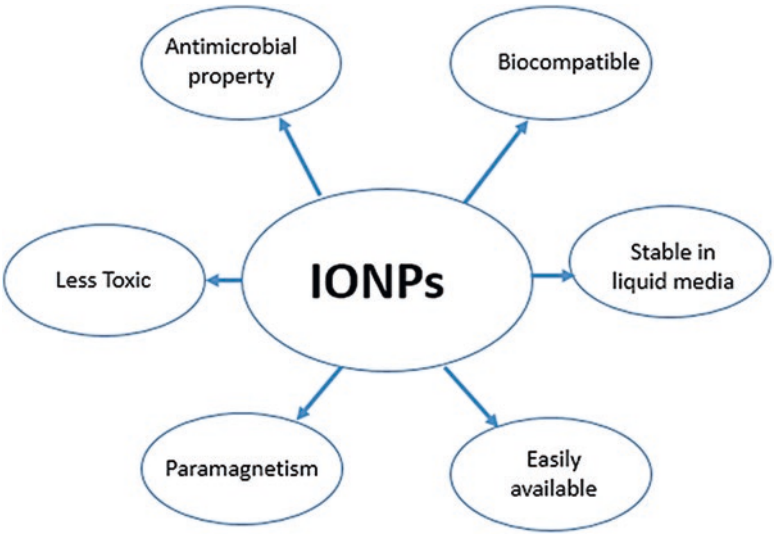


Fig. 7.2 Characteristic features of IONPs

et al., 2010), which has the ability to absorb radiation, is highly stable, and shows photo catalytic activity. Nano-sized zinc oxide exhibits cytotoxicity against cancer cells. Zinc oxide has a peculiar feature of generating reactive oxygen species (ROS) which can be suitably tailored to regulate cell death (Iv et al., 2015). Doping nanoparticles of zinc oxide with metals like iron enhances its ability to induce ROS, which leads to the effective killing of cancer cells.

The major biomedical applications of IONPs are given in Fig. 7.3. They include tissue engineering and bioseparation, drug and gene delivery, magnetic cell sorting, contrast agents in magnetic resonance imaging (MRI), magnetic particle imaging (MPI), hyperthermia therapy, especially for cancer treatment, immunoassays, and stem cell tracking (Boyer et al., 2010; Kaur et al., 2021). A very special application of IONPs is that it acts as an enhanced contrasting agent for MRI imaging.

7.2.2 Synthesis of Metal Oxide Nanoparticles

IONPs can be synthesized by physicochemical or biological routes (Kaur et al., 2021). Chemical synthesis methods are co-precipitation, flow injection, microemulsion, sol-gel, electrochemical deposition, hydrothermal and thermal decomposition methods (Walter et al., 2015), whereas physical methods are milling, grinding, and thermal ablation (Kaur et al., 2021). The popularly used synthesis method of IONPs is the co-precipitation process. This process offers a major control in size, shape, strength, and magnetic properties of the particle. It also provides an opportunity to conduct in-situ functionalization (Boyer et al., 2010). The decomposition of metallic precursors at high temperatures is another method. The nature of the precursor

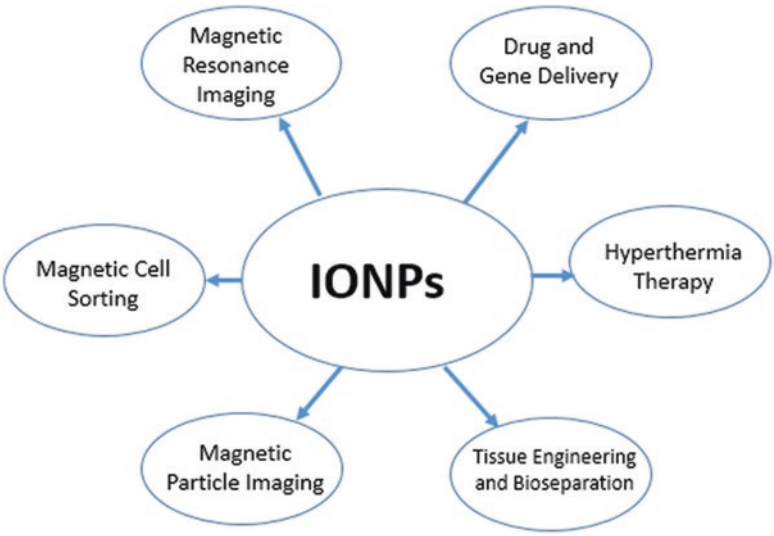


Fig. 7.3 Biomedical applications of IONPs

and operating condition decide the size of the particle, and the shape can be optimized by regulating the process conditions.

Flame spray pyrolysis can be used for the synthesis of metal oxides of iron, zirconia, silica, titanium nanoparticles, and their composites. It produces ultrafine-sized nanoparticles with fewer impurities. Scale-up of the process for large quantity manufacture is an advantage of this method. In microemulsion systems (Dadfar et al., 2019), controlled growth of nanoparticles is ensured in the dispersed phase which acts as a reactor. Proper control of the size of the particle and improved dispersity are ensured in this method. Sol-gel method produces monodispersed particles with high yield at room temperature. Further treatment at high temperatures is needed for obtaining crystal structures (Dadfar et al., 2019). Moreover, a downstreaming process is required for purification after the synthesis. In electrochemical method, an electric current is passed between a metal anode and a cathode placed in an electrolyte solution. Anode oxidizes to form metal ions and the cathode reduces the metal ions to metal in the presence of a stabilizer which acts as the coating.

A green, safe and environmental friendly approach for the synthesis of metal oxide nanoparticles is by biological means. The generalized methodology for biosynthesis and its mechanism is the one in which the plant extracts or microbes are treated with metallic precursors followed by downstreaming processes (Mejías et al., 2010). Microbial extracellular synthesis is advantageous than intracellular processes as it eliminates the requirement of downstreaming operations for purification (Kaur et al., 2021). The mechanism of biosynthesis of nanoparticles is bioreduction which happens in 4 stages namely activation, nucleation, growth, and termination stages (Kaur et al., 2021), as depicted in Fig. 7.4. In the activation stage, biomolecules present in plant extracts or microbes reduce the metallic precursors. In

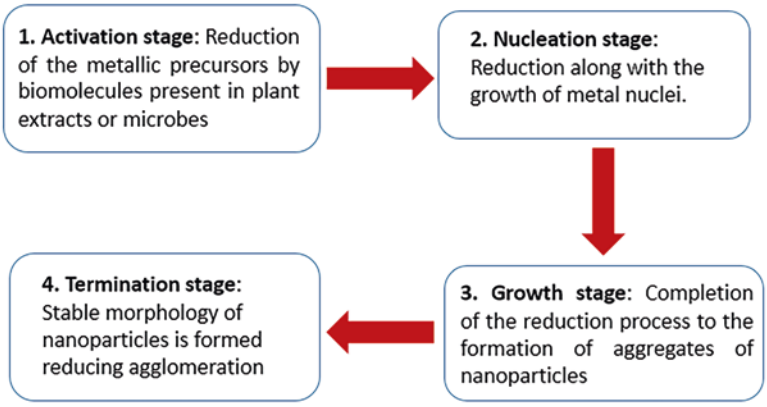


Fig. 7.4 Mechanism of biosynthesis of nanoparticles by bioreduction

the second stage, reduction along with the growth of metal nuclei happens. The growth stage completes the reduction process to the formation of aggregates of nanoparticles. Finally, in the termination stage, a stable morphology of nanoparticles is formed reducing agglomeration.

7.2.3 Physical and Chemical Characterization of Iron Oxide Nanoparticles

Different analytical techniques are prevailing to evaluate the physicochemical characterization of nanoparticles. These techniques help us to understand the specific properties like size, shape, morphology, stability, compositions, etc., of nanoparticles (Dadfar et al., 2019; Kanagasubbulakshmi & Kadirvelu, 2017). It also help to optimize the synthesis procedure according to the requirement. Estimation of size, size distribution, and surface-to-volume ratio of nanoparticles can be evaluated with X-ray scattering techniques. Infrared spectroscopy is used to identify the chemicals present in the particles. Compositional analysis can be conducted using inductively coupled plasma mass spectroscopy and flame atomic absorption spectrometry. A measure of the colloidal stability and aggregation nature of nanoparticles is obtained by nanoparticle tracking analysis and dynamic light scattering (DLS) technique. DLS also provide the hydrodynamic behavior and size distribution of particles. Composition and crystalline structure can be analyzed at the atomic scale with the selected area crystalline diffraction pattern obtained from high-resolution transmission electron microscopy. Scanning electron microscopy can be used to evaluate the morphological characteristics. Three-dimensional magnification of core size distribution is provided by atomic force microscopy. Crystalline structure can also be analyzed with X-ray diffraction patterns and X-ray scattering techniques (Dadfar et al., 2019; Rahman et al., 2011). Functional group analysis can be conducted with

nuclear magnetic resonance spectroscopy (NMR), Fourier transform infrared spectroscopy (FT-IR), and X-ray photoelectron spectroscopy. Energy-dispersive X-ray spectroscopy is used for elemental analysis.

7.2.4 Functionalization Strategies

Nanoparticles, as such, tend to agglomerate in biological medium forming settlements at various sites and adsorption to bio-entities leading to toxicity. Selective coating of proteins on nanoparticle surfaces results in the phenomena of protein corona (Torrisi et al., 2014). Better harnessing of their properties especially for in vivo applications can be achieved by different modification techniques like functionalization or coating. It involves the addition of materials to the surface of nanoparticles to form a stable morphology with mono-dispersed materials in a biological medium. Moreover, these modifications can improve the encapsulation efficiency of materials to drugs or genes leading to their targeted delivery to specific sites. Magnetic properties of the nanoparticles can be customized by incorporating cobalt, nickel, or manganese (Boyer et al., 2010).

Functionalization is usually done with organic compounds and polymers. Organic molecules can be grafted to the nanoparticles' surface by in situ functionalization technique where the coating is done during the synthesis of particles, or by post-synthesis functionalization technique where the coating is done after the synthesis of nanoparticles (Walter et al., 2015). Coating with polymers enhances the stabilization of colloidal particles through steric effect and provides surface functionality (Boyer et al., 2010). This can be done with grafting "onto" or grafting "from" techniques. These methods are involved in the stabilization of IONPs using functional diblock polymers, monofunctional polymer chains, and encapsulations (Boyer et al., 2010). Direct grafting can be done by exposing the molecule to be grafted to the suspension of nanoparticles. Kadirvelu and Kanagasubbulakshmi synthesized magnetic IONPs through the green route successfully and found that alcoholic and acetic functional groups are present in the nanoparticles (Kanagasubbulakshmi & Kadirvelu, 2017). This makes them hydrophilic and hence further modification is not required for stabilization. Guyen et al. used orthogonal click reactions for the preparation of multifunctional, well-dispersed IONPs with antifouling characteristics (N'Guyen et al., 2013). Carboxylic acids show high affinity with the surface of nanoparticles and thus modification with such groups helps to form stable suspensions (Liu et al., 2007). Stability can be enhanced by the modification of sulphates and carboxylates (Liu et al., 2007). Surface coating with natural polymers like dextran and chitosan enhances the biocompatibility and hydrophilicity of compounds (Liu et al., 2007; Wang et al., 2013). Synthetic polymer grafting with polyethylene glycol also provides enhanced biocompatibility, dispersion, and biodistribution of nanoparticles and increases blood circulation time. The longer chain length and density of polyethylene glycol reduce the tendency of the protein to adsorb on the nanoparticle surface (Walter et al., 2015). Dendrimers

or dendrons are used for the functionalization of nanoparticles of gold, polymers, carbon, iron oxide, and quantum dots (Walter et al., 2015). Research shows that the magnetic resonance imaging properties of IONPs are improved when functionalized with dendrons. Safi et al. investigated the nature of coating agents for functionalization of IONPs and observed that carboxylated polymers with higher molecular weight exhibit enhanced colloidal stability compared to low molecular weight ligands (Safi et al., 2011). Thi et al. used heparin-poloxamer to modify superparamagnetic iron oxide nanoparticles (SPIONs) and found successful attachment of both the particles (Hoang et al., 2019). They also conducted studies on the targeted delivery of anticancer drugs and observed targeted and controlled delivery of the drug with the prepared SPIONs. Multifunctional chlorotoxin-conjugated SPIONs were successfully used for the tumor-targeted drug delivery (Sun et al., 2008). Another challenging problem is the infection caused by the formation of biofilms on the medical devices implanted inside the human body. The biofilms formed by certain bacterial strains are resistant to antibiotics. Leuba et al. (2013) investigated the carboxylated functionalized SPIONs for the reduction of biofilm formed by *Staphylococcus aureus* and found that the material could successfully disrupt the biofilms due to the small size and the magnetic nature of SPIONs. Some of the important and popularly used functionalization methods are summarized in Table 7.1 (Walter et al., 2015).

7.3 Advances of Metal Oxide Nanoparticles in Nanomedicine and Diagnostics

Metal oxide nanomaterials can be applied in biosensing, image probing like MRI, tomography, drug delivery, theranostics applications like cancer diagnostics, hyperthermia, photothermal therapy, gene delivery applications, brain tumor detection, and characterization, etc. Conventional treatments of chronic diseases like cancer

Table 7.1 Functionalization Methods

Functionalization methods	Description
In situ functionalization	Functionalization is done during the synthesis of NPs by mixing with precursors
Post-synthesis functionalization	Functionalization and synthesis are done separately.
Direct grafting method	Functionalization is done by introducing the molecules in the suspension of NPs.
Grafting by ligand exchange process	NPs and the molecule to be grafted are mixed in organic solvent. The ligand will be replaced after functionalization and NPs will be transferred into water
Grafting by ligand exchange process with phase transfer	Molecules to be grafted are dissolved in the aqueous phase, and the NPs are dispersed in organic solvent. NP will be transferred from the organic phase to the aqueous phase under stirring

have many side effects. The treatment methods, which include chemotherapy and radiation therapy, while killing the diseased cells, cause damage to the healthy cells also. Nanotechnology aided treatment methods help to improve tumor treatment, by effectively targeting the drugs to diseased cells and regulating the release of drugs (Liu et al., 2007).

7.3.1 Imaging Techniques and Brain Tumor Detection

Magnetic resonance images are widely used in the detection of disorders or abnormal growth in all parts of the body. Magnetic contrast agents like IONPs, SPIONs, doped quantum dots, etc., play a major role in sensitively detecting ailments. MRI imaging with IONPs can be used for stem cell therapies (Vallabani & Singh, 2018). IONPs modified with dopamine and encapsulated with human serum albumin act as excellent contrasting agents for MRI. A study (Vallabani & Singh, 2018) demonstrated the synthesis of IONP coated with silica in the core and shell with a gold layer. This composite functional material finds application in an imaging mode. The resolution of an image depends on its modality. Multimodal imaging has unique benefits in comparison with single modality which helps in the confirmation of diagnosis of disorders. Another imaging technique, CT scan (computed tomography), is used to scan internal injuries in body parts like blood vessels, bones, etc. Gold-coated IONPs act as better contrasting agents for CT, MRI, and ultrasound (US) images. A hybrid imaging technique is developed with the combination of CT, MRI, and US to form positron emission tomographic (PET) imaging. It produces images with better resolution. A combination of PET images, CT scans, and MRI will give a detailed analysis of anatomy with high-resolution and sensitivity. This helps in the easy detection of ailments like cancer and also helps in the identification of targeted sites for delivery. The characteristics and material properties of IONPs allow them to tune the kinetics and also the easy surface modification, make them an attractive option as a multimodal agent. IONP-enabled multimodal imaging is given in Fig. 7.5.

Citrate-coated IONPs exhibit better imaging capability. IONPs doped with gadolinium and coated with polyethylene glycol act as contrasting agents and exhibit darker and brighter contrasting MRI. Oleic acid-coated iron oxide nanoparticles modified with polymers show superior tumor imaging properties. Vascular diseases can be diagnosed with magnetic resonance angiography (Vangijzegem et al., 2019). Superparamagnetic IONPs with ultra-small size ranges are used to enhance the contrast of magnetic resonance. Studies on blood-brain barrier and detection of brain tumors can be effectively done using contrast-enhanced MRI. Superparamagnetic IONPs play a vital role in the detection of brain tumor possibility and development (Dadfar et al., 2019; Vallabani & Singh, 2018).

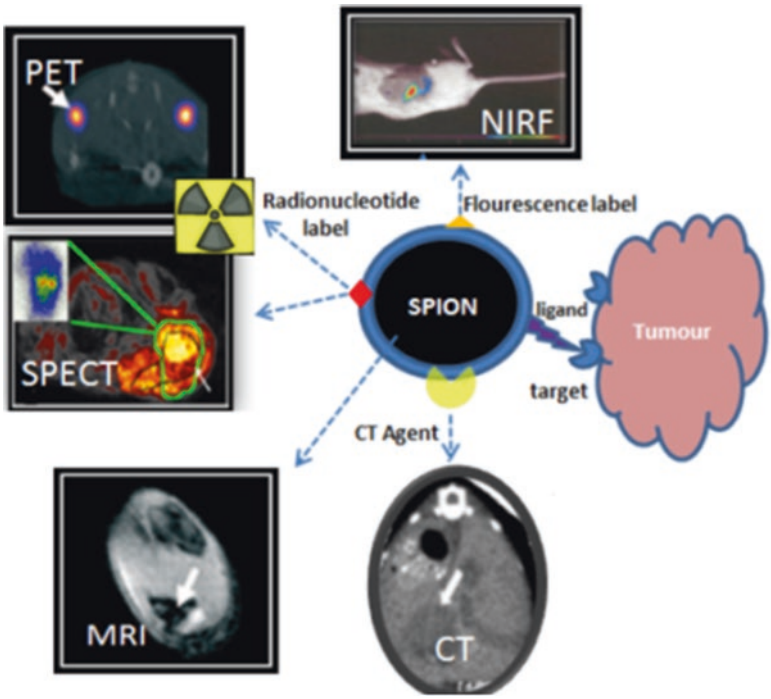


Fig. 7.5 Concept of IONP enabled multimodal imaging (Thomas et al., 2013). (Copyright at MDPI, 2013)

7.3.2 Cancer Diagnostics and Therapy

Early-stage detection of cancer is possible with imaging techniques using IONPs and SPIONs as MRI contrast agents (Zhu et al., 2017). IONPs conjugated with peptides or antibodies accumulate in cancer tissues and provide high-contrast detection of tumors by MRI. IONPs associated with pH-responsive ligands when targeted to tumor cells produce MRI signals under acidic conditions. This detects even very small tumors of 3 mm diameter (Zhu et al., 2017). MRI detects nanoparticle drug delivery and tumor size.

Hyperthermia is a phenomenon of developing temperature near a tumor growth using microwaves, radio waves, etc. The side effects of cancer treatment can be eliminated by combining radiation therapy and chemotherapy with hyperthermia (Vallabani & Singh, 2018). Cancer cells are very much susceptible to temperature, while healthy cells are not. Hence hyperthermia technique is used to kill cancer cells by creating temperature differences at specific tumor sites. Crystalline IONPs coated with polymer and with anti-biofouling properties can be used for photothermal therapy of cancer (Vallabani & Singh, 2018). ZnO nanoparticles can be used for the early-stage detection of cancer. Polymethyl methacrylate capped ZnO

nanoparticles can be used as biomarkers that can detect very low concentrations of cancer cells even with high sensitivity.

7.3.3 *Drug and Gene Delivery Applications*

The ability of nanoparticles to act as drug carriers have many advantages like targeting the tumor cells while leaving the healthy cells, controlling the dosages, reducing the frequency of dosages, as the medicine is released in a controlled manner for a longer time, minimizing side effects, enhancing drug stability and minimizing degradation (Soetaert et al., 2020). IONPs coated with derivatives of starch and phosphate groups loaded with the drug induce a strong magnetic fields at tumor sites. Accumulation of drugs at tumor sites for a longer time ensures prolonged and controlled release of drugs, thereby exhibiting high antitumor activity. Drugs can be conjugated into IONPs or encapsulated into a polymer coating for targeted delivery. The controlled release of a drug using superparamagnetic IONPs (SPION) coated with polymer is depicted in Fig. 7.6.

IONPs modified with albumin can effectively perform the delivery of cancer drugs than conventional treatment with the drug alone (Zhu et al., 2017). IONPs coated with other agents and combined with drugs will accumulate cytotoxicity in tumor cells. This damages the tumors alone without causing damage to the healthy cells.

Gene delivery is a phenomenon of the delivery of nucleic acid for the therapy of diseases (Vallabani & Singh, 2018). The transport of genes to the targeted sites is effected with the help of functionalized metal oxide nanoparticles, especially IONPs. Simultaneous imaging and gene delivery can be used for the treatment of diseases with IONPs loaded with chitosan and linoleic acid. ZnO nanostructures coated with silica and modified with amino groups can be effectively used for gene delivery (Rasmussen et al., 2010). Simultaneous imaging and gene delivery is

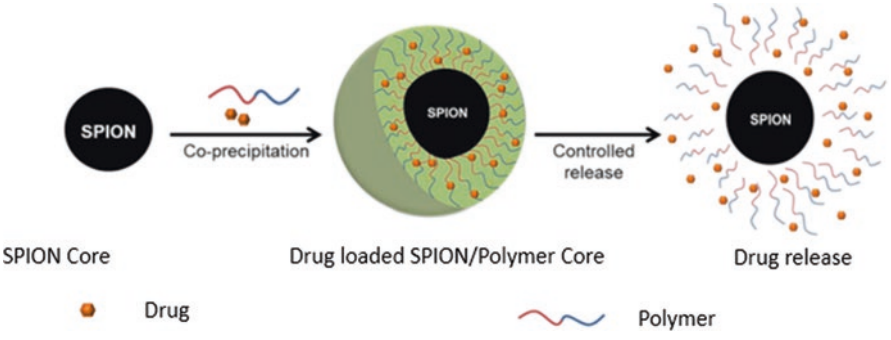


Fig. 7.6 Controlled release of drug with SPION/Polymer core (Hoang et al., 2019). (Copyright at MDPI, 2019)

exhibited by modified ZnO quantum dot structures. These can be used for targeted gene delivery for cancer theranostics.

7.4 Assessment of Safety Aspects of Metal Oxide Nanoparticles

The in vitro analysis of different metal oxide nanoparticles has successfully proved its potential in biomedical applications. The direct cellular interaction of nanoparticles offers advantages as well as disadvantages during in vivo applications. The high specific surface area of NPs during miniaturization imparts both exotic characteristics and toxic effects. This may lead to many issues and challenges which requires a crucial assessment of the potential toxic effect of nanomaterials on biological entities (Sun et al., 2010). The critical evaluation of genotoxicity, neurotoxicity, immunotoxicity, toxicokinetics, biodistribution, degradation, etc. are to be conducted through nanotoxicology studies. The degradation rate of NPs determines the duration of their toxic effects (Soenen & De Cuyper, 2010). Yang et al. studied the toxic effect of IONPs on human fibroblasts and found that surface functionalization of IONPs induced slight modification in the toxic effects towards normal and unhealthy fibroblasts (Yang et al., 2013). Studies show that coating of IONPs with polymers alters its toxic effect and it has specific consequences for different cells (Soenen & De Cuyper, 2010). Aminosilane-coated IONPs were synthesized by Sun et al. (2013). The particles are nontoxic to all types of cells examined at lower concentrations. It is also observed that at higher concentrations toxicity depends on the surface coating and not on the concentration of IONPs. Extensive research should be conducted in this area to assess the interaction of nanomaterials with cells for deriving its adverse effect or toxic nature, elucidating the mechanism of interaction and minimizing the toxicological nature of nanomaterials during in vivo applications (Tombácz et al., 2015). The assessment of undesirable biological consequences is inevitable to harness the interesting and promising applications of nanotechnology, especially in the biomedical field. Hong et al. observed that the functionalization of superparamagnetic IONPs induces alteration in their cytotoxic and genotoxic nature (Hong et al., 2011). It is also found that low concentrations of materials are not cytotoxic or genotoxic to fibroblasts.

7.5 Conclusion and Future Perspectives

Functionalization can enhance the unique properties of metal oxide nanomaterials which makes them fascinating with plenty of medicinal applications. Synthesis procedures have to be optimized to enhance and maintain biocompatibility, physicochemical, biological, surface, and magnetic properties. Treatment strategies must be

adopted for the early-stage detection and treatment of chronic diseases, especially cancer. Functionalized metal oxide nanoparticles can be used for multimodal imaging which helps in detection of diseases. Targeted drug delivery to diseased cells can be done by protecting healthy cells and tissues. Controlled drug release prevents the heavy and frequent dosage of medicines. It is necessary to evaluate the risk associated with the usage of extremely small particles for clinical applications. A deeper understanding of the mechanism of interaction between nanoparticles and biomolecules is inevitable so that effective nanoformulations can be designed for specific applications. Material scientists and cell biologists should work collaboratively to carefully analyze the interactions to translate the research findings into clinical applications.

References

Boyer, C., Whittaker, M. R., et al. (2010). The design and utility of polymer-stabilized iron-oxide nanoparticles for nanomedicine applications. *NPG Asia Materials, 2*(1), 23–30.

Dadfar, S. M., Roemhild, K., et al. (2019). Iron oxide nanoparticles: Diagnostic, therapeutic and theranostic applications. *Advanced Drug Delivery Reviews, 138*, 302–325.

Hoang Thi, T. T., Nguyen Tran, D. H., et al. (2019). Functional magnetic core-shell system-based iron oxide nanoparticle coated with biocompatible copolymer for anticancer drug delivery. *Pharmaceutics, 11*(3), 120.

Hong, S. C., Lee, J. H., et al. (2011). Subtle cytotoxicity and genotoxicity differences in superparamagnetic iron oxide nanoparticles coated with various functional groups. *International Journal of Nanomedicine, 6*, 3219.

Iv, M., Telischak, N., et al. (2015). Clinical applications of iron oxide nanoparticles for magnetic resonance imaging of brain tumors. *Nanomedicine, 10*(6), 993–1018.

Kanagasubbulakshmi, S., & Kadirvelu, K. (2017). Green synthesis of iron oxide nanoparticles using Lagenaria siceraria and evaluation of its antimicrobial activity. *Defence Life Science Journal, 2*(4), 422–427.

Kaur, K., Sidhu, A. K., et al. (2021). Green synthesis: An eco-friendly route for the synthesis of iron oxide nanoparticles. *Frontiers in Nanotechnology, 3*, 655062.

Leuba, K. D., Durmus, N. G., et al. (2013). Carboxylate functionalized superparamagnetic iron oxide nanoparticles (SPION) for the reduction of S. aureus growth post biofilm formation. *International Journal of Nanomedicine, 8*, 731.

Liu, Y., Miyoshi, H., et al. (2007). Nanomedicine for drug delivery and imaging: A promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. *International Journal of Cancer, 120*(12), 2527–2537.

Mejías, R., Pérez-Yagüe, S., et al. (2010). Liver and brain imaging through dimercaptosuccinic acid-coated iron oxide nanoparticles. *Nanomedicine, 5*(3), 397–408.

N'Guyen, T. T., Duong, H. T., et al. (2013). Functional iron oxide magnetic nanoparticles with hyperthermia-induced drug release ability by using a combination of orthogonal click reactions. *Angewandte Chemie, 125*(52), 14402–14406.

Nikolova, M. P., & Chavali, M. S. (2020). Metal oxide nanoparticles as biomedical materials. *Biomimetics, 5*(2), 27.

Rahman, M. M., Khan, S. B., et al. (2011). Iron oxide nanoparticles. *Nanomaterials, 3*, 43–67.

Rasmussen, J. W., Martinez, E., et al. (2010). Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. *Expert Opinion on Drug Delivery, 7*(9), 1063–1077.

Safi, M., Courtois, J., et al. (2011). The effects of aggregation and protein corona on the cellular internalization of iron oxide nanoparticles. *Biomaterials, 32*(35), 9353–9363.

Soenen, S. J., & De Cuyper, M. (2010). Assessing iron oxide nanoparticle toxicity in vitro: Current status and future prospects. *Nanomedicine, 5*(8), 1261–1275.

Soetaert, F., Korangath, P., et al. (2020). Cancer therapy with iron oxide nanoparticles: Agents of thermal and immune therapies. *Advanced Drug Delivery Reviews, 163*, 65–83.

Sun, C., Fang, C., et al. (2008). *Tumor-targeted drug delivery and MRI contrast enhancement by chlorotoxin-conjugated iron oxide nanoparticles* (Vol. 3, p. 495).

Sun, C., Du, K., et al. (2010). PEG-mediated synthesis of highly dispersive multifunctional superparamagnetic nanoparticles: Their physicochemical properties and function in vivo. *ACS Nano, 4*(4), 2402–2410.

Sun, Z., Yathindranath, V., et al. (2013). Characterization of cellular uptake and toxicity of aminosilane-coated iron oxide nanoparticles with different charges in central nervous system-relevant cell culture models. *International Journal of Nanomedicine, 8*, 961.

Thomas, R., Park, I. K., et al. (2013). Magnetic iron oxide nanoparticles for multimodal imaging and therapy of cancer. *International Journal of Molecular Sciences, 14*(8), 15910–15930.

Tombácz, E., Turcu, R., et al. (2015). Magnetic iron oxide nanoparticles: Recent trends in design and synthesis of magnetoresponsive nanosystems. *Biochemical and Biophysical Research Communications, 468*(3), 442–453.

Torrisi, V., Graillot, A., et al. (2014). Preventing corona effects: Multiphosphonic acid poly (ethylene glycol) copolymers for stable stealth iron oxide nanoparticles. *Biomacromolecules, 15*(8), 3171–3179.

Valdiglesias, V., Fernández-Bertólez, N., et al. (2016). Are iron oxide nanoparticles safe? Current knowledge and future perspectives. *Journal of Trace Elements in Medicine and Biology, 38*, 53–63.

Vallabani, N. V., & Singh, S. (2018). Recent advances and future prospects of iron oxide nanoparticles in biomedicine and diagnostics. *3 Biotech, 8*(6), 1–23.

Vangijzegem, T., Stanicki, D., et al. (2019). Magnetic iron oxide nanoparticles for drug delivery: Applications and characteristics. *Expert Opinion on Drug Delivery, 16*(1), 69–78.

Walter, A., Garofalo, A., et al. (2015). Functionalization strategies and dendronization of iron oxide nanoparticles. *Nanotechnology Reviews, 4*(6), 581–593.

Wang, Y. X., Xuan, S., et al. (2013). Recent advances in superparamagnetic iron oxide nanoparticles for cellular imaging and targeted therapy research. *Current Pharmaceutical Design, 19*(37), 6575–6593.

Yang, W. J., Lee, J. H., et al. (2013). Difference between toxicities of iron oxide magnetic nanoparticles with various surface-functional groups against human normal fibroblasts and fibrosarcoma cells. *Materials, 6*(10), 4689–4706.

Zhu, L., Zhou, Z., et al. (2017). Magnetic nanoparticles for precision oncology: Theranostic magnetic iron oxide nanoparticles for image-guided and targeted cancer therapy. *Nanomedicine, 12*(1), 73–87.

Chapter 8
Recent Advancement of Nanostructured Materials for Clinical Challenges in Vaccinology

Mohammad Harun-Ur-Rashid, Israt Jahan, and Abu Bin Imran

8.1 Introduction

Despite the surprisingly impressive success of vaccines in controlling and eradicating communicable diseases, there persists numerous globally catastrophic diseases without fully preventive vaccines, especially malaria, flu, AIDS (human immunodeficiency virus, HIV), hepatitis, and tuberculosis. Nanotechnology-based strategies have been developed both to fabricate advanced vaccines to control and eliminate these diseases and to clear the way for their worldwide administration. The limitations of why a particular pathogen may create difficulties for designing and developing vaccines are distinctive and connected to the coexisting history of humans and pathogens; however, there are usually issues that could be successfully addressed through the effective implementation of nanotechnology products such as nanostructured materials. Due to technological advancement, conventional materials, as well as bulk materials, have been replaced by advanced nanostructured and nanoengineered materials such as nanocomposite gels (Chowdhury et al., 2015; Harun-Ur-Rashid & Imran, 2019; Rezaul Karim et al., 2020), nanomedicine (Yang et al., 2022; Guo et al., 2021; Sun et al., 2021; Zheng et al., 2021; Cheng et al., 2021; Wei et al.,

M. Harun-Ur-Rashid
Department of Chemistry, International University of Business Agriculture and Technology, Dhaka, Bangladesh

I. Jahan
Department of Cell Physiology, Graduate School of Medicine, Nagoya University, Nagoya, Japan

A. B. Imran (✉)
Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Department of Chemistry, Bangladesh University of Engineering and Technology, Dhaka, Bangladesh
e-mail: abimran@chem.buet.ac.bd

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_8

2021), molecular machines (Imran et al., 2019), nanobiosensors (Harun-Ur-Rashid et al., 2022), and polymer nanocomposite drugs (Díez-Pascual, 2022; Shen et al., 2021). These structurally engineered nanomaterials have been introduced and investigated extensively to find out solutions for currently existing problems and to address and overcome the present challenges and limitations in many different sectors, including building and construction (Janczarek et al., 2022; Quazi & Park, 2022), automobile (Harun-Ur-Rashid et al., 2023a, b; Imran & Susan, 2022), aviation and space (Pathak & Dhakate, 2022), optics (Halali et al., 2020), packaging (Dey et al., 2022), textiles (Perera et al., 2022), electronics (Mo, 2022), energy (Adegoke & Maxakato, 2022), catalysis (Zeng et al., 2022), agriculture (An et al., 2022), food (Wu & Mu, 2022), cosmetics (Fauzi et al., 2022), environment (Wang et al., 2022), pharmaceuticals (Sridharan et al., 2022), biomedical, and health (Derakhshi et al., 2022).

A successful vaccine will require uplifting immunologic reactions that vary from immunologic reaction raised by natural infection. Nanostructured materials, with their specific compositions, basic adaptable construction, and nanoscale size allowing the involvement of major immunologic routes, unitedly facilitate the repeated design procedures essential to detect such preventive immunologic responses and attain them with expected reliability. Nanostructured materials also serve as approaches for engineering the transfer of the major vaccine components to specific immune cells and key tissues such as lymphoid tissues. They might be highly polyvalent, enhancing their involvement in the immune response system (Fries et al., 2021). Vaccines, prepared from nanostructured materials or nanoparticles (NPs) that serve as antigen transfer vehicles composed of lipidic, proteic, polymeric, metallic, or graphene, are termed nanovaccines. In nanovaccines, such NPs are commonly functionalized with the antigen through surface modification or encapsulation treatment. Covalent bonds or intermolecular forces of attractions arbitrate the unification of the antigen to the incorporated NPs. When the nanomaterials are intended for biomedical purposes, as illustrated in Fig. 8.1, some of the issues are to be taken care of such as drug toxicity, bioavailability, organ specificity, drug stability and solubility, and entire safety. The development and advancement of nanostructured materials as drug carriers have attracted researchers and commercial communities because of their outstanding characteristics such as better chemical and biological stability, greater carrier capacity, suitability for incorporating both hydrophobic and hydrophilic substances, and regulated drug delivery ability.

Nanostructured materials are widely used in various sectors of nanovaccines, cancer therapy, biomolecule detection, and regenerative medicine because of their biological, physical, and chemical characteristics, including flexibility, strength, performance, durability, surface morphology, surface zeta potential values, surface charge, and potential antimicrobial activity, which has been schematically represented in Fig. 8.2 (Foyez & Imran, 2022; Rudramurthy & Swamy, 2018). The rapid success of nanomaterials in biomedical applications has created a perception that nanomedicine is the "savior" of mankind. Nonetheless, the successful global deployment of nanomedicine or nanovaccines that we observe is the consequence of extensive research, design, improvement, and optimization of products, which must be commemorated with additional funding for further development. Conversely, the

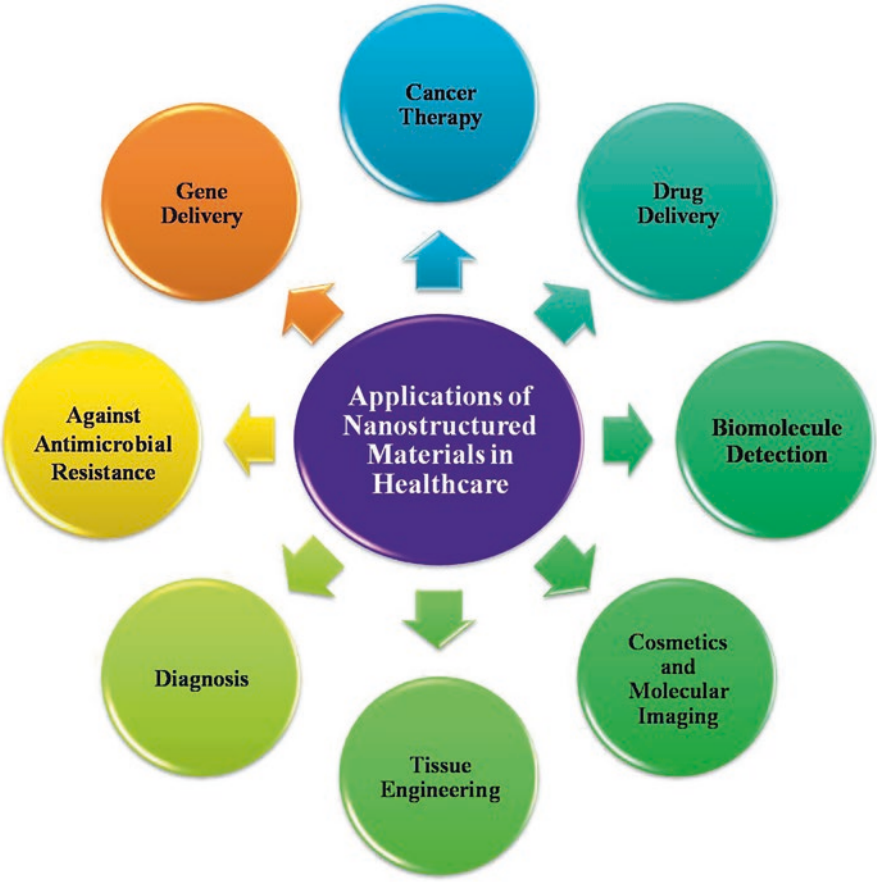


Fig. 8.1 Applications of nanostructured materials in various biomedical and healthcare sectors. (The figure has been reproduced with permission from ref. Rudramurthy and Swamy (2018). Copyright@2018, Springer)

oversimplified aggrandizement of nanomedicine or nanovaccines needs to be circumvented. Deliberation, awareness, and prospective thinking must triumph in managing the pandemic situation. This chapter will especially focus on the achievements of nanostructured materials in nanovaccine applications and the remaining clinical challenges in nanovaccinology.

8.2 Nanostructured Materials-Based Nanovaccines

The immunologic reaction system is an integrated network of cells, tissues, and organs that act as the safeguard of the body against diseases. The immune system comprises inborn and adaptive immunities. Adaptive immunity is capable of detecting a pathogenic component and evolving a durable impression of it. The aim of

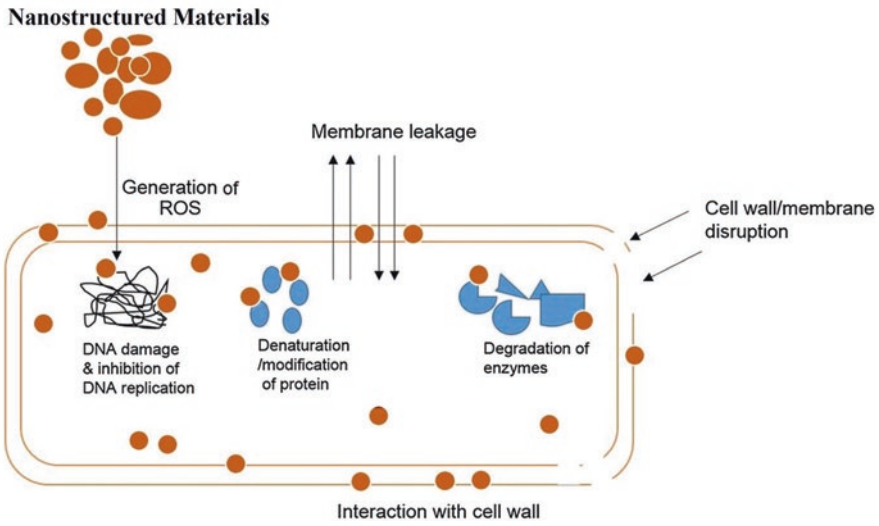


Fig. 8.2 Schematic representation of the antimicrobial activity of nanostructured materials. (The figure has been reproduced with permission from ref. Rudramurthy and Swamy (2018). Copyright@2018, Springer)

vaccination is to teach the adaptive immune system to either create immunological reminiscence before infection or to detect ongoing disease (Koff, 2016). Although the development of prophylactic vaccines in the case of deadly communicable diseases such as anthrax, smallpox, and plague has made a very remarkable contribution to healthcare. Recently, modern vaccines have shown great effectiveness in the treatment of incurable diseases like HIV infection, cancer, and type I diabetes (Greenwood, 2014). Nanovaccines have been designed and developed to overcome the limitations of conventional vaccines as well as to provide smart modulation to facilitate superior efficacy by increasing the stability of antigens, improving immunogenicity, specifying targeted delivery, and delaying the release of drugs (Azharuddin et al., 2022). Nanostructured materials present in nanovaccines provide effective protection to antigens and adjuvants against proteolytic and enzymatic degradation (Bishop et al., 2015). Nanostructured materials can induce both antibody-dependent and cell-arbitrated immune responses due to their idiosyncratic physicochemical properties (Fig. 8.3). In addition, they assist in transferring the drug in targeted areas and can promisingly load multiple antigenic components into a single scaffold. Nonetheless, an excellent adjustment of NPs physical characteristics, for instance, size, shape, and surface charge may lead to a great improvement in the durability of antigen that enhances cell-regulated immunity.

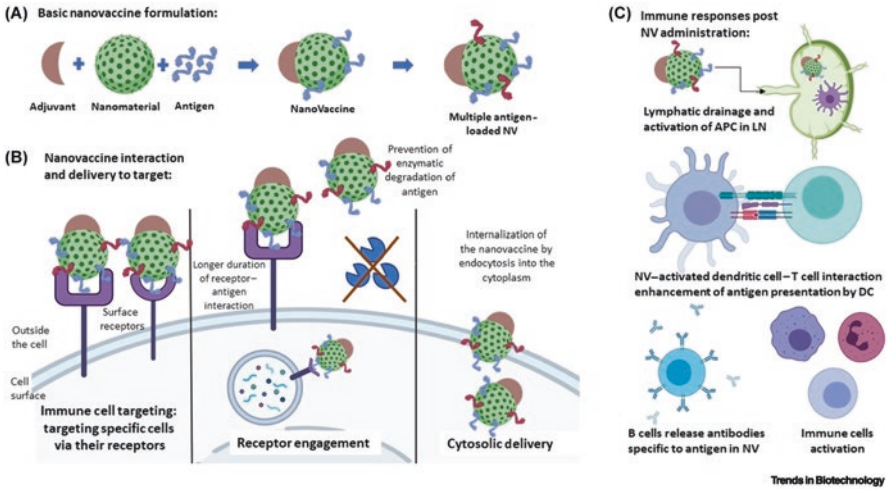


Fig. 8.3 The basic mechanisms of nanovaccines and their importance. Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; LN, lymph node; NP, nanoparticle; NV, nanovaccine. (The figure has been reproduced from the ref. Azharuddin et al. (2022). Copyright@2022, Elsevier)

8.2.1 Key Features of Nanovaccines

Traditional vaccines based on weakened or deactivated pathogens may have the potential risk of incorporating live pathogens and the incompetence to bring out a satisfactory level of an immune response, thus motivating the introduction of novel vaccines. With the advancement of nanotechnology, nanostructured materials-based vaccines (nanovaccines) have been fabricated to overcome the limitations of conventional vaccines as well as to provide advanced-level treatment. A nanovaccine should have some key features (illustrated in Fig. 8.4), such as enhanced immunogenicity, extended antigen stability, sustained release capability, and targeted delivery. Antigens present in the vaccine will be protected from enzymatic degradation since the NPs have a protective nature. NPs are immunogenic and capable of enhancing the immune response against the targeted antigen. One of the major features of nanovaccines is targeted delivery, which facilitates the transfer of antigen to specific sites and thereby minimizes harmful side effects. Enhanced activation of both humoral and cell-mediated immune responses can be achieved by the application of nanovaccines. Different types of antigens can be effectively loaded into a single NP that creates the opportunity to treat a wide range of pathogens as well as diseases. Nanovaccines can persist for an extended period of time without any change or degradation and thus offer enough opportunity for APCs to trigger the immune response.

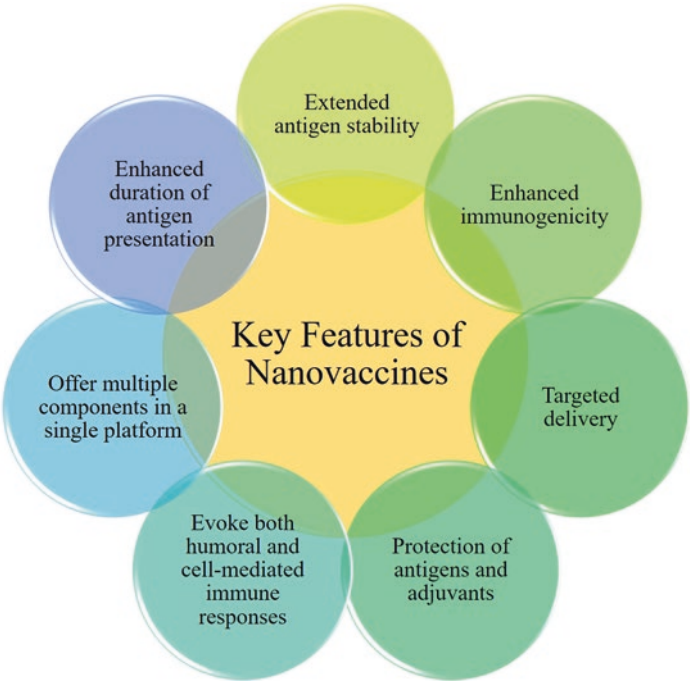


Fig. 8.4 The expected key features of nanovaccines

8.2.2 Types of Nanostructured Materials Used in Nanovaccines

Nanostructured materials such as metallic NPs, carbon nanomaterials, liposomes, silica and magnetic NPs, micelles, polymeric nanocomposites, dendrimers, protein NPs, and so on, utilized in nanovaccines preparation, act as suitable vehicles for antigens due to their nanoscale size that is comparable to the size of pathogens. They are also capable of loading and delivering active biomolecules. Gold NPs (AuNPs) have been employed in nanovaccines against influenza (Tao et al., 2014), malaria (Kumar et al., 2015), and cancer (Ahn et al., 2014). Though the gradual accumulation of nanostructured materials is a safety concern, which required more specific investigations, inorganic NPs such as carbon nanotubes (Hassan et al., 2019), silica NPs (Bancos et al., 2014), and magnetic NPs (Guo et al., 2015). Polymeric nanostructured materials including polylactide-co-glycolic acid (PLGA) copolymers, micelles, dendrimers, chitosan, protein, and liposomes are widely employed in the formulation of nanovaccines.

8.2.3 *Impact of NPs Size on Immunogenicity*

The size of NPs employed in nanovaccines effectively controls the activation of the immensity of immune response and thereby affects the performance of the vaccine. Commonly, it is found that smaller particles are more potential for selected drug delivery systems due to their greater ability to overcome biological barriers (Mumper et al., 2003). However, this trend is not true for all conditions, for example, 1000 nm size bovine serum albumin (BSA)-loaded PLGA particles elicit stronger serum IgG response than 200–500 nm size BSA NPs (Gutierro et al., 2002). Table 8.1 summarizes the effect of NPs size on the immune response.

Table 8.1 The effect of NPs size on immunological reactions (Azharuddin et al., 2022)

Size (nm)	Material	Context	Immunological reactions
1.5	Gold	*Listeria*	AuNP–LLO (listeriolysin O peptide) plus Advax™ adjuvant induced LLO-specific T cell immunity and protection against *Listeria* challenge
2–50	Gold	Foot and mouth diseases	Specific antibodies were induced by 2, 5, 8, 12, and 17 nm FMDV plus cysteine (pFMDV)–AuNP conjugates. Maximal antibody titer was generated with 8–17 nm conjugates
10–100, 60–350, 400–2500	Bilosome	`Influenza	Larger bilosome particles with influenza A antigens elicited immune responses that had a significantly greater Th1 bias than the small particles
12	Gold	`Influenza	Matrix 2 protein (M2e)–AuNP conjugates induced M2e-specific IgG serum antibodies
20–123	Polystyrene	Respiratory syncytial virus (RSV)	IFN-γ induction from CD8 T cells was limited to 40–49 nm beads, whereasCD4 T cell activation and IL-4 were induced by 93–123 nm beads
30–200	Polystyrene	Tumor	Nanobeads of 40–50 nm effectively induced cellular responses by activating$CD8^+$ T cells with IFN-γ production
40	Gold	Tetanus toxoid	Enhanced tetanus toxoid (TT)-specific IgG (34.53×) and IgA (43.75×) was elicited by TT-ARE-CsAuNPs
100, 500	PLGA	Nicotine	The 100 nm particles induced significantly higher antibodies than the 500 nm particles
200, 500, 1000	PLGA	Bovine serum albumin	A greater IgG response was elicited by 1000 nm particle than by 200–500 nm particles
200–600	PLA	Hepatitis B virus	Hepatitis B virus surface antigen (HBsAg) encapsulated in 2–8 μm particles generated more antibodies than 200–600 nm particles
220, 660, 1990	PMMA Eudragit®	HIV	HIV TAT protein modified NPs of 220 or 630 nm elicit strong TAT-specific cellular immune response but weaker anti-TAT antibody response than NPs of1.99 μm

Antigens transferred by NPs are embodied through multiple endocytic routes. In addition, the charge and functionalization techniques of selected molecules can make the transfer of antigens to APCs for antigen dispensing. Cationic NPs are incorporated by APCs more quickly and assist the transfer of antigens inside the cells by way of endosomal escape (Gao et al., 2019). Some of NPs such as cationic dendrimers loaded with antigens exhibit improved delivery performance of antigens to dendritic cells (DCs), and stimulate DCs including the discharge of cytokines such as IL-12 and IL-1β at the same time (Lu et al., 2015). DCs perform a critical role in the harmonization of the natural and accommodative immune system by antigen uptaking, processing, and dispensing of epitopes to naive T cells (illustrated in Fig. 8.5). Currently used vaccines are exogenic to the cells, which is why DCs play an important role in vaccine-mediated immune responses shown by cell against any diseases.

Different organic nanostructured materials are used to formulate nanovaccines for the safe keeping and transporting of active ingredients. For example, two vaccines for COVID-19 utilize lipid nanoparticles (LNP) for transferring the mRNA that systematizes to detect S-protein (spike protein) of SARS-CoV-2 where NPs function as nanocarriers (NCs) having the size ranging from 50 to 200 nm (Guerrini et al., 2022). Nucleic acid or protein-based nanovaccine, illustrated in Fig. 8.6a, comprises several components like polyethylene glycol (PEG)-lipids, ionizable lipids, structural lipids, and cholesterol. These nanovaccines are designed and developed to bring out functional and dynamic immune responses capable of generating specific antibodies against pathogens. The intramuscular (IM) administration of COVID-19 nanovaccines confirms an effective biodistribution and builds local reactogenicity that provides entire immunogenicity. After IM injection, the nanovaccine reaches the lymph nodes (Fig. 8.6b, (1) and (2)). Eventually, the objective of all nanovaccines is the well-controlled delivery of the antigen inside the cell (Fig. 8.6c) for triggering T-cell to support B-cell antibody generation, illustrated in Fig. 8.6b, (6).

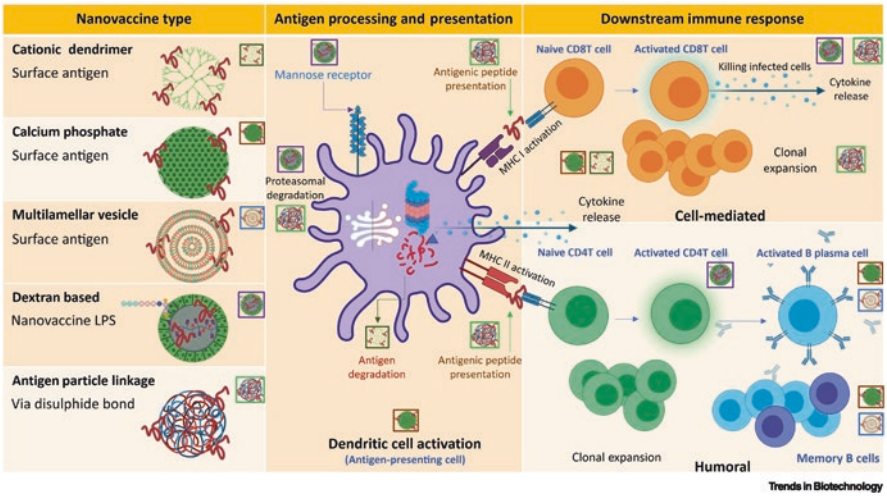


Fig. 8.5 The mode of action of nanovaccines. (The figure has been reproduced from the ref. Azharuddin et al. (2022). Copyright@2022, Elsevier)

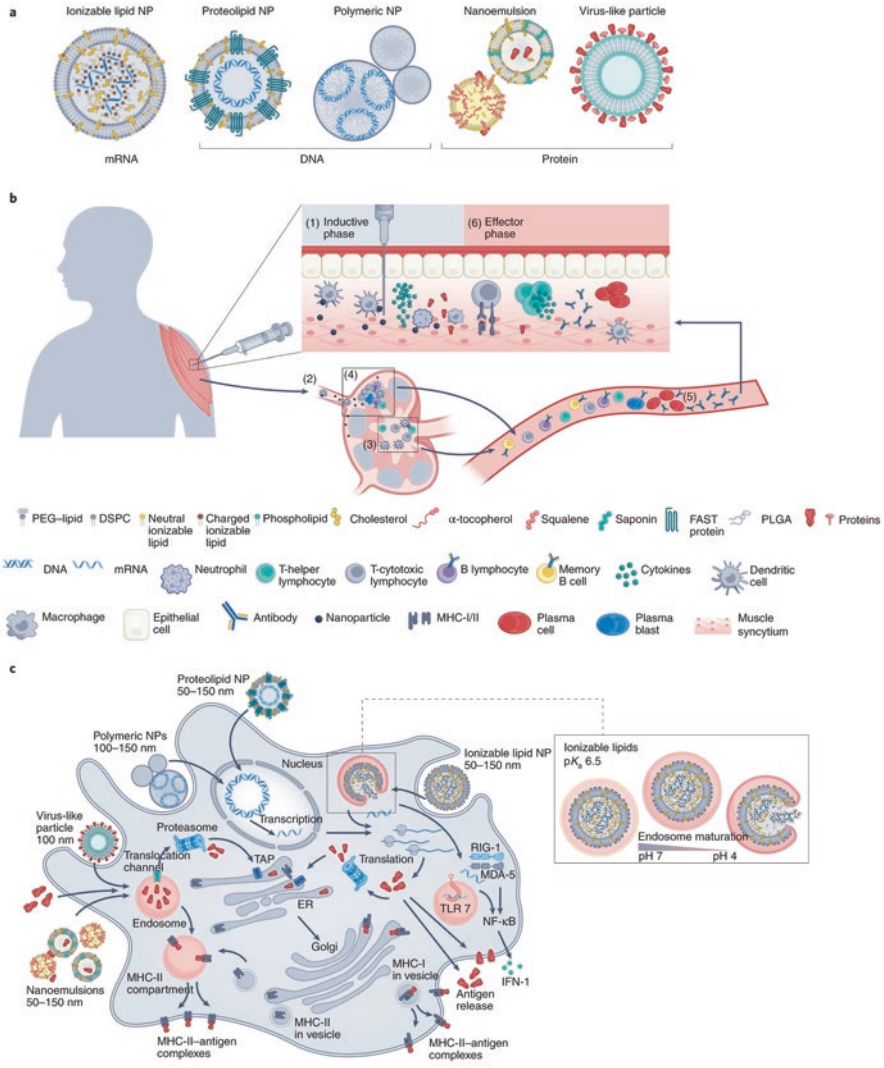


Fig. 8.6 Sketch of the nanovaccine components essential for the treatment of COVID-19, immunomodulatory features, and intracellular destination. (The figure has been reproduced with permission from ref. Guerrini et al. (2022).Copyright@2022, Springer Nature)

The modified metal oxide-based nanostructured materials are used for formulating antitumor vaccines (Chattopadhyay et al., 2016). The cobalt oxide (CoO) nanomaterials, carefully modified by N-phosphonomethyliminodiacetic acid (PMIDA), induce an antitumor immune response (illustrated in Fig. 8.7). The metal oxide nanovaccine can activate macrophage (MΦ) evidenced by tumor necrotic factor alpha (TNF-α) and interferon-gamma (IFN-γ)-level increment.

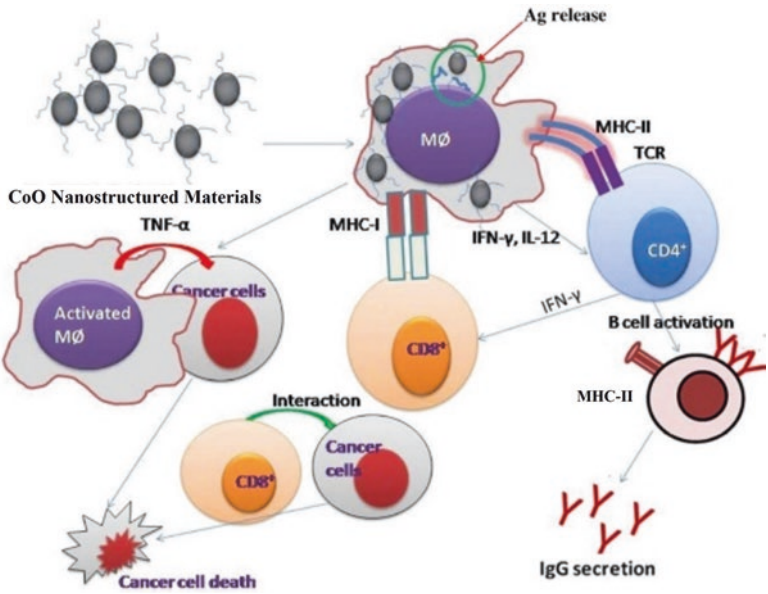


Fig. 8.7 Probable mechanism of PMIDA-modified CoO-based nanostructured materials as anti-tumor vaccine. (The figure has been reproduced with permission from ref. Chattopadhyay et al. (2016). Copyright@2016, Elsevier)

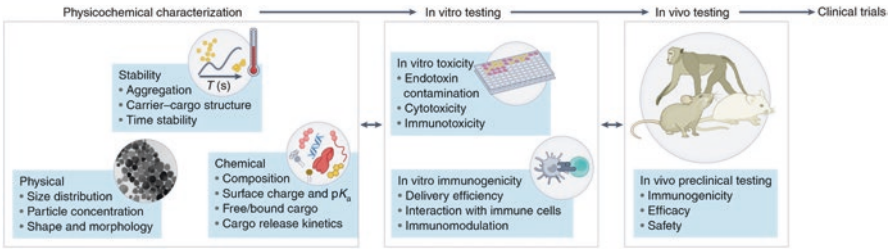


Fig. 8.8 Characterization strategy for nanovaccines. Combination of assays: physical, chemical, stability, in vitro immunogenicity, in vitro toxicology, and in vivo preclinical testing. (The figure has been reproduced with permission from ref. Guerrini et al. (2022). Copyright@2022, Springer Nature)

8.3 Characterization of Nanostructured Materials for Nanovaccines

The characterization of nanostructured materials should be conducted thoroughly in order to evaluate the properties, efficacy, and safety of nanovaccines before clinical practice by assessing stability, physical, and chemical characteristics. Before clinical trials, all the assessments must be performed by in vivo and in vitro testing (Fig. 8.8). Particle-size distribution of nanomaterials influences and determines

biodistribution and immunomodulation of the nanocarriers and active ingredients of nanovaccines. LNP-mRNA NPs of 64 nm and 146 nm both are able to activate immunoglobulin G (IgG) titer in mice. However, the activation performance of 146 nm particles is better than that of particle size 64 nm. Dynamic light scattering (DLS) or multiangle light scattering (MLS) methods are preferably employed to determine particle size, which is very effective for primary screening. Other approaches like nanoparticle tracking, ultracentrifugation, tunable resistive pulse sensing, and transmission electron microscopy (TEM) may provide the required data for the optimization and selection of suitable nanostructured materials for effective nanovaccine formulation. Chemical characterization of nanostructured materials is essential for safe and successful nanovaccine preparation. This process requires meticulous sample preparation and analytical procedures. Commonly used liquid chromatography (LC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) spectroscopy can provide the required data to evaluate chemical characterization. Electrophoresis coupled with capillary electrophoresis and MS or MS detector can provide information on protein integrity and molecular weight, concentration, disulfide bonds, aggregation, and glycosylation of nucleic acid for accomplishing protein sequence and post-translational modifications.

8.4 Stability Testing of Nanostructured Materials

The transportation and storage of nanovaccines are a great concern. Advanced technology is applied to manufacture stable nanovaccine that can withstand higher temperatures. Protein-based nanovaccine may lose its potency due to protein antigen unfolding. To avoid this degradation, the thermal decomposition and stability of protein antigens can be tracked by differential scanning calorimetry (DSC) and circular dichroism. So all the qualities of the nanomaterials must be evaluated and monitored at different timeframes by putting the samples under practical transportation, storage, and application conditions. The transformation from laboratory to practical applications proceeds through batch-to-batch constant assessment. Various nanostructured materials and their diverse physicochemical characteristics may alter the efficiency of nanovaccines. So, it is essential to appropriately select nanomaterial for serving specific quality for manufacturing nanovaccine with reproducible potency, safety, and bioavailability.

8.4.1 In Vitro Immunostimulation and Toxicology Testing

The composition, modification, and optimization of nanostructured materials affect the in vitro delivery effectiveness, immune cell interaction, and immunomodulatory characteristics of nanovaccines. Nanomaterials play a vital role in activating antigen-presenting cells (APCs) that regulate induction and initiation of

immunologic reactions. The antigen transportation and interaction of NPs are crucial and must be examined to optimize the nanovaccine for initiating cellular and humoral responses. Currently, unique methods are used to investigate the immunologic reaction in vitro. For example, tissue engineering can provide the platform to regenerate an in vitro model of human organs that replace the living models to study the working principles of nanoparticle aggregation and to conduct a toxicological assessment (Cupedo et al., 2012) and immunological investigation (Wagar et al., 2021).

Toxicological profiling of nanostructured materials proposed for nanovaccines formulation is an essential step in biocompatibility assessment. Toxicological estimation can be conducted in vitro by applying the approved standards (ISO 29701:2010, ISO 10993-22, and ASTM E2526-08), though specific and appropriate target organs or cells are highly appreciated. Specific and appropriate target cells, including immune cell subtypes (T cell, B lymphocyte, and human monocyte), blood cells (peripheral mononuclear), and entire blood cells, should be recommended to establish clear exposure–response relationships (Crist et al., 2013; Haile et al., 2017; Camera et al., 2021). More specifically, peripheral blood cells (especially mononucleates) are felicitous cells for a micronucleus study that is a prerequisite for the risk estimation of any kind of nanostructured materials selected as components and excipients for nanovaccines formulation. Usually, nanovaccines are administered IM, and they interact with blood. So, blood immunotoxicity and hematotoxicity of nanomaterials are required to be estimated by the following standard in vitro test techniques (ISO/TR 10993-22:2017, ISO 10993-4, and ASTM E2524-08) developed for nanostructured materials used for biomedical and healthcare purposes. Systematic dose-response assessment is typically worthy for bioformulations because of prospective hypersensitivity responses (Szebeni & Moghimi, 2009).

8.4.2 In Vivo Preclinical Testing

The tenacity and biodistribution of nanostructured materials are determined by in vivo imaging methods that facilitate the optimization of nanovaccines (Pardi et al., 2015; Tan et al., 2020; Ciabattini et al., 2021). The potency of nanovaccine depends on antibody neutralization. In vivo preclinical testing is conducted in mice to investigate the safety and protective role since no united safety gateway is designed and constructed for humans. Challenge-protection investigations for tracking the impact of the defection of the pathogen in aimed organs and any changes that occur in the body linked to pathological conditions need to be studied.

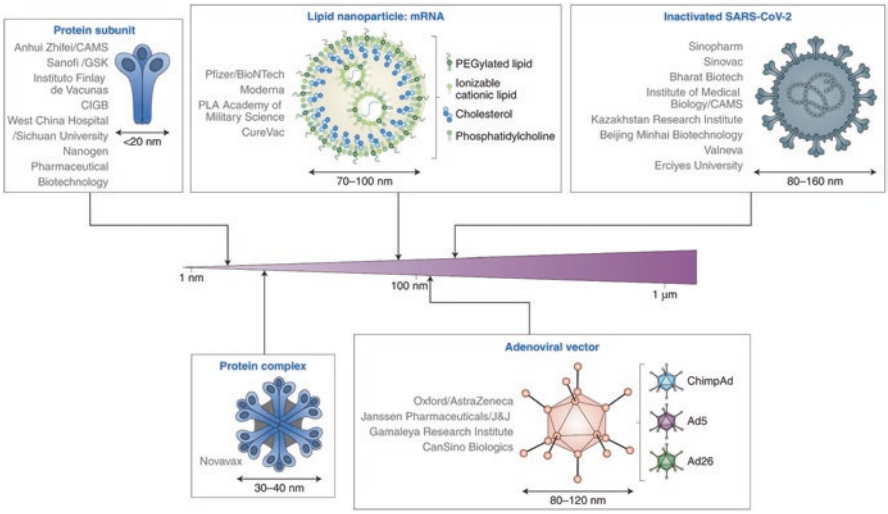


Fig. 8.9 Some nanovaccines for COVID-19 treatment. (The figure has been reproduced from ref. Kisby et al. (2021).Copyright@2021, Springer Nature)

8.5 Achievements of Nanovaccines and Remaining Challenges

Notably, most vaccines depend on natural or synthetic vector systems composed of nanostructured materials (Kisby et al., 2021). Almost all vaccine candidates in Fig. 8.9 fall in between the nanosize range. The advancement of nanostructured materials has created the opportunity to produce an endurable and efficient mRNA transfer mechanism composed of complete and perfect LNPs from decade-old liposome research (Kon et al., 2022; Higuchi et al., 2022; Albertsen et al., 2022). Now, the mRNA-LNPs based nanovaccines are more versatile, powerful, stable, and effective. Adenovirus particles stay persistently within the nanostructured materials and can be engineered to facilitate a suitable platform with intrinsic immunogenicity for effective vaccination. This type of vector's thermal stability is superior to that of the mRNA systems (Ripoll et al., 2022; Li et al., 2021).

Though there is a remarkable advancement in nanotechnology-based vaccines, some challenges still exist and need to be addressed. The thermal stability of mRNA cargo is poor. So, additional optimization of the nanostructured materials is required to be most pertinent for administration, create target-oriented immune activation, and extend the effect's duration. Sometimes, higher production cost is an issue for underdeveloped or developing countries. So, effective but low-priced alternatives should be introduced to the market. Ambiguous durability, duration of immunoprotection, and the reason for hypersensitivity of nanovaccines are required to be addressed and clarified wherever necessary. Further development is essential for selecting and optimizing adjuvants and antigens to improve the efficacy of nanovaccines (Fries et al., 2021).

8.6 Nanovaccines in Clinical Use and in Clinical Trials

Very few nanovaccines have been successfully transformed from the laboratory version to the clinical version. Among these clinical versions mostly trigger humoral responses only; however, it is critical to design and develop vaccines that are able to produce robust cellular responses against cancer and other infectious diseases. Vaxfectin® is a cationic liposomal nanovaccine that is under clinical trials at present. Vaxfectin® has been successfully employed for the treatment of herpes simplex virus type 2 (HSV-2) as well as influenza virus (H5N1). One more clinical version of nanovaccine is Inflexal® V, which has been utilized to treat influenza. For the treatment of cancer, another nanovaccine called Stimulax® is currently administered. Recently, substantial focus has been given to nanostructured materials for the development of potential vaccines for the control and eradication of COVID-19 (shown in Fig. 8.10).

In addition to COVID-19, the utilization of nanovaccines in the treatment of various diseases is quite common indeed. Many of such vaccines have been approved

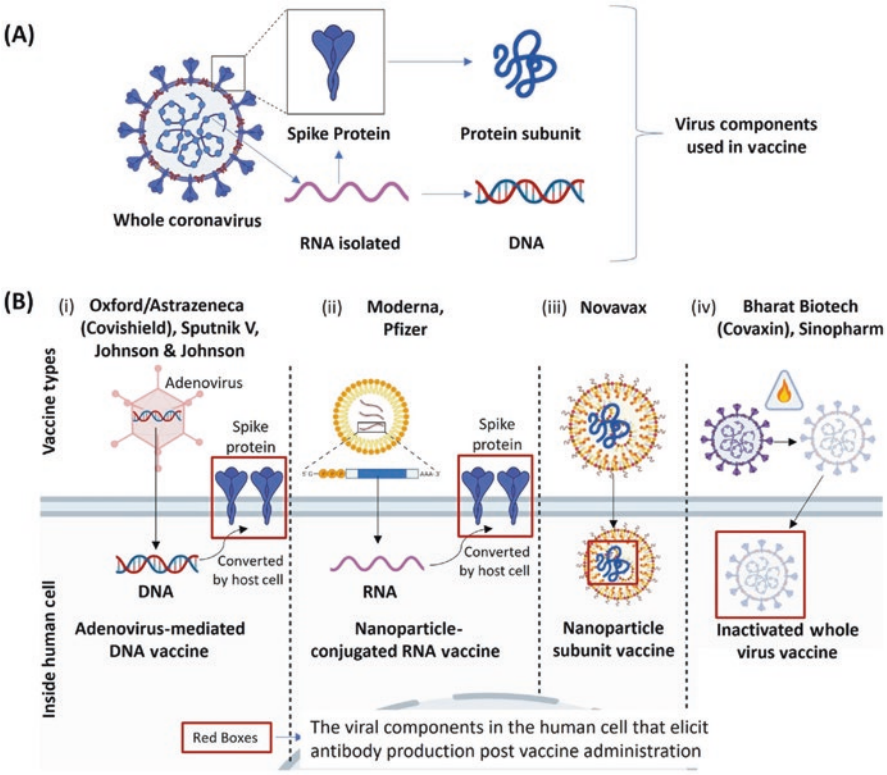


Fig. 8.10 Strategies for the development of nanovaccines against SARS-CoV-2. (The figure has been reproduced from the ref. Azharuddin et al. (2022). Copyright@2022, Elsevier)

by the respective authorities including the European Medicines Agency (EMA), FDA, and so on while many are under clinical trials at present. A list of such vaccines is provided in Table 8.2.

8.7 Future of Nanovaccines

Variations in the effectiveness of immunization have been observed across different demographic groups, including different age groups (such as young or adult individuals), patients with diabetes or without diabetes, males and females, and other categories during the development of various COVID-19 vaccines. The involvement of nanotechnology can offer an arrangement of the latest strategies to potentially develop a periodic vaccine where one infection may potentially facilitate other infections. For instance, influenza infection can induce bacterial super-infection and pneumonia. Similarly, coinfection with influenza A virus can intensify the infection created by SARS-CoV-2. Still, there are scopes to design and develop more effective and versatile nanovaccines with multiple epitopes and/or adjuvants to elicit a wide range of immune responses. Nanotechnology products may offer the best possible non-viral strategy to enclose and transfer nucleic acids. However, the thermal instability of vaccines remains an unsettled issue. It is undeniable that the natural immune system is uniquely composed of different individuals, and a general purpose approach is not a sustainable solution, where nanovaccines may play a pivotal role in the development of a new candidate of personalized vaccines for multifaceted and sustainable protection against catastrophic diseases. Figure 8.11 schematically illustrates the idea of future nanovaccines.

8.8 Comparative Study of NPs Suitable for Vaccine Development

The key features of the nanostructured materials used for the fabrication of nanovaccine have been condensed in Table 8.3 (Rosales-Mendoza & González-Ortega, 2019). The following table summarizes the required information such as the ease of synthesis, price, biocompatibility, FDA approval for medical use, and potentiality for utilization in clinical trials regarding the nanostructured intent to use in nanovaccine formulation. Gold NPs (AuNPs) have been potential applications in drug delivery, sensing, and imaging since they were first synthesized in 1951. PLGA NPs are basically utilized in drug delivery systems since their approval by the US FDA for biomedical implementations. In the vaccinology field, PLGA NPs as drug delivery systems in parenteral administration have been accepted by the US FDA and EMA (Nimesh, 2013). AuNPs-based vaccines are manufactured for the treatment of tumors (Trabbic et al., 2021). Chitosan-functionalized AuNPs (CsAuNPs) have

Table 8.2 The list of nanovaccines that are already approved or under clinical trial phase (Azharuddin et al., 2022)

Institution	Vaccine	Antigen	NPs	Trial stage
Moderna and NIAID	mRNA-1273 LNP	mRNA-1273 mRNA	LNP with mRNA encapsulated	Phase I/II/III
BioNTech and Pfizer	mRNA BNT162b2	mRNA encoding the trimerized RBD ofSARS-CoV-2	LNP with mRNA encapsulated	Phase I/II (UTRN) Phase I/II (Germany) Phase II/III (USA) Phase I (Japan)
Novavax	NVX-CoV2373	Full-length SARS-CoV-2 S glycoprotein	Recombinant glycoprotein NP saponin-based Matrix-M1 adjuvant	Phase I/II/III
Imperial College, London Acuitas Therapeutics, Vancouver	LNP-nCoV saRNA ARCT-021	saRNA and pre-fusion stabilized SARS-CoV-2 S protein	LNP with saRNA encapsulated	
Suzhou Abogen Biosciences Walvax Biotechnology and People's Liberation Army	ARCoV	mRNA encoding RBD of SARS-CoV-2 S glycoprotein	LNP with mRNA encapsulated	Phase I
Novavax		ARS-CoV S protein and influenza M1 protein	SARS-CoV VLP nanovaccine	Preclinical
Imophoron and Bristol University		Multiepitope display	VLP ADDomer™	Preclinical
Crucell	Inflexal®V	Influenza	Virosome with influenza virus surface antigens (hemagglutinin and neuraminidase)	Phase III completed
Crucell	Epaxal®	Hepatitis A	Virosome with inactivated virus particles	Phase III completed
Merck	Gardasil®9	HPV	Capsomere (major capsid protein L1)	Completed
Dendreon Pharmaceuticals	Provenge (Sipuleucel-T)	Prostate cancer	Each dose of contains a minimum of 50 million autologous CD54* cells activated with PAP-GM-CSF	Phase III completed

(continued)

Table 8.2 (continued)

Institution	Vaccine	Antigen	NPs	Trial stage
Novavax	NanoFlu™	Influenza	Recombinant HA protein on Tween 80 NP with Matrix-M adjuvant	Phase III
Novavax	EBOV GP Vaccine	Ebola	2014 Guinea Ebola virus recombinant glycoprotein on Tween 80 NP with/without Matrix-M adjuvant	Phase I
DAIDS/NIAID/NIH	MPER-656	HIV	HIV-1 gp41 membrane proximal external region (MPER) with liposomes	Phase I
BioNTech	W_ova1	Ovarian cancer	Liposome-formulated mRNAs. Three ovarian cancer tumor-associated antigens in combination with (neo-) adjuvant chemotherapy	Phase I
ImmunoVaccine Technologies	DPX-0907	Ovarian, breast, and prostate cancer	Liposomes with seven tumor-specific HLA-A2-restricted peptides, a universal T helper peptide, and a polynucleotide adjuvant in Montanide ISA51 VG	Phase I
Merck	Tecemotide	Multiple myeloma	Liposomes with tecemotide lipopeptide and 3-*O*-deacyl-4′-monophosphoryl lipid adjuvant	Phase II
Cascadian Therapeutics	ONT-10	Solid tumor	Liposomal MUC1 cancer vaccine	Phase I
XEME Biopharma	Oncoquest™	Follicular lymphoma Chronic lymphocytic leukemia	Liposomes containing autologous tumor-derived antigen and IL-2	Phase I/II
Lipotek Pty	Lipovaxin-MM	Metastatic melanoma	Multicomponent liposomes containing tumor antigens (gp100, tyrosinase, and melanA/MART-1) with DC-targeting moiety DMS-5000	Phase I

Abbreviations: *ARE Asparagus racemosus* extract, *CsAuNPs* chitosan-functionalized AuNPs, *F* RSV fusion protein, *HA* influenza virus hemagglutinin, *HPV* humanpapillomavirus, *LNP* lipid nanoparticle, *melanA/MART-1* melanoma antigen recognized by T cells, *MUC1* mucin 1, *NSCLC* non-small cell lung cancer, *PAP-GM-CSF* pulmonary alveolar proteinosis granulocyte macrophage colony-stimulating factor, *RBD* receptor-binding domain, *RSV* respiratory syncytial virus, *S* SARS-CoV-19 spikeprotein, *saRNA* self-amplifying mRNA, *VLP* vaccine-like particle

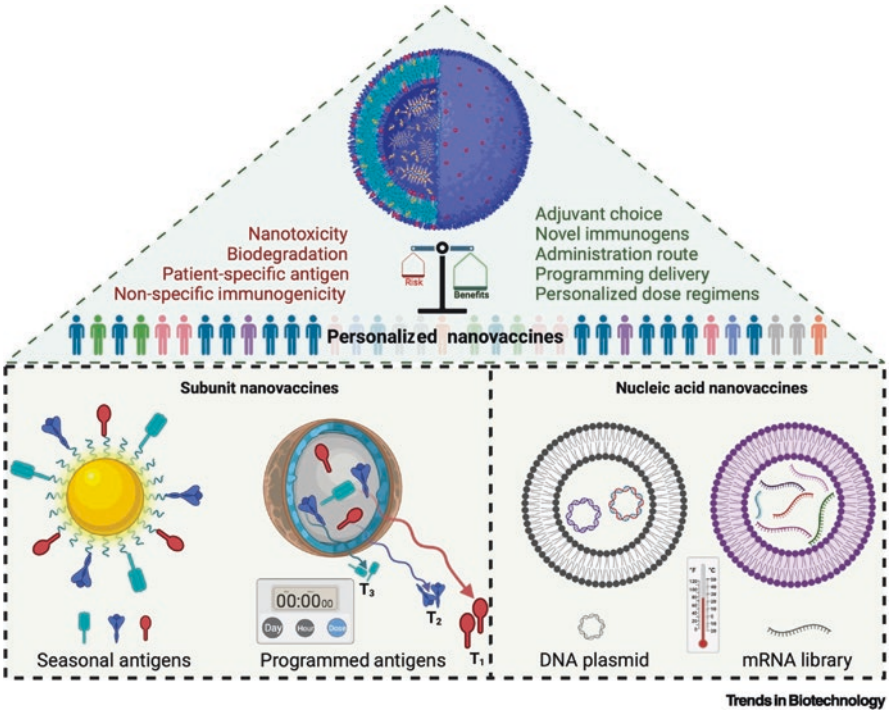


Fig. 8.11 The future of nanovaccines: personalized vaccines from nanostructured materials. (The figure has been reproduced from the ref. Azharuddin et al. (2022). Copyright@2022, Elsevier)

Table 8.3 Comparative analysis of the nanostructured materials employed in the design and development of nanovaccine (Rosales-Mendoza & González-Ortega, 2019)

Nanomaterial	Ease of synthesis	Cost	Biocompatibility	FDA approval for medical use	Used in clinical trials
Gold	Simple	High	Moderate	No	No
PLGA	Moderate	High	High	Yes	Yes
Silica	Moderate	Medium	Moderate	No	No
Carbon Nanotubes	Moderate	Low	Moderate	No	No
Chitosan	Simple	Medium	High	No	Yes
Liposomes	Hard	High	High	Yes	Yes
Nanogels	Simple	Medium	High	No	Yes
Virus-like particles	Hard	Low	High	Yes	Yes

been used for the oral delivery of tetanus toxoid (TT) where the NPs are 40 nm in diameter (Barhate et al., 2014). In this case, soluble triterpene glycosides processed from *Quillaja saponaria* (QS) have been used as adjuvants for the treatment of tetanus. AuNP-based nanovaccine has been formulated for the treatment of influenza

(Wang et al., 2018). Recombinant trimetric influenza A/Aichi/2/68 (H3N2) hemagglutinin (HA) has been combined with 18 nm AuNPs through a metal-chelating chemical process.

Most nanovaccines adsorbed in PLGA NPs are intended to treat human and animal diseases (Gu et al., 2019; Chudina et al., 2015). PLGA NPs containing HBsAg having trehalose and $Mg(OH)_2$ as stabilizers have been incorporated in vaccines for oral immunization (Mishra et al., 2011). PLGA NPs have been utilized to formulate the *Helicobacter pylori* vaccine (Tan et al., 2017).

Silica NPs can be altered chemically or physically to incorporate antigens or adjuvants. The toxicity of silica NPs is still a controversy. Hollow mesoporous silica nanoparticles (HMSNPs) have been used to formulate vaccines for cancer (Lee et al., 2020) and tuberculosis treatment (Montalvo-Quirós et al., 2020). Currently, a vaccine candidate against enterohemorrhagic *E. coli* O157:H7 is being developed by utilizing the EspA protein produced in recombinant *E. coli* as the selected antigen. rEspA has been entrapped onto Silica NPs having a diameter of 96 nm (Hajizade et al., 2018).

Carbon nanomaterials especially carbon nanotubes have been used as drug-delivery vehicles since these materials can be functionalized to introduce carboxylic (–COOH) or amino ($–NH_2$) groups for attaching antigens, adjuvants, or ligands to formulate nanovaccines (Holmannova et al., 2022; Bavandpour et al., 2020; Sawutdeechaikul et al., 2019).

Chitosan is a polymer that contains positively charged moieties derived from the d-glucosamine units. It is produced commercially by deacetylating chitin, which is the structural component of the exoskeleton of shrimp and crab, using NaOH (Rinaudo, 2006). Chitosan is nontoxic, biodegradable, and biocompatible since it is derived from natural sources; however, it is not approved by the US FDA. Chitosan NPs (CsNPs) have been extensively studied as drug delivery systems, especially for protein and gene delivery purposes. CsNPs containing nanovaccines have been investigated for the delivery of antigens and proteins especially for intranasal and oral immunization. Multiple attempts have already been taken and CsNPs-composed vaccines have been developed for the treatment of COVID-19 (Safer & Leporatti, 2021), E. coli involved diseases (Mohammed et al., 2021), Rift Valley Fever or tetanus (Gao et al., 2021), and Avian Coronavirus (Lopes et al., 2021).

Nanogels are common hydrogel NPs that are stimuli-responsive and smart nanostructured materials. They have been employed for diverse biomedical applications including nanovaccinology applications to transfer proteins or oligonucleotides-based antigens (Basu et al., 2021). Injectable sustained-release hydrogel NPs based vaccines have been formulated for COVID-19 treatment from Cowpea mosaic virus (CPMV), a plant virus (Nkanga et al., 2022). CPMV is a potential immunogenic adjuvant that is very much promising for the development of nanovaccines against infectious diseases and cancers. Hydrogel NPs modified with polyethylenimine functionalized graphene oxide (GO)-based RNA nanovaccines have been reported for sustainable cancer immunization (Yin et al., 2022). Supramolecular polymer hydrogel NPs have been employed to enhance the performance of influenza vaccines (Roth et al., 2021).

Virus-like particles (VLPs) are considered common platforms for vaccine development. Currently, multiple vaccines including hepatitis B virus (HBV) and human papillomavirus (HPV) vaccines are produced and marketed worldwide. An expandable, durable, and highly immunogenic VLP-based nanovaccine effective against SARS-CoV-2 has been developed by genetically fusing the receptor-binding motif (RBM) of the spike protein from SARS-CoV-2 into cucumber mosaic virus ($CuMV_{TT}$) (Mohsen et al., 2022).

8.9 Perspectives and Opportunities for Nanostructured Materials in Vaccine Arena

In general, each nanostructured material has both pros and cons and an extensive study of each offers a major advance in vaccine development. For the appropriate implementation of nanostructured materials as a successful candidate for vaccine formulation, adequate information such as exploiting immunology advances, employing bio-nanofabrication methods, and expanding the use of nanocomposites is crucial to select the best-fitting nanomaterial that is capable of serving the intended objectives. Understanding the immunologic reaction mechanism is essential to design and develop a successful vaccine. The immune system associated with protective and therapeutic effects is a complex biological network. The recent advancements in our understanding of regulatory T-cells have established a reliable platform for vaccine development.

The implementation of bio-nanofabrication may facilitate the vaccine formulation pathways with the help of nanostructured materials obtained through biocompatible synthetic routes. Numerous methods already have been established for the fabrication of metallic NPs using bioextracts from plants and algae. In the case of bio-nanofabrication approach, plant extracts act as natural reducing agents for biocompatible NPs synthesis that may be suitable for nanovaccine formulation. Bio-nanofabrication is successfully implemented to synthesize multiple metal and metal oxide NPs including Se, Ag, Au, ZnO, and TiO_2 (Agarwal et al., 2019). Microorganisms like fungi, bacteria, and yeast are also potential candidates for the synthesis of NPs applicable in vaccine formulation (Ahmed et al., 2017; Hulkoti & Taranath, 2014).

Expanding the utilization of nanocomposites (NCs) composed of complex combinations of nanostructured materials can result in extraordinary physicochemical features, leading to novel functional characteristics. In vaccine sectors, several composites have been explored, for instance, AgNPs/silica (Zhao et al., 2016), AuNPs/silica (Nguyen & Shen, 2016), and poly(glycerol adipate-co-ω-pentadecalactone) (PGA-co-PDL) polymeric nanoparticles (NPs) within L-leucine microcarriers (Rodrigues et al., 2018). Synthesis of polymer NCs may open possibilities to create multifunctional nanostructured materials having expected immunogenic activity, which may establish a strategy for future nanovaccines.

8.10 Concluding Remarks

Majority of the population is not familiar with the utilization of nanotechnology products. As nanovaccines are prepared using nanostructured material vectors, it may be challenging for people to readily accept multiple doses. The unfamiliarity with nanovaccines may lead to immoderate perspectives and conspiracy theories. That is why awareness, rumination, comprehension, and easy transmission of the scientific and clinical data produced from unparalleled and deliberate manifestation of nanostructured materials to the public are required. It should always be remembered and practiced that the safety of patients should never be compromised. The development of nanovaccines relies on the perspectives of regulators, ethics reviewers, inventors, and investors in nanotechnology for its introduction in biomedical applications. The outstanding development in the designing of antigen may provide multifunctional platforms of nanostructured materials for specifying immune responses that are effective and appropriate for protection against cancer, HIV/AIDS, malaria, TB, COVID-19, and many other infectious diseases. Once the proper and specific immunogens are identified, multiple platforms may provide nanovaccines with better thermal stability and environment friendly to facilitate distribution throughout the most resource-limited areas of the world.

Acknowledgments A.B. Imran gratefully acknowledges the financial support from Committee for Advanced Studies and Research (CASR) in BUET.

References

Adegoke, K. A., & Maxakato, N. W. (2022). Porous metal oxide electrocatalytic nanomaterials for energy conversion: Oxygen defects and selection techniques. *Coordination Chemistry Reviews, 457*, 214389.

Agarwal, H., Nakara, A., & Shanmugam, V. K. (2019). Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: A review. *Biomedicine & Pharmacotherapy, 109*, 2561–2572.

Ahmed, S., Chaudhry, S. A., & Ikram, S. (2017). A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: A prospect towards green chemistry. *Journal of Photochemistry and Photobiology. B, 166*, 272–284.

Ahn, S., Lee, I.-H., Sukmo, K., Kim, D., Choi, M., Saw, P. E., Shin, E.-C., & Jon, S. (2014). Gold nanoparticles displaying tumor-associated self-antigens as a potential vaccine for cancer immunotherapy. *Advanced Healthcare Materials, 3*, 1194–1199.

Albertsen, C. H., Kulkarni, J., Witzigmann, D., Lind, M., Petersson, K., & Simonsen, J. B. (2022). The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *Advanced Drug Delivery Reviews*, 114416.

An, C., Sun, C., Li, N., Huang, B., Jiang, J., Shen, Y., Wang, C., Zhao, X., Cui, B., Wang, C., Li, X., Zhan, S., Gao, F., Zeng, Z., Cui, H., & Wang, Y. (2022). Nanomaterials and nanotechnology for the delivery of agrochemicals: Strategies towards sustainable agriculture. *Journal of Nanbiotechnology, 20*(1), 1–19.

Azharuddin, M., Zhu, G. H., Sengupta, A., Hinkula, J., Slater, N. K., & Patra, H. K. (2022). Nano toolbox in immune modulation and nanovaccines. *Trends in Biotechnology, 40*(10), 1195–1212.

Bancos, S., Stevens, D. L., & Tyner, K. M. (2014). Effect of silica and gold nanoparticles on macrophage proliferation, activation markers, cytokine production, and phagocytosis in vitro. *International Journal of Nanomedicine, 10*, 183–206.

Barhate, G., Gautam, M., Gairola, S., Jadhav, S., & Pokharkar, V. (2014). Enhanced mucosal immune responses against tetanus toxoid using novel delivery system comprised of chitosan functionalized gold nanoparticles and botanical adjuvant: Characterization, immunogenicity, and stability assessment. *Journal of Pharmaceutical Sciences, 103*(11), 3448–3456.

Basu, P., Saha, N., Saha, T., & Saha, P. (2021). Polymeric hydrogel based systems for vaccine delivery: A review. *Polymer, 230*, 124088.

Bavandpour, A. K., Bakhshi, B., & Najar-Peerayeh, S. (2020). The roles of mesoporous silica and carbon nanoparticles in antigen stability and intensity of immune response against recombinant subunit B of cholera toxin in a rabbit animal model. *International Journal of Pharmaceutics, 573*, 118868.

Bishop, C. J., Kozielski, K. L., & Green, J. J. (2015). Exploring the role of polymer structure on intracellular nucleic acid delivery via polymeric nanoparticles. *Journal of Controlled Release, 219*, 488–499.

Camera, G. D., Lipsa, D., Mehn, D., Italiani, P., Boraschi, D., & Gioria, S. (2021). A step-by-step approach to improve clinical translation of liposome-based nanomaterials, a focus on innate immune and inflammatory responses. *International Journal of Molecular Sciences, 22*(2), 820.

Chattopadhyay, S., Dash, S. K., Mandal, D., Das, B., Tripathy, S., Dey, A., Pramanik, P., & Roy, S. (2016). Metal based nanoparticles as cancer antigen delivery vehicles for macrophage based antitumor vaccine. *Vaccine, 34*, 957–967.

Cheng, X., Xu, H. D., Ran, H. H., Liang, G., & Wu, F. G. (2021). Glutathione-depleting nanomedicines for synergistic cancer therapy. *ACS Nano, 15*(5), 8039–8068.

Chowdhury, A. N., Shapter, J., & Imran, A. B. (Eds.) (2015) in *Innovations in nanomaterials*. Nova Science Publishers, Inc. ISBN: 978-1-63483-572-5.

Chudina, T., Labyntsev, A., Manoilov, K., Kolybo, D., & Komisarenko, S. (2015). Cellobiose-coatedpoly(lactide-co-glycolide) particles loaded with diphtheria toxoid for *per os* immunization. *Croatian Medical Journal, 56*(2), 85–93.

Ciabattini, A., Pastore, G., Fiorino, F., Polvere, J., Lucchesi, S., Pettini, E., & Medaglini, D. (2021). Evidence of SARS-CoV-2-specific memory B cells six months after vaccination with the BNT162b2 mRNA vaccine. *Frontiers in Immunology, 12*.

Crist, R. M., Grossman, J. H., Patri, A. K., Stern, S. T., Dobrovolskaia, M. A., Adiseshaiah, P. P., Clogston, J. D., & McNeil, S. E. (2013). Common pitfalls in nanotechnology: Lessons learned from NCI's Nanotechnology Characterization Laboratory. *Integrative Biology, 5*(1), 66–73.

Cupedo, T., Stroock, A. D., & Coles, M. C. (2012). Application of tissue engineering to the immune system: Development of artificial lymph nodes. *Frontiers in Immunology, 3*, 3389.

Derakhshi, M., Daemi, S., Shahini, P., Habibzadeh, A., Mostafavi, E., & Ashkarran, A. A. (2022). Two-dimensional nanomaterials beyond graphene for biomedical applications. *Journal of Functional Biomaterials, 13*(1), 27.

Dey, A., Pandey, G., & Rawtani, D. (2022). Functionalized nanomaterials driven antimicrobial food packaging: A technological advancement in food science. *Food Control, 131*, 108469.

Díez-Pascual, A. M. (2022). Surface engineering of nanomaterials with polymers, biomolecules, and small ligands for nanomedicine. *Materials, 15*(9), 3251.

Fauzi, M. B., Smandri, A., Amirrah, I. N., Kamaruzaman, N., Salleh, A., Mazlan, Z., Sallehuddin, N., Zulkiflee, I., Jian, L. X., & No, F. M. (2022). Nanomaterials for aging and cosmeceutical applications. In *Food, medical, and environmental applications of nanomaterials* (pp. 455–472). Elsevier.

Foyez, T., & Imran, A. B. (2022). Nanotechnology in vaccine development and constraints. In K. Pal (Ed.), *Nanovaccinology outbreak as targeted therapeutics* (pp. 1–20). Wiley-Scrivener Publisher.

Fries, C. N., Curvino, E. J., Chen, J. L., Permar, S. R., Fouda, G. G., & Collier, J. H. (2021). Advances in nanomaterial vaccine strategies to address infectious diseases impacting global health. *Nature Nanotechnology, 16*(4), 1–4.

Gao, S., Yang, D., Fang, Y., Lin, X., Jin, X., Wang, Q., Wang, X., Ke, L., & Shi, K. (2019). Engineering nanoparticles for targeted remodeling of the tumor microenvironment to improve cancer immunotherapy. *Theranostics, 9*, 126–151.

Gao, X., Liu, N., Wang, Z., Gao, J., Zhang, H., Li, M., Du, Y., Gao, X., & Zheng, A. (2021). Development and optimization of chitosan nanoparticle-based intranasal vaccine carrier. *Molecules, 27*(1), 204.

Greenwood, B. (2014). The contribution of vaccination to global health: Past, present and future. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*, 20130433.

Gu, P., Wusiman, A., Zhang, Y., Liu, Z., Bo, R., Hu, Y., Liu, J., & Wang, D. (2019). Rational design of PLGA nanoparticle vaccine delivery systems to improve immune responses. *Molecular Pharmaceutics, 16*(12), 5000–5012.

Guerrini, G., Magrì, D., Gioria, S., Medaglini, D., & Calzolai, L. (2022). Characterization of nanoparticles-based vaccines for COVID-19. *Nature Nanotechnology, 16*, 1–7.

Guo, Y., Wang, D., Song, Q., Wu, T., Zhuang, X., Bao, Y., Kong, M., Qi, Y., Tan, S., & Zhang, Z. (2015). Erythrocyte membrane-enveloped polymeric nanoparticles as nanovaccine for induction of antitumor immunity against melanoma. *ACS Nano, 9*, 6918–6933.

Guo, Y., Liu, Y., Wu, W., Ling, D., Zhang, Q., Zhao, P., & Hu, X. (2021). Indoleamine 2, 3-dioxygenase (Ido) inhibitors and their nanomedicines for cancer immunotherapy. *Biomaterials, 276*, 121018.

Gutierro, I., Hernandez, R. M., Igartua, M., Gascon, A. R., & Pedraz, J. L. (2002). Size dependent immune response after subcutaneous, oral and intranasal administration of BSA loaded nanospheres. *Vaccine, 21*, 67–77.

Haile, L. A., Polumuri, S. K., Rao, R., Kelley-Baker, L., Kryndushkin, D., Rajaiah, R., Israely, T., Rao, V. A., & Verthelyi, D. (2017). Cell based assay identifies TLR2 and TLR4 stimulating impurities in interferon beta. *Scientific Reports, 7*(1), 1–11.

Hajizade, A., Salmanian, A. H., Amani, J., Ebrahimi, F., & Arpanaei, A. (2018). EspA-loaded mesoporoussilica nanoparticles can efficiently protect animal model against enterohaemorrhagic E. coliO157: H7. *Artificial Cells, Nanomedicine, and Biotechnology, 46*(Suppl 3), S1067–S1075.

Halali, V. V., Sanjayan, C. G., Suvina, V., Sakar, M., & Balakrishna, R. G. (2020). Perovskite nanomaterials as optical and electrochemical sensors. *Inorganic Chemistry Frontiers, 7*(14), 2702–2725.

Harun-Ur-Rashid, M., & Imran, A. B. (2019). Superabsorbent hydrogels from carboxymethyl cellulose. In I. H. Mondal (Ed.), *Carboxymethyl cellulose. Volume I: Synthesis and characterization* (pp. 159–182). Nova Science Publishers.

Harun-Ur-Rashid, M., Foyez, T., Jahan, I., Pal, K., & Imran, A. B. (2022). Rapid diagnosis of COVID-19 via nano-biosensor-implemented biomedical utilization: A systematic review. *RSC Advances, 12*(15), 9445–9465.

Harun-Ur-Rashid, M., Foyez, T., & Imran, A. B. (2023a). Emerging nanomaterials in Automobile Sector. In N. B. Singh, M. A. B. H. Susan, & R. G. Chaudhary (Eds.), *Emerging nanomaterials and their impact on society in the 21st century*. Materials Research Forum LLC.

Harun-Ur-Rashid, M., Imran, A. B., & Susan, M. A. B. H. (2023b). Green polymer nanocomposites in automotive and packaging industries. *Current Pharmaceutical Biotechnology, 24*(1), 145–163.

Hassan, H. A. F. M., Diebold, S. S., Smyth, L. A., Walters, A. A., Lombardi, G., & Al-Jamal, K. T. (2019). Application of carbon nanotubes in cancer vaccines: Achievements, challenges and chances. *Journal of Controlled Release, 297*, 79–90.

Higuchi, A., Sung, T. C., Wang, T., Ling, Q. D., Kumar, S. S., Hsu, S. T., & Umezawa, A. (2022). Material design for next-generation mrna vaccines using lipid nanoparticles. *Polymer Reviews*, 1–43.

Holmannova, D., Borsky, P., Svadlakova, T., Borska, L., & Fiala, Z. (2022). Carbon nanoparticles and their biomedical applications. *Applied Sciences, 12*(15), 7865.

Hulkoti, N. I., & Taranath, T. C. (2014). Biosynthesis of nanoparticles using microbes- a review. *Colloids and Surfaces. B, Biointerfaces, 121*, 474–483.

Imran, A. B., & Susan, M. A. B. H. (2022). Natural fiber-reinforced nanocomposites in automotive industry. In H. Song, T. Nguyen, G. Yasin, N. Singh, & R. Gupta (Eds.), *Nanocomposites for automotive application* (1st ed., pp. 85–103). Elsevier.

Imran, A. B., Harun-Ur-Rashid, M., & Takeoka, Y. (2019). Polyrotaxane actuators. In *Soft actuators* (pp. 81–147). Springer.

Janczarek, M., Klapiszewski, Ł., Jędrzejczak, P., Klapiszewska, I., Ślosarczyk, A., & Jesionowski, T. (2022). Progress of functionalized TiO2-based nanomaterials in the construction industry: A comprehensive review. *Chemical Engineering Journal, 430*, 132062.

Kisby, T., Yilmazer, A., & Kostarelos, K. (2021). Reasons for success and lessons learnt from nanoscale vaccines against COVID-19. *Nature Nanotechnology, 16*(8), 843–850.

Koff, W. C. (2016). A shot at AIDS. *Current Opinion in Biotechnology, 42*, 147–151.

Kon, E., Elia, U., & Peer, D. (2022). Principles for designing an optimal mRNA lipid nanoparticle vaccine. *Current Opinion in Biotechnology, 73*, 329–336.

Kumar, R., Ray, P. C., Datta, D., Bansal, G. P., Angov, E., & Kumar, N. (2015). Nanovaccines for malaria using Plasmodium falciparum antigen Pfs25 attached gold nanoparticles. *Vaccine, 33*, 5064–5071.

Lee, J. Y., Kim, M. K., Nguyen, T. L., & Kim, J. (2020). Hollow mesoporous silica nanoparticles with extra-large mesopores for enhanced cancer vaccine. *ACS Applied Material Interfaces, 12*(31), 34658–34666.

Li, J., Men, K., Gao, Y., Wu, J., Lei, S., Yang, Y., & Pan, H. (2021). Single micelle vectors based on lipid/block copolymer compositions as mRNA formulations for efficient cancer immunogene therapy. *Molecular Pharmaceutics, 18*(11), 4029–4045.

Lopes, P. D., Okino, C. H., Fernando, F. S., Pavani, C., Mariguela, V. C., Montassier, M. D., & Montassier, H. J. (2021). Comparative evaluation of immune responses and protection of chitosan nanoparticles and oil-emulsion adjuvants in avian coronavirus inactivated vaccines in chickens. *Vaccine, 9*(12), 1457.

Lu, F., Mencia, A., Bi, L., Taylor, A., Yao, Y., & HogenEsch, H. (2015). Dendrimer-like alpha-d-glucan nanoparticles activate dendritic cells and are effective vaccine adjuvants. *Journal of Controlled Release, 204*, 51–59.

Mishra, N., Tiwari, S., Vaidya, B., Agrawal, G. P., & Vyas, S. P. (2011). Lectin anchored PLGA nanoparticles for oral mucosal immunization against hepatitis B. *Journal of Drug Targeting, 19*(1), 67–78.

Mo, R. (2022). Functional nanomaterial-based flexible electronics. *Coatings, 12*(6), 809.

Mohammed, G. M., ElZorkany, H. E., Farroh, K. Y., Abd El-Aziz, W. R., & Elshoky, H. A. (2021). Potential improvement of the immune response of chickens against E. coli vaccine by using two forms of chitosan nanoparticles. *International Journal of Biological Macromolecules, 167*, 395–404.

Mohsen, M. O., Balke, I., Zinkhan, S., Zeltina, V., Liu, X., Chang, X., Krenger, P. S., Plattner, K., Gharailoo, Z., Vogt, A. C., & Augusto, G. (2022). A scalable and highly immunogenic virus-like particle-based vaccine against SARS-CoV-2. *Allergy, 77*(1), 243–257.

Montalvo-Quirós, S., Vallet-Regí, M., Palacios, A., Anguita, J., Prados-Rosales, R. C., González, B., & Luque-Garcia, J. L. (2020). Mesoporous silica nanoparticles as a potential platform for vaccine development against tuberculosis. *Pharmaceutics, 12*, 1218.

Mumper, R. J., Cui, Z., & Oyewumi, M. O. (2003). Nanotemplate engineering of cell specific nanoparticles. *Journal of Dispersion Science and Technology, 24*, 569–588.

Nguyen, H. T., & Shen, H. (2016). The effect of PEGylation on the stimulation of IL-1β by gold (Au) nanoshell/silica core nanoparticles. *Journal of Materials Chemistry B, 4*(9), 1650–1659.

Nimesh, S. (2013). Poly(D,L-lactide-co-glycolide)-based nanoparticles. In S. Nimesh (Ed.), *Woodhead Publishing series in biomedicine, gene therapy* (pp. 309–329). Woodhead Publishing.

Nkanga, C. I., Ortega-Rivera, O. A., Shin, M. D., Moreno-Gonzalez, M. A., & Steinmetz, N. F. (2022). Injectable slow-release hydrogel formulation of a plant virus-based COVID-19 vaccine candidate. *Biomacromolecules, 23*(4), 1812–1825.

Pardi, N., Tuyishime, S., Muramatsu, H., Kariko, K., Mui, B. L., Tam, Y. K., Madden, T. D., Hope, M. J., & Weissman, D. (2015). Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *Journal of Controlled Release, 217*, 345–351.

Pathak, A. K., & Dhakate, S. R. (2022). Carbon nanomaterial-carbon fiber hybrid composite for lightweight structural composites in the aerospace industry: Synthesis, processing, and properties. In *Advanced composites in aerospace engineering applications* (pp. 445–470). Springer.

Perera, S., Wijesekara, D., Thiripuranathar, G., & Menaa, F. (2022). The use of nanoparticles to enhance performance in the textile industry-A concise review. *Current Nanoscience, 18*(3), 319–335.

Quazi, M. Z., & Park, N. (2022). Nanohydrogels: Advanced polymeric nanomaterials in the era of nanotechnology for robust functionalization and cumulative applications. *International Journal of Molecular Sciences, 23*(4), 1943.

Rezaul Karim, M., Harun-Ur-Rashid, M., & Imran, A. B. (2020). Highly stretchable hydrogel using vinyl modified narrow dispersed silica particles as cross-linker. *ChemistrySelect, 5*(34), 10556–10561.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science, 31*(7), 603–632.

Ripoll, M., Bernard, M. C., Vaure, C., Bazin, E., Commandeur, S., Perkov, V., & Haensler, J. (2022). An imidazole modified lipid confers enhanced mRNA-LNP stability and strong immunization properties in mice and non-human primates. *Biomaterials, 286*, 121570.

Rodrigues, T. C., Oliveira, M. L. S., Soares-Schanoski, A., Chavez-Rico, S. L., Figueiredo, D. B., Gonçalves, V. M., Ferreira, D. M., Kunda, N. K., Saleem, I. Y., & Miyaji, E. N. (2018). Mucosal immunization with PspA (Pneumococcal surface protein A)-adsorbed nanoparticles targeting the lungs for protection against pneumococcal infection. *PLoS One, 13*(1), e0191692.

Rosales-Mendoza, S., & González-Ortega, O. (Eds.). (2019). Perspectives for the field of nanovaccines. In *Nanovaccines*. Springer Nature, pp. 319–326.

Roth, G. A., Saouaf, O. M., Smith, A. A., Gale, E. C., Hernández, M. A., Idoyaga, J., & Appel, E. A. (2021). Prolonged Codelivery of hemagglutinin and a TLR7/8 agonist in a supramolecular polymer–nanoparticle hydrogel enhances potency and breadth of influenza vaccination. *ACS Biomaterials Science & Engineering, 7*(5), 1889–1899.

Rudramurthy, G. R., & Swamy, M. K. (2018). Potential applications of engineered nanoparticles in medicine and biology: An update. *Journal of Biological Inorganic Chemistry, 23*(8), 1185–1204.

Safer, A. M., & Leporatti, S. (2021). Chitosan nanoparticles for antiviral drug delivery: A novel route for COVID-19 treatment. *International Journal of Nanomedicine, 16*, 8141.

Sawutdeechaikul, P., Jiangchareon, B., Wanichwecharungruang, S., & Palaga, T. (2019). Oxidized carbon nanoparticles as an effective protein antigen delivery system targeting the cell-mediated immune response. *International Journal of Nanomedicine, 14*, 4867.

Shen, S., Fan, D., Yuan, Y., Ma, X., Zhao, J., & Yang, J. (2021). An ultrasmall infinite coordination polymer nanomedicine-composited biomimetic hydrogel for programmed dressing-chemo-low level laser combination therapy of burn wounds. *Chemical Engineering Journal, 426*, 130610.

Sridharan, R., Monisha, B., Kumar, P. S., & Gayatahri, K. V. (2022). Carbon nanomaterials and its applications in pharmaceuticals: A brief review. *Chemosphere, 294*, 133731.

Sun, Y., Jiang, X., Liu, Y., Liu, D., Chen, C., Lu, C., & Liu, J. (2021). Recent advances in Cu (II)/Cu (I)-MOFs based nano-platforms for developing new nanomedicines. *Journal of Inorganic Biochemistry, 225*, 111599.

Szebeni, J., & Moghimi, S. M. (2009). Liposome triggering of innate immune responses: A perspective on benefits and adverse reactions. *Journal of Liposome Research, 19*, 85–90.

Tan, Z., Liu, W., Liu, H., Li, C., Zhang, Y., Meng, X., Tang, T., Xi, T., & Xing, Y. (2017). Oral helicobacter pylorivaccine-encapsulated acid-resistant HP55/PLGA nanoparticles promote immune protection. *European Journal of Pharmaceutics and Biopharmaceutics, 111*, 33–43.

Tan, C. W., Chia, W. N., Qin, X., Liu, P., Chen, M. I. C., Tiu, C., Hu, Z., Chen, V. C., Young, B. E., Sia, W. R., Tan, Y. J., Foo, R., Yi, Y., Lye, D. C., Anderson, D. E., & Wang, L. F. (2020).

A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. *Nature Biotechnology, 38*(9), 1073–1078.

Tao, W., Ziemer, K. S., & Gill, H. S. (2014). Gold nanoparticle–M2e conjugate coformulated with CpG induces protective immunity against influenza A virus. *Nanomedicine, 9*, 237–251.

Trabbic, K. R., Kleski, K. A., & Barchi, J. J., Jr. (2021). Stable gold-nanoparticle-based vaccine for the targeted delivery of tumor-associated glycopeptide antigens. *ACS Bio & Med Chem Au, 1*(1), 31–43.

Wagar, L. E., et al. (2021). Modeling human adaptive immune responses with tonsil organoids. *Nature Medicine, 27*, 125–135.

Wang, C., Zhu, W., Luo, Y., & Wang, B. Z. (2018). Gold nanoparticles conjugating recombinant influenzahemagglutinin trimers and flagellin enhanced mucosal cellular immunity. *Nanomedicine, 14*(4), 1349–1360.

Wang, Q., Liu, S., Liu, J., Sun, J., Zhang, Z., & Zhu, Q. (2022). Sustainable cellulose nanomaterials for environmental remediation-achieving clean air, water, and energy: A review. *Carbohydrate Polymers, 285*, 119251.

Wei, L., Chen, J., & Ding, J. (2021). Sequentially stimuli-responsive anticancer nanomedicine. *Nanomedicine, 16*(4), 261–264.

Wu, H., & Mu, W. (2022). Application prospects and opportunities of inorganic nanomaterials for enzyme immobilization in the food processing industry. *Current Opinion in Food Science*, 100909.

Yang, K., Yang, Z., Yu, G., Nie, Z., Wang, R., & Chen, X. (2022). Polyprodrug nanomedicines: An emerging paradigm for cancer therapy. *Advanced Materials, 34*(6), 2107434.

Yin, Y., Li, X., Ma, H., Zhang, J., Yu, D., Zhao, R., Yu, S., Nie, G., & Wang, H. (2022). In situ transforming RNA nanovaccines from polyethylenimine functionalized graphene oxide hydrogel for durable cancer immunotherapy. *Nano Letters, 21*(5), 2224–2231.

Zeng, W., Zhang, H., Yuan, X., Chen, T., Pei, Z., & Ji, X. (2022). Two-dimensional nanomaterial-based catalytic medicine: Theories, advanced catalyst and system design. *Advanced Drug Delivery Reviews*, 114241.

Zhao, K., Rong, G., Hao, Y., Yu, L., Kang, H., Wang, X., Wang, X., Jin, Z., Ren, Z., & Li, Z. (2016). IgA response and protection following nasal vaccination of chickens with Newcastle disease virus DNA vaccine nanoencapsulated with Ag@SiO2 hollow nanoparticles. *Scientific Reports, 6*, 25720.

Zheng, C., Li, M., & Ding, J. (2021). Challenges and opportunities of nanomedicines in clinical translation. *Bio Integration, 2*(2), 57–60.

Chapter 9
Flexibility in the Design of Nanomedicine Using Biomimetic Immunomodulatory

Archana S. Patil, Rajashree S. Masareddy, and Priyanka P. Patil

9.1 Overview of Immune System and Immunomodulators

From invertebrates to humans, the immune system (IS) is essential to the health of all living things and can either prevent or cause disease. The immune system is a sophisticated, interconnected system of cells, tissues, organs, and soluble mediators that protects the body from outside threats to its integrity. The majority of the IS's cells, such as neutrophils, macrophages, and monocytes, are phagocytic. Pathogens and foreign substances can be absorbed and digested by these cells.

The body's normal immune response to infections and cancers, as well as autoimmunity, are mediated by lymphocytes, the second-most numerous cells in the IS (Yatim & Lakkis, 2015). They can be divided into two groups known as T- and B-cells. Following haematopoiesis, common hematopoietic stem cells (HSCs) in the bone marrow give rise to all immune cells. Lymphocytes multiply and diversify exponentially as the immune response is activated. B cells develop into plasma cells, which are a type of antibody factory that release hundreds of antibodies into the bloodstream, while T cells differentiate into numerous subgroups with various specialties (Yatim & Lakkis, 2015).

Two traditional categories of the immune response are innate and adaptive immunity, which serve various and diverse roles in the immunological defence responses.

A. S. Patil (✉) · R. S. Masareddy
Department of Pharmaceutics, KLE College of Pharmacy Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, India

P. P. Patil
Department of Pharmacology and Toxicology, KLE College of Pharmacy Belagavi, KLE Academy of Higher Education and Research (KAHER), Belagavi, Karnataka, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_9

- A short-term memoryless innate immune system that offers a quick but insufficient defence against a foreign insult (Netea et al., 2011).
- Long-lived lymphocytes (memory cells) and their highly specialised receptors are part of an adaptive immune response, which is an antigen-specific system (Pancer & Cooper, 2006).
- Unbalanced immune responses can be the cause of a wide range of problems, including allergies, autoimmune diseases, immunosuppression, and AIDS, despite their high effectiveness and specificity (Yatim & Lakkis, 2015; Lerner et al., 2016).

Epidemiological data show that immunological disorders are becoming more prevalent nowadays, which has led to the development of a specific class of chemicals called immunomodulators that can either stimulate or decrease the immune response in diseases involving the immune system. While immunosuppressive medicines are used to reduce the immune response in many immunological-mediated disorders, immunostimulatory therapies may be useful for treating infections, immunodeficiency, and cancer (i.e., in organ transplantation and autoimmune diseases). The creation of new vaccines, therapies for autoimmune illnesses and allergies, regenerative medicine techniques, and immunotherapies for cancer are just a few biomedical applications where attempts to boost, decrease, or qualitatively change the immune response are crucial.

Since the beginning of time, people have been known to get ideas and inspiration from the natural world and its surroundings. This practise is known as "biomimetics," which is derived from the Greek words "bios" (life) and "mimesis" (to copy). It is the most sophisticated approach for applying biological principles—which underlie the structures, morphology, and performance characteristics of biological entities—to man-made designs or models in order to determine the most efficient way to tackle current issues through revolutionary urban design and innovative information technologies. Using structural and genetic methods, researchers are only now learning about the fine ways through which proteins, nucleic acids, metal ions, carbohydrates, and steroids interact with one another (Perera & Coppens, 2019). Researchers create materials with improved properties such as peptide-functionalised gold nanoparticles (NPs), protein-functionalised nanoparticles, and carbohydrate-functionalised nanoparticles by studying and simulating the complex biological structures and processes (Speck & Speck, 2019). This can offer solutions to basic issues in cell biology, biophysics, pharmacology, medicine, and more.

The three types of biomimetic systems are biological (Fig. 9.1), bio-hybrid, and synthetic. Natural biological molecules like proteins, DNA, and RNA, as well as synthetic biomolecules assembled or synthesised by biological systems, including synthetic amino acids created by genetic engineering, are the building blocks of biological structures. Materials that mix synthetic elements (such as metal particles, polymeric chains, and so on) with organic living molecules make up the biohybrid structures. Last but not least, synthetic elements are materials based on artificial building blocks, such as artificial amino acids and synthetic polymers (i.e., prepared in vitro such as solid-phase synthesised peptides). Where the underlying molecular

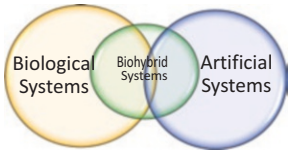


Fig. 9.1 Biomimetic system classification: The three types of biomimetic systems are biological, synthetic, and biohybrid. The interface between artificial and biological systems is where biohybrid systems live. Applications of biomimetic systems in drug delivery

principles are known, one can use and mimic biological processes and interactions to develop biomimetic systems.

Nanomedicine and nano delivery systems, which use materials in the nanoscale range, offer new technological advancements in the development of new and revolutionary pharmaceuticals as well as in the reformulation of currently available drugs to boost their efficacy, improve delivery, and reduce adverse effects. Nanomaterials' potential to more easily pass through biological barriers, persistence in the environment and the body, toxic qualities as well as their physicochemical properties that can cause changes to pharmacokinetics, including the absorption, distribution, elimination, and metabolism, are all causes for concern. Nanotechnology offers many benefits in the treatment of chronic human diseases by delivering precise drugs to designated areas and targets. It has also been shown to bridge the gap between biological and physical sciences by employing nanostructures and nanophases in a range of scientific domains (Liu et al., 2009). Nanoparticles have spurred the discipline of biomedicine, which includes medication delivery, nanobiotechnology, biosensors, and tissue engineering (Mirza & Siddiqui, 2014).

- To achieve their drug delivery goal of achieving the therapeutic concentration of a specific medication at the site of disorder while limiting off-target effects, nanoparticles (NPs) must complete the following crucial requirements.
- NPs need to circulate with enough time to get to the desired location (Yoo et al., 2010).
- NPs need to be able to only affect diseased tissue while insensitive to healthy tissues unaffected (Moghimi et al., 2001; Friedman et al., 2013).
- NPs must be synthesised from a biodegradable material that can be eliminated from the body safely (Naahidi et al., 2013).
- NPs engage with the complex biological environment of the human body.

Numerous immunotherapeutic approaches have produced outstanding results in the therapy of a variety of diseases (Gordon et al., 2014), however immune regulatory drugs' performances can be harmed by significant immune-mediated toxicity, poor solubility, and loss of bioactivity on prolonged circulation (Shen et al., 2018). It is encouraging to note that nanotechnology has the potential to address the issues at hand and so produce the anticipated therapeutic outcome. Studies have revealed that the nanoplatforms exhibit a variety of beneficial characteristics, including;

1. The simultaneous administration of antigens and adjuvants to intracellular spaces or antigen-presenting cells (APCs) (Tazaki et al., 2018).
2. Extended half-lives of molecules carrying bioactive payload by preventing their enzymatic oxidation during blood circulation (Kim et al., 2018).
3. Enhanced permeability and retention (EPR) effect, which is size-dependent, results in increased accumulation in tumour tissues (Xu et al., 2015; Li et al., 2016).
4. Surface modification to target particular cells or tissues (Ding et al., 2013; Chen et al., 2017).
5. Stimuli-sensitive behaviour for secure drug distribution and safe trafficking (Zhang et al., 2018a; Xu et al., 2017; Gao et al., 2019).
6. Higher tolerated doses of medication due to decreased buildup at tissues and organs that are off-target (Musetti & Huang, 2018).
7. Antigen and costimulatory surface interaction Antigen and costimulatory molecule surface coupling to create artificial APCs (aAPCs) with strong T-cell activation potential (Steenblock & Fahmy, 2008).
8. A variety of medication delivery methods, such as subcutaneous microneedle patch delivery or intranasal administration (Tazaki et al., 2018; Kim et al., 2012; Yang et al., 2017)
9. How artificial nanoparticles' inherent immunomodulatory properties work (Chahal et al., 2016; Li et al., 2018).

9.2 Nanoparticles for Immunostimulation

Immunotherapy is the idea that a disease can be treated by either stimulating or inhibiting the immune system. Engineering of immunostimulatory nanoparticles and immunosuppressive nanoparticles based on functional nanoplatforms, and their applications in the treatment of various diseases by regulating immune-related cells, cytokines, and enzymes.

9.3 Emergence of Biomimetic Nanoparticles

Specifically, nanomedicine has witnessed the evolution of multiple NP generations during the past few decades. Scientists have created significant progress in enhancing the medicinal effectiveness of these platforms with each iteration.

NPs in their early years as a generation (Fig. 9.2) were spent developing them with the sole goal of limiting interactions with the body's biological components while passively transporting NPs from site of injection to the illness location. This first generation of NPs was created primarily to test various chemical contents, non-fouling coatings, and sizes (Faraji & Wipf, 2009; Albanese et al., 2012). But it soon became clear that it was difficult to create NPs that are fully unaffected by the in vivo

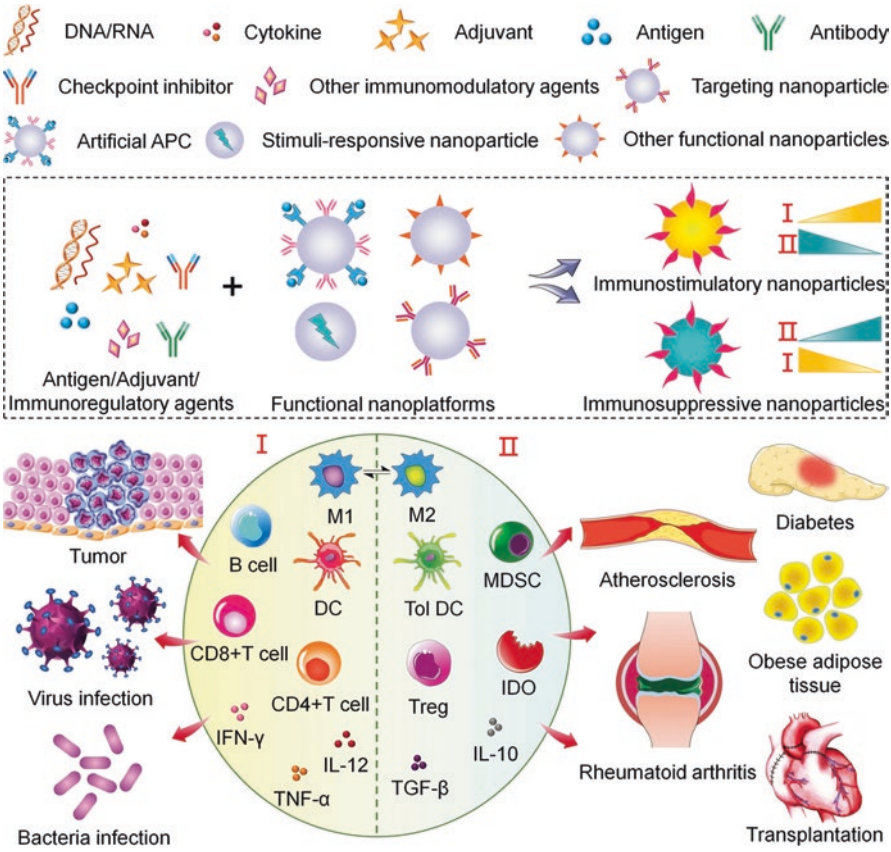


Fig. 9.2 Generations of nanoparticles. Early incarnations of the particles had non-fouling coatings to stop them from interacting with the cells they came into contact with in vivo and were biologically inactive. The subsequent generation of nanoparticles then evolved into active targeting molecules, allowing them to travel to the site of the disease and interact with the surrounding environment. The third generation of cell membrane-based biomimetic nanoparticles uses complete cell membrane or membrane protein functionalisation onto synthetic carriers to imitate the surface characteristics of real cells made with Biorender (Sushnitha et al., 2020)

environment. As a result, the second group of NPs began to concentrate on more specialised, bioactive carriers. These delivery systems were created specifically to allow drugs to access the specific ailment and lessen quasi biodistribution (Mout et al., 2012). Utilising binding ligands, such as peptides, antibodies, and small compounds, was a frequent technique (Friedman et al., 2013). This developing tendency in surface functionalisation was first seen in early attempts to direct active contact between a particle and the cells around it at the nano-bio interface. This second generation of NPs, in contrast to the first, consisted of particles with signals imprinted on their surfaces that allowed them to operate as a mediator in cell interactions. This approach has two variations that handle the two sides of the coin in this scenario of communicating with immune cells. NPs were functionalised with markers on one

side, reducing MPS uptake and clearance (Zhou & Dai, 2018). However, studies have also shown how the addition of affinity ligands makes it possible for NPs to target the area while activating the immune cells that are already present there (Chen et al., 2012; Schmid et al., 2017). Despite using these compounds has shown expected outcomes, by affixing them as solo molecules in their non-native state can prevent them from performing to their full potential. These molecules' arrangement and density on the surface of NP can alter as a result of the conjugation chemistries employed to bind them, changing or completely eliminating their function (Rambukwella et al., 2018).

Because the second generation of active targeted NPs has shortcomings, scientists turned to nature for ideas when creating NP formulations for particular uses. Here, we have witnessed the rise of the third phase NPs, known as biomimetic NPs, which imitate the characteristics of nature to improve their in vivo therapeutic benefits. In addition, a group of biomimetic NPs focused on mimicking the behaviour and function of real cells has arisen to improve these biomimetic NPs' ability to interact and connect with the biological environment. Biomaterials can also be created as instruments to influence the tissue, cell, and molecular interactions that affect immune cells in order to provide fresh insight on the operation of the immune system, just like in other fields of cell biology. This emerging field of immune engineering utilising biomaterials is producing innovative and potentially effective new approaches for vaccination, cancer immunotherapy, the therapy of autoimmune diseases, and the development of organ transplant tolerance. With this technology at their disposal, researchers have investigated the potential therapeutic uses for these biomimetic NPs.

Biomimetic nanoparticles based on cell membranes have become one method for achieving targeted medication administration by active association and interaction with the biological environment. "The surface features of NPs control their in vivo fate at the nano-bio interface, which serves as the primary interface for communication exchange. The interactions at the nano-bio interface, which is the area where the nanoparticle surface comes into direct contact with its surrounding biological environment, regulate the interaction between immune cells and NPs (Nel et al., 2009). This procedure is especially important while circulation because firstly an immune cell interacts with the NP surface. In the succeeding sequence of interactions at this nano-bio interface, direct and indirect signalling cues are used to regulate how the immune cell would respond to their presence in the bloodstream". As a result, the NP surface's physicochemical characteristics and composition considerably influence how the immune system perceives them and, consequently, can control their capacity to transcend the immune system's biological barriers (Wang & Wang, 2014; Liu & Tang, 2017).

9.4 Disease Applications of Biomimetic Nanoparticles

Immunostimulatory therapy should be utilised to immune system activation in the treatment of cancer and infectious diseases in order to identify non-self antigens, eradicate them, and build sustainable results for various disorders. Contrarily,

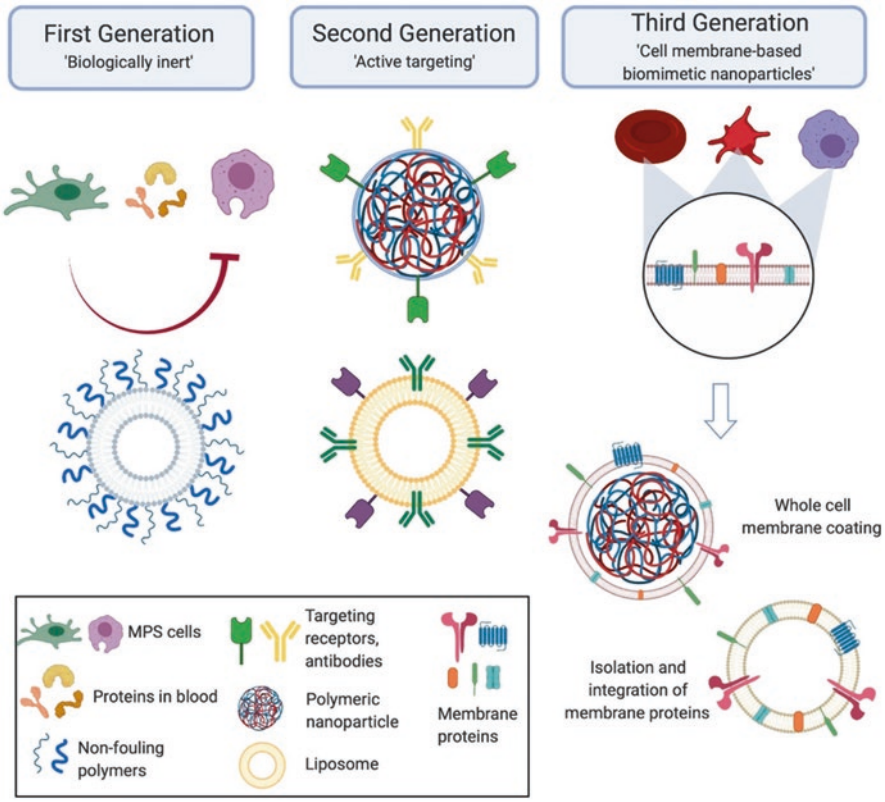


Fig. 9.3 Applications for treating numerous diseases by controlling cytokines, immune-related cells, and enzymes using immunosuppressive nanoparticles built on functional nanoplatforms (Feng et al., 2019)

immunosuppressive medication is required to reduce immune response and create specific immune tolerance for overactive immune response in disorders like rheumatoid arthritis (RA), atherosclerosis, diabetes, transplantation, and obesity (Fig. 9.3). Some immunotherapeutic approaches that have demonstrated impressive results in the treatment of different diseases are listed below;

9.4.1 Cancer

Biomimetic nanoparticles (NPs) based on cell membranes that target tumours have techniques that imitate numerous native cell types. The expression of "don't eat me" markers such as CD47 was used by RBC-based NPs, to accelerate circulation times and get to the target tumour without being affected by MPS. Similar techniques have been utilised to impart these properties to NPs using leukocyte membranes.

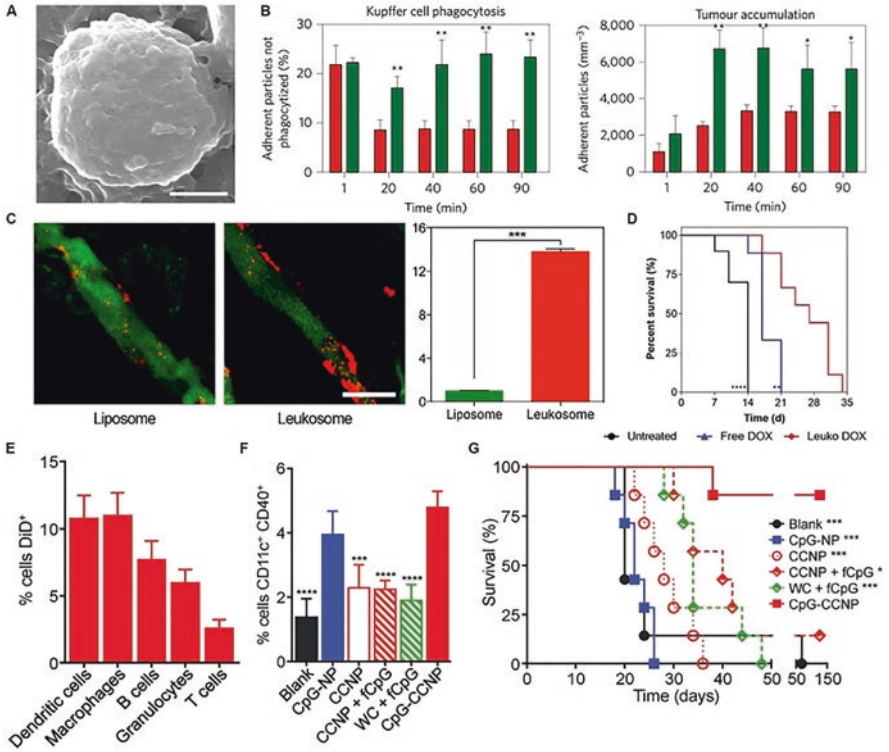


Fig. 9.4 Targeting tumours with bioinspired nanoparticles. Leukocyte mimicking nanoparticles exhibit decreased mononuclear phagocyte system uptake and enhanced tumour targeting in (**a**, **b**). Leukocyte membrane (LVV) is covering a porous silica nanoparticle in (**a**) SEM imagery. Scale bar: 1 mm (**b**) When compared to bare nanoparticles, LLV showed decreased absorption by Kupffer cells (left) and enhanced targeting to melanoma tumours (right). Leukocyte-based liposomes (Leukosomes), which have a stronger affinity for inflammatory tumour vasculature, are (**c**). Scale bar: 50 μm (**d**) Extended lifespan of tumour-bearing mice following administration of doxorubicin-loaded leukosomes. CpG-encapsulated nanoparticles with melanoma cell coating (**e**–**g**) for immunotherapy (**e**) Immune cells' in vitro uptake of CpG-CCNPs (**f**) In vivo maturation of dendritic cells after exposure to NPs and other controls (**g**) Mice inoculated with CpG-CCNPs and other control formulations both survived overall. (With permission, the images in (a, b) have been copied from (Parodi et al., 2013). Images in (c) have been copied from with permission (Martinez et al., 2018). With permission, the photo in (d) has been copied from (Molinaro et al., 2020). With permission, the images in (e–g) have been copied from (Kroll et al., 2017))

The circulating monocytes do not interact with these biomimetic NPs and identify them for removal by the MPS because they resemble native immune cells in appearance (Parodi et al., 2013; Corbo et al., 2017a) (Fig. 9.4a, b). Due to this, these NPs have a higher chance of reaching the tumour since they imitate the circulatory behaviour of these cells. Along with using these natural coatings for NPs to target the tumour more effectively while in circulation, researchers have also taken

advantage of these membrane-based NPs. For instance, when compared to naked liposomes, integrating liposomes with the leukocyte membrane proteins demonstrated a 14-times enhancement in attraction to inflammatory vasculature associated with triple-negative breast cancer tumours (Martinez et al., 2018) (Fig. 9.4c).

It has been found that the superior targeting is caused by "the presence of leukocyte proteins like LFA-1 and Mac-1. These essential signals give these NPs the potential to act like natural leukocytes that target regions of inflammation, and blocking these proteins on the NPs greatly decreased their ability to selectively accumulate within the tumour (Martinez et al., 2018). This leukocyte-based NP was also demonstrated to enhance doxorubicin administration in two cancer models, melanoma and breast cancer, leading to an improvement (64% and 142%, respectively) in median survival over untreated mice (Molinaro et al., 2020) (Fig. 9.4d). As a result, these NPs imitated the targeting capabilities of leukocytes to target the tumour and deliver the enclosed payload". Others have used activated platelet membranes coated silica NPs to target the circulating tumour cells (CTCs) responsible for the development of metastatic disease by utilising the connections between tumour cells and platelets (Li et al., 2016).

Another study discovered that primary breast cancer tumours accumulated more platelet-coated nanovesicles that were functionalised with TRAIL ('tumour necrosis factor-related apoptosis-inducing ligand') and loaded with the chemotherapy drug doxorubicin (Hu et al., 2015a). The effectiveness of employing cancer cell membrane-coated NPs was demonstrated, for instance, polymeric NPs coated with 4 T1 breast cancer cell membrane, which showed increased homotypic tumour targeting and longer circulation times (Sun et al., 2016). In this case, as the protein profiles of the NPs and cancer cells were similar, the cancer cell was able to recognise and internalise the NP. These studies demonstrate the mechanism of biomimetic NPs perform earlier generations by making use of natural cellular surface characteristics to evade clearance by immune cells that prevent tumour formation and interact directly with the cancer cells.

Another study has shown that the PD-1/PD-L1 immune inhibitory axis, which is the target of immune checkpoint drugs that have received clinical approval, can be disrupted using cancer nanovesicles (Zhang et al., 2018b). Studies also looked into the possibility of using biomimetic NPs as cancer vaccines, whereby the administration of the NP guards against the growth of a tumour when exposed to tumour cells. The same was demonstrated in a study, where pro-inflammatory cytokines were secreted in vitro by immune cells employing PLGA NPs coated with cancer cell membranes as an antigen-presenting material and an immunological adjuvant (Kroll et al., 2017). The study showed that these particles were picked up by a variety of immune cells and were also able to increase Dendritic cell (DC) maturation and overall lifespan of mice by 60% during the course of 5 months using a mouse melanoma model (Fig. 9.4e–g). Additionally, this biomimetic NP platform proved to be an excellent cancer vaccination and treatment option for existing tumours.

9.4.2 Cardiovascular Disease

Cell membrane-based biomimetic NPs can be developed to treat and target several aspects of the pathophysiology of cardiovascular diseases. Researchers have developed novel technologies that imitate the behaviour of innate cells while controlling the inflammatory response common to all of these by drawing inspiration from how native cells behave in this illness scenario.

Myocardial infarction, high blood pressure, and stroke are just a few of the ailments that fall within the broad category of cardiovascular diseases. These conditions are all connected to the heart and blood vessels' regular activities (Stewart et al., 2017). High levels of inflammation have been a defining feature of the pathophysiology of cardiovascular disease from its origin (Golia et al., 2014). However, an accumulation of atherosclerotic plaque is the root cause of many of these disorders (Bobryshev et al., 2016). In a healthy state, lipid and macrophage buildup is resisted by artery walls. However, factors that cause atherosclerosis, such as obesity, hypertension, and high saturated fat diet, start the development of adhesion molecules, which then allow lipids to enter the arterial wall and draw leukocytes to the damaged area; for these applications, biomimetic NPs based on cell membrane have been employed primarily to imitate different cell membranes, such as those of leukocytes platelets and protein complexes crucial for cardiovascular health, such as high-density lipoprotein (HDL) (Park et al., 2020). The body uses HDL, a native lipid carrier "NP, to move lipids with a natural affinity towards atherosclerotic plaque (Feig et al., 2014). Such interactions aid in the movement of macrophagial cholesterol that have accumulated plaque to the liver for processing. This molecule serves as a model complex whose functions NPs can imitate in order to enhance the pathophysiology related to plaques".

Additionally, researchers used artificial HDL-mimicking NPs to stop the growth of atherosclerotic plaque macrophages in an in vivo study with advanced atherosclerotic plaques (Tang et al., 2018). This consequently resulted in a 45% reduction in macrophage proliferation in the aortic roots throughout the course of the 8-week treatment period, a reduction in the inflammatory gene expression, and a reduction in atherosclerosis (Fig. 9.5a, b). Similar to normal HDL, these NPs changed the flow of cholesterol to the liver and prevented the growth of macrophages that feed atherosclerotic plaque.

Additionally, platelets have been strongly associated with the onset of cardiovascular disease and have shown a preference for adhering to blood vessel injury (Kinlough-Rathbone et al., 1983). Using this behaviour as the basis for targeting, NPs coated with platelet-membrane have been created using a freeze and thaw procedure, and after which the extracted membranes were adhered to PLGA cores (Hu et al., 2015a). In a model of rat coronary stenosis, these biomimetic NPs showed enhanced binding to damaged arteries in addition to inhibiting the growth of neointima (i.e., the creation of scar tissue).

NPs that imitate leukocytes and RBCs have also been created to transport therapeutic compounds to help treat cardiovascular disorders. For instance, in a model of

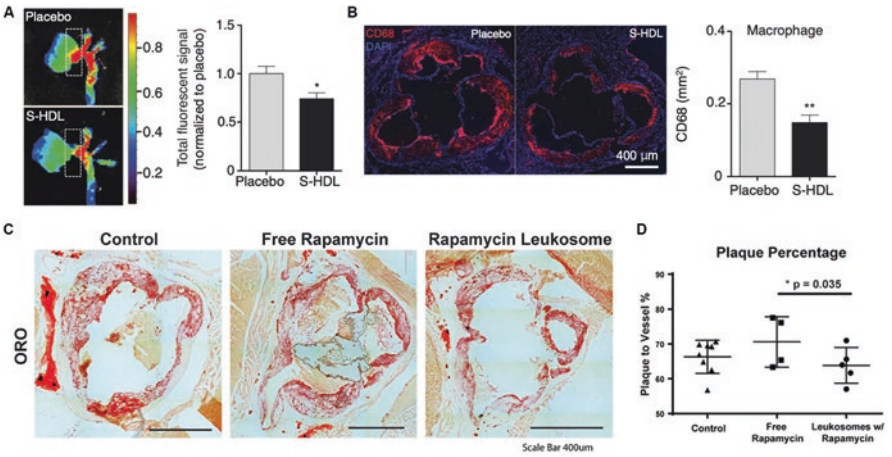


Fig. 9.5 Cardiovascular disease treatments using biomimetic nanoparticles. (**a**, **b**) Mice with atherosclerotic arteries have less plaque buildup and less macrophage infiltration (red) in the aortic roots when HDL-mimicking nanoparticles are used. (**c**, **d**) Leukocyte-based nanoparticles infused with rapamycin to cure atherosclerosis. (**c**) Mice with atherosclerosis treated with or without nanoparticles had lipid deposition stained with oil red O in their aortas. (**d**) Measurement of the plaque area in the image with the vessels. (With permission, the images in (a, b) have been copied from (Tang et al., 2015). Images in (c, d) have been copied from with the permission of (Boada et al., 2020))

cerebral artery occlusion, the circulation of a neuroprotective medication was prolonged by dextran polymer NPs coated with RBC membrane while ischaemic brain damage was reduced (Lv et al., 2018). In a mouse model, NPs based on leukocyte which were loaded with Rapamycin similarly showed enhanced accumulation in atherosclerotic plaques, lowering macrophage proliferation and reducing local inflammation (Boada et al., 2020). Additionally, the vessels' plaque load was decreased as a result of Rapamycin release from these particles (Fig. 9.5c, d). In this instance, the leukocyte proteins were included into the NP to increase targeting of the inflamed location as well as to cause effects that were anti-inflammatory and reduced the localised inflammation at the disease site (Boada et al., 2020).

9.4.3 Infectious Disease

An innovative family of medications that treat infections with three main strategies—targeting the infection's source, neutralising the pathogens' mechanisms for inactivating natural immune defences, and modulating the immune cells which are responsible for anti-pathogen response has been made possible by biomimetic NPs based on cell membrane. To reach this level of targeted precision, NPs that resemble epithelial cells, platelets, and also bacteria themselves have been utilised. For instance, it has been demonstrated that bacteria can invade platelets and cause

platelet aggregation (Fitzgerald et al., 2006). However, platelets are essential to the host's defence mechanism; excessive activation can result in the formation of difficult-to-treat thrombi, which can serve as a haven for germs that are immune to the host's defences. Utilising this characteristic of bacteria, investigators formulated platelet-coated nanoparticles to efficiently transport antibiotics (Hu et al., 2015b). Mice treated with these biomimetic NPs and systemically exposed to a methicillin-resistant strain of bacteria showed significant antibacterial efficacy. In other methods, antibiotics have been delivered through stomach epithelial cell membrane nanoparticles (Angsantikul et al., 2018). This strategy is particularly new because the NPs delivered the surface antigens that the bacteria would ordinarily recognise on the host's cells. The bacteria unintentionally ingest these NPs containing fatal antibiotics due to the identification of these particular proteins on the NP surface. Another strategy is to employ biomimetic NPs to bind the target locations to stop bacteria from adhering to the host's cells in a competitive manner (Zhang et al., 2019).This approach was demonstrated to be successful in a study, H. pylori bacteria were wrapped in polymeric nanoparticles (NPs) to prevent the bacteria from sticking to the stomach lining (Fig. 9.6a, b). Binding sites which were typically used by the pathogen to colonise and cause infection were occupied by bacteria that mimicked NPs. In vitro binding of H. pylori to intestinal cells was reduced by these biomimetic NPs by a factor of six, while in vivo, bacterial colonisation of murine stomach tissue was diminished by over 50%. These illustrations show how the biomimetic NPs' surface characteristics deftly mediate contact with the target pathogen or block the pathogen's interacting with the host cells, which eventually results in the bacterium's death.

Cell membrane-based biomimetic NPs have been investigated as toxin-neutralising platforms to shield immune cells from apoptosis and provide them the capacity to neutralise the pathogen (Fang et al., 2015). This strategy has been demonstrated largely employing RBC-coated NPs due to their long durations of circulation and their capacity to interact with the pathogens in the circulation. Multiple pore-forming toxins, including melittin, a-hemolysin, and streptolysin-O were demonstrated to be sequestered by polymeric NPs that were coated around the membranes of RBCs, protecting hemolysis cells (Hu et al., 2013). Additionally, these NPs, also known as "nanosponges," did not transport these poisons to host cells, illustrating the platform is generally safe.

It has also been demonstrated that biomimetic NPs based on cell membranes can alter the immune response required to treat an infection (Angsantikul et al., 2015). This was demonstrated in sepsis models, which occur when the infection spreads beyond the local tissue and results in systemic organ dysfunction, in particular (Delano & Ward, 2016).

By embedding macrophage membrane proteins into a liposome formulation, Molinaro et al. were able to minimise the impact of proinflammatory genes like IL-1ß and TNF-a while enhancing the expression of anti-inflammatory genes like TGF-ß and IL-10 (Molinaro et al., 2019) (Fig. 9.6e, f). Despite the fact that it ought to be emphasised that earlier research has demonstrated the ability of pure, artificial NPs in the therapy of sepsis, this research has only focused on physicochemical properties as a mechanism of interacting with the surroundings (Casey et al., 2019).

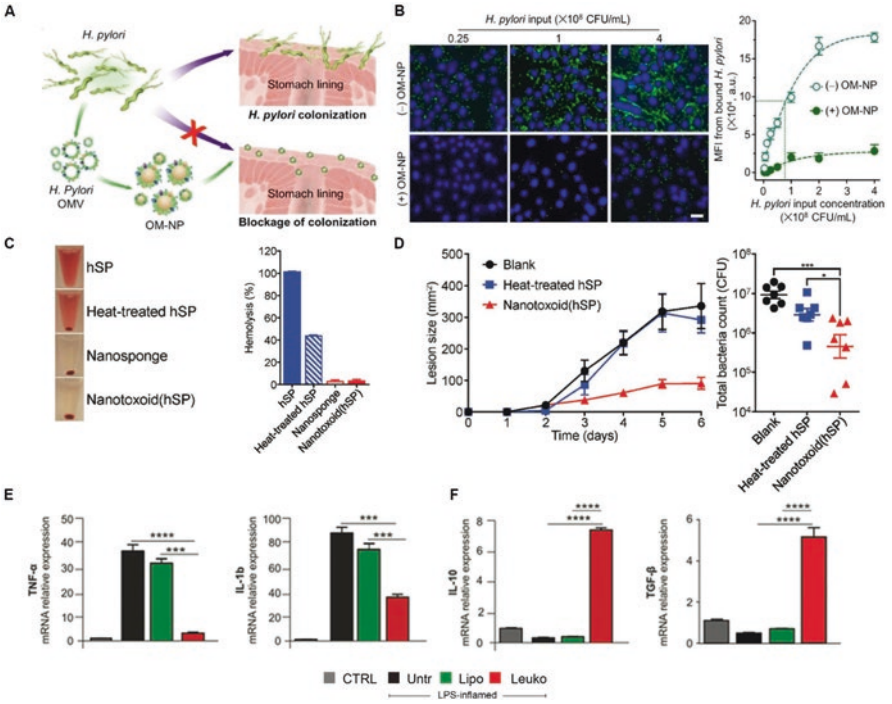


Fig. 9.6 To destroy, neutralise, and control the immune response to pathogens in infectious diseases, biomimetic nanoparticles are used. Nanoparticles with bacterial membrane coatings hinder binding to host cells (**a**, **b**). (**a**) Diagram showing the use of bacterial nanoparticles (NPs) to stop *H. pylori* from colonising stomach tissue. (**b**) Confocal pictures of *H. pylori* (green) adhering to gastric epithelial cells (blue) with or without treatment with NPs, and quantification of those images (right). Scale bar: 25 m (**c**, **d**) RBC-coated nanosponges (Nanotoxoid hSP) as bacterial infection toxoid vaccinations Following NP treatment, sample pictures (left) and haemolysis quantification are shown in (**c**). (**d**) Mice vaccinated with NPs and controls showed differences in lesion size (on the left) and overall bacterial count (on the right). (**e**, **f**) Using liposomes that imitate leukocytes to treat sepsis resulted in decreased expression of pro-inflammatory genes (**e**) and increased expression of anti-inflammatory genes (**f**). (With permission, the images in (a, b) have been copied from (Zhang et al., 2019) Images in (c, d) have been copied from with the permission of (Wei et al., 2017). With permission, the images in (e, f) have been copied from (Molinaro et al., 2019))

9.4.4 Autoimmune Disease

Numerous illnesses fall under the umbrella of autoimmune disorders, including rheumatoid arthritis, systemic lupus, and type 1 diabetes (Theofilopoulos et al., 2017). These illnesses are characterised by autoimmunity, a condition in which the immune system starts to begin to fight the body's own cells in a variety of ways, such as by producing antibodies against them (Wang et al., 2015). These illnesses are also characterised by a persistent inflammatory state in which the immune system keeps trying to fix the harm that has been done. However, these illnesses are

now thought to be incurable. “Research using biomimetic cell membrane-based NPs has demonstrated the growing potential these technologies offer to intervene and mediate the behaviour of the immune system in several illness states. Biomimetic NPs have been demonstrated to mimic native cells, which are capable of resolving inflammation and healing tissue damage and to act as binding decoys for systems that cause the chronic inflammatory state”. Engineered leukocyte membranes imitating NPs were developed to bind to inflamed mucosal tissue by overexpressing a4b7, a key integrin protein on T-lymphocytes in order to exploit the processes of T-cell activation in the course of IBD pathogenesis (Berlin et al., 1993). These “specialised leukosomes” showed more firm adherence to inflamed endothelia as a result of this overexpression. Additionally, these biomimetic NPs enhanced the crypt shape, decreased CD45+ immune cells, and inhibited edoema in DSS-induced IBD mice (Corbo et al., 2017b). It was hypothesised that the therapeutic effects seen after therapy with these customised NPs resulted from NPs binding to receptors that would normally be bound by the immune cells causing this disorder. With RBC-mimicking NPs, this method was also used to illustrate how to remove pathogenic antibodies. These RBC-based biomimetic NPs were very effective at acting as binding stooges for antibodies that would otherwise bind to native RBCs and mark them for extravascular haemolysis (Copp et al., 2014). In a model of induced anaemia, RBC numbers and haemoglobin levels returned to normal in mice given these RBC-NPs. The RBC count was reduced by 60% in mice that did not get the NPs, and the levels of haemoglobin were reduced by twofold. Finally, it has been demonstrated that neutrophil-mimicking NPs have important effects on rheumatoid arthritis treatment. In actuality, these NPs reduced joint degeneration and suppressed proinflammatory cytokines in two mouse models of arthritis. These results highlight the adaptability of biomimetic NPs in focusing on and fine-tuning the underlying pathways that underlie and fuel a variety of autoimmune disorders.

9.4.5 Vaccination

A vaccine consists of an antigen, which serves as the immune system’s target, and an adjuvant, which is injected along with the antigen to boost the immune system’s reaction. Nanoparticles (NPs) have recently drawn a lot of attention as vaccine delivery systems. In addition to improved immunogenicity and antigen stability, nanovaccine formulations also offer targeted distribution and protracted release. Additionally, NPs aid in preventing the antigen and adjuvant from being prematurely degraded by enzymatic and proteolytic processes (Bishop et al., 2015). Although NPs have the benefits listed above, they also have drawbacks, including an unfavourable interaction with the reticuloendothelial system and a lack of colloidal stability under physiological conditions caused by protein corona forms (RES) (Corbo et al., 2016, 2017c).

A unique type of nanoparticles known as biomimetic NPs effectively avoids unfavourable interactions with immune cells like RES and prolongs blood circulation

Table 9.1 Some of the reported biomimetic nanovaccines and their applications

Nanoparticles	Components	Application	References
Liposomes	PLGA NPs with lipid antigens	Malarial vaccine delivery	Moon et al. (2012)
	Liposome-polycation-DNA NPs	DNA vaccine delivery	Li et al. (1998)
VLPs	Genetically modified VLP	Anti-viral protection	Wu et al. (2012)
	Avian retrovirus with Gag fusion proteins	Intracellular protein delivery	Kaczmarczyk et al. (2011)
Self-assembling proteins	Hollow vault protein	Self-assembling protein with Flagellin scaffold	Champion et al. (2009)

(Angsantikul et al., 2015; Gao et al., 2013; Rao, 2013). When given to the body, these nano vaccines' carrier NPs, which resemble biological membranes, allow for longer circulation and the evasion of immunological reactions (Vijayan et al., 2018). A different kind of biomimetic carrier system with a "core-shell" shape is a cell-membrane-coated NP. A thin layer of plasma membrane serves as the shell, with the NP acting as the hydrophobic core (Hu et al., 2012) (Table 9.1).

An extruded polymeric NP was covered with red blood cell (RBC) membranes to create the first membrane-coated nanoparticles, according to Hu et al (Hu et al., 2012). The creation of membrane-coated NPs has made use of a variety of membranes from various sources, including RBCs (Hu et al., 2012; Gao et al., 2017) leukocytes (Wei et al., 2019; He et al., 2016, 2018), cytotoxic T-cells (Wei et al., 2018). Self-assembling proteins can also be utilised to make biomimetic nanovaccines since they have excellent symmetry and stability and can be structurally organised into particles with diameters between 10 and 150 nm (Kang et al., 2009). Due to their capacity to self-assemble and deploy into a specific shape that replicates the architecture of real microbes, these self-assembling protein NPs serve a variety of physiological functions and are chosen as vaccine carriers (Castón & Carrascosa, 2013).

9.5 Conclusion and Future Prospects

The area of biomimetic nanoparticle engineering has made enormous strides in the previous 10 years and is currently under rapid development. The bioinspired nanoparticles have a variety of functions, involving increased accumulation at infected locations, extended circulation, and less off-target effects in healthy tissues. They do this by utilising the many transport and translocation strategies that viruses and mammalian cells have devised. Therefore, thorough anti-infective investigations are required to verify the efficacy and long-term safety concerns of emerging bioengineered nanotherapies. The cellular and molecular events that dominate the in vivo pharmacokinetic and biopharmaceutical profiles of the biomimetic

nanotherapies now being produced should also be the subject of mechanistic studies. In light of recent developments in the life sciences and biological as well as the modernisation of nanotechnology, it is essential to increase the variety and utility of biomimetic nanoplatforms. Other state-of-the-art technologies, such as materials genome, artificial intelligence, and computational design can be incorporated to find more effective and beneficial nanoparticles based on bioengineering methodologies.

Bibliography

Albanese, A., Tang, P. S., & Chan, W. C. W. (2012). *Annual Review of Biomedical Engineering, 14*, 1–16.

Angsantikul, P., Thamphiwatana, S., Gao, W., & Zhang, L. (2015). *Vaccine, 3*, 814–828.

Angsantikul, P., Thamphiwatana, S., Zhang, Q., Spiekermann, K., Zhuang, J., Fang, R. H., Gao, W., Obonyo, M., & Zhang, L. (2018). *Advances in Therapy, 1*, 1800016.

Berlin, C., Berg, E. L., Briskin, M. J., Andrew, D. P., Kilshaw, P. J., Holzmann, B., Weissman, I. L., Hamann, A., & Butcher, E. C. (1993). *Cell, 74*, 185–195.

Bishop, C. J., Kozielski, K. L., & Green, J. J. (2015). *Journal of Controlled Release, 219*, 488–499.

Boada, C., Zinger, A., Tsao, C., Zhao, P., Martinez, J. O., Hartman, K., Naoi, T., Sukhovershin, R., Sushnitha, M., Molinaro, R., Trachtenberg, B., Cooke, J. P., & Tasciotti, E. (2020). *Circulation Research, 126*, 25–37.

Bobryshev, Y. V., Ivanova, E. A., Chistiakov, D. A., Nikiforov, N. G., & Orekhov, A. N. (2016). *BioMed Research International, 2016*, 1–13.

Casey, L. M., Kakade, S., Decker, J. T., Rose, J. A., Deans, K., Shea, L. D., & Pearson, R. M. (2019). *Biomaterials, 218*, 119333.

Castón, J. R., & Carrascosa, J. L. (2013). In M. G. Mateu (Ed.), *Structure and physics of viruses* (Vol. 68, pp. 53–75). Springer Netherlands.

Chahal, J. S., Khan, O. F., Cooper, C. L., McPartlan, J. S., Tsosie, J. K., Tilley, L. D., Sidik, S. M., Lourido, S., Langer, R., Bavari, S., Ploegh, H. L., & Anderson, D. G. (2016). *Proceedings of the National Academy of Sciences, 113*(29), E4133–E4142. https://doi.org/10.1073/pnas.1600299113

Champion, C. I., Kickhoefer, V. A., Liu, G., Moniz, R. J., Freed, A. S., Bergmann, L. L., Vaccari, D., Raval-Fernandes, S., Chan, A. M., Rome, L. H., & Kelly, K. A. (2009). *PLoS One, 4*, e5409.

Chen, W. C., Kawasaki, N., Nycholat, C. M., Han, S., Pilotte, J., Crocker, P. R., & Paulson, J. C. (2012). *PLoS One, 7*, e39039.

Chen, T., Shen, H.-M., Deng, Z.-Y., Yang, Z.-Z., Zhao, R.-L., Wang, L., Feng, Z.-P., Liu, C., Li, W.-H., & Liu, Z.-J. (2017). *Molecular Medicine Reports, 15*, 2057–2066.

Copp, J. A., Fang, R. H., Luk, B. T., Hu, C.-M. J., Gao, W., Zhang, K., & Zhang, L. (2014). *Proceedings of the National Academy of Sciences, 111*, 13481–13486.

Corbo, C., Molinaro, R., Parodi, A., Toledano Furman, N. E., Salvatore, F., & Tasciotti, E. (2016). *Nanomedicine, 11*, 81–100.

Corbo, C., Molinaro, R., Taraballi, F., Toledano Furman, N. E., Hartman, K. A., Sherman, M. B., De Rosa, E., Kirui, D. K., Salvatore, F., & Tasciotti, E. (2017a). *ACS Nano, 11*, 3262–3273.

Corbo, C., Cromer, W. E., Molinaro, R., Toledano Furman, N. E., Hartman, K. A., De Rosa, E., Boada, C., Wang, X., Zawieja, D. C., Agostini, M., Salvatore, F., Abraham, B. P., & Tasciotti, E. (2017b). *Nanoscale, 9*, 14581–14591.

Corbo, C., Molinaro, R., Tabatabaei, M., Farokhzad, O. C., & Mahmoudi, M. (2017c). *Biomaterials Science, 5*, 378–387.

Delano, M. J., & Ward, P. A. (2016). *Immunological Reviews, 274*, 330–353.

Ding, J., Xiao, C., Li, Y., Cheng, Y., Wang, N., He, C., Zhuang, X., Zhu, X., & Chen, X. (2013). *Journal of Controlled Release, 169*, 193–203.
Fang, R. H., Luk, B. T., Hu, C.-M. J., & Zhang, L. (2015). *Advanced Drug Delivery Reviews, 90*, 69–80.
Faraji, A. H., & Wipf, P. (2009). *Bioorganic & Medicinal Chemistry, 17*, 2950–2962.
Feig, J. E., Hewing, B., Smith, J. D., Hazen, S. L., & Fisher, E. A. (2014). *Circulation Research, 114*, 205–213.
Feng, X., Xu, W., Li, Z., Song, W., Ding, J., & Chen, X. (2019). *Advancement of Science, 6*, 1900101.
Fitzgerald, J. R., Foster, T. J., & Cox, D. (2006). *Nature Reviews. Microbiology, 4*, 445–457.
Friedman, A., Claypool, S., & Liu, R. (2013). *Current Pharmaceutical Design, 19*, 6315–6329.
Gao, W., Hu, C.-M. J., Fang, R. H., Luk, B. T., Su, J., & Zhang, L. (2013). *Advanced Materials, 25*, 3549–3553.
Gao, M., Liang, C., Song, X., Chen, Q., Jin, Q., Wang, C., & Liu, Z. (2017). *Advanced Materials, 29*, 1701429.
Gao, S., Tang, G., Hua, D., Xiong, R., Han, J., Jiang, S., Zhang, Q., & Huang, C. (2019). *Journal of Materials Chemistry B, 7*, 709–729.
Golia, E., Limongelli, G., Natale, F., Fimiani, F., Maddaloni, V., Pariggiano, I., Bianchi, R., Crisci, M., D'Acierno, L., Giordano, R., Di Palma, G., Conte, M., Golino, P., Russo, M. G., Calabrò, R., & Calabrò, P. (2014). *Current Atherosclerosis Reports, 16*, 435.
Gordon, J. R., Ma, Y., Churchman, L., Gordon, S. A., & Dawicki, W. (2014). *Frontiers in Immunology, 5*. https://doi.org/10.3389/fimmu.2014.00007
He, W., Frueh, J., Wu, Z., & He, Q. (2016). *ACS Applied Materials & Interfaces, 8*, 4407–4415.
He, H., Guo, C., Wang, J., Korzun, W. J., Wang, X.-Y., Ghosh, S., & Yang, H. (2018). *Nano Letters, 18*, 6164–6174.
Hu, C.-M. J., Fang, R. H., & Zhang, L. (2012). *Advanced Healthcare Materials, 1*, 537–547.
Hu, C.-M. J., Fang, R. H., Copp, J., Luk, B. T., & Zhang, L. (2013). *Nature Nanotechnology, 8*, 336–340.
Hu, Q., Sun, W., Qian, C., Wang, C., Bomba, H. N., & Gu, Z. (2015a). *Advanced Materials, 27*, 7043–7050.
Hu, C.-M. J., Fang, R. H., Wang, K.-C., Luk, B. T., Thamphiwatana, S., Dehaini, D., Nguyen, P., Angsantikul, P., Wen, C. H., Kroll, A. V., Carpenter, C., Ramesh, M., Qu, V., Patel, S. H., Zhu, J., Shi, W., Hofman, F. M., Chen, T. C., Gao, W., Zhang, K., Chien, S., & Zhang, L. (2015b). *Nature, 526*, 118–121.
Kaczmarczyk, S. J., Sitaraman, K., Young, H. A., Hughes, S. H., & Chatterjee, D. K. (2011). *Proceedings of the National Academy of Sciences, 108*, 16998–17003.
Kang, S. M., Pushko, P., Bright, R. A., Smith, G., & Compans, R. W. (2009). In R. W. Compans & W. A. Orenstein (Eds.), *Vaccines for pandemic influenza* (Vol. 333, pp. 269–289). Springer Berlin Heidelberg.
Kim, Y.-C., Park, J.-H., & Prausnitz, M. R. (2012). *Advanced Drug Delivery Reviews, 64*, 1547–1568.
Kim, H., Niu, L., Larson, P., Kucaba, T. A., Murphy, K. A., James, B. R., Ferguson, D. M., Griffith, T. S., & Panyam, J. (2018). *Biomaterials, 164*, 38–53.
Kinlough-Rathbone, R. L., Packham, M. A., & Mustard, J. F. (1983). *Arterioscler. Official Journal of the American Heart Association Inc, 3*, 529–546.
Kroll, A. V., Fang, R. H., Jiang, Y., Zhou, J., Wei, X., Yu, C. L., Gao, J., Luk, B. T., Dehaini, D., Gao, W., & Zhang, L. (2017). *Advanced Materials, 29*, 1703969.
Lerner, A., Jeremias, P., & Matthias, T. (2016). *International Journal of Celiac Disease, 3*, 151–155.
Li, S., Rizzo, M., Bhattacharya, S., & Huang, L. (1998). *Gene Therapy, 5*, 930–937.
Li, J., Ai, Y., Wang, L., Bu, P., Sharkey, C. C., Wu, Q., Wun, B., Roy, S., Shen, X., & King, M. R. (2016). *Biomaterials, 76*, 52–65.

Li, S., Feng, X., Wang, J., He, L., Wang, C., Ding, J., & Chen, X. (2018). *Nano Research, 11*, 5769–5786.
Liu, X.-Q., & Tang, R.-Z. (2017). *Drug Delivery, 24*, 1–15.
Liu, Z., Tabakman, S., Welsher, K., & Dai, H. (2009). *Nano Research, 2*, 85–120.
Lv, W., Xu, J., Wang, X., Li, X., Xu, Q., & Xin, H. (2018). *ACS Nano, 12*, 5417–5426.
Martinez, J. O., Molinaro, R., Hartman, K. A., Boada, C., Sukhovershin, R., De Rosa, E., Kuri, D., Zhang, S., Evangelopoulos, M., Carter, A. M., Bibb, J. A., Cooke, J. P., & Tasciotti, E. (2018). *Theranostics, 8*, 1131–1145.
Mirza, A. Z., & Siddiqui, F. A. (2014). *International Nano Letters, 4*, 94.
Moghimi, S. M., Hunter, A. C., & Murray, J. C. (2001). *Pharmacological Reviews, 53*, 283–318.
Molinaro, R., Pastò, A., Corbo, C., Taraballi, F., Giordano, F., Martinez, J. O., Zhao, P., Wang, X., Zinger, A., Boada, C., Hartman, K. A., & Tasciotti, E. (2019). *Nanoscale, 11*, 13576–13586.
Molinaro, R., Martinez, J. O., Zinger, A., De Vita, A., Storci, G., Arrighetti, N., De Rosa, E., Hartman, K. A., Basu, N., Taghipour, N., Corbo, C., & Tasciotti, E. (2020). *Biomaterials Science, 8*, 333–341.
Moon, J. J., Suh, H., Polhemus, M. E., Ockenhouse, C. F., Yadava, A., & Irvine, D. J. (2012). *PLoS One, 7*, e31472.
Mout, R., Moyano, D. F., Rana, S., & Rotello, V. M. (2012). *Chemical Society Reviews, 41*, 2539.
Musetti, S., & Huang, L. (2018). *ACS Nano, 12*, 11740–11755.
Naahidi, S., Jafari, M., Edalat, F., Raymond, K., Khademhosseini, A., & Chen, P. (2013). *Journal of Controlled Release, 166*, 182–194.
Nel, A. E., Mädler, L., Velegol, D., Xia, T., Hoek, E. M. V., Somasundaran, P., Klaessig, F., Castranova, V., & Thompson, M. (2009). *Nature Materials, 8*, 543–557.
Netea, M. G., Quintin, J., & van der Meer, J. W. M. (2011). *Cell Host & Microbe, 9*, 355–361.
Pancer, Z., & Cooper, M. D. (2006). *Annual Review of Immunology, 24*, 497–518.
Park, J. H., Dehaini, D., Zhou, J., Holay, M., Fang, R. H., & Zhang, L. (2020). *Nanoscale Horizons, 5*, 25–42.
Parodi, A., Quattrocchi, N., van de Ven, A. L., Chiappini, C., Evangelopoulos, M., Martinez, J. O., Brown, B. S., Khaled, S. Z., Yazdi, I. K., Enzo, M. V., Isenhart, L., Ferrari, M., & Tasciotti, E. (2013). *Nature Nanotechnology, 8*, 61–68.
Perera, A. S., & Coppens, M.-O. (2019). *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 377*, 20180268.
Rambukwella, M., Sakthivel, N. A., Delcamp, J. H., Sementa, L., Fortunelli, A., & Dass, A. (2018). *Frontiers in Chemistry, 6*, 330.
Rao, M. (2013). *Tissue Engineering Regenerative Medicine, 10*, 223–229.
Schmid, D., Park, C. G., Hartl, C. A., Subedi, N., Cartwright, A. N., Puerto, R. B., Zheng, Y., Maiarana, J., Freeman, G. J., Wucherpfennig, K. W., Irvine, D. J., & Goldberg, M. S. (2017). *Nature Communications, 8*, 1747.
Shen, Y., Hao, T., Ou, S., Hu, C., & Chen, L. (2018). *MedChemComm, 9*, 226–238.
Speck, O., & Speck, T. (2019). *Biomimetics, 4*, 26.
Steenblock, E. R., & Fahmy, T. M. (2008). *Molecular Therapy, 16*, 765–772.
Stewart, J., Manmathan, G., & Wilkinson, P. (2017). *JRSM Cardiovascular Disease, 6*, 204800401668721.
Sun, H., Su, J., Meng, Q., Yin, Q., Chen, L., Gu, W., Zhang, P., Zhang, Z., Yu, H., Wang, S., & Li, Y. (2016). *Advanced Materials, 28*, 9581–9588.
Sushnitha, M., Evangelopoulos, M., Tasciotti, E., & Taraballi, F. (2020). *Frontiers in Bioengineering and Biotechnology, 8*, 627.
Tang, J., Lobatto, M. E., Hassing, L., van der Staay, S., van Rijs, S. M., Calcagno, C., Braza, M. S., Baxter, S., Fay, F., Sanchez-Gaytan, B. L., Duivenvoorden, R., Sager, H. B., Astudillo, Y. M., Leong, W., Ramachandran, S., Storm, G., Pérez-Medina, C., Reiner, T., Cormode, D. P., Strijkers, G. J., Stroes, E. S. G., Swirski, F. K., Nahrendorf, M., Fisher, E. A., Fayad, Z. A., & Mulder, W. J. M. (2015). *Science Advances, 1*, e1400223.

Tang, J., Su, T., Huang, K., Dinh, P.-U., Wang, Z., Vandergriff, A., Hensley, M. T., Cores, J., Allen, T., Li, T., Sproul, E., Mihalko, E., Lobo, L. J., Ruterbories, L., Lynch, A., Brown, A., Caranasos, T. G., Shen, D., Stouffer, G. A., Gu, Z., Zhang, J., & Cheng, K. (2018). *Nature Biomedical Engineering, 2*, 17–26.

Tazaki, T., Tabata, K., Ainai, A., Ohara, Y., Kobayashi, S., Ninomiya, T., Orba, Y., Mitomo, H., Nakano, T., Hasegawa, H., Ijiro, K., Sawa, H., Suzuki, T., & Niikura, K. (2018). *RSC Advances, 8*, 16527–16536.

Theofilopoulos, A. N., Kono, D. H., & Baccala, R. (2017). *Nature Immunology, 18*, 716–724.

Vijayan, V., Uthaman, S., & Park, I.-K. (2018). *Polymers, 10*, 983.

Wang, E. C., & Wang, A. Z. (2014). *Integrative Biology, 6*, 9–26.

Wang, L., Wang, F.-S., & Gershwin, M. E. (2015). *Journal of Internal Medicine, 278*, 369–395.

Wei, X., Gao, J., Wang, F., Ying, M., Angsantikul, P., Kroll, A. V., Zhou, J., Gao, W., Lu, W., Fang, R. H., & Zhang, L. (2017). *Advanced Materials, 29*, 1701644.

Wei, X., Zhang, G., Ran, D., Krishnan, N., Fang, R. H., Gao, W., Spector, S. A., & Zhang, L. (2018). *Advanced Materials, 30*, 1802233.

Wei, X., Ran, D., Campeau, A., Xiao, C., Zhou, J., Dehaini, D., Jiang, Y., Kroll, A. V., Zhang, Q., Gao, W., Gonzalez, D. J., Fang, R. H., & Zhang, L. (2019). *Nano Letters, 19*, 4760–4769.

Wu, C.-Y., Yeh, Y.-C., Chan, J.-T., Yang, Y.-C., Yang, J.-R., Liu, M.-T., Wu, H.-S., & Hsiao, P.-W. (2012). *PLoS One, 7*, e42363.

Xu, W., Ding, J., Li, L., Xiao, C., Zhuang, X., & Chen, X. (2015). *Chemical Communications, 51*, 6812–6815.

Xu, W., Ding, J., & Chen, X. (2017). *Biomacromolecules, 18*, 3291–3301.

Yang, H.-W., Ye, L., Guo, X. D., Yang, C., Compans, R. W., & Prausnitz, M. R. (2017). *Advanced Healthcare Materials, 6*, 1600750.

Yatim, K. M., & Lakkis, F. G. (2015). *Clinical Journal of the American Society of Nephrology, 10*, 1274–1281.

Yoo, J.-W., Chambers, E., & Mitragotri, S. (2010). *Current Pharmaceutical Design, 16*, 2298–2307.

Zhang, Y., Cai, L., Li, D., Lao, Y.-H., Liu, D., Li, M., Ding, J., & Chen, X. (2018a). *Nano Research, 11*, 4806–4822.

Zhang, X., Wang, C., Wang, J., Hu, Q., Langworthy, B., Ye, Y., Sun, W., Lin, J., Wang, T., Fine, J., Cheng, H., Dotti, G., Huang, P., & Gu, Z. (2018b). *Advanced Materials, 30*, 1707112.

Zhang, Y., Chen, Y., Lo, C., Zhuang, J., Angsantikul, P., Zhang, Q., Wei, X., Zhou, Z., Obonyo, M., Fang, R. H., Gao, W., & Zhang, L. (2019). *Angewandte Chemie, International Edition, 58*, 11404–11408.

Zhou, Y., & Dai, Z. (2018). *Chemistry–An Asian Journal, 13*, 3333–3340.

Chapter 10
Green Nanotechnology Revolution in Biomedical Application and Treatments

Lavanya Nallasamy, Girija Sangari Murugavelu, Santhosh Ganesh, Praveen Kumar Nandhakumar, Deepika Krishnamoorthy, Sriram Chandrasekaran, and Leeba Balan

10.1 Introduction

Green engineering and green chemistry are fundamental to green nanotechnology. Wherever possible, less material and renewable inputs are used to limit the usage of energy and fuel. Green nanotechnology greatly helps to the sustainability of the environment and human health through the creation of nanomaterials and nano-products (Verma et al., 2019). These green synthesized nanostructures have been successfully used as biological agents by providing significant performance improvements over their conventionally manufactured equivalents. Better clearance from the organisms and compatibility are a few of these improvements, as well as a mild synthetic technique that is both affordable and environmentally friendly (Cruz et al., 2020). The green synthesis techniques for nanoparticles are simpler, less expensive, more efficient, and environment friendly than chemical or microbe-mediated synthesis. Most importantly, environmentally benign synthesis methods paved way for reliable nanotechnology. Although there are many challenges and issues with green nanotechnology, this does not diminish the benefits of a green and sustainable approach (Khan, 2020).

L. Nallasamy · G. S. Murugavelu · D. Krishnamoorthy
Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

S. Ganesh
Department of Biotechnology, National Institute of Technology (NIT)-Warangal, Hanamkonda, Telangana, India

P. K. Nandhakumar
Department of Biotechnology, Bennett University, Greater Noida, Uttar Pradesh, India

S. Chandrasekaran · L. Balan (✉)
Bionyme Laboratories Pvt Ltd, Chennai, Tamil Nadu, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_10

A large-scale, less-polluted product has been made possible by the conflation of AgNPs employing a variety of sources, including bacteria, fungus, algae, and shops, as a result of the exploding fashion ability of green technologies. In order to manage size and help agglomeration, a circumscribing agent/ stabilizer, factory excerpts, incentive, or bacteria are generally used in the environmentally benign and biocompatible process of " green conflation" (Ahmad et al., 2019). Green chemistry is defined as "the utilisation of the twelve principles that reduces and eliminates the use or generation of hazardous substances in the design, production, and application of chemical products and processes," whereas green nanotechnology focuses on the development and application of products in an environmentally friendly manner (Karn & Bergeson, 2009). The application of environmental technology against monitoring and assessment, pollution prevention and control and remediation and restoration is the key component of green technology affecting both production and consumption of the materials. It is analytical to reduce or eliminate the harmful compounds to the environment by adapting certain human activities, which helps to reduce the harm on the environment (Ayaz et al., 2014). Green chemistry offers solutions for converting biological systems to environmentally friendly synthesis methods while avoiding any related toxicity.

The field of green nanotechnology is a recent effort taken by the researches all over the world to make use of nature's ability to reduce the risk of nanomaterials usage posing an environmental treat to the human health conditions. It also seeks to encourage the replacement of current products with new nano-products that are more environmentally responsible over the course of their lifetimes (Nasrollahzadeh et al., 2019). With product design and the use of safer materials, it is possible to develop nanomaterials to be more environmentally friendly, hence reducing any potential dangers (Maksimović & Omanović-Mikličanin, 2017). Green nanotechnology has the potential to save hundreds of millions of lives every year, so it should be made widely accessible (Ikhajiagbe et al., 2021). A low-cost, environmentally friendly agriculture technology called green nanotechnology has the potential to increase output while using fewer pesticides. Uses in agriculture include improving plant nutrient uptake, administering nano-pesticides to specific target areas and detecting diseases (Igiebor et al., 2023). This will enable people to become pervasive and deliver fully automated goods and services for individuals, serving as a cornerstone of social, technological, and economic development (Aithal & Aithal, 2021). Water treatment, climate change, renewable energy, solar energy conversion, and pollution reduction all present significant environmental dangers. These issues can be addressed with the help of the green nanoparticles. Given that it results in more stable and environmentally friendly final products and is highly repeatable, sustainable, and energy-efficient, green synthesis of nanoparticles is recognized as an enhanced and distinctive technique in this regard (Bharti et al., 2022).

10.2 Role of Gold Particles

Biological molecules including proteins and nucleic acids can interact with gold nanoparticles due to their high surface area to volume ratio (Thipe et al., 2022). Their usage in several biological applications, including medication administration, cancer treatment, and diagnostic imaging, is a result of this feature. To selectively attach to cancer cells and transport therapeutic drugs to the location of the tumor, for instance, gold nanoparticles can be functionalized with targeting molecules such as antibodies or peptides (Alhumaydhi, 2022; Hammami & Alabdallah, 2021). Another usage for gold nanoparticles is in catalysis, where they serve as catalysts for various chemical processes because of their special electrical and surface characteristics (Aslan & Pérez-Luna, 2004). Gold nanoparticles are suitable for carbon monoxide sensors because they can accelerate the oxidation of carbon monoxide to carbon dioxide at ambient temperature (Menazea & Mostafa, 2020).

Historically, poisonous and dangerous compounds like sodium borohydride and chloroauric acid have been used in chemical processes to create gold nanoparticles (Raji et al., 2020). Yet, new developments in green nanotechnology have prompted the creation of environmentally friendly processes that manufacture gold nanoparticles using natural sources including plant extracts and microorganisms (Menazea & Awwad, 2020). Overall, gold nanoparticles are a promising nanomaterial with special qualities that make them beneficial in a variety of biomedical, electrical, and energy-related applications (Krishnan et al., 2016).

10.3 Green Nanotechnology in Drug Delivery Approach

Green nanotechnology is a promising solution that involves the utilization of environmentally friendly processes, living organisms, and biomolecules for the production of nanomaterials (Medina-Cruz et al., 2020). Drug delivery systems (DDS) are widely researched and developed to improve the effectiveness and delivery of active pharmaceutical compounds, including drugs, vaccines, antibodies, enzymes, peptides, and proteins (Kanwar et al., 2019). The field of "green nanomedicine" has emerged as a result of the impact of green and environmentally friendly chemistry on the nanotechnology-driven drug delivery sector. Research has demonstrated that among the various green nanotechnology-driven drug delivery systems, nanometal particles, polymers, and biological materials have received the most attention. Moreover, the development of revolutionary materials for green nanodrug delivery systems is underway, utilizing environmentally friendly chemical reactions or natural biomaterials like plant extracts and microorganisms. The use of green chemistry principles and eco-friendly synthesis techniques with minimal side effects is being prioritized in the design, synthesis, and application of these drug delivery systems (Patel et al., 2022).

Green nanotechnology is a field that combines engineering and green chemistry principles to develop eco-friendly and safe nanoparticles without using toxic chemicals. This approach offers various types of delivery systems, including quantum dots, metallic and mesoporous silica nanoparticles, organic polymeric dendrimers, solid lipid nanoparticles, and metal-organic frameworks (MOFs), without any associated toxicity. Green nanodrug delivery systems have already produced innovative materials that revolutionize the field by utilizing environmentally friendly chemical reactions or natural materials (Nabipour and Hu (2020). A drug delivery system based on nanotechnology offers an integrated platform for therapy, which improves the physicochemical properties of drugs, combats multidrug resistance, and reduces drug-related toxicity (Rajwar et al., 2023).

The field of nanotechnology has emerged as a highly active area of research due to the wide range of applications, including catalysis, sensing, electronics, photonics, and medicine. In recent decades, there has been significant attention on the synthesis of nanoparticles. Since the nineteenth century, scientists have recognized the potential of biological organisms to reduce metal precursors, although the underlying mechanisms are not yet fully understood. Researchers have turned to biological methods for nanoparticle synthesis because of their success in using natural reduction, capping, and stabilizing agents, as well as their ability to avoid harmful chemicals and high energy consumption (Khan et al., 2022a, b). The combination of biosensing platforms with drug delivery systems has resulted in efficient treatment approaches for biomedical applications. Nanotechnology, which involves the manipulation of materials at the nanoscale (1–100 nm), has significantly improved these biosensing and drug delivery systems by leveraging the unique properties exhibited by these materials (Noah & Ndangili, 2022).

10.4 Green Nanotechnology Evaluation of Therapeutic Efficacy

Plants have garnered considerable interest as a source for creating nanomaterials due to the presence of phytochemicals such as alkaloids, terpenes, saponins, phenols, alcohols, and proteins that act as reducing and capping agents. By isolating these phytochemicals, the reproducibility of size and shape-controlled nanomaterials can be improved. These bioactive nanomaterials have a diverse range of biomedical and pharmaceutical applications (Saravanan et al., 2021). The technology aims to design reactions with high efficiency, utilize renewable materials and energy sources, use safe solvents and reactants, and minimize waste production. Green nanotechnology applies the 12 principles of green chemistry to develop new nanomaterials that provide economic, social, health, and environmental benefits (Jahangirian et al., 2017). Depending on the components used for synthesis or

structural aspects, nanoparticles can be categorized as polymeric nanoparticles, liposomes, dendrimers, micelles, and inorganic nanoparticles. The fabrication methods and properties of nanoparticles play a crucial role in their applications and utility (Chenthamara et al., 2019).

To address human health-related diseases, the development of new and reliable therapeutic drug delivery systems (DDS) is crucial. Nanotechnology-based DDS with advanced features have been designed for biomedical, pharmaceutical, and cosmeceutical applications. These DDS have shown enhanced bioactivity, bioavailability, drug efficacy, targeted delivery, and improved safety compared to traditional drug formulations. This novel technology has the potential to overcome the limitations of traditional drug designs (MN Iqbal et al., 2016). Targeted therapies have shown great promise in reducing off-target toxicity and overcoming certain limitations. Nevertheless, biological barriers within the human body continue to pose significant obstacles to the successful delivery of these therapies (Khan et al., 2022a, b).

10.5 Application of Green Nanotechnology in Biomedicine

Nanotechnology has numerous applications in various fields, such as biomaterials, nanomedicines, nanoelectronics, environment, agriculture, and industries. It has been widely used in healthcare for disease diagnosis and treatment, drug delivery, and novel drug formulations.

10.5.1 Applications of Cobalt and Cobalt Oxide NPs

Cobalt and cobalt oxide nanoparticles (NPs) have various biomedical applications because of their distinctive antioxidant, antimicrobial, antifungal, anticancer, larvicidal, antileishmanial, anticholinergic, wound healing, and anti-diabetic properties. Researchers have recently developed an environmentally friendly, secure, straightforward method that uses biotic resources like plant extract, microorganisms, algae, and other biomolecules like starch and gelatin. These are naturally occurring cobalt and oxide nanoparticles (NPs) have more benefits than other physicochemical methods (Waris et al., 2021). Cancer is the second leading cause of human dysphoria, after cardiovascular disease. CoNPs demonstrated promising anticancer properties. Synthesized cobalt oxide NPs from *Euphorbia tirucalli* extract showed anti-proliferative activity against MCF-7 breast cancer cell lines. Investigations into the potential of green synthesized CoNPs for wound healing revealed that the ointment form of CoNPs has significant potential (Hou et al., 2020; Khalil et al., 2020).

10.5.2 Applications of Gold and Silver Nanoparticles

Chemotherapy and radiotherapy, two common methods for treating cancer, have a number of negative side effects, including drug toxicity, a lack of specificity, unpredictability, and drug resistance. AgNPs produced from the *Aerva javanica* extract combined with the anticancer drug (gefitinib) had greater apoptotic potency on the MCF-7 cells. The delivery of gefitinib by AgNPs increased drug efficacy while decreasing side effects. AgNPs conjugated with anti-seizure medications were used to treat infections of the central nervous system caused by brain-eating amoebae (*Naegleria flowleri*) outside of the field of oncology. Anti-seizure drugs (diazepam, phenobarbitone, and phenytoin) that are known to cross the blood-brain barrier were added to the surface of AgNPs as capping agents and demonstrated general anti-amoebic activity against both trophozoite and cyst phases (Simon et al., 2022).

AgNPs were synthesized using various plant extracts and AgNPs produced by *A. calamus* (rhizome) exhibited increased activity against A431 carcinoma cells among those extract-mediated AgNPs (Nayak et al., 2015). AgNPs mediated by *Artemisia vulgaris* extract have biofilm reduction and anthelmintic activity. This activity demonstrated the paralytic effect and could be used to treat biofilm formed by multidrug-resistant pathogens (Ejaz et al., 2018).

10.5.3 Green Microwave Synthesis of ZnO and CeO_2 Nanorods for Infectious Diseases Control

In the microwave-assisted hydrothermal method of green synthesis, the *Olea europaea* leaf extract is used as a natural medicinal capping agent to control the shape and size of CeO_2 nanorods and ZnO nanorods. ZnO and CeO_2 nanorods synthesized from green materials have an average crystallite size of about 15 nm. ZnO and CeO_2 nanorods were tested against clinical pathogens for their antimicrobial activity. Antimicrobial and anti-tumor activities were superior against hepatocellular carcinoma cell lines. ZnO and CeO_2 nanomaterials can be effectively synthesized by the green microwave process for many biomedical uses (Gharbia et al., 2022).

10.5.4 Applications of Tin Oxide Nanoparticles

Tin oxide nanoparticles have received a great deal of attention due to their numerous applications. It can be used for antibacterial, antifungal, antiviral, anticancer, antioxidant, drug delivery, and a variety of other biomedical applications. SnO2 NPs were synthesized using a sugar apple (*Annona squamosa*) peel extract-mediated process and their cytotoxicity against a hepatocellular carcinoma cell line was investigated ($HepG_2$). The results show that at the tested concentrations, SnO2

nanoparticles have moderate cytotoxicity against hepatocellular carcinoma ($HepG_2$) (Roopan et al., 2015).

10.5.5 Platinum Nanoparticles on Biomedicine

Platinum nanoparticles have the potential to revolutionize cancer treatment. PtNPs mediated by plant extracts containing essential oils, acids, alkaloids, and phytoncides are the most promising, with efficacy in various types of cancer. *Aloe vera, Catharanthus roseus, Capsicum annuum, Ocimum sanctum,* and many other medicinal plants have shown to have anticancer properties. The combined effect of platinum nanoparticles capped by various herb compounds may enhance the anticancer effect while eliminating the metal's toxic effect (Mikhailova, 2022).

10.6 Applications of Green Synthesized Nanoparticles in Biomedical Engineering

10.6.1 Bioimaging

Green synthesized nanoparticles have also shown potential in bioimaging. Thanks to tailored surface properties and controlled release patterns, nanotechnology has emerged as a promising strategy for diagnosis. Magnetic nanoparticles, such as iron oxide can be used as contrast agents in imaging techniques such as MRI, CT, and PET (Computed and positron emission tomography) (Hoskins, 2014).The iron oxide magnetic NPs are also shown to be efficient in the early diagnosis of malaria (Lyberopoulou et al., 2015). AgNPs synthesized from the extract of *Achyranthes aspera* can be utilized as a sensor for the detection of thiocyanate ions that may be present in contaminated water (Praveena & Kumar, 2015). AgNPs can also be used for sensing melamine in milk to detect possible adulteration (Varun et al., 2016). Gold nanoparticles synthesized using green methods from *Osmundaria obtusiloba* are used in bioimaging and cancer therapy due to their good optical properties (Rojas-Perez et al., 2015).

10.6.2 Tissue Engineering

Green synthesized nanoparticles have received much attention in tissue engineering applications due to their unique physicochemical properties. Nanoparticles can be used as scaffolds to support cell growth and tissue regeneration, for example, gold nanoparticle-integrated scaffolds have shown superior physical and chemical

properties that make them a promising tool for tissue engineering (11). Green synthesized zinc oxide nanoparticles extracted from Artemisia annua have been found to enhance the proliferation, differentiation, and mineralization of osteoblasts, which are responsible for bone formation. Thus, ZnO NPs show a great promise in the treatment of bone deformities like osteoporosis (12). Meanwhile, green synthesized chitosan-silver nanoparticles have been shown to have antimicrobial properties and can promote wound re-epithelialization and tissue regeneration, making them ideal for enhancing wound healing (13).

10.6.3 Antibacterial Activity

Antibiotic resistance has become a major concern in modern medicine, since high usage of antibiotics in recent times has led to the development of bacteria that are resistant to commercially available antibiotic drugs. Particularly, the most significant bacteria, Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) have become resilient to drugs (Ruparelia et al., 2018). Therefore, there is a need to develop better bactericidal agents with better efficacy. Metallic nanoparticles have proven to be a better substitute for antibiotics. Silver nanoparticles which were developed using a green approach with *Ganoderma lucidum* (GL) extract showed strong antibacterial activity against several strains of bacteria such as *B. subtilis*, *B. cereus*, *P. aeruginosa*, *E. coli,* and *S. aureus*. Similarly, Zinc oxide nanoparticles synthesized using an extract of Phoenix roebelenii leaves demonstrated significant bactericidal effect toward several gram-positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and gram-negative (*Escherichia coli and Salmonella typhi*) pathogenic bacteria (Aldeen et al., 2022). Nanoparticles exhibit their mechanism of action against bacterial strains by inhibiting cell wall synthesis, breaking down the cell wall and cell membrane, inducing oxidative stress via reactive oxygen species within the cell, and causing protein and DNA denaturation (Slavin et al., 2017).

10.7 Conclusion

Green-synthesized NPs show great promise, opening up new prospects not only in biomedical research, but also in energy and environmental research. Metal and metal oxide nanoparticles that are synthesized through green protocols have been shown to have several benefits, including being biocompatible, lucrative, and effective. Additionally, these nanoparticles have demonstrated notable physical, chemical, and biological properties that can be effectively utilized in various pharmaceutical and biomedical applications. Furthermore, the side effects of current treatments are becoming a significant concern. However, the potential of the metal nanoparticles still remains untapped for the most part as there are too many candidates of plant

species that satisfy the requirements for nanoparticle synthesis. To address this issue, the world is turning to new treatment options, and the development of green nanotechnology appears promising. The release of such NPs into the environment might cause odd behaviors; therefore, more scientific research is required in order to thoroughly study the properties of biologically synthesized NPs.

References

Ahmad, S., Munir, S., Zeb, N., Ullah, A., Khan, B., Ali, J., & Ali, S. (2019). Green nanotechnology: A review on green synthesis of silver nanoparticles-An ecofriendly approach. *International Journal of Nanomedicine*, 5087–5107.

Aithal, S., & Aithal, P. S. (2021). Green nanotechnology innovations to realize UN sustainable development goals 2030. *International Journal of Applied Engineering and Management Letters (IJAEML), 5*(2), 96–105.

Aldeen, T. S., Ahmed Mohamed, H. E., & Maaza, M. (2022). ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity. *Journal of Physics and Chemistry of Solids, 160*, 110313.

Alhumaydhi, F. A. (2022). Green synthesis of gold nanoparticles using extract of Pistacia chinensis and their in vitro and in vivo biological activities. *Journal of Nanomaterials, 2022*, 1.

Aslan, K., & Pérez-Luna, V. H. (2004). Quenched emission of fluorescence by ligand functionalized gold nanoparticles. *Journal of Fluorescence, 14*, 401–405.

Ayaz Ahmed, K. B., Subramanian, S., Sivasubramanian, A., Veerappan, G., & Veerappan, A. (2014). Preparation of gold nanoparticles using Salicornia brachiata plant extract and evaluation of catalytic and antibacterial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 130*, 54–58.

Bharti, B., Kumar, R., Kumar, H., Li, H., Zha, X., & Ouyang, F. (2022). Advanced applications and current status of green nanotechnology in the environmental industry. In *Green functionalized nanomaterials for environmental applications* (pp. 303–340). Elsevier.

Chenthamara, D., Subramaniam, S., Ramakrishnan, S. G., Krishnaswamy, S., Essa, M. M., Lin, F. H., & Qoronfleh, M. W. (2019). Therapeutic efficacy of nanoparticles and routes of administration. *Biomaterials Research, 23*(1), 1–29.

Cruz, D. M., Mostafavi, E., Vernet-Crua, A., Barabadi, H., Shah, V., Cholula-Díaz, J. L., et al. (2020). Green nanotechnology-based zinc oxide (ZnO) nanomaterials for biomedical applications: A review. *Journal of Physics: Materials, 3*(3), 034005.

Ejaz, K., et al. (2018). Biofilm reduction, cell proliferation, anthelmintic and cytotoxicity effect of green synthesised silver nanoparticle using Artemisia vulgaris extract. *IET Nanobiotechnology, 12*(1), 71–77.

Gharbia, N., Elsabbagh, S., Saleh, A., & Hafez, H. (2022). Green microwave synthesis of ZnO and CeO2 nanorods for infectious diseases control and biomedical applications. *AMB Express, 12*(1), 1–13.

Hammami, I., & Alabdallah, N. M. (2021). Gold nanoparticles: Synthesis properties and applications. *Journal of King Saud University-Science, 33*(7), 101560.

Hoskins, C. (2014). The use of iron oxide nanoparticles for pancreatic cancer therapy. *Journal of Nanomedicine Research, 1*(1).

Hou, H., Mahdavi, B., Paydarfard, S., Zangeneh, M. M., Zangeneh, A., Sadeghian, N., et al. (2020). Novel green synthesis and antioxidant, cytotoxicity, antimicrobial, antidiabetic, anticholinergics, and wound healing properties of cobalt nanoparticles containing Ziziphora clinopodioides Lam leaves extract. *Scientific Reports, 10*(1), 1–9.

Igiebor, F. A., Ikhajiagbe, B., & Asia, M. (2023). Green nanotechnology: A modern tool for sustainable agriculture in Nigeria–A review. *International Journal of Horticultural Science and Technology, 10*(3), 269–286.

Ikhajiagbe, B., Igiebor, F. A., & Ogwu, C. (2021). Growth and yield performances of rice (Oryza sativa var. "nerica") after exposure to biosynthesized nanoparticles. *Bulletin of the National Research Centre, 45*(62), 1–13.

Iqbal H, M. N., Rodriguez A, M. V., Khandia, R., Munjal, A., & Dhama, K. (2016). Recent trends in nanotechnology-based drugs and formulations for targeted therapeutic delivery. *Recent Patents on Inflammation & Allergy Drug Discovery, 10*(2), 86–93.

Jahangirian, H., Lemraski, E. G., Webster, T. J., Rafiee-Moghaddam, R., & Abdollahi, Y. (2017). A review of drug delivery systems based on nanotechnology and green chemistry: Green nanomedicine. *International Journal of Nanomedicine, 12*, 2957.

Kanwar, R., Rathee, J., Salunke, D. B., & Mehta, S. K. (2019). Green nanotechnology-driven drug delivery assemblies. *ACS Omega, 4*(5), 8804–8815.

Karn, B. P., & Bergeson, L. L. (2009). Green nanotechnology: Straddling promise and uncertainty. *Natural Resources & Environment, 24*, 9.

Khalil, A. T., Ovais, M., Ullah, I., Ali, M., Shinwari, Z. K., & Maaza, M. (2020). Physical properties, biological applications and biocompatibility studies on biosynthesized single phase cobalt oxide (Co3O4) nanoparticles via Sageretia thea (Osbeck.). *Arabian Journal of Chemistry, 13*(1), 606–619.

Khan, S. H. (2020). Green nanotechnology for the environment and sustainable development. In *Green materials for wastewater treatment* (pp. 13–46). Springer.

Khan, F., Shariq, M., Asif, M., Siddiqui, M. A., Malan, P., & Ahmad, F. (2022a). Green nanotechnology: Plant-mediated nanoparticle synthesis and application. *Nanomaterials, 12*(4), 673.

Khan, M. I., Batool, F., Kalsoom, F., Gao, S., Ali, R., Wang, W., Kazmi, A., Lianliang, L., Wang, G., & Bilal, M. (2022b). Trends in Nanotechnology to improve therapeutic efficacy across special structures. *OpenNano, 7*, 100049.

Krishnan, V., Bupesh, G., Manikandan, E., Thanigai, A. K., Magesh, S., Kalyanaraman, R., & Maaza, M. (2016). Green synthesis of silver nanoparticles using Piper nigrum concoction and its anticancer activity against MCF-7 and Hep-2 cell lines. *Journal of Antimicrobial Chemotherapy, 2*(3), 2472–1212.

Lyberopoulou, A., Efstathopoulos, E. P., & Gazouli, M. (2015). Nanodiagnostic and nanotherapeutic molecular platforms for cancer management. *Journal of Cancer Research Updates, 4*(4), 153–162.

Maksimović, M., & Omanović-Mikličanin, E. (2017). Towards green nanotechnology: Maximizing benefits and minimizing harm. In *CMBEBIH 2017: Proceedings of the international conference on medical and biological engineering 2017* (pp. 164–170). Springer Singapore.

Medina-Cruz, D., Mostafavi, E., Vernet-Crua, A., Cheng, J., Shah, V., Cholula-Diaz, J. L., Guisbiers, G., Tao, J., García-Martín, J. M., & Webster, T. J. (2020). Green nanotechnology-based drug delivery systems for osteogenic disorders. *Expert Opinion on Drug Delivery, 17*(3), 341–356.

Menazea, A. A., & Awwad, N. S. (2020). Antibacterial activity of TiO2 doped ZnO composite synthesized via laser ablation route for antimicrobial application. *Journal of Materials Research and Technology, 9*(4), 9434–9441.

Menazea, A. A., & Mostafa, A. M. (2020). Ag doped CuO thin film prepared via pulsed laser deposition for 4-nitrophenol degradation. *Journal of Environmental Chemical Engineering, 8*(5), 104104.

Mikhailova, E. O. (2022). Green synthesis of platinum nanoparticles for biomedical applications. *Journal of Functional Biomaterials, 13*, 260. https://doi.org/10.3390/jfb13040260

Nabipour, H., & Hu, Y. (2020). Sustainable drug delivery systems through green nanotechnology. In *Nanoengineered biomaterials for advanced drug delivery* (pp. 61–89). Elsevier.

Nasrollahzadeh, M., Sajjadi, M., Sajadi, S. M., & Issaabadi, Z. (2019). Green nanotechnology. In *Interface science and technology* (Vol. 28, pp. 145–198). Elsevier.

Nayak, D., et al. (2015). Biologically synthesised silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. *Journal of Colloid and Interface Science, 457*, 329–338.

Noah, N. M., & Ndangili, P. M. (2022). Green synthesis of nanomaterials from sustainable materials for biosensors and drug delivery. *Sensors International, 3*, 100166.

Patel, M., Patel, J., & Dayaramani, R. (2022). Green-nanotechnology-driven drug delivery systems. In *Sustainable nanotechnology: Strategies, products, and applications* (pp. 117–133). John Wiley & Sons.

Praveena, V. D., & Kumar, K. V. (2015). A thiocyanate sensor based on ecofriendly silver nanoparticles thin film composite. *International Journal of Innovative Science, Engineering and Technology, 2*, 741–752.

Raji, V., Pal, K., Zaheer, T., Kalarikkal, N., Sabu, T., de Souza, F. G., & Asiya, S. I. (2020). Gold nanoparticles against respiratory diseases: Oncogenic and viral pathogens review. *Therapeutic Delivery, 11*(8), 521.

Rajwar, T. K., Pradhan, D., Halder, J., Rai, V. K., Kar, B., Ghosh, G., & Rath, G. (2023). Opportunity in nanomedicine to counter the challenges of current drug delivery approaches used for the treatment of malaria; a review. *Journal of Drug Targeting*, 1–23.

Rojas-Pérez, A., Adorno, L., Cordero, M., Ruiz, A., Mercado-Díaz, Z., Rodríguez, A., et al. (2015). Biosynthesis of gold nanoparticles using Osmudaria Obtusiloba extract and their potential use in optical sensing applications. *Austin Journal of Biosensors & Bioelectronics, 1*(3), 1014.

Roopan, S. M., Kumar, S. H. S., Madhumitha, G., & Suthindhiran, K. (2015). Biogenic-production of SnO2 nanoparticles and its cytotoxic effect against hepatocellular carcinoma cell line (HepG2). *Applied Biochemistry and Biotechnology, 175*, 1567–1567.

Ruparelia, J. P., Chatterjee, A. K., Duttagupta, S. P., & Mukherji, S. (2018). Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomaterialia, 4*(3), 707–716.

Saravanan, M., Barabadi, H., & Vahidi, H. (2021). Green nanotechnology: Isolation of bioactive molecules and modified approach of biosynthesis. In *Biogenic nanoparticles for cancer theranostics* (pp. 101–122). Elsevier.

Simon, S., Sibuyi, N. R. S., Fadaka, A. O., Meyer, S., Josephs, J., Onani, M. O., Meyer, M., & Madiehe, A. M. (2022). Biomedical applications of plant extract-synthesized silver nanoparticles. *Biomedicine, 10*(11), 2792.

Slavin, Y. N., Asnis, J., Häfeli, U. O., & Bach, H. (2017). Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. *Journal of Nanobiotechnology, 15*, 65.

Thipe, V. C., Karikachery, A. R., Cakilkaya, P., Farooq, U., Genedy, H. H., Kaeokhamloed, N., Phan, D.-H., Rezwan, R., Tezcan, G., Roger, E., & Katti, K. V. (2022). Green nanotechnology—an innovative pathway towards biocompatible and medically relevant gold nanoparticles. *Journal of Drug Delivery Science and Technology, 70*, 103256.

Varun, S., Kiruba Daniel, S. C. G., & Gorthi, S. S. (2016). Rapid sensing of melamine in milk by interference green synthesis of silver nanoparticles. *Materials Science and Engineering: C, 74*, 253–258.

Verma, A., Gautam, S. P., Bansal, K. K., Prabhakar, N., & Rosenholm, J. M. (2019). Green nanotechnology: Advancement in phytoformulation research. *Medicine, 6*(1), 39.

Waris, A., Din, M., Ali, A., Afridi, S., Baset, A., Khan, A. U., & Ali, M. (2021). Green fabrication of Co and Co_3O_4 nanoparticles and their biomedical applications: A review. *Open Life Sciences, 16*(1), 14–30. https://doi.org/10.1515/biol-2021-0003

Chapter 11
Nanoparticles Function as Delivery Systems for Immune Potentiation

Sakshi Thakur, Vishal Mutreja, and Ajay Sharma

11.1 Introduction

Nanotechnology entails functional systems at the molecular level. Such systems have distinctive electrical, optical, physicochemical and biological characteristics that make them interesting candidates for applications in fields such as materials science and biomedicine. In biomedicine, drug delivery systems (DDSs) entail the administration of therapeutic or pharmaceutical components to a precise area of the body with better efficacy and safety (Hong et al., 2020). Delivering therapeutic components to the specific targeted site or cells is a noteworthy requirement for curing several ailments. A conventional DDS is sometimes characterized by a lack of selectivity, poor biodistribution and limited efficacy. Therefore, the development of drug delivery techniques could strategically use nanotechnology to expand the drug market. Nanoparticles (NPs) are colloidal nanocarriers (NCs) of synthetic or semi-synthetic polymers with a size of 1–100 nm, which are helpful in addressing concerns about the delivery of both modern and conventional drugs (Sur et al., 2019). NPs have been shown to possess better flexibility in accessing deep molecular targeting tissues and in regulating drug release (Karuppusamy & Venkatesan, 2017). When formulated properly, nanodrug particles can have greater adherence to biological surfaces, better saturation solubility, quick dissolution and resistance to settling, all of which contribute to a faster beginning of therapeutic action and higher bioavailability. In addition, the nanostructure's surface contains the vast bulk of its molecules (Bamrungsap et al., 2012). Most of the molecules within a nanostructure are found on the particle's surface, maximizing the delivery and loading tendency of cargoes such as various therapeutic drugs, polynucleotides, enzymes, proteins and genes to specific tissues or cells. Different types of nanostructures, including NPs,

S. Thakur · V. Mutreja · A. Sharma (✉)
Department of Chemistry, Chandigarh University, NH-95, Chandigarh–Ludhiana Highway, Mohali, Punjab, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_11

nanocomposites, nanotubes and nanofibres, effectively aid in the screening and treatment of a wide range of disorders (Baskar et al., 2017a, b; Chamundeeswari et al., 2013; Verma et al., 2012, 2013). Moreover, NCs with optimal biological and physicochemical properties could be applicable for delivering presently available bioactive chemicals, as cells can absorb them more readily than larger molecules (Saman & Iqbal, 2012; Zahin et al., 2020; Wilczewska et al., 2012). This chapter elaborates the different classes of colloidal NCs, which play a significant role in DDSs and are applied as a suitable factor for biological applications.

11.2 Nanocarriers as Drug Delivery Systems

NCs are the colloidal particle system of NPs that are frequently employed to carry therapeutic agents or any other compounds to a targeted site (Qian et al., 2012). As the size of microcapillaries in a body is 200 nm, NCs should be of a size less than 200 nm for their therapeutic applications in the body (Singh & Lillard, 2009). NCs are inactive and typically regarded as a safe medium and thus offer good biocompatibility, fewer side effects and many other physicochemical features (Kingsley et al., 2006), depending on their composition, shape and surface (Sun et al., 2014). As a result, they have a broad spectrum of drug delivery. Several types of NCs have been reported to exhibit remarkable site-specific drug delivery (Mishra et al., 2010), including applications such as enhanced pharmacokinetics and biodistribution, enhanced solubility and stability, toxicity reduction and sustainability.

11.2.1 Types of NCs and Their Classification

NCs possess a high surface-to-volume ratio and are categorized as inorganic, organic and hybrid NCs, which are further distributed into various classes (Fig. 11.1).

11.3 Inorganic NCs

Inorganic NCs have been classified into various types, including gold NPs (AuNPs), ceramic and superparamagnetic NCs, quantum dots (QDs), mesoporous silica, carbon nanotubes (CNTs), etc. (Fig. 11.2). These NCs have enormous applications in therapeutics and pharmacology, including biosensing, diagnostics, bioimaging, cell labelling, biocatalysis and gene delivery and targeting (Santos et al., 2014). Inorganic NCs also exhibit various other clinical applications, namely the treatment of tumours (Shi et al., 2020), chronic myelogenous leukaemia (Ghosn et al., 2019), inflammatory disease (Prosperi et al., 2017) and many others. In addition, the modification of the size and arrangement of inorganic NCs can lead to remarkable plasmonic and

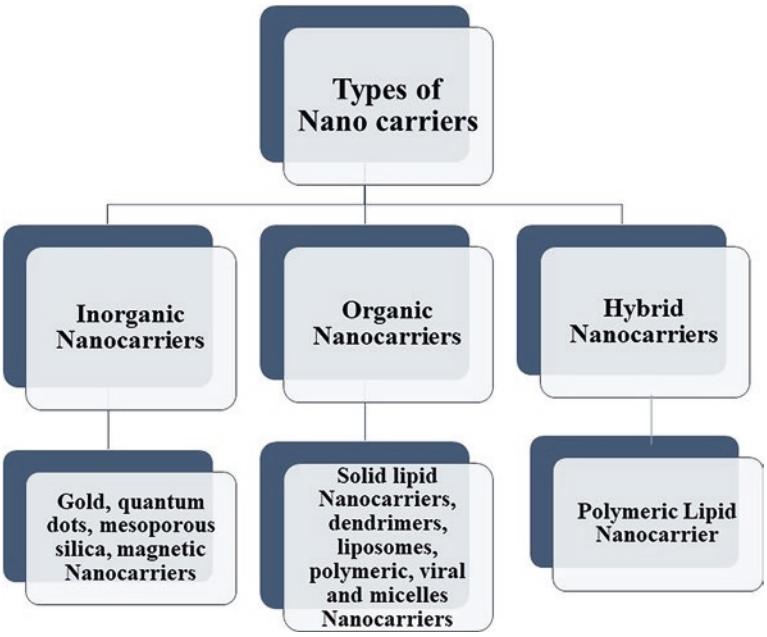


Fig. 11.1 NCs and their types

optical properties (Goldenberg et al., 2020; Gellini & Feis, 2021). Moreover, its composition with heavy metals could raise significant drawbacks which can lead to chronic health diseases (Ma et al., 2015).

11.3.1 Gold NCs

AuNPs are inorganic NCs in which the inner core contains the gold atom and its surface has negative groups. A monolayer of surface ligands can easily functionalize the surface for active targeting. AuNPs have surface biofunctionalization with biomolecules, including proteins, carboxylic acid, enzymes and so on (Mohammed & Al-Gawhari, 2020). These NPs display low toxicity and high surface area and thus show greater drug-loading tendency. Due to the uniform dispersity of AuNPs, they can reach the active targeted site and thus provide new delivery strategies. AuNPs have shown tremendous biomedical applications in optics, chemotherapy, photoacoustic imaging, gene delivery, photothermal therapy, etc. Furthermore, due to their optical properties, biomolecules such as proteins, enzymes, peptides, carbohydrates, genes and fluorophores can be attached to AuNPs, thus making possible the effective delivery of AuNPs within the cell (Khandelia et al., 2013). Developments in the pharmacokinetics, pharmacology and biodistribution of AuNPs are also imperative for enhancing their applications in medicinal drugs (Wang et al., 2004; Qian et al., 2008; García, 2011; Lu et al., 2010; Huang et al., 2006).

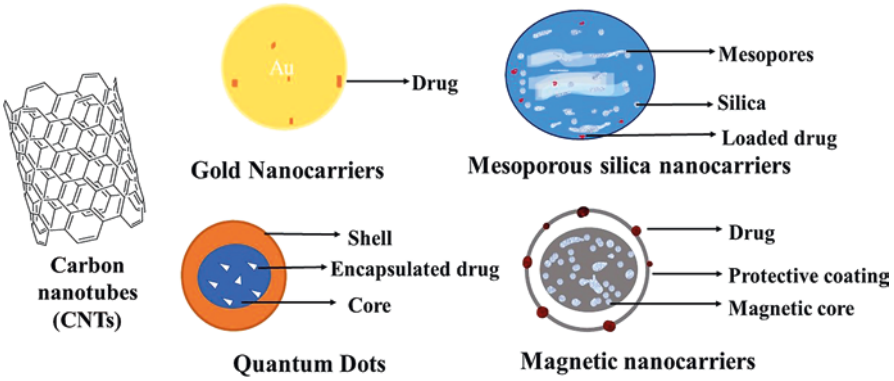


Fig. 11.2 Various classes of inorganic drug NCs

11.3.2 Ceramic NCs

Ceramic NCs are made of inorganic materials having pore-like properties, such as titania, silica and alumina (Medina et al., 2007; Nutter & Ratts, 1973). Silica has been proven to have better features owing to its biocompatibility, easy synthesis and surface modification (Bottini et al., 2007; Ohulchanskyy et al., 2007). Well-understood silane chemistry also makes it easier for drugs to cross-link with silica particles (Slowing et al., 2007). Furthermore, NCs of mesoporous silica are porous structures with a two-dimensional network of several mesopores, which resembles a honeycomb. Recent studies have shown that these NCs show exclusive biocompatibility in pharmacological applications as compared to amorphous silica materials of low biocompatibility (Descalzo et al., 2006; Trewyn et al., 2007). To deliver drug molecules at levels that are pharmacologically efficacious after the vehicle has been localized in the cytoplasm, it is preferable to have effectual control over their release. To accomplish this, it is advantageous to be able to selectively functionalize the internal nanochannel surface of mesoporous silica and their exterior particle surfaces (Angelos et al., 2007). To attain tissue specificity, the mesoporous silica surface can be modified with cell-specific moieties, such as organic compounds, peptides, antibodies and aptamers. Furthermore, versatile DDSs can be created using optical and magnetic contrast agents (Slowing et al., 2008).

11.3.3 Carbon-Based NCs

Carbon-based NCs have a tube-like assembly of carbon atoms. CNTs are considered carbon-based nanocarriers, which act as an excellent source of delivering drugs, due to their unique biological and physical–chemical features. CNTs belong

to the family of fullerenes, which are made by wrapping graphene sheets into a tube-like shape (Bianco, 2004). CNTs are suitable for numerous applications due to their high surface area with ultralight weight, nano-sized needle structure, high aspect ratio and thermal, mechanical, electrical and distinctive chemical properties (Ng et al., 2016; O'Regan & Gratzer, 1991). Moreover, their surface modification, structural flexibility and stability make them effective agents for destroying cancer cells. According to that theory, anti-cancer medications like paclitaxel are frequently encapsulated in or linked to functionalized carbon nanotubes (Liu et al., 2008; Thiruvengadam et al., 2021).

11.3.4 Quantum Dots

Elements such as Te, Se, Zn, As, P and so on are included in QD formulation and are considered energy carriers (Corrocher et al., 1975). The emission of light in the ultraviolet (UV) region is dependent on the quantum dot's size; for example, small-size QDs (~2 nm) lead to the emission of blue fluorescence, whereas large-size QDs (~5 nm) emit red fluorescence. Their optical quality sets them apart from other organic dyes, and thus, they can be utilized for cell imaging. For instance, the in vivo targeting of rat tumour vasculature uses a quantum dot–peptide conjugate (Åkerman et al., 2002). In addition, QDs are known for their effectiveness as delivery and reporting systems (Christian et al., 2003; Derfus et al., 2007). In the charge transfer process, these colloidal nanocrystals are used as an energy transfer quencher (Medintz et al., 2009), chemiluminescence resonance energy transfer acceptors (Freeman et al., 2011) and quantum dot–fluorescence resonance energy transfer system (Geißler et al., 2010).

11.3.5 Magnetic NCs

Magnetic NCs have shown an extensive range of applications for the diagnosis and treatment of diseases that pose risks to human life, such as cancer and neurological and cardiovascular conditions (Stergar et al., 2019; Abulibdeh et al., 2019; Almessiere et al., 2018a). These NCs work by magnetic absorption of specific tissues. They consist of supermagnetic and magnetic susceptibility and super-saturation properties (Üzek et al., 2019; Almessiere et al., 2018b; Advanced C, 2022). In contrast to metal oxide NPs, metal NPs are often more magnetic. They are used in biosensing. Among superparamagnetic and paramagnetic NPs, the former are more susceptible to magnetic fields than the latter. These NCs show good biocompatibility and offer good ease of surface modification and are considered for use in biomedical and industrial applications (Kianfar, 2021).

11.3.6 Mesoporous NCs

Mesoporous NCs have a porous honeycomb-like structure, which makes it possible to incorporate more drug molecules into them. These NCs have been applied in the biomedical industry due to their accessibility and simplicity. Both hydrophilic and aquaphobic drugs can bind to a ligand for targeted drug administration and can be encapsulated by mesoporous NCs (Li et al., 2017). Mesoporous silica possesses thermochemical properties and shows good biocompatibility, a large porous volume, a high surface area and drug-loading capacity (Wang et al., 2015). Some of the anti-cancer drugs such as camptothecin and methotrexate are proficiently distributed by using mesoporous silica.

11.4 Organic NCs

Organic NCs possess good drug-loading capability, biocompatibility and less toxicity. The first-generation NCs were basic excipients called polymeric NCs (PNCs) and liposomes that were used for drug delivery. Moreover, liposomes and micelles can amass at the specific spot due to their improved permeability and retention impact (Peng et al., 2020). Figure 11.3 shows the various classes of organic drug NCs.

11.4.1 Solid Lipid NCs (SLNCs)

Solid lipid NCs (SLNCs) are submicron spherical colloidal carriers with a typical size of nearly 40–1000 nm. SLNCs are composed of solid biodegradable lipids and biocompatible material (Liu et al., 2010). These are non-toxic alternative lipophilic colloidal drug carriers (Yadav et al., 2013). SLNCs are formed by dispersing melted solid lipids in water, followed by their stabilization with the addition of emulsifiers through the process of high-pressure homogenization or microemulsification (Yadav et al., 2013; Malam et al., 2009). Mono-, di- or triglycerides; steroids; free fatty alcohol or acids; and wax are some of the solid lipids used for the production of SLNCs, as shown in Fig. 11.4 (Torchilin, 2011; Rouco et al., 2020; Mäder & Mehnert, 2004).

SLNCs can be classified into two types: solid lipid NPs (SLNPs) and nanostructured lipid carriers (NLCs) (Naseri et al., 2015; Schwarz et al., 1994). Solid lipids are the major components of SLNPs, whereas NLCs contain solid and liquid lipids (Müller et al., 2002). SLNPs can carry both micro- and macro-molecules (protein and peptides) (Mu & Holm, 2018), using appropriate excipients and adopting suitable method of formulation or preparation, whereas NLCs are designed to improve the shortcomings of SLNCs (Das & Chaudhury, 2011; Kim et al., 2005). The drug

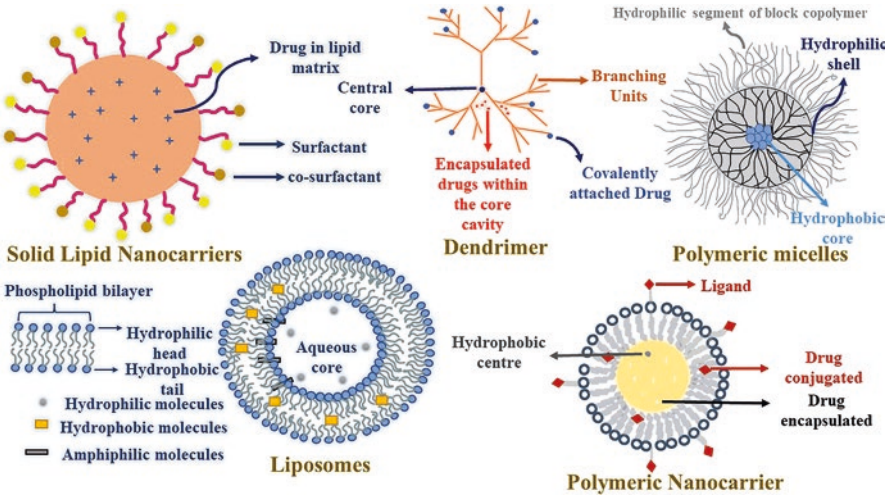


Fig. 11.3 Various classes of organic drug NCs

loading and release profile are both significantly impacted by the change in the lipid composition of SLNCs (Das et al., 2012; Balguri et al., 2016). Molecular drugs can be integrated into the matrix, shell or core of the solid lipid depending on the manufacturing conditions and conformation. Due to their versatility, SLNCs can overcome the limitations of conventional chemotherapy (Hallan et al., 2016). SLNCs, when loaded with curcumin, have also been investigated for breast cancer treatment (Wang et al., 2018). Furthermore, ionic and hydrophilic anti-cancer drugs can now be added to lipophilic drugs using SLNCs. These can also be utilized in parenteral and oral drug delivery (Chamundeeswari et al., 2019). SLNCs have provided value-added advantages as drug carriers in the field of pharmaceutics (Yaghmur & Mu, 2021).

11.4.2 Liposomes

Liposomes are the colloidal spherical structure made up of self-assembled phospholipids or amphiphilic lipid molecules (Guimarães et al., 2021; Sebaaly et al., 2016). Liposomal NCs possess a size of 50–100 nm. The liposomal membrane is composed of lamellas, that is, unilamellar or multilamellar lipid bilayers, forming a spherical vesicle (Nisini et al., 2018; Laouini et al., 2012). The lipid bilayers serve as the vehicles for hydrophilic and lipophilic drug delivery at the specific site. However, in systemic circulation, these molecules possess limited half-life. Therefore, polymeric molecules like polyethylene glycol (PEG) can be used to coat liposomes to create PEGylated liposomes or stealth liposomes. The stealth liposomes can evade the reticulate endothelial system owing to their long stability in

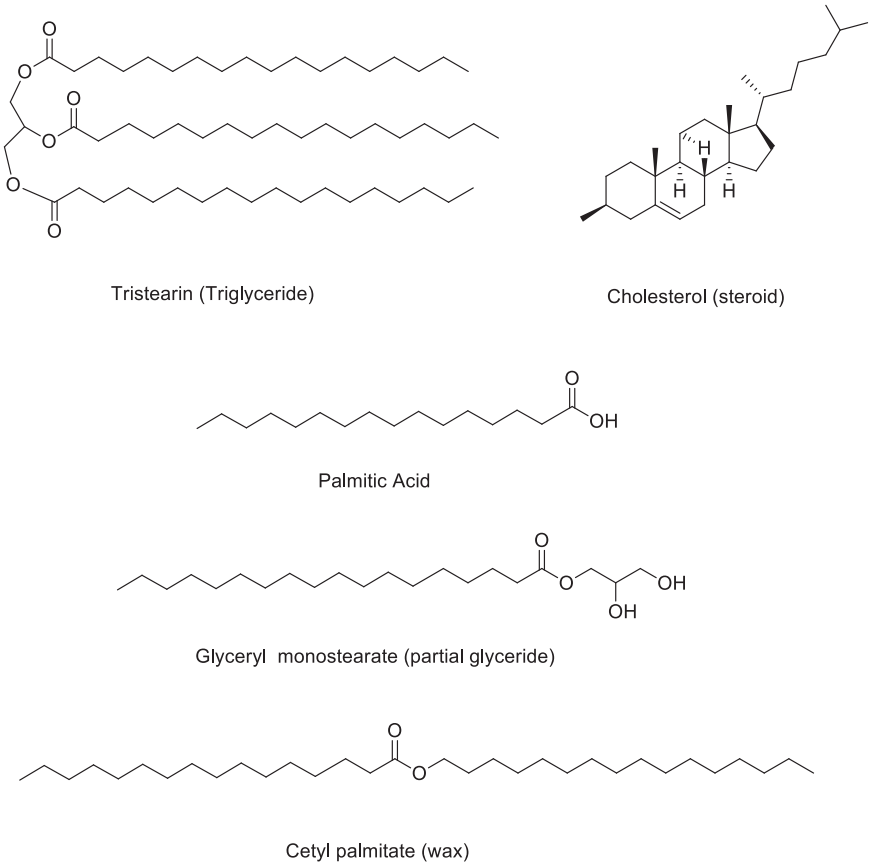


Fig. 11.4 Chemical structure of SLNCs

blood, which results in producing sustained drug release (Torchilin, 2000). Liposomes ultimately enhance the biodistribution and pharmacokinetics of incorporated drug molecules (Wang et al., 2012). Moreover, because of their structural versatility, biocompatibility and non-immunogenic nature, they are well sought as a good drug delivery agent. The amphiphilic nature of phospholipids in solution is similar to that of natural cell membranes, and this results in an effective interaction of liposomes with mammalian cell membranes to promote cellular absorption (Laouini et al., 2012). Liposomes are capable of carrying large drug payloads and have a wide range of physicochemical properties (Sercombe et al., 2015). Liposomes have enhanced biomedical and therapeutic properties that enable the biodistribution of drugs to the target site in vivo (Hua & Wu, 2013; Ding et al., 2006).

11.4.3 Polymeric Micelles (PMs)

Polymeric micelles (PMs) are the multifunctional NPs (10–100 nm) formed by the spontaneous association of di- or tri-block polymeric components (copolymers) or synthetic amphiphilic surfactants in an aqueous milieu to form micelle core–shell structures. A micelle's hydrophobic inner core is enclosed by a shell of hydrophilic polymers such as polyethylene glycol. The hydrophobic inner core contains amphiphilic and poorly water-soluble drugs, whereas the hydrophilic shell stabilizes the core. However, the hydrophilic shell of PMs allows solubility in aqueous media and modulates in vivo pharmacokinetics (Begines et al., 2020; Majumder et al., 2020). Until now, various drug components can be incorporated in PMs via covalent/chemical attachment (Wu et al., 2012) or physical attachment (Din et al., 2017; Batrakova et al., 1996; Nakanishi et al., 2001). PMs can be prepared by oil-in-water emulsion, dialysis, cosolvent evaporation, freeze-drying and solvent evaporation methods (Rapoport, 2007). PMs have been thought of as suitable NCs for the controlled release of biomedical drug delivery (Kaur et al., 2022). Anti-cancer medications are aquaphobic, and PMs can entrap these aquaphobic drug components within their core, which ultimately increases their water solubility. Drugs can be loaded into micellar systems with efficiency and ease via physical entrapment. Several anticancer medications, such as doxorubicin and paclitaxel, have been physically trapped for ultrasonic delivery in PMs (Rapoport et al., 1999a, b, 2000). In addition, PM-based nucleic acid carriers have been studied as nucleic acid therapeutics permit for therapeutic modulation of gene expression (Jarak et al., 2021; Toscanini et al., 2021; Howard et al., 2006).

11.4.4 Dendrimers

Dendrimers are multivalent globular nanoscale macromolecules with an initiator core in the centre, forming a star- or tree-like shape (Pawar et al., 2020), with a size of 1–10 nm. They have active terminal groups and provide a high range of surface functionality. Dendrimers are made up of nucleotides, amino acids and sugar molecules. The core cavities encapsulate the drug molecules within them via chemical interaction, hydrophobic bonds and hydrogen (H) bonds or are attached to the active terminal groups by covalent bonds. This class of NCs is used to encapsulate drugs like rifampicin, which are further used for the treatment of tuberculosis due to their structural applications (Mignani et al., 2018). Numerous anti-cancer medications, including dox and cisplatin, coupled with dendrimers create improved anti-cancer activity (Lai et al., 2007). Dendrimers play a significant role in DDS which include Oral drug delivery, transdermal drug delivery, ocular drug delivery, targeted gene delivery, and anticancer drug delivery (Mathur et al., 2015).

11.4.5 Polymeric Nanocarriers

Polymeric NCs (PNCs) are made from biodegradable synthetic polymers, semi-synthetic polymers or natural polymers. Figures 11.5 and 11.6 show the different classes and chemical structures of synthetic PNCs, respectively (Avramović et al., 2020; Wang et al., 2009). The solid colloidal PNCs can act as a reservoir type for nanocapsules that diffuse or encapsulate the molecular drugs in the matrix of polymers, that is, nanospheres (Prabhu et al., 2015). PNCs undergo a polymerization reaction involving several monomer units (Calzoni et al.; Zhu & Liao, 2015). When compared to other NCs, PNCs provide better stability, good drug payload tendency, adequate half-life in systemic circulation and prolonged drug release. To target cancerous cells, an anti-cancer medicine like dox is encapsulated inside PNCs. Physicochemical changes in the polymeric source can improve the regulated release of the medication. Multifunctional PNCs can also be made, which allow for the inclusion of various medications within them (Zhu & Liao, 2015; Nelemans & Gurevich, 2020). PNCs allow the active and passive modes of drug delivery, provide high concentration of drug delivery and preserve constancy of volatile pharmaceutical agents (López-Dávila et al., 2012).

11.5 Hybrid NCs

The combination of more than two organic and inorganic NCs, either organic–organic or inorganic–inorganic NCs, are termed as hybrid NCs. These NCs overcome all the disadvantages of the individual ones by incorporating two or more NPs

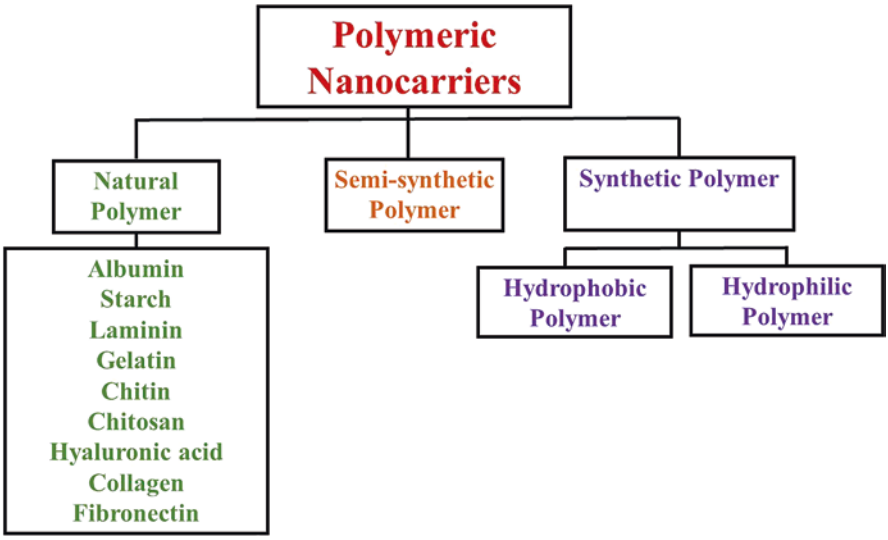


Fig. 11.5 Different classes of PNCs

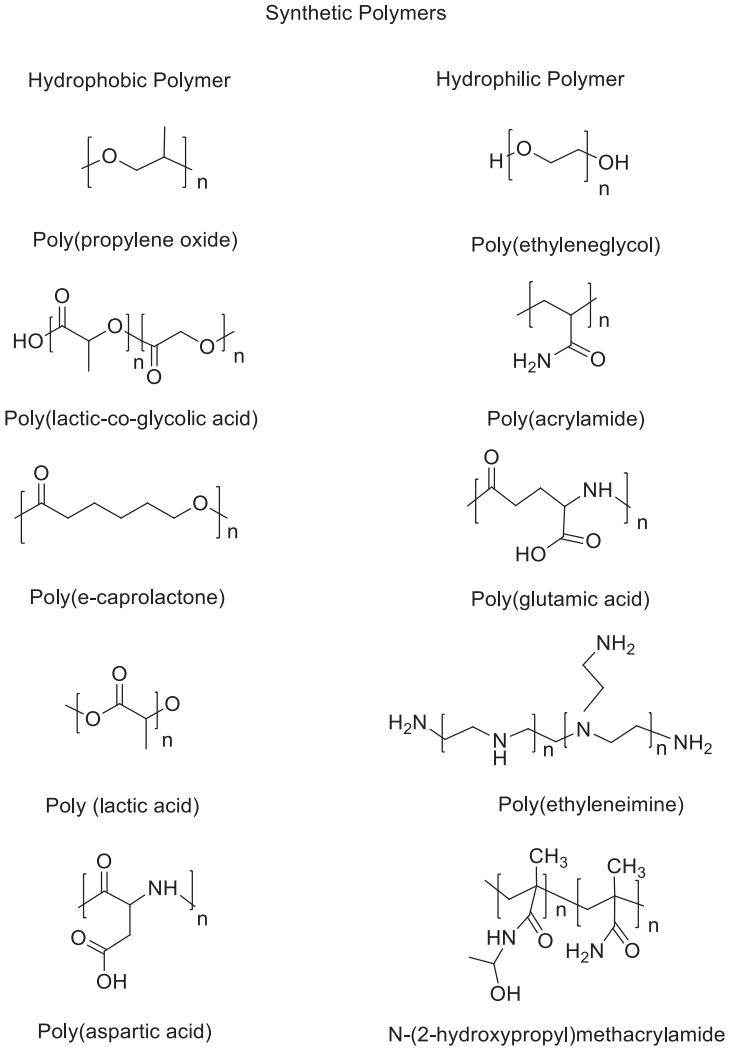


Fig. 11.6 Chemical structure of synthetic PNCs

together (Qian et al., 2012). This can be seen in the case of liposomes. Being unstable, liposomes are easily removed from the bloodstream, which makes them less effective. On the other hand, using hybrid liposomes can solve such problems. Further, these are classified into lipid polymer hybrid and ceramic polymer hybrid (Peer et al., 2007). During the selection of NPs to form hybrid NCs, various parameters should be followed such as drug type to be conjugated, site of action, physiological barrier while delivering the drugs and stability and solubility of the NCs. These hybrid NCs offer larger bioavailability of therapeutic substances with fewer adverse effects. Some of the most important examples of hybrid NCs are mesoporous silica NP–lipid bilayer hybrid NC system, which is useful for intracellular

delivery of zoledronic acid with a high retention rate in breast cancer and prevention of the premature release of drug (Desai et al., 2017).

11.6 Safety Concerns and Future Perspectives

With advancements in the field of nanotechnology, a new field has emerged which is nanotoxicology. As the name suggests, nanotoxicology is concerned with the toxic effect of nanomaterials on biological systems. Some of the studies have shown that nanomaterials lead to the formation of free radicals that can further damage our brain cells and can also cause unnecessary penetration through the epidermis. This can lead to many other toxic effects on the biological system (Shvedova et al., 2011; Niemeier et al., 2006; Oberdörster et al., n.d.; Lovrić et al., 2005). This is a serious obstacle confronted by pharmaceutical companies during NP formulation and encapsulation. Nanomaterials have great potential as nanomedicines because of their many advantages. Thus, a well-defined database is required to be followed by the experts, which should contain information about the nano DDS, storage, handling protocols and most importantly its toxicology towards biological systems (Kirchner et al., 2005; Choi et al., 2007; Chang et al., 2007). Moreover, a detailed investigation over the issues related to their shape, size, production and surface characterization should be performed to enhance their bioavailability and long-term advantages.

11.7 Conclusion

Pharmaceutical nanocarriers including micelles, nano emulsions, liposomes, PMs, NPs, etc., exhibit a wide range of beneficial properties, which includes longevity in blood, enabling their desired concentration in pathophysiological areas with the compromised vascular system; precise affected site targeting (due to various targeting ligands linked to the NCs surface), and many more. NCs have shown high efficacy and biocompatibility compared to traditional DDSs. Potential for creating extremely effective and precise systems for medications, diagnostics and genetic agents using pharmaceutical nanocarriers is practically limitless. Thus, the combination of nanotechnology and medicinal drugs has produced an offspring that is expected to lead to significant advancements in the treatment of several ailments. It is crucial to comprehend how the biodistribution of NCs affects the body's intricate biological network and mass transport throughout the compartmental boundaries. In addition, the development of a toxicological catalogue to facilitate safety and hazard assessments is essential for the healthy progress of this area. A well-defined database can be of great importance to formulate more nanodrugs. In a nutshell, NCs are the future of DDS owing to their specificity and effectiveness in treating a wide range of diseases, as well as their numerous therapeutic uses.

References

Abulibdeh, N., Kumar, K. V., Karthika, C., Jarin, T., Gopi, A., & Bouzidi, A. (2019). Exploring magnetic fluid sensor using dual circular core elliptical cladding photonic crystal fiber. *Results in Physics, 13*(February), 4–5.

Advanced C. Pe Off r ic s e o Phone: Education formation Foreign Languages. Research Areas Published journal articles indexed by a n d. 2022;2022.

Åkerman, M. E., Chan, W. C. W., Laakkonen, P., Bhatia, S. N., & Ruoslahti, E. (2002). Nanocrystal targeting in vivo, *99*(20), 12617–12621.

Almessiere, M. A., Slimani, Y., & Baykal, A. (2018a). Author's accepted manuscript. *Ceramics International [Internet]*. https://doi.org/10.1016/j.ceramint.2018.09.272

Almessiere, M. A., Slimani, Y., & Baykal, A. (2018b). Structural, morphological and magnetic properties of hard/soft SrFe12-xVxO19/(Ni0.5Mn0.5Fe2O4)y nanocomposites: Effect of vanadium substitution. *Journal of Alloys and Compounds, 767*, 966–975.

Angelos, S., Johansson, E., Stoddart, J. F., & Zink, J. I. (2007). Mesostructured silica supports for functional materials and molecular machines. *Advanced Functional Materials, 17*(14), 2261–2271.

Avramović, N., Mandić, B., Savić-Radojević, A., & Simić, T. (2020). Polymeric nanocarriers of drug delivery systems in cancer therapy. *Pharmaceutics, 12*(4), 1–17.

Balguri, S. P., Adelli, G. R., & Majumdar, S. (2016). Topical ophthalmic lipid nanoparticle formulations (SLN, NLC) of indomethacin for delivery to the posterior segment ocular tissues. *European Journal of Pharmaceutics and Biopharmaceutics [Internet], 109*, 224–235. https://doi.org/10.1016/j.ejpb.2016.10.015

Bamrungsap, S., Zhao, Z., Chen, T., Wang, L., Li, C., Fu, T., et al. (2012). Nanotechnology in therapeutics: A focus on nanoparticles as a drug delivery system. Review. *Carbohydrate Polymers* [Internet], *1*(1), 71–88. Available from: https://doi.org/10.1016/j.nano.2010.07.004%5Cn., http://linkinghub.elsevier.com/retrieve/pii/S1818087616300502%5Cn, https://doi.org/10.1016/j.carbpol.2016.06.026%5Cn, http://www.cancerjournal.net/article.asp?issn=0973-1482%5Cnyear=2014%5Cnvolume=10%5Cnissue=

Baskar, G., Lalitha, K., Garrick, B. G., & Chamundeeswari, M. (2017a). Conjugation, labeling and characterization of asparaginase bound silver nanoparticles for anticancer applications. *Indian Journal of Experimental Biology, 55*(7), 421–426.

Baskar, G., Bikku George, G., & Chamundeeswari, M. (2017b). Synthesis and characterization of asparaginase bound silver nanocomposite against ovarian cancer cell line A2780 and lung cancer cell line A549. *Journal of Inorganic and Organometallic Polymers and Materials [Internet], 27*(1), 87–94. https://doi.org/10.1007/s10904-016-0448-x

Batrakova, E. V., Dorodnych, T. Y., Klinskii, E. Y., Kliushnenkova, E. N., Shemchukova, O. B., Goncharova, O. N., et al. (1996). Anthracycline antibiotics non-covalently incorporated into the block copolymer micelles: In vivo evaluation of anti-cancer activity. *British Journal of Cancer, 74*(10), 1545–1552.

Begines, B., Ortiz, T., Pérez-Aranda, M., Martínez, G., Merinero, M., Argüelles-Arias, F., et al. (2020). Polymeric nanoparticles for drug delivery: Recent developments and future prospects. *Nanomaterials, 10*(7), 1–41.

Bianco, A. (2004). Carbon nanotubes for the delivery of therapeutic molecules. *Expert Opinion on Drug Delivery, 1*(1), 57–65.

Bottini, M., D'Annibale, F., Magrini, A., Cerignoli, F., Arimura, Y., Dawson, M. I., et al. (2007). Quantum dot-doped silica nanoparticles as probes for targeting of T-lymphocytes. *International Journal of Nanomedicine [Internet], 2*(2), 227–233. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17722550%0A, http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2673975

Calzoni, E., Cesaretti, A., Polchi, A., Michele, A. D., Tancini, B., & Emiliani, C. Biocompatible polymer nanoparticles for drug delivery applications in cancer and neurodegenerative disorder therapies. *Journal of Functional Biomaterials, 10*, 1–15.

Chamundeeswari, M., Sastry, T. P., Lakhsmi, B. S., Senthil, V., & Agostinelli, E. (2013). Iron nanoparticles from animal blood for cellular imaging and targeted delivery for cancer treatment. *Biochimica et Biophysica Acta (BBA) - General Subjects, 1830*(4), 3005–3010.

Chamundeeswari, M., Jeslin, J., & Verma, M. L. (2019). Nanocarriers for drug delivery applications. *Environmental Chemistry Letters [Internet], 17*(2), 849–865. https://doi.org/10.1007/s10311-018-00841-1

Chang, J. S., Chang, K. L. B., Hwang, D. F., & Kong, Z. L. (2007). In vitro cytotoxicitiy of silica nanoparticles at high concentrations strongly depends on the metabolic activity type of the cell line. *Environmental Science and Technology, 41*(6), 2064–2068.

Choi, A. O., Ju, S. J., Desbarats, J., Lovrić, J., & Maysinger, D. (2007). Quantum dot-induced cell death involves Fas upregulation and lipid peroxidation in human neuroblastoma cells. *Journal of Nanobiotechnology, 5*, 1–13.

Christian, S., Pilch, J., Akerman, M. E., Porkka, K., Laakkonen, P., & Ruoslahti, E. (2003). Nucleolin expressed at the cell surface is a marker of endothelial cells in angiogenic blood vessels*, 163*(4), 871–878.

Corrocher, R., Tedesco, F., Rabusin, P., & De Sandre, G. (1975). Effect of human erythrocyte stromata on complement activation. *British Journal of Haematology, 29*, 235–241.

Das, S., & Chaudhury, A. (2011). Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. *AAPS PharmSciTech, 12*(1), 62–76.

Das, S., Ng, W. K., & Tan, R. B. H. (2012). Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): Development, characterizations and comparative evaluations of clotrimazole-loaded SLNs and NLCs? *European Journal of Pharmaceutical Sciences [Internet], 47*(1), 139–151. https://doi.org/10.1016/j.ejps.2012.05.010

Derfus, A. M., Chen, A. A., Min, D., Ruoslahti, E., & Bhatia, S. N. (2007). Targeted quantum dot conjugates for siRNA delivery*, 18*, 1391–1396.

Desai, D., Zhang, J., Sandholm, J., Lehtimäki, J., Grönroos, T., Tuomela, J., et al. (2017). Lipid bilayer-gated mesoporous silica nanocarriers for tumor-targeted delivery of zoledronic acid in vivo. *Molecular Pharmaceutics, 14*(9), 3218–3227.

Descalzo, A. B., Martínez-Máñez, R., Sancenón, F., Hoffmann, K., & Rurack, K. (2006). The supramolecular chemistry of organic-inorganic hybrid materials. *Angewandte Chemie - International Edition, 45*(36), 5924–5948.

Din, F. U., Aman, W., Ullah, I., Qureshi, O. S., Mustapha, O., et al. (2017). Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *International Journal of Nanomedicine, 12*, 7291–7309.

Ding, B. S., Dziubla, T., Shuvaev, V. V., Muro, S., & Muzykantov, V. R. (2006). Advanced drug delivery systems that target the vascular endothelium. *Molecular Interventions [Internet]*. [cited 2022 Aug 8], *6*(2), 98–112. Available from: https://pubmed.ncbi.nlm.nih.gov/16565472/

Freeman, R., Liu, X., & Willner, I. (2011). Chemiluminescent and Chemiluminescence Resonance Energy Transfer (CRET) detection of DNA, metal ions, and aptamer À substrate complexes using hemin / G-quadruplexes and CdSe / ZnS quantum dots. *Journal of the American Chemical Society, 133*, 11597–11604.

García, M. A. (2011). Surface plasmons in metallic nanoparticles: Fundamentals and applications. *Journal of Physics D: Applied Physics, 44*(8), 283001.

Geißler, D., Charbonni, L. J., Ziessel, R. F., Butlin, N. G., Löhmannsröben, H., & Hildebrandt, N. (2010). Quantum dot biosensors for ultrasensitive multiplexed diagnostics. *Angewandte Chemie International Edition., 49*, 1396–1401.

Gellini, C., & Feis, A. (2021). Optothermal properties of plasmonic inorganic nanoparticles for photoacoustic applications. *Photoacoustics, 23*, 100281.

Ghosn, Y., Kamareddine, M. H., Tawk, A., Elia, C., El Mahmoud, A., Terro, K., et al. (2019). Inorganic nanoparticles as drug delivery systems and their potential role in the treatment of chronic myelogenous leukaemia. *Technology in Cancer Research & Treatment, 18*, 1–12.

Goldenberg, L. M., Köhler, M., Kahle, O., & Dreyer, C. (2020). Impact of inorganic nanoparticles on optical properties of low refractive index waveguiding polymers. *Optical Materials Express, 10*(11), 2987.

Guimarães, D., Cavaco-Paulo, A., & Nogueira, E. (2021). Design of liposomes as drug delivery system for therapeutic applications. *International Journal of Pharmaceutics, 601*(March).

Hallan, S. S., Kaur, P., Kaur, V., Mishra, N., & Vaidya, B. (2016). Lipid polymer hybrid as emerging tool in nanocarriers for oral drug delivery. *Artificial Cells, Nanomedicine and Biotechnology, 44*(1), 334–349.

Hong, S., Choi, D. W., Kim, H. N., Park, C. G., Lee, W., & Park, H. H. (2020). Protein-based nanoparticles as drug delivery systems. *Pharmaceutics, 12*(7), 1–28.

Howard, B., Gao, Z., Lee, S. W., Seo, M. H., & Rapoport, N. (2006). Ultrasound-enhanced chemotherapy of drug-resistant breast cancer tumors by micellar-encapsulated paclitaxel. *American Journal of Drug Delivery, 4*(2), 97–104.

Hua, S., & Wu, S. Y. (2013). The use of lipid-based nanocarriers for targeted pain therapies, *4*(November), 1–7.

Huang, X., Li, L., Qian, H., Dong, C., & Ren, J. (2006). A resonance energy transfer between chemiluminescent donors and luminescent quantum-dots as acceptors (CRET). *Angewandte Chemie - International Edition, 45*(31), 5140–5143.

Jarak, I., Pereira-Silva, M., Santos, A. C., Veiga, F., Cabral, H., & Figueiras, A. (2021). Multifunctional polymeric micelle-based nucleic acid delivery: Current advances and future perspectives. *Applied Materials Today, 25*, 101217.

Karuppusamy, C., & Venkatesan, P. (2017). Role of nanoparticles in drug delivery system: A comprehensive review. *Journal of Pharmaceutical Sciences and Research, 9*(3), 318–325.

Kaur, J., Gulati, M., Jha, N. K., Disouza, J., Patravale, V., Dua, K., et al. (2022). Recent advances in developing polymeric micelles for treating cancer: Breakthroughs and bottlenecks in their clinical translation. *Drug Discovery Today, 27*(5), 1495–1512.

Khandelia, R., Jaiswal, A., Ghosh, S. S., & Chattopadhyay, A. (2013). Gold nanoparticle-protein agglomerates as versatile nanocarriers for drug delivery. *Small, 9*(20), 3494–3505.

Kianfar, E. (2021). Magnetic nanoparticles in targeted drug delivery: A review. *Journal of Superconductivity and Novel Magnetism [Internet], 34*(7), 1709–1735. https://doi.org/10.1007/s10948-021-05932-9

Kim, B. D., Na, K., & Choi, H. K. (2005). Preparation and characterization of solid lipid nanoparticles (SLN) made of cacao butter and curdlan. *European Journal of Pharmaceutical Sciences, 24*(2–3), 199–205.

Kingsley, J. D., Dou, H., Morehead, J., Rabinow, B., Gendelman, H. E., & Destache, C. J. (2006). Nanotechnology: A focus on nanoparticles as a drug delivery system. *Journal of Neuroimmune Pharmacology, 1*(3), 340–350.

Kirchner, C., Liedl, T., Kudera, S., Pellegrino, T., Javier, A. M., Gaub, H. E., et al. (2005). Cytotoxicity of colloidal CdSe and CdSe/ZnS nanoparticles. *Nano Letters, 5*(2), 331–338.

Lai, P. S., Lou, P. J., Peng, C. L., Pai, C. L., Yen, W. N., Huang, M. Y., et al. (2007). Doxorubicin delivery by polyamidoamine dendrimer conjugation and photochemical internalization for cancer therapy. *Journal of Controlled Release, 122*(1), 39–46.

Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C., & Fessi, H. (2012). Preparation, characterization and applications of liposomes: State of the art. *Journal of Colloid Science and Biotechnology, 1*(2), 147–168.

Li, Y., Li, N., Pan, W., Yu, Z., Yang, L., & Tang, B. (2017). Hollow mesoporous silica nanoparticles with tunable structures for controlled drug delivery. *ACS Applied Materials & Interfaces, 9*, 2123.

Liu, Z., Chen, K., Davis, C., Sherlock, S., Cao, Q., Chen, X., et al. (2008). Drug delivery with carbon nanotubes for in vivo cancer treatment. *Cancer Research, 68*(16), 6652–6660.

Liu, D., Liu, C., Zou, W., & Zhang, N. (2010). Enhanced gastrointestinal absorption of N3-O-toluylfluorouracil by cationic solid lipid nanoparticles. *Journal of Nanoparticle Research, 12*(3), 975–984.

López-Dávila, V., Seifalian, A. M., & Loizidou, M. (2012). Organic nanocarriers for cancer drug delivery. *Current Opinion in Pharmacology, 12*(Figure 1), 414–419.

Lovrić, J., Bazzi, H. S., Cuie, Y., Fortin, G. R. A., Winnik, F. M., & Maysinger, D. (2005). Differences in subcellular distribution and toxicity of green and red emitting CdTe quantum dots. *Journal of Molecular Medicine, 83*(5), 377–385.

Lu, W., Huang, Q., Ku, G., Wen, X., Zhou, M., Guzatov, D., et al. (2010). Photoacoustic imaging of living mouse brain vasculature using hollow gold nanospheres. *Biomaterials, 31*(9), 2617–2626.

Ma, P., Xiao, H., Li, C., Dai, Y., Cheng, Z., Hou, Z., et al. (2015). Inorganic nanocarriers for platinum drug delivery. *Materials Today [Internet], 18*(10), 554–564. https://doi.org/10.1016/j.mattod.2015.05.017

Mäder, K., & Mehnert, W. (2004). Solid lipid nanoparticles - Concepts, procedures, and physicochemical aspects. In *Lipospheres in drug targets and delivery: Approaches, methods, and applications*, 1–22 p.

Majumder, N., Das, N. G., & Das, S. K. (2020). Polymeric micelles for anticancer drug delivery. *Therapeutic Delivery, 11*(10), 613–635.

Malam, Y., Loizidou, M., & Seifalian, A. M. (2009). Liposomes and nanoparticles: Nanosized vehicles for drug delivery in cancer. *Trends in Pharmacological Sciences, 30*(11), 592–599.

Mathur, V., Satrawala, Y., & Rajput, M. S. (2015). Dendrimers: A review Dendrimers (January 2010).

Medina, C., Santos-Martinez, M. J., Radomski, A., Corrigan, O. I., & Radomski, M. W. (2007). Nanoparticles: Pharmacological and toxicological significance. *British Journal of Pharmacology, 150*(5), 552–558.

Medintz, I. L., Farrell, D., Susumu, K., Trammell, S. A., Deschamps, J. R., Brunel, F. M., et al. (2009). Multiplex charge-transfer interactions between quantum dots and peptide-bridged ruthenium complexes*, 81*(12), 4831–4839.

Mignani, S., Tripathi, R. P., Chen, L., Caminade, A. M., Shi, X., & Majoral, J. P. (2018). New ways to treat tuberculosis using dendrimers as nanocarriers. *Pharmaceutics, 10*(3).

Mishra, B., Patel, B. B., & Tiwari, S. (2010). Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. *Nanomedicine: Nanotechnology, Biology and Medicine, 6*(1), 9–24.

Mohammed, I. A., & Al-Gawhari, F. J. (2020). Gold nanoparticle: Synthesis, functionalization, enhancement, drug delivery and therapy: A review. *Systematic Reviews in Pharmacy, 11*(6), 888–910.

Mu, H., & Holm, R. (2018). Solid lipid nanocarriers in drug delivery: Characterization and design. *Expert Opinion on Drug Delivery [Internet], 15*(8), 771–785. https://doi.org/10.1080/17425247.2018.1504018

Müller, R. H., Radtke, M., & Wissing, S. A. (2002). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced Drug Delivery Reviews, 54*(SUPPL), 131–155.

Nakanishi, T., Fukushima, S., Okamoto, K., Suzuki, M., Matsumura, Y., Yokoyama, M., et al. (2001). Development of the polymer micelle carrier system for doxorubicin. *Journal of Controlled Release, 74*(1–3), 295–302.

Naseri, N., Valizadeh, H., & Zakeri-Milani, P. (2015). Solid lipid nanoparticles and nanostructured lipid carriers: Structure, preparation and application. *Advanced Pharmaceutical Bulletin [Internet], 5*(3), 305–313. Available from: http://apb.tbzmed.ac.ir

Nelemans, L. C., & Gurevich, L. (2020). Drug delivery with polymeric nanocarriers — Cellular uptake mechanisms. *Materials, 13*(Figure 1), 366.

Ng, C. M., Loh, H. S., Muthoosamy, K., Sridewi, N., & Manickam, S. (2016). Conjugation of insulin onto the sidewalls of single-walled carbon nanotubes through functionalization and diimide-activated amidation. *International Journal of Nanomedicine, 11*, 1607–1614.

Niemeier, U., Granier, C., Kornblueh, L., Walters, S., & Brasseur, G. P. (2006). Global impact of road traffic on atmospheric chemical composition and on ozone climate forcing. *Journal of Geophysical Research Atmospheres, 111*(9), 4326–4335.

Nisini, R., Poerio, N., Mariotti, S., De Santis, F., & Fraziano, M. (2018). The multirole of liposomes in therapy and prevention of infectious diseases. *Frontiers in Immunology, 9*(FEB).

Nutter, D. O., & Ratts, T. (1973). Direct actions of prostaglandins E1, A1, and F2α on myocardial contraction. *Prostaglandins, 3*(3), 323–336.

O'Regan, B., & Gratzer, M. (1991). © 19 9 1 Nature Publishing Group 그라첼꺼. *Nature, 354*, 737–740.

Oberdörster, G., Oberdörster, E., & Oberdörster, J. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles supplemental web sections. *Environmental Medicine*.

Ohulchanskyy, T. Y., Roy, I., Goswami, L. N., Chen, Y., Bergey, E. J., Pandey, R. K., et al. (2007). Organically modified silica nanoparticles with covalently incorporated photosensitizer for photodynamic therapy of cancer. *Nano Letters, 7*(9), 2835–2842.

Pawar, R. S., Dimri, M., Maithani, A., & Luv, K. (2020). Asian Journal of Pharmaceutical Research and Development. *The application of Marine Natural Products (MNPS) in anti-Covid-19 therapeutics, 8*(6), 77–80.

Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R., & Langer, R. (2007). 84 Nat nanotech 2007 R Langer Nanocarriers as an emerging platform for cancer therapy.pdf. *Nature Nanotechnology, 2*, 751–760.

Peng, Y., Bariwal, J., Kumar, V., Tan, C., & Mahato, R. I. (2020). Organic nanocarriers for delivery and targeting of therapeutic agents for cancer treatment. *Advanced Therapeutics, 3*(2), 1–45.

Prabhu, R. H., Patravale, V. B., & Joshi, M. D. (2015). Polymeric nanoparticles for targeted treatment in oncology: Current insights. *International Journal of Nanomedicine, 10*, 1001–1018.

Prosperi, D., Colombo, M., Zanoni, I., & Granucci, F. (2017 Dec 1). Drug nanocarriers to treat autoimmunity and chronic inflammatory diseases. *Seminars in Immunology, 34*, 61–67.

Qian, X., Peng, X. H., Ansari, D. O., Yin-Goen, Q., Chen, G. Z., Shin, D. M., et al. (2008). In vivo tumor targeting and spectroscopic detection with surface-enhanced Raman nanoparticle tags. *Nature Biotechnology, 26*(1), 83–90.

Qian, W. Y., Sun, D. M., Zhu, R. R., Du, X. L., Liu, H., & Wang, S. L. (2012). pH-sensitive strontium carbonate nanoparticles as new anticancer vehicles for controlled etoposide release. *International Journal of Nanomedicine, 7*, 5781–5792.

Rapoport, N. (2007). Physical stimuli-responsive polymeric micelles for anti-cancer drug delivery. *Progress in Polymer Science, 32*(8–9), 962–990.

Rapoport, N. Y., Herron, J. N., Pitt, W. G., & Pitina, L. (1999a). Micellar delivery of doxorubicin and its paramagnetic analog, ruboxyl, to HL-60 cells: effect of micelle structure and ultrasound on the intracellular drug uptake. *Journal of Controlled Release, 58*(2), 153–162.

Rapoport, N., Smirnov, A. I., Pitt, W. G., & Timoshin, A. A. (1999b). Bioreduction of tempone and spin-labeled gentamicin by gram-negative bacteria: Kinetics and effect of ultrasound. *Archives of Biochemistry and Biophysics, 362*(2), 233–241.

Rapoport, N., Marin, A. P., & Timoshin, A. A. (2000). Effect of a polymeric surfactant on electron transport in HL-60 cells. *Archives of Biochemistry and Biophysics, 384*(1), 100–108.

Rouco, H., Diaz-Rodriguez, P., Remuñán-López, C., & Landin, M. (2020). Recent advances in solid lipid nanoparticles formulation and clinical applications. *Nanomaterials for Clinical Applications, 1*, 213–247.

Saman RA, Iqbal M. Nanotechnology-based drug delivery systems: Past, present and future. 2012;175–185.

Santos, H. A., Bimbo, L. M., Peltonen, L., & Hirvonen, J. (2014). Inorganic nanoparticles in targeted drug delivery and imaging. [cited 2022 Aug 22], 571–613. Available from: https://pureportal.strath.ac.uk/en/publications/inorganic-nanoparticles-in-targeted-drug-delivery-and-imaging

Schwarz, C., Mehnert, W., Lucks, J. S., & Müller, R. H. (1994). Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. *Journal of Controlled Release, 30*(1), 83–96.

Sebaaly, C., Greige-Gerges, H., Stainmesse, S., Fessi, H., & Charcosset, C. (2016). Effect of composition, hydrogenation of phospholipids and lyophilization on the characteristics of eugenol-loaded liposomes prepared by ethanol injection method. *Food Bioscience, 15*, 1–10.

Sercombe, L., Veerati, T., Moheimani, F., Wu, S. Y., Sood, A. K., & Hua, S. (2015). Advances and challenges of liposome assisted drug delivery. *Frontiers in Pharmacology, 6*(DEC), 1–13.

Shi, Z., Zhou, Y., Fan, T., Lin, Y., Zhang, H., & Mei, L. (2020). Inorganic nano-carriers based smart drug delivery systems for tumor therapy. *Smart Materials in Medicine, 1*, 32–47.

Shvedova, A., Castranova, V., Kisin, E., Murray, A., Gandelsman, V., & Baron, P. (2011). Current issues exposure to carbon nanotube material: Assessment of nanotube cytotoxicity using human keratinocyte cells. *Journal of Toxocology and Environmental Health, Part A, 66*(June 2012), 1909–1926.

Singh, R., & Lillard, J. W. (2009). Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology, 86*(3), 215–223.

Slowing, I. I., Trewyn, B. G., & Lin, V. S. Y. (2007). Mesoporous silica nanoparticles for intracellular delivery of membrane-impermeable proteins. *Journal of the American Chemical Society, 129*(28), 8845–8849.

Slowing, I. I., Vivero-Escoto, J. L., Wu, C. W., & Lin, V. S. Y. (2008). Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. *Advanced Drug Delivery Reviews, 60*(11), 1278–1288.

Stergar, J., Ban, I., & Maver, U. (2019). The potential biomedical application of NiCu magnetic nanoparticles. *Magnetochemistry, 5*(4).

Sun, T., Zhang, Y. S., Pang, B., Hyun, D. C., Yang, M., & Xia, Y. (2014). Engineered nanoparticles for drug delivery in cancer therapy. *Angewandte Chemie - International Edition., 53*(46), 12320–12364.

Sur, S., Rathore, A., Dave, V., Reddy, K. R., Chouhan, R. S., & Sadhu, V. (2019). Recent developments in functionalized polymer nanoparticles for efficient drug delivery system. *Nano-Structures and Nano-Objects [Internet], 20*, 100397. https://doi.org/10.1016/j.nanoso.2019.100397

Thiruvengadam, M., Rajakumar, G., Swetha, V., Ansari, M. A., Alghamdi, S., Almehmadi, M., et al. (2021). Recent insights and multifactorial applications of carbon nanotubes. *Micromachines, 12*(12).

Torchilin, V. P. (2000). Drug targeting. *European Journal of Pharmaceutical Science, 11*(SUPPL. 2), S81–S91.

Torchilin, V. (2011). Tumor delivery of macromolecular drugs based on the EPR effect. *Advanced Drug Delivery Reviews, 63*(3), 131–135.

Toscanini, M. A., Limeres, M. J., Garrido, A. V., Cagel, M., Bernabeu, E., Moretton, M. A., et al. (2021). Polymeric micelles and nanomedicines: Shaping the future of next generation therapeutic strategies for infectious diseases. *Journal of Drug Delivery Science and Technology, 66*, 102927.

Trewyn, B. G., Slowing, I. I., Giri, S., Chen, H. T., & Lin, V. S. Y. (2007). Synthesis and functionalization of a mesoporous silica nanoparticle based on the sol-gel process and applications in controlled release. *Accounts of Chemical Research, 40*(9), 846–853.

Üzek, R., Sari, E., & Merkoçi, A. (2019). Optical-based (Bio) sensing systems using magnetic nanoparticles. *Magnetochemistry, 5*(4), 1–25.

Verma, M. L., Barrow, C. J., & Puri, M. (2012). Nanobiotechnology as a novel paradigm for enzyme immobilisation and stabilisation with potential applications in biodiesel production. *Applied Microbiology and Biotechnology* [Internet]. [cited 2022 Aug 4], *97*(1), 23–39. Available from: https://link.springer.com/article/10.1007/s00253-012-4535-9

Verma, M. L., Chaudhary, R., Tsuzuki, T., Barrow, C. J., & Puri, M. (2013). Immobilization of β-glucosidase on a magnetic nanoparticle improves thermostability: Application in cellobiose hydrolysis. *Bioresource Technology, 135*, 2–6.

Wang, Y., Xie, X., Wang, X., Ku, G., Gill, K. L., O'Neal, D. P., et al. (2004). Photoacoustic tomography of a nanoshell contrast agent in the in vivo rat brain. *Nano Letters* [Internet]. [cited 2022 Aug 22], *4*(9), 1689–1692. Available from: https://pubs.acs.org/doi/abs/10.1021/nl049126a

Wang, X., Wang, Y., Chen, Z. G., & Shin, D. M. (2009). Advances of cancer therapy by nanotechnology. *Cancer Research and Treatment, 41*(1), 1.

Wang, A. Z., Langer, R., & Farokhzad, O. C. (2012). Nanoparticle delivery of cancer drugs. *Annual Review of Medicine, 63*, 185–198.

Wang, Y., Zhao, Q., Han, N., Bai, L., Li, J., Liu, J., et al. (2015). Mesoporous silica nanoparticles in drug delivery and biomedical applications. *Nanomedicine: Nanotechnology, Biology, and Medicine [Internet], 11*(2), 313–327. https://doi.org/10.1016/j.nano.2014.09.014

Wang, W., Chen, T., Xu, H., Ren, B., Cheng, X., Qi, R., et al. (2018). Curcumin-loaded solid lipid nanoparticles enhanced anticancer efficiency in breast cancer. *Molecules, 23*(7), 1–13.

Wilczewska, A. Z., Niemirowicz, K., Markiewicz, K. H., & Car, H. (2012). Nanoparticles as drug delivery systems. *Pharmacological Reports [Internet]., 64*(5), 1020–1037. https://doi.org/10.1016/S1734-1140(12)70901-5

Wu, Z., Zeng, X., Zhang, Y., Feliu, N., Lundberg, P., Fadeel, B., et al. (2012). Linear-dendritic polymeric amphiphiles as carriers of doxorubicin-In vitro evaluation of biocompatibility and drug delivery. *Journal of Polymer Science, Part A: Polymer Chemistry, 50*(2), 217–226.

Yadav, N., Khatak, S., & Singh Sara, U. V. (2013). Solid lipid nanoparticles- A review. *International Journal of Applied Pharmaceutics, 5*(2), 8–18.

Yaghmur, A., & Mu, H. (2021). Recent advances in drug delivery applications of cubosomes, hexosomes, and solid lipid nanoparticles. *Acta Pharmaceutica Sinica B, 11*(4), 871–885.

Zahin, N., Anwar, R., Tewari, D., Kabir, M. T., Sajid, A., Mathew, B., et al. (2020). Nanoparticles and its biomedical applications in health and diseases: special focus on drug delivery. *Environmental Science and Pollution Research, 27*(16), 19151–19168.

Zhu, Y., & Liao, L. (2015). Applications of nanoparticles for anticancer drug deliver: A review, *15*(7), 4753–4773.

Chapter 12
Gold Nanoparticle Preparation for Antibodies and Optimization Against Infections

Jyothy G. Vijayan

Abbreviations

BSA	Bovine Serum Albumin
CNTs	Carbon nanotubes
DLS	Dynamic light scattering
GNPs	Gold nanoparticles
HIV	Human Immuno Deficiency Virus
P	phosphorous
pDNA	Plasmid DNA
PEG	Polyethylene glycol
S	Sulfur
SEM	Scanning electron microscope
SiRNA	Small interfering RNA
TEM	Transmission electron microscopy

12.1 Introduction

Gold nanoparticle (GNP) bioconjugation has emerged as an important method for the advanced identification of pathogens (Pissuwan et al., 2010). Unique optical and surface properties and consistency make GNPs an unavoidable vessel for bioconjugation. Many scientists have studied the antibody bioconjugation of GNP to apply as a detector to find specific type of bacteria. Recently, antibody-conjugated GNPs have been synthesized for the detection of protein markers via tissue imaging and immune capture assays. In antibody-conjugated GNPs, antigens were tagged and noted from gold ions generated by laser ionization mass spectroscopy (El-Sayed & Huang, 2005). According to the researchers, GNPs can be applied as adjuvants to

J. G. Vijayan (✉)
Department of Chemistry, M.S. Ramaiah University of Applied Sciences, Bengaluru, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_12

increase the effectiveness of vaccines (Dykman, 2020). It has been done by stimulating antigen-presenting cells and controlled antigen release. According to the reports, GNPs have been applied to synthesize antibodies and vaccines against more than 45 types of pathogens of viral, bacterial, and parasitic infections. GNPs as carriers and as adjuvants help increase the immune response and support vaccine design. GNPs have been used as antigen carriers for immunization and vaccination in the last few decades. Due to their low toxicity, simple preparation, and unique physicochemical properties, they are applied in the biomedical field. According to the earlier reports, adjuvant characteristics are inherited in GNPs. This chapter discusses their synthesis, functionalization, and applications (Fig. 12.1).

12.2 Synthesis and Properties of GNPs for the Synthesis of Antibodies and Vaccine Development

12.2.1 Synthesis of GNPs

There are two methods for synthesizing GNPs: the top-down and bottom-up methods.

12.2.1.1 Top-Down Approach

It is a subtractive method, beginning with the segregation of bulk materials and ending with self-assembled nanoscale objects, for example, micropatterning and photolithography (Fu et al., 2018). The top-down approach is fast, but the main concern

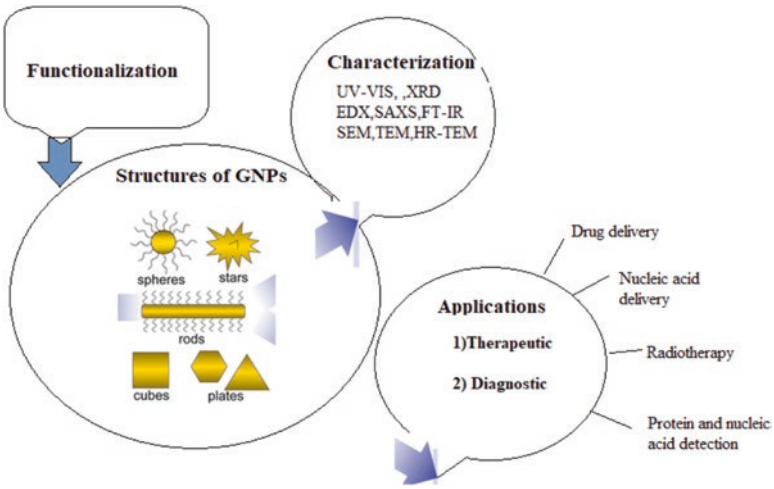


Fig. 12.1 Structure, characterization, functionalization, and applications of GNPs

is about achieving a uniform size during the synthesis. Recently developed microcontact deprinting overcomes the limitation. It is a fast and cheap method to synthesize NPs on a wide range of substrates. Other methods such as pyrolysis, lithography, radiation-induced method, and thermolysis are also used for synthesis.

12.2.1.2 Bottom-Up Approach

The bottom-up approach is an emerging method. It involves three types of syntheses.

I. *Physical Approach*: It involves microwave irradiation, ultrasound irradiation, ultraviolet irradiation, laser ablation, ion implantation, and gamma irradiation (Bandi et al., 2023).
II. *Chemical Approach*: It involves chemical irradiation of metal ions in solution by adding stabilizing agents, chemical agents, etc. Stabilizing agents include sodium borohydride, sodium hydroxide, lithium aluminum hydroxide, and ethylene glycol (Huang & Yang, 2004).
III. *Biological Approach:* In this approach, intracellular and extracellular extracts of eukaryotic and prokaryotic cells and various extracts from the parts of the plants are used (Patil & Kim, 2017).

12.2.2 Properties of GNPs

GNPs are used to synthesize antigen carrier systems for immunization. They are easy to synthesize and exhibit unique physicochemical properties. GNPs are used in different therapeutics and other applications as they can convert electromagnetic radiation to heat. GNPs display a unique surface area, size, and shape. They also possess high uptake efficiency (Jain et al., 2006). Therefore, they can penetrate into blood vessels and tissue barriers and reach the targeted sites. GNPs possess a large surface area and can be effectively applied in biomedical applications. They are applied in drug delivery, therapeutics, photothermal, and gene transfer agents. GNPs possess properties such as biocompatibility and can be easily conjugated with other main biological materials. In recent decades, they have been used in designing vaccines and other functionalizations. The main advantage of using GNPs in vaccine development is their ease of usage and functionalization only. These vaccines exhibit promising outcomes and can be used to treat diseases ranging from infections to cancers. Due to their biocompatibility, they can be used for in vitro and in vivo applications (Jia et al., 2017). When GNPs are allowed to bind with biomolecules, a new version of facile tracking of desired targets in aqueous samples can be obtained.

GNPs are applicable as an efficient antigen carrier system for immunization. Size, shape, geometry, and functionalization affect GNP functions. GNPs have unique properties such as biocompatibility and large surface area. They deliver

antigens to dendritic cells. This facilitates the immune response. GNPs are less toxic and inert in nature. They can be easily modified with proteins, peptides, and enzymes. For vaccine development, GNPs are used as adjuvants to enhance the immune response. GNPs show less toxicity in mammals (Murphy et al., 2008). They can be used as vaccine delivery vehicles. This is because of their biocompatibility and easy functionalization with antigens.

12.3 Functionalization of GNPs

GNPs are functionalized in numerous ways, which helps to design antibodies in vaccine development or in drug delivery systems. In general, GNPs covalently bind to the drug through cleavable bonds. The method used for drug delivery is very important. More important is the modification of the monolayer of GNPs. Presently, functionalization of GNPs includes the addition of functionalization of groups such as polyethylene glycol (PEG), bovine serum albumin (BSA), oligonucleotide, albumin, amino acid, and antibiotics (Tiwari et al., 2011). In PEGylation, GNPs are conjugated with PEG alone or with some molecules to confirm the cellulose uptake of GNPs effectively. Functionalized GNPs are very efficiently used for targeted drug delivery. Similarly, GNPs are conjugated with peptide/amino acids for targeted delivery systems. They include aspartic acid, lysine, glutamic acid, phenylalanine, and L-cysteine (Wangoo et al., 2008). Ammonium ions in the amino acids exhibit higher targeted drug delivery. Functionalization of GNPs with different molecules (Fig. 12.2) helps to increase the multilevel characteristics, physicochemical characteristics, etc.

GNPs have more applications in vaccinology. They can act as adjuvants and delivery agents and enhance the immune response with less toxicity (Table 12.1).

12.4 Characterization of GNPs Functionalized with Antibiotics

GNP characterization can be observed by a visual color change. The characterization of GNPs is done by two methods: (a) spectroscopic methods and (b) microscopy methods. Spectroscopic methods include techniques such as ultraviolet–visible (UV/Vis) spectrophotometry, X-Ray diffraction (XRD) analysis, energy-dispersive X-ray spectroscopy (EDXS), small-angle X-ray scattering (SAXS), and Fourier transform infrared (FT-IR) spectroscopy (Balasubramanian et al., 2010). Microscopic methods involve scanning electron microscopy (SEM), tunneling electron microscopy (TEM), high-resolution TEM (HR-TEM), and atomic force microscopy (AFM). FT-IR spectroscopy was used to analyze the nature of binding of antibiotics to GNPs. Dynamic light scattering is used to analyze the size

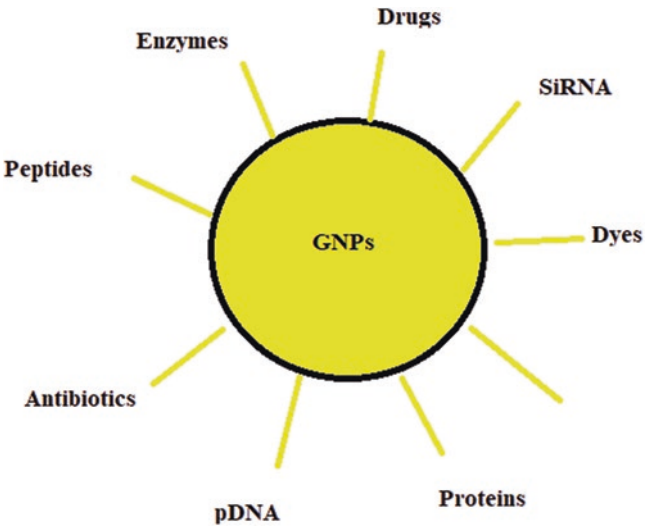


Fig. 12.2 Functionalization of GNPs

Table 12.1 Morphology and application of functionalized GNPs

Morphology of GNPs	Application	Experiment	Functionalization	References
Sphere	Adjuvant	In vivo	Polyinosinic/polycytidylic acid (PolyIC)	Tazaki et al. (2018)
Sphere	Bacterial infection	In vivo	Oligosaccharides	Safari et al. (2012)
Sphere	Cancer	In vivo	Disaccharides	Brinas et al. (2012)
Sphere	Viral	In vivo	Proteins	Tao et al. (2014)
Sphere	Parasitic	In vivo	CHrPfs25	Kumar et al. (2015)

distribution of GNPs, and the purity and the crystalline nature are confirmed by XRD analysis. It also provides the data to find the particle size. Chemical composition of GNPs is confirmed by EDXS. EDXS and SAXS are used to measure the interparticle distance of GNPs. They help in tumor imaging and tissue engineering. Surface morphology, shape, and size of the particle are analyzed by SEM, TEM, and AFM. SEM gives information about the particle at the nanoscale, and TEM gives information about the material layer composition, size, volume, and shape of NPs. HR-TEM is used to measure the size, shape, and crystalline structure of NPs. AFM gives information about the topography of NPs.

12.5 Applications of GNPS

Vaccination plays an important role in the protection of the immune system against different infections caused by pathogens. Here mainly used diagnosis method is called the immunochemical method (Dykman & Bogatyrev, 2007). It completely depends on the use of pathogen-specific antibodies. Recently, the most demanded antigen nanocarrier for vaccination and immunization has been only GNPs. It is mainly due to their low toxicity, high specificity, and ease of synthesis. GNPs are used to synthesize antibodies and vaccines against bacterial, viral, and parasitic infections (Carabineiro, 2017),

12.5.1 Designing Antibacterial Vaccines

Due to the emergence of drug resistance in bacterial pathogens, researchers are trying to find an alternative for antibiotics or new antibacterial agents (Roshmi et al., 2015). The development of nanoparticle-based therapeutic medications helps to provide treatment for drug-resistant bacteria. They include gold, silver, titanium, copper, and zinc oxide (ZnO). GNPs are mostly preferred due to their surface properties, low toxicity, high stability, optical properties, etc. They are successfully applied against bacterial and fungal infections (Santhakumar & Koperuncholan, 2019). GNPs are good drug carriers due to their antibacterial properties. They display photothermal effects that can kill bacteria within a short span of time. Surface functionalization of GNPs increases their properties, and it also increases their antibacterial characteristics (Vijayakumar & Ganesan, 2012). Antibacterial drugs can bind with GNPs through covalent and noncovalent bonds and act as antibacterial agents that display high antibacterial properties. The main advantage of GNPs is that, at certain concentrations, they did not show any toxic effects on normal cells. GNPs also exhibit antibacterial properties against multidrug-resistant bacteria.

12.5.2 Use of GNPs in Antiviral Immunization

GNPs are a major tool of virologists and are used for the synthesis of antiviral vaccines.

12.5.2.1 Human Immunodeficiency Virus (HIV)

For treating HIV, 2G12 antibiotics called GP120 are used. GNPs were efficient to bind to this antibiotic. GNPs carried with thiol-terminated oligosaccharides have been used for synthesizing HIV vaccines (Dykman, 2020).

12.5.2.2 Hepatitis B

For this HBs Antigen DNA coated with GNP was injected to the epidemic cells for the cure. GNPs act as adjuvants and help to trigger fast antibody production (Negahdari et al., 2019).

12.5.2.3 Hepatitis C

The hepatitis C vaccine was synthesized by using plasmonic GNPs activated by an electrical discharge. It helps to increase pore formation on the cell membrane (Paul et al., 2014).

12.5.2.4 Dengue

GNPs in different sizes such as 20 nm, 40 nm, and 80 nm are used against dengue viruses (Fig. 12.3). GNP conjugation with small interfering ribonucleic acid (siRNA) helps to promote the stability and delivery of siRNA and develops a high immune response (Sengupta et al., 2022).

12.5.3 Application of GNPs in Anti-parasitic Immunization

GNP-based antibiotics and vaccines in parasitic diseases are still being explored. In general, parasitic infections are caused by *Plasmodium falciparum* and cause deaths worldwide. GNP-conjugated antigens with GNP nanovaccine formation help to treat this type of infection.

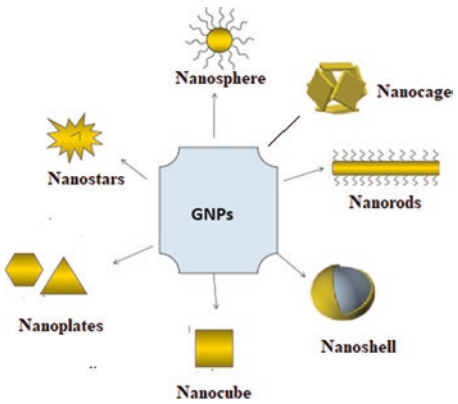


Fig. 12.3 Different shapes of GNPs

12.5.4 Therapeutics

The main issue is bacterial resistance toward antibacterial drugs. GNPs are taken up by immune cells because of their strong cell affinity. These GNPs specifically deliver at the infected area, enhancing bacterial inhibition, and cause damage to the microbial pathogens. GNPs are used as antioxidants too. They block the formation of reactive oxygen species (ROS) and increase the antioxidant activity of defensive enzymes. For cancer therapy, photothermal treatment (PTT) is used wherein GNPs are embedded within the tumor and produce heat in response to the laser effect. PTT is an important method for cancer treatment (Riley & Day, 2017).

12.6 Recent Developments in the Experimental and Theoretical Areas of Antibiotic GNP Functionalization

Bacterial resistance is a major issue in global public health concerns. It leads to finding new antibacterial agents accordingly. In recent decades, nanomaterials have been proven to be highly effective for antibacterial applications. GNPs have got a special attention due to their less toxicity, easy fabrication, and biological inertness. Now, major studies are related to antibiotic functionalized GNPs and their advantages toward antibiotic-resistant bacterial cells. It was found that antibiotic functionalization helps to increase the photothermal stability (Pattani & Tunnell, 2012).

Antibiotics are used against bacterial infections. They either prevent or kill bacterial cells, for example, in diabetic patients, chemopatients, and arthritis patients (El Domany et al., 2018; Sekar et al., 2022; Lima & Reis, 2015). Bacterial cells develop resistance towards some antibiotics, which is a large threat to the human health. The main reason behind antibiotic resistance is the misuse and overuse of antibiotics and the absence of new drugs. Variation in antibiotic concentration is also the reason behind antibiotic resistance, which causes changes in gene expression, horizontal gene transfer (HGT), etc. (Chen et al., 2014; Das & Patra, 2017; Shwartz et al., 2017).

12.7 Application of GNPs in Medicine and Antibiotic Developments

Colloidal GNPs are very efficient in the application of biological and medical studies. There are other nanomaterials that can be used as antibacterial agents. They include ZnO, titanium dioxide (TiO_2), silver, etc. (Zhang et al., 2008; Xu et al., 2020; Kim et al., 2016). GNPs are highly preferred due to their good functionalization with thiol-, amino-, and carboxyl-containing antibiotic molecules (Lin et al.,

2004; Ghosh et al., 2008; Liu et al., 2005). Real GNPs do not show any antibacterial activity. Surface functionalization with different molecules makes them good antibacterial agents. This functionalization involves two approaches: ex situ and in situ (Table 12.2).

12.8 Drug Delivery by GNPs

Nanomedicine exhibits good clinical performance with less tissue toxicity. GNPs are very efficient nanocarriers for different drugs such as plasmid deoxyribonucleic acid (pDNA), siRNA, proteins, and peptides (Song et al., 2010; Kim et al., 2013; Huo et al., 2014). The surface area of GNPs is increased by the functionalization of GNPs with different methods such as carboxylation, amination, and sulfonation (Chen et al., 2010; Garaiova et al., 2021).

12.8.1 Plasmid Deoxyribonucleic Acid (pDNA) Vector Delivery

Here, GNPs are conjugated with functionalized DNA. These DNA–GNP conjugates are very efficient as drug carriers and applied in biosensors and drug delivery (Ross et al., 2012; Miao et al., 2017; Tatumi & Fujihara, 2005). DNA is covalently bonded with GNPs, which enriches their stability. Functionalization of GNPs with

Table 12.2 Characteristics of ex situ and in situ functionalization

Ex situ	In situ
Grafting of antibiotic-functionalized GNPs proceeds by two or more than two steps. Conjugation is continued by either Au–N interactions, Au–S interactions, electrostatic interactions, adsorption, or coupling reaction. Au–S thiol-containing ligand interactions are possible as the deprotonated sulfhydryl group makes the Au–thiolate bond between Au and S. In Au–N interactions, the amino group of the antibiotic is involved in conjugation with GNPs. Electrostatic adsorption method involves the attraction of counter ions by Coulombic forces. Physical adsorption is a reversible process where the adsorbates are attached to the GNP surface by van der Waals forces, for example, neomycin-conjugated GNPs.	Antibiotic molecules are mixed during GNP preparation In situ grafting is carried out in two ways. The first step is in situ reduction or capping by antibiotic molecule. It is a one-step, green, and less labor-intensive method for the synthesis of antibiotic-conjugated GNPs. This method mainly depends on some parameters such as pH, concentration, temperature, antibiotic gold precursor molar ratio, etc. This molar ratio leads to the change in the shape of the GNPs. The second method is in situ reduction by reducing agents and capping by antibiotic molecule. This is a one-step process. In this method, separate reducing agents are required along with antibiotics.

oligonucleotides helps to develop NP dimers and trimers that contain a sequence drug carrier pattern (Xu et al., 2006; Giljohann et al., 2007; Pei et al., 2012). The GNP–DNA covalent linkage helps to increase their stability under DNA denaturing.

12.8.2 Ribonucleic Acid (RNA) Delivery

RNA is used against HIV, cancer, etc. Cancer has been treated by micro-RNA–GNP drug delivery to the targeted affected area (Sánchez-Visedo et al., 2020; Yin et al., 2016; Eissa et al., 2014). GNPs are used to deliver RNA to the targeted site. Small interfering RNA (SiRNA) delivery needs a vector system that has a specific target system and excellent biocompatibility (Lytton-Jean et al., 2011). GNPs help to cover siRNA from enzymatic degradation and mediate gene silencing. Peptides can be easily conjugated to Au–S bonds and can be used effectively due to low toxicity (Rajchakit & Sarojini, 2017).

12.9 Advantages of GNPs as Future Smart Materials

Some of the major advantages of using GNPs in biomedical field include the following:

- Simple and easy synthesis.
- Easy surface functionalization to graft and modify an array of ligands for multiple functionalities such as targeted delivery.
- For the application of contrast imaging and thermal ablation, the physicochemical properties of gold core are ideal.
- Well-engineered GNPs encompass the capability for early disease identification.

12.10 Limitations of GNPs

GNPs are considered promising materials for nanovaccine development. The major concern associated with nanomaterials is their biosafety. GNPs are nonbiodegradable products that cause many side effects. Literature reports suggest that the disruption of cellular metabolism is due to the precipitation of GNPs in cells and their organelles. GNPs can be surface-functionalized easily. However, it harms their histocompatibility. Therefore, GNP variants must be characterized individually before being applied in therapeutics and clinical applications. Positively and negatively charged GNPs are harmful to the DNA and other cell membranes. The melting point of GNPs changes with the variation in the size of the particles. GNPs have high

efficiency in the field of application in drug delivery but have serious side effects too. This includes nonspecific targeting, negatively affecting the host immune system.

12.11 Conclusions and Future Applications

In this chapter, we have analyzed the synthesis, properties, functionalization, and application of GNPs in the synthesis of antibodies against infections. GNPs are very efficient for the development of antibodies and carriers for drug delivery due to their special characteristics such as tunable size and shape, easy functionalization, and less toxicity. There are many methods and possibilities for tuning the surface of GNPs, including PEGylation, with amino acids, peptides, and antibodies. In this chapter, we mainly discussed the efficiency of GNPs for vaccine development for defeating deadly infections. GNPs are safely used to treat many diseases and for the development of antibiotics ranging from infections to cancers. GNPs possess inherent adjuvant potential. They exhibit a higher surface area-to-volume ratio. GNPs display multifunctionalization properties. There are still gaps in studies related to the influence of GNPs and their association with photothermal stability. GNPs can be modified effectively to improve antibacterial, antiviral, and antiparasitic properties. They can be used to synthesize antibodies and vaccines against different pathogens. Among the GNPs available in different shapes such as nanorods, nanocages, nanocubes, nanospheres, nanoprisms, nanostars, and nanoclusters, nanospheres are the best with a high antigen-carrying capacity. In general, antibody proliferation happens while immunization with GNP–antigen conjugates. It enhances the secretion of cytokines. Hence, GNPs that have inherent adjuvant efficiency can be used as effective tools in the design of antibiotics against infectious diseases.

GNPs are highly considered for the development of vaccines and other therapeutic purposes. However, before being widely applied, certain issues are to be addressed. There is an essential need to analyze the influence of GNPs on immune cells. Similarly, the impact of GNPs on off-target cells and organs should be analyzed, and GNPs with polymer coating and application should also be analyzed. GNPs with thermal and optical properties in inflammatory responses should be studied.

References

Balasubramanian, S. K., Yang, L., Yung, L., et al. (2010). Characterization, purification, and stability of gold nanoparticles. *Biomaterials, 31*(34), 9023–9030.

Bandi, R., Dadigala, R., & Alle, M. (2023). Emerging role of gold nanoparticles for healthier crop plants growth and enhanced yield. In *Engineered nanomaterials for sustainable agricultural production, soil improvement and stress management* (pp. 125–143). Academic.

Brinas, R. P., Sundgren, A., Sahoo, P., et al. (2012). Design and synthesis of multifunctional gold nanoparticles bearing tumor-associated glycopeptide antigens as potential cancer vaccines. *Bioconjugate Chemistry, 23*(8), 1513–1523.

Carabineiro, S. A. (2017). Applications of gold nanoparticles in nanomedicine: recent advances in vaccines. *Molecules, 22*(5), 857.

Chen, Y. S., Hung, Y. C., Lin, W. H., et al. (2010). Assessment of gold nanoparticles as a size-dependent vaccine carrier for enhancing the antibody response against synthetic foot-and-mouth disease virus peptide. *Nanotechnology, 21*(19), 195101.

Chen, C. W., Hsu, C. Y., & Lai, S. M. (2014). Metal nanobullets for multidrug resistant bacteria and biofilms. *Advanced Drug Delivery Reviews, 78*, 88–104.

Das, B., & Patra, S. (2017). Antimicrobials: meeting the challenges of antibiotic resistance through nanotechnology. In *Nanostructures for antimicrobial therapy* (pp. 1–22). Elsevier.

Dykman, L. A. (2020). Gold nanoparticles for preparation of antibodies and vaccines against infectious diseases. *Expert Review of Vaccines, 19*(5), 465–477.

Dykman, L. A., & Bogatyrev, V. A. (2007). Gold nanoparticles: preparation, functionalisation and applications in biochemistry and immunochemistry. *Russian Chemical Reviews, 76*(2), 181.

Eissa, S., Shawky, S. M., Matboli, M., et al. (2014). Direct detection of unamplified hepatoma upregulated protein RNA in urine using gold nanoparticles for bladder cancer diagnosis. *Clinical Biochemistry, 47*(1–2), 104–110.

El Domany, E. B., Essam, T. M., Ahmed, A. E., et al. (2018). Biosynthesis physico-chemical optimization of gold nanoparticles as anti-cancer and synergetic antimicrobial activity using Pleurotus ostreatus fungus. *Journal of Applied Pharmaceutical Science, 8*(5), 119–128.

El-Sayed, I. H., & Huang, X. (2005). Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer. *Nano Letters, 5*(5), 829–834.

Fu, X., Cai, J., Zhang, X., et al. (2018). Top-down fabrication of shape-controlled, monodisperse nanoparticles for biomedical applications. *Advanced Drug Delivery Reviews, 132*, 169–187.

Garaiova, Z., Melikishvili, S., Michlewska, S., et al. (2021). Dendronized gold nanoparticles as carriers for gp160 (HIV-1) peptides: Biophysical insight into complex formation. *Langmuir, 37*(4), 1542–1550.

Ghosh, P. S., Kim, C. K., Han, G., et al. (2008). Efficient gene delivery vectors by tuning the surface charge density of amino acid-functionalized gold nanoparticles. *ACS Nano, 2*(11), 2213–2218.

Giljohann, D. A., Seferos, D. S., & Patel, P. C. (2007). Oligonucleotide loading determines cellular uptake of DNA-modified gold nanoparticles. *Nano Letters, 7*(12), 3818–3821.

Huang, H., & Yang, X. (2004). Synthesis of polysaccharide-stabilized gold and silver nanoparticles: a green method. *Carbohydrate Research, 339*(15), 2627–2631.

Huo, S., Jin, S., Ma, X., et al. (2014). Ultrasmall gold nanoparticles as carriers for nucleus-based gene therapy due to size-dependent nuclear entry. *ACS Nano, 8*(6), 5852–5862.

Jain, P. K., Lee, K. S., El-Sayed, I. H., et al. (2006). Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *The Journal of Physical Chemistry. B, 110*(14), 7238–7248.

Jia, Y. P., Ma, B. Y., Wei, X. W., et al. (2017). The in vitro and in vivo toxicity of gold nanoparticles. *Chinese Chemical Letters, 28*(4), 691–702.

Kim, J., Park, J., Kim, H., et al. (2013). Transfection and intracellular trafficking properties of carbon dot-gold nanoparticle molecular assembly conjugated with PEI-pDNA. *Biomaterials, 34*(29), 7168–7180.

Kim, T., Braun, G. B., She, Z. G., et al. (2016). Composite Porous silicon–silver nanoparticles as theranostic antibacterial agents. *ACS Applied Materials & Interfaces, 8*(44), 30449–30457.

Kumar, R., Ray, P. C., Datta, D., Bansal, G. P., et al. (2015). Nanovaccines for malaria using Plasmodium falciparum antigen Pfs25 attached gold nanoparticles. *Vaccine, 33*(39), 5064–5071.

Lima, A. C., & Reis, S. (2015). Temperature-responsive polymeric nanospheres containing methotrexate and gold nanoparticles: A multi-drug system for theranostic in rheumatoid arthritis. *Colloids and Surfaces, B: Biointerfaces, 133*, 378–387.

Lin, S. Y., Tsai, Y. T., Chen, C. C., Lin, C. M., & Chen, C. H. (2004). Two-step functionalization of neutral and positively charged thiols onto citrate-stabilized Au nanoparticles. *The Journal of Physical Chemistry. B, 108*(7), 2134–2139.

Liu, S., Zhang, Z., & Wang, Y. (2005). Surface-functionalized silica-coated gold nanoparticles and their bioapplications. *Talanta, 67*(3), 456–461.

Lytton-Jean, A. K., Langer, R., & Anderson, D. G. (2011). Five years of siRNA delivery: spotlight on gold nanoparticles. *Small, 7*(14), 1932–1937.

Miao, P., Tang, Y., & Mao, Z. (2017). Adamantane derivatives functionalized gold nanoparticles for colorimetric detection of MiRNA. *Particle and Particle Systems Characterization, 34*(6), 1600405.

Murphy, C. J., Gole, A. M., Stone, J. W., et al. (2008). Gold nanoparticles in biology: Beyond toxicity to cellular imaging. *Accounts of Chemical Research, 41*(12), 1721–1730.

Negahdari, B., Darvishi, M., & Saeedi, A. (2019). Gold nanoparticles and hepatitis B virus. *Artificial Cells, Nanomedicine and Biotechnology, 47*(1), 455–461.

Patil, M. P., & Kim, G. D. (2017). Eco-friendly approach for nanoparticles synthesis and mechanism behind antibacterial activity of silver and anticancer activity of gold nanoparticles. *Applied Microbiology and Biotechnology, 101*(1), 79–92.

Pattani, V. P., & Tunnell, J. W. (2012). Nanoparticle-mediated photothermal therapy: A comparative study of heating for different particle types. *Lasers in Surgery and Medicine, 44*(8), 675–684.

Paul, A. M., Shi, Y., & Acharya. (2014). Delivery of antiviral small interfering RNA with gold nanoparticles inhibits dengue virus infection in vitro. *The Journal of General Virology, 95*(Pt 8), 1712.

Pei, H., Li, F., Wan, Y., et al. (2012). Designed diblock oligonucleotide for the synthesis of spatially isolated and highly hybridizable functionalization of DNA–gold nanoparticle nanoconjugates. *Journal of the American Chemical Society, 134*(29), 11876–11879.

Pissuwan, D., Cortie, C., Valenzuela, S. M., et al. (2010). Functionalised gold nanoparticles for controlling pathogenic bacteria. *Trends in Biotechnology, 28*(4), 207–213.

Rajchakit, U., & Sarojini, V. (2017). Recent developments in antimicrobial-peptide-conjugated gold nanoparticles. *Bioconjugate Chemistry, 28*(11), 2673–2686.

Riley, R. S., & Day, E. S. (2017). Gold nanoparticle-mediated photothermal therapy: applications and opportunities for multimodal cancer treatment. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 9*(4), 1449.

Roshmi, T., Soumya, K. R., & Jyothis, M. (2015). Effect of biofabricated gold nanoparticle-based antibiotic conjugates on minimum inhibitory concentration of bacterial isolates of clinical origin. *Gold Bulletin, 48*(1), 63–71.

Ross, R. D., Cole, L. E., & Roeder, R. K. (2012). Relative binding affinity of carboxylate-, phosphonate-, and bisphosphonate-functionalized gold nanoparticles targeted to damaged bone tissue. *Journal of Nanoparticle Research, 14*(10), 1–11.

Safari, D., Marradi, M., Chiodo, F., et al. (2012). Gold nanoparticles as carriers for a synthetic Streptococcus pneumoniae type 14 conjugate vaccine. *Nanomedicine Journal, 7*(5), 651–662.

Sánchez-Visedo, A., Gallego, B., Royo, L. J., et al. (2020). Visual detection of microRNA146a by using RNA-functionalized gold nanoparticles. *Microchimica Acta, 187*(3), 1–9.

Santhakumar, M., & Koperuncholan, M. (2019). Gold nano-drug design for antimicrobial activity. *Life Science Informatics Publications-Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences, 5*(2), 720–731.

Sekar, V., Al-Ansari, M. M., Narenkumar, J., et al. (2022). Synthesis of gold nanoparticles (AuNPs) with improved anti-diabetic, antioxidant and anti-microbial activity from Physalis minima. *Journal of King Saud University - Science, 34*(6), 102197.

Sengupta, A., Azharuddin, M., & Al-Otaibi, N. (2022). Efficacy and immune response elicited by gold nanoparticle-based nanovaccines against infectious diseases. *Vaccines, 10*, 505.

Shwartz, A., Betzer, O., Kronfeld, N., et al. (2017). Therapeutic effect of astroglia-like mesenchymal stem cells expressing glutamate transporter in a genetic rat model of depression. *Theranostics, 7*(10), 2690.

Song, W. J., Du, J. Z., Sun, T. M., et al. (2010). Gold nanoparticles capped with polyethyleneimine for enhanced siRNA delivery. *Smal, 6*(2), 239–246.

Tao, W., Ziemer, K. S., & Gill, H. S. (2014). Gold nanoparticle–M2e conjugate coformulated with CpG induces protective immunity against influenza A virus. *Nanomedicine Journal, 9*(2), 237–251.

Tatumi, R., & Fujihara, H. (2005). Remarkably stable gold nanoparticles functionalized with a zwitterionic liquid based on imidazolium sulfonate in a high concentration of aqueous electrolyte and ionic liquid. *Chemical Communications*, (1), 83–85.

Tazaki, T., Tabata, K., Ainai, A., Ohara, Y., et al. (2018). Shape-dependent adjuvanticity of nanoparticle-conjugated RNA adjuvants for intranasal inactivated influenza vaccines. *RSC Advances, 8*(30), 16527–16536.

Tiwari, P. M., Vig, K., Dennis, V. A., & Singh, S. R. (2011). Functionalized gold nanoparticles and their biomedical applications. *Journal of Nanomaterials, 1*(1), 31–63.

Vijayakumar, S., & Ganesan, S. (2012). Gold nanoparticles as an HIV entry inhibitor. *Current HIV Research, 10*(8), 643–646.

Wangoo, N., Bhasin, K. K., & Mehta, S. K. (2008). Synthesis and capping of water-dispersed gold nanoparticles by an amino acid: Bioconjugation and binding studies. *Journal of Colloid and Interface Science, 323*(2), 247–254.

Xu, X., Rosi, N. L., Wang, Y., et al. (2006). Asymmetric functionalization of gold nanoparticles with oligonucleotides. *Journal of the American Chemical Society, 128*(29), 9286–9287.

Xu, C., Zheng, J., & Wu, A. (2020). Antibacterial applications of TiO_2 nanoparticles. In *Applications in nanobiotechnology and nanomedicine* (pp. 105–132).

Yin, H., Zhou, Y., Li, B., et al. (2016). Photoelectrochemical immunosensor for microRNA detection based on gold nanoparticles-functionalized g-C3N4 and anti-DNA: RNA antibody. *Sensors and Actuators B: Chemical, 222*, 1119–1126.

Zhang, L., Ding, Y., Povey, M., & York, D. (2008). ZnO nanofluids–A potential antibacterial agent. *Progress in Natural Science, 18*(8), 939–944.

Chapter 13
Emerging Vaccine for the Treatment of Cancer via Nanotechnology

Tahmina Foyez, Yesmin Begum, and Abu Bin Imran

13.1 Introduction

Modern vaccinations have improved public health, saved lives by inducing immunity against infections, and bestowed passive immunity through antibodies or lymphocytes. Using the patient's immune system, cancer immunotherapy possesses high specificity and low toxicity in order to kill tumors and prevent cancer recurrence (Palucka & Banchereau, 2012; Wang et al., 2017). In the last decade, the US Food and Drug Administration (FDA) has authorized a substantial number of immunotherapies for clinical use, and many more are undergoing clinical or preclinical testing (Wang & Wang, 2017). Cytokine treatments (Berraondo et al., 2019; Rosenberg, 2014), immune checkpoint blockades (ICBs) (Auslander et al., 2018; Pardoll, 2012), chimeric antigen receptor T-cell (CAR-T) therapy (Adachi et al., 2018; Neelapu et al., 2018; Wu et al., 2019), and cancer vaccination (Banchereau & Palucka, 2018; Knutson & Mittendorf, 2015) are all potential immunotherapies for cancer that may produce immunity against tumors with less off-target consequences. Cancer vaccines trigger immune responses, generate robust immunity, and prolong immunological memory to suppress tumor growth, metastasis, and recurrence (X. Li & Bu, 2017; Sahin & Türeci, 2018; Thomas & Prendergast, 2016). Vaccines

T. Foyez (✉)
Department of Hematology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Department of Pharmacy, United International University, Dhaka, Bangladesh
e-mail: tfoyez@unc.edu

Y. Begum
Department of Pharmacy, Southeast University, Dhaka, Bangladesh

A. B. Imran
Department of Chemistry, Bangladesh University of Engineering and Technology, Dhaka, Bangladesh

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_13

are exceptional in providing precise, long-term immunity against prevalent infectious diseases. The development of therapeutic cancer vaccines based on nanomaterial delivery vehicles, such as polymeric, lipid, inorganic, and bio-inspired vehicles, often provides unique and attractive benefits (Foyez & Imran, 2022; Harun-Ur-Rashid et al., 2022). Cancer vaccines based on nanoparticles consist of nanovaccine core, loading, and delivery (Fig. 13.1), which have key advantages over conventional immunizations. First, the tumor immune microenvironment (TIME) may specifically activate polymeric nanovaccines, which encourages the transfer of antigens into the antigen-presenting cells (APCs) located in lymph nodes (LNs) (Ding et al., 2020; Duong et al., 2018; Knight et al., 2019). Second, utilizing in situ conjugation, the innate ability of endogenous nanocarriers to target LNs can be exploited to attach molecular vaccines to LNs (An & Liu, 2017; Lin et al., 2018). Third, lipid-based nanoparticles may improve the formulation and mass production of cancer vaccines (Chiu et al., 2015). Effective cancer immunotherapy has made use of biomimetic cell membrane–derived nanovaccines (Fang et al., 2014; Schudel et al., 2020). The primary objective of cancer vaccines is to activate the power of the immune system as a living therapeutic agent, with the intention of recognizing tumor antigens and eliminating tumor cells. By using nanotechnologies, cancer vaccines can eventually achieve their ultimate goal. Nanovaccines have flexible features, including multivalent target delivery to lymphoid tissues and particular immune cells, multistage stimulation control release of immune components, and essential immune pathway interaction (Eppler & Jewell, 2020; Tang et al., 2021). There has already been effective validation in the clinic of cancer immunotherapy,

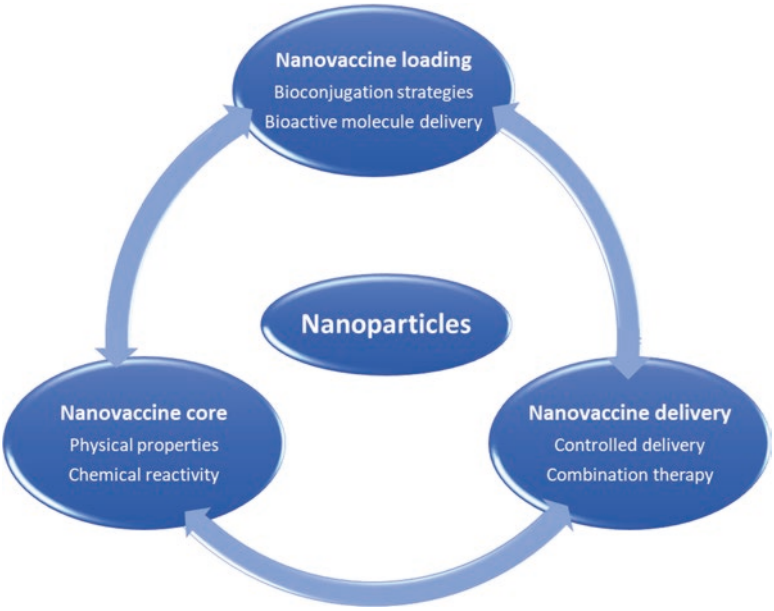


Fig. 13.1 The role of nanoparticles in nanovaccine programming

notably immune checkpoint blockade (ICB) and chimeric antigen receptor T-cell (CAR-T) immunotherapy. However, the low response rates from patients and off-target side effects of nanotechnologies show that there are still many problems to solve before these technologies are implemented successfully (Kingwell, 2019; McLeod et al., 2020). Antigen release, presentation, immune cell activation, infiltration of T cell into the tumor microenvironment, and specific detection and eradication of tumor cells are all parts of an anticancer immune response (Chen & Mellman, 2013). Without any step, the effective antitumor immune response would ultimately collapse. As a result, cancer immunotherapy has evolved from targeting only one stage of the cancer immune system to targeting the whole process. Vaccine nanotechnology may aid in developing more successful techniques and platforms for activating antitumor immunity. Nanovaccine is created by encapsulating tumor antigens in nanoparticles of synthetic polymer. It is injected, transporting tumor-associated proteins to lymph nodes. The vaccination promotes the body's immune defense system by activating an adaptor protein that stimulates the generation of T cells that target tumors and destroy cancer cells. Combining nanovaccines with radiation or other immunotherapy techniques may increase their antitumor efficacy. This chapter focuses mostly on nanoparticles used in cancer vaccines and provides an overview of the most recent developments in the field. In addition, it explores the crucial roles played by immune system activation mechanisms, immune component combination tactics, and various delivery strategies in vaccination.

13.2 Mechanism of Cancer Vaccines

Cancer vaccines have become a great promising therapy, but their practical use is hindered by the ineffectiveness of the immunological cascade of vaccines. Antigen recognition, internalization, coencapsulation of antigen and adjuvant, LN-targeted transport, and subsequent release of antigen and cross-presentation to T cells (Fig. 13.2) (Jiang et al., 2017; Zhu et al., 2017) are the five key phases in the cascade. Both immunologic adjuvants and tumor antigens are essential elements of vaccines for effectively generating an anticancer immune response. Despite the fact that different tumor antigens slightly increase the immune response when administered alone (Xu et al., 2020), nanomaterials may encapsulate antigens and adjuvants in nanovaccine systems via electrostatic, hydrophobic, and covalent interactions (Warrier et al., 2019). Traditional vaccination systems cannot perform well due to encapsulating vaccine components effectively, but nanomaterials with 200-nm particles may enable nanovaccine access to LNs (Liu et al., 2020). Different APCs, like dendritic cells (DCs), macrophages, and B cells are abundant in LNs and play a significant role in the presentation of antigens to T cells. APCs have several receptors for vaccine endocytosis, which may be utilized to produce targeted nanovaccines (Hu et al., 2020). As dendritic cells have the majority of mannose receptors, mannose-containing nanomaterials provide efficient drug delivery into DCs (He et al., 2015). After APCs internalize nanovaccines, endosome migration should be

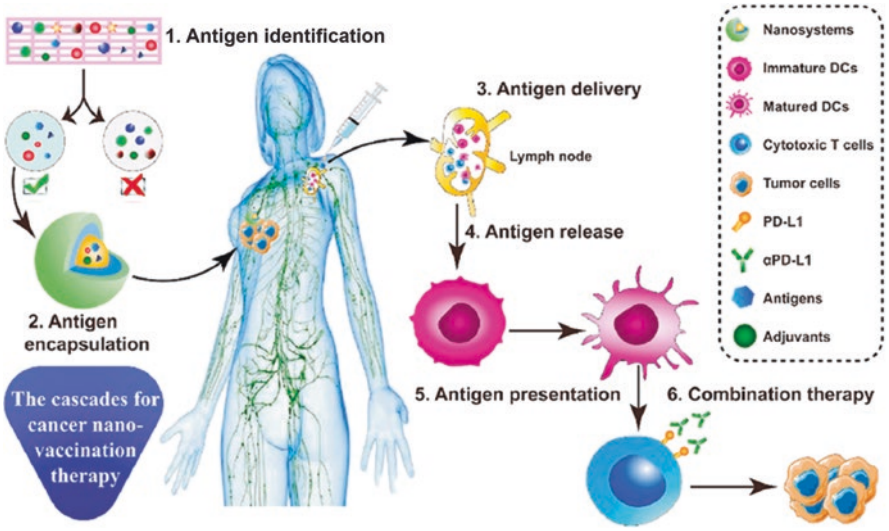


Fig. 13.2 Schematic representation of cancer vaccine pathways, which includes antigen detection, encapsulation, transport, release, T-cell presentation, and combination therapy. (Reproduced from Chen et al. (2021) with permission)

addressed for antigen and adjuvant release. Within endosomes, the acidic microenvironment can trigger the activation of a variety of acid-responsive polymeric nanomicelles, which can then rupture the endosome membrane (Feng et al., 2019). Antigens and adjuvants induce DC maturation, and matured DCs cross-present antigens and produce cytokines to activate effector T cells. Antigen cross-presentation has two key mechanisms: Lysosomal proteases break down antigens after internalization, and peptides are loaded onto major histocompatibility complex (MHC) class I molecules for antigen cross-presentation (Tang-Huau et al., 2018). Ingested antigens are transferred from lysosomes to the cytoplasm and destroyed. The generated new peptides are transported to the endoplasmic reticulum (ER) for cross-presentation (Grotzke et al., 2017).

13.3 Cancer Vaccine Composition

Cancer Vaccine include antigens, nanoadjuvants, and nanocarriers (Fig. 13.3). Tumor antigens are exclusively presented on the MHC molecules in tumor cells. Endogenous cancer antigens cannot elicit therapeutic immune responses due to immunodeficiency and immune evasion. By delivering exogenous tumor-relevant antigens, cancer vaccines enhance antigen-specific anticancer immune responses (Mellman et al., 2011). Both the cancer cells and healthy cells may express tumor-associated antigens (TAAs), and different types of cancer cells may express different TAAs (Fioretti et al., 2010). Cancer testis (CT) antigens are TAAs expressed

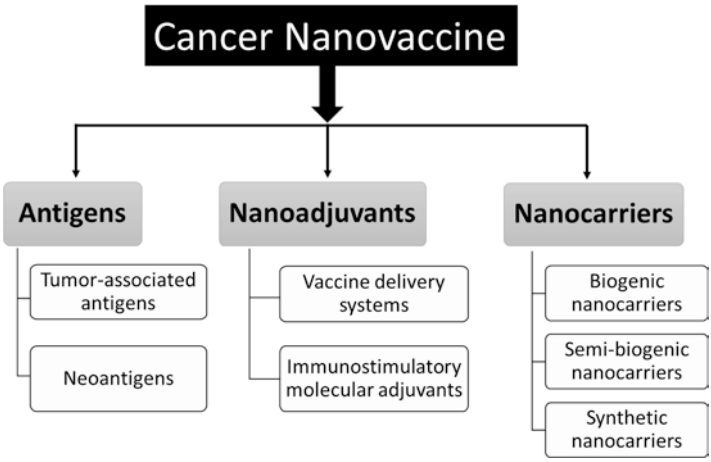


Fig. 13.3 Composition of cancer vaccines

exclusively in cancer cells but not normal adult cells. HER2/neu, breast, and ovarian cancer oncoprotein are typical TAAs (Ross & Fletcher, 1998). Neoantigens are produced from random somatic mutations or aberrant gene expression in tumor cells (Coulie et al., 1995). They are only expressed in tumor cells, not in normal cells (Parmiani et al., 2007).

There are two types of adjuvants: (1) vaccine delivery systems that may expose antigens to the immune system more effectively and regulate the release and storage of antigens, such as emulsions, mineral salts, liposomes, and virosomes (Felnerova et al., 2004; Schwaninger et al., 2004), and (2) immune adjuvants that potentially stimulate the immune system and boost antigen-induced immune responses, such as toll-like receptor (TLR) agonists, costimulatory ligands, and cytokines (Montomoli et al., 2011). Immune cells such as APCs are activated by immunostimulatory adjuvants, enhancing antigen-specific immune responses. Nanoparticles make an effective delivery system for subunit vaccinations. The delivery of vaccines in cancer immunotherapy has been done using a variety of nanomaterials. The current developments of nanocarrier-based cancer immunotherapy vaccines will be addressed in the subsequent sections.

Biogenic nanocarriers can be biodegradable, biocompatible, and low-toxic. Exosomes and outer membrane vesicles (OMVs) are typical biogenic nanocarriers. Exosomes with a size of 30–150 nm are ideal vaccine carriers. T cells, tumor cells, B cells, and APCs produce exosomes (Théry et al., 2002). Exosomes can be immunostimulatory or immunosuppressive, indicating a promising role in tumor or autoimmune disease immunotherapy (Tan et al., 2010). OMVs with a size of about 50–250 nm are ideal for lymph node homing and intracellular transport into APCs. Gram-negative bacteria produce OMVs to interact with one another and other microbes (Lin et al., 2018; Wang et al., 2019b).

Semisynthetic nanocarriers are made up of both biogenic and synthetic parts. When designed well, semisynthetic nanocarriers can have some of the properties of biogenic nanocarriers, such as biocompatibility and low toxicity. They can also have some of the properties of synthetic nanoparticles, such as being easy to make on a large scale and repeatable. There are three types of semisynthetic nanocarriers like membrane-coated nanocarriers, virus-like particles (VLPs), and endogenous protein-based nanocarriers. Nanoparticles coated with cancer cell membrane antigens offer a platform for cancer vaccines (Fang et al., 2014). Cell membrane camouflage-based nanovaccines can be widely used in clinical applications and allow the easy design of adjuvants and cell membranes. These investigations show that cell membrane-coated nanoparticles are promising nanovaccines. VLPs are semisynthetic nanocarriers with cancer vaccine potential that are noninfectious because they lack viral deoxyribonucleic acid (DNA). VLPs are rapidly taken up by APCs; hence, engineered antigens on VLP-based nanovaccines can stimulate long-lasting adaptive immune responses (Roy & Noad, 2008). Endogenous proteins are preferred for vaccine delivery to lymphoid tissues and APCs. Albumin-binding lipid coupled with subunit vaccines enhanced immunostimulation in lymph nodes. Immunomodulation effectiveness led to cancer therapy efficiency (Liu et al., 2014).

Synthetic nanocarriers use synthetic nanomaterials as vaccine carriers. Polymer nanoparticles, liposomes, and inorganic nanoparticles are used in nanovaccines. Poly(lactide-co-glycolide) (PLGA) has been researched for use in cancer vaccine delivery. The size, solubility, and stability of PLGA nanoparticles can be customized to enhance their effectiveness in this area. Compared to molecular antigens, antigen-loaded polymer nanovaccines have been shown to be more efficient in boosting T-cell responses (Rietscher et al., 2016). Nanovaccines also use liposomes, a biodegradable phospholipid bilayer. Immune cells may readily swallow inorganic nanocarriers. Inorganic nanoparticles conjugated to TAAs decrease murine tumor development antigen-specifically (Ahn et al., 2014; Meng et al., 2008) (Fig. 13.4).

13.4 Nanomaterials for Cancer Vaccine Delivery

Antigen- and adjuvant-based cancer vaccines have gained popularity due to their ease of manufacture and safety (Calvo Tardón et al., 2019; Xu & Moyle, 2018). These cancer vaccines target tumor cells by generating antigen-specific antitumor immune responses and overcome immune tolerance (Aiga et al., 2020; Wang et al., 2019c). Nanomaterials provide intriguing ways to solve cancer vaccine delivery problems (Innovations in Nanomaterials, 2015). The following section will address stimuli-activatable nanomaterials, self-adjuvant nanomaterials, and modularly assembled nanomaterials to transport nanovaccine into LNs for enhanced cancer therapy.

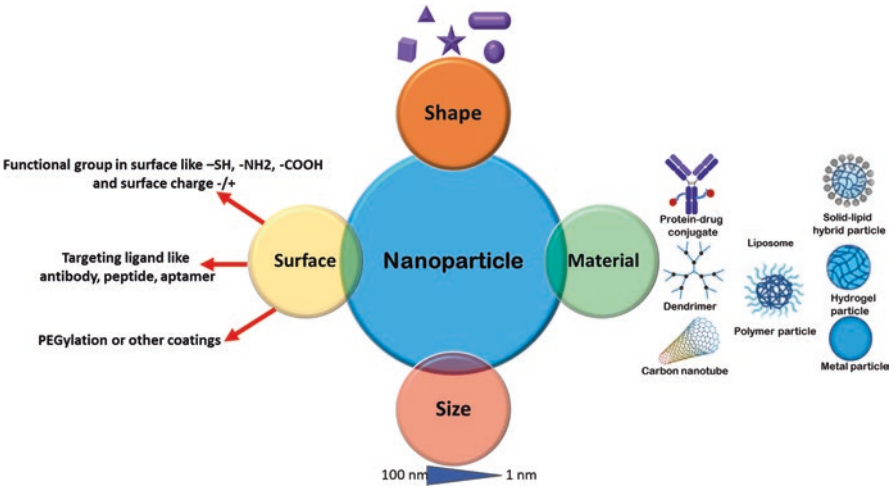


Fig. 13.4 Nanovaccines designed through physical adsorption, chemical conjugation, encapsulation, or physical mixing of antigens with different nanoparticles

13.4.1 Stimuli-Activatable Cancer Vaccines

Stimuli-activatable polymeric nanoparticles have been used to treat cancer (Imran, 2020; Imran et al., 2010; Lyu & Pu, 2017; Zhang et al., 2020). Researchers created thermally activatable nanomaterials for cancer immunotherapy (Li et al., 2019; Zhang & Pu, 2020). These nanomaterials may undergo structural transition or dissociation under appropriate stimulation to regulate medication distribution and release (Mura et al., 2013; Wang et al., 2019a). Loading a TLR agonist into a semiconducting nanocore created a polymeric nanoadjuvant. Irradiation not only ablates tumors but also induces cell death, promoting DC maturation and anticancer immune responses (Sun et al., 2021). Stimuli-activatable nanomaterials have also increased the therapeutic effectiveness of cancer vaccines in LNs (Lin et al., 2019). Exogenous cues have been used to construct smart cancer vaccines. A thermally activatable gel nanosystem for combinatorial cancer treatment has been developed.

13.4.2 Self-Adjuvant Cancer Vaccines

Nanomaterials have also been shown to have intrinsic adjuvant qualities that stimulate cytokine production, activate the immunological sensing system, and prompt cell death to produce antigens against tumors (Chen et al., 2016; Zhong et al., 2019). Xia et al. focused on enhancing the efficacy of cancer vaccinations by improving the flexibility and the movement of antigens (Xia et al., 2018). Mannosylated nanomaterials show much promise in cancer therapy because they have effects on specific

cells, modify the microenvironment, and boost immunity. (Hu et al., 2018). Based on these findings, a number of different glycosylation compounds have been developed as nanovaccines (Buonsanti et al., 2016; Kightlinger et al., 2019).

13.4.3 Modularly Assembled Cancer Vaccines

Cancer vaccines typically involve the combination of antigens and adjuvants into nanomaterials through the use of covalent bonds. Nanovaccines have a particular difficulty eliciting antitumor immune responses because the physicochemical properties of different antigens might vary significantly from one another (Hailemichael et al., 2013; Xi et al., 2018). In recent years, modularly constructed nanomaterials based on noncovalent conjugation have garnered much attention as customizable platforms for nanovaccines (Forner et al., 2020; Lou et al., 2019). There are several different ways in which the therapeutic effects of vaccines based on antigen peptides and adjuvants could be improved, such as (i) making a series of nanomaterials that can be activated by stimuli to deliver antigens and adjuvants to target cells quickly and on demand, (ii) creating powerful self-adjuvant nanomaterials that can be linked to antigen peptides to trigger a combinational immune response against cancer, and (iii) the modularly assembled approach that is also used to construct universal and flexible nanovaccine platforms, which are used to alleviate difficulties with standard formulations of nanovaccines. Despite the availability of these promising techniques to boost the therapeutic efficacy of classic antigen peptide-/adjuvant-based cancer vaccines, certain difficulties remain before their clinical translation. First, certain stimuli activatable nanomaterials' raw materials, preparation processes, and conditions are too difficult for mass manufacturing. Second, inadequate homogeneity, stability, and unexplained in vivo metabolic processes of certain nanovaccine formulations limit their clinical application. In addition to concentrating on nanomaterials, we also need to develop efficient methodologies to uncover patient-specific antigen peptides and potent immune. Recent advancements in antigen peptide-/adjuvant-based nanovaccines may enhance clinical cancer therapy results (Table 13.1).

13.5 DNA- or RNA-Based Nanovaccines

Nucleic acid–based vaccines are easy to make, safe, and versatile and may generate cellular and humoral immune responses (Fan et al., 2018; Yang et al., 2014). Despite these promising findings, nucleic acid–based cancer vaccines have not advanced much. The fast development of nanomaterials has suggested realistic techniques to promote nucleic acid as cancer vaccines (Lindsay et al., 2019; Mascola & Fauci, 2020). Nucleic acid–based cancer vaccines increase immune responses by releasing DNA or RNA into immune cells and expressing encoded antigens. Virus-like nanoparticles, mesoporous silica, and lipid-based nanomaterials have transferred

Table 13.1 Nanotechnology-based cancer vaccines that have been clinically tested and approved

Type	Formulation	Clinical status	Application
Ribonucleic acid (RNA)-based nanovaccine	RNA expressed with liposomes named Lipo-MERIT (Kranz et al., 2016)	Phase I/II	Melanoma
Messenger RNA (mRNA)-based nanovaccine	Lipid nanoparticle-encapsulated mRNA-based therapeutic encoding OX40L T-cell costimulator, interleukin (IL)-23, and IL-36γ proinflammatory cytokines (Patel et al., 2020)	Phase I	Solid tumors and lymphoma
mRNA-based nanovaccine	Lipid-encapsulated mRNA-based cancer vaccine encoding neoantigens (Burris III et al., 2019)	Phase I	Melanoma, bladder carcinoma
Protein-based nanovaccine	Antigen-Specific Cancer Immunotherapeutic (ASCI), a recombinant HER2 protein combined with the immunological adjuvant AS15, containing monophosphoryl lipid (MPL), Quillaja saponaria (QS-21), cytosine-phosphate-guanine (CpG), and liposome (Hamilton et al., 2012)	Phase I	Metastatic breast cancer
Polymer-based nanovaccine	SN38, the active metabolite of irinotecan (CPT-11) is encapsulated in polymeric nanoparticles (poly(lactic acid)–poly(ethylene glycol) [PLA-PEG]) (Nguyen et al., 2018)	Preclinical	Neuroblastoma
Liposome-based nanovaccine	Liposome-encapsulated peptide vaccine consists of a synthetic peptide derived from the mucin 1 (MUC-1) antigen (Wu et al., 2011)	Phase III	Lung cancer
Peptide-based nanovaccine	Six-transmembrane epithelial antigen of the prostate (STEAP) peptide-loaded PLGA nanoparticles (Chen et al., 2019)	Preclinical	Prostate cancer
Peptide-based nanovaccine	Heat shock protein (HSP) is encapsulated in polymeric nanoparticles (Shevtsov & Multhoff, 2016)	Phase I	Gastric carcinoma
VLP-based nanovaccine	VLP-based vaccine has been developed by combining immunopeptide (Mohsen et al., 2019)	Preclinical	Melanoma
Biadjuvant nanovaccine	MC38 colorectal cancer cell–specific neoantigen (ADP-dependent glucokinase [ADPGK]) are encapsulated into nanoparticles (Ni et al., 2020)	Preclinical	Colorectal cancer
Poly(lactic-co-glycolic acid) -based nanovaccine	Nanoparticles are loaded with the model protein/antigen, ovalbumin (OVA) (Rietscher et al., 2016)	Preclinical	Colon cancer

(continued)

Table 13.1 (continued)

Type	Formulation	Clinical status	Application
PLGA nanoparticles	Nanovaccine loaded with only water-soluble components (Ma et al., 2021)	Preclinical	Melanoma, lung cancer, and triple-negative breast cancer
E7- indocyanine green (ICG)-bovine serum albumin (BSA) nanoparticles	BSA with the E7 antigen and then encapsulating the photosensitizer and adjuvant (Zhang et al., 2022)	Preclinical	Cervical cancer
Biomimetic nanoparticles	Nanoparticles consist of ultrasmall Cu2-x Se nanoparticles (Wang et al., 2022)	Preclinical	Glioblastoma
Self-assembling nanovaccines	Peptide and polysaccharide antigens can be connected to the nanoparticles (Pan et al., 2020)	Preclinical	T-lymphoma

nucleic acid to LNs to enhance cancer vaccination results. There is a reason to believe that the combination of nanovaccines based on nucleic acids and chemotherapy could be a promising course of action.

RNA may express antigens and adjuvants to activate APCs (McNamara et al., 2015; Reinhard et al., 2020). Nanomaterial-based RNA cancer vaccines have developed rapidly. They prevent ribonucleases from damaging RNA molecules and make it easier for RNA to be delivered into APCs (Granot-Matok et al., 2019; Kowalski et al., 2019). RNA nanovaccines based on nanomaterials employ viral-like nanoparticles as carriers. Lipid-based nanomaterials are used to transfer antigen-encoding RNA to LNs.

DNA-based vaccines for cancer treatment have proven to be problematic in preclinical studies because of inadequate LNs-targeting plasmid DNA (pDNA) distribution. pDNA delivery is a developing technology for improving DNA vaccines. In recent years, nanomaterial-based techniques have presented innovative ways to increase pDNA delivery (Farris et al., 2017). For instance, thiol-ligand-modified gold nanoparticles are capable of directing DNA-based cancer vaccines (Gulla et al., 2019).

13.6 Cancer Vaccines Based on Biomimetic Nanomaterials

The advancement of nanotechnology provides unique ways to build cancer vaccines. Viral, lipid-based, and polymeric nanoparticles transport antigens and adjuvants to APCs (Pardi et al., 2015). To increase the safety and efficiency of nanovaccines, biomimetic materials have been used to manufacture multifunctional nanomaterials (Li et al., 2020). Natural and membrane fusion nanomaterials, tumor cell-derived nanovesicles, and artificial APCs (aAPCs) are examples of biomimetic nanomaterials for the administration of cancer vaccines.

Exogenous nanomaterials are less effective at improving immune responses against cancer and reducing harmful side effects than natural nanomaterials. Exosomes (Liao et al., 2019), OMVs (Huang et al., 2020), and proteins have been examined and shown to transport medications to target areas. Exosomes are nano-sized extracellular vesicles with the potential to facilitate effective intercellular interactions (Huang et al., 2018). Proteins and vector ligands on exosomes may bind to target cells to facilitate medication delivery. DCs-derived exosomes might serve as a cancer vaccine (Lu et al., 2017). Moreover, fused cells (FCs) may develop greater LN homing potential to improve antigen presentation by T-cells. aAPCs is a biomimetic nanovaccine that replicates the biological function of natural APCs to elicit a tumor-specific immune response without the need for antigens and adjuvants. aAPC-derived biomimetic nanovaccines use iron oxide nanoparticle-based magnetic nanoclusters as biomimetic nanomaterials (Hickey et al., 2020). In conclusion, biomimetic nanomaterials may increase the therapeutic efficacy of cancer vaccines and might be used to codeliver vaccine components with chemotherapeutic medicines.

13.7 Conclusion

The recent growth of nanostructured materials opened up new ways to make nanomedicine and vaccines. Here, we studied nanomaterials to deliver cancer vaccinations efficiently. Cancer vaccines boost adaptive immunity to detect and target tumor cells safely and potently, addressing the high toxicity and insufficient targeting of current cancer therapies. Recent advances in nanotechnology have introduced new ways to enhance clinical standard modalities using various nanomaterials. Certain polymeric nanoparticles release vaccine components on demand when stimulated. Among the currently known adjuvants, cytosine-phosphate-guanine oligodeoxynucleotide (CpG ODN) is the most efficient immune stimulant, which may be identified by toll-like receptor 9 (TLR9) to activate humoral and cellular immunity for cancer prevention and treatment. Cationic and ionizable nanoparticles may boost proinflammatory cytokines because of their immunogenic characteristics. It is also important to note that mRNA-based nanovaccine would hold a considerable promise in the treatment of cancer. Liposomes and lipid nanoparticles have been playing major roles in the clinical application of nanovaccine. Physicochemical characteristics, biointerfacing, quality control, and cost-effectiveness must be addressed for nanovaccine clinical translation. By analyzing the nanovaccines already available and under clinical trials, we would gain insight into the potential future of this field. Emerging vaccination tactics have hurdles, but nanotechnology and material science developments may improve their clinical translation for cancer immunotherapy.

Acknowledgments T. Foyez cordially acknowledges the support received from United International University. A.B. Imran is grateful to the Committee for Advanced Studies and Research (CASR) of Bangladesh University of Engineering and Technology for their financial support.

References

Adachi, K., Kano, Y., Nagai, T., Okuyama, N., Sakoda, Y., & Tamada, K. (2018). IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nature Biotechnology, 36*(4), 346–351. https://doi.org/10.1038/nbt.4086

Ahn, S., Lee, I. H., Kang, S., Kim, D., Choi, M., Saw, P. E., et al. (2014). Gold nanoparticles displaying tumor-associated self-antigens as a potential vaccine for cancer immunotherapy. *Advanced Healthcare Materials, 3*(8), 1194–1199. https://doi.org/10.1002/adhm.201300597

Aiga, T., Manabe, Y., Ito, K., Chang, T. C., Kabayama, K., Ohshima, S., et al. (2020). Immunological evaluation of co-assembling a lipidated peptide antigen and lipophilic adjuvants: Self-adjuvanting anti-breast-cancer vaccine candidates. *Angewandte Chemie (International Ed. in English), 59*(40), 17705–17711. https://doi.org/10.1002/anie.202007999

An, M., & Liu, H. (2017). Dissolving microneedle arrays for transdermal delivery of amphiphilic vaccines. *Small, 13*(26). https://doi.org/10.1002/smll.201700164

Auslander, N., Zhang, G., Lee, J. S., Frederick, D. T., Miao, B., Moll, T., et al. (2018). Robust prediction of response to immune checkpoint blockade therapy in metastatic melanoma. *Nature Medicine, 24*(10), 1545–1549. https://doi.org/10.1038/s41591-018-0157-9

Banchereau, J., & Palucka, K. (2018). Immunotherapy: Cancer vaccines on the move. *Nature Reviews. Clinical Oncology, 15*(1), 9–10. https://doi.org/10.1038/nrclinonc.2017.149

Berraondo, P., Sanmamed, M. F., Ochoa, M. C., Etxeberria, I., Aznar, M. A., Pérez-Gracia, J. L., et al. (2019). Cytokines in clinical cancer immunotherapy. *British Journal of Cancer, 120*(1), 6–15. https://doi.org/10.1038/s41416-018-0328-y

Burris III H. A., Patel, M. R., Cho, D. C., Clarke, J. M., Gutierrez, M., Zaks, T. Z. et al. (2019). A phase 1, open-label, multicenter study to assess the safety, tolerability, and immunogenicity of mRNA-4157 alone in subjects with resected solid tumors and in combination with pembrolizumab in subjects with unresectable solid tumors (Keynote-603). In.: American Society of Clinical Oncology.

Buonsanti, C., Balocchi, C., Harfouche, C., Corrente, F., Galli Stampino, L., Mancini, F., et al. (2016). Novel adjuvant Alum-TLR7 significantly potentiates immune response to glycoconjugate vaccines. *Scientific Reports, 6*, 29063. https://doi.org/10.1038/srep29063

Calvo Tardón, M., Allard, M., Dutoit, V., Dietrich, P. Y., & Walker, P. R. (2019). Peptides as cancer vaccines. *Current Opinion in Pharmacology, 47*, 20–26. https://doi.org/10.1016/j.coph.2019.01.007

Chen, D. S., & Mellman, I. (2013). Oncology meets immunology: the cancer-immunity cycle. *Immunity, 39*(1), 1–10. https://doi.org/10.1016/j.immuni.2013.07.012

Chen, Q., Xu, L., Liang, C., Wang, C., Peng, R., & Liu, Z. (2016). Photothermal therapy with immune-adjuvant nanoparticles together with checkpoint blockade for effective cancer immunotherapy. *Nature Communications, 7*, 13193. https://doi.org/10.1038/ncomms13193

Chen, Q., Bao, Y., Burner, D., Kaushal, S., Zhang, Y., Mendoza, T., Bouvet, M., Ozkan, C., Minev, B., & Ma., W. (2019). Tumor growth inhibition by mSTEAP peptide nanovaccine inducing augmented CD8+ T cell immune responses. *Drug Delivery and Translational Research, 9*(6), 1095–1105.

Chen, F., Wang, Y., Gao, J., Saeed, M., Li, T., Wang, W., & Yu, H. (2021). Nanobiomaterial-based vaccination immunotherapy of cancer. *Biomaterials, 270*, 120709. https://doi.org/10.1016/j.biomaterials.2021.120709

Chiu, Y. C., Gammon, J. M., Andorko, J. I., Tostanoski, L. H., & Jewell, C. M. (2015). Modular vaccine design using carrier-free capsules assembled from polyionic immune signals. *ACS Biomaterials Science & Engineering, 1*(12), 1200–1205. https://doi.org/10.1021/acsbiomaterials.5b00375

Coulie, P. G., Lehmann, F., Lethé, B., Herman, J., Lurquin, C., Andrawiss, M., & Boon, T. (1995). A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. *Proceedings of the National Academy of Sciences of the United States of America, 92*(17), 7976–7980. https://doi.org/10.1073/pnas.92.17.7976

Ding, B., Zheng, P., Jiang, F., Zhao, Y., Wang, M., Chang, M., et al. (2020). MnO(x) nanospikes as nanoadjuvants and immunogenic cell death drugs with enhanced antitumor immunity and antimetastatic effect. *Angewandte Chemie (International Ed. in English), 59*(38), 16381–16384. https://doi.org/10.1002/anie.202005111

Duong, H. T. T., Yin, Y., Thambi, T., Nguyen, T. L., Giang Phan, V. H., Lee, M. S., et al. (2018). Smart vaccine delivery based on microneedle arrays decorated with ultra-pH-responsive copolymers for cancer immunotherapy. *Biomaterials, 185*, 13–24. https://doi.org/10.1016/j.biomaterials.2018.09.008

Eppler, H. B., & Jewell, C. M. (2020). Biomaterials as tools to decode immunity. *Advanced Materials, 32*(13), e1903367. https://doi.org/10.1002/adma.201903367

Fan, Y. N., Li, M., Luo, Y. L., Chen, Q., Wang, L., Zhang, H. B., et al. (2018). Cationic lipid-assisted nanoparticles for delivery of mRNA cancer vaccine. *Biomaterials Science, 6*(11), 3009–3018. https://doi.org/10.1039/c8bm00908b

Fang, R. H., Hu, C. M., Luk, B. T., Gao, W., Copp, J. A., Tai, Y., et al. (2014). Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery. *Nano Letters, 14*(4), 2181–2188. https://doi.org/10.1021/nl500618u

Farris, E., Brown, D. M., Ramer-Tait, A. E., & Pannier, A. K. (2017). Chitosan-zein nano-in-microparticles capable of mediating in vivo transgene expression following oral delivery. *Journal of Controlled Release, 249*, 150–161. https://doi.org/10.1016/j.jconrel.2017.01.035

Felnerova, D., Viret, J. F., Glück, R., & Moser, C. (2004). Liposomes and virosomes as delivery systems for antigens, nucleic acids and drugs. *Current Opinion in Biotechnology, 15*(6), 518–529. https://doi.org/10.1016/j.copbio.2004.10.005

Feng, B., Hou, B., Xu, Z., Saeed, M., Yu, H., & Li, Y. (2019). Self-amplified drug delivery with light-inducible nanocargoes to enhance cancer immunotherapy. *Advanced Materials, 31*(40), e1902960. https://doi.org/10.1002/adma.201902960

Fioretti, D., Iurescia, S., Fazio, V. M., & Rinaldi, M. (2010). DNA vaccines: developing new strategies against cancer. *Journal of Biomedicine & Biotechnology, 2010*, 174378. https://doi.org/10.1155/2010/174378

Forner, M., Defaus, S., & Andreu, D. (2020). Peptide-based multiepitopic vaccine platforms via click reactions. *The Journal of Organic Chemistry, 85*(3), 1626–1634. https://doi.org/10.1021/acs.joc.9b02798

Foyez, T., & Imran, A. B. (2022). Nanotechnology in vaccine development and constraints. In *Nanovaccinology as targeted therapeutics* (pp. 1–20).

Granot-Matok, Y., Kon, E., Dammes, N., Mechtinger, G., & Peer, D. (2019). Therapeutic mRNA delivery to leukocytes. *Journal of Controlled Release, 305*, 165–175. https://doi.org/10.1016/j.jconrel.2019.05.032

Grotzke, J. E., Kozik, P., Morel, J. D., Impens, F., Pietrosemoli, N., Cresswell, P., et al. (2017). Sec61 blockade by mycolactone inhibits antigen cross-presentation independently of endosome-to-cytosol export. *Proceedings of the National Academy of Sciences of the United States of America, 114*(29), E5910–e5919. https://doi.org/10.1073/pnas.1705242114

Gulla, S. K., Rao, B. R., Moku, G., Jinka, S., Nimmu, N. V., Khalid, S., et al. (2019). In vivo targeting of DNA vaccines to dendritic cells using functionalized gold nanoparticles. *Biomaterials Science, 7*(3), 773–788. https://doi.org/10.1039/c8bm01272e

Hailemichael, Y., Dai, Z., Jaffarzad, N., Ye, Y., Medina, M. A., Huang, X. F., et al. (2013). Persistent antigen at vaccination sites induces tumor-specific $CD8^+$ T cell sequestration, dysfunction and deletion. *Nature Medicine, 19*(4), 465–472. https://doi.org/10.1038/nm.3105

Hamilton, E., Blackwell, K., Hobeika, A. C., Clay, T. M., Broadwater, G., Ren, X.-R., et al. (2012). Phase I clinical trial of HER2-specific immunotherapy with concomitant HER2 kinase inhibition, *10*(1), 1–9.

Harun-Ur-Rashid, M., Foyez, T., Jahan, I., Pal, K., & Imran, A. B. (2022). Rapid diagnosis of COVID-19 via nano-biosensor-implemented biomedical utilization: a systematic review. *RSC Advances, 12*(15), 9445–9465. https://doi.org/10.1039/D2RA01293F

He, L. Z., Weidlick, J., Sisson, C., Marsh, H. C., & Keler, T. (2015). Toll-like receptor agonists shape the immune responses to a mannose receptor-targeted cancer vaccine. *Cellular & Molecular Immunology, 12*(6), 719–728. https://doi.org/10.1038/cmi.2014.100

Hickey, J. W., Isser, A., Salathe, S. F., Gee, K. M., Hsiao, M. H., Shaikh, W., et al. (2020). Adaptive nanoparticle platforms for high throughput expansion and detection of antigen-specific T cells. *Nano Letters, 20*(9), 6289–6298. https://doi.org/10.1021/acs.nanolett.0c01511

Hu, J., Wei, P., Seeberger, P. H., & Yin, J. (2018). Mannose-functionalized nanoscaffolds for targeted delivery in biomedical applications. *Chemistry, an Asian Journal, 13*(22), 3448–3459. https://doi.org/10.1002/asia.201801088

Hu, Y., Lin, L., Chen, J., Maruyama, A., Tian, H., & Chen, X. (2020). Synergistic tumor immunological strategy by combining tumor nanovaccine with gene-mediated extracellular matrix scavenger. *Biomaterials, 252*, 120114. https://doi.org/10.1016/j.biomaterials.2020.120114

Huang, Y., Liu, K., Li, Q., Yao, Y., & Wang, Y. (2018). Exosomes function in tumor immune microenvironment. *Advances in Experimental Medicine and Biology, 1056*, 109–122. https://doi.org/10.1007/978-3-319-74470-4_7

Huang, W., Zhang, Q., Li, W., Yuan, M., Zhou, J., Hua, L., et al. (2020). Development of novel nanoantibiotics using an outer membrane vesicle-based drug efflux mechanism. *Journal of Controlled Release, 317*, 1–22. https://doi.org/10.1016/j.jconrel.2019.11.017

Imran, A. B. (2020). Design, development, characterization and application of smart polymeric hydrogel. In M. A. Mellal (Ed.), *Manufacturing systems: Recent progress and future directions* (pp. 187–218). Nova Science Publishers, Inc.

Imran, A. B., Seki, T., & Takeoka, Y. (2010). Recent advances in hydrogels in terms of fast stimuli responsiveness and superior mechanical performance. *Polymer Journal, 42*(11), 839–851%@ 1349-0540.

Innovations in Nanomaterials. (2015). (A.-N. Chowdhury, J. Shapter, & A. B. Imran Eds.). Nova Science Publishers, Inc.

Jiang, H., Wang, Q., & Sun, X. (2017). Lymph node targeting strategies to improve vaccination efficacy. *Journal of Controlled Release, 267*, 47–56. https://doi.org/10.1016/j.jconrel.2017.08.009

Kightlinger, W., Duncker, K. E., Ramesh, A., Thames, A. H., Natarajan, A., Stark, J. C., et al. (2019). A cell-free biosynthesis platform for modular construction of protein glycosylation pathways. *Nature Communications, 10*(1), 5404. https://doi.org/10.1038/s41467-019-12024-9

Kingwell, K. (2019). Uncoupling resistance to cancer immunotherapy. *Nature Reviews. Drug Discovery, 18*(3), 171. https://doi.org/10.1038/d41573-019-00025-8

Knight, F. C., Gilchuk, P., Kumar, A., Becker, K. W., Sevimli, S., Jacobson, M. E., et al. (2019). Mucosal immunization with a pH-responsive nanoparticle vaccine induces protective CD8(+) lung-resident memory T cells. *ACS Nano, 13*(10), 10939–10960. https://doi.org/10.1021/acsnano.9b00326

Knutson, K. L., & Mittendorf, E. A. (2015). Cancer vaccines in the new era of cancer immunotherapy. *Vaccine, 33*(51), 7376. https://doi.org/10.1016/j.vaccine.2015.09.086

Kowalski, P. S., Rudra, A., Miao, L., & Anderson, D. G. (2019). Delivering the messenger: Advances in technologies for therapeutic mRNA delivery. *Molecular Therapy, 27*(4), 710–728. https://doi.org/10.1016/j.ymthe.2019.02.012

Kranz, L. M., Diken, M., Haas, H., Kreiter, S., Loquai, C., Reuter, K. C., et al. (2016). Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. *Nature, 534*(7607):396–401.

Li, X., & Bu, X. (2017). Progress in vaccine therapies for breast cancer. *Advances in Experimental Medicine and Biology, 1026*, 315–330. https://doi.org/10.1007/978-981-10-6020-5_15

Li, J., Cui, D., Huang, J., He, S., Yang, Z., Zhang, Y., et al. (2019). Organic semiconducting pronanostimulants for near-infrared photoactivatable cancer immunotherapy. *Angewandte Chemie (International Ed. in English), 58*(36), 12680–12687. https://doi.org/10.1002/anie.201906288

Li, X., Cai, X., Zhang, Z., Ding, Y., Ma, R., Huang, F., et al. (2020). Mimetic heat shock protein mediated immune process to enhance cancer immunotherapy. *Nano Letters, 20*(6), 4454–4463. https://doi.org/10.1021/acs.nanolett.0c01230

Liao, W., Du, Y., Zhang, C., Pan, F., Yao, Y., Zhang, T., & Peng, Q. (2019). Exosomes: The next generation of endogenous nanomaterials for advanced drug delivery and therapy. *Acta Biomaterialia, 86*, 1–14. https://doi.org/10.1016/j.actbio.2018.12.045

Lin, L. C., Chattopadhyay, S., Lin, J. C., & Hu, C. J. (2018). Advances and opportunities in nanoparticle- and nanomaterial-based vaccines against bacterial infections. *Advanced Healthcare Materials, 7*(13), e1701395. https://doi.org/10.1002/adhm.201701395

Lin, L. C., Huang, C. Y., Yao, B. Y., Lin, J. C., Agrawal, A., Algaissi, A., et al. (2019). Viromimetic STING agonist-loaded hollow polymeric nanoparticles for safe and effective vaccination against middle east respiratory syndrome coronavirus. *Advanced Functional Materials, 29*(28), 1807616. https://doi.org/10.1002/adfm.201807616

Lindsay, K. E., Bhosle, S. M., Zurla, C., Beyersdorf, J., Rogers, K. A., Vanover, D., et al. (2019). Visualization of early events in mRNA vaccine delivery in non-human primates via PET-CT and near-infrared imaging. *Nature Biomedical Engineering, 3*(5), 371–380. https://doi.org/10.1038/s41551-019-0378-3

Liu, H., Moynihan, K. D., Zheng, Y., Szeto, G. L., Li, A. V., Huang, B., et al. (2014). Structure-based programming of lymph-node targeting in molecular vaccines. *Nature, 507*(7493), 519–522. https://doi.org/10.1038/nature12978

Liu, X., Feng, Z., Wang, C., Su, Q., Song, H., Zhang, C., et al. (2020). Co-localized delivery of nanomedicine and nanovaccine augments the postoperative cancer immunotherapy by amplifying T-cell responses. *Biomaterials, 230*, 119649. https://doi.org/10.1016/j.biomaterials.2019.119649

Lou, B., De Beuckelaer, A., Boonstra, E., Li, D., De Geest, B. G., De Koker, S., et al. (2019). Modular core-shell polymeric nanoparticles mimicking viral structures for vaccination. *Journal of Controlled Release, 293*, 48–62. https://doi.org/10.1016/j.jconrel.2018.11.006

Lu, Z., Zuo, B., Jing, R., Gao, X., Rao, Q., Liu, Z., et al. (2017). Dendritic cell-derived exosomes elicit tumor regression in autochthonous hepatocellular carcinoma mouse models. *Journal of Hepatology, 67*(4), 739–748. https://doi.org/10.1016/j.jhep.2017.05.019

Lyu, Y., & Pu, K. (2017). Recent advances of activatable molecular probes based on semiconducting polymer nanoparticles in sensing and imaging. *Advances Science (Weinh), 4*(6), 1600481. https://doi.org/10.1002/advs.201600481

Ma, L., Diao, L., Peng, Z., Jia, Y., Xie, H., Li, B., et al. (2021). Immunotherapy and prevention of cancer by nanovaccines loaded with whole-cell components of tumor tissues or cells. *Advanced Materials* (Deerfield Beach, Fla), *33*(43):e2104849.

Mascola, J. R., & Fauci, A. S. (2020). Novel vaccine technologies for the 21st century. *Nature Reviews. Immunology, 20*(2), 87–88. https://doi.org/10.1038/s41577-019-0243-3

McLeod, H. L., Mariam, A., Schveder, K. A., & Rotroff, D. M. (2020). Assessment of adverse events and their ability to discriminate response to anti-PD-1/PD-L1 antibody immunotherapy. *Journal of Clinical Oncology, 38*(1), 103–104. https://doi.org/10.1200/jco.19.01712

McNamara, M. A., Nair, S. K., & Holl, E. K. (2015). RNA-based vaccines in cancer immunotherapy. *Journal of Immunology Research, 2015*, 794528. https://doi.org/10.1155/2015/794528

Mellman, I., Coukos, G., & Dranoff, G. (2011). Cancer immunotherapy comes of age. *Nature, 480*(7378), 480–489. https://doi.org/10.1038/nature10673

Meng, J., Meng, J., Duan, J., Kong, H., Li, L., Wang, C., et al. (2008). Carbon nanotubes conjugated to tumor lysate protein enhance the efficacy of an antitumor immunotherapy. *Small, 4*(9), 1364–1370. https://doi.org/10.1002/smll.200701059

Mohsen, M. O., Vogel, M., Riether, C., Muller, J., Salatino, S., Ternette, N., et al. (2019). Targeting mutated plus germline epitopes confers pre-clinical efficacy of an instantly formulated cancer nano-vaccine. 10, 1015.

Montomoli, E., Piccirella, S., Khadang, B., Mennitto, E., Camerini, R., & De Rosa, A. (2011). Current adjuvants and new perspectives in vaccine formulation. *Expert Review of Vaccines, 10*(7), 1053–1061. https://doi.org/10.1586/erv.11.48

Mura, S., Nicolas, J., & Couvreur, P. (2013). Stimuli-responsive nanocarriers for drug delivery. *Nature Materials, 12*(11), 991–1003. https://doi.org/10.1038/nmat3776

Neelapu, S. S., Tummala, S., Kebriaei, P., Wierda, W., Gutierrez, C., Locke, F. L., et al. (2018). Chimeric antigen receptor T-cell therapy - Assessment and management of toxicities. *Nature Reviews. Clinical Oncology, 15*(1), 47–62. https://doi.org/10.1038/nrclinonc.2017.148

Nguyen, F., Alferiev, I., Guan, P., Guerrero, D. T., Kolla, V., Moorthy, G. S., et al. (2018). Enhanced Intratumoral delivery of SN38 as a Tocopherol Oxyacetate Prodrug using nanoparticles in a Neuroblastoma Xenograft model. *Clinical Cancer Research, 24*(11), 2585–2593.

Ni, Q., Zhang, F., Liu, Y., Wang, Z., Yu, G., Liang, B., et al. (2020). A bi-adjuvant nanovaccine that potentiates immunogenicity of neoantigen for combination immunotherapy of colorectal cancer. *6*(12), eaaw6071.

Palucka, K., & Banchereau, J. (2012). Cancer immunotherapy via dendritic cells. *Nature Reviews. Cancer, 12*(4), 265–277. https://doi.org/10.1038/nrc3258

Pan, C., Wu, J., Qing, S., Zhang, X., Zhang, L., Yue, H., et al. (2020). Biosynthesis of Self-Assembled Proteinaceous Nanoparticles for Vaccination. *Advanced Materials* (Deerfield Beach, Fla), *32*(42), e2002940.

Patel, M. R., Bauer, T. M., Jimeno, A., Wang, D., LoRusso, P., & Do, K. T., et al. (2020). A phase I study of mRNA-2752, a lipid nanoparticle encapsulating mRNAs encoding human OX40L, IL-23, and IL-36γ, for intratumoral (iTu) injection alone and in combination with durvalumab. In.: American Society of Clinical Oncology.

Pardi, N., Tuyishime, S., Muramatsu, H., Kariko, K., Mui, B. L., Tam, Y. K., et al. (2015). Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *Journal of Controlled Release, 217*, 345–351. https://doi.org/10.1016/j.jconrel.2015.08.007

Pardoll, D. M. (2012). The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews. Cancer, 12*(4), 252–264. https://doi.org/10.1038/nrc3239

Parmiani, G., De Filippo, A., Novellino, L., & Castelli, C. (2007). Unique human tumor antigens: Immunobiology and use in clinical trials. *Journal of Immunology, 178*(4), 1975–1979. https://doi.org/10.4049/jimmunol.178.4.1975

Reinhard, K., Rengstl, B., Oehm, P., Michel, K., Billmeier, A., Hayduk, N., et al. (2020). An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors. *Science, 367*(6476), 446–453. https://doi.org/10.1126/science.aay5967

Rietscher, R., Schröder, M., Janke, J., Czaplewska, J., Gottschaldt, M., Scherließ, R., et al. (2016). Antigen delivery via hydrophilic PEG-b-PAGE-b-PLGA nanoparticles boosts vaccination induced T cell immunity. *European Journal of Pharmaceutics and Biopharmaceutics, 102*, 20–31. https://doi.org/10.1016/j.ejpb.2016.02.014

Rosenberg, S. A. (2014). IL-2: the first effective immunotherapy for human cancer. *Journal of Immunology, 192*(12), 5451–5458. https://doi.org/10.4049/jimmunol.1490019

Ross, J. S., & Fletcher, J. A. (1998). The HER-2/neu oncogene in breast cancer: Prognostic factor, predictive factor, and target for therapy. *The Oncologist, 3*(4), 237–252.

Roy, P., & Noad, R. (2008). Virus-like particles as a vaccine delivery system: myths and facts. *Human Vaccines, 4*(1), 5–12. https://doi.org/10.4161/hv.4.1.5559

Sahin, U., & Türeci, Ö. (2018). Personalized vaccines for cancer immunotherapy. *Science, 359*(6382), 1355–1360. https://doi.org/10.1126/science.aar7112

Schudel, A., Chapman, A. P., Yau, M. K., Higginson, C. J., Francis, D. M., Manspeaker, M. P., et al. (2020). Programmable multistage drug delivery to lymph nodes. *Nature Nanotechnology, 15*(6), 491–499. https://doi.org/10.1038/s41565-020-0679-4

Schwaninger, R., Waelti, E., Zajac, P., Wetterwald, A., Mueller, D., & Gimmi, C. D. (2004). Virosomes as new carrier system for cancer vaccines. *Cancer Immunology, Immunotherapy, 53*(11), 1005–1017. https://doi.org/10.1007/s00262-004-0545-5

Shevtsov, M., & Multhoff, G. (2016). Heat shock protein–peptide and HSP-Based immunotherapies for the treatment of cancer. 7.

Sun, H., Yu, T., Li, X., Lei, Y., Li, J., Wang, X., et al. (2021). Second near-infrared photothermal-amplified immunotherapy using photoactivatable composite nanostimulators. *Journal of Nanobiotechnology, 19*(1), 433. https://doi.org/10.1186/s12951-021-01197-5

Tan, A., De La Peña, H., & Seifalian, A. M. (2010). The application of exosomes as a nanoscale cancer vaccine. *International Journal of Nanomedicine, 5*, 889–900. https://doi.org/10.2147/ijn.S13402

Tang, Z., Xiao, Y., Kong, N., Liu, C., Chen, W., Huang, X., et al. (2021). Nano-bio interfaces effect of two-dimensional nanomaterials and their applications in cancer immunotherapy. *Acta Pharmaceutica Sinica B, 11*(11), 3447–3464. https://doi.org/10.1016/j.apsb.2021.05.004

Tang-Huau, T. L., Gueguen, P., Goudot, C., Durand, M., Bohec, M., Baulande, S., et al. (2018). Human in vivo-generated monocyte-derived dendritic cells and macrophages cross-present antigens through a vacuolar pathway. *Nature Communications, 9*(1), 2570. https://doi.org/10.1038/s41467-018-04985-0

Théry, C., Duban, L., Segura, E., Véron, P., Lantz, O., & Amigorena, S. (2002). Indirect activation of naïve CD4+ T cells by dendritic cell-derived exosomes. *Nature Immunology, 3*(12), 1156–1162. https://doi.org/10.1038/ni854

Thomas, S., & Prendergast, G. C. (2016). Cancer vaccines: A brief overview. *Methods in Molecular Biology, 1403*, 755–761. https://doi.org/10.1007/978-1-4939-3387-7_43

Wang, R. F., & Wang, H. Y. (2017). Immune targets and neoantigens for cancer immunotherapy and precision medicine. *Cell Research, 27*(1), 11–37. https://doi.org/10.1038/cr.2016.155

Wang, C., Sun, W., Ye, Y., Hu, Q., Bomba, H. N., & Gu, Z. (2017). In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy. *Nature Biomedical Engineering, 1*(2), 1–10.

Wang, M., Zhai, Y., Ye, H., Lv, Q., Sun, B., Luo, C., et al. (2019a). High co-loading capacity and stimuli-responsive release based on cascade reaction of self-destructive polymer for improved chemo-photodynamic therapy. *ACS Nano, 13*(6), 7010–7023. https://doi.org/10.1021/acsnano.9b02096

Wang, S., Gao, J., & Wang, Z. (2019b). Outer membrane vesicles for vaccination and targeted drug delivery. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 11*(2), e1523. https://doi.org/10.1002/wnan.1523

Wang, Z., Xu, L., Yu, H., Lv, P., Lei, Z., Zeng, Y., et al. (2019c). Ferritin nanocage-based antigen delivery nanoplatforms: Epitope engineering for peptide vaccine design. *Biomaterials Science, 7*(5), 1794–1800. https://doi.org/10.1039/c9bm00098d

Wang, T., Zhang, H., Qiu, W., Han, Y., Liu, H., & Li, Z. (2022). Biomimetic nanoparticles directly remodel immunosuppressive microenvironment for boosting glioblastoma immunotherapy. *Bioactive Materials, 16*, 418–432.

Warrier, V. U., Makandar, A. I., Garg, M., Sethi, G., Kant, R., Pal, J. K., et al. (2019). Engineering anti-cancer nanovaccine based on antigen cross-presentation. *Bioscience Reports, 39*(10). https://doi.org/10.1042/bsr20193220

Wu, Y. -L., Park, K., Soo, R. A., Sun, Y., Tyroller, K., Wages, D., et al. (2011). INSPIRE: A phase III study of the BLP25 liposome vaccine (L-BLP25) in Asian patients with unresectable stage III non-small cell lung cancer. *BMC Cancer, 11*(1):430.

Wu, C., Zhang, L., Brockman, Q. R., Zhan, F., & Chen, L. (2019). Chimeric antigen receptor T cell therapies for multiple myeloma. *Journal of Hematology & Oncology, 12*(1), 120. https://doi.org/10.1186/s13045-019-0823-5

Xi, X., Zhang, L., Lu, G., Gao, X., Wei, W., & Ma, G. (2018). Lymph node-targeting nanovaccine through antigen-CpG self-assembly potentiates cytotoxic T cell activation. *Journal of Immunology Research, 2018*, 3714960. https://doi.org/10.1155/2018/3714960

Xia, Y., Wu, J., Wei, W., Du, Y., Wan, T., Ma, X., et al. (2018). Exploiting the pliability and lateral mobility of Pickering emulsion for enhanced vaccination. *Nature Materials, 17*(2), 187–194. https://doi.org/10.1038/nmat5057

Xu, Z., & Moyle, P. M. (2018). Bioconjugation approaches to producing subunit vaccines composed of protein or peptide antigens and covalently attached toll-like receptor ligands. *Bioconjugate Chemistry, 29*(3), 572–586. https://doi.org/10.1021/acs.bioconjchem.7b00478

Xu, J., Lv, J., Zhuang, Q., Yang, Z., Cao, Z., Xu, L., et al. (2020). A general strategy towards personalized nanovaccines based on fluoropolymers for post-surgical cancer immunotherapy. *Nature Nanotechnology, 15*(12), 1043–1052. https://doi.org/10.1038/s41565-020-00781-4

Yang, B., Jeang, J., Yang, A., Wu, T. C., & Hung, C. F. (2014). DNA vaccine for cancer immunotherapy. *Human Vaccines & Immunotherapeutics, 10*(11), 3153–3164. https://doi.org/10.4161/21645515.2014.980686

Zhang, C., & Pu, K. (2020). Molecular and nanoengineering approaches towards activatable cancer immunotherapy. *Chemical Society Reviews, 49*(13), 4234–4253. https://doi.org/10.1039/c9cs00773c

Zhang, Y., Xu, C., Yang, X., & Pu, K. (2020). Photoactivatable protherapeutic nanomedicine for cancer. *Advanced Materials, 32*(34), e2002661. https://doi.org/10.1002/adma.202002661

Zhang, L., Wang, K., Huang, Y., Zhang, H., Zhou, L., Li, A., et al. (2022). Photosensitizer-induced HPV16 E7 nanovaccines for cervical cancer immunotherapy. *Biomaterials, 282*, 121411.

Zhong, X., Zhang, Y., Tan, L., Zheng, T., Hou, Y., Hong, X., et al. (2019). An aluminum adjuvant-integrated nano-MOF as antigen delivery system to induce strong humoral and cellular immune responses. *Journal of Controlled Release, 300*, 81–92. https://doi.org/10.1016/j.jconrel.2019.02.035

Zhu, G., Zhang, F., Ni, Q., Niu, G., & Chen, X. (2017). Efficient nanovaccine delivery in cancer immunotherapy. *ACS Nano, 11*(3), 2387–2392. https://doi.org/10.1021/acsnano.7b00978

Chapter 14
Nanostructured Materials–Enabled Biosensors for Drug Delivery and Medical Diagnosis

Bhagavathsingh Jebasingh, A. R. Sneha, K. M. Thushara, Shaji Ephrin, and B. Anna Benedict

14.1 Introduction

Sensors have become an integral part of our daily lives, transforming mundane tasks into smart living. Their importance in the overall Internet of things (IOT) picture cannot be overstated. Leland C. Clark known as the "father of biosensors" invented the first biosensor for oxygen detection in 1956. He also invented an oxygen electrode that bears his name: "Clark electrode." (Heineman et al., 2006). Demonstration of an amperometric enzyme electrode by Leland C. Clark in 1962 for the detection of glucose was followed by the discovery of the first potentiometric biosensor to detect urea by Guilbault and Montalvo in 1969 (Guilbault & Montalvo Jr, 1969). Eventually, Yellow Spring Instruments (YSI) developed the first commercial biosensor in 1975. Biosensors are classified according to the biological compound used, such as antibiotics, enzymes, nucleic acids, or cells, or according to the type of transducer used, such as optical, electrochemical, piezoelectric, or mass-based transducers. They are classified into catalytic and affinity biosensors based on the mode of interaction between both the analyst and the biological materials in the biosensor (Nguyen et al., 2019a). A catalytic biosensor is a type of biosensor where

B. Jebasingh (✉) · K. M. Thushara · S. Ephrin
Department of Applied Chemistry, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India
e-mail: jebasinghb@karunya.edu

A. R. Sneha
Centre for Nanosciences and Genomics, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India

B. Anna Benedict
Department of Chemistry, Panimalar Institute of Technology, Poonthanmalli, Chennai, Tamil Nadu, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_14

the interactions between the analyte and the sensing element led to the formation of a biochemical reaction product, whereas in affinity biosensor, the interactions result in analyte binding onto the transducer. These biosensors have a wide range of applications in drug delivery and medical diagnosis (Yazdi et al., 2020). In drug delivery, many progresses have been made in the development of efficient lab-on-a-chip devices. Biosensor-enabled drug delivery systems have received extensive research, particularly for the treatment of persistent illnesses like diabetes, cardiovascular disease (CVD), and cancer, where usual precise administration of drugs and continuous monitoring are important (Singh et al., 2021). In addition, there are various drug delivery systems including pH-dependent drug delivery, hypoxia-sensing reactive oxygen species–responsive drug delivery that responds to the presence of reactive oxygen species and enzyme-responsive drug delivery that can be done with biosensing technologies. These devices will definitely become more functional and more compact with higher controllability in terms of targeted drug delivery as in the field of nanobiotechnology. Nanostructured materials have shown great potential for biosensing applications due to their unique physical, chemical, electronic, thermophysical, optical, and mechanical properties. Moreover, their small size allows for greater mobility within the human body compared to larger materials. The integration of intelligent nanomotors that can sense and deliver therapeutic agents at the cellular level could bring a significant breakthrough in chemotherapy in the near future (Cicha et al., 2022). Nanoparticle-based drug design has been thoroughly investigated and is a highly sophisticated technology in the realm of nanoscale applications. This technology offers immense potential benefits, such as the capacity to modify various drug properties, including drug release profiles, bioavailability, diffusivity, solubility, and immunogenicity (Patra et al., 2018). Nanoparticle-based drug design has the potential to enhance drug administration routes, minimize toxicity, improve drug distribution, reduce side effects, and prolong the drug's life span. In addition, the use of biosensing systems that can identify and release biomarkers associated with regenerative medicine and diseases has revolutionized the precise treatment of chronic illnesses. However, the application of biosensors faces certain challenges, including their sensitivity, small size, and low detection limits. Overcoming these limitations, nanostructured materials–embedded biosensor systems pave the way for the integration of multiple intelligent biomaterials, both inorganic and organic, with drugs of varying physical and chemical properties. This approach promises to improve disease diagnosis, monitoring, treatment, and management in the future (Naresh & Lee, 2021).

14.1.1 What Are Biosensors?

A biosensor is an advanced analytical device that has the ability to convert a biological response into an electrical signal that can be detected. Typically, biosensors consist of two main components: a molecular recognition element, which is the biological component, and a physicochemical detector component, which is the

transducer. The recognition element, which is immobilized on the transducer's surface, interacts with the target molecules (Fig. 14.1). The transducer then captures the signal produced by this interaction and transforms it into measurable signals. These signals can be used to determine the quantity of analyte present in the sample. Biosensors, which utilize a combination of physical and chemical sensing techniques, were first named by Cammann.

14.1.2 Classification of Biosensors

Based on the two elements present, a sensing element and a transducer, the pioneers Clarke and Lyons in the late 1960s classified biosensors into following types (shown in Fig. 14.2).

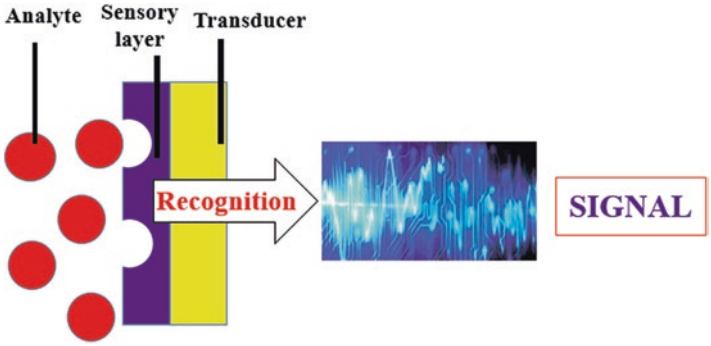


Fig. 14.1 Schematic representation of biosensors

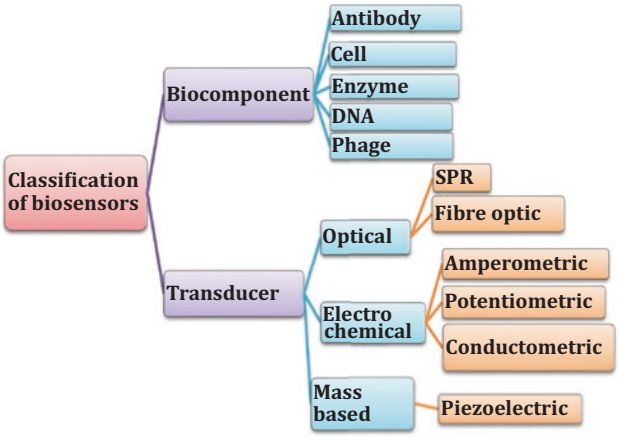


Fig. 14.2 Classification of biosensors

Type of sensors	Characteristics	Example
Active sensor	External power source is required to operate	Global positioning system (GPS) sensors and radar sensors
Passive sensor	They generate their own electric signals	Thermal sensors, metal detecting, and electric field sensing
Contact sensor	They require physical contact with their stimulus	Temperature and strain gauge sensors
Noncontact sensor	No physical contact is required	Optical and magnetic sensors
Absolute sensor	They provide an absolute reading of stimulus to mimic its name	Thermistor always reads the accurate temperature
Relative sensor	They measure a fixed or variable measurement	Thermocouple, where the temperature difference is measured
Analog sensor	They make continuous analogue output signals that are proportional to their measurement	Pressure sensors, light sensors, and sound sensors
Digital sensor	They convert the data transmission, digitally	Digital accelerometers, pressure sensors, and temperature sensors

14.1.3 Why Nanostructured Materials–Based Biosensors?

Nanomaterials play a principal role toward the high performance of a biosensor. Having the greatest surface area to volume ratio, nanomaterials provide greater sensitivity and stability, and they are specifically used for developing electrochemical and optical biosensors. Biocompatibility of nanomaterials is an important factor in creating a biosensor to investigate biomolecules such as bacteria, deoxyribonucleic acid (DNA), viruses, and so on (Malhotra & Ali, 2018). Immobilization of analytes using nanomaterials is essential to boost the detection limit with real-time analysis. A wide range of nanostructured materials such as gold, silver, copper, silicon, graphene, and carbon nanotubes (CNTs) are used for developing biosensor immobilization (Vigneshvar et al., 2016). They are considered as critical components having potential advantages in drug delivery and biomedicine. Nanostructured materials–enabled biosensors improve analyte detection signal by enhancing electrode active surface area, improving enzyme loading, and increasing election transfer and are thus frequently used in the fabrication of enzymatic biosensors (Cheraghi et al., 2022). They can bind with any other materials due to electrical insulation, high specific surface area, biocompatibility, catalytic properties, the presence of oxygenated functional groups, and so on. Therefore, nanostructured materials–based biosensors were discovered to be efficient and superior to other sensors.

14.1.4 Evaluation of Biosensors

The efficiency of biosensors is determined by the following characteristics:

1. Sensitivity: Sensitivity of a biosensor refers to how it responds to changes in the concentration of biological analytes per unit. A biosensor with a higher limit of detection (LOD) is more sensitive. To obtain more precise sensitivity readings, biosensor users typically rely on the linear part of the calibration curve.
2. Selectivity: It is the desired feature of the sensor to respond only to the target analyte from the other interference molecules. It is the ratio of its best response to its second-best response and is one of the most crucial figures of merit in sensing.
3. Stability: Sensor stability is of crucial importance for commercial success. Stability characteristics related to shelf life, continuous use stability, and reusability must be investigated and reported.
4. Response time: It refers to the duration it takes for the device to show its ultimate response as a result of a sequence of changes in analyte concentration. Factors that can influence response time include the concentration of the sample being analyzed, temperature, thickness and permeability of the bioreceptor, and the geometry and agitation rate of the analyzing mixture.
5. Reproducibility: It refers to the sensor's capacity or ability to produce the same output for an equivalent number of inputs applied over a given time period. A biosensor with improved repetition and reproducibility is more reliable. The reproducibility of the sensor's output can be used to determine its accuracy.

The following are to be considered for biosensor development:

1. Choosing an appropriate bioreceptor or biorecognition entity.
2. Method of immobilization selection.
3. Choosing and designing a suitable transducer material.
4. Biosensor design for measurement range, linearity, and interference minimization.
5. Biosensor packaging into a complete unit.
6. Assessing the biosensor's effectiveness.

14.2 Biosensors for Drug Delivery

Biosensor-mediated drug delivery systems have received much attention, especially in the treatment of persistent diseases such as diabetes, cancer, and cardiovascular disease (CVD), where frequent drug administration and management and continuous monitoring are important. These biosensors are either implanted into the body or administered orally. Biosensor is indeed an analytical device with two major components: a biorecognition element that recognizes the target analyte and a transducer that transforms the molecular recognition results into a detectable electrical

signal. Biosensors detect and monitor particular physiochemical changes in the body with high specificity and sensitivity by employing biomolecules such as enzymes, proteins, peptides, antibodies, or nucleic acids such as DNA and ribonucleic acid (RNA) as a biorecognition element known as a bioreceptor (Bazin et al., 2016). It paves way for both monitoring and precisely delivering the drug to the target area. In case of cancer, this sensor delivers drug exactly to the target cells (cancer cells) without affecting the growth of normal cells. As a matter of fact, they are site-specific and precise, allowing for the controlled or genetically programmed release of therapeutic agents. Furthermore, two-dimensional nanomaterials can effectively attack tumor cells in drug delivery by controlling drug release and increasing cell absorption. One good drug delivery medium is MXene (Lei & Guo, 2022). In case of diabetic patients, continuous glucose monitoring is important, which could be done using wearable biosensors (Wang et al., 2022). Insulin can also be administered orally using a novel therapeutic biosensor, such as a self-orienting millimeter scale applicator (SOMA). Recently, smart self-propelled nanomotors and micromotors are being used in the development of drug delivery systems, particularly for cancer treatment (Okeyo et al., 2021). Among the various other types of DNA nanostructured materials, the tetrahedron architecture, having good mechanical properties and functionalities applicable for drug loading, has been used widely in biomedical applications such as drug delivery and in biosensing applications (Chi et al., 2020). Over the years, progress has been made to enhance the situation, particularly in approaches to direct the aggregation and accumulation of drug delivery vehicles to appropriate locations and with controlled release mechanism. Microcapsules, for example, are commonly used as a first generation of drug delivery systems for the controlled release of proteins, peptides, or pharmaceuticals within the body. They can release active molecules at a fair rate, but they cannot pinpoint the particular site of action. In second-generation systems, environmentally sensitive (e.g., temperature-, pH-, or pressure sensitive) microcapsules, nanocapsules, or magnetic spheres are used as delivery vehicles. When a particular signal is received, such as a predetermined temperature or pH, these drug delivery devices will release their payload. Third-generation systems rely on drug-carrying micromatrices or nanomatrices or shells that are functionalized with specialized bioreceptors for target tracking.

14.2.1 Limit of Detection (LOD)

The detection limit is the least effective of analyte that a sensor can reliably detect repeatedly while sustaining its sensitivity rate. It varies from assay to assay, but factors such as working reagents or materials, operator proficiency, storage, processing and diagnostic assay steps, sample quality, and volume all have an impact on detection performance. The methods for determining LOD are determined by the following factors: analytic or manual method using the appropriate measuring equipment,

instrumental technique attributes and sample preparation capability, and sensor responsiveness and noise floor of the read-out system.

14.2.2 Application of Biosensors

Glucose oxidase electrode–based biosensor helps in analysis of glucose in blood samples, thereby detecting the blood sugar level (Bruen et al., 2017). Uric acid biosensor detects clinical abnormalities and diseases (Tvorynska et al., 2021). Silicon biosensors have a wide range of applications in bioimaging, biosensing, and cancer therapy (Ji et al., 2018). Nanomaterials-based biosensors are used in biomedicine, site-specific drug delivery, biomolecular immobilization, etc. Biosensors that are genetically encoded or fluorescence-tagged are used to study biological processes and molecular systems within cells. Wearable electrochemical biosensors are employed for the monitoring of metabolites and nutrients. Biosensors are used for the detection of pathogens (bacteria: *Escherichia coli*) and virus in food (Castillo-Henríquez et al., 2020), clinical analysis and diagnosis of disease, general healthcare monitoring, industrial processing and monitoring, screening for disease, veterinary and agricultural applications, environmental pollution control, remote sensing of airborne bacteria, determining levels of toxic substances before and after bioremediation, etc (Fig. 14.3)

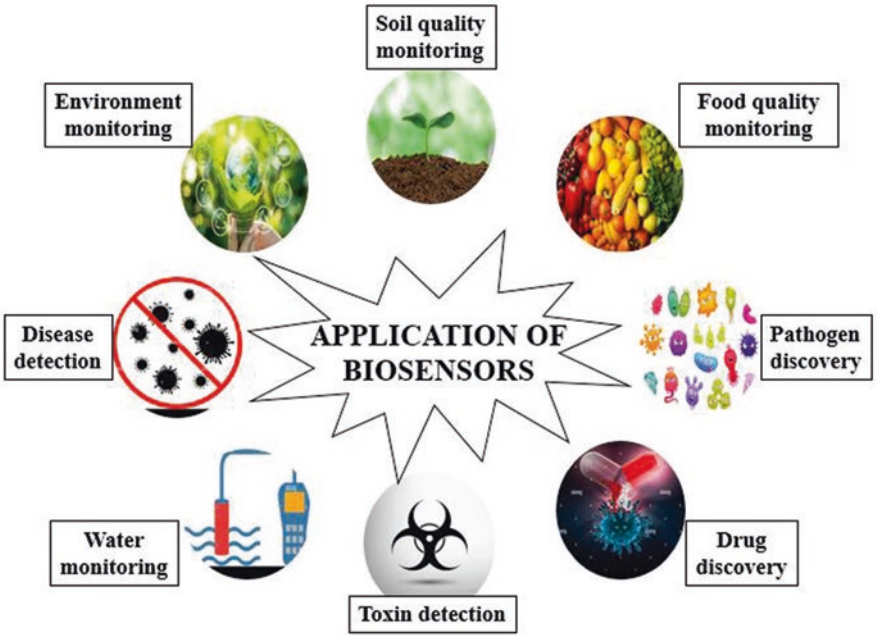


Fig. 14.3 Applications of biosensors

14.3 Nanostructured Materials–Based Biosensors for Medical Diagnosis

14.3.1 Enzyme-Based Biosensors

Enzyme-based biosensors have become a common technique for quantitative and qualitative analysis of a wide range of target analytes in biomedicine, food quality control, agriculture, and environmental and pharmaceutical industries (Rocchitta et al., 2016). The earliest proposed biosensor was the enzyme-based biosensor. It was a glucose sensor proposed by Clark in 1962. The biological component used here is an enzyme. Enzymes are biological macromolecules that have high selectivity and catalytic performance and are responsible for the acceleration of biochemical processes under mild conditions. The glucose sensor is a well-known marketed enzyme-based biosensor. They have substantial advantages over conventional approaches in terms of sensitivity and selectivity, high throughput mobility, and the possibility to be employed for real-time disease analysis and monitoring. In addition, the high specificity of enzymes improves the capacity to detect the limit of detection. In the health-care sector, the most commonly utilized enzyme-based biosensors are those intended for glucose, cholesterol, glutamate, urea, and lactate. The incorporation of inorganic nanoparticles (e.g., silver, platinum, gold, and indium tin oxide) and other nanocomposites as electrode materials is one of the most promising directions in enzyme biosensor (Malhotra & Ali, 2018). Chitosan, a nontoxic chemically inert biopolymer with excellent adhesion qualities, is one of the most appealing electrode coverings in terms of biocompatibility. The assembly or immobilization of the enzyme on the electrode surface is critical in this form of biosensor, and if done incorrectly, the accessibility of the active site, the enzyme's reusability, and the stability over time may be compromised. The primary strategies for immobilization are adsorption, cross-linking, covalent bonding, encapsulation, and entrapment (Nguyen et al., 2019b). Although absorption is simple and affordable, the enzymes have a weak interaction with the support. Because it produces a stable combination between the enzyme and the support, covalent bonding is the most commonly utilized approach. The selection of support material is required to provide selectivity and stability and even to boost enzyme performance. The support material should also be stable, inert, and resistant. A few limitations, such as the quick loss of enzyme activity due to contacts with the electrode surface caused by the employment of enzymes, limit the life span of a biosensor to a few weeks. However, if the enzyme is stabilized with the support, the sensor's life span will be extended. As a result, they have a wide range of uses in food, medicine, and environmental monitoring. Artificial peroxidase nanozyme–enzyme-based lactate biosensors have been produced and are likely to find a widespread application in the development of oxidase-based biosensors (Vokhmyanina et al., 2020) (Fig. 14.4).

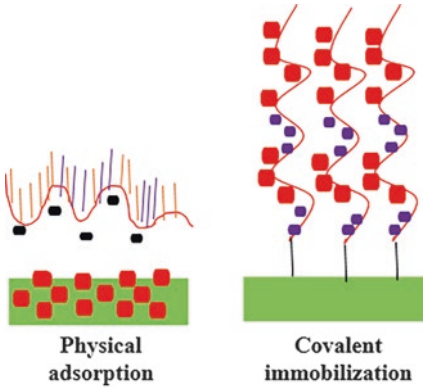


Fig. 14.4 Immobilization techniques for the fabrication of biosensors

14.3.2 Antibody (Ab)-Based Biosensors

An antibody-based biosensor also called immunosensor is made out of antigen or immunizer as a bioreceptor for clinical diagnosis. A broad range of applications including the usage of monoclonal and polyclonal antibodies have been highlighted. These sensors can provide a reliable, sensitive, and timely analysis. Antibodies are perfect biorecognition elements that the body produces in response to an antigen (a foreign molecule or pathogen). They are Y-shaped and have a role in the immune system's defense process. Monoclonal antibody (mAb) technology, also known as hybridoma technology, has transformed the use of Ab as research tools for the prevention, detection, and treatment of certain diseases. Unlike polyclonal antibodies, monoclonal antibodies are antigen-specific. Köhler and Milstein pioneered the hybridoma technique in 1975 (Kohler & Milstein, 1975). In this technology, myeloma cells and B cells (B lymphocytes) from spleen are fused together by using polyethylene glycol (PEG). Then, the fused cells or hybridoma is transferred to hypoxanthine and thymidine (HAT) medium. Only the cells that have hypoxanthine guanine phosphoribosyltransferase (HGPRT) gene can grow and survive in HAT medium. B cells are HGPRT+ cells. Hence, only the myeloma cells that got fused with B cells survive and grow, whereas the unfused myeloma cells die as they are devoid of HGPRT gene (Lu et al., 2020). Monoclonal antibodies that have been cultured are then used in biosensing to detect analytes such as food and environmental toxins, biowarfare agents, disease markers (biomarkers), and illicit substances. In most cases, the quality of the recombinant antibodies is the most important factor. This includes the potential to be genetically changed to improve immobilization, selectivity, and sensitivity. It has a wide variety of applications in the detection of pathogens and bacterial, fungal, and viral cells, which cause considerable heath issues to human, and it compromises the agricultural yield.

14.3.3 Immobilization-Based Biosensors

Immobilization-enabled sensors include enzyme-based, antibody-based, cell-based, and DNA biosensors. Because of the relative instability of the mobile enzyme and the difficulties in active recovery of the antibody or enzyme, the methods of enzyme immobilization are extremely important in these types of biosensors. Adsorption is a simple immobilization approach that relies on weak bonds such as van der Waal's forces, hydrophobic interactions, and electrostatic interactions. The next immobilization method is covalent bonding, which provides a stable bond between the enzyme/antibody and the support. The benefits of covalent immobilization include great self-assembled monolayer (SAM) homogeneity and superior control over the amount of immobilized enzyme. To enhance substrate capture potential and retain functional conformation, enzymes must be immobilized in their native form in the immobilization technique. Immobilized enzyme–based biosensors are employed in a variety of applications, including food safety monitoring, biomedical application, tissue engineering, industrial bioprocess monitoring, and pollution detection (Mehrotra, 2016).

14.3.4 Electrochemical Biosensors

Physical, chemical, and biological reactions are turned into electrical signals via transducers in electrochemical biosensors. The electrochemical properties of the transducer define the property of the sensor, which is known as an electrochemical biosensor (Biosensors & Bioelectronics). The classic invention of glucose oxidase–based biosensors (Clark & Lyons, 1962) is the first in the line of electrochemical biosensor discovery. Typically, they are created by altering the surface of electrodes with biomaterials such as enzymes, antibodies, or DNA. The output signals are changes in the physicochemical properties of electroactive substances such as voltage, current, resistance, or superficial charge caused by redox reactions on the electrode surface. Recently, a biosensor based on hemin-functionalized reduced graphene oxide (rGO) that provides good analytical performance was proposed (Zou et al., 2015). rGO shows high catalytic activity and sensitivity due to its larger surface area. They are frequently used as the chemical reactions may lead to changes in the measurement of ions or electrons, which have an effect on electrical parameters of solution. Nanomaterials-based electrochemical biosensors are efficient for antibiotics detection owing to their privileged merits including selectivity, high sensitivity, easy operation, reliability, simplicity, flexible application, low cost, and the amenability of integration into multifunctional analytical tools (Pollap & Kochana, 2019). Electrochemical biosensors are classified into amperometric, voltammetric, impedimetric, and photoelectrochemical biosensors based on the different electrochemical types of transducers. Electrochemical biosensors assess the levels of antioxidants and reactive oxygen species; have more stable output, high sensitivity, and

fast response; and suffer from lesser interferences. They are used in the detection of cancer cells and bacteria; sensing of uric acid, glucose and cholesterol, biological materials, such as enzymes, cells, specific ligands, and tissues, and nonbiological matrixes; and in the detection and quantification of six major pharmaceutical chemicals, including antiinflammatory, antidepressant, antibacterial, antiviral, antifungal, and anticancer medications (Qian et al., 2021).

14.4 Conclusion

Biosensor technology provides several distinct advantages over other traditional analytical methods, including real-time operation, higher sensitivity, lower cost, ease of operation, low detection limits, and downsizing. Concurrent detection and quantification can also take place without any prior derivatization or separation. Furthermore, current developments point to integration and miniaturization in autonomously controlled clinical instruments and lab-on-a-chip devices, which could simplify and extend clinical monitoring to bedside patient and home testing. In the future, the development of these technologies will very definitely be scaled up to the industrial level, allowing for significant benefits to be derived from fundamental studies in biosensors and bioelectrochemistry. Attempts are being undertaken to improve the accuracy, resolution, and miniaturization of biosensors for biomolecule detection and to preprocess microfluidics and samples. Biosensors are expected to meet a number of unmet needs in the medication delivery and medical diagnostic industries due to their portability and quick turnaround time. Smart biosensors that can detect minute concentrations of a desired analyte are emerging as the characteristics of nanomaterials and their dimensions at the nanoscale level advance. Biomolecules such as single-stranded DNA (ssDNA), antibodies, enzymes, or cells can be connected with nanostructured materials via surface chemistry to create an appropriate bio-nano interface capable of detecting a minute concentration of target molecules of interest. Biosensor technology now powers a large portion of the mobile and wearable device industries. While the revolution has already begun, advances in biosensor technology are on the verge of opening up many intriguing new possibilities.

References

Bazin, I., Tria, S., Hayat, A., & Marty, J. (2016). New biorecognition molecules in biosensors for the detection of toxins. *Biosensors and Bioelectronics, 87*, 285. https://doi.org/10.1016/j.bios.2016.06.083

Biosensors & Bioelectronics has an open access companion journal Biosensors & Bioelectronics:X. To submit to Biosensors & Bioelectronics:X. https://www.editorialmanager.com/BIOSX/default.aspx

Bruen, D., Delaney, C., Florea, L., & Diamond, D. (2017). Glucose sensing for diabetes monitoring: Recent developments. *Sensors (Basel), 17*(8), 1866. https://doi.org/10.3390/s17081866

Castillo-Henríquez, L., Brenes-Acuña, M., Castro-Rojas, A., Cordero-Salmerón, R., Lopretti-Correa, M., & Vega-Baudrit, J. R. (2020). Biosensors for the detection of bacterial and viral clinical pathogens. *Sensors (Basel), 20*(23), 6926. https://doi.org/10.3390/s20236926

Cheraghi, S., Taher, M. A., Karimi-Maleh, H., Karimi, F., Shabani-Nooshabadi, M., Alizadeh, M., Al-Othman, A., Erk, N., Raman, P. K. Y., & Karaman, C. (2022). Novel enzymatic graphene oxide based biosensor for the detection of glutathione in biological body fluids. *Chemosphere, 287*(Part 2), 132187, ISSN 0045-6535. https://doi.org/10.1016/j.chemosphere.2021.132187

Chi, Q., Yang, Z., Xu, K., Wang, C., & Liang, H. (2020 Jan 28). DNA nanostructure as an efficient drug delivery platform for immunotherapy. *Frontiers in Pharmacology, 10*, 1585. https://doi.org/10.3389/fphar.2019.01585

Cicha, I., Priefer, R., Severino, P., Souto, E. B., & Jain, S. (2022 Aug 29). Biosensor-integrated drug delivery systems as new materials for biomedical applications. *Biomolecules, 12*(9), 1198. https://doi.org/10.3390/biom12091198

Clark, L. C., & Lyons, C. (1962). Electrode systems for continuous monitoring in cardiovascular surgery. *Annals of the New York Academy of Sciences, 102*, 29–45. https://doi.org/10.1111/j.1749-6632.1962.tb13623.x

Guilbault, G. G., & Montalvo, J. G., Jr. (1969). Urea-specific enzyme electrode. *Journal of the American Chemical Society, 91*, 2164–2165. https://doi.org/10.1021/ja01036a083

Heineman, W. R., Jensen, W. B., & Clark, L. C., Jr. (2006). Biosensor. *Bioelectron, 21*, 1403–1404. https://doi.org/10.1016/j.bios.2005.12.005

Ji, X., Wang, H., Song, B., Chu, B., & He, Y. (2018). Silicon nanomaterials for biosensing and bioimaging analysis. *Frontiers in Chemistry, 6*, 38. https://doi.org/10.3389/fchem.2018.00038

Kohler, G., & Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature, 256*, 495–497.

Lei, Z.-L., & Guo, B. (2022). 2D Material-based optical biosensor: Status and prospect. *Advanced Science, 9*, 2102924. https://doi.org/10.1002/advs.202102924

Lu, R. M., Hwang, Y. C., Liu, I. J., et al. (2020). Development of therapeutic antibodies for the treatment of diseases. *Journal of Biomedical Science, 27*, 1. https://doi.org/10.1186/s12929-019-0592-z

Malhotra, B. D., & Ali, M. A. (2018). Nanomaterials in biosensors: Fundamentals and applications. *Nanomaterials for Biosensors*, 1–74. https://doi.org/10.1016/B978-0-323-44923-6.00001-7

Mehrotra, P. (2016). Biosensors and their applications - A review. *Journal of Oral Biology and Craniofacial Research, 6*(2), 153–159. https://doi.org/10.1016/j.jobcr.2015.12.002

Naresh, V., & Lee, N. (2021). A review on biosensors and recent development of nanostructured materials-enabled biosensors. *Sensors, 21*, 1109. https://doi.org/10.3390/s21041109

Nguyen, H. H., Lee, S. H., Lee, U. J., Fermin, C. D., & Kim, M. (2019a Jan 2). Immobilized enzymes in biosensor applications. *Materials (Basel), 12*(1), 121. https://doi.org/10.3390/ma12010121

Nguyen, H. H., Lee, S. H., Lee, U. J., Fermin, C. D., & Kim, M. (2019b). Immobilized enzymes in biosensor applications. *Materials (Basel), 12*(1), 121. https://doi.org/10.3390/ma12010121

Okeyo, P. O., Rajendran, S. T., Zór, K., & Boisen, A. (2021). Sensing technologies and experimental platforms for the characterization of advanced oral drug delivery systems. *Advanced Drug Delivery Reviews, 176*, 113850, ISSN 0169-409X. https://doi.org/10.1016/j.addr.2021.113850

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta-Torres, L. S., Diaz-Torres, L. A., Grillo, R., Swamy, M. K., Sharma, S., Habtemariam, S., & Shin, H. S. (2018). Nano based drug delivery systems: Recent developments and future prospects. *Journal of Nanobiotechnology, 16*(1), 71. https://doi.org/10.1186/s12951-018-0392-8

Pollap, A., & Kochana, J. (2019). Electrochemical immunosensors for antibiotic detection. *Biosensors, 9*, 61. https://doi.org/10.3390/bios9020061

Qian, L., Durairaj, S., Prins, S., & Chen, A. (2021). Nanomaterial-based electrochemical sensors and biosensors for the detection of pharmaceutical compounds. *Biosensors and Bioelectronics, 175*, 112836. https://doi.org/10.1016/j.bios.2020.112836

Rocchitta, G., Spanu, A., Babudieri, S., Latte, G., Madeddu, G., Galleri, G., Nuvoli, S., Bagella, P., Demartis, M. I., Fiore, V., Manetti, R., & Serra, P. A. (2016). Enzyme biosensors for biomedical applications: Strategies for safeguarding analytical performances in biological fluids. *Sensors, 16*, 780. https://doi.org/10.3390/s16060780

Singh, A., Sharma, A., Ahmed, A., Sundramoorthy, A. K., Furukawa, H., Arya, S., & Khosla, A. (2021). Recent advances in electrochemical biosensors: Applications, challenges, and future scope. *Biosensors, 11*, 336. https://doi.org/10.3390/bios11090336

Tvorynska, S., Barek, J., & Josypčuk, B. (2021). Flow amperometric uric acid biosensors based on different enzymatic mini-reactors: A comparative study of uricase immobilization. *Sensors and Actuators B: Chemical, 344*, 130252, ISSN 0925-4005. https://doi.org/10.1016/j.snb.2021.130252

Vigneshvar, S., Sudhakumari, C. C., Senthilkumaran, B., & Prakash, H. (2016). Recent advances in biosensor technology for potential applications - An overview. *Frontiers in Bioengineering and Biotechnology, 4*, 11. https://doi.org/10.3389/fbioe.2016.00011

Vokhmyanina, D. V., Andreeva, K. D., Komkova, M. A., Kaeyakina, E., & E., & Karyakin.A.A. (2020). Artificial peroxidase nanoenzyme-based lactate biosensor. *Talanta, 208*, 120393. https://doi.org/10.1016/j.talanta.2019.120393

Wang, M., Yang, Y., Min, J., et al. (2022). A wearable electrochemical biosensor for the monitoring of metabolites and nutrients. *Nature Biomedical Engineering, 6*, 1225–1235. https://doi.org/10.1038/s41551-022-00916-z

Yazdi, M. K., Zarrintaj, P., Bagheri, B., Kim, Y. C., Ganjali, M. R., & Saeb, M. R. (2020). 32 - Na Mozafari. In *Woodhead Publishing Series in Biomaterials, nanoengineered biomaterials for advanced drug delivery* (pp. 767–779). Elsevier. https://doi.org/10.1016/B978-0-08-102985-5.00032-2

Zou, H. L., Li, B. L., Luo, H. Q., & Li, N. B. (2015). A novel electrochemical biosensor based on hemin functionalized graphene oxide sheets for simultaneous determination of ascorbic acid, dopamine and uric acid. *Sensors and Actuators B: Chemical, 207*(Part A), 535–541, ISSN 0925-4005. https://doi.org/10.1016/j.snb.2014.10.121

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

C. Pushpalatha, S. V. Sowmya, Dominic Augustine, Chhaya Kumar, K. V. Bharkavy, S. Jithya, V. S. Gayathri, Arshiya Shakir, and Reshma Dhodwad

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,

C. Pushpalatha (✉) · C. Kumar · K. V. Bharkavy · S. Jithya · V. S. Gayathri · A. Shakir
R. Dhodwad
Department of Pedodontics and Preventive Dentistry, Faculty of Dental Sciences, M. S. Ramaiah University of Applied Sciences, Bengaluru, India

S. V. Sowmya · D. Augustine
Department of Oral Pathology and Microbiology, Faculty of Dental Sciences, M.S. Ramaiah University of Applied Sciences, Bengaluru, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_15

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.

References

Afarid, M., Mahmoodi, S., & Baghban, R. (2022). Recent achievements in nano-based technologies for ocular disease diagnosis and treatment, review and update. *Journal of Nanobiotechnology, 20*, 361. https://doi.org/10.1186/s12951-022-01567-7

Ahmed, S., Chaudhry, S. A., & Ikram, S. (2017). A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: A prospect towards green chemistry. *Journal of Photochemistry and Photobiology B: Biology, 166*, 272–284.

Ahmed, R., et al. (2019). A public BCR present in a unique dual-receptor-expressing lymphocyte from type 1 diabetes patients encodes a potent T cell autoantigen. *Cell, 177*(6), 1583–1599.

Aljabali, A. A., et al. (2020). Albumin nano-encapsulation of piceatannol enhances its anticancer potential in colon cancer via downregulation of nuclear p65 and HIF-1α. *Cancers, 12*(1), 113. https://doi.org/10.3390/cancers12010113

Aqel, A., et al. (2012). Carbon nanotubes, science and technology part (I) structure, synthesis and characterisation. *Arabian Journal of Chemistry, 5*(1), 1–23. https://doi.org/10.1016/j.arabjc.2010.08.022

Asadi, K., & Gholami, A. (2021). Virosome-based nanovaccines; a promising bioinspiration and biomimetic approach for preventing viral diseases: A review. *International Journal of Biological Macromolecules, 182*, 648. https://doi.org/10.1016/j.ijbiomac.2021.04.005

Azharuddin, M., et al. (2022). Nano toolbox in immune modulation and nanovaccines. *Trends in Biotechnology* [Preprint].

Baetke, S. C., Lammers, T., & Kiessling, F. (2015). Applications of nanoparticles for diagnosis and therapy of cancer. *The British Journal of Radiology, 88*(1054), 20150207. https://doi.org/10.1259/bjr.20150207

Ball, J. M., et al. (1998). Oral immunization with recombinant norwalk virus-like particles induces a systemic and mucosal immune response in mice. *Journal of Virology, 72*(2), 1345–1353.

Banzhoff, A., et al. (2008). MF59®-adjuvanted vaccines for seasonal and pandemic influenza prophylaxis. *Influenza and Other Respiratory Viruses, 2*(6), 243–249. https://doi.org/10.1111/j.1750-2659.2008.00059.x

Bhavsar, M. D., & Amiji, M. M. (2007). Polymeric nano-and microparticle technologies for oral gene delivery. *Expert Opinion on Drug Delivery, 4*(3), 197–213.

Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. *International Journal of Nanomedicine, 10*, 975. https://doi.org/10.2147/IJN.S68861

CALDERon-GARCIDUEnas, L, et al. (2004). Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. *Toxicologic Pathology, 32*(6), 650–658.

Crampton, H. L., & Simanek, E. E. (2007). Dendrimers as drug delivery vehicles: Non-covalent interactions of bioactive compounds with dendrimers. *Polymer International, 56*(4), 489–496. https://doi.org/10.1002/pi.2230

Cruz, L. J., et al. (2014). Tracking targeted bimodal nanovaccines: immune responses and routing in cells, tissue, and whole organism. *Molecular Pharmaceutics, 11*(12), 4299–4313.

Cui, R., Han, Z., & Zhu, J.-J. (2011). Helical carbon nanotubes: Intrinsic peroxidase catalytic activity and its application for biocatalysis and biosensing. *Chemistry - A European Journal, 17*(34), 9377–9384. https://doi.org/10.1002/chem.201100478

Dar, P., et al. (2013). Montanide ISA (TM) 201 adjuvanted FMD vaccine induces improved immune responses and protection in cattle. *Vaccine, 31*. https://doi.org/10.1016/j.vaccine.2013.05.078

Das, I., et al. (2017). Biocompatible chitosan nanoparticles as an efficient delivery vehicle for Mycobacterium tuberculosis lipids to induce potent cytokines and antibody response through activation of γδ T cells in mice. *Nanotechnology, 28*(16). https://doi.org/10.1088/1361-6528/aa60fd

De Jong, W. H., & Borm, P. J. (2008). Drug delivery and nanoparticles: Applications and hazards. *International Journal of Nanomedicine, 3*(2), 133.

Demento, S. L., Cui, W., Criscione, J. M., Stern, E., Tulipan, J., Kaech, S. M., & Fahmy, T. M. (2012). Role of sustained antigen release from nanoparticle vaccines in shaping the T cell memory phenotype. *Biomaterials, 33*(19), 4957–4964. https://doi.org/10.1016/j.biomaterials.2012.03.041

Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews, 281*(1), 8–27.

Dobrovolskaia, M. A., & McNeil, S. E. (2016). *Handbook of immunological properties of engineered nanomaterials* (In 3 Volumes). World Scientific.

Dykman, L. A. (2020). Gold nanoparticles for preparation of antibodies and vaccines against infectious diseases. *Expert Review of Vaccines*, 1–13. https://doi.org/10.1080/14760584.2020.1758070

El Sayed, M., Niazy, M. A., & Farouk, H. F. (2022). Evaluation of remineralizing potential of cranberry and chitosan on demineralized dentin (An in Vitro Study). *Al-Azhar Dental Journal for Girls, 9*(1), 83–93. https://doi.org/10.21608/adjg.2021.74882.1359

Fang, M., et al. (2012). Quantum dots for cancer research: Current status, remaining issues, and future perspectives. *Cancer Biology & Medicine, 9*(3), 151–163. https://doi.org/10.7497/j.issn.2095-3941.2012.03.001

Feng, C., et al. (2022a). Emerging vaccine nanotechnology: From defense against infection to sniping cancer. *Acta Pharmaceutica Sinica B, 12*(5), 2206–2223. https://doi.org/10.1016/j.apsb.2021.12.021

Feng, C., et al. (2022b). Emerging vaccine nanotechnology: From defense against infection to sniping cancer. *Acta Pharmaceutica Sinica B* [Preprint].

Gao, W., et al. (2013). Liposome-like nanostructures for drug delivery. *Journal of Materials Chemistry. B, Materials for Biology and Medicine, 1*(48). https://doi.org/10.1039/C3TB21238F

Gao, W., et al. (2015). Modulating antibacterial immunity via bacterial membrane-coated nanoparticles. *Nano Letters, 15*(2), 1403–1409. https://doi.org/10.1021/nl504798g

Gao, Y., Wijewardhana, C., & Mann, J. F. S. (2018). Virus-like particle, liposome, and polymeric particle-based vaccines against HIV-1. *Frontiers in Immunology, 9*. https://doi.org/10.3389/fimmu.2018.00345

Gheibi Hayat, S. M., & Darroudi, M. (2019). Nanovaccine: A novel approach in immunization. *Journal of Cellular Physiology, 234*(8), 12530–12536.

Gonzalez-Aramundiz, J. V., et al. (2012). Nanovaccines: nanocarriers for antigen delivery. *Biologie aujourd'hui, 206*(4), 249–261.

Gregory, A. E., Titball, R., & Williamson, D. (2013). Vaccine delivery using nanoparticles. *Frontiers in Cellular and Infection Microbiology, 3*. https://doi.org/10.3389/fcimb.2013.00013

Gupta, A. K., et al. (2007). Recent advances on surface engineering of magnetic iron oxide nanoparticles and their biomedical applications.

Han, J., et al. (2018). Polymer-based nanomaterials and applications for vaccines and drugs. *Polymers, 10*(1), 31. https://doi.org/10.3390/polym10010031

Han, X., et al. (2022). Biomaterial-assisted biotherapy: A brief review of biomaterials used in drug delivery, vaccine development, gene therapy, and stem cell therapy. *Bioactive Materials, 17*, 29–48. https://doi.org/10.1016/j.bioactmat.2022.01.011

Hasan, A., et al. (2018). Nanoparticles in tissue engineering: Applications, challenges and prospects. *International Journal of Nanomedicine, 13*, 5637. https://doi.org/10.2147/IJN.S153758

Holm, C. K., et al. (2012). Virus-cell fusion as a trigger of innate immunity dependent on the adaptor STING. *Nature Immunology, 13*(8), 737–743.

Jackson, T. C., Patani, B. O., & Ekpa, D. E. (2017). Nanotechnology in diagnosis: A review. *Advances in Nanoparticles, 6*(3), 93–102.

Jain, K. K. (2003). Nanodiagnostics: Application of nanotechnology in molecular diagnostics. *Expert Review of Molecular Diagnostics, 3*(2), 153–161.

Jeevanandam, J., et al. (2018). Review on nanoparticles and nanostructured materials: History, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology, 9*, 1050–1074. https://doi.org/10.3762/bjnano.9.98

Jing, Z., et al. (2022). Nanomedicines and nanomaterials for cancer therapy: Progress, challenge and perspectives. *Chemical Engineering Journal, 446*, 137147. https://doi.org/10.1016/j.cej.2022.137147

Junghanns, J.-U. A. H., & Müller, R. H. (2008). Nanocrystal technology, drug delivery and clinical applications. *International Journal of Nanomedicine, 3*(3), 295–310.

Karch, C. P., & Matyas, G. R. (2021). The current and future role of nanovaccines in HIV-1 vaccine development. *Expert Review of Vaccines, 20*(8), 935–944. https://doi.org/10.1080/14760584.2021.1945448

Kendall, M. (2006). Engineering of needle-free physical methods to target epidermal cells for DNA vaccination. *Vaccine, 24*(21), 4651–4656.

Keshari, R., et al. (2019). Nanotechnology -Emerging weapon in Health care system.

Kiessling, F., et al. (2014). Nanoparticles for imaging: Top or flop? *Radiology, 273*(1), 10.

Korupalli, C., et al. (2019). Single-injecting, bioinspired nanocomposite hydrogel that can recruit host immune cells in situ to elicit potent and long-lasting humoral immune responses. *Biomaterials, 216*, 119268.

Kulkarni, P., & Vaidya, K. (2010). Liposomes: A novel drug delivery system. *International Journal of Current Pharmaceutical Review and Research, 3*.

Kushnir, N., Streatfield, S. J., & Yusibov, V. (2012). Virus-like particles as a highly efficient vaccine platform: Diversity of targets and production systems and advances in clinical development. *Vaccine, 31*(1), 58. https://doi.org/10.1016/j.vaccine.2012.10.083

Lin, F. (2015). *Development of gold nanoparticle-based antigen delivery platform for vaccines against HIV-1*. Doctor of Philosophy. Iowa State University, Digital Repository. https://doi.org/10.31274/etd-180810-4094

López-Sagaseta, J., et al. (2015). Self-assembling protein nanoparticles in the design of vaccines. *Computational and Structural Biotechnology Journal, 14*, 58–68. https://doi.org/10.1016/j.csbj.2015.11.001

Lu, Z.-R., Ye, F., & Vaidya, A. (2007). Polymer platforms for drug delivery and biomedical imaging. *Journal of Controlled Release, 122*(3), 269–277.

Lyons, J. G., et al. (2020). Nanostructured biomaterials for bone regeneration. *Frontiers in Bioengineering and Biotechnology, 8*, 922. https://doi.org/10.3389/fbioe.2020.00922

MacDonald, K. N., Piret, J. M., & Levings, M. K. (2019). Methods to manufacture regulatory T cells for cell therapy. *Clinical & Experimental Immunology, 197*(1), 52–63.

Madaan, K., et al. (2014). Dendrimers in drug delivery and targeting: Drug-dendrimer interactions and toxicity issues. *Journal of Pharmacy & Bioallied Sciences, 6*(3), 139–150. https://doi.org/10.4103/0975-7406.130965

Manceau, C., et al. (2021). Biomarker in active surveillance for prostate cancer: A systematic review. *Cancers, 13*(17). https://doi.org/10.3390/cancers13174251

Mitchell, M. J., et al. (2021). Engineering precision nanoparticles for drug delivery. *Nature Reviews Drug Discovery, 20*(2), 101–124. https://doi.org/10.1038/s41573-020-0090-8

Mody, K., et al. (2013). Mesoporous silica nanoparticles as antigen carriers and adjuvants for vaccine delivery. *Nanoscale, 5*. https://doi.org/10.1039/c3nr00357d

Mondal, J., et al. (2022). Carbon nanotube and its derived nanomaterials based high performance biosensing platform. *Biosensors, 12*(9), 731. https://doi.org/10.3390/bios12090731

Nandedkar, T. D. (2009). Nanovaccines: Recent developments in vaccination. *Journal of Biosciences, 34*(6), 995–1003.

Nandedkar, T. D. (2012). Nanotechnology a path to nanovaccine. *International Journal of Pharma and Bio Sciences, 3*(2), 290–292.

Nasir, A. (2009). Nanotechnology in vaccine development: A step forward. *Journal of Investigative Dermatology, 129*(5), 1055–1059.

Negahdari, B., Darvishi, M., & Saeedi, A. A. (2019). Gold nanoparticles and hepatitis B virus. *Artificial Cells, Nanomedicine, and Biotechnology, 47*(1), 455–461.

Nel, A., et al. (2006). Toxic potential of materials at the nanolevel. *Science, 311*(5761), 622–627. https://doi.org/10.1126/science.1114397

Ni, Q., et al. (2020). A bi-adjuvant nanovaccine that potentiates immunogenicity of neoantigen for combination immunotherapy of colorectal cancer. *Science Advances, 6*(12), eaaw6071. https://doi.org/10.1126/sciadv.aaw6071

Nicol, P. A. E., & Lachmann, P. J. (1973). The alternate pathway of complement activation. *Immunology, 24*(2), 259–275.

Nooraei, S., et al. (2021). Virus-like particles: Preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. *Journal of Nanobiotechnology, 19*(1), 59. https://doi.org/10.1186/s12951-021-00806-7

Oberdörster, G., Oberdörster, E., & Oberdörster, J. (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspectives, 113*(7), 823–839.

Oun, A. A., Shankar, S., & Rhim, J.-W. (2020). Multifunctional nanocellulose/metal and metal oxide nanoparticle hybrid nanomaterials. *Critical Reviews in Food Science and Nutrition, 60*(3), 435–460. https://doi.org/10.1080/10408398.2018.1536966

Patra, J. K., et al. (2018). Nano based drug delivery systems: Recent developments and future prospects. *Journal of Nanobiotechnology, 16*. https://doi.org/10.1186/s12951-018-0392-8

Poon, W., et al. (2019). Elimination pathways of nanoparticles. *ACS Nano, 13*(5), 5785–5798.

Pusic, K., et al. (2013). Iron oxide nanoparticles as a clinically acceptable delivery platform for a recombinant blood-stage human malaria vaccine. *FASEB Journal: official Publication of the Federation of American Societies for Experimental Biology, 27*(3), 1153–1166. https://doi.org/10.1096/fj.12-218362

Renu, S., et al. (2020). Oral deliverable mucoadhesive chitosan-salmonella subunit nanovaccine for layer chickens. *International Journal of Nanomedicine, 15*, 761–777. https://doi.org/10.2147/IJN.S238445

Rezaei, R., et al. (2019). The role of nanomaterials in the treatment of diseases and their effects on the immune system. *Open Access Macedonian Journal of Medical Sciences, 7*(11), 1884.

Rosales-Mendoza, S., & González-Ortega, O. (2019). *Nanovaccines*. Springer.

Saadeh, Y., & Vyas, D. (2014). Nanorobotic applications in medicine: Current proposals and designs. *American Journal of Robotic Surgery, 1*(1), 4–11. https://doi.org/10.1166/ajrs.2014.1010

Scheinberg, D. A., et al. (2013). Carbon nanotubes as vaccine scaffolds. *Advanced Drug Delivery Reviews, 65*(15). https://doi.org/10.1016/j.addr.2013.07.013

Schwendener, R. A. (2014). Liposomes as vaccine delivery systems: A review of the recent advances. *Therapeutic Advances in Vaccines, 2*(6), 159–182. https://doi.org/10.1177/2051013614541440

Sengupta, A., et al. (2022). Efficacy and immune response elicited by gold nanoparticle-based nanovaccines against infectious diseases. *Vaccine, 10*(4), 505.

Shadjou, N., & Hasanzadeh, M. (2015). Silica-based mesostructured nanomaterials for use in bone tissues engineering: Recent progress. *Materials Science and Engineering: C, 55*. https://doi.org/10.1016/j.msec.2015.05.027

Sharma, S., et al. (2009). Pharmaceutical aspects of intranasal delivery of vaccines using particulate systems. *Journal of Pharmaceutical Sciences, 98*(3), 812–843.

Shaw, S. Y., et al. (2008). Perturbational profiling of nanomaterial biologic activity. *Proceedings of the National Academy of Sciences, 105*(21), 7387–7392.

Shen, H., et al. (2006). Enhanced and prolonged cross-presentation following endosomal escape of exogenous antigens encapsulated in biodegradable nanoparticles. *Immunology, 117*(1), 78–88. https://doi.org/10.1111/j.1365-2567.2005.02268.x

Shen, Y., et al. (2018). Applications and perspectives of nanomaterials in novel vaccine development. *MedChemComm, 9*(2), 226. https://doi.org/10.1039/c7md00158d

Shetty, N. J., Swati, P., & David, K. (2013). Nanorobots: Future in dentistry. *The Saudi Dental Journal, 25*(2), 49–52. https://doi.org/10.1016/j.sdentj.2012.12.002

Sreeharsha, N., et al. (2022). Multifunctional mesoporous silica nanoparticles for oral drug delivery. *Coatings, 12*(3), 358. https://doi.org/10.3390/coatings12030358

Sun, H.-X., Xie, Y., & Ye, Y.-P. (2009). ISCOMs and ISCOMATRIX. *Vaccine, 27*(33), 4388–4401. https://doi.org/10.1016/j.vaccine.2009.05.032

Sundararaman, S. K., & Barbie, D. A. (2018). Tumor cGAMP awakens the natural killers. *Immunity, 49*(4), 585–587. https://doi.org/10.1016/j.immuni.2018.10.001

Tandale, P., et al. (2021). Fluorescent quantum dots: An insight on synthesis and potential biological application as drug carrier in cancer. *Biochemistry and Biophysics Reports, 26*, 100962. https://doi.org/10.1016/j.bbrep.2021.100962

Tao, W., & Gill, H. S. (2015). M2e-immobilized gold nanoparticles as influenza A vaccine: Role of soluble M2e and longevity of protection. *Vaccine, 33*(20), 2307–2315. https://doi.org/10.1016/j.vaccine.2015.03.063

Tao, W., Ziemer, K. S., & Gill, H. S. (2014). Gold nanoparticle–M2e conjugate coformulated with CpG induces protective immunity against influenza A virus. *Nanomedicine, 9*(2), 237–251. https://doi.org/10.2217/nnm.13.58

Tayeb, H. H., et al. (2021). Nanoemulsions: Formulation, characterization, biological fate, and potential role against COVID-19 and other viral outbreaks. *Colloid and Interface Science Communications, 45*, 100533. https://doi.org/10.1016/j.colcom.2021.100533

Vu, C.-A., & Chen, W.-Y. (2019). Field-effect transistor biosensors for biomedical applications: Recent advances and future prospects. *Sensors (Basel, Switzerland), 19*(19). https://doi.org/10.3390/s19194214

Wang, X., Ishida, T., & Kiwada, H. (2007). Anti-PEG IgM elicited by injection of liposomes is involved in the enhanced blood clearance of a subsequent dose of PEGylated liposomes. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 119*(2), 236–244. https://doi.org/10.1016/j.jconrel.2007.02.010

Wang, N., Chen, M., & Wang, T. (2019). Liposomes used as a vaccine adjuvant-delivery system: From basics to clinical immunization. *Journal of Controlled Release, 303*, 130–150. https://doi.org/10.1016/j.jconrel.2019.04.025

Yaqoob, A. A., et al. (2020). Recent advances in metal decorated nanomaterials and their various biological applications: A review *Frontiers in Chemistry*, 8. https://www.frontiersin.org/articles/10.3389/fchem.2020.00341. Accessed 18 Sept 2022.

Yezdani, U., et al. (2018). Application of nanotechnology in diagnosis and treatment of various diseases and its future advances in medicine. *World Journal of Pharmacy and Pharmaceutical Sciences, 7*(11), 1611–1633.

Youssef, F. S., et al. (2019). Application of some nanoparticles in the field of veterinary medicine. *International Journal of Veterinary Science and Medicine, 7*(1), 78–93. https://doi.org/10.1080/23144599.2019.1691379

Ys, C., et al. (2010). Assessment of gold nanoparticles as a size-dependent vaccine carrier for enhancing the antibody response against synthetic foot-and-mouth disease virus peptide. *Nanotechnology, 21*(19). https://doi.org/10.1088/0957-4484/21/19/195101

Zeinali, M., et al. (2009). Immunological and cytotoxicological characterization of tuberculin purified protein derivative (PPD) conjugated to single-walled carbon nanotubes. *Immunology Letters, 126*(1), 48–53. https://doi.org/10.1016/j.imlet.2009.07.012

Zhang, C., et al. (2022). Applications and biocompatibility of mesoporous silica nanocarriers in the field of medicine. *Frontiers in Pharmacology, 13*, 829796. https://doi.org/10.3389/fphar.2022.829796

Zhao, L., et al. (2014). Nanoparticle vaccines. *Vaccine, 32*(3), 327–337. https://doi.org/10.1016/j.vaccine.2013.11.069

Zhu, M., Wang, R., & Nie, G. (2014). Applications of nanomaterials as vaccine adjuvants. *Human Vaccines & Immunotherapeutics, 10*(9), 2761–2774. https://doi.org/10.4161/hv.29589

Zhu, X.-Y., et al. (2021). Novel nanofluidic cells based on nanowires and nanotubes for advanced chemical and bio-sensing applications. *Nanomaterials, 11*(1), 90. https://doi.org/10.3390/nano11010090

Chapter 16
Green Nanotechnology Approaches in Vaccinology: Advantages and Disadvantages in Biomedical Sciences

Shubhankhi Dey and Gajendra B. Singh

16.1 Introduction

Vaccines have been a life-saviour ever since its introduction in the 1900s. With the rise of deadly epidemics around the world, the need for the development of vaccines has increased significantly. Hence, by the end of 1940s, successful vaccines against pertussis, diphtheria, tetanus and smallpox had given a glimpse of hope to combat against the major epidemics and in a few years resulted in constriction and elimination of respective viral breakouts (Pollard & Bijker, 2021). Subsequently, specialized research in the field of vaccines and vaccination had acquired a substantial importance. Vaccines against polio, influenza, measles, etc., have also been developed, paving way for more advanced and effective vaccines in the future. However, as the history stands witness, vaccine development is recognized as a tedious process that consumes almost more than a decade for successful completion of all stages of development (Han, 2015). The reason for this long tenure is the phases of clinical trials that are followed by the exploratory phase, which is considered the pioneering phase of development. In the beginning, preclinical assessment of vaccine development is accomplished, during which the vaccine is tested on cell culture systems proceeding to tissue cultures and then to animal models for assessing immunogenicity and safety of it. Depending on the results achieved after animal model trials, further human trials are performed. This includes similar assessment for safety and immunogenicity starting from smaller groups of people and gradually moving towards larger groups. The entire process is summed up in three phases of human clinical trials for approval.

Even though the development of vaccines is the need of the hour, researchers often encounter challenges, especially in case of trials on the international level due

S. Dey · G. B. Singh (✉)
University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India
e-mail: gajendra.singh@cumail.in

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_16

to the differences in rules prevailing in different countries. Apart from this, finding progressive trial sites is also a challenging task. Lack of infrastructure, finding the correct dosage that can suffice efficacy globally and lack of skilled personnel may also emerge as notable limitations against successful vaccination development process (Drury et al., 2019).

Vaccines have been one of the most significant achievements in the history of medical science. Vaccination had emerged as one of the most effective methods to combat life-threatening infectious diseases. Although over the years scientists constantly strive towards developing more and more vaccines, most of them are inefficient in the process. This is because most of the vaccines that have already been designed are characterized with lower immunogenicity (Kim et al., 2014). Besides, the existing vaccines have also been reported to have higher toxicity and lower in vivo stability (Kheirollahpour et al., 2020). In most cases, vaccination requires multiple follow-up administration of doses and cold chains. Moreover, the vaccines previously developed were futile in providing prolonged immune responses against the causative agent. Hence, currently, scientists are approaching adopting nanovaccines as a better and more advanced alternative to the existing conventional vaccines.

Thus, this chapter discusses nanovaccines and the types of nanovaccines that are currently being considered in vaccinology. Brief discussion has been provided regarding the importance of green nanotechnology in the field of vaccinology and their benefits as an advanced future alternative.

16.2 Nanovaccine

Nanoparticle (NP)-based vaccines ranging in the size between 10 and 1000 nm have shown exceptional physicochemical properties and a potential for enabling drug delivery. Particles like biocompatible nanoparticles, exosomes and liposomes in the above-mentioned range can be considered effective vaccine delivery systems. Such nanovaccines offer more stability and are able to enhance the bioavailability of antigens, messenger ribonucleic acid (mRNA) and deoxyribonucleic acid (DNA) in an encapsulated form (Pardi et al., 2018; Sharma et al., 2020). Nanovaccines have the potential to overcome the limitations of subunit vaccines due to their customized surface chemistry, adjustable size, serum stability and enhanced controlling capacity (Yıldız & Ünver, 2022; Zhao et al., 2014). NP-based vaccines are capable of entrapping antigens or enabling surface attachment, thereby facilitating subcellular trafficking. Thus, these can induce Th1 type immunity in the host organism, which can effectively combat against intracellular pathogenic viral species such as *Trypanosoma*, *Mycobacterium*, *Leishmania* and human immunodeficiency virus (HIV) (Fries et al., 2021; Petkar et al., 2021).

16.2.1 Green Nanotechnology in Vaccines

The implementation of nanotechnology in vaccines has paved a pathway for the implementation of advanced therapeutic measures. Currently, for the preparation of vaccines, scientists have been preferring green synthesis methods with the help of biocompatible compounds for reduced toxicity and better bioavailability (Shah et al., 2015). Green synthesis involves the implementation of plant derivatives, proteins, microbes, etc. Such compounds act not only as reducing agents in the nanoparticle synthesis but also as capping agents in most cases. Specific capping agents for metal or nonmetal nanoparticles are selected, which not only enhance their biomedical properties but also adhere to their additive properties (Schröfel et al., 2014; Singh et al., 2016). These, when implemented in the case of nanovaccines, specific biological components are used. Thus, nanovaccines made up of liposomes or viral protein components carry antigenic components that thereby help in successfully enhancing the immunity in the hosts. The green synthesis method of metallic nanoparticles has been represented diagrammatically in Fig. 16.1.

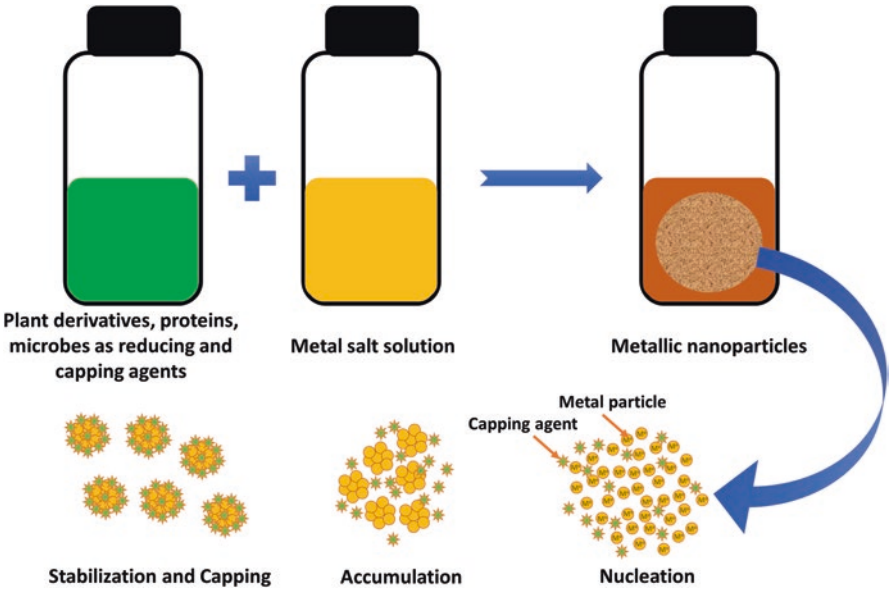


Fig. 16.1 Green synthesis of metallic nanoparticles

16.2.2 Factors Determining the Successful Development of Nanovaccines

For the successful development of vaccines, three important factors need to be given primary importance: (i) a good antigen for the activation of immunity, (ii) an immune adjuvant for the costimulation of the innate immunity and (iii) a carrier system for receiving the antigen-presenting cells (APC) (Facciolà et al., 2019). Any vaccine is developed keeping these factors in mind. However, for the preparation of nanovaccine, the properties of nanoparticles should be considered alongside. The size, charge, distribution and shape of the nanoparticle play an important role in the development of nanovaccines. This is because studies conducted on several cell lines have shown some to be highly specific to allowing only microscale particles. Apart from this, the size of nanoparticles also plays an important role in determining the immune responses. As per the study conducted by Hirosue et al. (2010), the type 1 $CD4^+$ and $CD8^+$ cells are stimulated by nanoparticles that belong to the range below 500 nm, whereas the type 2 $CD4^+$ cells can be stimulated for antibody production by particles of size greater than 500 nm. Hence, the physicochemical properties of the nanoparticles used for vaccine development play a crucial role in determining the efficacy of the nanovaccines.

16.2.3 Nanocarriers for Vaccine Delivery

In the hunt for developing effective solutions that can combat against some of the major life-threatening viruses in the world, nanotechnology has been highly successful. Considering the wide range of applications of nanotechnology, it was also implemented in the field of vaccinology. Implementing nanotechnology in vaccinology has resulted in upgraded forms of vaccine development overcoming several underlying issues (Kallon et al., 2021). The advanced form of treatment has been reported to have higher immunogenicity, prolonged stability and better efficacy. The type of nanostructures involved plays a vital role, as the intrinsic properties of the nanocarriers become a deciding factor in the therapeutic approach. Various nanocarriers used for the synthesis of nanovaccines have been depicted in Fig. 16.2.

16.2.3.1 Organic Nanocarriers

16.2.3.1.1 Exosome Vaccines

Exosome vaccines have played an important role in cancer immunotherapy. Exosomes used for the treatment of cancer have shown promising results in activating anticancer immune response. Cells that are detected as malignant secrete an increased number of exosomes that are infused with tumour-inducing antigens. According to the study conducted by Chen et al. (2018), proteinaceous checkpoints

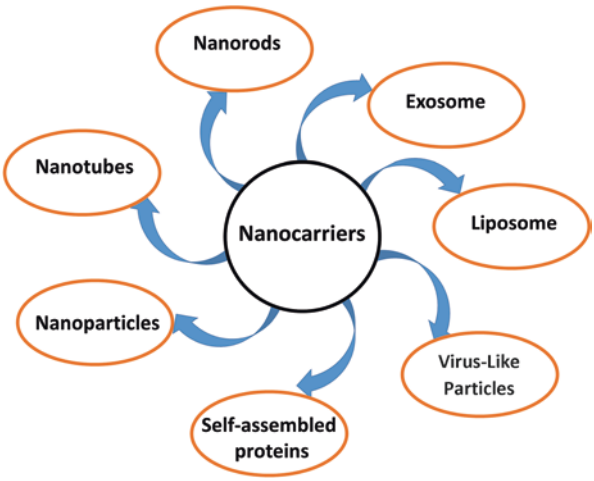


Fig. 16.2 Nanocarriers used for the synthesis of nanovaccines

such as programmed death-ligand 1 (PD-L1) have been detected on the surface of exosomes that are freshly budding from the malignant cells. These after interacting with the programmed death-1 (PD-1) receptors present on the cytotoxic T cells do not allow the destruction of the tumour cells. However, as per the present research, the tumour-derived exosomes (TEXs) and the dendritic cell (DC)-derived exosomes (DEXs) have shown promising results in the development of anticancer vaccines used for therapeutic purposes (Naseri et al., 2020).

To develop exosome vaccines, scientists are using reverse engineering technology to find cures for cancers. As per reports, TEXs have shown reversal ability to tumorigenesis and immunosuppressing ability. TEX vaccines under preclinical trials have shown significant results in stimulating immune responses against cancer causing elements in breast cancer, metastatic carcinoma and leukemic models (Naseri et al., 2020). Besides, several immune-cell derived exosomes artificially engineered with neoantigens may have therapeutic anticancerous properties (Shenoda & Ajit, 2016).

16.2.3.1.2 Liposome Vaccines

Due to their high immunogenicity (Zahednezhad et al., 2019), liposomes are being used as potential drug delivery systems and gene transporters. Liposomes are recognized as spherical in shape, lipid bilayers, with an internal cavity that serves as carriers of biologically active compounds (van der Koog et al., 2022). Measuring between 50 and 1000 nm, liposomes may have one or more than one concentric membrane. The biophysical and physicochemical properties of liposomes are flexible (Kim & Jeong, 2021), which make them useful for drug or vaccine delivery systems, fabrication, biosensors and diagnosis (Nakhaei et al., 2021). Hence, in the

field of biomedicine, liposomes are being incorporated as nanovaccines in the form of nanostructures (Vijayan et al., 2019; Moon et al., 2011). Implementing liposomes in the nano form enhances their intrinsic properties and also makes them more bioavailable with an increased efficiency.

Liposome vaccines have reported comparatively lower reactogenicity. These flexible and biodegradable components act as adjuvants that strengthen the vaccine's immune response. This occurs as the antigen gets embedded inside the aqueous core or absorbed on the membrane for transportation. However, there are several factors that determine the proficiency of the liposomes as a reliable vaccine. In this, the lamellarity of the liposome, size, bilayer fluidity, surface charge and immunostimulatory lipid addition play a crucial role as, either singly or jointly, they determine the cumulative function of liposomes as a vaccine.

16.2.3.1.3 Virus-Like Particles (VLPs)

Nanoparticles formed by the self-assembly of protein capsid devoid of the genetic material, categorized as virus-like particles (VLPs), have been effectively implemented in the development of viral vaccines (Cappelli et al., 2022; Kushnir et al., 2012). Derived from different types of viruses through different cellular systems (plant, yeast, baculovirus, *Escherichia coli*, etc.) and cell-free systems, the VLPs can range in size between 20 and 800 nm (Lin et al., 2022; Pushko et al., 2013). VLPs naturally mimic the size of the viruses and have a repetitive structural order. These particles have high immunity boosting ability without the negative property of inducing viral infection. Thus, self-assembly of such rapidly processing nanoparticles induces a faster and a long-term immune response in the host, even when there is an absence of adjuvant (Jeong & Seong, 2017). Considering such factors, scientists have been striving towards developing VLP-based vaccines. The ones that are already available in the market have been derived by self-assembly of viral proteins extracted from the virus against which the vaccine is being developed. Several VLP-based vaccines are currently under the various trial phases, while researchers are constantly developing more (Khan et al., 2022; Kushnir et al., 2012).

VLP-based vaccines have been under intensive research for the last few decades. In 1986, the very first VLP-based vaccine, with the ability of stimulating the activation of $CD8^+$ and $CD4^+$ T cells, was designed against the highly pathogenic hepatitis B. It also paved way for future alternatives for viral infections like hepatitis E virus (HEV) and human papillomavirus. VLP-based vaccine for hepatitis B contains surface antigen of hepatitis B (HBsAg) that is formed through recombinant yeast cells. Such yeast-based VLPs also comprise non-glycosylated, hydrophobic S-proteins, lipids (derived from the host cell) and disulphide bonds for structure stabilization (Souri et al., 2022; Roldão et al., 2010). Thus, it can be recognized as a lipoprotein having a hydrophobic fluid core surrounded by a rigid lipid layer that is meant for absorbing the protein moiety (Zhao et al., 2013).

Another ribonucleic acid (RNA)-based viral infection of hepatitis E has been recognized as one of the leading chronic infections and can be fatal, especially in

the first trimester of pregnancy (Yang et al., 2018). Extensive studies have been done, henceforth, on hepatitis E virus, its seroprevalence, core structure and viral potential, and several new vaccines were designed with different protein composition. In this regard, hepatitis E-based VLPs have also been designed by self-assembling core protein, ORF2. The model was the first of its kind designed at that time, and thus, the potential of the vaccine had thereafter been thoroughly assessed and were then subjected to necessary clinical trials (Cid & Bolívar, 2021). Besides the direct administration, Li et al. (2005) used the p239 protein and successfully developed hepatitis E-based VLP in bacteria through recombinant DNA technology (RDT). Successful prevention against the viral infection was obtained in both the cases. A similar study was conducted by Go et al. (2021) where the HEV VLP–based vaccines were designed by expressing the HEV-3 capsid protein, p239 of pigs or swine.

After being able to successfully contain and prevent HBV infection, scientists had thereafter shifted to the HPV, which was not only widespread but also life-threatening. Hence, VLP-based vaccine against human papillomavirus (HPV) was approved in 2006 for successful human implementation (Cutts et al., 2007). For this, the role of the virus was intensively studied. HPV belonged to the group of oncogenic viruses, which was responsible for causing lesions in several skin layers and the mucous membranes, which may or may not be malignant. In the present days, HPV is specifically characterized with cervical malignancy and can be categorized into typical and atypical cancer types. The former is associated with malignancies in the vagina, anus, vulva, oropharynx and penis, whereas the latter is associated with cancer detected in the prostate, rectum or bladder region. This has been restricted up to a certain extent after the advent of the VLP-based vaccines. Studies reveal that aligned vaccination has been able to reduce the widespread cases of cervical cancer approximately 90% (Kavanagh et al., 2017). The HPV vaccines are also designed by self-assembly of 72 pentameric components of the viral capsid (L1 protein) in yeast cells via RDT. Following such mechanism, three VLP-based HPV vaccines have been approved by the US Food and Drug Administration (FDA): The first vaccine was developed in 2006 when a tetravalent vaccine was developed, the second bivalent vaccine was developed in 2009, and a third nonavalent vaccine was approved in 2014 (Brown et al., 2021; Chaturvedi et al., 2011).

16.2.3.1.4 Self-Assembled Proteins

Self-assembly means a procedure of forming organized structures from an unorganized system of components that were already existing. The process may occur without any external interference but by means of local or internal interaction (Chung et al., 2015). When the organized structures are formed of disorganized proteins, those are known as self-assembly of proteins. Although similar to the VLPs, self-assembled proteins differ in the fact that it is not composed of the viral components. Self-assembling proteins such as those of ferritin and major vault protein (MVP) have shown promising therapeutic approaches. Ferritin could form

consecutive spheres measuring up to 10 nm, which when combined with the influenza hemagglutinin (HA) protein produced eight trimeric spikes of HA, which showed an enhanced immune response compared with conventional vaccines (Iyer et al., 2022; Kanekiyo et al., 2013).

The major vault protein is an intracellular, ubiquitously occurring protein; self-assembling from 96 copies results in the formation of a nanoparticle mimicking the shape of a barrel of the dimension of 70 nm × 40 nm. Antigens are first attached to the active domain of the MVP, which is then wrapped inside the self-assembled structure. These proteins have shown successful results in delivering the major outer membrane protein (MOMP) of *Chlamydia* sp. for the active stimulation of the mucosal immunity (Reddy, 2022; Champion et al., 2009).

16.2.3.2 Inorganic Nanoparticles as Vaccines

16.2.3.2.1 Calcium Phosphate Nanoparticle

One of the prime reasons why calcium phosphate nanoparticles have been explored for their vaccine-carrying capacity is because of their biodegradable nature and structural attributes (Temchura et al., 2014). Calcium phosphate nanoparticles have a unique feature of naturally releasing the encapsulated components into the cell even if deprived of any external factor. This occurs due to the change in pH encountered by the nanoparticles after being phagocytized inside the cell (from neutral pH outside the cell to acidic pH inside the cell). In addition, calcium nanoparticles are also preferred because of their biocompatibility and stability. The proficiency of calcium phosphate nanoparticle as stable nanocarriers was proven by Morgan et al. (2008) when a suitably modified carbon phosphate nanoparticle with a size of 80 nm carrying genetic components was tested in liver both in vivo and in vitro. In both the cases, the nanoparticles were able to prevent the encapsulated genetic components from being degraded by deoxyribonuclease (DNase) in both the conditions provided. This motivated the scientists to research its vaccine-carrying potential as well. Thus, several trials, to estimate the potential of the nanoparticles as adjuvants for immune responses in the mucosal region and for DNA vaccines, have also been successfully performed (Zhao et al., 2021; Knuschke et al., 2014).

16.2.3.2.2 Gold Nanoparticle

Due to their intrinsic therapeutic properties, gold nanoparticles are recognized as an important component in the field of nanotechnology. The versatility of the gold nanoparticles resides in the flexibility of their shape as they can be moulded into cubical shape and spherical or cylindrical rod shape according to their applicability. Besides shape, the length of gold nanoparticles ranging in size between 1 and 150 nm also plays a role in regulating the augmentation of the immune responses through various cytokine pathways (Cai et al., 2022; Niikura et al., 2013). Although

gold nanoparticles have been used in several occasions in the biomedical field, it has also been proven as effective carriers for antigen conjugates of influenza virus and for HIV (Facciolà et al., 2017). Xu et al. (2012) experimented with three different components used as surface coatings on gold nanorods. The compounds used are polyethyleneimine (PEI), cetyltrimethylammonium bromide (CTAB) and poly(diallyldimethylammonium chloride) (PDDAC), among which gold-based PEI or PDDAC nanorods showed the successful activation of APCs, along with T-cell proliferation, leading to an increase in both humoral immunity and cellular immunity. Thus, gold-based nanoparticles as vaccine carriers showed better efficacy as compared to treatments with naked HIV-1 envelope plasmid DNA.

Several experiments have been performed by scientists to test the extent of therapeutic potential of gold nanoparticles. In a separate study, a specific ovalbumin 323–339 peptide–D-glucose complex was designed based on gold glyconanoparticles carrying a tetrasaccharide epitope of the *Streptococcus pneumoniae* type 14 capsular polysaccharide (Pn14PS) (Colombo et al., 2018). The complex when administered on mice induced the activation of Th cell by generating more cytokine and anti-Pn14PS IgG antibodies and thereby showed an enhanced immune response.

16.2.3.2.3 Carbon Nanoparticle

Since carbon is an essential cellular component, several scientists have researched its applicability as vaccines. Extensive research has been done to assess its application as a suitable antiviral agent in the form of a nanoparticle, either as a carrier or as an adjuvant (Serrano-Aroca et al., 2021). Carbon nanoparticles may have a wide variation in the physiochemical properties owing to the differences in the structure of the nanoparticles. This also regulates the antigen-carrying capacity of the nanoparticles and boosts their immune responses via specific surface modifications (Liu et al., 2014). Although carbon nanoparticles of different shapes have been designed, they in the form of nanotubes have garnered a distinct attention. Carbon nanotubes (CNTs) are made up of graphene sheets that are rolled into hollow cylindrical shapes. CNTs can either be single-walled, known as single-walled carbon nanotubes (SWCNTs), or be multiwalled by forming concentric sheets (with more than one sheet, up to 50 sheets), known as multiwalled carbon nanotubes (MWCNT) (Rozhina et al., 2021; De Volder et al., 2013). Where the former can measure between 0.5 and 1.5 nm, the latter may measure nearly 100 nm. The size of the carbon nanotubes is important to consider as the functionality and applicability of the carbon nanotubes vary accordingly.

Hence, in 2003, Pantarotto et al. conducted an experiment with the viral peptides of hand-foot-mouth disease (Hoang et al., 2019). In this study, the enveloped viral proteins were covalently attached to the carbon nanotubes. It was observed that the structure of the epitope continued to maintain its immunogenicity even after getting attached to the carbon nanotubes, and the viral protein–CNT complexes were able to successfully release IgG for neutralizing the infection.

In an experiment performed by Hassan et al. in 2016, multiwalled antigen conjugates of different lengths and varied surface charges were used, and their applicability was tested. The results showed a boost in the cellular uptake level with reduced levels of negative changes in the multiwalled carbon nanotubes, which also resulted in enhanced immunity. Hence, it was proved that the surface properties of the carbon nanotubes played a role in regulating their efficacy as vaccine carriers both in vivo and in vitro.

16.2.3.2.4 Silica Nanoparticles (SiNPs)

Silica nanoparticles (SiNPs) are primarily preferred in therapeutic approaches where specific targeting of tumours is needed. However, recent researches have also discovered their excellent applicability as vaccine carriers or as drug delivery agents (Yu et al., 2015). The most striking feature of the silica nanoparticles is that they can stimulate immune response both in vivo and in vitro without any antigenic conjugation (Hou et al., 2022; Wibowo et al., 2014). With necessary modification, silica nanoparticles have proven to be efficient in cellular interaction, improving cell recognition, biomolecule absorption and uptake of necessary cellular components. Silica nanoparticles with versatile applications were also developed so as to enhance the effectiveness of the particles. Thus, mesoporous silica nanoparticles (MSNs) ranging in size between 50 and 200 nm were developed, which could serve both as adjuvants and as nanocarriers for antigen delivery (Ahmed et al., 2022; Chen et al., 2012). To understand their potential, Mody et al. in 2016 tested SiNPs in the form of vesicles against bovine viral diarrhoea virus (BVDV), which resulted in restricted release of the BVDV antigen that was a codon-optimized E2 peptide, thereby preventing the viral infection.

16.3 Biomedical Applications of Nanovaccines

Vaccines have emerged as a medical necessity for not only combating against life-threatening viral infections and diseases but also preventing them. Over the years, scientists have strived towards developing prophylactic vaccines that have successfully helped in almost eradicating diseases like tetanus and smallpox and are continuously striving towards eliminating viral infections like hepatitis (Soriano et al., 2022). However, with the increase in the number of life-threatening diseases, the need for advanced vaccines is on the rise. Thus, therapeutic vaccines that can not only provide life-long immunity against specific diseases but also modulate immune responses are the need of the hour. To obtain reliable solutions, scientists are now shifting towards nanovaccines for an advanced approach against viral diseases.

Nanoparticles are being highly implemented in several sectors of biomedicine. Besides being used in bioimaging, antibiotics, etc., its recent addition as an alternative for conventional vaccines has been praiseworthy. The extent of applications of

nanovaccines is still being studied, and the potential of nanoparticle vaccines as an immune system stimulant is being stressed on. Nanovaccines are being recognized for their ability to enhance both the adaptive immune system and modulating innate immunity (Luo et al., 2017). These are easily retained in the lymph nodes, show better loading of antigen and require lesser dosage in terms of both the frequency and the amount to be administered. With the help of suitable nanoparticles loaded with different foreign antigens, overcoming immunotolerance has also been possible, which thereby diminishes the need for booster doses. Thus, their potential as a therapeutic agent has been appreciated for preventing cancer, tuberculosis, influenza, acquired immunodeficiency syndrome (AIDS) and malaria (Bhardwaj et al., 2020).

Owing to the specific antigen-targeting ability, nanoparticles have proven to be a significant therapeutic approach in the treatment of cancer. Nanocarriers like polymers, liposomes and nanospheres have shown successful results in the delivery of vaccine antigen, thereby stabilizing the anti-tumour T cells. At present, nanovaccines are highly implemented as drug delivery agents in chemotherapies for tumour cell targeting. Current researches are including formulations with dendritic cells, which have proven to be efficient as APCs for the treatment of cancer. These dendritic formulations function by providing selective delivery meant only for specific antigens targeting lymphoid organs, thereby progressing the time period for cytotoxic T-cell immune response. Besides, scientists are also focusing on formulating nanovaccines in combination with T cells for the improvement of adoptive T-cell therapy (Xiao et al., 2021; Fan & Moon, 2015; Park et al., 2013).

The efficacy of nanovaccines has not been limited to humans, but it has also been implemented in animals. The application of nanotechnology in veterinary sciences has been a breakthrough in improving the health of several animals due to the development of effective vaccines and targeted drug delivery. For instance, VGX Animal Health, Inc. has devised ways for inducing both cellular and humoral immune response that has led to the formulation of vaccines meant either for oral ingestion or for intradermal and intramuscular injection. Such drugs are often administered in the form of biobullets available with polyethylene glycol (PEG) coating and are used in the vaccination against *Brucella abortus* in bisons (Malek-Khatabi et al., 2022) or as cancer therapy in dogs (Zhou et al., 2022).

With myriad changes in both biotic and abiotic factors on the earth, sudden advents of noble viruses that may take the shape of epidemic and pandemic are on the rise. Thus, effective vaccination to combat different types of infectious diseases is the need of the hour. Since nanoparticles exhibit successful antiviral properties (Gurunathan et al., 2020), green synthesized nanoparticles are optimally tested for their antiviral efficacy. For instance, in a study conducted by Neuhaus et al. in 2014 included double-adjuvant vaccine with HAC1 (H1N1 influenza hemagglutinin influenza antigen), c-di-GMP (bis-(3′,5′)-cyclic dimeric guanosine monophosphate, mucosal vaccine adjuvant) and silica nanoparticle where the latter acted as the drug delivery system. Vaccine administration was done in the lower lungs of mice model where effective antigen response was observed along with the

reactivation of both systemic and local immune response in order to protect against the influenza virus.

Even today malaria is one of the dreaded diseases, which is responsible of deaths globally. To combat against the high rise of malaria, a vaccine was introduced which arrests the parasite, *Plasmodium falciparum*, in its sexual stage. Among the many tested, Pfs25 was recognized as a promising one (Yadav et al., 2018). However, in order to obtain an immune response over an extended period of time, the combination with gold nanoparticles has yielded promising results. In the study conducted by Kumar et al. (2015) the use of codon-harmonized recombinant Pfs25 (CHrPfs25) with gold nanoparticles acting as delivery agents resulted in enhanced immunogenicity, thereby providing a promising therapeutic approach against malaria. Biomedical applications of nanovaccines are illustrated in Fig. 16.3.

16.4 Advantages and Disadvantages of Nanovaccines

With researchers constantly striving towards developing a better alternative, nanovaccines are emerging as a suitable option. Specifically, nanovaccines are being observed to have an upper hand over traditional vaccines for several reasons. For

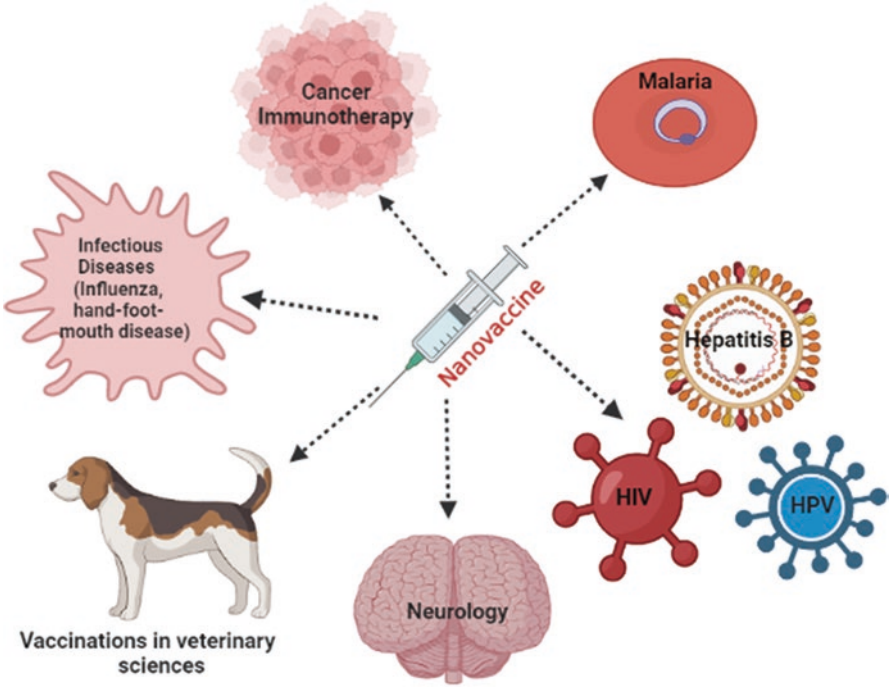


Fig. 16.3 Biomedical applications of nanovaccine. (Image created by BioRender.com)

instance, the administration of traditional vaccines requires the whole body to partake, whereas, in case of nanovaccines, specific infection-encountered areas and source of origination are targeted. Besides, nanosystems ensure proper maintenance of the antigen integrity by increasing stability and preventing degradation. This is possible by increasing the rate of solubility of such compounds that are hydrophobic in nature, thereby enduring better administration. Moreover, nanovaccines also require a reduced dose as compared to traditional vaccine doses (Stammers et al., 2013; Shahbazi & Santos, 2015). One of the distinguished advantages of nanovaccines is their complementing size with that of the cellular components, which facilitates better penetration via endocytosis. Nanovaccines are also capable of targeted drug delivery if coated with antibodies meant for targeting cell-specific receptors and enhance antigen absorption by APCs. Nanovaccines have the ability of cross-presenting antigens with the help of major histocompatibility complex class I (MHC I), thereby activating both the cell's immune system and humoral immunity and thus displaying better efficacy than conventional vaccines (Hirosue et al., 2010; Baljon & Wilson, 2022). Some other notable advantages of the nanovaccine are that the administration of the nanovaccine requires neither a booster dose nor a continuous maintenance of the cold chain. Nanovaccines are capable of creating active targets and are known to have increased longevity in the bloodstream due to greater stability (Gheibi Hayat & Darroudi, 2019).

However, there are several limitations related to the production and use of nanovaccine such as chemical composition, capping agent used and nanoparticle morphology. Toxicity is recognized as one of the major limitations of nanoparticles. It is an unwanted side effect that needs to be minimised if not completely eliminated, for an enhanced efficacy of nanoparticles. The size of the nanoparticle and the zeta potential determine the toxicity of the nanovaccines (Rasmussen et al., 2020). Hence, nanovaccines with lower toxicity and higher stability are preferred for human use. Besides toxicity, nanoparticles inside the body do have access to tissues they pass through. Furthermore, achieving a sterile laboratory condition during scaling-up is another challenge that the nano-industry is currently facing and needs to be solved for future productions at the industrial level (Shahbazi & Santos, 2015).

16.5 Conclusions, Outlook and Future Aspects

Implementation of nanotechnology in vaccinology offers a promising solution to several medical issues. The new generation of vaccines is developed using nanoparticles as potential drug delivery agents and immunostimulants with fewer side effects. The use of nanoparticles in vaccine development enhances the antigen uptake from the antigen-presenting cells. The efficacy of nanoparticle-based nanovaccines is primarily determined by the size, shape and charge of the particles used, which consecutively affects the adjuvanticity, immune system stimulation, antigenicity and inflammatory responses inside the host.

With every progressing day, newer preclinical reports are obtained, which suggest that cationic liposomes and poly(lactic-co-glycolic acid) (PLGA) yield better efficacy in terms of nanovaccines that have also shown future possibility of overcoming drawbacks related to subunit vaccines and live-attenuated vaccines. Nanovaccines are designed based on the physicochemical properties and optimized according to the purpose and target of the cell or organ inside the host. These have gained due attention for their enhanced bioavailability, biocompatibility and biodegradability. These cost-effective formulations of nanovaccines have shown potential therapeutic results against malaria, influenza virus, hepatitis B, HIV and cancer, thereby paving way for future breakthrough. Besides, these are comparatively safer than conventional vaccines as the nanovaccines have low toxicity and higher stability in the bloodstream. However, an extensive study about the physiochemical properties and the binding or functioning inside the host environment is still required. This is because nanovaccines are still a new addition to both the fields of nanotechnology and vaccinology, and a vast amount of studies is still required to assess probable vaccine components in order to eradicate existing life-threatening diseases. Nanovaccines as potential drug delivery agents have provided several groundbreaking therapeutic approaches in both humans and animals; however, an in-depth research regarding feasible alternatives in the form of nanovaccines, along with their preclinical trials, to eliminate risk factors should be continued.

References

Ahmed, H., Gomte, S. S., Prabakaran, A., Agrawal, M., & Alexander, A. (2022). Biomedical applications of mesoporous silica nanoparticles as a drug delivery carrier. *Journal of Drug Delivery Science and Technology, 76*, 103729.

Baljon, J. J., & Wilson, J. T. (2022). Bioinspired vaccines to enhance MHC class-I antigen cross-presentation. *Current Opinion in Immunology, 77*, 102215.

Bhardwaj, P., Bhatia, E., Sharma, S., Ahamad, N., & Banerjee, R. (2020). Advancements in prophylactic and therapeutic nanovaccines. *Acta Biomaterialia, 108*, 1–21.

Brown, D. R., Joura, E. A., Yen, G. P., Kothari, S., Luxembourg, A., Saah, A., Walia, A., Perez, G., Khoury, H., Badgley, D., & Stanley, M. (2021). Systematic literature review of cross-protective effect of HPV vaccines based on data from randomized clinical trials and real-world evidence. *Vaccine, 39*(16), 2224–2236.

Cai, F., Li, S., Huang, H., Iqbal, J., Wang, C., & Jiang, X. (2022). Green synthesis of gold nanoparticles for immune response regulation: Mechanisms, applications, and perspectives. *Journal of Biomedical Materials Research Part A, 110*(2), 424–442.

Cappelli, L., Cinelli, P., Giusti, F., Ferlenghi, I., Utrio-Lanfaloni, S., Wahome, N., Bottomley, M. J., Maione, D., & Cozzi, R. (2022). Self-assembling protein nanoparticles and virus like particles correctly display β-barrel from meningococcal factor H-binding protein through genetic fusion. *PLoS One, 17*(9), e0273322.

Champion, C. I., Kickhoefer, V. A., Liu, G., Moniz, R. J., Freed, A. S., Bergmann, L. L., Vaccari, D., Raval-Fernandes, S., Chan, A. M., Rome, L. H., & Kelly, K. A. (2009). A vault nanoparticle vaccine induces protective mucosal immunity. *PLoS One, 4*(4), e5409.

Chaturvedi, A. K., Engels, E. A., Pfeiffer, R. M., Hernandez, B. Y., Xiao, W., Kim, E., Jiang, B., Goodman, M. T., Sibug-Saber, M., Cozen, W., & Liu, L. (2011). Human papillomavirus

and rising oropharyngeal cancer incidence in the United States. *Journal of Clinical Oncology, 29*(32), 4294.

Chen, K., Zhang, J., & Gu, H. (2012). Dissolution from inside: A unique degradation behaviour of core–shell magnetic mesoporous silica nanoparticles and the effect of polyethyleneimine coating. *Journal of Materials Chemistry, 22*(41), 22005–22012.

Chen, G., Huang, A. C., Zhang, W., Zhang, G., Wu, M., Xu, W., Yu, Z., Yang, J., Wang, B., Sun, H., & Xia, H. (2018). Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature, 560*(7718), 382–386.

Chung, E. J., Mlinar, L. B., Sugimoto, M. J., Nord, K., Roman, B. B., & Tirrell, M. (2015). In vivo biodistribution and clearance of peptide amphiphile micelles. *Nanomedicine: Nanotechnology, Biology and Medicine, 11*(2), 479–487.

Cid, R., & Bolívar, J. (2021). Platforms for production of protein-based vaccines: From classical to next-generation strategies. *Biomolecules, 11*(8), 1072.

Colombo, C., Pitirollo, O., & Lay, L. (2018). Recent advances in the synthesis of glycoconjugates for vaccine development. *Molecules, 23*(7), 1712.

Cutts, F. T., Franceschi, S., Goldie, S., Castellsague, X. D., De Sanjose, S., Garnett, G., Edmunds, W. J., Claeys, P., Goldenthal, K. L., Harper, D. M., & Markowitz, L. (2007). Human papillomavirus and HPV vaccines: A review. *Bulletin of the World Health Organization, 85*, 719–726.

De Volder, M. F., Tawfick, S. H., Baughman, R. H., & Hart, A. J. (2013). Carbon nanotubes: Present and future commercial applications. *Science, 339*(6119), 535–539.

Drury, G., Jolliffe, S., & Mukhopadhyay, T. K. (2019). Process mapping of vaccines: Understanding the limitations in current response to emerging epidemic threats. *Vaccine, 37*(17), 2415–2421.

Facciolà, A., Venanzi Rullo, E., Ceccarelli, M., D'aleo, F., Di Rosa, M., Pinzone, M. R., Condorelli, F., Visalli, G., Picerno, I., Fisichella, R., & Nunnari, G. (2017). Kaposi's sarcoma in HIV-infected patients in the era of new antiretrovirals. *European Review for Medical and Pharmacological Sciences, 21*(24), 5868–5869.

Facciolà, A., Visalli, G., Laganà, P., La Fauci, V., Squeri, R., Pellicanò, G. F., Nunnari, G., Trovato, M., & Di Pietro, A. (2019). The new era of vaccines: The "nanovaccinology". *European Review for Medical and Pharmacological Sciences, 23*(16), 7163–7182.

Fan, Y., & Moon, J. J. (2015). Nanoparticle drug delivery systems designed to improve cancer vaccines and immunotherapy. *Vaccine, 3*(3), 662–685.

Fries, C. N., Curvino, E. J., Chen, J. L., Permar, S. R., Fouda, G. G., & Collier, J. H. (2021). Advances in nanomaterial vaccine strategies to address infectious diseases impacting global health. *Nature Nanotechnology, 16*(4), 1–14.

Gheibi Hayat, S. M., & Darroudi, M. (2019). Nanovaccine: A novel approach in immunization. *Journal of Cellular Physiology, 234*(8), 12530–12536.

Go, H. J., Park, B. J., Ahn, H. S., Kim, D. H., Kim, D. Y., Kim, J. H., Lee, J. B., Park, S. Y., Song, C. S., Lee, S. W., & Choi, Y. K. (2021). Pigs immunized with the virus-like particle vaccine are protected against the hepatitis E-3 virus. *Vaccine, 9*(11), 1265.

Gurunathan, S., Qasim, M., Choi, Y., Do, J. T., Park, C., Hong, K., Kim, J. H., & Song, H. (2020). Antiviral potential of nanoparticles—Can nanoparticles fight against coronaviruses? *Nanomaterials, 10*(9), 1645.

Han, S. (2015). Clinical vaccine development. *Clinical and Experimental Vaccine Research, 4*(1), 46–53.

Hassan, H. A., Smyth, L., Rubio, N., Ratnasothy, K., Wang, J. T. W., Bansal, S. S., Summers, H. D., Diebold, S. S., Lombardi, G., & Al-Jamal, K. T. (2016). Carbon nanotubes' surface chemistry determines their potency as vaccine nanocarriers in vitro and in vivo. *Journal of Controlled Release, 225*, 205–216.

Hirosue, S., Kourtis, I. C., van der Vlies, A. J., Hubbell, J. A., & Swartz, M. A. (2010). Antigen delivery to dendritic cells by poly (propylene sulfide) nanoparticles with disulfide conjugated peptides: Cross-presentation and T cell activation. *Vaccine, 28*(50), 7897–7906.

Hoang, C. Q., Nguyen, T. T. T., Ho, N. X., Nguyen, H. D., Nguyen, A. B., Nguyen, T. H. T., Phan, H. C., & Phan, L. T. (2019). Transmission and serotype features of hand foot mouth disease in household contacts in Dong Thap, Vietnam. *BMC Infectious Diseases, 19*(1), 1–10.

Hou, F., Teng, Z., Ru, J., Liu, H., Li, J., Zhang, Y., Sun, S., & Guo, H. (2022). Flower-like mesoporous silica nanoparticles as an antigen delivery platform to promote systemic immune response. *Nanomedicine: Nanotechnology, Biology and Medicine, 42*, 102541.

Iyer, S., Yadav, R., Agarwal, S., Tripathi, S., & Agarwal, R. (2022). Bioengineering strategies for developing vaccines against respiratory viral diseases. *Clinical Microbiology Reviews, 35*(1), e00123–e00121.

Jeong, H., & Seong, B. L. (2017). Exploiting virus-like particles as innovative vaccines against emerging viral infections. *Journal of Microbiology, 55*(3), 220–230.

Kallon, S., Samir, S., & Goonetilleke, N. (2021). Vaccines: Underlying principles of design and testing. *Clinical Pharmacology & Therapeutics, 109*(4), 987–999.

Kanekiyo, M., Wei, C. J., Yassine, H. M., McTamney, P. M., Boyington, J. C., Whittle, J. R., Rao, S. S., Kong, W. P., Wang, L., & Nabel, G. J. (2013). Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. *Nature, 499*(7456), 102–106.

Kavanagh, K., Pollock, K. G., Cuschieri, K., Palmer, T., Cameron, R. L., Watt, C., Bhatia, R., Moore, C., Cubie, H., Cruickshank, M., & Robertson, C. (2017). Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: A 7-year cross-sectional study. *The Lancet Infectious Diseases, 17*(12), 1293–1302.

Khan, W. H., Ahmad, R., Khan, N., & Ansari, M. A. (2022). SARS-CoV-2 variants associated challenges in the ongoing vaccination of COVID-19.

Kheirollahpour, M., Mehrabi, M., Dounighi, N. M., Mohammadi, M., & Masoudi, A. (2020). Nanoparticles and vaccine development. *Pharmaceutical Nanotechnology, 8*(1), 6–21.

Kim, E. M., & Jeong, H. J. (2021). Liposomes: Biomedical applications. *Chonnam Medical Journal, 57*(1), 27.

Kim, M. G., Park, J. Y., Shon, Y., Kim, G., Shim, G., & Oh, Y. K. (2014). Nanotechnology and vaccine development. *Asian Journal of Pharmaceutical Sciences, 9*(5), 227–235.

Knuschke, T., Bayer, W., Rotan, O., Sokolova, V., Wadwa, M., Kirschning, C. J., Hansen, W., Dittmer, U., Epple, M., Buer, J., & Westendorf, A. M. (2014). Prophylactic and therapeutic vaccination with a nanoparticle-based peptide vaccine induces efficient protective immunity during acute and chronic retroviral infection. *Nanomedicine: Nanotechnology, Biology and Medicine, 10*(8), 1787–1798.

Kumar, R., Ray, P. C., Datta, D., Bansal, G. P., Angov, E., & Kumar, N. (2015). Nanovaccines for malaria using Plasmodium falciparum antigen Pfs25 attached gold nanoparticles. *Vaccine, 33*(39), 5064–5071.

Kushnir, N., Streatfield, S. J., & Yusibov, V. (2012). Virus-like particles as a highly efficient vaccine platform: Diversity of targets and production systems and advances in clinical development. *Vaccine, 31*(1), 58–83.

Li, S. W., Zhang, J., He, Z. Q., Gu, Y., Liu, R. S., Lin, J., Chen, Y. X., Ng, M. H., & Xia, N. S. (2005). Mutational analysis of essential interactions involved in the assembly of hepatitis E virus capsid. *Journal of Biological Chemistry, 280*(5), 3400–3406.

Lin, X., Li, W., Wen, Y., Su, L., & Zhang, X. (2022). Aggregation-induced emission (AIE)-based nanocomposites for intracellular biological process monitoring and photodynamic therapy. *Biomaterials, 287*, 121603.

Liu, Y., Xu, Y., Tian, Y., Chen, C., Wang, C., & Jiang, X. (2014). Functional nanomaterials can optimize the efficacy of vaccines. *Small, 10*(22), 4505–4520.

Luo, M., Samandi, L. Z., Wang, Z., Chen, Z. J., & Gao, J. (2017). Synthetic nanovaccines for immunotherapy. *Journal of Controlled Release, 263*, 200–210.

Malek-Khatabi, A., Tabandeh, Z., Nouri, A., Mozayan, E., Sartorius, R., Rahimi, S., & Jamaledin, R. (2022). Long-term vaccine delivery and immunological responses using biodegradable polymer-based carriers. *ACS Applied Bio Materials, 5*, 5015.

Moon, J. J., Suh, H., Bershteyn, A., Stephan, M. T., Liu, H., Huang, B., Sohail, M., Luo, S., Ho Um, S., Khant, H., & Goodwin, J. T. (2011). Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. *Nature Materials, 10*(3), 243–251.

Morgan, T. T., Muddana, H. S., Altinoglu, E. I., Rouse, S. M., Tabakovic, A., Tabouillot, T., Russin, T. J., Shanmugavelandy, S. S., Butler, P. J., Eklund, P. C., & Yun, J. K. (2008). Encapsulation of organic molecules in calcium phosphate nanocomposite particles for intracellular imaging and drug delivery. *Nano Letters, 8*(12), 4108–4115.

Nakhaei, P., Margiana, R., Bokov, D. O., Abdelbasset, W. K., Jadidi Kouhbanani, M. A., Varma, R. S., Marofi, F., Jarahian, M., & Beheshtkhoo, N. (2021). Liposomes: Structure, biomedical applications, and stability parameters with emphasis on cholesterol. *Frontiers in Bioengineering and Biotechnology, 9*, 748.

Naseri, M., Bozorgmehr, M., Zöller, M., Ranaei Pirmardan, E., & Madjd, Z. (2020). Tumor-derived exosomes: The next generation of promising cell-free vaccines in cancer immunotherapy. *Oncoimmunology, 9*(1), 1779991.

Neuhaus, V., Chichester, J. A., Ebensen, T., Schwarz, K., Hartman, C. E., Shoji, Y., Guzmán, C. A., Yusibov, V., Sewald, K., & Braun, A. (2014). A new adjuvanted nanoparticle-based H1N1 influenza vaccine induced antigen-specific local mucosal and systemic immune responses after administration into the lung. *Vaccine, 32*(26), 3216–3222.

Niikura, K., Matsunaga, T., Suzuki, T., Kobayashi, S., Yamaguchi, H., Orba, Y., Kawaguchi, A., Hasegawa, H., Kajino, K., Ninomiya, T., & Ijiro, K. (2013). Gold nanoparticles as a vaccine platform: Influence of size and shape on immunological responses in vitro and in vivo. *ACS Nano, 7*(5), 3926–3938.

Pantarotto, D., Partidos, C. D., Graff, R., Hoebeke, J., Briand, J. P., Prato, M., & Bianco, A. (2003). Synthesis, structural characterization, and immunological properties of carbon nanotubes functionalized with peptides. *Journal of the American Chemical Society, 125*(20), 6160–6164.

Pardi, N., Hogan, M. J., Porter, F. W., & Weissman, D. (2018). mRNA vaccines—a new era in vaccinology. *Nature Reviews Drug Discovery, 17*(4), 261–279.

Park, Y. M., Lee, S. J., Kim, Y. S., Lee, M. H., Cha, G. S., Jung, I. D., Kang, T. H., & Han, H. D. (2013). Nanoparticle-based vaccine delivery for cancer immunotherapy. *Immune Network, 13*(5), 177–183.

Petkar, K. C., Patil, S. M., Chavhan, S. S., Kaneko, K., Sawant, K. K., Kunda, N. K., & Saleem, I. Y. (2021). An overview of nanocarrier-based adjuvants for vaccine delivery. *Pharmaceutics, 13*(4), 455.

Pollard, A. J., & Bijker, E. M. (2021). A guide to vaccinology: From basic principles to new developments. *Nature Reviews Immunology, 21*(2), 83–100.

Pushko, P., Pumpens, P., & Grens, E. (2013). Development of virus-like particle technology from small highly symmetric to large complex virus-like particle structures. *Intervirology, 56*(3), 141–165.

Rasmussen, M. K., Pedersen, J. N., & Marie, R. (2020). Size and surface charge characterization of nanoparticles with a salt gradient. *Nature Communications, 11*(1), 1–8.

Reddy, B. M. (2022). Nanomedicine and Nanovaccinology tools in targeted drug delivery. *Nanovaccinology as Targeted Therapeutics*, 21–52.

Roldão, A., Mellado, M. C. M., Castilho, L. R., Carrondo, M. J., & Alves, P. M. (2010). Virus-like particles in vaccine development. *Expert Review of Vaccines, 9*(10), 1149–1176.

Rozhina, E., Batasheva, S., Miftakhova, R., Yan, X., Vikulina, A., Volodkin, D., & Fakhrullin, R. (2021). Comparative cytotoxicity of kaolinite, halloysite, multiwalled carbon nanotubes and graphene oxide. *Applied Clay Science, 205*, 106041.

Schröfel, A., Kratošová, G., Šafařík, I., Šafaříková, M., Raška, I., & Shor, L. M. (2014). Applications of biosynthesized metallic nanoparticles–a review. *Acta Biomaterialia, 10*(10), 4023–4042.

Serrano-Aroca, Á., Takayama, K., Tuñón-Molina, A., Seyran, M., Hassan, S. S., Pal Choudhury, P., Uversky, V. N., Lundstrom, K., Adadi, P., Palù, G., & Aljabali, A. A. (2021). Carbon-based

nanomaterials: Promising antiviral agents to combat COVID-19 in the microbial-resistant era. *ACS Nano, 15*(5), 8069–8086.

Shah, M., Fawcett, D., Sharma, S., Tripathy, S. K., & Poinern, G. E. J. (2015). Green synthesis of metallic nanoparticles via biological entities. *Materials, 8*(11), 7278–7308.

Shahbazi, M. A., & Santos, H. A. (2015). Revolutionary impact of nanovaccines on immunotherapy. *New Horizons in Translational Medicine, 2*(2), 44–50.

Sharma, J., Shepardson, K., Johns, L. L., Wellham, J., Avera, J., Schwarz, B., Rynda-Apple, A., & Douglas, T. (2020). A self-adjuvanted, modular, antigenic VLP for rapid response to influenza virus variability. *ACS Applied Materials & Interfaces, 12*(16), 18211–18224.

Shenoda, B. B., & Ajit, S. K. (2016). Modulation of immune responses by exosomes derived from antigen-presenting cells. *Clinical Medicine Insights: Pathology, 9*, CPath-S39925.

Singh, P., Kim, Y. J., Zhang, D., & Yang, D. C. (2016). Biological synthesis of nanoparticles from plants and microorganisms. *Trends in Biotechnology, 34*(7), 588–599.

Soriano, V., Edagwa, B., Mendoza, C. D., Barreiro, P., Corral, O., Treviño, A., & Gendelman, H. E. (2022). Ultra-long-acting antivirals as chemical vaccines to prevent viral diseases. *Future Microbiology*, (0), 887.

Souri, M., Chiani, M., Farhangi, A., Mehrabi, M. R., Nourouzian, D., Raahemifar, K., & Soltani, M. (2022). Anti-COVID-19 nanomaterials: Directions to improve prevention, diagnosis, and treatment. *Nanomaterials, 12*(5), 783.

Stammers, T. S., Erden, Y. J., & Hunt, G. (2013). The promise and challenge of nanovaccines and the question of global equity. *Nanotechnology Perceptions, 9*, 16.

Temchura, V. V., Kozlova, D., Sokolova, V., Überla, K., & Epple, M. (2014). Targeting and activation of antigen-specific B-cells by calcium phosphate nanoparticles loaded with protein antigen. *Biomaterials, 35*(23), 6098–6105.

van der Koog, L., Gandek, T. B., & Nagelkerke, A. (2022). Liposomes and extracellular vesicles as drug delivery systems: A comparison of composition, pharmacokinetics, and functionalization. *Advanced Healthcare Materials, 11*(5), 2100639.

Vijayan, V., Mohapatra, A., Uthaman, S., & Park, I. K. (2019). Recent advances in nanovaccines using biomimetic immunomodulatory materials. *Pharmaceutics, 11*(10), 534.

Wibowo, N., Chuan, Y. P., Seth, A., Cordoba, Y., Lua, L. H., & Middelberg, A. P. (2014). Co-administration of non-carrier nanoparticles boosts antigen immune response without requiring protein conjugation. *Vaccine, 32*(29), 3664–3669.

Xiao, P., Wang, J., Fang, L., Zhao, Z., Sun, X., Liu, X., Cao, H., Zhang, P., Wang, D., & Li, Y. (2021). Nanovaccine-mediated cell selective delivery of neoantigens potentiating adoptive dendritic cell transfer for personalized immunization. *Advanced Functional Materials, 31*(36), 2104068.

Xu, L., Liu, Y., Chen, Z., Li, W., Liu, Y., Wang, L., Liu, Y., Wu, X., Ji, Y., Zhao, Y., & Ma, L. (2012). Surface-engineered gold nanorods: Promising DNA vaccine adjuvant for HIV-1 treatment. *Nano Letters, 12*(4), 2003–2012.

Yadav, H. K., Dibi, M., Mohammad, A., & Srouji, A. E. (2018). Nanovaccines formulation and applications-a review. *Journal of Drug Delivery Science and Technology, 44*, 380–387.

Yang, C., Yu, W., Bi, Y., Long, F., Li, Y., Wei, D., Hao, X., Situ, J., Zhao, Y., & Huang, F. (2018). Increased oestradiol in hepatitis E virus-infected pregnant women promotes viral replication. *Journal of Viral Hepatitis, 25*(6), 742–751.

Yıldız, Ş., & Ünver, Y. (2022). Nanomaterial-based vaccination to ameliorate the challenges of traditional vaccination, icaens. In *3rd international conference on applied engineering and natural sciences*.

Yu, Z., Xu, Q., Dong, C., Lee, S. S., Gao, L., Li, Y., D'Ortenzio, M., & Wu, J. (2015). Self-assembling peptide nanofibrous hydrogel as a versatile drug delivery platform. *Current Pharmaceutical Design, 21*(29), 4342–4354.

Zahednezhad, F., Saadat, M., Valizadeh, H., Zakeri-Milani, P., & Baradaran, B. (2019). Liposome and immune system interplay: Challenges and potentials. *Journal of Controlled Release, 305*, 194–209.

Zhao, Q., Li, S., Yu, H., Xia, N., & Modis, Y. (2013). Virus-like particle-based human vaccines: Quality assessment based on structural and functional properties. *Trends in Biotechnology, 31*(11), 654–663.

Zhao, L., Seth, A., Wibowo, N., Zhao, C. X., Mitter, N., Yu, C., & Middelberg, A. P. (2014). Nanoparticle vaccines. *Vaccine, 32*(3), 327–337.

Zhao, P., Wang, C., Ding, J., Zhao, C., Xia, Y., Hu, Y., Zhang, L., Zhou, Y., Zhao, J., & Fang, R. (2021). Evaluation of immunoprotective effects of recombinant protein and DNA vaccine based on Eimeria tenella surface antigen 16 and 22 in vivo. *Parasitology Research, 120*(5), 1861–1871.

Zhou, J., Miyamoto, Y., Ihara, S., Kroll, A. V., Nieskens, N., Tran, V. N., Hanson, E. M., Fang, R. H., Zhang, L., & Eckmann, L. (2022). Codelivery of antigens and adjuvant in polymeric nanoparticles coated with native parasite membranes induces protective mucosal immunity against Giardia lamblia. *The Journal of Infectious Diseases, 226*, 319.

Chapter 17
Clinical Applications of Nanovaccine Formulation Technology Market Research

Sunil Kumar, Attuluri Vamsi Kumar, and Hardeep Kaur

17.1 Introduction

The most efficient method of preventing infectious illnesses is vaccination; hence, it is one of the main concerns for human health. In the last 200 years, immunizations have contributed to the eradication of smallpox globally, the eradication of poliomyelitis in most countries and the decline in the morbidity and mortality of a variety of vaccine-preventable diseases (VPDs). Nevertheless, vaccination reluctance is growing in many nations, particularly as a result of unfounded worries about hypothetical vaccine side effects and a lack of confidence in the efficacy and safety of vaccines (Case & Desmond, 2016; Vaccine Hesitancy: An Overview on Parents' Opinions about Vaccination and Possible Reasons of Vaccine Refusal - PubMed, n.d.-a). Hence, there have been occasional VPD outbreaks in various regions of the world (Danesh-Bahreini et al., 2011; Facciola et al., 2019; Nevagi et al., n.d.; Plebanski Geoffrey Pietersz et al., 2004), and several international studies have shown that important public health objectives such as the eradication of poliomyelitis and congenital rubella are still not being met (Bai et al., 2021; Huang et al., 2020; Kalkanidis et al., n.d.; Skwarczynski & Nanomedicine, 2014). Unexpectedly, vaccine reluctance affects even health-care personnel who frequently have insufficient or no vaccinations against the primary VPDs due to concerns about the safety and effectiveness

S. Kumar · A. V. Kumar
Department of Medical Lab Technology Chandigarh University, University Institute of Allied Health Science, Mohali, Punjab, India

H. Kaur (✉)
Department of Biotechnology, University Institute of Biotechnology, Chandigarh University, Mohali, India

Department of Chemistry, University Institute of Sciences, Chandigarh University, Mohali, India
e-mail: haardeep.uibt@cumail.in

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_17

of the vaccines. The number of potential vaccines is steadily growing since vaccine research is continually changing (Facciolà et al., 2019; Feng et al., 2022; J. Liu et al., 2020). The majority of potential vaccines, however, have an essential composition that is often defined by a reduced immunogenicity and an inability to elicit strong and durable immune responses (Adabi et al., 2017; Dong et al., 2020; Mendes et al., 2018). Therefore, we require adjuvants and novel delivery mechanisms that increase the immunogenicity of vaccines in order to acquire contemporary, effective vaccinations. The findings of this study are seen in the light of technological innovations intended to lessen the impact of infectious illnesses in all of their forms. Richard P. Feynman, a renowned physicist, introduced the first concept for nanotechnology in 1959 (Healy et al., 2014; Nevagi et al., n.d.; Zhang et al., n.d.-a). The word "nanotechnology" was first used by Eric Drexler in his book *Engines of Creation* in 1986 to characterize the method of molecular manufacturing and some of its consequences. The development of nanotechnology has significant industrial and scholarly applications. Nanotechnology has several industrials aspects of potential needs and significant utilizations. Since the early 1990s, nanotechnology has been a component of traditional scientific ideas with possible medicinal applications. Since then, several "nanostructures," including nanoparticles, nanorods (NRs), nanospheres, nanofibres and nanotubes, among others, have been taken into consideration for use in various biological systems (Saunders, 2009). Nanotechnology, according to the National Nanotechnology Initiative, is the study of structures having a size between 1 and 1000 nm in at least one dimension and changeable composition, size, form and surface properties (Couvreur & Vauthier, 2006; Moghimi et al., 2005). Due to their size resemblance to some biological components, nanoparticles can enter live cells via the endocytosis process, namely pinocytosis (Treuel et al., 2013). As transporters and deliverers of biologically active substances, these structures are changing various medical sectors, including diagnosis, prevention and therapy. In fact, a few licensed nano-sized vaccinations and medication delivery methods are causing a true revolution in the treatment and prevention of diseases (Couvreur & Vauthier, 2006; Dobrovolskaia & McNeil, 2007; Maurer et al., 2005; Treuel et al., 2013). Researchers have created and tested natural and man-made materials as drug delivery carriers that can take in exogenous drugs and self-assemble into nanoparticles (NPs) under aqueous settings (Fang et al., 2016; Yu et al., 2015, 2016a, b). Numerous studies have been conducted to evaluate nanoparticles' biocompatibility and potential cellular toxicity, in addition to studies on potential medicinal uses of them (Eltayeb et al., 2016; Fang et al., 2016; Trovato et al., 2018; Visalli, Currò, et al., 2017a; Visalli et al., 2017b). The ability to overcome some challenges and create efficient vaccinations is being made possible by the use of nanotechnology in vaccine development (Eltayeb et al., 2016; Liao et al., 2014; Visalli, Currò, et al., 2017a). Particularly, nanoparticles being used as delivery systems for vaccine components can play a significant role in the creation of novel vaccines by enhancing the host's immune responses and by having the potential to reach certain cellular regions due to their small size. We can tell the difference between a therapeutic and a preventative nanovaccinology (Bolhassani et al., 2011;

Chackerian, 2010; Hamdy et al., 2011; Liao et al., 2014). Although therapeutic nanovaccinology is mostly focused on the treatment of cancer, it is also being researched as a potential treatment for other illnesses or ailments, including Alzheimer's disease, hypertension and nicotine addiction (Tissot et al., 2008). Instead, prophylactic nanovaccinology focuses on the prevention of a number of infectious illnesses. Prophylactic nanovaccines have received approval for use in humans in a small number of cases, and several are now being investigated in clinical or preclinical trials (Chackerian, 2010; Correia-Pinto et al., 2013; Gregory et al., 2013; Kushnir et al., 2012). The immune system is exposed to vaccine antigen in the same way as a pathogen would by placing it on the surface or by encapsulating it inside the nanoparticles (Kalkanidis et al., n.d.; Zhang et al., n.d.-a). The following three elements are crucial for an efficient and effective vaccine: A good antigen is necessary to activate immunity, followed by an immune adjuvant to costimulate the innate immune system and a carrier system that enable the two earlier elements to attain the antigen-presenting cells (APCs). To accomplish these objectives, it is necessary to consider the composition, size, surface characteristics, biodistribution and immunostimulatory potential of the used nanostructures during the construction of nanoparticles (Niu et al., 2017; Wen & Meng, 2014; Ye et al., 2014). According to certain research, nanoparticles facilitate the cellular entrance of the vaccine components by endocytosis, particularly pinocytosis, which increases the efficacy of vaccines (Niu et al., 2017; Wen & Meng, 2014; Ye et al., 2014). The size, physicochemical components and changes in the materials of nanoparticles must be fully understood in order to maximize their application in vaccinology, as these factors impact their biological effects "in vivo". Shape and dosage of the nanoparticles are other aspects to be taken into account when discussing vaccinations based on nanoparticles (Zhang et al., n.d.-b). As a result, varied needs call for the production of vaccine nanoparticles with a variety of compositions, sizes, forms and surface qualities. In this chapter, we have given a broad overview of the types, synthesis techniques, characteristics, features and uses of nanotechnology in the manufacturing of vaccines (Wen & Collier, 2015).

17.2 Potential Vaccine Delivery Vehicles: Different Types of Nanoparticles

Different kinds of nanoparticles are under consideration as potential carriers of vaccination antigens. In particular, some studies have concentrated on the creation of synthetic delivery methods based on nanoparticles in the viral size range (20–200 nm) that shield antigen from degradation, improve its presentation and make it easier for professional APCs to take it up. The main components of these systems include virus-like particles, proteins that can fold into themselves, micelles, liposomes, inorganic nanoparticles and polymers.

17.3 Virus-Like Particles (VLPs)

The creation of viral vaccines has already employed virus-like particles (VLPs), which are non-infectious, self-assembling nanoparticles made of a structured protein capsid that lacks a genetic material. Because VLPs are furnished with an exterior viral shell that is distinguished by repeating epitopes and the immune system instantly identifies them as non-self-structures, they are dazzling nanoparticles. As a result, VLPs and viruses both have the positive ability to aggressively drive immune responses, but they both do not have the negative ability to cause infection. Even in the absence of an adjuvant, the naturally resembling size, the repeated structural order and the quick and efficient processing of these nanoparticles cause host immune responses to be elicited (Bachmann & Jennings, 2010; Jeong & Seong, 2017; Noad & Roy, 2003; Zhang et al., 2000).

VLPs typically range in size from 20 to 800 nm can originate from a variety of viruses and can be created utilizing a variety of cell systems, including cell-free systems (L. F. Zhang et al., 2000). *Escherichia coli*, yeasts, baculovirus, mammalian, plant and cell-free systems have shown the activities like VLPs (Pattenden et al., 2005; Zeltins, 2013). Moreover, these viruses can be produced with technologies using different cell systems. Figure 17.1 shows the manufacturing scheme of virus-like particles for the nanovaccine development.

The production of VLPs is carried out via an in vivo technique, with the first stage being the direct spontaneous assembly of viral capsid proteins inside the expression cell vector. The newly formed particles are then to be cleaned of impurities that have been integrated into the cells and that have attached to the surface in a subsequent phase.

It is often necessary to dismantle nanoparticles and then reassemble them in order to get well-purified VLPs. A more recent and growing method, however, involves processing cell-free in vitro assemblies (Facciolà et al., 2022; Lu et al., 2013; Sánchez-Rodríguez et al., 2012). The typical "assemble-then-purify" paradigm is reversed in this procedure.

Specifically, the capsid proteins are isolated from contaminants after expression and then put back together in vitro. In this manner, after VLPs are assembled into

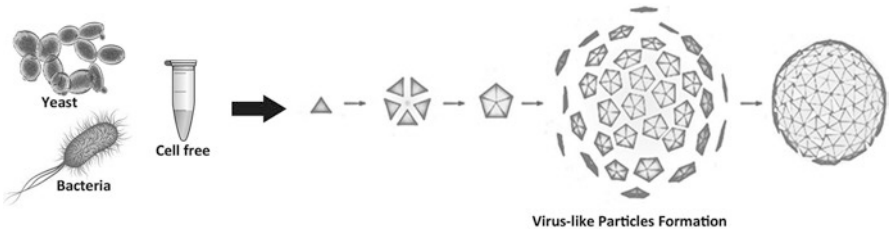


Fig. 17.1 The manufacturing scheme of VLPs for further study and understanding. (Image adapted from *The new era of vaccines: the "nanovaccinology"* (Facciolà et al., 2022; Lu et al., Page No. 7165))

the cells, there is no need to deconstruct them. However, there are more methods for creating VLPs (Pushko et al., 2013; Visalli, Currò, et al., 2017a; Zhao et al., 2013).

The self-assembled viral protein particles used in currently available VLP-based vaccinations come from the virus against which the vaccine was developed. In addition, target antigens connected to the surface and coming from unrelated viruses can also be carried by VLPs (Ionescu et al., 2006; Lua et al., 2014; Tissot et al., 2010). Since several VLP-based vaccinations are currently undergoing clinical or preclinical testing, we may expect a rise in the number of these vaccines (Greiner et al., 2012; Roldão et al., 2010).

17.4 Hepatitis B Virus (HBV) Vaccine Based on VLP

Hepatitis B surface antigen (HBsAg), generated in yeast using recombinant deoxyribonucleic acid (DNA) technology, was the first VLP-based vaccination developed and marketed for human use in 1986. The 22-nm spherical VLPs produced by the recombinant HBsAg may be further adsorbed on an aluminium hydroxide gel (Greiner et al., 2012). A total of 70 molecules of S protein and host cell–derived lipids (30–50%) are also present in these nanoparticles. Intramolecular and intermolecular disulphide linkages maintain the structure. Unlike naturally occurring mammalian-derived HBsAg particles, the S protein in these yeast-derived nanoparticles is extremely hydrophobic and not glycosylated. In addition, it has a tight relationship with lipids and is in charge of HBsAg VLPs' antigenic properties. As a result, these VLPs may be thought of as lipoproteins made up of an internal core that is hydrophobic and more fluid and a well-organized, somewhat stiff lipid surface. The protein components are present on the outside portion and the one inserted into the inner core is absorbed in this lipid substance (Greiner et al., 2010). It has been demonstrated that this vaccination has the ability to promote CD4+ and CD8+ T-cell activation (Zhao et al., 2013).

17.5 VLP-Based Vaccine for Human Papillomavirus (HPV)

The human papillomavirus (HPV) vaccine was the second VLP-based vaccination to be authorized for human use in 2006, following the HBV vaccine (Cutts et al., 2007). There are various carcinogenic viruses, including HPVs, connected with cutaneous and mucous membrane lesions, both benign and malignant. These days, it is well recognized how these viruses contribute to the development of cervical cancer and a number of other typical (anal, oropharyngeal, penile, vulvar and vaginal) and atypical malignancies (prostate, bladder and rectal) (Ceccarelli et al., 2018; Gillison, D'Souza, et al., 2008b; Jørgensen & Jensen, 2020; Rezazadeh et al., 2009; Visalli et al., 2016a,b). In particular, the National Cancer Institute reported that HPVs are responsible for 75% of vaginal, 70% of oropharyngeal, 90% of anal and

practically all cervical cancers (Jørgensen & Jensen, 2020; Kavanagh et al., 2017). Due to these factors, an increase in HPV vaccination rates might significantly lessen the incidence of some cancers (Huh et al., 2017). In particular, the dissemination of HPV vaccination has the potential to cut the incidence of cervical cancer by up to 90% worldwide (Steinbrook, 2006).

This technique can also assist to lower health-care expenditures and worries about follow-up treatments by lowering the need for screening and medical care (particularly biopsies and invasive procedures). The foundation of HPV vaccines is a VLP made of 72 pentamers of L1, the main capsid protein of HPV, which is generated in yeast in vast amounts using recombinant DNA technology, purified and then mixed to create the vaccine (Garland & Smith, 2010). Three distinct HPV vaccines have received US Food and Drug Administration (FDA) approval to date: a tetravalent (2006), a bivalent (2009) and, most recently, a nonavalent vaccine (2014). All the three vaccines are effective in preventing infections brought on by the two common high-risk HPV (HR-HPV) types—16 and 18—that account for around 70% of cervical cancer cases and a significant portion of other HPV-related cancers (Chaturvedi et al., 2011; Gillison et al., 2008a). In addition, the tetravalent vaccination protects against infections brought on by HPV types 6 and 11, which account for 90% of genital warts (Koutsky et al., 2002). Last but not the least, the new nonavalent vaccination protects infections brought on by the same four HPV types included in earlier immunizations, plus an additional five HR-HPVs (Correia-Pinto et al., 2013; Iannazzo et al., 2017; Liao et al., 2016a; Ye et al., 2014; Yu et al., 2016a). These vaccines contain an adjuvant made of amorphous aluminium hydroxyphosphate sulphate (AAHS), aluminium hydroxide, AS04, monophosphoryl lipid A (MPLA) and nonavalent (Koutsky et al., 2002). Mathematical models have been used to examine the potential public health impact and cost-effectiveness of the nonavalent HPV vaccine in a variety of conditions including efficacy, cost and vaccination coverage. In particular, Largeron et al. showed that the widespread use of the nonavalent HPV vaccine in Germany extended to boys would be linked to a 24% decrease in the incidence of cervical cancer, 30% and 14% decreases in the incidence of anal cancer in men and women, respectively, and finally a million cases of genital warts avoided after 100 years. In addition, this plan would have an incremental cost-effectiveness ratio (ICER) of 22,987 euros for every quality-adjusted life year (QALY) gained. Despite these crucial data, various reported negative effects of HPV vaccinations have been linked with numerous scientific studies (Largeron et al., 2017).

17.6 Hepatitis E Vaccine Based on VLPs

Hepatitis E virus (HEV), a member of the Hepeviridae family, frequently results in a self-limited acute hepatitis with a mortality incidence of less than 0.1% in healthy persons and a chronic infection in those with weakened immune systems. However, if this virus is caught during pregnancy, particularly in the first trimester, it may be

very dangerous (Yang et al., 2018). According to the estimates, the HEV prevalence rate in the United States is around 9%, but in Europe, it ranges from 5% to 20% (Giudice, 2010, 2017; Horvatits et al., 2018; Yang et al., 2018). Numerous experiments have been carried out to acquire recombinant HEV VLPs since it is difficult to produce large quantities of viruses using cell cultures (Guu et al., 2009; Horvatits et al., 2018). Different systems have been developed in the earlier years to research HEV. The production of VLPs by the self-assembly of the core protein was one of the first models used to investigate the structure of the virus, its entry mechanism and its potential as a vaccine open reading frame 2 (ORF2). In fact, the HEV core protein's antigenicity was recognized quite early, and several approaches to develop a viable HEV vaccine have been investigated (Genovese et al., 2018). Rhesus monkeys injected with 110 g doses of the 56-kDa HEV capsid protein were 100% protected from developing hepatitis and 63% protected from infection after the first inoculation when challenged with an infectious dosage of HEV. In a later Phase II randomized, double-blind, placebo-controlled study in Nepal with 2,000 participants, the same vaccine based on the 56-kDa capsid protein gave an efficient protection against HEV (Shrestha et al., 2007). The second method is based on p239, a protein that was recombinantly generated in bacteria and purified under denaturing conditions in order to preserve the antigenic epitopes; p239 is a protein that contains the amino acids 368 to 606 of the capsid protein. In Rhesus monkeys, Li et al. investigated the efficacy of p239 as a potential vaccination candidate using prime/boost doses of 5 g, 10 g and 20 g. The challenge with HEV genotypes 1 and 4 resulted in protection for the immunized monkeys (Li et al., 2005). The vaccination provided complete protection for the monkeys. After receiving a viral dosage of HEV, it provided production from both hepatitis and infection. Using a viral dosage, the same percentage of effectiveness against hepatitis was attained. A randomized, controlled, Phase II clinical study with 457 adults using the same vaccine candidate revealed an encouraging 100% seroconversion rate in vaccinated subjects (Hartl et al., 2016). Finally, from August 2007 to June 2009, in China, a sizable randomized, double-blind, placebo-controlled, single-centre, Phase III clinical study was conducted. Half of the 112,604 participants in this research received the p239 vaccination, whereas the other half (the placebo group) received a three-dose schedule of the HBV vaccine (Purcell et al., 2003). According to the findings, the p239 vaccination was immunogenic, 100% effective and well-tolerated (Zhu et al., 2010). Following this experiment, the p239 vaccination was approved in 2012 in China. Despite the promising results, the vaccine was not licensed in other countries for a number of reasons, including the trial's failure to include participants under the age of 16 and older than 65 and the two main risk categories of pregnant women and immunocompromised patients. A bacterially manufactured self-assembling core protein (aa 439–617 of the capsid protein) called p179, which was initially tested on rabbits to determine its protective potential, is the third vaccination candidate to be put through clinical trials (Largeron et al., 2017; Genovese et al., 2018). The animals were given three different dosages of p179 (20 g, 30 g and 40 g), and although they were largely immune to the infection thanks to the generation of high titres of anti-HEV, some challenged rabbits did shed the virus. Following these findings in animals, a Chinese

Phase I trial including 120 individuals and a p179 vaccine was conducted in 2017 to examine the vaccine's effectiveness and potential negative effects. As a consequence, the vaccination was well accepted and seemed to be safe (Cao et al., 2017).

17.7 Proteins That Self-Assemble as Nanovaccine

Some supramolecular structures, like VLPs, have been created from proteins that self-assemble, and more recently, self-assembling systems carrying larger quantities of antigen have been investigated for the creation of nanoparticle-based vaccines (Cheetham et al., 2017). The main distinction between VLPs and other types of proteins is that self-assembled proteins are not created by viral components. Drug delivery methods using self-assembled peptide nanoparticles (SAPNs) have been investigated (Liao et al., 2016b; Sun et al., 2015). These nanoparticles are made up of two helical coil–coil domains connected together into a single peptide chain by a brief linker region. While the other creates a pentameric coiled coil1, one helix coils into a trimeric coil (Y. Yang et al., 2011). The physiochemical characteristics of nanostructures cause them to have a propensity for self-assembly in aqueous media. Amphiphilic peptides do not appear to be hazardous to the liver, spleen, kidney, bladder, colon, lung, or heart, according to Chung et al. (2015). It has been shown that APCs can more efficiently absorb amylopectin nanoparticles (APNPs) and present antigen epitopes compared to naked antigens (Nochi et al., 2010). In 2012, Wahome et al. conducted an in vivo experiment employing APNPs displaying two significant human immunodeficiency virus (HIV) epitopes on their surface, 4E10 and 2F5, which are major targets in the development of neutralizing antibodies. According to the findings, these nanostructures induced strong immune responses against these crucial HIV epitopes. A different method relies on the protein ferritin, which has the ability to self-assemble into a nearly spherical 10-nm shape (Wahome et al., 2012). In order to create a recombined protein that spontaneously assembled to form an octahedrally symmetric particle with eight trimeric hemagglutinin (HA) spikes, Kanekiyo et al. fused influenza virus hemagglutinin (HA) to ferritin (Kanekiyo et al., 2013). The researchers showed that compared to conventional influenza vaccination, these nanovaccines induce a stronger immune response. In addition, Lee et al. developed a potential therapeutic vaccination for the treatment of cancer by using engineered human ferritin nanoparticles to transport tumour antigens to lymph nodes. The researchers showed that compared to conventional influenza vaccination, these nanovaccines induce a stronger immune response. Additionally, Lee et al. developed a potential therapeutic vaccination for the treatment of cancer by using engineered human ferritin nanoparticles to transport tumour antigens to lymph nodes (Lee et al., 2016).

17.8 Micelles

Micelles are nanoparticles created when single amphiphilic (hydrophobic/hydrophilic) molecules spontaneously self-assemble in water, resulting in the production of two separate components: a hydrophobic core and a hydrophilic surface region known as the "corona" (Trimaille & Verrier, 2015). Micellar nanoparticles, which have historically been researched and exploited as drug delivery systems by encapsulating hydrophobic pharmaceuticals in the core, have also recently been investigated as adjuvants for the administration of vaccines (Isoglu et al., 2017). Micelles have two ways to deliver vaccine candida thanks to their chemical properties. The hydrophilic corona can first be covalently linked to antigens. This method has been used to link HIV antigens to micelles made of adjuvant-loaded polymer, resulting in an active antigen that can effectively excite APCs in vitro (Jiménez-Sánchez et al., 2015).

The creation of peptide amphiphiles (PAs), a kind of peptide-based biomaterials made up of peptides connected to hydrophobic alkyl tails that self-assemble into micellar structures in aqueous media, is the second method. In several investigations, PA micelles have been employed as vaccine candidates to enhance peptide immunogenicity (Barrett et al., 2016; Black et al., 2012; Trent et al., 2015). A number of PA micelle features, including size, shape and composition, are essential for enhancing these nanoparticles' ability to control immunological responses (Black et al., 2010; Liu & Irvine, 2015). For instance, it has been shown that cylindrical- or spherical-shaped PA micelles may effectively reach the lymph nodes because they contain the proper concentrations of antigen and amphiphilic adjuvants (Barrett et al., 2016; Liu & Irvine, 2015; Trent et al., 2011). A higher antigen-specific immunoglobulin G1 (IgG1) antibody response against group A streptococcus was induced in mice following subcutaneous inoculation using PAs in two separate studies (Barrett et al., 2016; Trent et al., 2015). Micelles, which are typically less than 100 nm in size, are able to facilitate the transport of antigen to APCs, particularly dendritic cells (DCs), which are more prevalent in lymph nodes than the periphery. These nanoparticles can interact with DCs at the injection site, but they can also migrate via the lymphatic system and reach lymph nodes, where they can help in germinal centre formation (Luo et al., 2015). Through the careful selection of hydrophilic corona segments, micelles can quickly acquire the necessary surface characteristics. Through the right design of the hydrophobic and hydrophilic parts, a number of additional immunostimulatory molecules, for example, Toll-like receptor ligands or mannose receptor ligands, may be easily incorporated in these nanoparticles to produce an increased activation of the DCs, which are crucial for the initiation of immune responses. In addition, micelles may swiftly identify endosome breakdown with the consequent release of peptide vaccines in the cytoplasm of cells, antigenic presentation and powerful cytotoxic T-cell responses in vivo by choosing certain hydrophobic molecules. Micelles therefore provide a special platform to improve the antigen's ability to stimulate the immune system (Keller et al., 2014).

17.9 Liposomes-Based Nanovaccine

In the development of vaccines, liposomes—spherical vesicles made of a double lipid coating around an aqueous core—have drawn a lot of attention (Moon et al., 2011). The number of layers in these nanostructures can be classified as unilamellar, multilamellar, or polymerized types (Schwendener, 2014). Due to their immunogenic potential, liposomes are particularly promising for applications in biomedicine field as drug delivery systems and gene transporters (Moghimi et al., 2005; Schwendener, 2014). Antigen in a liposome can travel in the aqueous core, be encapsulated in the lipid bilayer, or be absorbed on the surface. Surface adsorption, covalent lipid joining (before or after vesicle formation), non-covalent surface attachment, encapsulation, electrostatic interactions (with lipids of opposite charge), surface adsorption, and surface attachment can all result in antigen integration. Liposome functions as an adjuvant, enhancing immune responses and boosting the effectiveness of the vaccination. Low reactogenicity, biodegradability and flexibility are the characteristics of liposomal vaccines. When creating liposomal vaccines, several intrinsic aspects must be taken into account (Fig. 17.2).

In the past assessments, several factors that could influence the performance and efficacy of liposomes as potential vaccine candidates were analysed (Perrie et al., 2016; Watson et al., 2012). The size of the liposome, the lamellarity, the surface charge, the fluidity of the bilayer and the presence of immunostimulatory lipids are some of these variables. Compared to 500-nm vesicles, it has been demonstrated that vesicles with a diameter >2 m exhibiting a tuberculosis (TB) antigen are more efficient in inducing cell proliferation and low interleukin (IL)-10 production (Perrie et al., 2016). The liposomal vesicle's lamellar structure may potentially have an impact on immune responses. Using small unilamellar vesicles (SUVs) and large multilamellar vesicles (MLVs), Beck et al. investigated the effectiveness of a

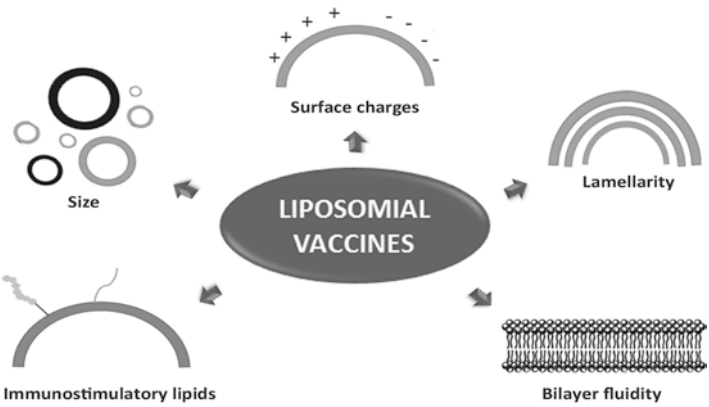


Fig. 17.2 (Facciolà et al., 2019, Page No. 7169). (This image was taken from a review article titled *"Nanovaccinology," the new era of vaccinations*). Designing a liposomal vaccination involves taking physical, chemical and morphological considerations

recombinant HIV protein known as CN54 gp140 (Henriksen-Lacey et al., 2011). The liposomes included various combinations of lipids and monophosphoryl lipid A (MPLA), with or without saponin QS21. The authors demonstrated that, in contrast to MLVs, SUVs lacking QS21 were able to elicit a Th2 cell–mediated response to the antigen due to a high antibody production. Because it can significantly impact the interaction of the antigen with the liposome (e.g. anionic antigens better associate with cationic lipids), the appropriate vaccine formulation depends critically on the liposomes' surface charge (Hussain et al., 2014). By substituting the neutral lipid distearoyl-sn-glycero-3-phosphocholine (DSPC) for the cationic lipid dimethyldioctadecylammonium (DDA), Hussain et al. found that the TB recombinant antigen H56 was decreased from 84% to 15%. A liposomal-based intra-nasal influenza vaccination model that contains hemagglutinin neuraminidase (HN) antigen coupled with outer protein and also investigated various monocationic, neutral and anionic lipids as well as the polycationic sphingolipid ceramide carbamoyl-spermine (CCS) (Joseph et al., 2006). In a murine model, the authors demonstrated that neutral and anionic lipid-based preparations were ineffective, while two monocationic-based liposomal formulations (containing 1,2-dimyristoyl-3-trimethylammonium propane [DMTAP] and strong local and systemic immune responses) were greatly activated by 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) (both Th1 and Th2). Finally, the significance of bilayer fluidity in the formulation of successful liposomal vaccines has been acknowledged and documented in several published articles by Kaur et al.; they have demonstrated the impact of cholesterol on the fluidity of the bilayer and, subsequently, the antigenic potential of liposome-based vaccines. Particularly, when cholesterol was elevated in DDA/trehalose-6,6′-dibehenate (TDB) liposomal formulations used for inoculated mice, reduced immunoglobulin G (IgG) production was found (Kaur et al., 2014).

This result might be due to the antigen being lost in fluidized liposomes with high cholesterol concentrations. In addition, the level of interferon gamma (IFN-γ) increased when cholesterol was absent from the lipid bilayer. For application in humans, a number of liposome-based vaccines against viral, bacterial, fungal and parasite illnesses have been proposed. In particular, viral liposomes, sometimes known as virosomes, have undergone extensive research. Virosomes are a kind of liposomes that include viral proteins on their surface that enable them to successfully bind to the target cells' membranes (Vaccine Hesitancy: Causes, Consequences, and a Call to Action - PubMed, n.d.-b).

The human respiratory system was modelled in a triple culture system; a research has recently been conducted to examine immunological responses to virosomes and liposomes and their internalization. Particularly, the culture model employed consisted of cells cultured alongside DCs and monocyte-derived macrophages (MDMs) that were produced from the epithelial cell line 16HBE. Both the nanoparticles were introduced to cells in order to assess the immunological responses. Virosomes were made using neutral lipids 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE). This made the membrane proteins of influenza A/Brisbane/59/2007 H1N1 soluble. There were no membrane proteins in the similar neutral lipids that produced liposomes that could

be soluble in influenza. The findings demonstrated that all cell types absorbed virosomes more effectively. Using flow cytometry and laser scanning microscopy, MDMs and DCs were shown to have the greatest capacity for internalization in monocultures and cocultures. Furthermore, in cocultures, DCs secreted higher quantities of cytokines including IL-1 and IL-8 and were subtly stimulated by liposomes and virosomes in monocultures. Finally, virosomes were internalized by epithelial cells at greater rates than liposomes (Blom et al., 2016).

17.10 Inorganic Nanoparticles

These are the nanocarriers with an inorganic solid nucleus that have been employed in vaccination research as adjuvants and antigen-carriers to boost immune responses. The rigid structure and controlled manufacture of these nanoparticles are their good traits; their frequent non-biodegradability is a drawback. Carbon, gold, silica and calcium nanoparticles are the inorganic nanoparticles that have been explored the most as vaccination platforms (He et al., 2016; Kalkanidis et al., 2006).

17.11 Nanoparticles of Carbon

Since carbon nanomaterials may be incorporated into a wide range of cell types, several studies have been conducted to assess their potential utility as adjuvants or carriers for various vaccines (Gottardi & Douradinha, 2013; Pantarotto et al., 2003; Scheinberg et al., 2013). The capacity of these nanosystems to transport antigens and elicit immunological responses is affected by a variety of structural and physical characteristics, including their surface modifications. Carbon nanotubes (CNTs) are among the carbon nanoparticles that have drawn the most interest due to their unique properties that make them useful in a variety of industrial applications (Liu et al., 2014a). Engineered nanoparticles known as CNTs are created when a thick graphene sheet is rolled up into a hollow cylinder (single-walled CNT [SWCNT]). The CNT will be created by concentric numerous sheets (from 2 to 50, joined together by van der Waals interactions); if there are more graphene sheets present, this CNT is known as the multiwalled CNT (MWCNT) (Pimentel et al., 2009) (Fig. 17.3).

Their diameter ranges between 0.4 and 2 nm and 2 and 100 nm, while their lengths extend from 100 to 1000 nm (De Volder et al., 2013).

The SWCNTs and MWCNTs both are measured in nanometres. Pantarotto et al. conducted some of the earliest studies employing CNTs as carriers for vaccine candidates in 2003 (Pantarotto et al., 2003). The hand, foot and mouth disease virus (HFMDV) envelope peptides were covalently attached to CNTs as the main experimental component of the study. The findings demonstrated that after being attached to CNTs, the epitope structure retained its immunogenic ability. In fact, animal

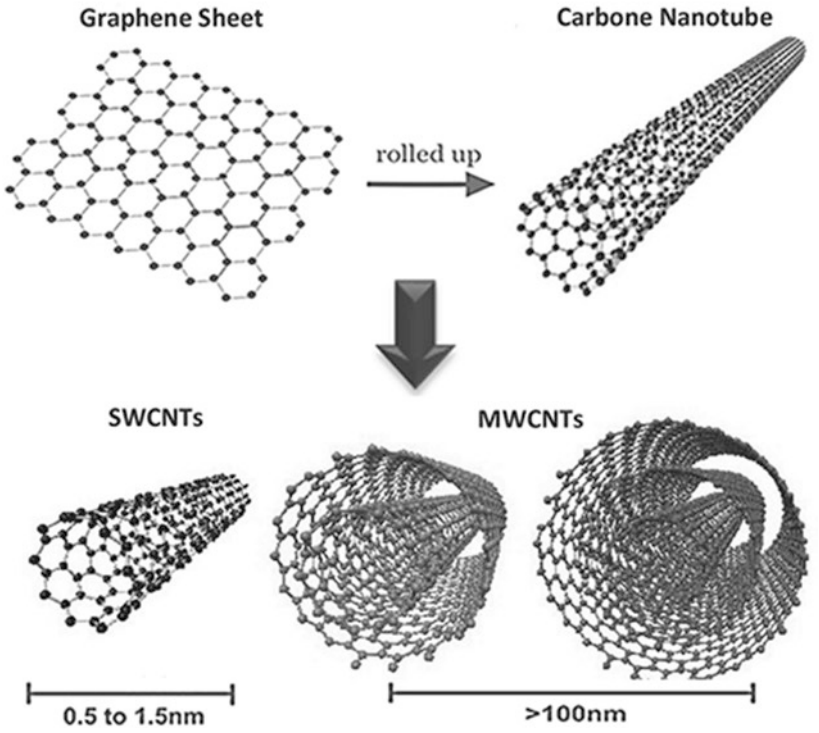


Fig. 17.3 Structure and types of carbon nanotubes (CNTs). (The images adapted from the review article titled *The new era of vaccines: the "nanovaccinology"* (Facciolà et al., 2019, Page No. 7170))

models showed that the CNT–viral protein complexes might induce particular neutralizing IgG immunological responses (Pantarotto et al., 2003). The immunological and cytotoxicological effects of tuberculin purified protein derivative (PPD) conjugated to SWCNTs were studied by Zeinali et al. in 2009. In this work, PPD, SWCNT–PPD conjugate and Bacillus Calmette–Guerin (BCG) in complete Freund's adjuvant (CFA) were given to male BALB/c mice as challenges. By monitoring the levels of Th1 (IFN-γ and IL-12) and Th2 cytokines, the induction of cellular immune responses was examined (IL-10 and IL-5). The researchers discovered that, whereas vaccination with BCG resulted in a mixed Th1/Th2 cytokine response, vaccination with PPD or PPD in CFA caused a Th2 cytokine response. In contrast, SWCNT–PPD mostly induced a Th1-type cytokine response without the risk of lethal effects (Tekewe et al., 2017).

In 2016, Hassan et al. showed that the surface characteristics of CNTs influence how effective they are as vaccine nanocarriers both in vitro and in vivo. In particular, the scientists altered the length and surface charge of MWCNTs–antigen conjugates to study the changes in DC internalization. The findings demonstrated that MWCNT–antigen conjugates' increased cellular uptake and subsequent stimulation

of immune responses are caused by a decrease in charge negativity. Finally, a research on the use of MWCNTs as viral DNA carriers for the induction of immune responses against dengue illness was conducted in 2016 by Calegari et al. (Hussain et al., 2014). In order to increase the expression of the tetravalent vaccine candidate (TVC) plasmid vector for dengue virus in vitro using Vero cells and carry out a parallel in vivo challenge through the intramuscular route in animals to evaluate the immunological responses, the study sought to compare carboxylated and non-carboxylated MWCNTs. The findings demonstrated MWCNTs' capacity to enter target cells and enter both the cytoplasm and the cell nucleus, increasing the number of B cells that produce antibodies in comparison with naked plasmid (Calegari et al., 2016).

17.12 Nanovaccines Based on Gold Nanoparticles (GNs)

Gold nanoparticles (GNs) are particles with dimensions ranging from 1 to 150 nm, a variety of shapes (rod, spherical, cubic, etc.) and the capacity to modify the surfaces by the attachment of residues from carbohydrates. The immunological characteristics of gold nanoparticles are primarily influenced by their size and structure, which improve immune response via several cytokine pathways (Niikura et al., 2013; Safari et al., 2012). In order to stimulate particular immune responses in mice, Safari et al. used gold glyconanoparticles displaying a synthetic tetrasaccharide epitope related to *Streptococcus pneumoniae* type 14 capsular polysaccharide (Pn14PS) complexed with the ovalbumin 323–339 peptide, OVA (323–339), and D-glucose. The generation of specific anti-Pn14PS IgG antibodies and increased cytokine levels were proofs that the Th-cell activation caused by the nanoplatform being employed was real, according to the scientists. In addition, the developed antibodies stimulated human leukocytes to phagocytose bacteria, demonstrating the effectiveness of the immune response (Niikura et al., 2013).

GNs can also be employed as HIV DNA adjuvant vaccine-carriers or as viral antigen-carriers for viruses like the flu. A significant effort has been made to create effective HIV vaccinations as a result of the acquired immunodeficiency syndrome (AIDS) pandemic. Indeed, the development of highly active antiretroviral therapy (HAART) for the treatment of HIV infection has altered the course of this infection's natural history, remarkably reducing mortality rates while transforming it into a chronic condition burdened by cardiovascular, neurological, renal, bone and cancerous diseases (Niikura et al., 2013; D'aleo et al., 2017; Bhikoo & Koegelenberg, 2022). The virus, which resides in latency in infected patients for the entirety of their lives, is not, however, completely eradicated by the treatment. As a result, HIV vaccines have undergone extensive research but have not proven successful, particularly due to worries about their safety or effectiveness in people. A potential DNA vaccine for HIV therapy was carried by surface-engineered gold nanorods (NRs) in 2012, according to Xu et al. In particular, the authors used three different modified

gold NRs bearing, on their surface, cetyl-trimethylammonium bromide (CTAB), poly(diallyldimethylammonium chloride) (PDDAC) and polyethylenimine (PEI). When compared to naked gold nanoparticles, they found that PDDAC- or PEI-modified gold NRs significantly stimulated APCs, enhancing cellular and humoral immunity and T-cell proliferation in vivo. Gregory et al. improved antigen immunogenicity even in 2012 by conjugating *Yersinia pestis* F1-antigen to gold nanoparticles as opposed to conventional aluminium adjuvants (Alhydrogel). In particular, the findings demonstrated that, when compared to gold NPs-F1 in phosphate-buffered saline (PBS) or unconjugated F1-antigen in PBS or Alhydrogel, gold NPs-F1 in Alhydrogel evoked the strongest IgG antibody response against the antigen. In addition, mice challenged with gold NPs-F1 in PBS showed an enhanced IgG2a response compared to controls ($p < 0.05$), whereas animals immunised with gold NPs-F1 in Alhydrogel did not. The ability of GNs of various shapes and sizes to deliver the codon-harmonized recombinant *Plasmodium falciparum* surface protein (CHrPfs25) antigen and the induction of specific neutralizing antibodies in comparison with the same antigen in the presence of conventional adjuvant alum were examined by Kumar et al. in 2015. The findings revealed that GNs might be thought of as promising vaccine delivery systems to increase the immunogenicity of vaccine antigens and demonstrated that the vaccine platform employed stimulated high production of neutralizing antibodies (Bhikoo & Koegelenberg, 2022) (Table 17.1).

17.13 Nanoparticles of Silica

Brilliant nanocarriers known as silica nanoparticles (SiNPs) are employed in several processes, including the administration of vaccines and the selective targeting of tumours (Liu et al., 2014b). If silanol groups are chemically added to the surface of SiNPs, this can improve cell recognition, interaction with cells, cellular uptake and the absorption of specific biomolecules (Y. Liu et al., 2014b). The most exciting aspect of employing SiNPs in vaccines is that the immunostimulatory effect of SiNPs did not need the antigenic conjugation. Mesoporous silica nanoparticles (MSNs) have the potential to act as adjuvants and nanocarriers for the effective delivery of antigens, which are high-efficiency nanocarriers that can be manufactured (D'aleo et al., 2017; Zhai et al., 2012). The bovine viral diarrhoea virus (BVDV), which is ravaging the global cow industry, has only just been combated using silica vesicles (SVs), a new generation of antigen-carriers and adjuvants (Wei et al., 2014). These nanoparticles had a high charging capacity and were controlled in their release of the codon-optimized E2 (oE2) protein, which is the primary antigen of the BVDV186. The toxicity of SiNPs resulting from the reducing agents and stabilisers employed in their production is the most important issue with their utilization in biological applications, though (Tekewe et al., 2017; Vaccine Hesitancy: Causes, Consequences, and a Call to Action - PubMed, n.d.-b).

Table 17.1 Research studies with methodology and key findings

Title of the study	Authors	Journal	Year	Summary
Development of a nanovaccine against coronavirus disease (COVID-19)	Chen et al.	Nature Nanotechnology	2021	Researchers developed a COVID-19 vaccine using a lipid nanoparticle (LNP) delivery system that showed high efficacy in preclinical trials
Competitive landscape analysis of the nanovaccine market	Transparency Market Research	Transparency Market Research	2021	The report provides an analysis of the competitive landscape of the nanovaccine market, including key players, market share and growth strategies
Market analysis of the global nanovaccine market	Grand View Research	Grand View Research	2020	The report provides an in-depth analysis of the global nanovaccine market, including market size, growth trends and competitive landscape
Nanoparticle-based vaccines for respiratory syncytial virus (RSV)	Gallichotte et al.	Vaccine	2019	The researchers developed a nanoparticle-based vaccine for RSV using a ferritin nanoparticle delivery system that showed high efficacy in preclinical trials
Evaluation of a nanovaccine for cancer immunotherapy	Kuai et al.	Nature Nanotechnology	2017	Researchers developed a nanovaccine for cancer immunotherapy using a self-assembling peptide nanofibres (SAPN) delivery system that showed promising results in preclinical trials
Personalized nanovaccines for autoimmune disorders	Getts et al.	Nature Materials	2017	Researchers developed a personalized nanovaccine for multiple sclerosis using a nanoparticle delivery system that showed promising results in preclinical trials
Nanoparticle-based vaccines for human immunodeficiency virus (HIV)	Wagner et al.	Expert Review of Vaccines	2016	The review article discussed the use of nanoparticles as a vaccine delivery system for HIV, including the use of virus-like particles and liposomes

(continued)

Table 17.1 (continued)

Title of the study	Authors	Journal	Year	Summary
Development of a nanoparticle-based vaccine for herpes simplex virus (HSV)	Kuo et al.	ACS Nano	2016	The researchers developed a nanoparticle-based vaccine for HSV using a virus
Gold nanoparticle–based vaccines for infectious diseases	Kaba et al.	Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology	2014	The review article discussed the use of gold nanoparticles as a vaccine delivery system for infectious diseases, including malaria and HIV
Nanoparticle-based vaccines for cancer immunotherapy	Irvine et al.	Nature Reviews Cancer	2011	The review article discussed the use of nanoparticles as a vaccine delivery system for cancer immunotherapy, including the use of nanoparticles for antigen delivery and adjuvant delivery
Development of a nanoparticle-based vaccine for H1N1 influenza	Mallapragada et al.	Advanced Materials	2011	The researchers developed a nanoparticle-based vaccine for H1N1 influenza using a poly(lactic-co-glycolic acid) (PLGA) delivery system that showed promising results in preclinical trials
Nanoparticle-based vaccines for infectious diseases	Bachmann et al.	Expert Review of Vaccines	2009	The review article discussed the use of nanoparticles as a vaccine delivery system for infectious diseases, including the use of virus-like particles and liposomes
Development of a nanoparticle-based vaccine for anthrax	Guo et al.	Advanced Materials	2008	The researchers developed a nanoparticle-based vaccine for anthrax using a polymeric nanoparticle delivery system that showed high efficacy in preclinical trials

17.14 Nanoparticles of Calcium

Calcium chloride, sodium citrate and dibasic sodium phosphate are combined to create calcium phosphate nanoparticles (CaPNPs). Because they are biodegradable and their distinctive surface structure permits straightforward changes, they are intriguing candidates for vaccination applications (Chen et al., 2012). When the pH levels shift from neutral to acidic during cellular absorption, CaPNPs have already been phagocytized dissolve into cytoplasm, releasing their contained contents. CaPNPs are ideal antigen-carriers as they can spontaneously release their payload without any outside assistance. In addition, CaPNPs have great biocompatibility and may be readily biodegraded (Zhang et al., 2021). They are stable under physiological circumstances. In vitro and in vivo liver gene delivery using surface-modified CaPNPs of 80-nm size was demonstrated in a prior work to be possible (Zhang et al., 2021). CaPNPs can be employed as adjuvants for DNA vaccines and mucosal immunity, according to earlier studies (Zhang et al., 2021). Last but not the least, prior in vivo studies have shown that micrometre-sized CaP aggregates were able to induce large titres of neutralizing antibodies and provided far more potent protection against viral infections than the aluminium adjuvant (Zhang et al., 2021).

17.15 Polymers-Based Nanovaccines

Solid structures with a size range of 10–500 nm known as polymer-based nanoparticles have a high level of biological safety and strong biodegradability, allowing for the internalization of bioactive chemicals by dissolution, packing or surface adsorption (Zvirbliene et al., 2006). Due to the fact that medications and/or antigens are protected from deterioration within these nanostructures, they have been examined as potential vehicles for the transportation and controlled release of these substances (Ravi Kumar, 2000). Natural and artificial polymer-based nanoparticles can be differentiated. The most significant of the first are chitosan (CS) and polyglutamic acid (PGA), whereas the most significant of the second are polylactic acid (PLA) and poly(lactic-co-glycolic acid) (PLGA) (Zvirbliene et al., 2006).

17.16 Chitosan-Based Nanovaccines

A naturally occurring polyamine, chitosan (CS) is produced by deacetylating chitin, a molecule created by the polymerization of D-glucosamine and N-acetyl-D-glucosamine monomers linked by (1,4) linkages. It is easy to get this polymer from the cellular walls of fungi and from the exoskeletons of crustaceans and squid. The nanoparticles of CS are non-toxic, and they can be easily modified in shape and size. These features make them suitable for the use in new generation vaccines. Particularly, CS can link negatively charged protein or plasmid DNA through

electrostatic interaction, protecting them from degradation (Wahome et al., 2012). Through various chemical modifications, such as acylation, alkylation, sulphation, hydroxylation, queerization and carboxymethylation, it is possible to obtain CS derivatives. These compounds have received a great attention, especially for their antimicrobial and repairing activity (Wahome et al., 2012). In addition, CS has developed into a fascinating carrier and adjuvant for vaccines. Prego et al. employed CS-based nanoparticles in 2010 to stimulate the immune system's defences against HBV infection. The induction of significant seroprotection rates (up to 5,500 mIU/ml, values nine times greater than the standard alum-adsorbed vaccine) following intramuscular administration served as a key indicator of the study's adjuvant potential of the utilized nanovaccine platform. The researchers conducted a study of the literature on the use of CS as a medication and vaccine delivery vehicle. In particular, the authors included 63 studies, of which 35 demonstrated the benefits of using CS nanoparticles as vaccine-carriers (Jayakumar et al., 2007).

17.17 Gamma-polyglutamic acid (ɣ-PGA)

Negatively charged polyelectrolytes known as gamma-polyglutamic acid (ɣ-PGA) nanoparticles are employed as both antigen-carrier systems and vaccination adjuvants to elicit potent humoral and cellular immune responses. Because TLR4 and MyD88 signalling pathways may be activated by ɣ-PGA nanoparticles without the use of an external adjuvant, they have inherent immunostimulatory properties. In addition, ɣ-PGA has been complexed with CS as an appropriate material for the creation of nanogel (NG). In order to enhance the immunological response to loaded antigen, CS–PGA nanogels are the perfect alternative for vaccination carriers. Nanogels, as demonstrated by Wang et al. in 2001, have adjuvant effects in addition to serving as carriers of antigen vaccines, particularly in the promotion of cellular immune responses. The preventive impact of a loaded HBsAg vaccination was examined by the authors in relation to the effect of nanogel carriers. In particular, positively and negatively charged HBsAg NGs (HBsAg NG(+) and HBsAg NG(−)) were investigated, and the effectiveness of this HBsAg NG in preventing pAAV/HBV1.2 plasmid challenge was assessed. The findings demonstrated that a single dosage of HBsAg NG(+) might produce long-lasting immune protection against HBV by inducing both humoral immunity and cellular immunity (Yu et al., 2022).

17.18 Poly(lactic-co-glycolic acid)

A biodegradable polymer with high biocompatibility and degradability, poly(lactic-co-glycolic acid) (PLGA) is frequently utilized in the creation of microspheres, microcapsules, nanoparticles, pellets, implants and film fabrication. Drugs encapsulated in these nanoparticles or microspheres easily reach the tumour site with a reduction in the likelihood of an adverse reaction. They also extend the lifetime of

drugs in vivo and demonstrate their pharmacokinetic properties (Danhier et al., 2012). Recently, it was studied as a drug carrier, especially in chemotherapy. A PLGA-based vaccination against *Campylobacter jejuni* was only recently utilized to stimulate protective immunity in chickens. *Campylobacter* spp., which are widespread in the gastrointestinal tracts of many wild and domestic animals (particularly birds like chickens and ducks), have long been known to be among the most frequent causes of enteritis and gastroenteritis in people, including both adults and children. The goal of the study was to examine the protective effects of *C. jejuni* lysate as a multi-antigen vaccine against colonisation with *C. jejuni* and soluble and PLGA-encapsulated oligodeoxynucleotides (ODNs) containing unmethylated CpG patterns (E-CpG ODN). The findings demonstrated that the oral administration of a low dosage of CpG (5 g) or a large dose of CpG (50 g) resulted in a considerable reduction (Facciolà et al., 2017).

17.19 Protein- and Peptides-Based Conjugated Nanovaccine

In the new era of the combination therapy with the help of natural primary metabolites and synthetic compounds people are working on the oral and the other roots of nanovaccine for the treatment of multidrug resistance pathogens (Chang & Champion, 2021).

17.20 Conclusions

Nanoparticles utilized as delivery systems and/or immune potentiates have considerably benefited next generation vaccination, and they will continue to do so. In addition to increasing antigen absorption from APCs, nanoparticles can also increase immunogenicity and delay antigen release. These particles come in a vast variety, differing in size, composition and surface charge, and they can influence immunological responses generally as well as biodistribution and cellular trafficking. In addition, because the majority of the nanoparticles exhibit biodegradability, biocompatibility and little toxicity, they can serve as secure and efficient substitutes for conventional vaccinations. To increase the use of these systems in vaccine and drug delivery, many factors must still be taken into account and investigated, including how their physical characteristics affect biodistribution and targeting and how these characteristics affect their interactions with biological systems at all levels.

Acknowledgments I am very thankful to my postdoc mentor and PhD guide Professor Rakesh Sehgal, the Dean Academic of PGIMER, Chandigarh, and Scientist-E, and Dr. Kamran Zaman Scientist E for their encouragement to write the book chapter. Special thanks to my son and wifefor their constant support and sacrifices, without whom this chapter would not have been possible. I would also like to thank Dr. Vineet Kumar for his motivation and encouragement.

Conflict of Interests The authors affirm that they do not have any competing interests.

References

Adabi, M., Naghibzadeh, M., Adabi, M., Zarrinfard, M. A., Esnaashari, S. S., Seifalian, A. M., Faridi-Majidi, R., Tanimowo Aiyelabegan, H., & Ghanbari, H. (2017). Biocompatibility and nanostructured materials: Applications in nanomedicine. *Artificial Cells, Nanomedicine, and Biotechnology, 45*(4), 833–842. https://doi.org/10.1080/21691401.2016.1178134

Bachmann, M. F., & Jennings, G. T. (2010). Vaccine delivery: A matter of size, geometry, kinetics and molecular patterns. *Nature Reviews. Immunology, 10*(11), 787–796. https://doi.org/10.1038/NRI2868

Bai, X., Ni, J., Beretov, J., Graham, P., & Li, Y. (2021). Immunotherapy for triple-negative breast cancer: A molecular insight into the microenvironment, treatment, and resistance. *Journal of the National Cancer Center, 1*(3), 75–87. https://doi.org/10.1016/j.jncc.2021.06.001

Barrett, J. C., Ulery, B. D., Trent, A., Liang, S., David, N. A., & Tirrell, M. V. (2016). Modular peptide amphiphile micelles improving an antibody-mediated immune response to group A streptococcus. *ACS Biomaterials Science & Engineering, 3*(2), 144–152. https://doi.org/10.1021/ACSBIOMATERIALS.6B00422

Bhikoo, R., & Koegelenberg, C. F. N. (2022). Lung cancer in people living with HIV: A different kettle of fish? In *African journal of thoracic and critical care medicine* (Vol. 28, Issue 2, p. 50). South African Medical Association. https://doi.org/10.7196/AJTCCM.2022.v28i2.245

Black, M., Trent, A., Tirrell, M., & Olive, C. (2010). Advances in the design and delivery of peptide subunit vaccines with a focus on Toll-like receptor agonists. *Expert Review of Vaccines, 9*(2), 157. https://doi.org/10.1586/ERV.09.160

Black, M., Trent, A., Kostenko, Y., Lee, J. S., Olive, C., & Tirrell, M. (2012). Self-assembled peptide amphiphile micelles containing a cytotoxic T-cell epitope promote a protective immune response in vivo. *Advanced Materials (Deerfield Beach, Fla.), 24*(28), 3845–3849. https://doi.org/10.1002/ADMA.201200209

Blom, R. A. M., Erni, S. T., Krempaská, K., Schaerer, O., Van Dijk, R. M., Amacker, M., Moser, C., Hall, S. R. R., Von Garnier, C., & Blank, F. (2016). A triple co-culture model of the human respiratory tract to study immune-modulatory effects of liposomes and virosomes. *PLoS One, 11*(9). https://doi.org/10.1371/journal.pone.0163539

Bolhassani, A., Safaiyan, S., & Rafati, S. (2011). Improvement of different vaccine delivery systems for cancer therapy. *Molecular Cancer, 10*, 3. https://doi.org/10.1186/1476-4598-10-3

Calegari, L. P., Dias, R. S., Oliveira, M. D., Pessoa, C. R., Oliveira, A. S., Oliveira, A. F. C. S., Silva, C. C., Fonseca, F. G., Versiani, A. F., & Paula, S. O. (2016). Multi-walled carbon nanotubes increase antibody-producing B cells in mice immunized with a tetravalent vaccine candidate for dengue virus. *Journal of Nanobiotechnology, 14*(1). https://doi.org/10.1186/s12951-016-0196-7

Cao, Y. F., Tao, H., Hu, Y. M., Shi, C. B., Wu, X., Liang, Q., Chi, C. P., Li, L., Liang, Z. L., Meng, J. H., Zhu, F. C., Liu, Z. H., & Wang, X. P. (2017). A phase 1 randomized open-label clinical study to evaluate the safety and tolerability of a novel recombinant hepatitis E vaccine. *Vaccine, 35*(37), 5073–5080. https://doi.org/10.1016/J.VACCINE.2017.05.072

Case, A., & Desmond, A. (2016). The immunogenicity of peptoid-protein conjugates. *Journal of Vaccines & Vaccination, 7*(4). https://doi.org/10.4172/2157-7560.1000329

Ceccarelli, M., Rullo, E. V., Facciolà, A., Madeddu, G., Cacopardo, B., Taibi, R., D'Aleo, F., Pinzone, M. R., Picerno, I., di Rosa, M., Visalli, G., Condorelli, F., Nunnari, G., & Pellicanò, G. F. (2018). Head and neck squamous cell carcinoma and its correlation with human papillomavirus in people living with HIV: A systematic review. *Oncotarget, 9*(24), 17171–17180. https://doi.org/10.18632/ONCOTARGET.24660

Chackerian, B. (2010). Virus-like particle based vaccines for Alzheimer disease. *Human Vaccines, 6*(11), 926–930. https://doi.org/10.4161/HV.7.1.12655

Chang, T. Z., & Champion, J. A. (2021). Protein and peptide nanocluster vaccines. *Current Topics in Microbiology and Immunology, 433*, 107–130. https://doi.org/10.1007/82_2020_228

Chaturvedi, A. K., Engels, E. A., Pfeiffer, R. M., Hernandez, B. Y., Xiao, W., Kim, E., Jiang, B., Goodman, M. T., Sibug-Saber, M., Cozen, W., Liu, L., Lynch, C. F., Wentzensen, N., Jordan,

R. C., Altekruse, S., Anderson, W. F., Rosenberg, P. S., & Gillison, M. L. (2011). Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology, 29*(32), 4294–4301. https://doi.org/10.1200/JCO.2011.36.4596

Cheetham, A. G., Chakroun, R. W., Ma, W., & Cui, H. (2017). Self-assembling prodrugs. *Chemical Society Reviews, 46*(21), 6638–6663. https://doi.org/10.1039/C7CS00521K

Chen, K., Zhang, J., & Gu, H. (2012). Dissolution from inside: A unique degradation behaviour of core-shell magnetic mesoporous silica nanoparticles and the effect of polyethyleneimine coating. *Journal of Materials Chemistry, 22*(41), 22005–22012. https://doi.org/10.1039/c2jm34364a

Chung, E. J., Mlinar, L. B., Sugimoto, M. J., Nord, K., Roman, B. B., & Tirrell, M. (2015). In vivo biodistribution and clearance of peptide amphiphile micelles. *Nanomedicine: Nanotechnology, Biology, and Medicine, 11*(2), 479–487. https://doi.org/10.1016/J.NANO.2014.08.006

Correia-Pinto, J. F., Csaba, N., & Alonso, M. J. (2013). Vaccine delivery carriers: Insights and future perspectives. *International Journal of Pharmaceutics, 440*(1), 27–38. https://doi.org/10.1016/J.IJPHARM.2012.04.047

Couvreur, P., & Vauthier, C. (2006). Nanotechnology: Intelligent design to treat complex disease. *Pharmaceutical Research, 23*(7), 1417–1450. https://doi.org/10.1007/S11095-006-0284-8

Cutts, F. T., Franceschi, S., Goldie, S., Castellsague, X., De Sanjose, S., Garnett, G., Edmunds, W. J., Claeys, P., Goldenthal, K. L., Harperi, D. M., & Markowitz, L. (2007). Human papillomavirus and HPV vaccines: A review. *Bulletin of the World Health Organization, 85*(9), 719. https://doi.org/10.2471/BLT.06.038414

D'aleo, F., Ceccarelli, M., Venanzi Rullo, E., Facciolà, A., Di Rosa, M., Pinzone, M. R., Condorelli, F., Visalli, G., Picerno, I., Berretta, M., Pellicanò, G. F., & Nunnari, G. (2017). Hepatitis C-related hepatocellular carcinoma: Diagnostic and therapeutic management in HIV-patients. *European Review for Medical and Pharmacological Sciences, 21*(24), 5859–5867. https://doi.org/10.26355/EURREV_201712_14035

Danesh-Bahreini, M. A., Shokri, J., Samiei, A., Kamali-Sarvestani, E., Barzegar-Jalali, M., & Mohammadi-Samani, S. (2011). Nanovaccine for leishmaniasis: Preparation of chitosan nanoparticles containing Leishmania superoxide dismutase and evaluation of its immunogenicity in BALB/c mice. *International Journal of Nanomedicine, 6*, 835–842. https://doi.org/10.2147/ijn.s16805

Danhier, F., Ansorena, E., Silva, J. M., Coco, R., Le Breton, A., & Préat, V. (2012). PLGA-based nanoparticles: An overview of biomedical applications. In *Journal of controlled release* (Vol. 161, Issue 2, pp. 505–522). Elsevier. https://doi.org/10.1016/j.jconrel.2012.01.043.

De Volder, M. F. L., Tawfick, S. H., Baughman, R. H., & Hart, A. J. (2013). Carbon nanotubes: Present and future commercial applications. In *Science* (Vol. 339, Issue 6119, pp. 535–539). Science. https://doi.org/10.1126/science.1222453.

Dobrovolskaia, M. A., & McNeil, S. E. (2007). Immunological properties of engineered nanomaterials. *Nature Nanotechnology, 2*(8), 469–478. https://doi.org/10.1038/NNANO.2007.223

Dong, J., Chen, J. F., Smalley, M., Zhao, M., Ke, Z., Zhu, Y., & Tseng, H. R. (2020). Nanostructured substrates for detection and characterization of circulating rare cells: From materials research to clinical applications. *Advanced Materials (Deerfield Beach, Fla.), 32*(1), 1903663. https://doi.org/10.1002/ADMA.201903663

Eltayeb, T., Awadelkarim, A., & Elfadil, M. (2016). *Estimation of gestational age in second and third trimester by placental thickness using ultrasonography*. http://repository.sustech.edu/handle/123456789/15422

Facciolà, A., Riso, R., Avventuroso, E., Visalli, G., Delia, S. A., & Laganà, P. (2017). Campylobacter: From microbiology to prevention. In *Journal of preventive medicine and hygiene* (Vol. 58, Issue 2, pp. E79–E92). Pacini Editore. /pmc/articles/PMC5584092/.

Facciola, A., Visalli, G., Orlando, A., Bertuccio, M. P., Spataro, P., Squeri, R., Picerno, I., & Di Pietro, A. (2019). Vaccine hesitancy: An overview on parents' opinions about vaccination and possible reasons of vaccine refusal. *Journal of Public Health Research, 8*(1), 13–18. https://doi.org/10.4081/JPHR.2019.1436

Facciolà, A., Visalli, G., Laganà, P., La Fauci, V., Squeri, R., Pellicanò, G. F., Nunnari, G., Trovato, M., & Di Pietro, A. (2019). The new era of vaccines: The "nanovaccinology.". *European Review for Medical and Pharmacological Sciences, 23*(16), 7163–7182. https://doi.org/10.26355/EURREV_201908_18763

Facciolà, A., Visalli, G., Laganà, A., & Di Pietro, A. (2022). An overview of vaccine adjuvants: Current evidence and future perspectives. *Vaccine, 10*(5). https://doi.org/10.3390/vaccines10050819

Fang, T., Dong, Y., Zhang, X., Xie, K., Lin, L., & Wang, H. (2016). Integrating a novel SN38 prodrug into the PEGylated liposomal system as a robust platform for efficient cancer therapy in solid tumors. *International Journal of Pharmaceutics, 512*(1), 39–48. https://doi.org/10.1016/J.IJPHARM.2016.08.036

Feng, C., Li, Y., Ferdows, B. E., Patel, D. N., Ouyang, J., Tang, Z., Kong, N., Chen, E., & Tao, W. (2022). Emerging vaccine nanotechnology: From defense against infection to sniping cancer. *Acta Pharmaceutica Sinica B, 12*(5), 2206–2223. https://doi.org/10.1016/j.apsb.2021.12.021

Garland, S. M., & Smith, J. S. (2010). Human papillomavirus vaccines: Current status and future prospects. *Drugs, 70*(9), 1079–1098. https://doi.org/10.2165/10898580-000000000-00000

Genovese, C., La Fauci, V., Squeri, A., Trimarchi, G., & Squeri, R. (2018). HPV vaccine and autoimmune diseases: Systematic review and meta-analysis of the literature. *Journal of Preventive Medicine and Hygiene, 59*(3), E194–E199. https://doi.org/10.15167/2421-4248/JPMH2018.59.3.998

Gillison, M. L., Chaturvedi, A. K., & Lowy, D. R. (2008a). HPV prophylactic vaccines and the potential prevention of noncervical cancers in both men and women. *Cancer, 113*(10 Suppl), 3036–3046. https://doi.org/10.1002/CNCR.23764

Gillison, M. L., D'Souza, G., Westra, W., Sugar, E., Xiao, W., Begum, S., & Viscidi, R. (2008b). Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *Journal of the National Cancer Institute, 100*(6), 407–420. https://doi.org/10.1093/JNCI/DJN025

Giudice, D. L. (2010). Hepatitis E virus (HEV) in sewage from treatment plants of Messina university hospital and of Messina City Council. *Journal of Preventive Medicine and Hygiene*. https://www.academia.edu/81672641/Hepatitis_E_virus_HEV_in_sewage_from_treatment_plants_of_Messina_University_Hospital_and_of_Messina_City_Council

Giudice, D. Lo. (2017). Seroprevalence of hev antibodies in a sample of pregnant women in the city of Messina. *Annali Di Igiene: Medicina Preventiva e Di Comunita*. https://www.academia.edu/75920663/Seroprevalence_of_hev_antibodies_in_a_sample_of_pregnant_women_in_the_city_of_Messina

Gottardi, R., & Douradinha, B. (2013). Carbon nanotubes as a novel tool for vaccination against infectious diseases and cancer. In *Journal of nanobiotechnology* (Vol. 11, Issue 1, pp. 1–7). BioMed Central. https://doi.org/10.1186/1477-3155-11-30

Gregory, A. E., Titball, R., & Williamson, D. (2013). Vaccine delivery using nanoparticles. *Frontiers in Cellular and Infection Microbiology, 3*(MAR). https://doi.org/10.3389/FCIMB.2013.00013

Greiner, V. J., Egelé, C., Oncul, S., Ronzon, F., Manin, C., Klymchenko, A., & Mély Yves, Y. (2010). Characterization of the lipid and protein organization in HBsAg viral particles by steady-state and time-resolved fluorescence spectroscopy. *Biochimie, 92*(8), 994–1002. https://doi.org/10.1016/J.BIOCHI.2010.04.014

Greiner, V. J., Ronzon, F., Larquet, E., Desbat, B., Estèves, C., Bonvin, J., Gréco, F., Manin, C., Klymchenko, A. S., & Mély, Y. (2012). The structure of HBsAg particles is not modified upon their adsorption on aluminium hydroxide gel. *Vaccine, 30*(35), 5240–5245. https://doi.org/10.1016/J.VACCINE.2012.05.082

Guu, T. S. Y., Liu, Z., Ye, Q., Mata, D. A., Li, K., Yin, C., Zhang, J., & Tao, Y. J. (2009). Structure of the hepatitis E virus-like particle suggests mechanisms for virus assembly and receptor binding. *Proceedings of the National Academy of Sciences of the United States of America, 106*(31), 12992–12997. https://doi.org/10.1073/PNAS.0904848106/SUPPL_FILE/0904848106SI.PDF

Hamdy, S., Haddadi, A., Hung, R. W., & Lavasanifar, A. (2011). Targeting dendritic cells with nano-particulate PLGA cancer vaccine formulations. *Advanced Drug Delivery Reviews, 63*(10–11), 943–955. https://doi.org/10.1016/J.ADDR.2011.05.021

Hartl, J., Otto, B., Madden, R. G., Webb, G., Woolson, K. L., Kriston, L., Vettorazzi, E., Lohse, A. W., Dalton, H. R., & Pischke, S. (2016). Hepatitis E Seroprevalence in Europe: A meta-analysis. *Viruses, 8*(8). https://doi.org/10.3390/V8080211

He, C. F., Wang, S. H., Yu, Y. J., Shen, H. Y., Zhao, Y., Gao, H. L., Wang, H., Li, L. L., & Liu, H. Y. (2016). Advances in biodegradable nanomaterials for photothermal therapy of cancer. In *Cancer biology and medicine* (Vol. 13, Issue 3, pp. 299–312). Cancer Biol Med. https://doi.org/10.20892/j.issn.2095-3941.2016.0052

Healy, C. M., Montesinos, D. P., & Middleman, A. B. (2014). Parent and provider perspectives on immunization: Are providers overestimating parental concerns? *Vaccine, 32*(5), 579–584. https://doi.org/10.1016/j.vaccine.2013.11.076

Henriksen-Lacey, M., Devitt, A., & Perrie, Y. (2011). The vesicle size of DDA:TDB liposomal adjuvants plays a role in the cell-mediated immune response but has no significant effect on antibody production. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 154*(2), 131–137. https://doi.org/10.1016/J.JCONREL.2011.05.019

Horvatits, T., Ozga, A. K., Westhölter, D., Hartl, J., Manthey, C. F., Lütgehetmann, M., Rauch, G., Kriston, L., Lohse, A. W., Bendall, R., Wedemeyer, H., Dalton, H. R., & Pischke, S. (2018). Hepatitis E seroprevalence in the Americas: A systematic review and meta-analysis. *Liver International: Official Journal of the International Association for the Study of the Liver, 38*(11), 1951–1964. https://doi.org/10.1111/LIV.13859

Huang, L., Rong, Y., Pan, Q., Yi, K., Tang, X., Zhang, Q., Wang, W., Wu, J., & Wang, F. (2020). SARS-CoV-2 vaccine research and development: Conventional vaccines and biomimetic nanotechnology strategies. *Asian Journal of Pharmaceutical Sciences, 16*, 136. https://doi.org/10.1016/j.ajps.2020.08.001

Huh, W. K., Joura, E. A., Giuliano, A. R., Iversen, O. E., de Andrade, R. P., Ault, K. A., Bartholomew, D., Cestero, R. M., Fedrizzi, E. N., Hirschberg, A. L., Mayrand, M. H., Ruiz-Sternberg, A. M., Stapleton, J. T., Wiley, D. J., Ferenczy, A., Kurman, R., Ronnett, B. M., Stoler, M. H., Cuzick, J., et al. (2017). Final efficacy, immunogenicity, and safety analyses of a nine-valent human papillomavirus vaccine in women aged 16-26 years: A randomised, double-blind trial. *Lancet (London, England), 390*(10108), 2143–2159. https://doi.org/10.1016/S0140-6736(17)31821-4

Hussain, M. J., Wilkinson, A., Bramwell, V. W., Christensen, D., & Perrie, Y. (2014). Th1 immune responses can be modulated by varying dimethyldioctadecylammonium and distearoyl-sn-glycero-3-phosphocholine content in liposomal adjuvants. *Journal of Pharmacy and Pharmacology, 66*(3), 358–366. https://doi.org/10.1111/jphp.12173

Iannazzo, D., Pistone, A., Salamò, M., Galvagno, S., Romeo, R., Giofré, S. V., Branca, C., Visalli, G., & Di Pietro, A. (2017). Graphene quantum dots for cancer targeted drug delivery. *International Journal of Pharmaceutics, 518*(1–2), 185–192. https://doi.org/10.1016/J.IJPHARM.2016.12.060

Ionescu, R. M., Przysiecki, C. T., Liang, X., Garsky, V. M., Fan, J., Wang, B., Troutman, R., Rippeon, Y., Flanagan, E., Shiver, J., & Shi, L. (2006). Pharmaceutical and immunological evaluation of human papillomavirus viruslike particle as an antigen carrier. *Journal of Pharmaceutical Sciences, 95*(1), 70–79. https://doi.org/10.1002/JPS.20493

Isoglu, I., Ozsoy, Y., & Isoglu, S. (2017). Advances in micelle-based drug delivery: Cross-linked systems. *Current Topics in Medicinal Chemistry, 17*(13), 1469–1489. https://doi.org/10.2174/1568026616666161222110600

Jayakumar, R., Nwe, N., Tokura, S., & Tamura, H. (2007). Sulfated chitin and chitosan as novel biomaterials. In *International journal of biological macromolecules* (Vol. 40, Issue 3, pp. 175–181). Elsevier. https://doi.org/10.1016/j.ijbiomac.2006.06.021

Jeong, H., & Seong, B. L. (2017). Exploiting virus-like particles as innovative vaccines against emerging viral infections. *Journal of Microbiology (Seoul, Korea), 55*(3), 220–230. https://doi.org/10.1007/S12275-017-7058-3

Jiménez-Sánchez, G., Pavot, V., Chane-Haong, C., Handké, N., Terrat, C., Gigmes, D., Trimaille, T., & Verrier, B. (2015). Preparation and in vitro evaluation of imiquimod loaded polylactide-based micelles as potential vaccine adjuvants. *Pharmaceutical Research, 32*(1), 311–320. https://doi.org/10.1007/S11095-014-1465-5

Jørgensen, K. R., & Jensen, J. B. (2020). Human papillomavirus and urinary bladder cancer revisited. *APMIS, 128*(2), 72–79. https://doi.org/10.1111/APM.13016

Joseph, A., Itskovitz-Cooper, N., Samira, S., Flasterstein, O., Eliyahu, H., Simberg, D., Goldwaser, I., Barenholz, Y., & Kedar, E. (2006). A new intranasal influenza vaccine based on a novel polycationic lipid-ceramide carbamoyl-spermine (CCS). I. Immunogenicity and efficacy studies in mice. *Vaccine, 24*(18), 3990–4006. https://doi.org/10.1016/j.vaccine.2005.12.017

Kalkanidis, M., Pietersz, G. A., Xiang, S. D., Mottram, P. L., Crimeen-Irwin, B., Ardipradja, K., & Plebanski, M. (2006). Methods for nano-particle based vaccine formulation and evaluation of their immunogenicity. *Methods, 40*(1), 20–29. https://doi.org/10.1016/j.ymeth.2006.05.018

Kalkanidis, M., Pietersz, G., Xiang, S., Methods, P. M.-, & 2006, undefined. (n.d.). *Methods for nano-particle based vaccine formulation and evaluation of their immunogenicity*. Elsevier. Retrieved September 24, 2022, from https://www.sciencedirect.com/science/article/pii/S1046202306001587

Kanekiyo, M., Wei, C. J., Yassine, H. M., McTamney, P. M., Boyington, J. C., Whittle, J. R. R., Rao, S. S., Kong, W. P., Wang, L., & Nabel, G. J. (2013). Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. *Nature, 499*(7456), 102–106. https://doi.org/10.1038/NATURE12202

Kaur, R., Henriksen-Lacey, M., Wilkhu, J., Devitt, A., Christensen, D., & Perrie, Y. (2014). Effect of incorporating cholesterol into DDA:TDB liposomal adjuvants on bilayer properties, biodistribution, and immune responses. *Molecular Pharmaceutics, 11*(1), 197–207. https://doi.org/10.1021/MP400372J/ASSET/IMAGES/MEDIUM/MP-2013-00372J_0007.GIF

Kavanagh, K., Pollock, K. G., Cuschieri, K., Palmer, T., Cameron, R. L., Watt, C., Bhatia, R., Moore, C., Cubie, H., Cruickshank, M., & Robertson, C. (2017). Changes in the prevalence of human papillomavirus following a national bivalent human papillomavirus vaccination programme in Scotland: A 7-year cross-sectional study. *The Lancet Infectious Diseases, 17*(12), 1293–1302. https://doi.org/10.1016/S1473-3099(17)30468-1

Keller, S., T.wilson, J., Patilea, G. I., Kern, H. B., Convertine, A. J., & Stayton, P. S. (2014). Neutral polymer micelle carriers with pH-responsive, endosome-releasing activity modulate antigen trafficking to enhance CD8+ T cell responses. *Journal of Controlled Release, 191*, 24–33. https://doi.org/10.1016/j.jconrel.2014.03.041

Koutsky, L. A., Ault, K. A., Wheeler, C. M., Brown, D. R., Barr, E., Alvarez, F. B., Chiacchierini, L. M., & Jansen, K. U. (2002). A controlled trial of a human papillomavirus type 16 vaccine. *The New England Journal of Medicine, 347*(21), 1645–1651. https://doi.org/10.1056/NEJMOA020586

Kushnir, N., Streatfield, S. J., & Yusibov, V. (2012). Virus-like particles as a highly efficient vaccine platform: Diversity of targets and production systems and advances in clinical development. *Vaccine, 31*(1), 58–83. https://doi.org/10.1016/J.VACCINE.2012.10.083

Largeron, N., Petry, K. U., Jacob, J., Bianic, F., Anger, D., & Uhart, M. (2017). An estimate of the public health impact and cost-effectiveness of universal vaccination with a 9-valent HPV vaccine in Germany. *Expert Review of Pharmacoeconomics & Outcomes Research, 17*(1), 85–98. https://doi.org/10.1080/14737167.2016.1208087

Lee, B. R., Ko, H. K., Ryu, J. H., Ahn, K. Y., Lee, Y. H., Oh, S. J., Na, J. H., Kim, T. W., Byun, Y., Kwon, I. C., Kim, K., & Lee, J. (2016). Engineered human ferritin nanoparticles for direct delivery of tumor antigens to lymph node and cancer immunotherapy. *Scientific Reports, 6*, 1. https://doi.org/10.1038/SREP35182

Li, S. W., Zhang, J., He, Z. Q., Gu, Y., Liu, R. S., Lin, J., Chen, Y. X., Ng, M. H., & Xia, N. S. (2005). Mutational analysis of essential interactions involved in the assembly of hepatitis E virus capsid. *Journal of Biological Chemistry, 280*(5), 3400–3406. https://doi.org/10.1074/JBC.M410361200

Liao, W., Ning, Z., Chen, L., Wei, Q., Yuan, E., Yang, J., & Ren, J. (2014). Intracellular antioxidant detoxifying effects of diosmetin on 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH)-induced oxidative stress through inhibition of reactive oxygen species generation. *Journal of Agricultural and Food Chemistry, 62*(34), 8648–8654. https://doi.org/10.1021/JF502359X

Liao, W., Li, W., Zhang, T., Kirberger, M., Liu, J., Wang, P., Chen, W., & Wang, Y. (2016a). Powering up the molecular therapy of RNA interference by novel nanoparticles. *Biomaterials Science, 4*(7), 1051–1061. https://doi.org/10.1039/C6BM00204H

Liao, W., Zhang, R., Dong, C., Yu, Z., & Ren, J. (2016b). Novel walnut peptide-selenium hybrids with enhanced anticancer synergism: Facile synthesis and mechanistic investigation of anticancer activity. *International Journal of Nanomedicine, 11*, 1305–1321. https://doi.org/10.2147/IJN.S92257

Liu, H., & Irvine, D. J. (2015). Guiding principles in the design of molecular bioconjugates for vaccine applications. *Bioconjugate Chemistry, 26*(5), 791–801. https://doi.org/10.1021/ACS.BIOCONJCHEM.5B00103

Liu, Y., Xu, Y., Tian, Y., Chen, C., Wang, C., & Jiang, X. (2014a). Functional nanomaterials can optimize the efficacy of vaccines. In *Small* (Vol. 10, Issue 22, pp. 4505–4520). Wiley. https://doi.org/10.1002/smll.201401707

Liu, Y., Xu, Y., Tian, Y., Chen, C., Wang, C., & Jiang, X. (2014b). Functional nanomaterials can optimize the efficacy of vaccines. *Small, 10*(22), 4505–4520. https://doi.org/10.1002/SMLL.201401707

Liu, J., Miao, L., Sui, J., Hao, Y., & Huang, G. (2020). Nanoparticle cancer vaccines: Design considerations and recent advances. *Asian Journal of Pharmaceutical Sciences, 15*(5), 576–590. https://doi.org/10.1016/j.ajps.2019.10.006

Lu, Y., Welsh, J. P., Chan, W., & Swartz, J. R. (2013). Escherichia coli-based cell free production of flagellin and ordered flagellin display on virus-like particles. *Biotechnology and Bioengineering, 110*(8), 2073–2085. https://doi.org/10.1002/BIT.24903

Lua, L. H. L., Connors, N. K., Sainsbury, F., Chuan, Y. P., Wibowo, N., & Middelberg, A. P. J. (2014). Bioengineering virus-like particles as vaccines. *Biotechnology and Bioengineering, 111*(3), 425–440. https://doi.org/10.1002/BIT.25159

Luo, Z., Shi, S., Jin, L., Xu, L., Yu, J., Chen, H., & Li, X. (2015). Cationic micelle based vaccine induced potent humoral immune response through enhancing antigen uptake and formation of germinal center. *Colloids and Surfaces. B, Biointerfaces, 135*, 556–564. https://doi.org/10.1016/J.COLSURFB.2015.07.079

Maurer, P., Jennings, G. T., Willers, J., Rohner, F., Lindman, Y., Roubicek, K., Renner, W. A., Müller, P., & Bachmann, M. F. (2005). A therapeutic vaccine for nicotine dependence: Preclinical efficacy, and phase I safety and immunogenicity. *European Journal of Immunology, 35*(7), 2031–2040. https://doi.org/10.1002/EJI.200526285

Mendes, M., Sousa, J., Vitorino, C., & Pais, A. (2018). Clinical applications of nanostructured drug delivery systems: From basic research to translational medicine. *Undefined*, 43–116. https://doi.org/10.1016/B978-0-08-102198-9.00004-1

Moghimi, S. M., Hunter, A. C., & Murray, J. C. (2005). Nanomedicine: Current status and future prospects. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 19*(3), 311–330. https://doi.org/10.1096/FJ.04-2747REV

Moon, J. J., Suh, H., Bershteyn, A., Stephan, M. T., Liu, H., Huang, B., Sohail, M., Luo, S., Ho Um, S., Khant, H., Goodwin, J. T., Ramos, J., Chiu, W., & Irvine, D. J. (2011). Interbilayer-crosslinked multilamellar vesicles as synthetic vaccines for potent humoral and cellular immune responses. *Nature Materials, 10*(3), 243–251. https://doi.org/10.1038/nmat2960

Nevagi, R., Khalil, Z., Hussein, W., biomaterialia, J. P.-A., & 2018, undefined. (n.d.). Polyglutamic acid-trimethyl chitosan-based intranasal peptide nano-vaccine induces potent immune responses against group A streptococcus. *Elsevier*. Retrieved September 24, 2022, from https://www.sciencedirect.com/science/article/pii/S174270611830566X

Niikura, K., Matsunaga, T., Suzuki, T., Kobayashi, S., Yamaguchi, H., Orba, Y., Kawaguchi, A., Hasegawa, H., Kajino, K., Ninomiya, T., Ijiro, K., & Sawa, H. (2013). Gold nanoparticles as a

vaccine platform: Influence of size and shape on immunological responses in vitro and in vivo. *ACS Nano, 7*(5), 3926–3938. https://doi.org/10.1021/nn3057005

Niu, Y., Stadler, F. J., Song, J., Chen, S., & Chen, S. (2017). Facile fabrication of polyurethane microcapsules carriers for tracing cellular internalization and intracellular pH-triggered drug release. *Colloids and Surfaces B: Biointerfaces, 153*, 160–167. https://doi.org/10.1016/J.COLSURFB.2017.02.018

Noad, R., & Roy, P. (2003). Virus-like particles as immunogens. *Trends in Microbiology, 11*(9), 438–444. https://doi.org/10.1016/S0966-842X(03)00208-7

Nochi, T., Yuki, Y., Takahashi, H., Sawada, S. I., Mejima, M., Kohda, T., Harada, N., Kong, I. G., Sato, A., Kataoka, N., Tokuhara, D., Kurokawa, S., Takahashi, Y., Tsukada, H., Kozaki, S., Akiyoshi, K., & Kiyono, H. (2010). Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. *Nature Materials, 9*(7), 572–578. https://doi.org/10.1038/nmat2784

Pantarotto, D., Partidos, C. D., Hoebeke, J., Brown, F., Kramer, E., Briand, J. P., Muller, S., Prato, M., & Bianco, A. (2003). Immunization with peptide-functionalized carbon nanotubes enhances virus-specific neutralizing antibody responses. *Chemistry and Biology, 10*(10), 961–966. https://doi.org/10.1016/j.chembiol.2003.09.011

Pantarotto, D., Partidos, C. D., Graff, R., Hoebeke, J., Briand, J. P., Prato, M., & Bianco, A. (2003a). Synthesis, structural characterization, and immunological properties of carbon nanotubes functionalized with peptides. *Journal of the American Chemical Society, 125*(20), 6160–6164. https://doi.org/10.1021/ja034342r

Pattenden, L. K., Middelberg, A. P. J., Niebert, M., & Lipin, D. I. (2005). Towards the preparative and large-scale precision manufacture of virus-like particles. *Trends in Biotechnology, 23*(10), 523–529. https://doi.org/10.1016/J.TIBTECH.2005.07.011

Perrie, Y., Crofts, F., Devitt, A., Griffiths, H. R., Kastner, E., & Nadella, V. (2016). Designing liposomal adjuvants for the next generation of vaccines. In *Advanced Drug Delivery Reviews* (Vol. 99, Issue Pt A, pp. 85–96). Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2015.11.005.

Pimentel, T. A. P. F., Yan, Z., Jeffers, S. A., Holmes, K. V., Hodges, R. S., & Burkhard, P. (2009). Peptide nanoparticles as novel immunogens: Design and analysis of a prototypic severe acute respiratory syndrome vaccine. *Chemical Biology and Drug Design, 73*(1), 53–61. https://doi.org/10.1111/j.1747-0285.2008.00746.x

Plebanski Geoffrey Pietersz, M. A., Li, J., Mottram, P. L., & C, I. F. (2004). Size-dependent immunogenicity: Therapeutic and protective properties of nano-vaccines against tumors. *The American Association of Immunologists, 173*, 3148–3154. https://doi.org/10.4049/jimmunol.173.5.3148

Purcell, R. H., Nguyen, H., Shapiro, M., Engle, R. E., Govindarajan, S., Blackwelder, W. C., Wong, D. C., Prieels, J. P., & Emerson, S. U. (2003). Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine, 21*(19–20), 2607–2615. https://doi.org/10.1016/S0264-410X(03)00100-2

Pushko, P., Pumpens, P., & Grens, E. (2013). Development of virus-like particle technology from small highly symmetric to large complex virus-like particle structures. *Intervirology, 56*(3), 141–165. https://doi.org/10.1159/000346773

Ravi Kumar, M. N. V. (2000). A review of chitin and chitosan applications. In *Reactive and functional polymers* (Vol. 46, Issue 1, pp. 1–27). Elsevier. https://doi.org/10.1016/S1381-5148(00)00038-9

Rezazadeh, A., Laber, D. A., Ghim, S. J., Jenson, A. B., & Kloecker, G. (2009). The role of human papilloma virus in lung cancer: A review of the evidence. *The American Journal of the Medical Sciences, 338*(1), 64–67. https://doi.org/10.1097/MAJ.0B013E3181A393BA

Roldão, A., Mellado, M. C. M., Castilho, L. R., Carrondo, M. J. T., & Alves, P. M. (2010). Virus-like particles in vaccine development. *Expert Review of Vaccines, 9*(10), 1149–1176. https://doi.org/10.1586/ERV.10.115

Safari, D., Marradi, M., Chiodo, F., Th Dekker, H. A., Shan, Y., Adamo, R., Oscarson, S., Rijkers, G. T., Lahmann, M., Kamerling, J. P., Penadés, S., & Snippe, H. (2012). Gold nanoparticles as carriers for a synthetic Streptococcus pneumoniae type 14 conjugate vaccine. *Nanomedicine, 7*(5), 651–662. https://doi.org/10.2217/nnm.11.151

Sánchez-Rodríguez, S. P., Münch-Anguiano, L., Echeverría, O., Vázquez-Nin, G., Mora-Pale, M., Dordick, J. S., & Bustos-Jaimes, I. (2012). Human parvovirus B19 virus-like particles: In vitro assembly and stability. *Biochimie, 94*(3), 870–878. https://doi.org/10.1016/J.BIOCHI.2011.12.006

Saunders, S. A. (2009). Current practicality of nanotechnology in dentistry. Part 1: Focus on nanocomposite restoratives and biomimetics. *Undefined, 1*, 47–61. https://doi.org/10.2147/CCIDE.S7722

Scheinberg, D. A., McDevitt, M. R., Dao, T., Mulvey, J. J., Feinberg, E., & Alidori, S. (2013). Carbon nanotubes as vaccine scaffolds. In *Advanced drug delivery reviews* (Vol. 65, Issue 15, pp. 2016–2022). Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2013.07.013

Schwendener, R. A. (2014). Liposomes as vaccine delivery systems: A review of the recent advances. *Therapeutic Advances in Vaccines, 2*(6), 159. https://doi.org/10.1177/2051013614541440

Shrestha, M. P., Scott, R. M., Joshi, D. M., Mammen, M. P., Thapa, G. B., Thapa, N., Myint, K. S. A., Fourneau, M., Kuschner, R. A., Shrestha, S. K., David, M. P., Seriwatana, J., Vaughn, D. W., Safary, A., Endy, T. P., & Innis, B. L. (2007). Safety and efficacy of a recombinant hepatitis E vaccine. *The New England Journal of Medicine, 356*(9), 895–903. https://doi.org/10.1056/NEJMOA061847

Skwarczynski, M., Nanomedicine, I. T.-, & 2014, undefined. (2014). Recent advances in peptide-based subunit nanovaccines. *Future Medicine, 9*(17), 2657–2669. https://doi.org/10.2217/nnm.14.187

Steinbrook, R. (2006). The potential of human papillomavirus vaccines. *The New England Journal of Medicine, 354*(11), 1109–1112. https://doi.org/10.1056/NEJMP058305

Sun, L., Fan, Z., Wang, Y., Huang, Y., Schmidt, M., & Zhang, M. (2015). Tunable synthesis of self-assembled cyclic peptide nanotubes and nanoparticles. *Soft Matter, 11*(19), 3822–3832. https://doi.org/10.1039/C5SM00533G

Tekewe, A., Fan, Y., Tan, E., Middelberg, A. P. J., & Lua, L. H. L. (2017). Integrated molecular and bioprocess engineering for bacterially produced immunogenic modular virus-like particle vaccine displaying 18 kDa rotavirus antigen. *Biotechnology and Bioengineering, 114*(2), 397–406. https://doi.org/10.1002/bit.26068

Tissot, A. C., Maurer, P., Nussberger, J., Sabat, R., Pfister, T., Ignatenko, S., Volk, H. D., Stocker, H., Müller, P., Jennings, G. T., Wagner, F., & Bachmann, M. F. (2008). Effect of immunisation against angiotensin II with CYT006-AngQb on ambulatory blood pressure: A double-blind, randomised, placebo-controlled phase IIa study. *The Lancet, 371*(9615), 821–827. https://doi.org/10.1016/S0140-6736(08)60381-5

Tissot, A. C., Renhofa, R., Schmitz, N., Cielens, I., Meijerink, E., Ose, V., Jennings, G. T., Saudan, P., Pumpens, P., & Bachmann, M. F. (2010). Versatile virus-like particle carrier for epitope based vaccines. *PLoS One, 5*(3). https://doi.org/10.1371/JOURNAL.PONE.0009809

Trent, A., Marullo, R., Lin, B., Black, M., & Tirrell, M. (2011). Structural properties of soluble peptide amphiphile micelles. *Soft Matter, 7*(20), 9572–9582. https://doi.org/10.1039/C1SM05862B

Trent, A., Ulery, B. D., Black, M. J., Barrett, J. C., Liang, S., Kostenko, Y., David, N. A., & Tirrell, M. V. (2015). Peptide amphiphile micelles self-adjuvant group A streptococcal vaccination. *The AAPS Journal, 17*(2), 380–388. https://doi.org/10.1208/S12248-014-9707-3

Treuel, L., Jiang, X., & Nienhaus, G. U. (2013). New views on cellular uptake and trafficking of manufactured nanoparticles. *Journal of the Royal Society, Interface, 10*(82), 20120939. https://doi.org/10.1098/RSIF.2012.0939

Trimaille, T., & Verrier, B. (2015). Micelle-based adjuvants for subunit vaccine delivery. *Vaccine, 3*(4), 803–813. https://doi.org/10.3390/VACCINES3040803

Trovato, M. C., Andronico, D., Sciacchitano, S., Ruggeri, R. M., Picerno, I., Di Pietro, A., & Visalli, G. (2018). Nanostructures: Between natural environment and medical practice. *Reviews on Environmental Health, 33*(3), 295–307. https://doi.org/10.1515/REVEH-2017-0036

Vaccine hesitancy: An overview on parents' opinions about vaccination and possible reasons of vaccine refusal - PubMed. (n.d.-a). Retrieved September 24, 2022, from https://pubmed.ncbi.nlm.nih.gov/30997357/

Vaccine Hesitancy: Causes, consequences, and a call to action - PubMed. (n.d.-b). Retrieved September 24, 2022, from https://pubmed.ncbi.nlm.nih.gov/26337116/

Visalli, G., Currò, M., Facciolà, A., Riso, R., Mondello, P., Laganà, P., Di Pietro, A., Picerno, I., & Spataro, P. (2016a). Prevalence of human papillomavirus in saliva of women with HPV genital lesions. *Infectious Agents and Cancer, 11*(1), 1–6. https://doi.org/10.1186/S13027-016-0096-3/FIGURES/2

Visalli, G., Riso, R., Facciolà, A., Mondello, P., Caruso, C., Picerno, I., Di Pietro, A., Spataro, P., & Bertuccio, M. P. (2016b). Higher levels of oxidative DNA damage in cervical cells are correlated with the grade of dysplasia and HPV infection. *Journal of Medical Virology, 88*(2), 336–344. https://doi.org/10.1002/JMV.24327

Visalli, G., Currò, M., Iannazzo, D., Pistone, A., Pruiti Ciarello, M., Acri, G., Testagrossa, B., Bertuccio, M. P., Squeri, R., & Di Pietro, A. (2017a). In vitro assessment of neurotoxicity and neuroinflammation of homemade MWCNTs. *Environmental Toxicology and Pharmacology, 56*, 121–128. https://doi.org/10.1016/J.ETAP.2017.09.005

Visalli, G., Facciolà, A., Iannazzo, D., Piperno, A., Pistone, A., & Di Pietro, A. (2017b). The role of the iron catalyst in the toxicity of multi-walled carbon nanotubes (MWCNTs). *Journal of Trace Elements in Medicine and Biology: Organ of the Society for Minerals and Trace Elements (GMS), 43*, 153–160. https://doi.org/10.1016/J.JTEMB.2017.01.005

Wahome, N., Pfeiffer, T., Ambiel, I., Yang, Y., Keppler, O. T., Bosch, V., & Burkhard, P. (2012). Conformation-specific display of 4E10 and 2F5 epitopes on self-assembling protein nanoparticles as a potential HIV vaccine. *Chemical Biology & Drug Design, 80*(3), 349–357. https://doi.org/10.1111/J.1747-0285.2012.01423.X

Watson, D. S., Endsley, A. N., & Huang, L. (2012). Design considerations for liposomal vaccines: Influence of formulation parameters on antibody and cell-mediated immune responses to liposome associated antigens. In *Vaccine* (Vol. 30, Issue 13, pp. 2256–2272). Vaccine. https://doi.org/10.1016/j.vaccine.2012.01.070

Wei, M., Zhang, X., Yu, H., Tang, Z. M., Wang, K., Li, Z., Zheng, Z., Li, S., Zhang, J., Xia, N., & Zhao, Q. (2014). Bacteria expressed hepatitis E virus capsid proteins maintain virion-like epitopes. *Vaccine, 32*(24), 2859–2865. https://doi.org/10.1016/j.vaccine.2014.02.025

Wen, Y., & Collier, J. H. (2015). Supramolecular peptide vaccines: Tuning adaptive immunity. *Current Opinion in Immunology, 35*, 73–79. https://doi.org/10.1016/J.COI.2015.06.007

Wen, Y., & Meng, W. S. (2014). Recent in vivo evidences of particle-based delivery of small-interfering RNA (siRNA) into solid tumors. *Journal of Pharmaceutical Innovation, 9*(2), 158–173. https://doi.org/10.1007/S12247-014-9183-4

Yang, Y., Dai, M., Wilson, T. M., Omelchenko, I., Klimek, J. E., Wilmarth, P. A., David, L. L., Nuttall, A. L., Gillespie, P. G., & Shi, X. (2011). Na+/K+-ATPase α1 identified as an abundant protein in the blood-labyrinth barrier that plays an essential role in the barrier integrity. *PLoS One, 6*(1). https://doi.org/10.1371/JOURNAL.PONE.0016547

Yang, C., Yu, W., Bi, Y., Long, F., Li, Y., Wei, D., Hao, X., Situ, J., Zhao, Y., & Huang, F. (2018). Increased oestradiol in hepatitis E virus-infected pregnant women promotes viral replication. *Journal of Viral Hepatitis, 25*(6), 742–751. https://doi.org/10.1111/JVH.12865

Ye, M., Tang, L., Luo, M., Zhou, J., Guo, B., Liu, Y., & Chen, B. (2014). Size- and time-dependent alteration in metabolic activities of human hepatic cytochrome P450 isozymes by gold nanoparticles via microsomal coincubations. *Nanoscale Research Letters, 9*(1). https://doi.org/10.1186/1556-276X-9-642

Yu, C., Qian, L., Uttamchandani, M., Li, L., & Yao, S. Q. (2015). Single-vehicular delivery of antagomir and small molecules to inhibit miR-122 function in hepatocellular carcinoma cells by using "smart" mesoporous silica nanoparticles. *Angewandte Chemie (International Ed. in English), 54*(36), 10574–10578. https://doi.org/10.1002/ANIE.201504913

Yu, Z., Cai, Z., Chen, Q., Liu, M., Ye, L., Ren, J., Liao, W., & Liu, S. (2016a). Engineering β-sheet peptide assemblies for biomedical applications. *Biomaterials Science, 4*(3), 365–374. https://doi.org/10.1039/C5BM00472A

Yu, Z., Yan, B., Gao, L., Dong, C., Zhong, J., D'Ortenzio, M., Nguyen, B., Seong Lee, S., Hu, X., & Liang, F. (2016b). Targeted delivery of bleomycin: A comprehensive anticancer review. *Current Cancer Drug Targets, 16*(6), 509–521. https://doi.org/10.2174/1568009616666151130213910

Yu, H. H., Deng, Q. P., Zheng, Q. H., Wang, Y., Shen, J., & Zhou, J. H. (2022). Hypericin nanoparticles for self-illuminated photodynamic cytotoxicity based on bioluminescence resonance energy transfer. *International Journal of Pharmaceutics, 620*, 121738. https://doi.org/10.1016/j.ijpharm.2022.121738

Zeltins, A. (2013). Construction and characterization of virus-like particles: A review. *Molecular Biotechnology, 53*(1), 92–107. https://doi.org/10.1007/S12033-012-9598-4

Zhai, W., He, C., Wu, L., Zhou, Y., Chen, H., Chang, J., & Zhang, H. (2012). Degradation of hollow mesoporous silica nanoparticles in human umbilical vein endothelial cells. *Journal of Biomedical Materials Research - Part B Applied Biomaterials, 100 B*(5), 1397–1403. https://doi.org/10.1002/jbm.b.32711

Zhang, L. F., Zhou, J., Chen, S., Cai, L. L., Bao, Q. Y., Zheng, F. Y., Lu, J. Q., Padmanabha, J., Hengst, K., Malcolm, K., & Frazer, I. H. (2000). HPV6b virus like particles are potent immunogens without adjuvant in man. *Vaccine, 18*(11–12), 1051–1058. https://doi.org/10.1016/S0264-410X(99)00351-5

Zhang, Q., Wang, W., Schmelzer, E., Gerlach, J., Liu, C., & Nettleship, I. (2021). The degradation behavior of calcium-rich hydroxyapatite foams in vitro. *Journal of Biomedical Materials Research - Part A, 109*(6), 859–868. https://doi.org/10.1002/jbm.a.37077

Zhang, Q., Hong, S., Dong, X., Zheng, D., Biomaterials, J. L.-, & 2022, undefined. (n.d.-a). Bioinspired nano-vaccine construction by antigen pre-degradation for boosting cancer personalized immunotherapy. *Elsevier*. Retrieved September 24, 2022, from https://www.sciencedirect.com/science/article/pii/S014296122200268X

Zhang, Q., Hong, S., Dong, X., Zheng, D., Biomaterials, J. L.-, 2022, undefined, Kalkanidis, M., Pietersz, G., Xiang, S., Methods, P. M.-, & 2006, undefined. (n.d.-b). Bioinspired nano-vaccine construction by antigen pre-degradation for boosting cancer personalized immunotherapy. *Elsevier*. Retrieved September 24, 2022, from https://www.sciencedirect.com/science/article/pii/S1046202306001587

Zhao, Q., Li, S., Yu, H., Xia, N., & Modis, Y. (2013). Virus-like particle-based human vaccines: Quality assessment based on structural and functional properties. *Trends in Biotechnology, 31*(11), 654–663. https://doi.org/10.1016/J.TIBTECH.2013.09.002

Zhu, F. C., Zhang, J., Zhang, X. F., Zhou, C., Wang, Z. Z., Huang, S. J., Wang, H., Yang, C. L., Jiang, H. M., Cai, J. P., Wang, Y. J., Ai, X., Hu, Y. M., Tang, Q., Yao, X., Yan, Q., Xian, Y. L., Wu, T., Li, Y. M., et al. (2010). Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: A large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet (London, England), 376*(9744), 895–902. https://doi.org/10.1016/S0140-6736(10)61030-6

Zvirbliene, A., Samonskyte, L., Gedvilaite, A., Voronkova, T., Ulrich, R., & Sasnauskas, K. (2006). Generation of monoclonal antibodies of desired specificity using chimeric polyomavirus-derived virus-like particles. *Journal of Immunological Methods, 311*(1–2), 57–70. https://doi.org/10.1016/j.jim.2006.01.007

Chapter 18
Dosing Strategies of Nanovaccines

Hardeep Kaur, Virender Kumar, Rakesh Kumar Marwaha, Gajendra Singh, and Davinder Kumar

18.1 Introduction

Vaccines are a complex mixture of biological substances. Common antigenic components of vaccines include proteins derived from viruses, bacteria and others: live attenuated viruses; killed or inactivated viruses; bacterial toxins such as components of tetanus toxin (TT) and diphtheria toxoid (DT); toxins produced by bacteria such as pertussis component; genes encoding viruses known as deoxyribonucleic acid (DNA) (viruses); yeast cells; and other microorganisms that produce toxins that can cause diseases (Vaccine Types | NIH, 2022; Wilson-Welder et al., 2009). Antigenic components of vaccines can contain all or part of any pathogen, but most vaccines have common vaccination strategy components; for example, they are often made from a combination of multiple antigens into one vaccine. Vaccines can protect against diseases (Pollard & Bijker, 2020). They do this by exposing the body to a portion of a virus or bacterium to encourage the immune system to make antibodies and fight off illness. Vaccines may also contain other components such as inactive ingredients, preservatives and stabilizers (Types of vaccines, 2022) (Fig. 18.1).

Biological molecules for treating cancer, inflammatory and infectious diseases and autoimmune diseases are generally derived from living organisms or produced by recombinant DNA technologies. Several types of molecules can be classified as

H. Kaur
Department of Biotechnology, University Institute of Biotechnology, Chandigarh University, Mohali, India

V. Kumar · G. Singh · D. Kumar (✉)
College of Pharmacy, PGIMS, University of Health Sciences, Rohtak, Haryana, India

R. K. Marwaha
Department of Pharmaceutical Sciences, Maharishi Dayanand University, Rohtak, Haryana, India

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_18

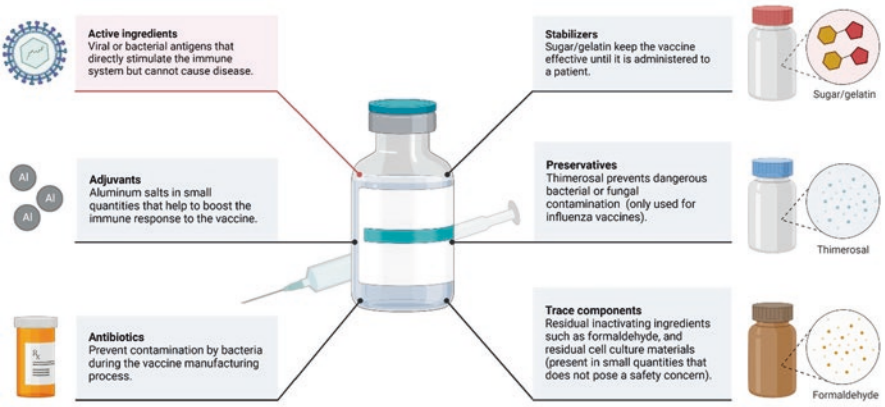


Fig. 18.1 Various components of the vaccine

biopharmaceuticals, along with growth hormones and purified or recombinant proteins (Dimitrov, 2012). In 2020–2022, these biomolecules gained much attention because of their attractive characteristics, specificity and potent therapeutic effects, resulting in high clinical success rates for approved products and increased biomolecule development (Gupta et al., 2017). In addition, the pharmaceutical industry generates billions of dollars from expanding marketed biomolecules. Many changes have been made to ensure compliance with regulatory requirements while optimizing and securing the high-scale production of biomolecules. Today, virtually all preparations in the biopharmaceuticals field are administered via parenteral routes, making formulation a major challenge for scientists (Taylor, 2015). Many studies focused their research on developing technologies and formulation strategies to deliver these molecules by alternative routes of administration with special attention to the oral route, always keeping the main formulation objective, that is, to ensure stability while formulating, during storage and till the administration of the patient.

In addition to the complicated structure and fragility of biomolecules, oral administration of these molecules also results in low bioavailability. Several literature reviews have already demonstrated the oral bioavailability of therapeutic proteins. While conventional vaccines cover the whole body, nanovaccines could target an area within the body where a disease or an infection originated. They stabilize various therapeutic agents, including peptides, proteins and nucleic acids, reducing vaccine doses and preserving antigen integrity. It is sometimes possible to correct hydrophobic compounds' solubility in a solution using nanoparticle systems so that they are suitable for parenteral administration through nanoparticle systems.

In addition, particle systems may have several advantages in mucosal immunity, like antigen protection against gastric and intestinal degradation (enzymes and acids) (Rodger & King, 2000; Homayun et al., 2019; Moeller & Jorgensen, 2008). It may also regulate the types of immunity induced by antigens and act as a reservoir for their controlled release. There is a possibility of a depot effect with these particles. They prevent the vaccine from spreading the antigen to the surface of the

injection site and release it gradually, allowing the vaccine to reach the immune cells for a longer period. Nanoparticles (NPs) can enter cells via endocytosis since their size is similar to cellular components.

Vaccines made with nanoparticles cross-present antigens through class I of the major histocompatibility complex and stimulate both humoral and cellular immune systems. In addition, antigen-specific antibodies can be used to cover nanovaccine particles to deliver targeted vaccines. Studies have shown that macrophages and dendritic cells can readily uptake cationic nanoparticles due to their positive charges, which are opposite to those on the membrane of dendritic cells to their high efficiency; they outperform conventional vaccines (Sarkar et al., 2019). There is no need for peripheral dendritic cells to move NPs towards lymph nodes. A nanovaccine also has the advantage of being sprayed in the nose, making it more convenient. With NPs, vaccines can reach cells more quickly and sometimes reach cells 30 times faster than with the vaccines alone. Studies have shown that nanoparticles are more effective in absorbing nutrients than microparticles (Turnis & Rooney, 2010).

18.2 Vaccine Adjuvants

The adjuvant concept is more than 80 years old, with the first adjuvant present in human vaccines, an aluminium salt (aluminium potassium sulphate, also known as alum). A new vaccine technology has spawned whether adjuvants need to be included in a new vaccine. Adjuvants are substances that can enhance and modulate the immunogenicity of the vaccine antigen, but they do not contain antigenic material. They may be coupled with the antigen (inactivated adjuvants) or antigenic components such as proteins and salts (Bonanni & Santos, 2011; Strugnell et al., 2011; di Pasquale et al., 2015; Zepp, 2010).

The recent shortage of novel influenza A (H1N1) pdm09 vaccines following the US Food and Drug Administration (FDA)'s decision to exclude subunit and inactivated poliovirus vaccines as candidates for pandemic response vaccines (PRVs) caused considerable concern among public health officials and stakeholders (Hawken & Troy, 2012). Adjuvants can be inactivated forms of older vaccines, such as alum, and newer substances, such as oils or squalene, called organic adjuvants. Different types of adjuvants and their uses in different vaccines are shown in Fig. 18.2**.**

18.2.1 Challenges of Oral Administration of Vaccines

Vaccines are one of the best ways to prevent disease, but they often fail. Nanotechnology is the most promising way to solve this problem because it allows us to create effective vaccines that can be given in a few doses and used for many years. Conventional vaccines are made by killing many cells and propagating them

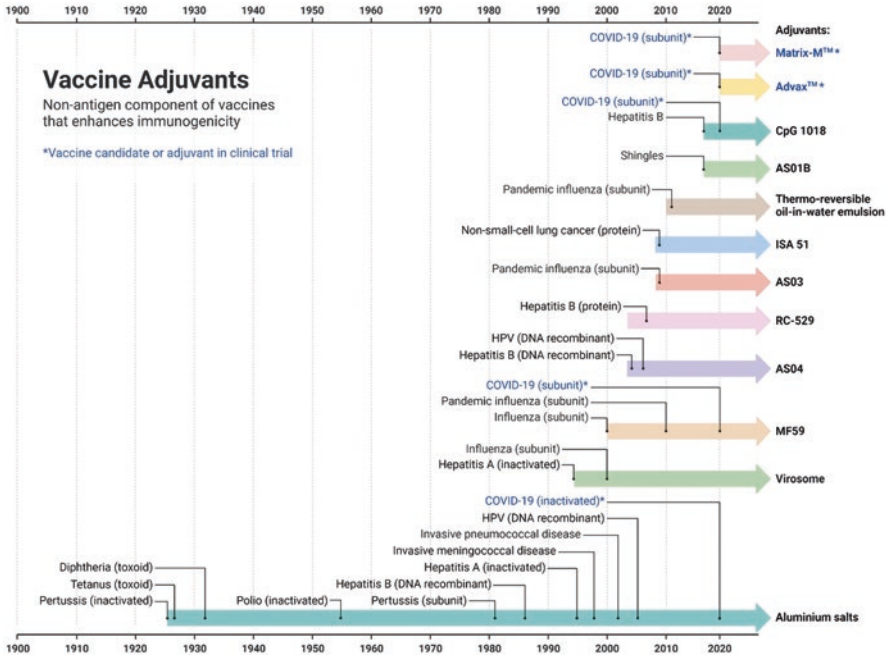


Fig. 18.2 Timeline of adjuvants used in human vaccines

in culture to generate antigens. Vaccine preparation depends on the host immune response to provoke an immune reaction, but not all strains induce antitumor and antibody responses equivalent or superior to the vaccines developed against wild-type viruses and bacteria. With the number of vaccines that need to be delivered at home, the need for setting up facilities with advanced technological infrastructure has been increasing (Lee, 2016).

Oral vaccines are the most convenient and available route of administration. The oral route is effective for delivering vaccines, which are normally biologically active substances produced locally in the mouth. Because of their convenience and practicality, they have become widely used in preventing infectious diseases. Oral vaccination strategies aim to prime the immune system by delivering subunit vaccines through many oral delivery systems. These vaccines can be divided into two broad categories: subunit vaccines and conjugate vaccines. Subunit oral vaccine formulations offer advantages over conventional parenteral routes in terms of safety and efficacy, ease of administration and convenience to patients. The most common products in this category are killed-virus-based vaccine formulations that include Ebola, Zika and MERS-CoV (Xie et al., 2020; Jhaveri & Torchilin, 2014; Hua, 2020; Alqahtani et al., 2021). Multiple routes can deliver oral vaccines against hepatitis C virus (HCV), but only a few of these are suitable for routine clinical use. The most popular route for delivering particles for proteinase K (PK)-resistant vaccine delivery is the oral route, although it has several drawbacks, including high pH, low

biological activity and dehydrating effects on both molecules and cells (Halliday et al., 2011). Among other strategies, it is necessary to consider the delivery system choice and its characteristics, including size, geometry, antigen loading and release kinetics capabilities and the ability to include functional molecules to improve their performance. However, there are several challenges to the oral administration of vaccines, such as transit time, safety and high cost. The primary challenge associated with administering vaccines is a lack of stability, making it difficult to administer to varying populations and high cost. Some controllable properties include size, geometry, antigen loading and release kinetic capabilities and finally the ability to include functional molecules to improve their performance (Vinarov et al., 2021). Tailoring these characteristics will prolong the residence time of immunogens, enable codelivery with antigens and adjuvants, boost their immunogenicity and target immune cells (specifically antigen-presenting cells [APCs]) for efficient transport, uptake and presentation. Passive vaccine delivery systems were developed to address these challenges. Microparticles and edible beads are used in passive vaccines (Coffman et al., 2010).

18.3 Nanotechnology and Nanovaccines

Nanotechnology has become a powerful tool in many fields of science since the discovery of electronics by Gabor in 1947. Nanoparticles carry many beneficial properties such as surface area, self-assembly and biointeractions. Nanotechnology compromises a material's size, shape and function using components with dimensions less than 100 nm in length, 50 nm in width and 1000 nm in thickness (Bayda et al., 2020). Nanoscale materials have many potential applications across industry and science, including within pharmaceuticals, cosmetics and fragrances. Nanotechnology allows scientists to create the world's smallest particles and make them last longer than normal. It has been used in vaccines and disease control, but some believe that this technology could produce drugs to fight cancer and other diseases (Boverhof et al., 2015; Jeevanandam et al., 2018).

Nanovaccines are being used in ways that could make an enormous difference to the health-care industry. Nanoscience and nanotechnology represent a revolutionary new field of medicine, and they have the potential to trigger a powerful immune response. Nanovaccines can combat diseases such as cancer and provide unique opportunities for treatment (Chauhan et al., 2020; Al-Halifa et al., 2019). For developing potential nanovaccines, researchers are looking at two different designs: the first technology is to use nanoparticles to carry an antigen (a particle attached to a protein), allowing it to be delivered through the bloodstream to body tissue at a higher rate than in conventional vaccines, and the second design is to use nanoparticles with attached antigens and deliver this directly through the bloodstream, bypassing any immune response. Nanoparticle-based vaccines have the potential to reduce lymphatic filtration, cause less tissue irritation and provide superior immunological memory than conventional vaccines (Reichmuth et al., 2016). Vaccine

delivery has historically been limited by a lack of a suitable vehicle for delivering nucleocapsid (NCt) nanoparticles.

Similarly, the natural lipids of the oleaginous mosquito egg yolk, lipid IVA and mixed esters of polyunsaturated fatty acids (MePS) are suitable vehicles for delivering NCT nanoparticles because they mimic the pH required for nanoparticle release and enhance corneal transfection due to their oil-like form and stability of pH. Delivering authentic NCT nanoparticles using an oleaginous vector demonstrated that nanoparticle-based vaccines are feasible and safe for humans. Future research will focus on identifying which nanoparticles are optimally suited for improving immune responses and generating antibodies (Mohan et al., 2013).

18.3.1 Nanovaccines and Their Applications

Tons of different formulations of nanovaccines have been developed, and synthetic nanoparticles stand out the most. The advantage of these nanoparticles is their ability to do more work than previous forms of vaccines (Pati et al., 2018), including (Fig. 18.3) the following:

1. They can deliver a significant number of molecules that can be targeted for recognition by the immune system.
2. They minimize undesired effects or side effects due to administration.

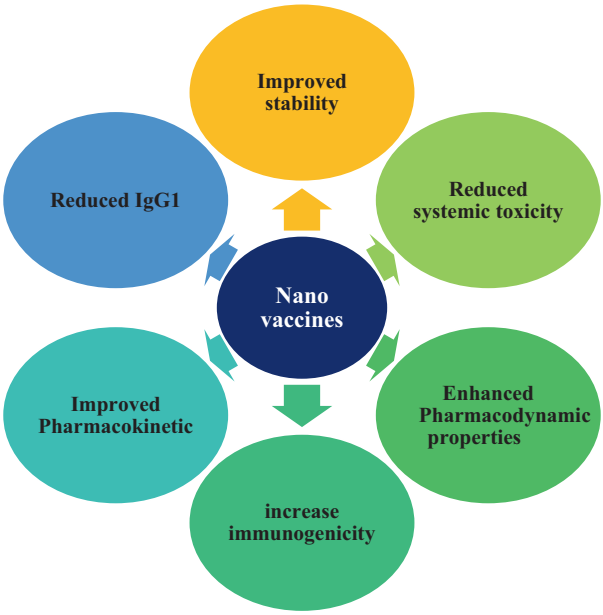


Fig. 18.3 Applications of nanovaccines

3. They have high selectivity and delivery efficiency as compared to traditional vaccines.
4. They deliver even greater numbers of molecules but are very small enough that they cannot elicit an immune response, and yet, they are still able to stimulate regulatory T cells (Tregs), which leads to better protection against infections.
5. Not only do these antibodies enhance and boost antibody production, but they also stop the overproduction of cytokines and cells in response to a viral infection, which results in faster recovery.

Nanoparticle vaccines are the latest evolution of vaccine delivery, opening up exciting new possibilities for the future of immunization. Nanoparticles are engineered to improve immunogenicity and decrease degradation by improving cross-linking, stabilizing antigen release, or adding an adjuvant effect.

The process of developing nanoparticle vaccines is similar to the method used for developing traditional vaccines, but the characteristics and design of these particles have significantly evolved. Nanoparticles deliver targeted genes to cancer patients through innovative and cheap drug delivery platforms (Diaz-Arévalo & Zeng, 2020). Nanoparticles are produced by chemically cross-linking protein antigens and carrier molecules to increase immunogenicity and decrease the degradation of the antigens. The properties of this vaccine include improved stability, reduced systemic toxicity, enhanced immune responses through IMMUNIN structural reversion (isolation of a single viral spike), reduced immunoglobulin G1 (IgG1) antibodies towards the carrier when exposed to an antigen and the ability to adsorb more antigens at the surface of nanoparticles than those in bulk (Kim et al., 2019; Yun & Cho, 2020).

18.3.2 Highlights of Polymeric/Particulate Vaccines

1. Polymeric/particulate vaccines are pharmaceutical products made from a non-protein subunit of the virus or bacterium and associated adjuvants. These can be either life, killed or inactive.
2. A polymeric/particulate vaccine contains both an antigen, a vehicle in which it is delivered to the immune system and a medium for delivering it. A polymer/particulate vaccine is made of a solid or liquid in the form of a micelle or hollow sphere, and then, the particles are released into the patient's body.
3. Particles can be any aspect of the formulation that makes up the vaccine, from encapsulated antigens to adjuvants like aluminium phosphate.
4. Polymeric vaccines contain antigenic proteins. The surface of the particulate carrier can be chemically modified to increase its immunogenicity.
5. Particulate vaccines are made from petroleum and particulate glass microfibres or synthetic polymer/tissue particles such as polylactide, poloxamer and povidone-K. Particulate vaccines have been developed to provide increased local immunity due to the long duration of immunity induced by the antibodies that bind to the antigen.

18.3.3 Single-Shot Vaccines

Single-shot vaccines result from engineering to catch diseases early and quickly. The vaccine takes on the illness and neutralizes it immediately without remembering to take another dose later. Single-shot vaccines have been live attenuated vaccines, killed vaccines and recombinant vaccines. The most common single-shot vaccine is H5N1 pandemic vaccine. The vaccine uses the knowledge that single-dose vaccines protect large mammals such as humans against infectious diseases caused by viruses. Single-shot vaccines, unlike multidose schedules, can be administered by a simple intramuscular injection. A novel Vernier pipette that allows accurate dispensing of precise volumes of liquids and lyophilization is a technology used to produce single-shot vaccines (Khademi et al., 2018). The production process of lyophilized vaccines, including developing a novel Vernier pipette, allows the accurate dispensing of the precise volume of liquids. The functional utility of lyophilized vaccines is often limited by low stability and the need to store them in a refrigerated environment. It utilizes the single-shot concept to produce the lyophilized vaccine product efficiently and quickly. Rather than relying on manual pouring techniques that are inefficient, time-consuming and prone to errors, this method relies on a novel Vernier pipette that allows precise dispensing (Bora et al., 2020).

18.4 Calculating Annual Vaccine Needs from the Size of the Target Population

The National Immunization Survey found that the population size of people who are 19 to 35 years old and have visited the doctor in the last year is 82 million. There are 86.6 million people aged 19–35 years who have seen a doctor in the past 12 months, and there are 92.5 million in this age group who have not been vaccinated against tetanus, diphtheria or pertussis (the three leading causes of a childhood illness) (Immunization Module, 2022) (Fig. 18.4).

The number of vaccines needed to achieve population coverage depends on the following two factors:

- The vaccine's age-specific target population.
- The total number of people required for a given population.

For example, if a vaccine is targeted for new-born immunization, all persons born in a given year must receive it during their first 18 months of life. Similarly, adults who have never received any vaccines will require a booster if they were born before 1980 and did not receive menses-based tetanus toxoid (Tdap) vaccination.

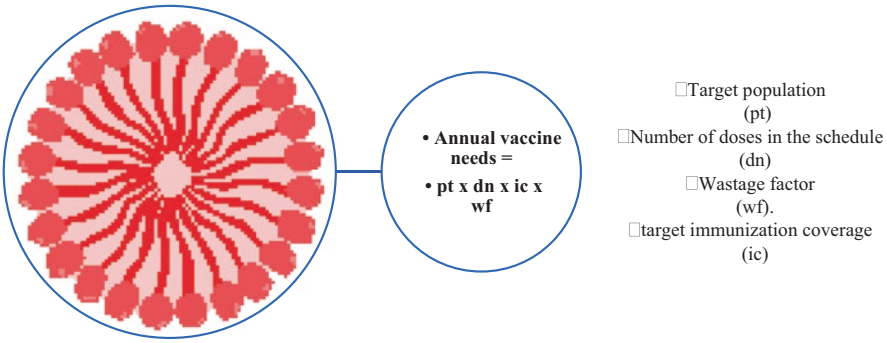


Fig. 18.4 Vaccine volume calculations

18.4.1 Determination of the Number of Doses for Nanovaccines

The number of doses included in a bank of vaccines can be determined by calculating the average number of doses contained in a set of medical supplies (Vu et al., 2021). The average number of doses supplied can be calculated using a simple procedure based on the following assumptions:

I. The ability of an individual to administer a certain dose is proportional to their weight or size.
II. No more than one dose may be available at any given time (either because there is only one unused dose or because it has been used).
III. It takes no more than 30 seconds per dose administered (this may be increased if the same needle is used repeatedly).
IV. Each person receives only one dose per month unless complications occur or there is some other reason for repetition.

Example To estimate the number of doses to include in a bank of vaccines, we need to know how many doses have been ordered and what their average price is. Consider a hypothetical vaccine with a list price of US$5 per dose. The manufacturer may estimate that 60% of children who receive the vaccine will be protected against disease, while 20% will not be protected. How much doses required will have to determine in a bank, multiply 60% by US$5/dose, plus 20% by US$5/dose, times 30%, which equals 13 times 20% equals 45 units × 3 units minus 18 units × 5 units equals 1.

18.5 Dosing Strategies and Their Importance

Nanoparticle-based vaccines represent one of the most important technologies in the emerging field of biodefense and personalized medicine. By using nanoparticles that are small enough to cross the blood–brain barrier, yet large enough to interact with the immune system, it may be possible to develop therapies against antigens that are able to elicit protective immunity without producing inflammation or neutralizing antibodies. The concept of dosing strategies for nanovaccines focuses on scientific and technological solutions for the preparation, size modification and future applications of nanovaccines. This is an important area as more focus is being directed towards nanoweapons by threat actors with increasing financial capabilities, speed and ease within which to synthesize compounds, manufacture them into weapons and dispense them. Nano criteria for dosing strategies of nanovaccines are more straightforward to enter into a computer than clinical doses. Safety concerns can be pursued with the traditional methodologies, but quantification of plasma levels comes at a high cost in terms of time and patient data collection (Zhou et al., 2020). The feasibility of delivering DNA-based vaccines within polymer nanoparticles has been demonstrated in rabbits, and information is available on the use of lipid nanoparticle–DNA conjugates as targeted delivery vehicles in mice. The ability to formulate a novel vaccine (containing an encapsulated antigen) that remains stable at high temperatures and pH has been developed (Semple et al., 2022). The nano criteria offer a powerful tool for improving vaccine delivery rapidly, specifically in situations where the scale, size or complexity of a trial is increased from clinical trials to large-scale trials on large populations. Nanocritic dose scaling reduces the costs associated with obtaining required information in the dosing regimen while improving safety standards and increasing efficiency. Smaller vials may allow for easier access to vaccine doses and more rapid delivery systems. Nanoparticle vaccines are also compatible with the current "cold chain" method of shipping vaccines, which involves refrigeration and holding at a temperature just above freezing (Semple et al., 2022; Liu et al., 2021).

References

Al-Halifa, S., Gauthier, L., Arpin, D., Bourgault, S., & Archambault, D. (2019). Nanoparticle-based vaccines against respiratory viruses. *Frontiers in Immunology, 10*(Jan), 22.

Alqahtani, M. S., Kazi, M., Alsenaidy, M. A., & Ahmad, M. Z. (2021). Advances in oral drug delivery. *Frontiers in Pharmacology, 12*, 62.

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., & Rizzolio, F. (2020). The history of nanoscience and nanotechnology: From chemical–physical applications to nanomedicine. *Molecules [Internet], 25*(1). Available from: /pmc/articles/PMC6982820/. [cited 2022 Sept 27].

Bonanni, P., & Santos, J. I. (2011). Vaccine evolution. *Perspectives in Vaccinology, 1*(1), 1–24.

Bora, M., Patel, C. L., Rajak, K. K., Verma, M. R., Yousuf, R. W., & Singh, R. P. (2020). Development of a process for upscaling and production of thermotolerant Peste-des-petits ruminants vaccine. *Virus [Internet], 31*(3), 357. Available from: /pmc/articles/PMC7459036/. [cited 2022 Sept 27].

Boverhof, D. R., Bramante, C. M., Butala, J. H., Clancy, S. F., Lafranconi, W. M., West, J., et al. (2015). Comparative assessment of nanomaterial definitions and safety evaluation considerations. *Regulatory Toxicology and Pharmacology, 73*(1), 137–150.

Chauhan, G., Madou, M. J., Kalra, S., Chopra, V., Ghosh, D., & Martinez-Chapa, S. O. (2020). Nanotechnology for COVID-19: Therapeutics and vaccine research. *ACS Nano, 14*(7), 7760–7782.

Coffman, R. L., Sher, A., & Seder, R. A. (2010). Vaccine adjuvants: Putting innate immunity to work. *Immunity [Internet], 33*(4), 492. Available from: /pmc/articles/PMC3420356/. [cited 2022 Sept 27].

di Pasquale, A., Preiss, S., da Silva, F. T., & Garçon, N. (2015). Vaccine adjuvants: From 1920 to 2015 and beyond. *Vaccines (Basel) [Internet], 3*(2), 320. Available from: /pmc/articles/PMC4494348/. [cited 2022 Sept 26].

Diaz-Arévalo, D., & Zeng, M. (2020). Nanoparticle-based vaccines: opportunities and limitations. *Nanopharmaceuticals [Internet]*, 135. Available from: /pmc/articles/PMC7153331/. [cited 2022 Sept 27].

Dimitrov, D. S. (2012). Therapeutic proteins. *Methods in Molecular Biology [Internet], 899*, 1. Available from: /pmc/articles/PMC6988726/. [cited 2022 Sept 26].

Gupta, V., Sengupta, M., Prakash, J., & Tripathy, B. C. (2017). Production of recombinant pharmaceutical proteins. *Basic and Applied Aspects of Biotechnology [Internet], 77*. Available from: /pmc/articles/PMC7120688/. [cited 2022 Sept 26].

Halliday, J., Klenerman, P., & Barnes, E. (2011). Vaccination for hepatitis C virus: closing in on an evasive target. *Expert Review of Vaccines [Internet], 10*(5), 659. Available from: /pmc/articles/PMC3112461/. [cited 2022 Sept 26].

Hawken J, Troy SB. Adjuvants and inactivated polio vaccine: A systematic review. Vaccine [Internet]. 2012 ;30(49):6971. Available from: /pmc/articles/PMC3529007/. [cited 2022 Sept 26].

Homayun, B., Lin, X., & Choi, H. J. (2019). Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. *Pharmaceutics [Internet], 11*(3) Available from: /pmc/articles/PMC6471246/. [cited 2022 Sept 26].

Hua, S. (2020). Advances in oral drug delivery for regional targeting in the gastrointestinal tract - influence of physiological, pathophysiological and pharmaceutical factors. *Frontiers in Pharmacology, 11*, 524.

Immunization Module: Vaccine supply and stock management: View as a single page [Internet]. [cited 2022 Sept 27]. Available from: https://www.open.edu/openlearncreate/mod/oucontent/view.php?id=53353&printable=1

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology [Internet], 9*(1), 1050. Available from: /pmc/articles/PMC5905289/. [cited 2022 Sept 27].

Jhaveri, A. M., & Torchilin, V. P. (2014). Multifunctional polymeric micelles for delivery of drugs and siRNA. *Frontiers in Pharmacology, 5*, 77.

Khademi, F., Derakhshan, M., Yousefi-Avarvand, A., & Tafaghodi, M. (2018). Potential of polymeric particles as future vaccine delivery systems/adjuvants for parenteral and non-parenteral immunization against tuberculosis: A systematic review. *Iranian Journal of Basic Medical Sciences [Internet], 21*(2), 116. Available from: /pmc/articles/PMC5811749/. [cited 2022 Sept 27].

Kim, C. G., Kye, Y. C., & Yun, C. H. (2019). The role of nano vaccine in cross-presentation of antigen-presenting cells for the activation of CD8+ T cell responses. *Pharmaceutics, 11*(11), 612.

Lee Ventola, C. (2016). Immunization in the United States: Recommendations, barriers, and measures to improve compliance: part 1: Childhood vaccinations. *Pharmacy and Therapeutics [Internet], 41*(7), 426. Available from: /pmc/articles/PMC4927017/. [cited 2022 Sept 26].

Liu, M., Li, Q., Lin, J., Lin, Y., & Hoffman, E. (2021). Innovative trial designs and analyses for vaccine clinical development. *Contemporary Clinical Trials [Internet], 100*, 106225. Available from: /pmc/articles/PMC7834363/. [cited 2022 Sept 27].

Moeller, E. H., & Jorgensen, L. (2008). Alternative routes of administration for systemic delivery of protein pharmaceuticals. *Drug Discovery Today: Technologies, 5*(2–3), e89.

Mohan, T., Verma, P., & Nageswara, R. D. (2013). Novel adjuvants & delivery vehicles for vaccines development: A road ahead. *The Indian Journal of Medical Research [Internet], 138*(5), 779. Available from: /pmc/articles/PMC3928709/. [cited 2022 Sept 27].

Pati, R., Shevtsov, M., & Sonawane, A. (2018). Nanoparticle vaccines against infectious diseases. *Frontiers in Immunology, 9*(Oct), 2224.

Pollard, A. J., & Bijker, E. M. (2020). A guide to vaccinology: From basic principles to new developments. *Nature Reviews Immunology, 21*(2), 83–100. Available from: https://www.nature.com/articles/s41577-020-00479-7. 21:2 [Internet]. 2020 Dec 22 [cited 2022 Sept 26].

Reichmuth, A. M., Oberli, M. A., Jeklenec, A., Langer, R., & Blankschtein, D. (2016). mRNA vaccine delivery using lipid nanoparticles. *Therapeutic Delivery [Internet], 7*(5), 319. Available from: /pmc/articles/PMC5439223/. [cited 2022 Sept 27].

Rodger, M. A., & King, L. (2000). Drawing up and administering intramuscular injections: A review of the literature. *Journal of Advanced Nursing, 31*(3), 574–582.

Sarkar, I., Garg, R., & van Drunen Littel-van den Hurk, S. (2019). Selection of adjuvants for vaccines targeting specific pathogens. *Expert Review of Vaccines [Internet], 18*(5), 505. Available from: /pmc/articles/PMC7103699/. [cited 2022 Sept 26].

Semple, S. C., Leone, R., Barbosa, C. J., Tam, Y. K., & Lin, P. J. C. (2022). Lipid nanoparticle delivery systems to enable mRNA-based therapeutics. *Pharmaceutics [Internet], 14*(2). Available from: /pmc/articles/PMC8876479/. [cited 2022 Sept 27].

Strugnell, R., Zepp, F., Cunningham, A., & Tantawichien, T. (2011). Vaccine antigens. *Perspectives in Vaccinology, 1*(1), 61–88.

Taylor, D. (2015). The pharmaceutical industry and the future of drug development. *Issues In Environmental Science and Technology [Internet], 2016-January*(41), 1–33. Available from: https://pubs.rsc.org/en/content/chapterhtml/2015/bk9781782622345-00001. [cited 2022 Sept 26].

Turnis, M. E., & Rooney, C. M. (2010). Enhancement of dendritic cells as vaccines for cancer. *Immunotherapy [Internet], 2*(6), 847. Available from: /pmc/articles/PMC3433954/. [cited 2022 Sept 26].

Types of vaccines | Immunisation Advisory Centre [Internet]. [cited 2022 Sept 26]. Available from: https://www.immune.org.nz/vaccines/vaccine-development/types-vaccines

Vaccine Types | NIH: National Institute of Allergy and Infectious Diseases [Internet]. [cited 2022 Sept 26]. Available from: https://www.niaid.nih.gov/research/vaccine-types

Vinarov, Z., Abrahamsson, B., Artursson, P., Batchelor, H., Berben, P., Bernkop-Schnürch, A., et al. (2021). Current challenges and future perspectives in oral absorption research: An opinion of the UNGAP network. *Advanced Drug Delivery Reviews, 171*, 289–331.

Vu, M. N., Kelly, H. G., Kent, S. J., & Wheatley, A. K. (2021). Current and future nanoparticle vaccines for COVID-19. *EBioMedicine [Internet], 74*, 103699. Available from: http://www.thelancet.com/article/S235239642100493X/fulltext. [cited 2022 Sept 27].

Wilson-Welder, J. H., Torres, M. P., Kipper, M. J., Mallapragada, S. K., Wannemuehler, M. J., & Narasimhan, B. (2009). Vaccine adjuvants: Current challenges and future approaches. *Journal of Pharmaceutical Sciences [Internet], 98*(4), 1278. Available from: /PMC/articles/PMC8092333/. [cited 2022 Sept 26].

Xie, J., Bi, Y., Zhang, H., Dong, S., Teng, L., Lee, R. J., et al. (2020). Cell-penetrating peptides in diagnosis and treatment of human diseases: From preclinical research to clinical application. *Frontiers in Pharmacology, 11*, 697.

Yun, C. H., & Cho, C. S. (2020). Nanoparticles to improve the efficacy of vaccines. *Pharmaceutics [Internet], 12*(5) Available from: /pmc/articles/PMC7284527/. [cited 2022 Sept 27].

Zepp, F. (2010). Principles of vaccine design-Lessons from nature. *Vaccine, 28*(Suppl 3), C14.

Zhou, J., Kroll, A., Holay, M., Fang, R. H., & Zhang, L. (2020). Biomimetic nanotechnology towards personalized vaccines. *Advanced Materials [Internet], 32*(13), e1901255. Available from: /pmc/articles/PMC6918015/. [cited 2022 Sept 27].

Chapter 19
Concluding Remarks on Target Nanomedicine: Present and Future Aspects

Tean Zaheer, Afshan Muneer, Amjad Islam Aqib, Kaushik Pal, Maheen Murtaza, Mahreen Fatima, Muhammad Saad, and Kashif Prince

19.1 Introduction

Since targeted and controlled medication delivery using nanomaterials has been well established, while nanomedicines application in Nanotechnology, is an emerging topic in the field of medical science. The advancement of this technique not only prompted innovations in the field of medical science but also made significant contributions to the fields of diagnostic biomarkers, molecular imaging, and biosensing (Hu et al., 2022). The tremendous progress toward nanomedicines has the potential to provide numerous benefits such as targeted ability, dose response, bioavailability, improved efficacy, and safety over conventional therapeutics. This development is

T. Zaheer
Department of Parasitology, University of Agriculture Faisalabad, Faisalabad, Pakistan

A. Muneer · M. Murtaza
Department of Zoology, Cholistan University of Veterinary and Animal Sciences Bahawalpur, Bahawalpur, Pakistan

A. I. Aqib (✉)
Department of Medicine, Cholistan University of Veterinary and Animal Sciences Bahawalpur, Bahawalpur, Pakistan
e-mail: amjadislamaqib@cuvas.edu.pk

K. Pal
University Centre for Research and Development (UCRD), Chandigarh University, Mohali, Punjab, India

M. Fatima
Faculty of Biosciences, Cholistan University of Veterinary and Animal Sciences Bahawalpur, Bahawalpur, Pakistan

M. Saad · K. Prince
Department of Theriogenology, Cholistan University of Veterinary and Animal Sciences Bahawalpur, Bahawalpur, Pakistan

K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6_19

the product of a collaborative effort on the part of research institutions, educational institutions, and a variety of pharmaceutical corporations, all of which supported and encouraged research in the field of medical science. The biggest advantage of using targeted nanomedicine over traditional medicine is that it can overcome the cellular/physical barrier and provide diagnostic applications at the nanoscale level. After the first report, several government industries, private institutes, and research centers started contributing to the development of this area with the allocation of heavy funds and the development of new research centers in universities (Jahan et al., 2017). Nanoparticles are noninvasive tools because of their unique magnetic, optical, physical, chemical, and structural properties for drug delivery, diagnostic imaging, tumor detection, etc. Nanoparticles are more valuable due to the uniqueness of their characters. These nanoparticles are categorized into organic nanoparticles (liposomes, micelles, and exosomes) and inorganic nanoparticles (gold nanoparticles, magnetic nanoparticles, silver nanoparticles, and silica nanoparticles), which are discussed in detail in this chapter. These particles occur naturally as the by-products of the combustion reactions and are produced purposely. The other applications of nanoparticles range from cosmetics to environmental preservation and air purification (Anselmo & Mitragotri, 2019). The field of nanomedicine diverts the focus of scientists to explore new horizons in the field of medicine. Although the use of nanoparticles as drug carriers in nanomedicine has been successfully discussed in vitro, there is still much to be done in vivo. The future of nanomedicine is looking quite bright, and it is moving forward quickly. A wide range of nanomaterials based on organic, inorganic, lipid, protein, or glycan compounds and on synthetic polymers have been employed to produce innovative cancer therapeutics. According to the clinicaltrials.gov registry, 1575 nanomedicine formulations (search terms "liposome," "nanoparticle," or "micelle") have been submitted for clinical trials (N.I.H, 2016). One-third of these or 1381 formulations are in the field of cancer therapy. It is essential for the development of nanomedicine as a tool for illness detection and treatment that the effectiveness and safety of nanoparticles (NPs) in biological systems are established. (Kolosnjaj-Tabi et al., 2015). Nanomedicine is not just restricted to colloidal substances and the technology used to test them in vivo. Advances in nanomedicine go beyond the idea of a "magic particle bullet." Nanomedicine could aid in tissue regeneration by developing novel scaffolds and surfaces for sensors, implantable technology, and electronics (i.e., regenerative medicine). Although many of these ideas are still in their infancy, several have already entered practical use (Peteiro-Cartelle et al., 2009).

19.2 The Worldwide Market of Nanomedicines

As a result, the biological sciences and healthcare are now referred to as "nanomedicine," and this application of nanotechnology is seen as a rapidly developing topic in the realm of nanotechnology. In the past few years, the US Food and Drug Administration (FDA) has established over a hundred nanomedicine-based

products (Farjadian et al., 2019). The US federal officials have provided more than 41.1 billion in resources in an initiative to verify the significance of nanotechnology. One of the most significant technologies to emerge within the next decade, according to a report, is nanotechnology. The Food and drug Administration (FDA) approved the 100 nanomedicines without elaborating on current market trends in this area (Varshosaz et al., 2017). The primary goal of pharmaceutical research is to develop modern chemical organizations with less influence and greater medical benefits (Farjadian et al., 2019). Targeting drugs using NPs offers several benefits for improving biological system interactions and reducing nonspecific toxins. Another major challenge for narcotics (drugs) transmitted by NPs is the synthesis using traditional physicochemical methods (Mofazzal Jahromi et al., 2019). Currently, it may take up to two decades for a narcotic to register on the market after major developments. Another issue is the requirement for highly qualified medical personnel who are specialists in their fields and are willing to spend a decade or more learning these skills for a single project (Farjadian et al., 2019). The market needs to be accurately assessed, and the risk ratio is high. Patents must be filed at every stage of the drug discovery and commercialization process to protect the wise property of creators and organizations and to prevent time and money from being wasted in court battles. However, the 20-year term of patent protection is less given the maximum amount of time needed to oversee and approve a drug, pass the necessary clinical trials, and eventually launch the most recent pharmaceutical to the market. Trade exemption period is up to 12 years or less. Therefore, the time available for a company to make a profit is sometimes shortened to risk important resources (Hamburg, 2012).

19.2.1 Status of Food and Drug Administration (FDA)–Approved Nanoparticles and Nanoformulations

The terms such as “nanotechnology,” “nanoscale,” “nanomaterials,” and “nanocrystalline” are not regulated by the FDA. These terms are commonly used in the engineering of materials that range in size from approximately 1 nm to at least one dimension of 100 nm (Zingg & Fischer, 2019). The FDA is a very significant regulator of the US government agencies. A primary goal of the Food and Drug Administration (FDA) is the safety and efficacy of medical devices, vaccines, medications for animals, and tobacco products that reach consumers. Across the globe, there is a lack of regulatory framework for nanotechnology (Bartoszewski & Sikorski, 2019). The FDA has not issued any specific guidelines for nanopharmaceuticals.

All nanopharmaceuticals were given the FDA approval prior to being on the market using existing rules without any additional testing (Macdonald & Williams-Jones, 2012). However, the new nanoformulations approval challenged the FDA’s regulatory framework. The FDA is submitting the products for market approval and

focusing on products in specific areas, including some that may include nanomaterials or nanomedicines. It is hard to judge how the nanoparticles will be organized. The FDA's review process can add complexity to the process by introducing size changes and potentially unexpected results at the nanoscale. Although the FDA presently uses the products, it is possible that not all nanoproducts will benefit from their use as it might be challenging to categorize them into one of the established traditional classes. The FDA creates additional challenges and reviews issues arising from these complications (Farjadian et al., 2019) (Table 19.1).

19.3 Types of Nanoformulations Used in Clinical Studies

19.3.1 Organic Nanoparticles

19.3.1.1 Liposomes

Liposomes are smaller round artificial vesicles. They are made from cholesterol and natural nontoxic phospholipids. They have a hydrophobic and hydrophilic role because of their size. They play an important role in drug delivery (Ferreira et al., 2021). The size of a liposome ranges from very small to large. The diameter of large vesicles is 2.5 μm, whereas that of smaller vesicles is 0.025 μm. In addition, liposomes can also have one- or two-layer membranes. Liposomes can be divided into three groups based on size: small, medium, and larger. The membrane layers can be unicellular vesicles (ULVs), oligolamellar vesicles (OLVs), and multilamellar vesicles (MLVs) (Rizwan et al., 2022). Smaller unicellular vesicles (SUVs), such as ULVs, contain a phospholipid boiler, but in terms of dimensions, they are less than 100 nm in size (Rizwan et al., 2022). OLVs are liposomes consisting of two to five vesicles that can be of the same or of different sizes. In the structure of the OLVs, all vesicles are covered in a big phospholipid boiler without being inward to each other. MLVs are primarily used to deliver hydrophobic agents (Allen & Cullis, 2013). Liposomal NPs are synthesized for several benefits: they can deliver both

Table 19.1 Nanocrystallines currently available in the market

Nanocrystals	Nanoparticle type	Years (FDA Approved)	Nanoparticles	References
Ostim®	Nanocrystalline	2004	Calcium hydroxyapatite	Siddiqui et al. (2018)
Emend®	Nanocrystalline	2003	Antiemetic	Andrews and Horn (2006)
Rapamune®	Nanocrystalline	2015	Calcium phosphate	Bobo et al. (2016)
Vitoss®	Nanocrystals	2003	β-tricalcium phosphate (B-TCP, $Ca_3[PO_4]_2$)	Alagona (2010)
TriCor®	Nanocrystals	2004	Triglyceride	Alagona (2010)

hydrophilic and hydrophobic molecules; their composition extends the half-life of circulation, protects treatment contents from the in vivo environment, and improves vascular permeability, biological distribution, and targeting. It is used for treating cancer, including breast, lung, gastric, ovarian, sarcoma, myeloma, leukemia, and lymphomas (Fig. 19.1).

19.3.1.2 Micelles

The basic shell-type nanoparticles made by the assembly of polymeric micelles, stop copolymers, have been widely recognized as assuring nanocarriers in the treatment of cancer target therapy (Viitala et al., 2019). These are colloidal particles that combine in water to create nanocapsules. Amphiphilic molecules self-assemble to create micelles, which are colloidal particles found in water (Sharma, 2018). Micelles have groups of polar heads that commonly make the outside look like the level of micelles. The heads of the micelle can be cationic, anionic, or neutral groups. The kind of amphiphilic molecule, micelles may be distributed in lipid micelles and polymeric micelles. One of the advantages of polymeric micelles is that the polymeric entity's isolated effect from the environment can prevent the degradation of chemical or biological stimuli-sensitive molecules (Adams et al., 2003). They can dissolve drugs that do not dissolve well in water or that do not like water. This makes the drugs more bioavailable. Exosomes inside the cells are boxes of exosomes called multivascular bodies (MVBs) that take fragments of cytoplasm and their contents in the vesicles to attach to the membrane. Exosomes are widely established to have a role in the exchange of live genetic material (Lemière et al.,

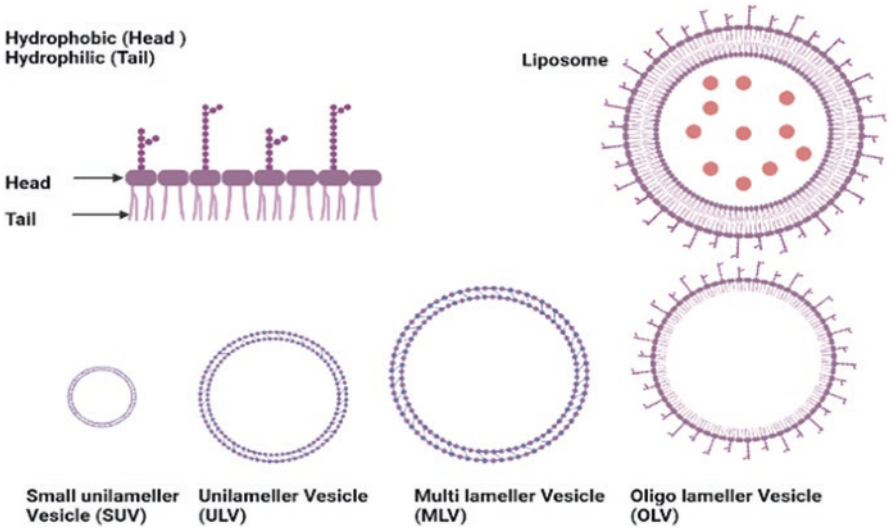


Fig. 19.1 Types of liposomes

2015). These biological characteristics include targeting exosomes that are thought to be essential for cancer growth, modifying exosomes as engineering and treatment tools, and discovering new biomarkers for early diagnosis (Sun et al., 2008). The treatment of myocardial ischemia–reperfusion and kidney injury, myocardial infection, muscle or bone degeneration, arthritis, nerve regeneration, multiple sclerosis, cancer, and neurological illnesses like Alzheimer's disease (AD) and Parkinson's disease are beneficial consequences of the exosome (Table 19.2).

19.3.2 Inorganic Nanoparticles

Research using inorganic nanoparticles, such as gold, iron oxide, silver, and silica, has been conducted in both medical and clinical studies to treat, diagnose, and detect a variety of diseases (Haque et al., 2022). In addition, many inorganic compounds that act as materials for making nanoparticles have long been used in clinics for different treatments (Palekar et al., 2015).

19.3.2.1 Gold Nanoparticles

The AuNPs are great metals known for their modern visual features inspired by the popularity of the localized surface plasmon resonance (LSPR) (Stafford et al., 2018). Plasmonic gold nanoparticles exhibit optical and photothermal features depending on unique size properties due to the collective duplication of free electrons in their conduction band. AuNPs have previously been instrumental in human well-being in the medical diagnostic sector and in several medical applications. As cancer, antibacterials, and AIDS research progresses, AuNPs are emerging as the most appealing and promising treatment option (Kumar et al., 2011).

Table 19.2 Different nanoparticle applications and drugs

NPs	Application	Drugs	References
Liposome	Cancer therapy	Doxil®, DaunoXome®, Depocyt®, Myocet®, Marqibo®, Onivyde®, and Amphotec®	Bulbake et al. (2017)
	Fungal infections	ABELCET®, AmBisome®, and Amphotec®	Bavli et al. (2021)
	Photodynamic therapy	VISUDYNE®	Bavli et al. (2021)
	Viral infections	Epaxal® and Inflexal®	Cech et al. (2011)
Micelles	Cancer treatment	Doxorubicin (DOX) and docetaxel	Bavli et al. (2021)
Exosomes	Cancer treatment	Cytotoxic	Bulbake et al. (2017)

19.3.2.2 Magnetic Nanoparticles

Magnetic elements including iron, cobalt, chromium, and manganese and nanoparticles relate to the bunch of nanotechnology-based mattes. Their biological applications include drug administration, magnetic resonance imaging (MRI), tissue healing, transfusion, and tissue targeting. These chemical processes for synthesizing AgNPs have issues with NP stabilizing and agglomeration and particle size and form. For organic and inorganic reducing agents, as well as stabilizing agents such polyvinylpyrrolidone (PVP), and silver precursors, AgNPs must be synthesized. Magnetic nanoparticles' shells are linked to drug molecules. Magnetic iron oxide nanoparticles have the benefit of being visible during magnetic resonance imaging, and drug-laden nanoparticles can also be seen via magnetic resonance imaging. Iron oxide magnetic particles are the most common type of MNPs. Iron oxide magnetic particles are covered in biosynchronized materials, which prevents the particles from building up, degrading biologically, or changing from their initial condition. This permits the bioactive agents to be trapped on the particle through attachment or absorption. Magnetic resonance imaging can detect them, and magnetic fields can be used to insert drug-laden nanoparticles (Kumar et al., 2011).

19.3.2.3 Silver Nanoparticles

AgNPs are used in nanomedicine because of their unique chemical and physical features and their biocompatibility. They are good drug delivery career candidates. It has been reported that AgNPs were effective nanoparticles in treating cancer and infection, so they had tumor-killing and antiseptic (Xing et al., 2019). In the other ocular field, AgNPs demonstrated the delivery of the treatment agents; they have been applying coating agents for lenses. In addition, AgNPs have demonstrated the effects of significant antiangiogenic, antioxidant, and anticataract in different cell culture systems and animal models for eye diseases, as when combined with natural plant extracts. The manufacture of AgNPs using various chemical techniques confronts difficulties such as particle size accumulation and stability (Iravani et al., 2014). Furthermore, MNPs have two additional advantages: first, they may be controlled by noncontact pressures; second, they can be tracked using MRI and targeted by nanodrug delivery systems (Giannaccini et al., 2014) (Fig. 19.2).

19.3.2.4 Silica Nanoparticles

Silica nanoparticles can absorb large amounts of drugs into their pores and have a particle size of 30–300 nm. The modern properties of SiNPs include large specific level area and size of the pores, size of controllable particles, and improved biocompatibility that make them the best nanoplatforms for biomedical applications and drug delivery. SiNPs have a large surface-to-volume ratio, ease of surface change, and a stable chemical structure (Giannaccini et al., 2014).

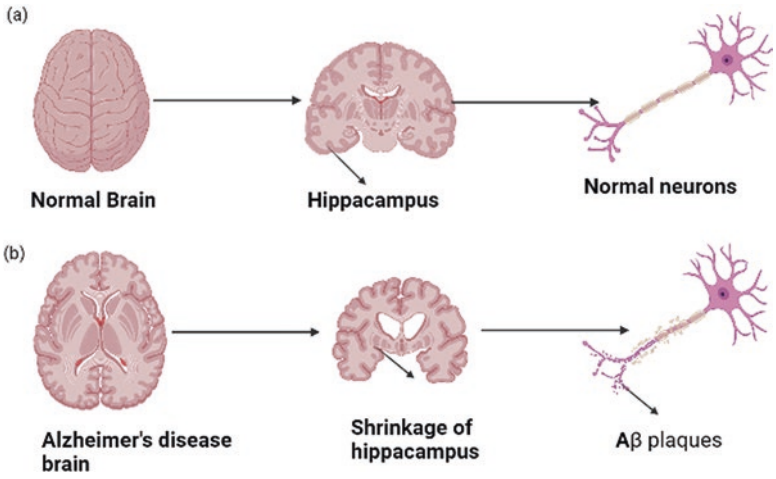


Fig. 19.2 (**a**) Healthy Brain healthy. (**b**) Diseased brain

19.4 Status of Nanomedicine in Various Diseases

19.4.1 Brain

Disorders including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, and stroke are spreading quickly due to the aging population. The United States has over 4.5 million patients with Alzheimer's disease, and medical and institutional care costs more than US$100 billion every year (Kabanov & Gendelman, 2007). Humans have always been affected by neurodegenerative disorders (NDDs). Currently, no definitive treatments exist, and the diagnosis of NDDs is made years after the disease has begun: symptoms are only detectable by neurologists in the late stages of the disease (Paulsen et al., 2013). Nanomedicine made a significant contribution to the fight against such devastating diseases. NP-based systems have been used to support and create new therapeutic approaches and to enhance the physical (solubility or ionization) and biopharmaceutical (bioavailability, biodistribution, toxicity, pharmacokinetics, and pharmacodynamics) aspects of recognized active compounds. Nevertheless, nanomedicine has certainly contributed to the most promising innovations in Alzheimer's disease (AD) research with early diagnosis methods. Because of the extremely low concentration of these substances, enzyme-linked immunosorbent assay (ELISA), for instance, is very challenging to carry out for the detection and quantification of AD biomarkers, Tau proteins, and A peptides in physiological fluids (Henriksen et al., 2014). Amplification of AD biomarker signals using NP-based procedures proved to be successful in overcoming such limitations. The immune-polymerase chain reaction or wave extinction assays have been used to quantify Tau proteins by gold nanoparticles (Stegurová et al., 2014) or

Rayleigh scattering assays (Neely et al., 2009) functionalized with tau-specific monoclonal antibody. Both NP-based methods were superior to ELISA methods in terms of their sensitivity.

19.4.2 Anticancer Agents

Anticancer agents can be treated with nanomedicines as an alternative treatment. With nanomedicines, specific site targeting is possible because of their small nanometer (nm) size, increasing their biological availability and reducing their toxic side effects. Moreover, consuming small amounts of medicines can reduce the cost of treatment (Rasool et al., 2022). The World Health Organization (WHO) estimated that cancer caused around 13% of all deaths in 2012. It is expected that, in the next two decades, the number of cancer cases may rise from 14 to 22 million. Toxicity must be restricted as a result of systemic exposure and the challenge of building resistance to anticancer agents (Kola & Landis, 2004). It is possible, however, that nanoparticles could solve the problems of solubility and stability associated with cancer drugs. As a result of the compound's water solubility, its bioavailability is limited, hampering the development of early anticancer drugs. The delivery and consumption of poorly soluble drugs are improved by encapsulating them inside a hydrophilic nanocarrier. Chemical stability is also improved. Drugs that are not pH-sensitive, such as doxorubicin, can be coupled with pH-sensitive nanoparticles to increase cellular absorption and intracellular drug release. As nanomedicine technologies advance, nanocarriers are developed to release their payloads upon commencement. Finally, the resistance of neoplasms to antitumor drugs is reduced through directed nanomedicine treatments. In general, focused intake and adenosine triphosphate (ATP)/multidrug resistance (MDR) outflow pump-driven excretion have no effect on specificity. The circulation period of a chemical can be increased using nanomedicine, facilitating the release of stimuli-responsive medicines and endocytic absorption. It is imperative to maintain a balance between the potential harms and benefits of cancer treatments. Nanotechnologies are intended to shift the balance in this direction. Nanomedicine products have several general schemes and modern practices that can improve the drug treatment index (Maitland & Schilsky, 2011). Several monotherapies have been created in the recent years and have been administered to a group of cancer patients, but there are only a few carriers that are specifically approved to target cancer cells (Olusanya et al., 2018). Individual markers serve as a key to personalized therapies because they can detect tumor types in different patients at particular stages of the disease, making it easier to predict results. In addition to identifying diagnostic and prognostic markers, nanoparticle technology can also be used to detect cancer cells in the body and to test the effectiveness of specific treatments. Several such systems use quantum dots, biocompositing, etc. There is great promise in the use of these techniques for detecting the underlying causes of a wide variety of cancers (Estella-Hermoso de Mendoza et al., 2009).

19.4.3 Tuberculosis

The most devastating infectious disease on the planet remains tuberculosis (TB). The diagnosis of latent tuberculosis infection (LTBI), extrapulmonary tuberculosis (EPTB), drug-resistant tuberculosis (DR-TB), HIV-associated tuberculosis (HIV-TB), and pediatric tuberculosis is still challenging in the developing countries. The spread of TB is mainly attributable to delayed or misdiagnosed cases, which contribute to its global spread. There is still no ideal diagnostic test for TB, and conventional methods remain essential for diagnosis, despite their limited diagnostic abilities (Gupta et al., 2020). Physiochemically (inert and nontoxic) and optically, gold nanoparticles (AuNPs) are ideal nanomaterials for clinical diagnosis, treatment, and other multidisciplinary research purposes. AuNPs' optical properties with antibodies or antigens, as well as with other biomolecules, make them useful for detecting various pathogens. In addition, the functional activity of AuNPs is not compromised after the immobilization of antigens (Sonawane & Nimse, 2016). Gold nanoparticles with surface functionalization enhance the antibody–antigen reaction, thereby increasing the immunoassay signal and thus increasing the test sensitivity (Kim et al., 2018). As a result, numerous samples can be tested simultaneously using a simple, low-cost assay. This assay is extremely specific and produces reliable results even from tiny amounts of mycobacterial deoxyribonucleic acid (DNA). By using gold nanoparticles modified with thiols, single-stranded DNA can be detected calorimetrically from test DNA samples (Cordeiro et al., 2016).

19.4.4 Malaria

All parasitic diseases have a negative impact on human health, but malaria has the greatest impact (Khan et al., 2019). According to the WHO, 214 million malaria infections and almost 438,000 malaria-related deaths occurred worldwide in 2015 (Gimenez et al., 2021). Due to the overuse of antimalarial drugs, such as chloroquine, parasites have devised a number of mechanisms to develop resistance to antimalarial drugs. Nanotechnologies can treat a wide range of parasitic infections, including diabetic foot ulcers, lymphatic filariasis, soil-transmitted helminthiasis, parasitic zoonoses, onchocerciasis, African trypanosomiasis, leishmaniasis, ectoparasitic skin infections, Chagas disease, malaria, and tuberculosis. Nanotechnology can eradicate malaria by developing successful therapeutic approaches to eliminate the malaria vector. It can also attack the parasite directly. Researchers have also used liposomes containing or paired with recombinant human tumor necrosis factor to successfully cure experimental cerebral malaria (ECM) in mice infected with *Plasmodium berghei* K173. *Plasmodium berghei*-infected mice were given polyethylene glycol (PEG)-coated halofantrine-loaded polylactic acid (PLA) nanocapsules to treat malaria, and cardiotoxicity was assessed. In a study of primaquine-loaded NPs, primaquine-loaded NPs operated better than non-primaquine-loaded NPs at

extending the lives of mice. Nanoparticles of varying sizes of albumin and gelatin were used to target the liver with the same molecule. A lipid nanoemulsion of primaquine (10–200 nm) was developed and demonstrated very good performance against *P. berghei* infection in Swiss albino mice in 2008. The delivery of malaria-specific antigens to targets has recently been improved using nanoprotein adjuvants. In mice, these adjuvants were conjugated with antigens of size 16–73 nm after injection and showed a better immune response against malaria when compared with the antigens alone (Scaria et al., 2017). Extrinsic protein adjuvants are rarely used because of their poor compatibility with vaccinations and antigens (Sia et al., 2021).

19.4.5 Human Immunodeficiency Virus (HIV)

Human immunodeficiency virus (HIV) infections have become increasingly devastating in the last few years. An estimated 7400 cases of HIV are diagnosed each day. HIV-positive patients have been found to live longer when they receive a combination of at least three antiretroviral (ARV) drugs, known as highly active antiretroviral therapy (HAART). Although HAART has contributed to reducing mortality rates in the developed countries, the situation continues to be bad in the developing countries with millions of people infected. To improve the HIV therapeutic situation, nanotechnology-based drug systems have been investigated. Nanosystems have a number of unique advantages in HIV therapeutics including improved bioavailability, water solubility, stability, and the ability to target HIV medicines. Polymeric micelles, liposomes, nanoparticles, liposomes, and dendrimers are the most common nanotechnology-based systems being explored for HIV treatment. As far as pharmaceutical drug delivery is concerned, nanotechnology represents a technological revolution. Nanotechnology's basic concept is to modulate the pharmacokinetics of incorporated molecules to efficiently remove HIV. An anti-HIV drug enclosed in a nanosystem is not governed by the properties of the drug but by the physical–chemical properties of the nanosystem, such as its surface-exposed molecules and electric charge, and by its size. By targeting drugs to HIV reservoirs and increasing the half-lives of the drugs, nanotechnology can be used to deliver ARV drugs to the tissues to cure HIV. Nanomedicines can exert antiviral efficacy by targeting different stages of the HIV replication cycle. Metal nanoparticles, dendrimers, and Bucky balls, for example, bind HIV enzymes or proteins, potentially limiting HIV reproduction through steric hindrance. Some inorganic metals (e.g., silver) have an antiviral impact when deposited in nanometer-sized macromolecules but not in bulk or atomic form (Sun et al., 2005).

19.5 Future Perspectives

19.5.1 Field of Public Health

Nanomedicine has a substantial impact on numerous elements of public health, including enhancing general health, improving quality of life, aging, and preventing and treating disease conditions. It can also refer to a community or social health issues such as vaccination, infection control, sanitation, environmental infection control, and early diagnosis and prevention of infectious disease (More et al., 2021). Recently, the FDA has not developed specific rules for products composed of nanomaterials. Nanomedicine will also impact the health-care system. Screening methods with high sensitivity and specificity to detect the disease can greatly improve the diagnosis and reduce the cost of healthcare. Initially, the use of nanomedicine by insurance providers may lead to resistance to payment for main treatments. The latest technologies are more costly than traditional medical interference, although newer methods are more effective. Nanomedicine can be used for disease prevention, diagnosis, and treatment. Social and physical environments influence public health with regard to environmental health. The climate and human health of engineered nanomaterials, and products and essential to discovering the process involved in making them. The nanomaterials environmental regulation falls down the control US Environmental Protection Agency (EPA)'s toxic substances. Although there are already more than 1000 nanomaterials-enabled user products on market, there is a difference in knowledge about their outcome and transportation within humans, and ecosystems (Feng et al., 2006).

19.5.1.1 Applications of Nanomedicines

Molecular imaging, drug delivery to specific parts of the body, and disease diagnostics are just a few of the promising medical uses of nanotechnology currently being studied and tested in clinical studies. Targeting drug delivery to diseased wounds is an important aspect of the drugs that are needed. Proper drug carriers are needed to deliver enough doses of medicine to the wound. While the chance to develop an effective nanotechnology-based drug delivery system is spread across all pharmaceutical treatment procedures for respiratory, central nervous system, and cardiovascular disorders, there remains a vital need in terms of finances and treatment. Many treatment agents have not been successful because of the restricted ability of the target to arrive at tissue. In addition, there are rapidly growing opportunities in increasing delivery systems for anticancer agents, hormones, and vaccines due to safety and utility flaws in traditional management practices. Novel medicinal substances to cure ailments in our pet population may be produced by the production and manipulation of new synthetic molecules. These novel substances, for instance, might protect animals from bacterial or viral infections and hasten wound healing. These novel molecules may also deliver medications and genes to cells, improving

the effectiveness of disease treatments. Nanopharmaceuticals will one day be used to treat most pet ailments. Drug delivery methods might benefit from new developments brought about by research in the field of nanopharmaceuticals. These systems affect how quickly medications or other chemicals are absorbed into the body, distributed throughout it, metabolized, and excreted. They must permit drug–target receptor binding and receptor–action regulation. The materials and manufacturing processes that can be employed in drug delivery devices are severely constrained. The drug delivery medium must be bioresorbable, compatible, and simple to bond with the medication. Stringent processing requirements must be adhered to during the production process and chemistry that nevertheless produces a cost-effective product without compromising the drug's quality.

Both diagnostic and therapeutic advancements are being made in the fascinating field of nanomedicine. In biomedical research, metallic and nanostructured particles can be used as helpful diagnostic tools to see how a cell is doing or how drugs are being distributed throughout the body. Magnetic resonance imaging (MRI) may be used to view magnetic nanoform metals in vivo at high concentrations, such as iron oxide (Ali et al., 2016). Through two-photon excitation or light activation, nanostructured particles may be made to fluoresce (Ajmal et al., 2015). In addition to these diagnostic nanoparticles, innovative molecular lab-on-a-chip technologies for qualitative and quantitative biological assessments have been developed. These lab-on-a-chip technologies are a desirable alternative because of their reduced analyte and reagent volume requirements, little waste production, and decreased wait times. Several microscale items are now on the market, whereas nanoscale products are only beginning to appear; for further details, see Wang et al. (2016) (Fig. 19.3).

19.5.1.2 Product Underdevelopment for Public Health

While many biocidal possibilities must still be tested in vivo through clinical studies and nutritional safety testing in compliance with federal regulations, it will take longer for nanoparticles to completely replace antibiotics in feed. Soon, it is likely that regular nanosupplements will be added to cattle feed to fortify it. Nanoparticles have previously been applied outside of animal products, such as in antiseptic wound dressings, and more will come. Nanoparticle cytotoxicity study in cancer cell lines and normal, healthy cell lines is critical for research of nanoparticles with anticancer properties. It may be inaccurate to just use cancer cells and assert that the under-research nanoparticle has anticancer characteristics, as the nanoparticle may be harmful to all types of cells. It is necessary to confirm the effects of nanoparticles in vivo through research done in vitro.

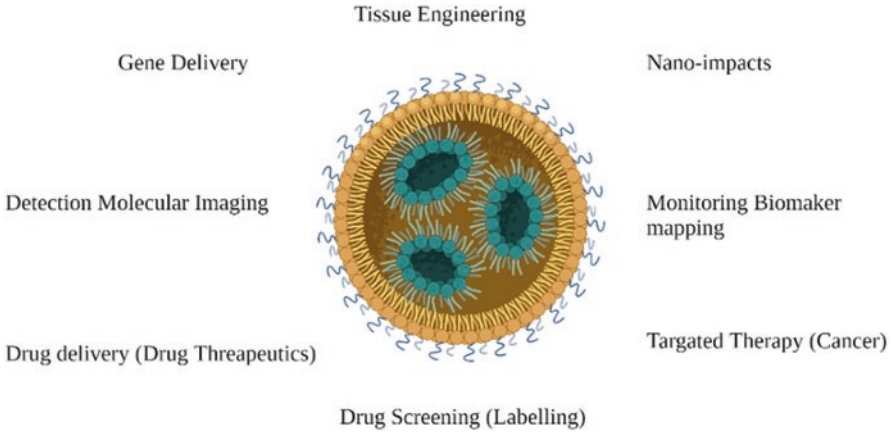


Fig. 19.3 Applications of nanomedicines

19.6 Conclusions, Outlook, and Future Aspects

Nanoparticles are a good candidate for future antifungal, antibacterial, antiviral, and anticancer medicines. The study of medical records exposes a protracted, uphill battle to advance human health, a battle that will ultimately result in a stunning victory: the abolition of disease and misery in the twenty-first century. If roughly ten billion people who have ever lived survived an average of 40 years and only experienced hardship and illness for 2% of their lives, then a significant cost of 70 trillion man-hours of human suffering will have been incurred to accomplish this. Due to their numerous significant triumphs over the past few decades, biotechnology and genetic engineering are very well-known. However, organizers of these techniques frequently ignore a postbiotechnology discipline that is just now starting to appear on the 2–3 decade and has the potential to virtually guarantee the elimination of biological senescence throughout the entire body and the perpetual maintenance of healthy mind and body with little to no unwanted medical side effects. This revolutionary technique integrates nanorobotics and molecular nanotechnology into healthcare. It will soon become abundantly evident that biotechnology in its whole is really a small part of nanotechnology, albeit an essential fraction. In actuality, rather than biotechnology, nanotechnology—the engineering and manufacturing of objects with atomic-scale precision—will rule the twenty-first century. One of humanity's grandest and most noble endeavors is poised to be completed. Earlier in the twenty-first century, a new medical paradigm known as nanomedicine will start to take shape as a result of our expanding capacity to quickly treat the majority of physically severe wounds, get rid of pathogens, and relieve suffering using molecular tools. A general definition of nanomedicine is the comprehensive monitoring, control, construction, repair, defense, and improvement in all human biological systems at the molecular level with the use of engineered nanodevices and nanostructures, molecular machine systems, and—in the end—nanorobots too small to be seen by the human eye.

19.6.1 Diagnosis

The cost of manufacturing of the advanced, high-tech tools required by medicine is currently extremely high (particularly medical research). Making tools from individual atoms allows for the construction of very minuscule implements. It will be possible to create sensors and perhaps complete nanobots that are so small that they can fit inside live cells. Because of the complexity of the human body, determining its state necessitates gathering a significant amount of data. There will even be real-time analysis of these data (crunched by integrated nanocomputers millions of times faster than current-day computers). They will build a complete model of the patient's body, monitor the patient's condition constantly, and employ a predictive approach to foresee the progression of the disease or other ailment and any future course. Based on the calculations of the probabilities of various potential therapies, the sensors/nanocomputers might even make recommendations. For the first time, collecting these data will be made possible even in routine diagnosis because of the tiny size and inexpensive cost of nanosensors. Real-time monitoring of a patient's systems makes it feasible to spot issues considerably earlier, enabling a more forceful and experimental approach to treatment. One affordable hand-held device will incorporate thousands of diagnostic testing. This will greatly improve diagnostic accuracy while decreasing malpractice and insurance liabilities. Many different kinds of nanoparticles, including liposomes, polymer nanoparticles (nanospheres and nanocapsules), solid lipid nanoparticles, nanocrystals, polymer therapeutics like dendrimers, fullerenes (most commonly C60 or Bucky ball, similar in size to hormones and peptide α-helices), and inorganic nanoparticles, can be used to deliver drugs and genes and probe DNA structures (e.g., gold and magnetic nanoparticles). Nanoparticles are the basic building blocks of nanotechnology and have a wide range of applications, such as fluorescent biological markers, protein markers, DNA structure probes, biological molecule separation and purification, improved magnetic resonance imaging, tumor heating, tissue engineering, drug and gene delivery, and many others. Second, it is also feasible to create magnetic nanoparticles with adjustable diameters between 2 and 30 nm that can bind to or interact with biological substances by coating them with biological molecules. If the particles are contained in a hydrogen matrix that is temperature-sensitive, the matrix will break down and release the agents when the temperature reaches a certain point. They can be programmed to deliver a payload to a specific area of the body, such as an anticancer medication or a group of radioactive atoms. The ability of the magnetic particles to be heated up and supplied with energy from the stimulating external field allows them to deliver dangerously high levels of thermal energy to the targeted tissues, such as tumors, making them effective hyperthermia agents (Abeer, 2012).

The only problem with the use of nanoparticles is toxicity to the tissues. Unfortunately, the knowledge regarding the toxicity of each nanoparticle is scarce, which is making it difficult to commercially launch these nanoparticles for public use. It is a dire need to study the toxicity of these nanoparticles in vivo in laboratories, and then, field trials must be conducted and reported before use in humans and

commercial preparations. The external routes of drug administration are relatively safe to use like skin applications, so certain products are being launched for dressing and wound treatment. Safety trials must be conducted before launching any product for cancer treatment because nanoparticles are toxic to both cancer and noncancer cells. Nanotechnology-based equipment can be used for analysis, imaging, detection, diagnosis and treatment, and clinical interventions, including cancer targeting, drug discovery, improving cell–material interactions, tissue engineering scaffolds, and gene delivery systems, and they offer novel approaches in the struggle against intractable diseases. It is anticipated that several cutting-edge nanoparticles and nanodevices would be used, having a hugely beneficial effect on human health. Nanotechnology has the potential to fundamentally change science, technology, and society over the next 10–20 years. This will offer a huge chance to advance human health in unique ways, especially by facilitating early disease detection and diagnosis and an accurate and efficient patient-specific therapy.

References

Abeer, S. (2012). Future medicine: Nanomedicine. *JIMSA, 25*(3), 187–192.

Adams, M., Lavasanifar, A., & Kwon, G. (2003). Amphiphilic block copolymers for drug delivery. *Journal of Pharmaceutical Sciences, 92*, 1343–1355. https://doi.org/10.1002/jps.10397

Ajmal, M., Yunus, U., Matin, A., & Haq, N. U. (2015). Synthesis, characterization and in vitro evaluation of methotrexate conjugated fluorescent carbon nanoparticles as drug delivery system for human lung cancer targeting. *Journal of Photochemistry and Photobiology B: Biology, 153*, 111–120.

Alagona, P. (2010). Fenofibric acid: A new fibrate approved for use in combination with statin for the treatments of mixed dyslipidemia. *Vascular Health and Risk Management, 6*, 351–362. https://doi.org/10.2147/VHRM.S6714

Ali, A., Zafar, H., Zia, M., et al. (2016). Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. *Nanotechnology, Science and Applications, 9*, 49–67. https://doi.org/10.2147/NSA.S99986

Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews, 65*(1), 36–48. https://doi.org/10.1016/j.addr.2012.09.037

Andrews, P. L. R., & Horn, C. C. (2006). Signals for nausea and emesis: Implications for models of upper gastrointestinal diseases. *Autonomic Neuroscience, 125*(1–2), 100–115. https://doi.org/10.1016/j.autneu.2006.01.008

Anselmo, A. C., & Mitragotri, S. (2019). Nanoparticles in the clinic: An update. *Bioengineering & Translational Medicine, 4*(3), e10143. https://doi.org/10.1002/btm2.10143

Bartoszewski, R., & Sikorski, A. F. (2019). Editorial focus: Understanding off-target effects as the key to successful RNAi therapy. *Cellular & Molecular Biology Letters, 24*, 69. https://doi.org/10.1186/s11658-019-0196-3

Bavli, Y., Rabie, M., Fellig, Y., Nevo, Y., & Barenholz, Y. (2021). Liposomal bupivacaine (Bupigel) demonstrates minimal local nerve toxicity in a rabbit functional model. *Pharmaceutics, 13*(2). https://doi.org/10.3390/pharmaceutics13020185

Bobo, D., Robinson, K. J., Islam, J., Thurecht, K. J., & Corrie, S. R. (2016). Nanoparticle-based medicines: A review of FDA-approved materials and clinical trials to date. *Pharmaceutical Research, 33*(10), 2373–2387. https://doi.org/10.1007/s11095-016-1958-5

Bulbake, U., Doppalapudi, S., Kommineni, N., & Khan, W. (2017). Liposomal formulations in clinical use: An updated review. *Pharmaceutics, 9*(2). https://doi.org/10.3390/pharmaceutics9020012

Cech, P. G., Aebi, T., Abdallah, M. S., et al. (2011). Virosome-formulated Plasmodium falciparum AMA-1 & CSP derived peptides as malaria vaccine: Randomized phase 1b trial in semi-immune adults & children. *PLoS One, 6*(7), e22273. https://doi.org/10.1371/journal.pone.0022273

Clinical Trials, N. I. H. (2016). gov, a service of the US National Institutes of Health. 2015.

Cordeiro, M., Ferreira Carlos, F., Pedrosa, P., Lopez, A., & Baptista, P. V. (2016). Gold nanoparticles for diagnostics: Advances towards points of care. *Diagnostics (Basel, Switzerland), 6*(4). https://doi.org/10.3390/diagnostics6040043

Estella-Hermoso de Mendoza, A., Campanero, M. A., Mollinedo, F., & Blanco-Prieto, M. J. (2009). Lipid nanomedicines for anticancer drug therapy. *Journal of Biomedical Nanotechnology, 5*(4), 323–343. https://doi.org/10.1166/jbn.2009.1042

Farjadian, F., Ghasemi, A., Gohari, O., Roointan, A., Karimi, M., & Hamblin, M. R. (2019). Nanopharmaceuticals and nanomedicines currently on the market: Challenges and opportunities. *Nanomedicine (London, England), 14*(1), 93–126. https://doi.org/10.2217/nnm-2018-0120

Feng, L., Qi, X. R., Zhou, X. J., et al. (2006). Pharmaceutical and immunological evaluation of a single-dose hepatitis B vaccine using PLGA microspheres. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 112*(1), 35–42. https://doi.org/10.1016/j.jconrel.2006.01.012

Ferreira, M., Ogren, M., Dias, J. N. R., et al. (2021). Liposomes as antibiotic delivery systems: A promising nanotechnological strategy against antimicrobial resistance. *Molecules, 26*(7). https://doi.org/10.3390/molecules26072047

Giannaccini, M., Giannini, M., Calatayud, M. P., et al. (2014). Magnetic nanoparticles as intraocular drug delivery system to target retinal pigmented epithelium (RPE). *International Journal of Molecular Sciences, 15*(1), 1590–1605. https://doi.org/10.3390/ijms15011590

Gimenez, A. M., Marques, R. F., Regiart, M., & Bargieri, D. Y. (2021). Diagnostic methods for non-falciparum malaria. *Frontiers in Cellular and Infection Microbiology, 11*. https://www.frontiersin.org/articles/10.3389/fcimb.2021.681063

Gupta, A., Singh, A., & Singh, S. Diagnosis of tuberculosis: Nanodiagnostics approaches. In: 2020.

Hamburg, M. A. (2012). Science and regulation. FDA's approach to regulation of products of nanotechnology. *Science, 336*(6079), 299–300. https://doi.org/10.1126/science.1205441

Haque, S. T., Banaszak Holl, M. M., & Chowdhury, E. H. (2022). Strategies to assemble therapeutic and imaging molecules into inorganic nanocarriers. *Frontiers of Materials Science, 16*(3), 1–44. https://doi.org/10.1007/s11706-022-0604-x

Henriksen, K., O'Bryant, S. E., Hampel, H., et al. (2014). The future of blood-based biomarkers for Alzheimer's disease. *Alzheimer's & Dementia, 10*(1), 115–131. https://doi.org/10.1016/j.jalz.2013.01.013

Hu, T., Gong, H., Xu, J., Huang, Y., Wu, F., & He, Z. (2022). Nanomedicines for overcoming cancer drug resistance. *Pharmaceutics, 14*(8). https://doi.org/10.3390/pharmaceutics14081606

Iravani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of silver nanoparticles: Chemical, physical and biological methods. *Research in Pharmaceutical Sciences, 9*(6), 385–406.

Jahan, S. T., Sadat, S. M. A., Walliser, M., & Haddadi, A. (2017). Targeted therapeutic nanoparticles: An immense promise to fight against cancer. *Journal of Drug Delivery, 2017*, 9090325. https://doi.org/10.1155/2017/9090325

Kabanov, A. V., & Gendelman, H. E. (2007). Nanomedicine in the diagnosis and therapy of neurodegenerative disorders. *Progress in Polymer Science, 32*(8–9), 1054–1082. https://doi.org/10.1016/j.progpolymsci.2007.05.014

Khan, S. U., Anjum, S. I., Ansari, M. J., et al. (2019). Antimicrobial potentials of medicinal plant's extract and their derived silver nanoparticles: A focus on honey bee pathogen. *Saudi Journal of Biological Sciences, 26*(7), 1815–1834. https://doi.org/10.1016/j.sjbs.2018.02.010

Kim, J., Oh, S. Y., Shukla, S., et al. (2018). Heteroassembled gold nanoparticles with sandwich-immunoassay LSPR chip format for rapid and sensitive detection of hepatitis B virus surface antigen (HBsAg). *Biosensors & Bioelectronics, 107*, 118–122. https://doi.org/10.1016/j.bios.2018.02.019

Kola, I., & Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nature Reviews. Drug Discovery, 3*(8), 711–715. https://doi.org/10.1038/nrd1470

Kolosnjaj-Tabi, J., Javed, Y., Lartigue, L., Volatron, J., Elgrabli, D., Marangon, I., et al. (2015). The one year fate of iron oxide coated gold nanoparticles in mice. *ACS Nano, 9*(8), 7925–7939.

Kumar, A., Boruah, B., & Liang, X. J. (2011). Gold nanoparticles: Promising nanomaterials for the diagnosis of cancer and HIV/AIDS. Hindawi Publ Corp. *Journal of Nanomaterials, 17*, 1. https://doi.org/10.1155/2011/202187

Lemière, J., Carvalho, K., & Sykes, C. (2015). Cell-sized liposomes that mimic cell motility and the cell cortex. *Methods in Cell Biology, 128*, 271–285. https://doi.org/10.1016/bs.mcb.2015.01.013

Macdonald, C., & Williams-Jones, B. (2012). Nothing new (ethically) under the sun: Policy & clinical implications of nanomedicine. *Bioéthique Online, 1*.

Maitland, M. L., & Schilsky, R. L. (2011). Clinical trials in the era of personalized oncology. *CA: A Cancer Journal for Clinicians, 61*(6), 365–381. https://doi.org/10.3322/caac.20135

Mofazzal Jahromi, M. A., Abdoli, A., Rahmanian, M., et al. (2019). Microfluidic brain-on-a-chip: Perspectives for mimicking neural system disorders. *Molecular Neurobiology, 56*(12), 8489–8512. https://doi.org/10.1007/s12035-019-01653-2

More, S. L., Kovochich, M., Lyons-Darden, T., Taylor, M., Schulte, A. M., & Madl, A. K. (2021). Review and evaluation of the potential health effects of oxidic nickel nanoparticles. *Nanomaterials (Basel, Switzerland), 11*(3). https://doi.org/10.3390/nano11030642

Neely, A., Perry, C., Varisli, B., et al. (2009). Ultrasensitive and highly selective detection of Alzheimer's disease biomarker using two-photon Rayleigh scattering properties of gold nanoparticle. *ACS Nano, 3*(9), 2834–2840. https://doi.org/10.1021/nn900813b

Olusanya, T. O. B., Haj Ahmad, R. R., Ibegbu, D. M., Smith, J. R., & Elkordy, A. A. (2018). Liposomal drug delivery systems and anticancer drugs. *Molecules, 23*(4). https://doi.org/10.3390/molecules23040907

Palekar, R. U., Jallouk, A. P., Lanza, G. M., Pan, H., & Wickline, S. A. (2015). Molecular imaging of atherosclerosis with nanoparticle-based fluorinated MRI contrast agents. *Nanomedicine (London, England), 10*(11), 1817–1832. https://doi.org/10.2217/nnm.15.26

Paulsen, J. S., Nance, M., Kim, J. I., et al. (2013). A review of quality of life after predictive testing for and earlier identification of neurodegenerative diseases. *Progress in Neurobiology, 110*, 2–28. https://doi.org/10.1016/j.pneurobio.2013.08.003

Peteiro-Cartelle, J., Rodriguez-Pedreira, M., Zhang, F., Gil, P. R., del Mercato, L. L., & Parak, W. J. (2009). One example on how colloidal nano-and microparticles could contribute to medicine. *Nanomedicine, 4*(8), 967–979.

Rasool, M., Malik, A., Waquar, S., et al. (2022). New challenges in the use of nanomedicine in cancer therapy. *Bioengineered, 13*(1), 759–773. https://doi.org/10.1080/21655979.2021.2012907

Rizwan, M., Hassan, M., Makinde, O. D., Bhatti, M. M., & Marin, M. (2022). Rheological modeling of metallic oxide nanoparticles containing non-Newtonian nanofluids and potential investigation of heat and mass flow characteristics. *Nanomaterials (Basel, Switzerland), 12*(7). https://doi.org/10.3390/nano12071237

Scaria, P. V., Chen, B., Rowe, C. G., Jones, D. S., Barnafo, E., Fischer, E. R., ... & Duffy, P. E. (2017). Proteinprotein conjugate nanoparticles for malaria antigen delivery and enhanced immunogenicity. *PLoS One, 12*(12), e0190312.

Sharma, R. (2018). Surfactants: Basics and versatility in food industries*, 2*, 17–29.

Sia, Z. R., Miller, M. S., & Lovell, J. F. (2021). Engineered nanoparticle applications for recombinant influenza vaccines. *Molecular Pharmaceutics, 18*(2), 576–592. https://doi.org/10.1021/acs.molpharmaceut.0c00383

Siddiqui, H. A., Pickering, K. L., & Mucalo, M. R. (2018). A review on the use of hydroxyapatite-carbonaceous structure composites in bone replacement materials for strengthening purposes. *Materials (Basel, Switzerland), 11*(10). https://doi.org/10.3390/ma11101813

Sonawane, M., & Nimse, S. B. (2016). Surface modification chemistries of materials used in diagnostic platforms with biomolecules. *Journal of Chemistry, 2016*, 1–19. https://doi.org/10.1155/2016/9241378

Stafford, S., Serrano García, R., & Gun'ko, Y. (2018). Multimodal magnetic-plasmonic nanoparticles for biomedical applications. *Applied Sciences, 8*, 97. https://doi.org/10.3390/app8010097

Stegurová, L., Dráberová, E., Bartos, A., Dráber, P., Rípová, D., & Dráber, P. (2014). Gold nanoparticle-based immuno-PCR for detection of tau protein in cerebrospinal fluid. *Journal of Immunological Methods, 406*, 137–142. https://doi.org/10.1016/j.jim.2014.03.007

Sun, R. W. Y., Chen, R., Chung, N. P. Y., Ho, C. M., Lin, C. L. S., & Che, C. M. (2005). Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chemical Communications*, (40), 5059–5061. https://doi.org/10.1039/b510984a

Sun, C., Lee, J. S. H., & Zhang, M. (2008). Magnetic nanoparticles in MR imaging and drug delivery. *Advanced Drug Delivery Reviews, 60*(11), 1252–1265. https://doi.org/10.1016/j.addr.2008.03.018

Varshosaz, J., Taymouri, S., Hamishehkar, H., Vatankhah, R., & Yaqoubi, S. (2017). Development of dry powder inhaler containing tadalafil-loaded PLGA nanoparticles. *Research in Pharmaceutical Sciences, 12*, 222. https://doi.org/10.4103/1735-5362.207203

Viitala, L., Pajari, S., Gentile, L., et al. (2019). Shape and phase transitions in a PEGylated phospholipid system. *Langmuir, 35*(11), 3999–4010. https://doi.org/10.1021/acs.langmuir.8b03829

Wang, S., Chinnasamy, T., Lifson, M. A., Inci, F., & Demirci, U. (2016). Flexible substrate-based devices for point-of-care diagnostics. *Trends in Biotechnology, 34*(11), 909–921. https://doi.org/10.1016/j.tibtech.2016.05.009

Xing, Y., Li, W., Wang, Q., et al. (2019). Antimicrobial nanoparticles incorporated in edible coatings and films for the preservation of fruits and vegetables. *Molecules, 24*(9). https://doi.org/10.3390/molecules24091695

Zingg, R., & Fischer, M. (2019). The consolidation of nanomedicine. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 11*(6), e1569. https://doi.org/10.1002/wnan.1569

Index

D. K. Pal (ed.), *Nanovaccinology*, https://doi.org/10.1007/978-3-031-35395-6