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

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2023

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

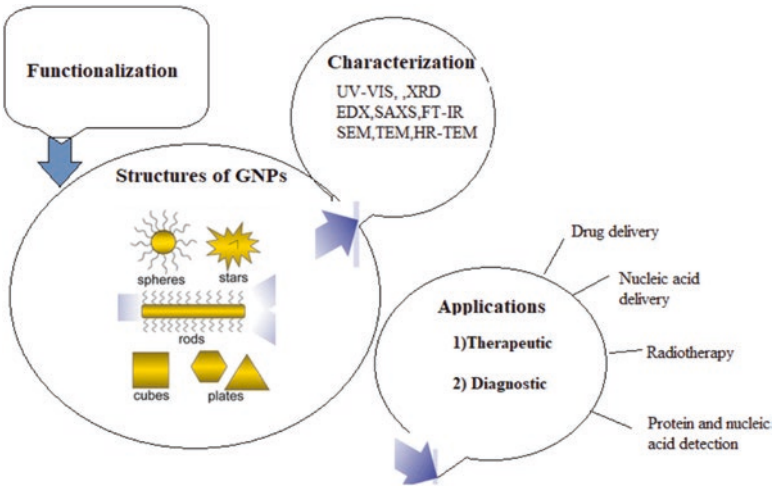

Fig. 12.1 Structure, characterization, functionalization, and applications of GNPs

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

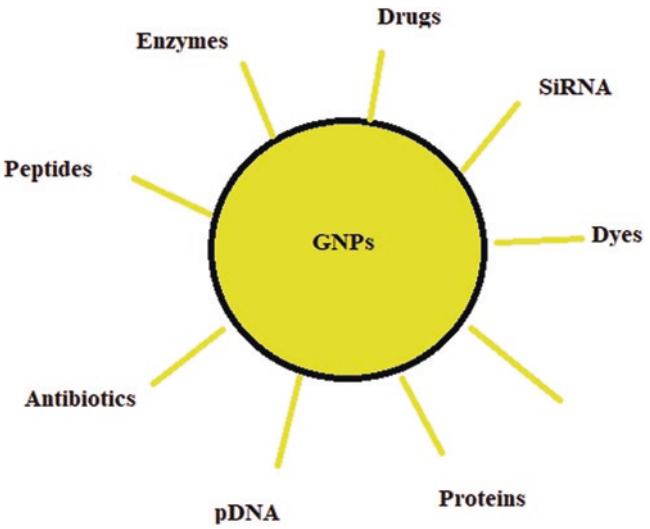

Fig. 12.2 Functionalization of GNPs

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.

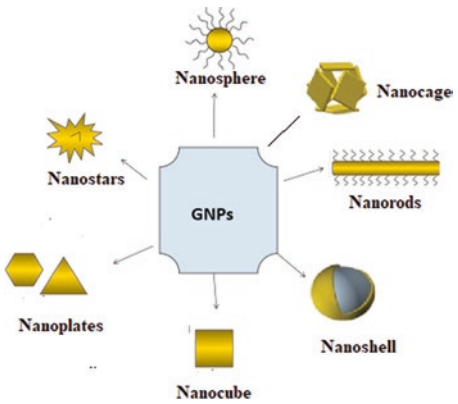

Fig. 12.3 Different shapes of GNPs

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.