Biomolecular-conjugated nanoparticles for biomedical applications

Prachi Goyal, Kamani Parmar, Sonika Gupta, Mukesh Sharma, M. P. Dobhal & Sambasiva Rao

1 Department of Biotechnology, Maharaj Vinayak Global University, Jaipur, Rajasthan (India)
2 Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan (India)
3 Department of Biotechnology, Acharya Nagarjuna University, Guntur, A.P. (India)

Received: April 2016, Accepted: May 2016, Published online: June 2016

Abstract

Biomolecular-conjugated nanoparticles (NP) demonstrate unique properties with wide-ranging applications in the diagnosis of infectious diseases as well as application in gene therapy and drug delivery therapies. The unique properties and utility of NP arise from a variety of attributes, including the similar size of nanoparticles and biomolecules. Biological functions depend primarily on units that have nanoscale dimensions, such as viruses, ribosomes, molecular motors and components of the extracellular matrix. In addition, engineered devices at the nanoscale are small enough to interact directly with sub-cellular compartments and to probe intracellular events. This review focuses on the methods of nanoparticle interaction with different biomolecules such as antibodies, DNA, lipids, and proteins. More specifically, there is discussion about bioconjugation linkage and a summary of potential biomedical applications of bio-conjugated nanoparticles as targeted drug delivery vehicles.

Keywords: Nanoparticles (NP), Therapy, Diagnosis, Bioconjugation, Biomolecular, Lipid, Protein, Antibody

Introduction

Nanobiotechnology is a new frontier in biology with applications in medicine. The use of nanomaterials in biotechnology merges material science and biology nanotechnology, a burgeoning field of research and development. The Development of efficient drug delivery systems has attracted great attention during the last two decades. In 1959, the Nobel Physicist, Richard Feynman, introduced the idea of nanotechnology [1]. The study of phenomena and fine-tuning of materials at molecular, atomic and macromolecular scales, where properties differ significantly from those at a larger scale [2].

Nanotechnology has provided the opportunity to deliver drugs to specific cells using nanoparticles with more useful behaviour and fewer side effects. Nanoparticles having a dimension below 0.1µm – 100 nm [3]. Years between the 1980s and early 1990s, saw a significant increase in the popularity of nanotechnology. Further developments include the use of nanoparticles for the delivery of drugs transverse the blood–brain barrier (BBB) and PEGylated nanoparticles through a prolonged blood circulation time [4]. From ancient times Swarna bhasma is also considered as a functional dosage form in the Ayurvedic treatment. In traditional medicine, especially in India, gold is utilized in the form of purified metallic fine powder as nanoparticles [5].
Considerable attention to the use of nanoparticles as drug carriers has been observed in the recent years due to their stability, biodegradability, and ease of preparation. The study of nanoparticles is currently an area of intense scientific interest because of several potential applications in biomedical fields [6].

**CLASSIFICATION OF NANOPARTICLES:**

**Organic nanoparticles:**
Nanocrystals are aggregates of hundreds or thousands of molecules which combine in crystal form. Nanocrystal technology may be utilized for many dosage forms in drug delivery. Quantum dots are also known as nanocrystals and are nanosized semiconductors that can emit light. Quantum dots are extensively used in cell labelling and imaging. Dendrimers are highly branched macromolecules whose size and shape can be exactly controlled. Dendrimers are most widely used in drug and gene delivery. A polymer nanoparticle consists of a biodegradable polymer [7]. The advantage of PNPs in drug delivery that they increase the stability of any pharmaceutical agent [8]. Liposomes are lipid based liquid crystals. Liposomes are used to deliver both hydrophobic and hydrophilic drugs. The first liposome drug formulation is Doxil, approved by food and drug administration (FDA) for the treatment of ovarian cancer and AIDS-related Kaposi’s sarcoma [9].

**Inorganic nanoparticles:**
Magnetic nanoparticles (MNPs) have interesting properties such as nonimmunogenicity, biocompatibility, and conjugation for cell specific targeting and imaging. They have been used in the treatment of cancer especially brain tumours [10]. Superparamagnetic iron oxide nanoparticles (SPION) are most widely used for biomedical applications (e.g., magnetic resonance imaging, drug delivery, immunoassay). The surface of SPION needs to be modified according to the biomolecules surface. Otherwise, biomolecules cannot bind and interact properly with the surface of nanoparticles. Gold nanoparticles have a unique shape and size dependent property. Several subtypes of gold nanoparticles are: gold nanospheres, nanocages, nanorods and nanoshells. Gold nanoparticles are less invasive, simple, non-toxic, and provide increased contrast for the diagnosis of cancer [11].

Biomolecular Engineering deals with the manipulation of several biomolecules. These include proteins, antibodies, DNA, enzymes, polymers, and lipids. These molecules are the basic building blocks of life [12]. Bio-conjugations are the purposeful and unique method to manipulate biomolecule mobility by chemical or physical means to achieve a desired property. “Bio-conjugation” refers to covalent derivatization of proteins, DNA, RNA, enzymes, antibodies, and polymers [13]. Bio-conjugation is a broad field of research and biomedical application in in vivo imaging, sensing and targeted drug delivery systems. The conjugation of nanoparticles with biomolecules such as protein DNA enzymes and antibodies can be achieved by using two different methods - direct covalent linkage and non-covalent interaction of particles and biomolecules [14]. The most favourable approach for the bioconjugation is covalent attachment [15]. This conjugation can also be done by chemisorption of the biomolecules to the nanoparticle surface or through the use of a heterobifunctional linker. Bifunctional linkers offer an adaptable method of bioconjugation [16].

**BIOCONJUGATION LINKAGE:**

**Covalent coupling:**
Covalent coupling is used for the immobilization of biomolecules when a very stable and active microsphere reagent is required. Reagent biomolecules are permanently bound with the help of microspheres and will not desorb / leach over time [17]. Polymeric or silica microspheres can be coupled to biomolecules through various surface chemistry methods such as polymeric – amino and carboxyl, hydrazide and chloromethyl, and silica – silanol, carboxyl [18]. Buffers such as phosphate buffered saline, borate buffer have also been used in covalent coupling [19]. Traditional strategies for covalent conjugation have poor control over the site of modification and experience loss of biological function of the targeted molecules [20]. The new methods of bioconjugation, such as diels- alder cycloaddition and Staudinger ligation, are most widely used in biological applications.

**Diels- alder cycloaddition:**
Used for immobilization of carbohydrate onto glass slide hydroquinone function groups. Diels – alder reaction between a trans-cyclooctene and tetrazine is more rapid and has much potential for bioconjugation [21].

**Staudinger ligation:**
Used for bioconjugation. Staudinger ligation is used for rapid and site specific immobilization of peptides and proteins. In this method an azide is reduced to an amine by a phosphine [22]. Common linkages for site specific bioconjugation are discussed below.
Linkage containing thioether:
In aqueous solution thiolates are potent nucleophiles. The derivatization of protein through the thiolate group of cysteine residue is a common method of bioconjugation [23]. Thiol reactive functional groups consist of idoacetamides, disulphide, and maleimides [24].

Linkage containing amide bond:
Amide bond has a half-life of 600 years in neutral solution at 25°C. The significant stability of amides makes them highly desirable for bioconjugation [25]. Staudinger ligation and native chemical ligation are two modern methods for generating amide linkage at a specific site in a protein [26].

Linkage containing carbon- nitrogen double bonds:
The synthesis of carbon nitrogen double bond through the condensation of nitrogen bases with ketones and aldehydes at neutral pH makes them attractive for bioconjugation [27]. When the nitrogen base is a hydrazine, hydrazones (C=N-N) are formed [28]. Oximes (C=N-O) are generated when the nitrogen base is an alkoxyamine [29].

**Pharmacokinetics of Bioconjugated Nanoparticles:**
The pharmacokinetics (PK) and tissue distribution of the nanoparticles largely define their therapeutic effect. Nanoparticle size, shape, and surface functionalization play an important role in pharmacokinetics. The natural clearance and excretion mechanisms of the human body provide a framework for the rational design of effective nanoparticles for use in medical therapies. There are strategies for overcoming barriers for intracellular delivery and drug release. Bioconjugated nanoparticles are successfully used in localization and drug delivery to specific biological targets; they bind with efficient evasion of the reticulo endothelial system [30]. Considerable attention has been given in recent years to the use of nanoparticles as drug carriers due to their stability, biodegradability and ease of preparation. Very small nanomaterial (1–20 nm) has long circulatory residence times and slower extravasation from the vasculature into interstitial spaces. This may cause slower attainment of the maximal volume of distribution, or even an altered volume of distribution when administered intravenously. They may, in fact, be better deliver drugs to tiny areas within the body. Nanoparticles are used in the control and sustained release of drugs throughout their transportation and delivery localization, altering organ distribution of the drugs and consequently clearance of the drugs [31]. The surface functionalization of nanoparticles with biomolecule based drug delivery has provided a new direction for modulating pharmacokinetics and significantly increases the efficacy of the targeted drug. The surface of nanomaterials can be ligated with an RGD peptide, an antibody, or an aptamer. Nanomaterials present a unique opportunity to deliver pharmaceutical agents to specific tissues. Bioconjugated nanoparticles are able to minimize drug degradation and loss, and increase drug availability to the target areas. Biomolecules like lipid have the ability to carry both hydrophobic and hydrophilic drugs simultaneously. Bioconjugated nanoparticle drug delivery is more efficient and less harmful due to target specific reactivity. Drug loaded nanoparticles administered by i.v. or i.p. routes and the respective pharmacokinetics and tissue distribution show good results. Recently, biodegradable polymeric micelles with a size of 10–200 nm have attracted attention as drug delivery nanocarriers, as they have shown remarkable therapeutic potential. Pharmacological properties of bioconjugated nanoparticles can be manipulated by changing the physical and chemical properties of biomolecules. They enhance permeability and retention, since they are more specific and have no side effects [32].

**Biomolecular Conjugated Nanoparticles: Method and Applications:**

**Antibody conjugation method:**
The conjugation of nanoparticles with antibodies merges the property of the nanoparticles with the specific and selective recognition ability of the antibody to antigens [33]. There are two ways to achieve specific targeting passive and active strategy. Passive targeting exploits the property of nanoparticle to be accumulated on the target (tumour site) due to the enhanced permeation and retention effect. In passive targeting, nanoparticles are injected near the artery where the nanoparticles are cook by the application of laser or magnetic field, feed the tumour and reduce the tumour size [34]. Active targeting is based on specific physical properties of the recognition moieties linked to the nanoparticles. Various biomolecules like apatamers, protein, nucleic acid, lipid, enzymes, etc., are used as a biocjugate to achieve active targeting. Antibodies are the best among all biomolecules, as they are highly specific and possess large diversity and recognition efficiency to their target [35].

**Ionic Interactions:**
The two reacting species have charged surfaces. These surfaces are used for binding together of both components to form a conjugate. This is a timesaving and simple process, but the conjugates
formed have poor stability. The interaction can easily be influenced by factors such as pH, ionic strength, and the concentration and nature of the solvent.

Apart from stability, there are problems facing the orientation of the antibodies on the nanoparticle surface for conjugates obtained by ionic interaction strategies [36]. By controlling unspecific ionic adsorption, it is possible to eliminate the problem associated with the orientation of functionalized Ab on the nanoparticles. A very suitable methodology to preserve the biological activity of Ab conjugated nanoparticles is a two step immobilization strategy which engages an initial rapid ionic adsorption of the Ab followed by a much slower covalent attachment of Ab in an orient way [37].

Imine Interactions:

This is the most promising strategy, because the interaction has a possibility to effect attachment to the Fc region of the antibody. The glycan moieties on the Fc region of the antibody can be oxidized to aldehyde using the common oxidizing agents like periodates (NaIO₄). The oxidation product can be easily reacted with the amine functionality of any reacting species to form a conjugate [38].

Antibody disulfide cleavage:

The cleavage of the antibody with enzymes like papain may help in conjugation, but it can cause changes in the tertiary structure of the antibody. Proteolytic enzyme Papain attacks the disulfide bond and splits the Ab into 3 fragments of equal size.

Two of these fragments are Fab- Fragments, which are identical and are able to bind antigens; the third fragment is Fc-Fragment which is different and is not capable of antigen binding. It results in reduced reactivity and conformational changes in the molecule’s tertiary structure [38]. (Figure 1).

Diagnostic Application:

In 2006 a global survey was conducted by the European Science and Technology Observatory. It shows that more than 150 companies are developing nanoscale therapeutic applications. To the date of this writing 24 nanotechnology-based therapeutic products have been approved for clinical use, with total sales exceeding 5.4 billion. Liposomal drugs and polymer–drug conjugates are the two dominant classes, accounting for more than 80% of the market.

Cell sorting and bioseparation are the main applications for the AbNP conjugates. Magnetic nanoparticles (MNPs) coupled with murine monoclonal antibodies specifically for human epithelial cells allow the identification and selection of a population containing particular tumour cells [39].

Gold nanoparticles have been conjugated to anti-prostate specific antibodies and can be used to quantify the amount of prostate specific antigen (FDA approved biomarker for prostate cancer diagnosis) [40].

Anti-Her-2 monoclonal antibody conjugated polymer fluorescent nanoparticles (PFPNPs) have been used to detect ovarian cancer cells with fluorescence microscopy imaging technology [41]. Anti-Her2 antibody conjugated superparamagnetic iron oxide nanoparticles have been used to target breast cancer cells. Targeted breast cancer cells can be detected by using magnetic relaxometry. Superconducting quantum interference device (SQUID) sensors are used in magnetic relaxometry. They are more specific and faster than mammography, since they detect only those particles bound to the targets, eliminating problems associated with signals from unbound particles [42].
Antibody conjugates- silver nanoparticle are often used as optical or electrochemical markers in applications like immunochemistry, lateral flow tests, biosensors and immunoassays [43].

Therapeutic Application:

The important and most desirable quality of nanoprobes is their functionalization for targeting specific molecular sites. It has been reported that conjugating the nanoparticles with antibodies is the most efficient way to target specific markers or onc proteins which are known to be overexpressed in the malignant cells. An example is the fabrication of engineered iron oxide magnetic nanoparticles (MNPs) functionalized with anti-human epidermal growth factor receptor type 2 (HER2) antibodies to target the tumour antigen HER2 [44].

Antibody-nanoparticle conjugates have been used widely in targeted drug delivery. For example, Polycefin has been synthesized with poly-(malic acid)-based nanoparticles. Two different monoclonal antibodies are covalently coupled to the same polycefin loaded nanoparticle. First, monoclonal anti-TFR is directly conjugated across the blood-tumour barrier, followed by monoclonal zC5 to target tumour cell surface-bound nucleosomes [45].

There is a potent application of Ab-NP in gene delivery and tissue repair. Four types of carriers are used to internalize DNA into cell- cationic lipid viral carriers and polymers, inorganic nanoparticles and recombinant protein. For targeting cell delivery, some of these carriers may be conjugated with antibody or Fabs [46].

Epidermal growth factor receptor (EGFR) targeted nanoparticles are developed by conjugating a single-chain anti-EGFR antibody (ScFvEGFR) to surface functionalized quantum dots (QDs) or magnetic iron oxide (IO) nanoparticles. ScFvEGFR can be successfully conjugated to nanoparticles for the targeted delivery of a therapeutic agent in lung cancer treatment [47].

DNA CONJUGATION METHOD:

DNA nanoparticle binding can be affected by groove binding complementary single strand DNA binding and electrostatic interactions [48]. DNA conjugated nanoparticles utilize complementary electrostatic interactions to promote high affinity of nanoparticle-DNA binding. Cationic ligand attached to the nanoparticle surface provides a complimentary surface for binding the negatively charged DNA [49].

DNA Sandwich hybridization assay (cross linking GNP aggregation assay) with DNA-GNP Conjugate:

This method involves the attachment of noncomplementary DNA oligonucleotide capped with thiol groups on the surface of two batches of 15nm GNPs. Then the DNA, which is complementary to the two grafted sequences, is added to dispersion, and a polymer network is formed. The condensed network of conjugated GNPs self assembles into aggregates associated with red to purple colour change. This cross linking method of DNA-Nanoparticle conjugate is used to detect DNA target sequence [50].

Rapid synthesis of stable and functional conjugates of DNA/gold nanoparticles:

In this method DNA conjugates to the gold nanoparticle in 2-3 h using tween 80 as a protective agent. Tween 80 helps to maintain the stability of functional DNA/AuNPs, temperature, pH, and freeze-thaw cycle. The DNA density on the surface of AuNPs is determined through a fluorescence based technique. DNA conjugated gold nanoparticles have broad application in medical diagnostics, biosensors and biological analysis [51].

Diagnostic Application:

DNA conjugated nanoparticles are used in the detection of long term transgenic activity in brain using bioluminescence imaging. BLI has been used in neurologic diagnosis to track stem cells and monitor the growth of implanted or spontaneous brain tumours [52].

Conjugation of DNA conjugate with iron oxide nanoparticles through maleimide coupling has been used in MRI and fluorescence-active imaging agents to diagnose several diseases [53]. SPION can be designed to serve as a novel dual purpose probe for the simultaneous delivery of DNA and siRNA to diagnose tumour cells [54]. DNA conjugated silver nanoparticles (AgNP) are used in the quantitative diagnosis of HIV DNA by using the sandwich method based on the strong Plasmon resonance scattering signals [55].

Therapeutic Application:

The recent successes of nanoparticle therapeutics have raised the interest of academic and industry investigators in the field of nanomedicine. This has resulted in the development of more complex nanoparticle systems over the past decade. These nanoparticles have shown therapeutic potential in almost every branch of medicine such as endocrinology, neurology, ophthalmology, cardiology, immunology, orthopaedics, pulmonary, and oncology.

DNA aptamers (have been used in targeted drug delivery. DNA aptamers conjugated on the surface of PEG-PLGA nanoparticles through an EDC/NHS technique are used for the treatment of infiltrative brain tumours such as gliomas [56].

DNAzyme conjugated iron oxide nanoparticles are used for the treatment of hepatitis C by inducing knockdown of the hepatitis C virus (HCV) gene, NS3. DNAzyme is an oligonucleotide which allows the cleavage of mRNA in a sequence-specific manner,
thus silencing the target gene [57]. Catalytic DNA molecules (DNAzymes) are conjugated to gold nanoparticles to regulate gene expression through an RNAi independent mechanism for the treatment of cancer. A gold nanoparticle extends the half-life of DNAzymes and protects them against nuclease degradation [58].

**LIPID-CONJUGATED NANOPARTICLES:**

Liposomes are small vesicles composed of a phospholipid bilayer that is able to encapsulate the active drug. Liposomal-encapsulated drugs are characterized by increased solubility, improved therapeutic index, prolonged duration of exposure, and selective delivery of entrapped drug to the site of action [59]. (Figure 2).

Lipid-polymer conjugated nanoparticles with a lipid bilayer or multilayer shell are prepared by a two-step synthesis method. Liposome is prepared by a sonication procedure, and a polymer core is formed by a high pressure homogenization and nanoprecipitation method. A thin lipid film is formed by evaporating the organic solvent, then an aqueous solution is added to rehydrate the lipids; after this the polymer nanoparticles are mixed together with liposomes by high speed vortexing or an extrusion method. Finally, the lipid film fuses onto the surface of the polymer core to form lipid-polymer conjugated nanoparticles [60].

Fig. 2 Diagram showing method and application of lipid conjugated nanoparticles.
Diagnostic Application:
Lipid conjugated nanoparticles such as liposomes. Micelles have been used as a multi modal MR contrast agent. Lipids are amphiphilic molecules with hydrophobic and hydrophobic sides. MR detectable and fluorescent amphiphilic molecules can be simply integrated with lipid conjugated nanoparticles [61].
Poly (lipid) - coated, highly luminescent silica nanoparticles have been used as fluorescent probes for biolabelling and cellular imaging [62].
Lipid polymer conjugated nanoparticle can be used for delivery of imaging agents like quantum dots (QDs) and iron oxide by encapsulating them inside the polymer core [63].

Therapeutic Applications:
Folic acid conjugated nanoparticles of mixed lipid monolayers are used in controlled and the targeted delivery of anti-cancer drug, Docetaxel (Taxotere). Docetaxel controls the targeting effect by adjusting the lipid component ratio of the mixed lipid monolayer and provides versatile targeting [64].
Transferrin (Tf) conjugated lipid coated PLGA nanoparticles have been used in the targeted drug delivery of aromatase inhibitors in the treatment of breast cancer. The specificity and efficiency of therapeutic delivery of aromatase inhibitors are improved by Tf-conjugated lipid-coated PLGA nanoparticles [65].
Lipid conjugated nanoparticles can be used in the targeted delivery of radiotherapy and chemotherapy agents for the treatment of prostate cancer. In this dual targeted delivery system, first anticancer drug docetaxel (encapsulated inside the polymer core by nanoprecipitation) and then radioisotope Indium are used [66].
Lipid drug conjugate nanoparticles are used in the targeted delivery of hydrophilic antitrypanosomal drug diminazene diaceturate to the brain for the treatment of sleeping sickness, which is caused by trypanosome brucei grambiense [67].
Lipid based emulsion nanoparticles are used in drug delivery. Currently BCS class II and class IV drugs are widely used in delivery systems because oral nanoemulsion develops bioavailability by increasing the solubility of hydrophobic drugs. It uses harmless edible materials such as GRAS-grade excipients and food-grade oils [68].

PROTEIN-CONJUGATION METHOD:
The selection of the protein bioconjugation method depends strictly on the physicochemical and biochemical properties of protein and nanoparticles. The most recent technique for coupling covalently nanoparticles to protein is based on the existence of specific and reactive functional groups on proteins such as amino–NH₂ (lysine), carboxylic acid–COOH (aspartic, glutamic), hydroxyl–OH (serine, tyrosine) and –SH (cysteine) [69].
Proteins can be chemically coupled according to different types of nanoparticles by using reagents such bifunctional cross-linker molecules.

Nanoparticles are classified according to functional groups such as carboxylic acid, hydroxyl, sulfydryl and amino groups. There are three common types of bioconjugation chemistry: (a) amine coupling of lysine amino acid residues, (b) sulfydryl coupling of the cysteine residue, and (c) photo chemically initiated free radical [70].
The covalent bioconjugation process can be summarized in: 1) Grafting the nanoparticles by the active functional groups. 2) Chemical activation of thiol groups on the protein side with a specific reductive agent. 3) Total removal of the excess reduction agent. 4) Post conjugation procedures such as removal of unbound protein/remnant excess. The disadvantage of the long experimental procedure of covalent bioconjugation is that it can affect protein structure and function, resulting in its partial denaturation [16]. (Figure 3).

Diagnostic Application:
Surface modification of superparamagnetic contrast agents with HIV-1 tat peptide is capable of intracellular magnetic labelling and non-invasive tracking of a large number of cell types with MRI [71].
Protein chips and nanomaterials can be used in tumour marker immunoassay. Electrochemical immunosensors have high sensitivity, and easy automation and miniaturization for the detection of tumour markers. Tumour markers play an important role in diagnosis and provide insights into the aetiology of cancer [72].
Supramolecular protein nanoparticles can be used in ultrasensitive detection of early markers of Type I diabetes during the early phase of pancreatic β-cell destruction [73].
Casein conjugated nanoparticles can be used as electrochemical biosensors for pathogenic bacteria detection [74].

Therapeutic Application:
Hyperlipidemia. This is a condition characterized by the lack of low density lipoprotein receptors in the hepatocyte. In this in vitro method biocompatible and biodegradable polylactide nanoparticles are covalently linked with apolipoproteinB-100 prior to their use for the treatment of hyperlipidemia [75].
Alzheimer. Curcumin is a polyphenolic compound. Coupling of curcumin encapsulated PLGA conjugated nanoparticles with Tet-1 peptide is used in the treatment of Alzheimer, because they have an affinity to neuron and have a retrograde transportable property. Curcumin encapsulated PLGA nanoparticles have an affinity to damage
amyloid aggregates demonstrating a noncytotoxic effect and antioxidative properties [76].

Cancer Therapy. Chlorotoxin bound SPION (superparamagnetic iron oxide nanoparticle) is currently being evaluated for application in cancer therapy and imaging. SPION of narrow sizes can be easily produced and coupled to proteins permit easy delivery into the cells [77].

Inflammatory Diseases. G protein-coupled adenosine receptors conjugated gold nanoparticles can be used in the treatment of cancer. A typical application is conjugation to gold carriers of small molecules (nonpeptide) GPCR, which are under examine for the treatment of cancer and inflammatory diseases [78].

CONCLUSIONS:

The application of nanotechnology to drug delivery has shown a significant impact on many areas with therapeutic applications. More than 20 nanoparticles currently are in clinical use, validating the ability of nanoparticles to improve the therapeutic index of drugs. Bioconjugation is presently a highly attractive platform for a diverse array of biological applications. Bioconjugated nanoparticles are undergoing rapid and significant development, thus making a wide variety of applications possible, especially in in vitro and in vivo diagnosis. Bioconjugated nanoparticles have great potential to convert poorly soluble and labile biologically active substance into promising deliverable drugs. However, greater understanding of the different mechanisms of biological interactions and particle engineering is still required. Further advances are needed to transform the concept of nanoparticle technology into a realistic practical application. It is expected that with the introduction of bit specific nanomaterials together with novel engineering approaches that result in optimally designed nanoparticles, it will be possible to increase the number of multifunctional nanoparticles that enter clinical usage in future. Overall, with continued research and development efforts, nanotechnology is expected to have a tremendous impact on medicine for decades to come.

![Fig. 3 Method and Application of protein conjugated nanoparticles.](image-url)
Acknowledgement:

The authors are indebted to M. Arrbueo, Universidad de Zaragoza, Spain, for providing some important literature and the comprehensive review of this manuscript. We also wish to express our thanks to Avi Domb, Hebrew University of Jerusalem, Israel for their critical comments and the comprehensive review of this manuscript. Mukesh Doble, IIT, Madras, Krishna Pramanika, NIT Rourkela, and Madhusudhan, M. C, CFTRI, Mysore for their critical comments and suggestion.

References