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Various methods of gold nanoparticles (GNPs) conjugation to antibodies

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ABSTRACT

The unique properties of gold nanoparticles, their rich surface chemistry, and low toxicity as well as easy methods of synthesis have promoted conjugation of the particles with numerous biomolecules for site-specific delivery. Gold nanoparticles have multiple applications including photoablation, diagnostic imaging, radiosensitization, vaccine development, antioxidant, and multifunctional drug-delivery vehicles.

These applications require an increasingly complex level of surface decoration in order to achieve efficacy, and limit off-target toxicity. This review will discuss the chemical and physical approaches commonly utilized in relation to surface decoration and the powerful system used to indicate success of the conjugation. Finally, we review the range of recent studies about covalent and noncovalent modes for conjugation of antibodies to the particle surface that aim to advance gold nanoparticle treatments and diagnostics toward the clinic.

Contents

1. Introduction

Nanotechnology suggests unique approaches to detect and regulate diversity of biomedical processes that take place at nanometer scale, and is expected to have a fundamental effect on biology and medicine. Nanoparticles the size of which falls into the range of biological molecules and structures have attracted much attention in recent years for their potential applications in biomedical research. Useful features can be incorporated into nanoparticles for manipulation or detection of biological structures and events [1,2]. (See Tables A.1 and A.2.)

Different characteristics of nanoparticles such as size, shape and surface charge have all been shown to strongly influence therapeutic and diagnostic efficiency by changing cell uptake and functional surface area. Nanoparticles are defined as particles between 1 and 1000 nm that have a range of unique properties including surface chemistry,

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Table A.1
Physical and chemical interactions between antibodies and gold nanoparticles surface in bio conjugation process.

Physical interactions	Chemical interactions
• Ionic interactions: between positively charged groups in antibodies and the negatively charged surface of the gold nanoparticles • Hydrophobic interactions: between hydrophobic parts of the antibody and the gold nanoparticles surface • Dative binding: between the gold conducting electrons and amino acid sulfur atoms of the antibody	• Chemisorption via thiol derivatives • Bifunctional linkers or mediator linkers: EDC/NHS chemistry • Adapter molecules: like Streptavidin andbiotin

size, shape-dependent electronic, and optical properties that support a variety of applications containing drug and gen delivery [3], bioimaging [4,5], vaccine development [6],biosensors, and therapies [7–11].

Bioconjugation simply involves the bond of biomolecules to nanoparticles by chemical or biological means, which render them ideal for clinical applications; it includes the conjugation of biologically active molecules to nanoparticles. The outcome is the combinations of beneficial properties [12].

The conjugation of different functionalized groups to nanoparticles is necessary for their stability, functionality, and biocompatibility and develops their application fields, and provides them with novel and improved properties [13]. A range of functionalized groupscan be attached to the nanoparticles including low molecular weight ligands [14], peptides [15,16], proteins [17], polysaccharides [18], polyunsaturated and saturated fatty acids [19], DNA [20], plasmids, and siRNA [21–24].

2. Unique synthetic properties of gold nanoparticles (GNPs)

By using simple and biocompatible chemistry, it is possible to obtain mono dispersed samples in the 5 nm to 50 nm size range [25]. This review will describe the various interactions and modes used to functionalize GNPs, and will provide a detailed overview of recent developments in bioconjugation.

2.1. Gold nanoparticles

Gold nanoparticles have been often studied due to their unique surface, chemical inertness, high electron density, and strong optical absorption. In recent decades, gold nanoparticles have been applied in genomics [3,26], clinical chemistry [27], vaccine development [28,29], immunoassay [30], biosensors [31], diagnosis, and micro organisms control [32], cancer-cell imaging, and drug delivery [33–35]. In addition, it has been reported that GNPs conjugated by prostate specific membrane antigen (PSMA) RNA aptamer after loading of doxorubicin can be useful as therapeutic agents for diagnosis and treating of prostate cancer [4]. It has also been reported that it is possible to control the interactions of GNPs with cell membranes in order to improve their cellular uptake while minimizing their toxicity byrigid change of their surface charges densities [36]. GNPs conjugated by PEG and antibodyhave also been used as effective agents for plasmonic photothermal therapy [37]. To develop observation of GNP based therapeutics or diagnostic agents, the surface decoration is principally esteemed necessary. Surface decoration or conjugation is commonly used to improve drug loading, decrease immunogenicity, decrease aggregation or increase stability, improve cellular uptake, actively target cancer cells, and tumors. The ligand molecules bound to the nanoparticles surface not only control the growth of the particles during synthesis, but also prevent the aggregation of the nanoparticles. The repulsive force between particles can, in principle, be due to electrostatic repulsion, steric exclusion, or a hydration layer on the surface [24].

The intricacy issues are associated with the conjunction of functional groups to gold nanoparticle surface and confirmation of bioconjugation.

Table A.2
Non-covalent and covalent modes between antibodies and gold nanoparticles surface in bioconjugation process.

Non-covalent modes	Covalent modes
• Ionic interactions • Hydrophobic interactions	• Chemisorption via thiol derivatives • Bifunctional linkers or mediator linkers: EDC/NHS chemistry • Adapter molecules: like Streptavidin andbiotin that formed the complex • Dative binding

2.2. Chemical synthesis of GNPs

A pointed understanding of the chemistry is needed to rightly synthesize and functionalize GNPs, and develops successful nanoparticle diagnostics and therapeutics. Gold nanoparticles have unique synthetic properties [25].

The most routine protocol for GNP synthesis is the Turkevich method [38]. Briefly, this method can be characterized as the reduction of gold chloride with sodium citrate. The gold nanoparticles have an absorption maximum at 529 nm, and TEM shows an average size of gold nanoparticles [38,39]. Other protocol for GNP synthesis is the Czech Patent No method. Briefly, chloroauric acid ($HAuCl_4$) was heated to boiling point. Then, aqueous solution of 1%trisodium citrate was added to the $HAuCl_4$ solution under rapid stirring and the GNPs were prepared by crystallization. The citrate ions added as reducing agent for GNPs formation and asstabilizing agent, preventing aggregation or agglomeration of GNPs [33]. The protocol for preparation of gold nanoparticles is wet chemical methods which are based on the reduction of gold salts by reagents such as sodium borohydride and ascorbic acid [40]. Penades prepared gold nanoparticles by reduction of Au(III) to Au(0) by $NaBH_4$ in the presence of a mixture of thiols [41].

The method reported by Wang and coworkersinvolves using atmospheric-pressure non-thermal microplasma for the synthesis of gold nanoparticles. Electrolyte solution consisting of Chloroauric acid ($HAuCl_4$) and sodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) in an electrochemical cell at room temperature was exposed to the microplasma. Once the microplasma was inflamed, the color of the $HAuCl_4$ solution altered gently from light yellow to pink, which displayed the formation of GNPs [42].

2.3. Conjugation of GNPs

If the aim is to use gold nanoparticles in biomedicine as diagnostic and therapeuticagents in cells or tissues, then, it is necessary to rightly choose the targeting component such as a monoclonal antibody (mAb), and the strategy to attach it on the surface of the particle [43].

GNPs conjugated byantibody orother functionalized groups have also been used as effective agents for diagnostic and therapeutic applications [37]. Physical and chemical interactions are used for attaching antibodies and other molecules to GNPs surface.

Physical Interaction between antibodies and gold nanoparticles depends on three phenomena: (a) ionic attraction between the negatively charged gold and the positively charged antibody; (b) hydrophobic attraction between the antibody and the gold surface; (c) dative binding between the gold conducting electrons and amino acid sulfur atoms of the antibody. Chemical interactions between antibodies and nanoparticle surface are achieved in the number of ways like (i) chemisorption via thiol derivatives; (ii) through the use of bifunctional linkers (iii), and through the use of adapter molecules like Streptavidin andbiotin [12,44].

For conjugation of antibodies to the gold particles, both covalent and non-covalent immobilization modes have been used [45]. The antibodies or other functionalized groups are nonspecifically adsorbed onto gold nanoparticles while still keeping the nanoparticles negatively charged, providing stability in colloidal solution [37]. In other words, the bioconjugation protocol uses non-covalent modes of binding based on a combination of electrostatic and hydrophobic interactions of the antibody and the gold surface [40]. Non-covalent technique is described as spontaneous absorption of antibodies onto the surface of citrate stabilized nanoparticles; there are several types of interactions that may occur in this process: hydrophobic interactions, ionic interactions and etc. Hydrophobic interactions are due to attraction between hydrophobic parts of the antibody and the metal surface that result in the formation of a non-covalent bond.Positively charged groups are abundant in antibodies i.e., positively charged amino acids and the N-terminal are present. Ionic interactions are formed between these groups and the negatively charged surface of the particles [44].In recent years, Raja Gopal Rayavarapu used this method to bind mouse monoclonal antibody specific to Human EGF Receptor 2 (HER2) to gold nanoparticles which were then used as contrast agents for optical imaging techniques ofbreast cancer [40]. Zhang and coworkers used non-covalent mode forconjugation of 5-aminolevulinic acid to gold nanoparticleswhich were then used for photodynamic therapy of cancer [46].

Sokolov used this method to bind *anti*-EGFR to gold nanospheres which were described as new class of molecular specific contrast agents for Real-Time Vital Optical Imaging of ovarian cancer cells based on gold nanoparticles [47]. El-Sayed also used non-covalent modes to bind *anti*-EGFR to gold nanoparticles in molecular biosensor techniques for the diagnosis of oral cancer cells in vivo and in vitro [39].

In the work by Peng Chen and coworkers, the antibody adsorbed onto the gold nanoparticles through a combination of ionic and hydrophobic interactions which were then used as electrochemical label [48].

Hydrophobic and ionic interactions between antibody and gold nanoparticle surface are illustrated in Fig. A.1.

Non-covalent modes have several major weaknesses; these include the necessity of a high concentration of antibodies for the preparation of antibody–gold particle conjugates, random orientation of antibodies at the gold nanoparticle surface, and due to their electrostatic attraction they are making the biological response difficult to control; the binding is impressed by changes in pH, and ultimately because antibodies are non-covalently conjugated to nanoparticles; they can be replaced by other molecules in biological samples [45].

Covalent modes are also used to bind functionalized groups to gold nanoparticle surface [45,49]. Gold nanoparticles can be used for direct conjugation with thiol group-containing bio-molecules such as antibodies, and other biomolecules. Dative binding is a physical interaction that may occur between antibody and GNPs surface. It is the formation of a covalent bond between the gold nanoparticle and free sulfhydryl groups of the antibody [44,51].

Covalent modes are also used to bind antibodies directly to the surface of nanoparticles; These modes are used as a mediator linker, or can take place via adapter molecules like avidin and biotin for the formation of the complex [45]. Functional groups present on the nanoparticle surface can be converted to other functional groups by bifunctional molecules. Particularly, in the case of nanoparticles dispersed in an aqueous media, the reaction conditions may harm the stability of the nanoparticles, so often rather mild reactions have to be selected like the ones applied for bioconjugation chemistry and a large number of bifunctional molecules are commercially accessible. Usually found carboxylic groups can be reacted with primary amines by means of a condensation reaction to yield amide bonds. For this reason, a water-soluble carbodiimide (e.g. EDC) is usually used. After forming an intermediate compound with the carboxylic moiety, the activated group is reactive toward primary amines. In the case of primary amines present on the particle surface, active ester compounds (*N*-hydroxy-succinimide; NHS) can be used to equally form amide bonds [24].

Thiolated polyethylene glycol (PEG-SH) is used to coat bare gold nanoparticles surfaces to decrease nonspecific interactions. The hydrophilic nature of PEG also increases the biocompatibility of the conjugate.

The PEGylated gold nanoparticles used were carboxyl terminated, providing a chemical group suitable for covalent binding. Although carboxyl groups do not spontaneously form bonds to antibodies, they can be chemically modified to serve this purpose. The coupling chemistry was EDC/NHS chemistry, which provides a covalent bond without the addition of a spacer. Upon exposure of EDC/NHS to the carboxyl groups reactive NHS esters are formed. When a primary amine group in an antibody (or another protein) comes in contact with the ester, a covalent bond is formed [52,53]. This reaction is illustrated in Fig. A.2.

The covalent binding of the thiol group to gold is used generally for nanoparticle surface decoration. Liao used long chainsuccinimidyl 6-[3′-[2-pyridyldithio]-propionamido] (LCSPDP) cross-linker hexanoate to bind antibody to gold nanorods. The hetero bifunctional cross-linker LC-SPDP was used to conjugate secondary antibodies to the nanorod surface before PEGylation.LC-SPDP consists of a pyridildithio group that binds to the gold nanorod surface, anda NHS ester which binds primary amines in the antibodies [54]. In the work by Loo and coworkers the *anti*-HER2 antibody was bound to a polyethylene glycol (PEG)linker

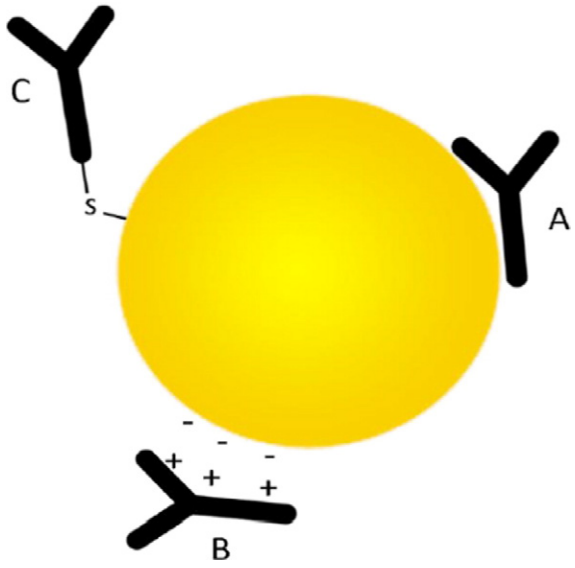

Fig. A.1. Hydrophobic and ionic interactions between antibody and gold nanoparticle surface. A) hydrophobic interaction B) ionic interaction C) a covalent bond is formed due to dative binding [25].

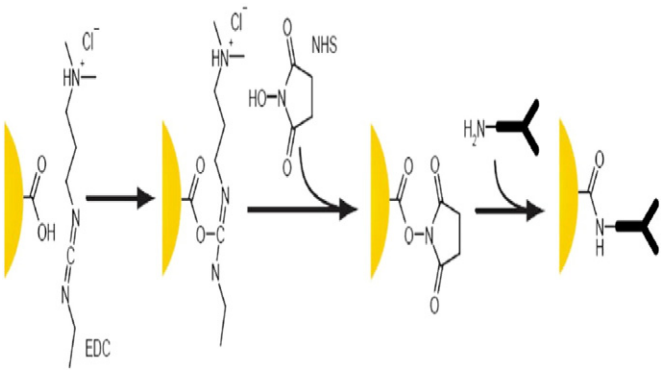

Fig. A.2. An antibody is coupled to a PEGylated gold nanoparticle through EDC/NHS chemistry. A reactive ester is created from a carboxyl group. Primary amines present in an introduced antibody react and covalently bind to the ester, without addition of a spacer [20].

[orthopyridyl disulfide-polyethyleneglycol-*N*-hydroxysuccinimide (OPSS-PEG-NHS), molecular weight of 2000] through a hydroxysuccinimide group(NHS). The antibody–PEG linker complex was then attached to the gold nanoshell surface through a sulfur containing group located at the distal end of the PEG linker [55]. Shamsipur and coworkers used this method to bind gold nanoparticles to multiwall carbon nanotubeionic liquid electrode, which were then used as a novel impedimetric immunosensor for low level detection of human serum albumin in biological fluids. For activation of colloidal gold nanoparticles (GNPs), GNPs solution was added to *N*-hydroxy succinimide (NHS) containing *N*-ethyl N-[3-dimethylaminopropyl] carbodiimide (EDC). The prepared GNPs/HDT/GNP@MW-CILE electrode was finally immersed into the antibody solution (Ab) to obtain Ab/GNP/HDT/GNP@MW-CILE electrode [50]. Xia and coworker used succinimidyl propionyl polyethylene glycoldisulfide (NHS-activated PEG) to conjugate antibodies to the surface of a gold nanocage. NHS-activated PEG was reacted with the primary amine of antibody; then, the PEG-antibody complex was bonded to the gold nanocage by breaking its internal disulfide bond and forming an Au-S linkage as shown in Fig. A.3. [56].

1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide, EDC is using as a mediator linker for conjugating antibody and other molecules to GNPs via covalent bonds.

Conjugation is achieved by forming a peptide bond between N-terminal primary amine of the protein and carboxylic acid groups of negatively charged GNPs utilizing 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide, EDC [57,58].

Dam and coworkers used covalent modes for attachment of oligonucleotides with secondary structure on gold nanoparticles. Nucleic acids thiolated to GNPs surface at pH = 1.7 because repulsion between the negatively charged oligonucleotides and the GNPs surface decreases [59]. Avidin–biotin complex also uses covalent modes to bind antibodies to GNPs [45,60]. Biotin is a molecule attached to avidin/streptavidin glycoproteins with high affinity, selectivity and specificity that is an example for the molecular key-and-lock system in biological processes as shown in Fig. A.4. This method has a major shortcoming that with increasing biotinylation nanoparticle surface becomes more and more hydrophobic and the biotinylated nanoparticles precipitate gradually in aqueous solution [61–64].

Carbodiimide chemistry enables covalent immobilization of antibodies to PEGylated gold nanoparticles. The covalent immobilization assembles a stronger bond and a more stable conjugate than physisorption [44].

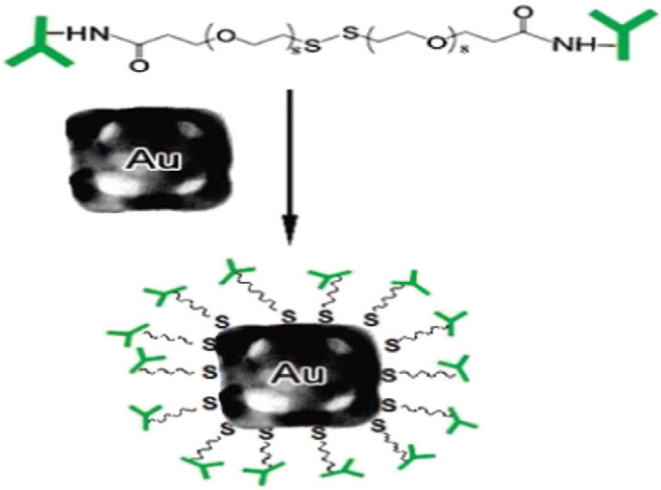

Fig. A.3. the NHS linker is used to conjugate antibodies to the surface of a gold nanocage [56].

2.4. Method and instruments for the confirmation of bioconjugation GNPs

Sol particle immunoassay(SPIA) relies on two important properties of gold sols: their characteristic bright red color, which remains approximately unchanged on adsorption of high-molecular weight compounds onto gold particles, and the noticeable change in the absorption spectrum, and thus in the sol color that attends particle aggregation; this change in the absorption spectrum is easily detected spectrophotometrically or visually. If the bio specific reaction continuation on the colloidal particle surface results in sol destabilization, the gold particles aggregate when polyclonal antibodies are used, and the process is called agglutination leading tofundamental changes in the absorption spectrum with a noticeable color change from red to blue or gray. It is possible to use the widely available spectrophotometers and colorimeters to assess the reaction outcome [66]. Several techniques were used to measure the amount of bound conjugatethat is colorimetry and carbon rod atomic absorption spectrophotometry (CRAAS) [67].

Longitudinal SPR is a tool for confirmation of biomoleculars conjugation to gold nanoparticle surface.Lowvariations in the environment around the GNRs can induce significant changes in the longitudinal SPR (LSPR) peak wavelength, which is much more sensitive than the transverse SPR (TSPR) peak wavelength observed from gold nanoparticles [68].

Leuvering used carbon rod atomic absorption spectrophotometry(CRAAS) to measure the bound amount of silver and goldconjugation [67].

Jans and coworkers used Dynamic Light Scattering as a powerful tool for confirmation of biomoleculars conjugation to gold nanoparticle surface by measuring the average particle size change of the assay solution [69].

Atomic force microscopy is a powerful tool for the structural investigation of biomolecular conjugation to GNPs surface and analysis of bioconjugation-based nanostructures [70].

Zhang used UV–Visible (Vis) spectrophotometer to measure the absorbance spectrum of the final solution and compare to the absorbance spectrum of the primary solution to investigate for possible aggregation of the solution [46]. Great changes in aggregation will make a color shift from red to blue or gray that is observable by eye [45]. The conjugation of GNPs can beconfirmed by using a transmission electron microscopy, and a particulate size description analyzer [46]. Confocal microscopy is also used for confirmation of bioconjugation [40]. The conjugation efficiency of the GNPs was confirmed qualitatively and quantitatively through gel electrophoresis, and critical flocculation concentration analysis [71].

3. Conclusion

Naturally, gold nanoparticles play a key role in future medicine by providing unique properties like rich surface chemistry, low toxicity, high electron density, and strong optical absorption. Understanding the bioconjugation, chemistry of functionalization of GNPs and detection system of conjugation, opens novel opportunities for their use in biomedicine. This review article mainly focuses on the chemical and physical interactions of bioconjugation on the surface of gold nanoparticles. These interactions discussed in this article formulate gold nanoparticles for various biological applications. The large surface to volume ratio is the unique property, which is useful for the conjugation of gold nanoparticles. Although multifunctional gold nanoparticles constructed by the conjugation of various targeting molecules are extensively investigated in the imaging and treatment of cancer cells and tumors, controversial reports about toxicological issues of functionalized nanoparticles and shortcoming in conjugation limit the clinical applications of GNPs formulations. Therefore, a number of issues such as in vivo and in vitro targeting efficiency, and toxicological issues of functionalized gold nanoparticles are under rigorous investigation.

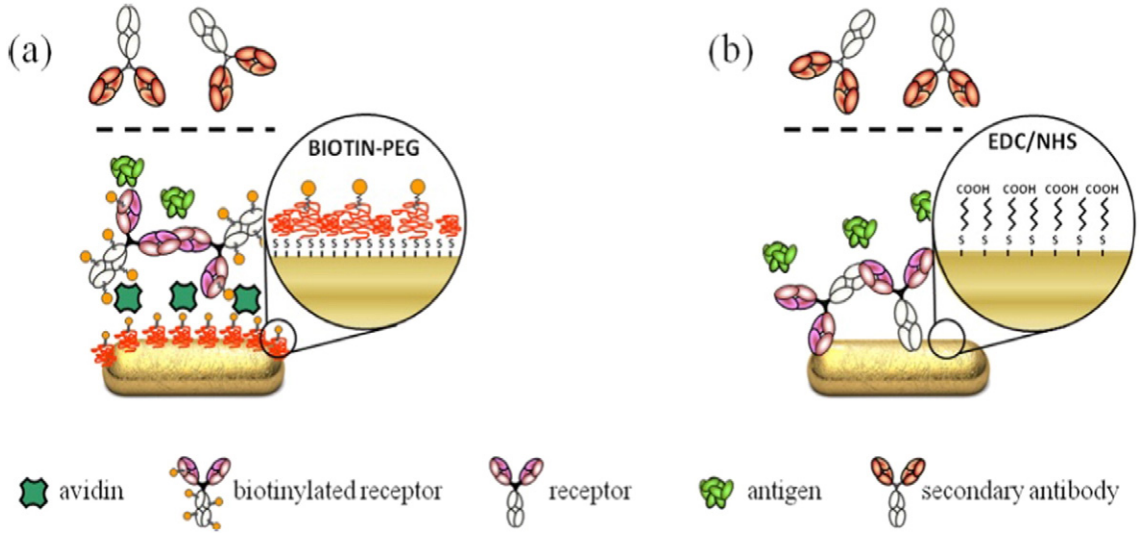

Fig. A.4. covalent modes of bioconjugation(a) a biotin–avidin is used to link the receptor and (b) carbodiimide chemistry is used for bioconjugation [65].

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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