Bio-Functionalized Metallic Nanoparticles with Applications in Medicine

30

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Contents

Abstract

Due to their special physicochemical, optical, and biological properties, noble metal nanoparticles have huge potential for application in many different biological and medical areas, such as highly sensitive diagnostic assay, thermal ablation, radiotherapy, or carriers for drugs and gene delivery. This chapter selectively reviews the bio-functionalization of metallic nanoparticles and their recent applications in medicine. The chapter is divided into four sections: "Introduction," "Bioconjugation of Metallic Nanoparticles," "Cancer Therapy," and "Gene Delivery." After a short introduction, we present few general strategies for bioconjugation of metallic nanoparticles: physisorption, physisorption using mediator molecules, covalent binding of biomolecules to cross-linkers, covalent binding of biomolecules to nanoparticles, and linking of biotinylated biomolecules to streptavidin-functionalized nanoparticles. The third section presents the recent advances in cancer therapy based on two strategies: passive targeting and antibody targeting, using functionalized gold nanoparticles. The fourth section describes the gene delivery process, by which *foreign DNA* is

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introduced into the host cells. The process typically involves the formation of transient pores or "holes" into the cell membrane, which allows the uptake of foreign material. The main aspects that are discussed about the gene delivery process are the *stealth character* and the *targeted recognition* of tissues.

Keywords
Bio-functionalization • Cancer therapy • Gene delivery • Metallic nanoparticles

Introduction

Therapeutic applications of nanoparticles are ranging from antimicrobial action gene, drug, and vaccine delivery to cancer therapy. Cancer, a complex disease that involves unregulated cell growth, is one of the leading causes of mortality in the modern world. Finding an efficient tool for treating cancer is a constant challenge for researchers. Effective treatments include surgery, radiotherapy, chemotherapy, hormone therapy, and immunotherapy. Chemotherapy – treatment with cytotoxic chemicals – is an efficient tool for cancer therapy, but the therapeutic efficacy of many drugs is seriously compromised by the low bioavailability and intrinsic toxicity, since most chemotherapeutics also kill the healthy cells. The synthesis of drugs that may differentiate the normal cells from the cancer ones is very difficult. Nanoparticles can be designed to overcome some of the disadvantages of the conventional therapies. The small dimensions of the nanoparticles allow them to pass through the weak membrane of the blood vessels, which supply the tumors, without the penetration of the healthy tissues. Therefore, by loading the particles with chemotherapy drugs, the tumor cells can be reached without damaging the healthy ones [1].

Noble metal nanoparticles (Au, Ag, or combination of both) have the potential to be used in many different biological and medical applications including highly sensitive diagnostic assay, thermal ablation, and radiotherapy enhancement [2] as well as carriers for drug and gene delivery [3]. Researchers are interested to study the intracellular traffic of nanoparticles and to determine the critical parameters for their efficient cellular uptake and retention.

Silver nanoparticles can be incorporated in several consumer products such as footwear, paints, cosmetics, and plastics due to their antibacterial properties. Since the size, shape, and composition of silver nanoparticles can significantly affect their efficacy, extensive research has been devoted to this field [4].

Gold nanoparticles have high chemical stability, good oxidation resistance, and biocompatibility and therefore can be used for diagnosis, therapeutic purposes, or as drug carriers. As in the case of silver nanoparticles, the properties of gold nanoparticles strongly depend on their size and shape. Therefore, there is an increased interest into the fabrication of gold nanostructures with specific shapes, through various methods [5, 6]. Although metal nanoparticles are innovative in therapy, imaging, and early diagnosis of several diseases, a special attention should be paid to their toxicity, due to their tendency to accumulate in the liver. For this reason, their removal from the body needs to be properly addressed.

A recent number of papers have dealt with the application of metal nanoparticles in medicine. Murthy reported about the role of nanoparticles in modern medicine and the environmental and social impact of their usage [7]. Tiwari et al. reviewed the recent advances in the field of functionalization of gold nanoparticles and their potential applications in medicine and biology [8]. Dykman and Khlebtsov wrote a critical review about the application of gold nanoparticles in biomedical diagnosis, photothermal and photodynamic therapies, as well as the delivery of target molecules [9].

Ravindran et al. reported about the synthesis of silver nanoparticles along with their antimicrobial and anti-inflammatory properties. In addition, they discussed the possibility to link various functionalities like protein- or nucleic acid-based recognition elements for bioconjugation of silver nanoparticles and for the application in the field of biomedicine [10].

Labouta and Schneider highlighted in a recent review the current applications of inorganic nanoparticles and discussed about the status of their skin penetration. They reported the results generated from experiments on human skin and include some recommendations for future research [11].

In this paper, we provide an overview on the bio-functionalization of metallic nanoparticles with potential applications in cancer therapy and gene delivery (see Table 1).

Bioconjugation of Metallic Nanoparticles

Bioconjugation is a procedure that links biomolecules to nanoparticles under mild (physiological) conditions [12]. Sometimes, nanoparticles cannot be directly attached to biomolecules, because their surface-chemical properties are not appropriate. In this case, they must be chemically changed so the bioconjugation reactions can successfully proceed.

Murcia and Naumann have presented few strategies to bioconjugate nanoparticles [13]. The main requirements, which must be fulfilled during bioconjugation, are as follows: (i) the biomolecule-nanoparticle link is stable in time; (ii) the biomolecular activity is preserved. In addition, it is of great interest to control the number of binding sites on the nanoparticle surface.

The easiest approach is via physisorption or noncovalent coupling, as shown in Fig. 1a. In this case, the biomolecular activity might be affected, and in addition, it is difficult to control the amount of bound molecules. A more complex approach is based on noncovalent coupling between biomolecules and nanoparticles, previously covered by a mediator, Fig. 1b. The presence of the mediator may help the binding of biomolecules in a proper orientation, which preserves the biomolecular activity.

A stronger coupling can be obtained for biomolecules with reactive groups like thiols or primary amines, which can be covalently bound with cross-linker molecules, as shown in Fig. 1c. In this case, the bond is more stable, but the multiple active sites on the target biomolecules might prevent a coupling with high specificity.

Table 1 Bio-functionalized metal nanoparticles and their potential applications

Type of nanoparticles	Potential applications	References
Bioconjugation of Au and Ag nanoparticles	Drug delivery, diagnostics, therapy, biosensors	[12–22]
Au nanoparticles	Radiofrequency thermal destruction of human gastrointestinal cancer cells	[32]
Au nanospheres and nanorods linked with thiol-functionalized PEG	In vivo/in vitro investigation of cancer therapy	[34–37]
Colloidal Au functionalized with PEG-SH molecules and recombinant human TNF-α	Treatment of cancer and indicator for immunodiagnostics	[42]
Au nanorods conjugated with anti-EGFR antibody or TNF-α protein	Therapy of cancer cells	[48–51]
Calsequestrin-functionalized Au nanoparticles	Detection of Ca^{2+}, useful in detecting and monitoring several diseases associated with hypercalcemia, such as malignant tumors	[52]
PEG-modified Au nanoparticles	Hepatocyte gene delivery and enhanced gene expression	[58]
Amine-functionalized Au nanoparticles	Intracellular delivery of small interfering RNAs	[59]
Au nanoparticles coated with lysine-based headgroups	Transfection vectors	[62]
Dendrimer-entrapped Au nanoparticles	Gene delivery	[64]
Au nanoparticles modified with 2-aminoethanethiol, 8-amino-1-octanethiol, and 11-amino-1-undecanethiol	Gene transfection	[65]
Poly-L-lysine with citrate-capped gold nanoparticles	Gene delivery	[66]
Au nanoparticles functionalized with single-stranded DNA	Deliver highly structured RNA aptamers into the nucleus of human cells	[67]
Au nanoparticles functionalized with cysteamine	Deliver unmodified microRNAs into living cells	[68]
Au nanoparticles functionalized with covalently attached oligonucleotides	Development of therapeutic and gene delivery systems	[69]

A facile approach is the chemical coupling of biomolecules directly to nanoparticles (Fig. 1d). This method is intensively used for attaching chemically modified oligonucleotides to nanoparticles, e.g., via thiol groups [14–16]. Finally, nanoparticles can be functionalized with streptavidin (or avidin) and then coupled with high specificity with biotinylated ligands or target biomolecules, like represented in Fig. 1e [17, 18].

The bioconjugation of metallic nanoparticles (gold, silver) is of high interest due to their multiple applications in medicine (drug delivery, diagnostics, and therapy) [19, 20] and biosensors [21, 22]. In particular, gold is extremely attractive due to its excellent biocompatibility. In addition, it does not require chemical modification

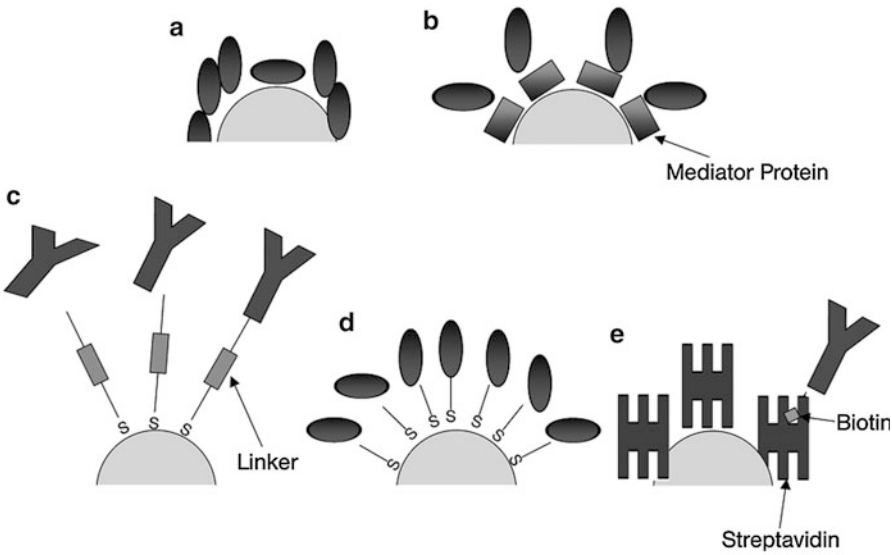

Fig. 1 Few strategies to bioconjugate nanoparticles: physisorption (**a**); physisorption using mediator molecules (**b**); covalent binding of biomolecules to cross-linkers (**c**); covalent binding of biomolecules to nanoparticles (**d**); high specificity binding of biotinylated biomolecules to nanoparticles functionalized with streptavidin (**e**) (Reproduced with kind permission from reference [13]. Copyright (2013) Wiley-VCH Verlag GmbH & Co. KGaA)

prior the bioconjugation. The nanoparticles of both metals can be prepared with very narrow size distribution, which is greatly advantageous for controlling the number of bound biomolecules. The biomolecules can be attached to these nanoparticles either by physisorption or by chemisorption.

Cancer Therapy

The recent advances in nanoscience and nanotechnology have evidenced the suitability of noble metal nanostructures in cancer therapy. This is due to their strong visible and near-infrared light absorption bands (*surface plasmon resonance band*, *SPR*) [23–28] which are more intense than those observed for the common laser phototherapy agents. As shown by Jain et al. [29], the absorption cross section of gold nanoparticles is up to five orders of magnitude stronger than that of rhodamine 6G dye molecule [30]. Based on these optical properties, a selective and efficient cancer therapy was developed, named *plasmonic photothermal therapy* (*PPTT*) [31].

In another study Gannon et al., [32] have reported that intracellular gold nanoparticles (GNP) favor the radiofrequency thermal destruction of human gastrointestinal cancer cells. Hep3B and Panc-1 cells were treated with 67 $\mu M \cdot L^{-1}$ GNPs and then exposed to external 13.56 MHz RF radiation. Both cell lines had markedly

higher rates of cell death than the control samples not treated with GNPs at all time points, as measured by PI-FACS ($p < 0.01$). In addition, they reported that cells treated with RF after a GNP dose of 1 $\mu M \cdot L^{-1}$ had no increased cytotoxicity compared with control cells grown only with media (no GNPs). Cells receiving 10 $\mu M \cdot L^{-1}$ GNPs had slightly, but not significantly, greater cytotoxicity compared to cells treated without GNPs.

Gold nanoparticles can be easily functionalized with tumor-targeting molecules. Two strategies are generally employed: the *passive targeting* and the *antibody targeting*. The *passive targeting* approach uses thiolated poly(ethylene)glycol (PEG) for functionalization of nanoparticles in order to increase their biostability and biocompatibility. This approach is also called *stealth technology* [33], and its main advantage consists in considerably reducing the interaction between nanoparticles and the immune system.

In order to obtain functionalized metallic nanoparticles with *stealth property* in a cellular environment, it is necessary to understand the interaction between protein and various surfaces. Generally, such interactions are noncovalent and include electrostatic, hydrophobic, and hydrogen bonding. Protein adsorption is most efficiently suppressed if the nanoparticle surface is neutral, hydrophilic, and highly dynamic. The functionalization of metallic nanoparticles with PEG chains strongly diminishes their interaction with proteins within the cellular environment. This is due to PEG molecules which are hydrophilic and electrically neutral (therefore hydrophobic and electrostatic interactions are minimized) and also highly dynamic in aqueous environment. Consequently, the formation of hydrogen bonding between the protein and polymer is completely suppressed.

However, if the nanoparticles are so well coated that they become invisible to the immune system, they also lose their ability to bind to specific receptors. This is a common disadvantage of other drug-based therapies, where the drug is not delivered to a specific site but is widely dispersed in the body.

Gold nanospheres and nanorods were linked with thiol-functionalized PEG and used for in vivo/in vitro investigation of cancer therapy [34, 35]. Experimental results (in vivo) have shown that PEG-linked nanoparticles are preferentially accumulated into tumor tissues due to the larger permeability of their blood vessels, compared with normal tissues. In addition, the tumor tissues have a longer retention time for large molecules, while normal tissues quickly expel them out.

O'Neal et al. [36] have used PEG-linked gold nanoshells for treating tumor-bearing mice. They reported that after NIR laser irradiation, all tumors were ablated and the mice were tumor-free for several months. The same treatment applied to mice which were not injected with nanoshells was less efficient, and consequently they were affected by tumor growth. Stern et al. [37] have reported that high dose of PEG nanoshells (8.5 μL/g) injected in mice that were subsequently irradiated with NIR laser leads to tumor necrosis and regression (93 %). Surprisingly, when a slightly lower dose was used (7 μL/g), the results showed only a tumor growth arrest at 21 days but no tumor ablation. Such differences may be explained by the fact that this method is a *passive targeting* one, based on the nonspecific accumulation of nanoshells in tumor. As mentioned before, tumor tissues are characterized by an

"enhanced permeability and retention (EPR) effect" [38] which favors the accumulation process.

The second approach named the *antibody targeting* tries to overcome the nonspecific adsorption, by modifying the surface of the nanoparticles with an antibody which is specific to biomarkers on the diseased cells [39–43]. This method is highly specific, due to the fact that the nanoparticles are targeted to assemble on the surface of a certain type of cancer cell. The anti-epidermal growth factor receptor (anti-EGFR) antibody is generally employed to detect cancerous cells that overexpress EGFR. Numerous papers have reported such studies for oral cancer cells [44, 45] and cervical cancer cells [46, 47].

Huang et al. [48] have used the *antibody-targeting* strategy for photothermal cancer therapy. Gold nanorods were conjugated with anti-EGFR antibody and then incubated with two cancerous oral epithelial cell lines: HOC313 clone8 and HSC3. The control experiment employed the noncancerous epithelial cell line (HaCat) that was also incubated with anti-EGFR–gold nanorods. They reported that lower laser fluence (about half) was necessary to kill the cancerous cells compared with normal cells. Such efficient ablation was due to the selective attachment of anti-EGFR–gold nanorods conjugates to the surface of the malignant cell that has overexpressed EGFR. Although this approach is without doubt more efficient than the *passive targeting* one, it also has its own limitations due to the potential activation of the normal host immune response.

Besides anti-EGFR antibodies, another tumor necrosis factor-alpha (TNF-α) protein has been successfully linked with gold nanoparticles and used for the therapy of cancer cells. Like EGFR, TNF-α is overexpressed in solid tumors and induces hemorrhagic necrosis in tumor tissues [49, 50].

The main advantage of TNF–AuNPs conjugates relates with the selective penetration of cancerous cells by the protein component, through receptor-mediated endocytosis (RME). RME is a process by which cells internalize molecules or viruses. The process depends on the interaction of that molecule with a specific binding protein in the cell membrane, called *receptor*.

Previous reports have shown that native TNF-α has low therapeutic effect and acute toxicity. After linking with gold nanoparticles, TNF-α exhibited a reduced toxicity and more importantly the conjugates were able to selectively accumulate in tumor vasculature [51].

Paciotti et al. [42] have used functionalized colloidal gold as a therapeutic for the treatment of cancer as well as an indicator for immunodiagnostics. The optimal combination consists of PEG–SH molecules and recombinant human TNF-α that are directly bound onto the surface of the gold nanoparticles (designated PT-cAu-TNF).

In vivo experiments have shown that after intravenous administration in tumor-bearing mice, PT-cAu-TNF rapidly accumulates in MC-38 colon carcinoma tumors. Little or no accumulation was detected in other healthy organs of the animals (livers, spleens). The marked color change of the tumor tissues indicates the accumulation of colloidal gold solution and was coincident with the active and tumor-specific sequestration of TNF-α. The results have shown that PT-cAu-TNF

was extremely efficient to reduce the tumor effects compared with native TNF-α. A maximal antitumor response was obtained at lower doses of PT-cAu-TNF drug.

Kim and Jon reviewed the diagnostic and therapeutic use of gold nanoparticles based on their research, emphasizing the applications in cancer therapy. Besides their unique physical and chemical properties, biocompatibility, ease of synthesis, and surface modification, gold nanoparticles have a higher X-ray absorption coefficient than iodine, being used as in vivo computed tomography (CT) contrast agent. A simple and rapid colorimetric method for the detection of Ca^{2+} with high specificity using calsequestrin-functionalized gold nanoparticles was developed. The technique is simple, rapid, and accurate, does not require specialized equipment, and may be useful in detecting and monitoring several diseases associated with hypercalcemia, such as malignant tumors [52].

Gene Delivery

Gene delivery is the process by which *foreign DNA* is introduced into host cells. Gene delivery is, for example, one of the steps necessary for gene therapy and the genetic modification of crops. The process typically involves the formation of transient pores or "holes" in the cell membrane, to allow the uptake of material. In the last years, methods like electroporation, sonoporation, and hydrodynamic injections were used for gene delivery [53–55]. In vivo electroporation has been used as a method to increase gene expression after DNA injection into various tissues: the skin, muscles, liver, and tumors [56, 57].

The most important aspects that have to be considered during gene delivery process are the *stealth character* and the *targeted recognition* of tissues. The *stealth character* prevents the interaction of DNA complexes with plasma proteins. In addition, it favors a prolonged circulation period of DNA in blood, which is essential for in vivo gene delivery.

Kawano et al. [58] have combined the use of PEG-modified gold nanoparticles with electroporation for hepatocyte gene delivery and enhanced gene expression. PEG–gold nanoparticles were functionalized with plasmid DNA (8.4 w/w ratio) and then intravenously injected into mice. About 20 % of gold nanoparticles were detected in blood at 120 min after injection, and 5 % of DNA strands were observed in blood after 5 min. By applying electroporation to a lobe of the liver following injection, significant gene expression was specifically observed in the pulsed lobe. The authors concluded that PEG–gold nanoparticles have weaker binding abilities for DNA than other cationic molecules, and therefore DNA can be easily released from complexes. In addition, the electrical pulses used in electroporation trigger the release of DNA. Such method can have clinical use, after optimization of electroporation parameters (e.g., number of pulses, strength, and frequency of current) in order to avoid tissue damage.

Lee et al. [59] have demonstrated the applicability of amine-functionalized gold nanoparticles for intracellular delivery of small interfering RNAs (siRNAs). The emerged interest for this method is based on the superior ability of siRNA to

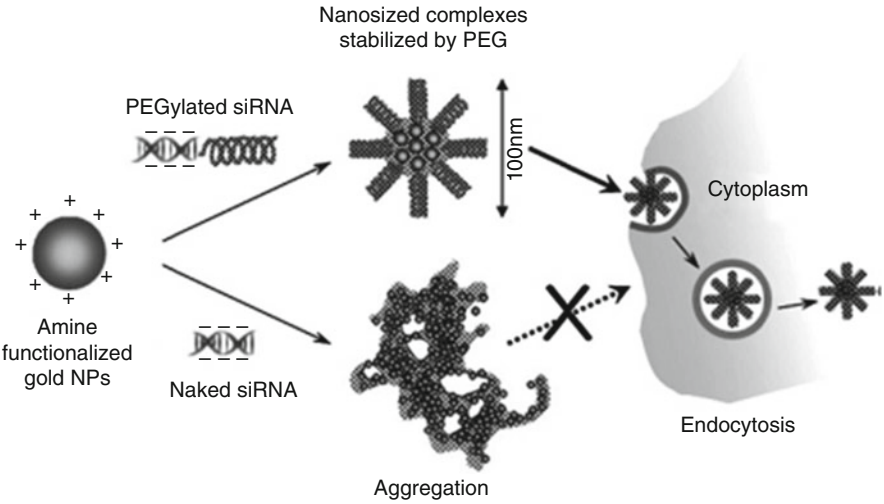

Fig. 2 Schematic illustration for polyelectrolyte complexes formed from amine-functionalized gold nanoparticles (AF–AuNPs) with siRNA and siRNA–PEG conjugate (Reprinted with kind permission from reference [59]. Copyright (2013) Elsevier)

induce catalytic destruction of its complementary mRNA target. Additionally, the method might have a variety of potential therapeutic applications for diseases that cannot be cured by conventional treatments. The most challenging goal that must be fulfilled is the safe and effective intracellular delivery of siRNA.

In their study, Lee et al. have reported that amine-functionalized gold nanoparticles form stable polyelectrolyte complexes (hydrodynamic diameter of 96.3 $\pm$ 25.9 nm) through electrostatic interactions with PEG-conjugated siRNA (Fig. 2).

The complexes have a disulfide link, which can be broken under reduced cytosol condition. By using confocal laser scanning microscopy, they have demonstrated that the complexes were efficiently internalized in human prostate carcinoma cells, favoring the intracellular uptake of siRNA. Additionally, they reported that siRNA/gold complexes significantly inhibited the expression of a target gene within the cells, without showing severe cytotoxicity.

Pissuwan et al. reported in a recent review about the advantages of using gold nanoparticles for drug and gene delivery. They also discuss the topics of surface modification and site specificity [60]. Knipe et al. gave a general review on some of the most widely used types of inorganic nanoparticles in theranostic applications, including magnetic nanoparticles, gold nanoparticles, and quantum dots [61].

Ghosh et al. demonstrated that by coating gold nanoparticles with lysine-based headgroups, effective transfection vectors can be produced (Fig. 3). The efficiency of DNA delivery strongly depended on the ability of headgroups to condense DNA and on the structure of these groups. The lysine dendron-functionalized gold nanoparticles were more effective by about 28 times than polylysine. They also

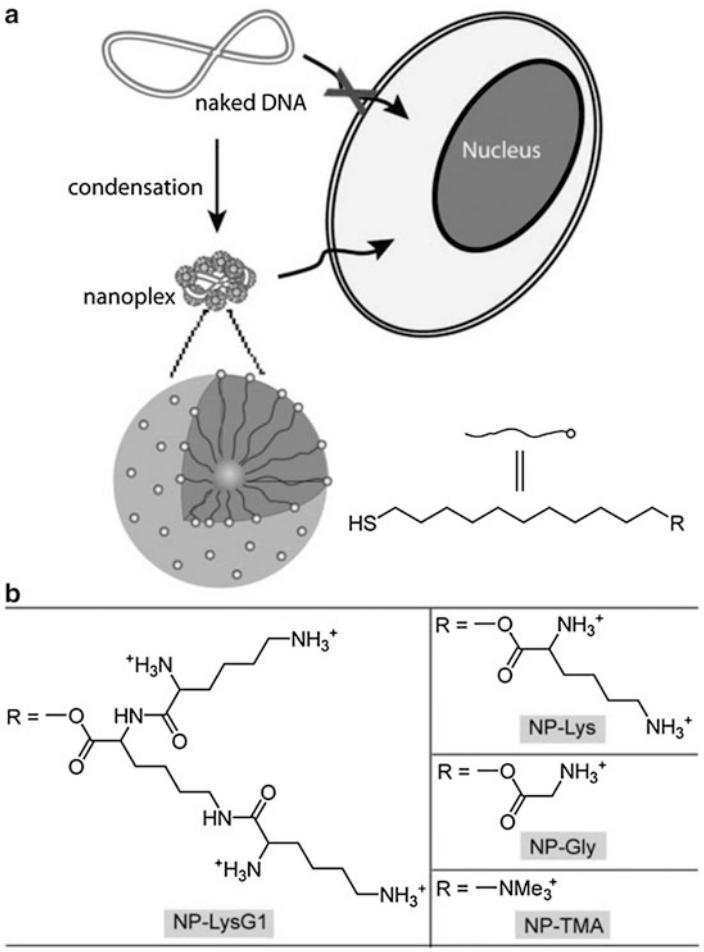

Fig. 3 Schematic illustration of the monolayer-protected gold nanoparticles used as transfection vectors (**a**); chemical structures of headgroups presented on the surface of the nanoparticles (**b**) (Reprinted with kind permission from reference [62]. Copyright (2013) American Chemical Society)

reported that amino acid-functionalized gold nanoparticles showed no cytotoxicity, when used as transfection agents [62].

The layer-by-layer technique was also employed to deliver small interfering RNA (siRNA) and plasmid DNA into cancer cells with charge-reversal polyelectrolyte-deposited gold nanoparticles, as carriers. The occurrence of the charge-reversal property of functional gold nanoparticles was confirmed by polyacrylamide gel electrophoresis measurements of siRNA. The charge reversal under acidic environment facilitates the escape of gold nanoparticle/nucleic acid complexes from endosome/lysosome and the release of functional nucleic acids into the

cytoplasm, making the nanoparticles a promising material for in vivo therapeutic applications [63].

Shan et al. reported a new gene delivery vector based on dendrimer-entrapped gold nanoparticles. In order to synthesize gold nanoparticles with various Au atom/dendrimer molar ratios (25:1, 50:1, 75:1, and 100:1), they used as templates amine-terminated generation five poly(amidoamine) dendrimers. Gel retardation assay, light scattering, zeta potential measurements, and atomic force microscopic imaging were used to characterize the formed dendrimer-entrapped gold nanoparticles. Their results showed that the appropriate composition of dendrimer-entrapped gold nanoparticles (25:1 M ratio) enabled enhanced gene delivery with efficiency of 100 times higher than that of the dendrimers without nanoparticles. They suggested that the entrapment of gold nanoparticles within the dendrimer templates helped to preserve the 3D spherical shape of dendrimers, enabling high compaction of DNA. An important observation was that dendrimer-entrapped gold nanoparticles had lower cytotoxicity compared with the dendrimers without nanoparticles [64].

Chen et al. prepared gold nanoparticles modified with 2-aminoethanethiol, 8-amino-1-octanethiol, and 11-amino-1-undecanethiol, by the reduction of $HAuCl_4$ with $NaBH_4$ in water or water/ethanol solvents, in the presence of the respective thiols. The surface charge of gold nanoparticles was changed from negative to positive, following the modification with thiol molecules. The cationic gold nanoparticles interacted with plasmid DNA, and their complex was used for gene transfection [65].

The interaction of poly-L-lysine with citrate-capped gold nanoparticles was also investigated for possible application of the functionalized gold nanoparticles, in gene delivery. Strong electrostatic interaction between polycationic chains of poly-L-lysine and citrate-capped gold nanoparticles was observed in weakly acidic to weakly alkaline solutions (pH 5–9). This interaction was evidenced by the bathochromic shift of the surface resonance plasmon band and by the strong increase of the resonance elastic light scattering intensity [66].

Ryou et al. have demonstrated that single-stranded DNA-functionalized Au nanoparticles can be used to deliver highly structured RNA aptamers into the nucleus of human cells, where they exert physiological effects by interacting with target molecules. Fluorescence microscopy analysis showed that the labeled aptamers were efficiently delivered into the cells [67].

Ghosh et al. developed cysteamine-functionalized gold nanoparticles to deliver unmodified microRNAs into living cells. Compared to the conventional liposome-mediated transfection, these cysteamine-functionalized gold nanoparticles are capable of delivering up to a 10–20-fold overexpression of mature microRNAs. The gold nanoparticle platform was able to release functional miRNAs that efficiently downregulate target genes and modulate the rate of proliferation, as it was showed by in vitro studies. The best formulation has the highest payload, lowest toxicity (98 % of cell viability following treatment), efficient uptake (96 % of cells took it), fastest endosomal escape, and increased half-lives (at least 5 days) [68].

Kim et al. reported that gold nanoparticles functionalized with covalently attached oligonucleotides activate the immune-related genes and pathways in human peripheral blood mononuclear cells. These oligonucleotide-modified gold nanoparticles can be applied in the development of therapeutic and gene delivery systems. Their results highlighted the need to study the potential harmful interactions between the engineered nanoparticle structures and the relevant biological system [69].

Conclusions

In this review we selectively presented few strategies for bioconjugation of noble metal nanoparticles along with the advantages of their usage in cancer therapy and gene delivery. Although there are many advances in these fields, the requirements for new technologies that will allow the earlier treatment of cancer or other diseases are still needed. Among the most interesting studies, we have outlined the development of a colloidal gold nanoparticle vector (thiol-derivatized PEG and recombinant human TNF, directly bound to the surface of gold nanoparticles) that targets the delivery of TNF to a solid tumor growing in mice (in vivo experiments). The proposed vector was very efficient in reducing the tumor burden compared with native TNF. Other studies have shown that the efficiency of both cancer therapy and gene delivery can be considerably enhanced by exploiting the active targeting strategy, which offer greater specificity and are also cost-effective.

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