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Review Article

GOLD NANOPARTICLES IN BIOMEDICAL APPLICATIONS

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ABSTRACT

The applications of gold nanoparticles in Biomedical field are reviewed in this paper. The physical and chemical properties of Gold nanoparticles are illustrated. In this paper we present physical, chemical, and biosynthesis methods of gold nanoparticles preparation. This review focus on various biomedical applications of gold nanoparticles

Keywords Gold nanoparticles, Physical properties, Biosynthesis, Biomedical applications

INTRODUCTION

In the field of nanotechnology, especially for biomedical applications, scientists and clinicians are combining their efforts to enable medical intervention at the molecular scale for diagnosis, prevention, and treatment of diseases. Particles with submicroscopic dimensions represent ideal candidates for penetrating the least-accessible compartments within tissues and cells, and offer the additional advantage of escaping from premature degradation and elimination from the body.[1]

Metal nanoparticles, with diameters ranging roughly between 1 and 100 nanometers, are natural bridges between molecules and extended solids. They are complex many-electron systems, where reduced size and quantum confinement of electrons and photons give birth to fascinating new effects, potentially tunable with particle size and shape. Metal nanoparticles attract strong interest both because they open up a new field in fundamental science and because of their potential technological applications.

Multifunctional nanoparticles (NPs) already have widespread biomedical applications[2,3], such as serving as carriers for targeted drug delivery and gene delivery[4], efficient contrast agents for molecular imaging in medical diagnostics[5], and therapeutic reagents for targeted photo thermal therapy.[6] Recently, NPs have proved to be useful in cancer therapy, allowing for effective and targeted drug delivery.[7, 8]

Gold is a rare metallic element with a melting point of 1064°C and a boiling point of 2970°C. Several properties of gold such as its excellent conductive properties and its inability to react with water or oxygen have made it very useful to mankind over time. During the 5th millennium B.C., the extraction of gold started near Varna (Bulgaria) and it is believed that "soluble" gold appeared around the 5th or 4th century B.C. in Egypt and China. The marvelous statue of Touthankamon, which was constructed around that time stands as proof. It was referred by different names such as soluble gold and drinkable gold, before the term "colloid" (from the French word, colle) was coined.[9]

Currently, Gold Nanoparticles (GNPs) are used in diagnostics, bioimaging, biosensors, photo thermal and photodynamic therapy as well as in drug/genetic material delivery.[10]

Gold Nanoparticles possesses many excellent properties, such as effortless reductive preparation, water solubility, high chemical stability, and significant biocompatibility and affinity. [11]

PHYSICAL AND CHEMICAL PROPERTIES OF GNPS:-

GNPs, as for other nonmaterial's, can be subjected to a detailed analysis of their optical and surface chemical properties. Optical properties of GNPs are normally used to determine the strategies of their applications. The interaction of GNPs with light is determined by their size, physical dimensions as well as environment. Oscillating electrical fields of light beams circulating near GNPs interact with free electrons causing a joint oscillation of electron charge which is in resonance with the respective frequency of visible light. These resonant oscillations are recognized as surface plasmons. A plasmon can be defined as a quantum of plasma oscillation. Normally, plasmons are collective oscillations of the free electron gas density at certain (for example optical) frequencies. Plasmons can also couple with a photon, thus creating another quasi-particle known as a plasma polariton.[12-14]

Most of the plasmon properties can be derived directly from Maxwell's equations since they stand a quantization of classical plasma oscillations.[12-14] The resonance and the optical properties of GNPs depend on their size. Both extinction (A_{ext}) and scattering efficiency (at 90° angle (I90(λ)) – these are the main optical characteristics of NPs) depend on their size. If we assume that the GNP has d=2a in an aqueous environment (their concentration is a constant value) where the refractive index equals $n_m(\lambda)$, and calculate its A_{ext} as well as I90(λ), the dependence of these parameters on particle size becomes obvious (see equations (1) and (2)).

$$A_{ext} = 0.651 \frac{cl}{\rho} \frac{Q_{ext}}{d} \qquad (1)$$

$$I_{90}(\lambda) = 0.651 \frac{cl^2}{\rho d} \left[\frac{16 S_{11(ka,\theta=90^0)}}{3(ka)^2} \right] \qquad (2)$$

In this case ρ – is the density of the metal, $Q_{ext} = C_{ext}/\pi a\,2$ – is the scattering efficiency factor, S11(ka, θ) – normalised scattering efficiency at the angle of 90°, $k = 2\pi n_m/\lambda$ – the wave number in the water. As such, smaller GNPs mostly absorb light while bigger particles tend to scatter it.[12-14] For example, in monodisperse GNPs with d<30 nm the surface plasmon resonance phenomenon causes absorption of light in the blue-green region of the spectrum (~450 nm) while red light (>680 nm) is reflected, resulting in a red colour. Particles with greater size (>50 nm) tend to absorb red while blue light is reflected, giving rise to a pale blue or purple colour. A simplified diagram representing dependence of the GNP colour on their size is presented in the Figure 1.

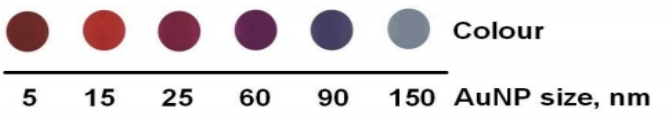

Figure 1. Dependence of the GNP colour on particle size

A recently discovered a phenomenon reported by Huang et al showed that GNPs smaller than 10 nm display unique advantages over NPs larger than 10 nm in terms of their ability to interact with living cells. It was found that the properties of the surface of smaller GNPs differ from those of larger nonmaterial and this is generally reflected in changes in the frequency and intensity of the corresponding surface plasmon resonance peaks.[15] Chemical and biological effects of GNPs are not only determined by their size and optical/resonance properties but also by their surface chemistry. Gold surfaces demonstrate high affinity to thiol groups and even to disulfides. The adsorption of thiols on Au(111) at low coverage could be considered as the simplest case where the influence of the van der Waals interactions are minimized. Au(111) undergoes a 23×√3 reconstruction where face centered cubic (fcc) domains alternate with hexagonal close-packed (hcp) domains, separated by smaller regions with Au atoms on bridge positions.[16, 17]

The sulphur of thiol groups and disulfides form semi-covalent interactions with gold with the strength of approximately 45kcal/mol.[16,17] This property of GNPs is used to coat them with different compounds. For example, PEGylation (coupling of the GNPs with polyethylene glycol (PEG)) of GNPs is normally performed through SH groups or dihydrolipoic acid (DHLA, Figure 2).[18]

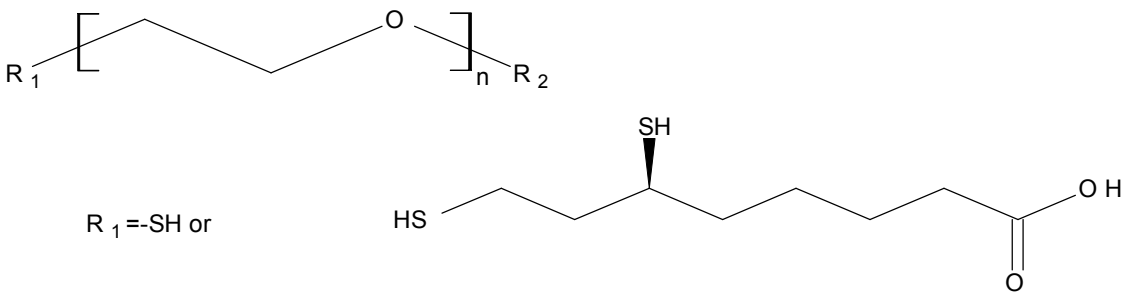

Figure 2 Structure of PEG used to couple with AuNPs.

Sulphur, contained in the two amino acids cysteine and methionine, was found to interact with gold surfaces.[16] Additionally, GNP surfaces can form weaker hydrophobic interactions with compounds such as lipopolysaccharide (LPS) – the main component of the cell wall of Gram-negative bacteria. [19]

VARIOUS METHODS OF SYNTHESIS GOLD NANOPARTICLES

Array Method

GNPs have been synthesized by an array of methods which mainly are based on the reduction of chloroauric acid in the presence of a stabilizing agent. The most commonly used method, the citrate synthesis method, includes reduction of chloroauric acid using trisodium citrate resulting into the formation of GNPs.[20, 21] The size of GNPs is determined mainly by the salt concentration, temperature and rate of addition of reactants resulting in size range of 10–25 nm. However, the size range of 1–100 nm or more can also be achieved by varying the salt concentration and temperature. Another widely used method employs toluene using the tetra-octanyl ammonium bromide as a phase transfer reagent.[22] Several modifications of the basic methods have resulted into an array of techniques to synthesize and manipulate these nanoparticles satiating the needs of a specific research objective.[23–25] Chemical reduction using L-Tryptophane as a reducing agent for ionic gold and polyethylene glycol was used to produce $AuCl4^-$ ions to provide higher stability and uniformity in size, shape, and particle distribution.[26]

Laser ablation method

Laser ablation method is used to produce gold nanoparticles by using the pulsed laser irradiation of gold target in water in the absence of any additives, at (532 nm, 10 ns, 10 Hz), or (266 nm) wavelengths.[27] Inert gas condensation can be used for the preparation of gold nanoparticles.[28] In this method, the gold nanoparticles as soon as they

are formed rapidly collide with inert gas in a low-pressure environment and thus smaller and controlled nanoparticles are formed. The advantage of these methods is the narrow particle size distribution of the produced gold nanoparticles, while its limitation is the need for expensive equipment. Other physical methods such as thermolysis of gold(I) complex at 180^0C for 5 h under nitrogen atmosphere,[29] radiolysis of gold salts in aqueous solution using c-irradiation-induced reduction in the field of a 60 Coc-ray source,[30] photochemistry, e.g. in the $HAuCl_4$ solution containing certain amounts of protective agent and acetone, the colloidal gold particles with an average diameter of 5 nm (r= 0.86) were prepared by UV 300 nm irradiation,[31] and sonochemistry using ultrasound-induced reduction of gold salts in aqueous solution[32-34] have been used to prepare a variety of gold nanoparticles.

Green Synthesis (Biosynthesis)

Gold nanoparticles (GNPs) can be produced using a multitude of chemical and physical processes; however, these approaches are often costly and have environmental risks associated with their production. To reduce the use of toxic chemicals used in typical GNP synthesis, researchers are actively investigating alternative synthesis methods using biological materials (proteins, polysaccharides, polyphenol, etc.) for green synthesis of gold nanoparticles[35-38]

A. Sustainable synthesis of GNPs using live shoots

Live English ivy shoots (Hedera Helix) were cut to lengths of 15 cm, leaving one attached leaf on the apical end of the stem. After sterilization and treatment with auxins, four shoots were placed into Magenta GA7 (MAG) boxes and held upright by placing them through holes cut into the lids. After 24 hours, the boxes were transported to a windowsill, where the Nanoparticle synthesis was conducted. To initiate nanoparticles synthesis, aqueous $HAuCl_4$ was added to the 50 ml of water present in the MAG boxes to achieve concentrations of 0, 0.025, 0.05, 0.1, 0.2, 0.5, 1 and 5 mM. The shoots were exposed to these concentrations for 24 hours, before the solution was removed to test for Nanoparticle production. After collecting the solution after 24 hours, fresh $HAuCl_4$ solution at the same concentration was added back to the MAG boxes. This method was repeated for the duration of the study. To concentrate any nanoparticles present in the solution, the solution was centrifuged at 14,000 rpm for 10 min. The supernatant was then removed, and DI water was added to the precipitate. This procedure was repeated three times to remove soluble factors present in the solution, including secreted proteins, polysaccharides, and excess $HAuCl_4$.

B. **Synthesis of GNPs using adventitious root extract**

Adventitious roots were homogenized in a minimal volume of water, creating a dense solution. This solution was centrifuged at 4,400 rpm for 5 min to remove large tissue debris from the homogenization. The resulting light brown supernatant was then transferred to dialysis tubing with a molecular weight cutoff value (MWCO) of 3.5 kDa, and dialyzed overnight against DI water. After dialysis, the solution outside of the dialysis tubing was collected and labeled as Solution I. The solution remaining in the tubing was then transferred to new tubing and dialyzed at 12 kDa. As indicated above, the solution outside of the tubing was collected and labeled Solution II, followed by a final dialysis through dialysis tubing with a cutoff value of 300 kDa. The final solution outside of the membrane was labeled as Solution III, and the solution remaining in the tubing was labeled Solution IV. Prior to analysis, the extracts were freeze dried and re-suspended in DI water. To synthesize gold nanoparticles from the ivy rootlet extract solutions, 500 µl of each solution (I-V) was transferred into a clean microfuge tube and aqueous HAuCl4 was added to a final concentration of 0.5 mM. The mixture was then vortexed, and reacted at room temperature. To concentrate the synthesized gold nanoparticles, the solutions were centrifuged and washed as described above.[39]

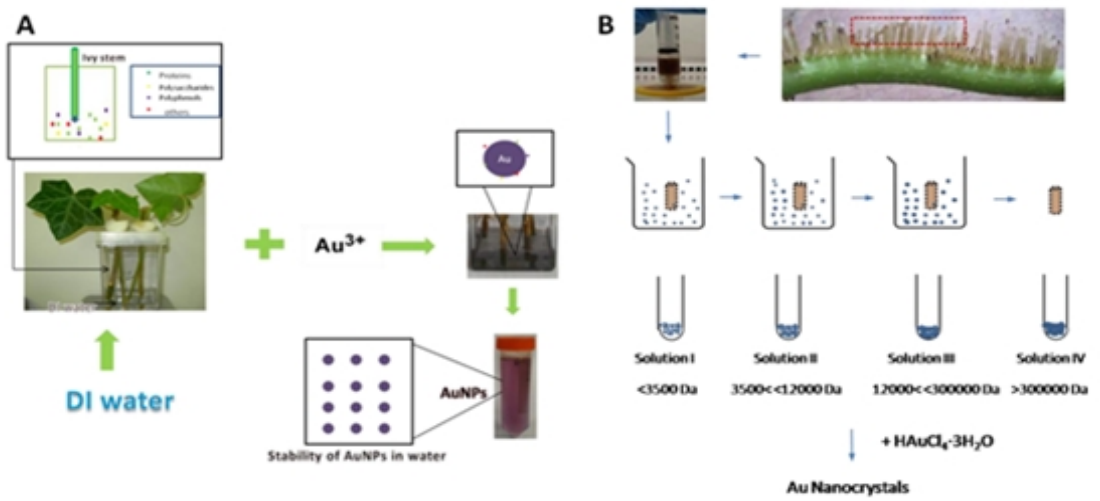

Figure 3.Schematic diagrams of two different methods used in this study[39]

4} **By Using Olive Leaf Extract**

For the synthesis of the gold nanoparticles, a certain volume of the olive leaf extract (0.1–6 ml) was added to the $HAuCl_4$~ $3H_2O$ solution and the volume was adjusted to 10 ml with de-ionized water. The final concentration of Au was 1.3×10^{-4} M. The reduction process of $Au3^+$ to Au nanoparticles was followed by the change in the color of the solution from yellow to violet to dark pink and green depending on the extract concentration. The nanoparticles prepared at different pH values, the Ph of the solutions (1.3×10^{-4} M $AuCl^-_4$ and 2 ml extract in 10 ml flask) were adjusted using 0.1 N HCl or 0.1 N NaOH solutions.[40]

5} By Using Seed

Seed-mediated growth where small particles produced by other techniques like irradiation were exploited as seeds and fresh Au(III) ions were reduced onto the surface of the seed particles by reducing

agents like ascorbic acid [41], use of reverse micelles which involves reduction of $HAuCl_4$ in sodium bis (2-ethylhexyl) sulfosuccinate /isooctane reverse micelles system using reducing agents like ascorbic acid [42],phase transfer reactions as a representative reaction in a novel water– cyclohexane two-phase system, the aqueous formaldehyde is transferred to cyclohexane phase via reaction with dodecylamine to form reductive intermediates in cyclohexane; the intermediates are capable of reducing gold ions in aqueous solution to form gold nanoparticles in cyclohexane solution at room temperature.[43]

6} **Biosynthesis By Using Fungus**

Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received considered attention due to a growing need to develop environmentally benign technologies in material syntheses. Sastry and coworkers have reported the extracellular synthesis of gold nanoparticles by fungus Fusarium oxysporum and actinomycete Thermomonospora sp., respectively.[44, 45] The same group has reported the intracellular synthesis of gold nanoparticles by fungus Verticillium sp. as well.[46] The reaction of $AuCl_4$ ions with the extract of geranium leaves and an endophytic fungus, Colletotrichum sp., present in the leaves, leads to the formation of GNPs.[47] Nair and Pradeep reported the growth of nanocrystals and nanoalloys using Lactobacillus.[48] Daizy has reported a successful attempt on the extract of Volvariella volvacea, an edible mushroom, as reducing and protecting agent for the synthesis of gold, silver and Au–Ag alloy nanoparticles. Gold nanoparticles of different sizes (20–150 nm) and shapes, from triangular to nearly spherical and hexagonal nanoprisms, are obtained by this novel method. The size and shape of gold nanoparticles are also found to depend on the temperature of the extract.[49] Gold nanoparticles of 20–100 nm diameter were synthesized within HEK-293 (human embryonic kidney), HeLa (human cervical cancer), SiHa (human cervical cancer), and SKNSH (human neuroblastoma) cells. Incubation of 1 mM tetrachloroaurate solution, prepared in phosphate buffered saline (PBS); pH 7.4, with human cells grown to~80% confluency yielded systematic growth of nanoparticles over a period of 96 h. The cells, stained due to nanoparticle growth, were adherent to the bottom of the wells of the tissue culture plates, with their morphology preserved, indicating that the cell membrane was intact. Transmission electron microscopy of ultrathin sections showed the presence of nanoparticles within the cytoplasm and in the nucleus, the latter being much smaller in dimension. Scanning near field microscopic images confirmed the growth of large particles within the cytoplasm. Normal cells gave UV–visible signatures of higher intensity than the cancer cells. Differences in the cellular metabolism of cancer and noncancerous cells were manifested, presumably in their ability to carry out the reduction process. The differential ability with which nanoparticles are synthesized by cancer and normal cells can have implications to cancer diagnostics.[50]

BIOMEDICAL APPLICATION

Biosensing and diagnostic

Gold nanoparticles are useful in the construction of electrochemical immunosensors where it plays a crucial role both in the enhancement of the electrochemical signal transducing the binding reaction of antigens at antibody immobilized surfaces and in the ability of increasing the amount of immobilized immunoreagents in a stable mode. Hepatitis B virus surface antigen was detected using electrochemical impedance spectroscopy (EIS) through immobilization of the antibody onto gold nanoparticles-modified 4-aminothiophenol self-assembled monolayer's.[51] Potentiometric and amperometric immunosensors for Hepatitis B virus surface antigen detection were also constructed by electrostatic adsorption of the antibody onto gold nanoparticles/tris (2, 2-bipyridyl) cobalt (III) multilayer films[52] or by immobilization of the antibody onto gold nanoparticles-modified thiol-containing sol–gel network[53] The antigen–antibody reaction is detected through measuring the changes in the electric potential before and after. Furthermore, a different strategy was used to fabricate an amperometric immunosensor based on immobilization of hepatitis B antibody on a gold electrode modified with gold nanoparticles and horseradish peroxidase[54] in order to avoid non-specific adsorption and also amplify the response of the antigen–antibody reaction. Also, a gold nanoparticles-based potentiometric immunosensor was developed for detecting diphtheria antigen and diphtherotoxin.[55] An electrode fabricated from gold nanoparticles was used
for the determination of aflatoxin B1 based on immunoreactions.[56]

Diagnosis of disease

Gold nanoparticles have been used in the assembly of electrochemical and amperometric biosensors for the diagnosis of patients with germ cell tumors and hepatocellular carcinoma. This is done by the detection of a tumor marker, alpha-fetoprotein (AFP), an oncofetal glycoprotein.[57] Carbohydrate antigen 19-9 (CA19-9) is one of the most important carbohydrate tumor markers expressed in many malignancies as pancreatic, colorectal, gastric and hepatic carcinomas.[58] Another carbohydrate antigen CA125 is also an important marker determined by nanogold immunosensors.[59] Carcinoembryonic antigen (CEA) is a well-known marker associated with the progression of colorectal tumors. PSA is prostate-specific antigen in prostate cancer.[60]

DNA-Modified Gold Nanoparticles as Biosensors

Gold nanoparticles-modified electrodes are used in the assembly of electrochemical DNA biosensors. They constitute useful analytical tools for sequence-specific DNA diagnosis and detection due to their inherent advantages of low cost, sensitivity and rapidity of response.[61] Pathogens, bacteria and viruses, can be detected via their unique, respective nucleic acid sequences.

Gold Nanoparticle in Therapeutics

Gold nanoparticles exploit their unique chemical and physical properties for transporting and unloading the pharmaceuticals. First, the gold core is essentially inert and is their ease of synthesis; monodisperse nanoparticles can be formed with core sizes. Ranging from 1 to 150 nm.[62] Second advantage is imparted by their ready functionalization, generally through thiol linkages. In addition, their photo physical proper- ties could trigger drug release at remote place.[63]

Drug delivery systems (DDSs) provide positive attributes to a 'free' drug by improving solubility, in vivo stability, and biodistribution. They can also alter unfavorable pharmacokinetics of some 'free' drugs. Moreover, huge loading of pharmaceuticals on DDSs can render 'drug reservoirs' for controlled and sustained release to maintain the drug.

A recent study has reported on the therapeutic ability of a novel cyclodextrin-covered gold nanoparticle (GNP) carrier for noncovalent encapsulation of an anti-cancer drug.[64] The surface of the GNPs was functionalized with cyclodextrin as a drug pocket, anti-epidermal growth factor receptor (anti-EGFR) antibody as a targeting moiety, and poly (ethylene glycol) (PEG) as an anti-fouling shell. β-Lapachone, an anti-cancer drug, was efficiently encapsulated into the hydrophobic cavity of cyclodextrin on the surface of the GNP carriers (GNP-1). The glutathione-mediated release of β-Lapachone from the surface of GNP-1 was demonstrated by an experiment with MCF-7 (low glutathione concentration) and A549 cells (high glutathione concentration).[64]

Gold nanoparticles in biomolecules delivery

In addition to the delivery of small molecules, tunable size and functionality of gold nanoparticles make them a useful scaffold for efficient recognition and delivery of biomolecules such as peptides, proteins, or nucleic acids like DNA or RNA. Efficient and safe nonviral gene delivery systems are a prerequisite for the clinical application of therapeutic genes. In some studies, it was reported that an enhancement of the transfection efficiency of plasmid DNA happened via the use of positively charged colloidal gold nanoparticles (GNPs). The results of this study suggested that the GNP/DNA complexes may harbor the potential for development into efficient and safe gene delivery vehicles.[65] Gold nanoparticles chemically modified with primary amine groups were developed as intracellular delivery vehicles for therapeutic small interfering RNA (siRNA).

Recent work has shown that the combination of phototherapy with conventional gene therapy offers a high possibility to improve the efficiency of gene delivery into cells.[66] For example, Niidome et al., (2006) have investigated the release of plasmid DNA from spherical gold nanoparticles after exposure to pulsed laser irradiation.[67]

Gold nanoparticles for protein deliveary

Gold nanoparticles can be novel nanocarriers of peptides and proteins of interest. Cationic tetraalkylammonium-functionalized GNPs recognize the surface of an anionic protein through complementary electrostatic interaction and inhibit its activity.[68] Pokharkar and coworkers have demonstrated functionalized gold nanoparticles as the carriers of insulin.[69] Chitosan-coated particles strongly adsorb insulin on their surface, and are effective for transmucosal delivery of insulin.

Gold nanoparticles for site-directed photothermal applications

Gold nanoparticles cause local heating when they are irradiated with light (800–1200 nm). El-Sayed et al. have recently reported about the potential use of GNPs in photothermal destruction of tumors.[70] Citrate-stabilized GNPs (core d= 30 nm) were coated with anti-EGFR (epidermal growth factor receptor) to target HSC3 cancer cells (human oral squamous cell carcinoma). The use of GNPs enhanced the efficacy of photothermal therapy by 20 times. In another approach, optically responsive delivery systems have been designed by incorporation of gold nanospheres into the shells of capsules.

Needle-free drug delivery

Gold-based technologies are also provide a unique needle-free delivery system, a technique that used gold nanoparticles and allowed vaccines to be delivered through the skin making use of the fact that small particles can pass through gaps between cells while large ones cannot.

Gold nanoparticles against HIV/AIDS

One of the most efficient usages of gold nanoparticles in recent years is detecting and fighting against HIV.

Mercury Control and Sensing

Nanotechnology can control and sense mercury using gold nanoparticles. Mercury is one of very toxic material that exists all over the world. Mercury can cause some diseases such as Alzheimer and autism. Almost over 100 tonnes of mercury finds its way into the atmosphere every year, mercury exit from some boilers in the utilities industry. Gold-based catalysts can provide a solution. Gold nanoparticles have considerable promise as mercury oxidation catalysts.

Improving Water and Air Quality

One of the most useful applications of gold nanoparticles is increasing water and air quality, Carbon monoxide is a colorless, odorless gas which is very toxic to humans. Gold nanoparticles provide a simple solution. Gold nanoparticles allow the oxidation of CO to carbon dioxide (CO_2) that transforms an acutely dangerous gas to a far less toxic substance. Recent years have seen a sharp rise in the use of noble metal nanoparticles for water purification and contaminant detection. Gold nanoparticles have also been shown to be efficient adsorbents for the removal of significant levels of mercury from drinking water.

Detection of Microorganisms

Detection of microorganisms can be achieved by several biochemicals, microbiological and molecular methods. Recent advances in the field of nanotechnology have made it possible to detect microorganisms by using nanoparticles functionalized with oligonucleotides complementary to the gene tags of the microorganisms. In one such study, oligonucleotides complementary to the unique sequences of the heat shock protein 70 (HSP 70) of Cryptosporidium parvum was used to functionalize GNPs, which could be used to detect the oocytes of Cryptosporidium in a colorimetric assay, offering a simple and robust method of molecular detection.[71]

GNPs were used to detect Salmonella enteritidis and Listeria monocytogenes, where GNPs deposited within the flagella and in the biofilm network.[72] Similarly, GNP–Poly (para-phenyleneethynylene) could efficiently identify both Gram-positive and negative bacteria based on the differential response by each bacteria.[73] In another study, GNPs functionalized with hairpin DNA was used to image live HEp-2 cells infected with Respiratory syncytial virus [74] Another immunoassay based on multi-functionalized GNPs was developed by using antibodies against protein A, a cell wall protein of the bacterium Staphylococcus aureus, to detect it in food samples.[75] A gold nanoparticle based chemiluminescence assay was designed for the detection of Staphylococcus enterotoxin B (SEB).[76] Antibody against SEB was bioconjugated to the GNPs through physical adsorption followed by adsorption of the complex on a polycarbonate surface. The SEB was then detected based on sandwich type ELISA and chemiluminescence signal arising from the secondary antibody. The method was found to be simple, easy and highly sensitive with a detection limit of ~0.01 ng/mL. Recent increase in the extent of antibiotic resistance in various microbial pathogens has made it necessary to design suitable methods for the detection of antibiotic resistant organisms. A simple colorimetric assay was developed using GNPs functionalized with β-lactam antibiotics.[77] Upon encounter with β-lactamase the GNPs can be made either to aggregate or disaggregate so as to give a visible color change depending upon the attached linker groups. For example, thiol group when used as linker between GNP and the antibiotic is cleaved making the GNPs disaggregate, resulting in a color change.

Enzyme Immobilization

GNPs have been used as immobilization matrices for enzymes. GNPs with a carboxyl terminated thiol group were functionalized through the attachment of the enzyme glucose oxidase.[78] The immobilized enzyme was found to be more stable thermally as compared to free enzyme. Such immobilized systems can be very useful in several biotechnological processes in food and environment fields. Hollow gold nanoshells entrapping horse radish peroxides have been synthesized for detection of small molecules which can enter the nanoshells.[79] This method helps the enzyme remain active in nanoshells, making it useful for various biotechnological applications.

Immunoassay

Various immunoassays have been designed using GNPs functionalized with antibodies such as human IgG and antibodies against pathogenic bacteria[80, 81] Immunosensors have been recently developed using single chain fragment variable recombinant antibodies (scFv) instead of traditional mono or polyclonal antibodies.

SNP Detection

Single nucleotide polymorphisms (SNPs) have by far been the most appropriate method for the detection of point mutations or polymorphisms in various genes, which can be easily, detected using complementary single stranded DNA molecules.

SNPs are often associated with disease detection including diabetes mellitus, β-thalassemia, etc. GNPs functionalized with single-strand-specific-nucleases have been used to detect SNPs.[82] Likewise, a simple colorimetric assay was developed using DNA functionalized GNPs to detect SNPs in the human p53 gene.[83] This was successfully used to detect 12 point mutations in the human p53 gene as compared to wild type method showing a simple approach towards the detection of altered nucleotide sequences. This method neither needs complicated modification of GNPs or DNA, nor additional requirement of DNA probes.

Metal Sensors

Development of an easy colorimetric assay to detect uranium has been achieved by using DNAzyme-GNPs system.[84] Traditionally, uranium in the environment is detected using complex biophysical techniques such as fluorimetry, ICP-MS and atomic absorption spectroscopy. However, these methods are difficult to be used on-site. DNAzyme-GNP system provides an alternative to the traditional methods. DNAzymes are catalytic DNA molecules developed in vitro with specific affinities to metal cofactors such as Uranyl (UO_2^{2+}) which is the most common bioavailable form of uranium. These biosensors were able to detect uranium in two ways, either by disassembly of DNAzyme functionalized GNPs in the presence of uranyl ions causing a visible color change from purple to red ("turn-on" method)or by "turn off" method which was based on different adsorption properties of single and double stranded DNA on GNPs in the presence of uranyl ions. The method was significant as it could detect uranyl below the maximum contamination limits determined by the US environmental protection agency.

SERS nanoparticles for in vivo multiplexed imaging

One element of design, when using SERS nanoparticles for molecular imaging, is the selection of Raman reporters. Different Raman reporters adsorbed on the rough gold surface provide different Raman spectra. This enables us to design SERS nanoparticles with more easily interpretable spectroscopic information. By simply changing the adsorbed Raman tags on the gold surface, different SERS nanoparticles [85] with a multiplexed imaging property can be created. In one study Gambhir and coworkers designed ten different SERS nanoparticles. Each one was composed of a gold core, a different Raman label and silica coating [85] Each SERS nanoparticles produced a distinct Raman spectrum in solution. The authors sought to test the bioavailability and the signal generating capability of these nanoprobes in vivo. After injecting the SERS nanoparticles subcutaneously (s.c) in nude mice, they obtained ten different optical signals consistent with those obtained in solution. The signals could be separated using spectroscopic information.

After identifying the brightest SERS nanoparticles, the authors tested their ability to read signals in deep tissue. Five of the brightest nanoparticles were administered intravenously (i.v.) in order to observe their spectral separation in the liver where they naturally accumulate. Each of these five SERS nanoparticles was both identifiable and resolvable in the liver using an optimized in vivo Raman system. The authors were also able to correlate the signal intensity with the injected dosage. Due to the signal enhancement achieved, nanoparticle accumulation in deep tissues could be measured semi-quantitatively. The authors concluded that simultaneous noninvasive imaging of multiple diseases would be possible by combining the ultra sensitivity of Raman spectroscopy with the multiplexing properties of SERS. This work is important for molecular imaging as it delivers information about multiple different anatomical or physiologic phenomena by using the same nanoparticle template

In Vivo Molecular Targeting Of Cancer Markers

In vivo administration of nanoparticles for tumor imaging or therapy utilizes either of two targeting methods, active or passive. In active targeting, nanoparticles are functionalized with a targeting moiety for receptor mediated uptake by over-expressed surface antigens on cancer cells.[86] In passive targeting, uptake is achieved by nanoparticle escape through leaky vasculature - an enhanced permeability and retention effect (EPR).[87-89] Both of these methods have been widely used. However some researchers report that active targeting results in better therapeutic effect due to receptor-mediated uptake[90, 91] Others prefer passive targeting since active targeting risks loss of the probe to the reticuloendothelial system (RES) whenever incorporated ligands on the nanoparticle surface bind to blood proteins nonspecifically.[92, 93]

In one study authors used active targeting to specifically direct gold nanoparticles into human tumor xenografts, subcutaneously implanted in nude mice. The authors reported that active targeting achieved more nanoparticle accumulation in the tumor they examined.[94] In this study, gold nanoparticles functionalized with Raman labels were used for SERS imaging in order to validate the nanoparticle accumulation in the tumor. The nanoparticles were synthesized with gold ion salts. After nanoparticles with an optimum size for SERS were obtained, they were engineered as SERS probes. The nanoparticles were mixed with Raman reporter molecules, which were then covered with polyethylene glycol PEG molecules for: (a) protecting the nanoparticles from aggregating; (b) sealing the Raman tags onto the gold surface; (c) providing a terminal functional group for further functionalization with a targeting moiety; and (d) increasing the circulation time in the blood stream.

As a last step, nanoparticles were functionalized with ScFv B10, an antibody fragment specific for human EGFR. The nanoparticles were first incubated with EGFR-positive cancer cells (Tu686) and EGFR-negative cancer cells (human non-small cell lung carcinoma NCIH520). The EGFR-positive cells showed internalization of nanoparticles, which was validated by SERS. However, EGFR-negative cells did not show any detectable SERS signal. After validating the detection of SERS signal from the nanoparticles taken up by cancer cells, the authors moved to a mouse model. Nude mice were implanted with Tu686 tumor cells, injected subcutaneously into the flank. Nanoparticles with or without targeting ligands were injected systemically into the mice. After the nanoparticle injection, tumors were monitored with an in vivo SERS imaging system. The tumors of the mice with targeted-nanoparticle administration showed strong SERS signals, which suggested a successful delivery of probe to the tumor. However tumors in the mice with nontargeted-nanoparticle injections did not show any readable SERS signal. The authors presented the first and only in vivo molecular imaging study of tumor biomarkers using SERS. This report has been a cornerstone in the translation of SERS into a noninvasive molecular imaging modality for detection of different carcinomas.

SERS/MRI nanoprobes for in vivo multimodal imaging

Designing contrast agents for multimodal imaging is an emerging and important field. Any given imaging modality could be powerful in certain respects and weaken others. Therefore combining two or more modalities may allow the offsetting of one modality's weakness with the strength of another. For instance MRI is a very powerful biomedical imaging modality in terms of image resolution. However MRI suffers from poor sensitivity and has a low detection limit. High dosages of contrast agents are required to obtain useful image contrast. SERS, on the other hand, has poor resolution but very high sensitivity, which can identify single molecules. Hence a combination of MRI and SERS should constitute an imaging modality with high resolution and multiplexed sensitivity.

Preclinical endoscopy imaging

Even though enhanced Raman spectroscopy is a powerful and promising method for preclinical imaging, the challenge of limited light penetration in deep tissues cannot be overlooked.

Therefore in one recent study the authors proposed to use Raman spectroscopy for gathering in vivo or ex vivo information by coupling Raman spectroscopy with endoscopy. This approach potentially addresses the challenge of limited light penetration in Raman imaging.

Imaging zebrafish embryos with SERS nanoparticles

Cell labeling is a useful tool for following embryonic development and differentiation. In one recent study, researchers engineered a gold SERS nanoprobe for this purpose. The authors showed that detection and imaging of zebrafish embryos at the one-cell stage, with microinjected nanoprobes, is possible.[95] In this study gold nanoparticles 40 nm in diameter were used as the SERS template. This size produced the greatest signal enhancement capability of all sizes tested.

Ex vivo imaging applications

Molecular imaging of EGFR by SERS nanoparticles

EGFR is over-expressed in most human colon cancers. In one recent study researchers synthesized multimodal SERS nanoparticles for monitoring colon tumors with different EGFR expression levels.[96] The nanoparticles were targeted to EGFR using antibodies, which have nanomolar binding affinity for the antigen.

Imaging tissue biopsies with immuno-SERS microscopy

In one recent study researchers engineered 60 nm sized gold/silver nanostars encoded with Raman active reporters. These nanoparticles were used for molecular imaging of a tumor suppressor marker, utilizing a new modality dubbed immune-SERS microscopy [97] The star-shaped nanoparticles were chosen because (a) their Plasmon bands are observed in the red region of the spectrum with minimal interference from tissue auto fluorescence; and (b) the star shape has sharp tips and edges, which enhance the Raman signals remarkably.

fGNPs for Targeted Delivery

fGNPs have been used to target drugs and biomolecules to specific cell types and organelles such as the nucleus or mitochondria. GNPs functionalized with PEG and 3-mercaptopropionic acid was shown to penetrate the nucleus of HeLa cells without causing severe cytotoxicity and hence can be used as a nuclear drug delivery carrier[98] Similarly, GNPs encapsulated by liposomes have been studied for their cellular targeting and uptake capacity while carrying drugs or other cargos[99] Intracellular uptake of GNPs as small as 1.4 nm has been shown to enhance internalization by 1000-fold. Such nanoparticles harbor significant potential to be used as gene delivery vehicles, drug-carriers and carriers for other biomolecules.

CONCLUSION

Gold nanoparticles have been synthesized by various methods. It is extraordinary molecular carrier for targeting, intracellular delivery of huge array of Biomolecules. Gold nanoparticles were conjugated with cancer seeking peptides to impart target specificity in hybrid gold nanoparticles for their potential applications in cancer imaging and therapy. Their plasmonic properties and surface chemistry allow them to form stable interactions with biologically active compounds. Gold nanoparticles have useful as Biosensors as well as in gene delivery, drug delivery and Bioimaging.

Without affecting these many potential applications, there is still there is still a pressing need to determine any long term toxicological effects of GNPs in order to determine their biocompatibility.

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