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Review Article

APPLICATIONS OF GOLD NANOPARTICLES IN CLINICAL MEDICINE

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ABSTRACT

Gold based nanoparticles, owing to their unique optical properties at the nanoscale, biocompatibility, and rich surface chemistry, have attracted considerable attention from the material science and biomedical community. Although colloidal gold has been used since ancient times in various therapeutic applications, a better understanding of the physical attributes of nanosized gold has led to a burst of research activity in the past twenty years related to their synthesis and biomedical applications. Novel strategies for the synthesis of nanosized gold of various shapes and morphologies have been put forward, which includes nanospheres, nanorods, nanocages, nanoshells, etc. Both the absorption and scattering properties of nanosized gold, along with their facile conjugation with different biomolecules, has been exploited in the development of a number of bioanalytical assays and protocols, related to the quantification of nucleic acids, proteins, other small biomolecules, etc. In addition, gold nanoparticles are being increasingly used for *in vivo* imaging applications for the early diagnosis of cancer. Finally, gold nanoparticles are playing a significant role in nanotherapeutic applications, either as drug carriers or as the drug itself in light-activated photothermal therapy. Several gold-nanoparticle based Bioanalytical assays and therapeutic applications are in the active clinical development phase. This chapter will provide examples of the various biomedical applications of gold nanoparticles with potential for clinical translation.

Keywords: Gold nanoparticles, Theranostics, SPR, Colorimetric biosensing, SERC, *In vitro* diagnostics, PTT, PDT

INTRODUCTION

In the realm of nanotechnology and nanomedicine, gold nano-structures are undoubtedly the most extensively investigated ones owing to their versatility [1-4]. This versatility stems from a number of beneficial characteristics that gold possesses in the nano-dimension. These include their unique optical, electronic and catalytic properties, biocompatibility, non-immunogenicity, antimicrobial properties, and facile surface chemistry. Colloidal gold has been used since ancient times as a coloring material owing to their vibrant, size and concentration-dependent multiple colors (yellow, ruby red, orange, maroon, etc), with the glass decoration in churches being a prominent example. Colloidal gold was also used extensively in ancient times for medicinal applications, such as the 'elixir' drink used in ancient Egypt as what was believed to bea 'rejuvenative potion', powdered gold mixed with drinks for the treatment of sore limbs (arthritis) in medieval Europe, for the treatment of alcoholism and other addictions in nineteenth-century United States, etc. In modern times, with the growing awareness of the importance of nanotechnology in medicine and healthcare, the applications of gold nanoparticles have increased exponentially, from the analysis of body fluids and cells for the identification of biochemical signatures indicative of diseases to the diagnosis and targeted treatment of cancer. In general, nanoparticulate gold has both diagnostic and therapeutic capabilities, two beneficial features that scientists are currently trying to merge together into what is known as 'theranostic' medicine [5, 6].

This book chapter will first briefly explain the physical principles underlying the unique properties of nanosized gold, followed by their various applications in medicine, with emphasis on their potential for clinical translation. Owing to the vast literature available on the topic of various biomedical applications of nanosized gold, only selected examples have been provided here; the authors sincerely regret any unintentional exclusion. The authors have also referred to informative review articles and book chapters available on several sub-topics at suitable sections within this chapter. The various synthetic methodologies developed for the synthesis of a myriad of gold nanostructures, such as nanospheres, nanorods, nanoshells, nanocages, etc are not discussed in this chapter; readers are suggested to consult some excellent reviews available on this topic [7, 8].

Physical features

Several inorganic materials such as gold, some semiconductors, iron oxide, etc. are known to possess certain unique physical properties when their dimensions are reduced to the nanoscale. These physical properties, which are absent in their respective bulk counterparts, emanates from the principle of quantum confinement, which means their size is confined within the quantum domain [9, 10]. For nanosized gold, the unique properties are electronic and optical, the latter receiving more attention owing to their widespread applications in both non-biological and biological disciplines [11]. The bright, tunable color of colloidal gold stems directly from this optical property, which is known as the localized surface plasmon resonance (LSPR) effect. Here, upon irradiation with light, the conduction band electrons in nanosized gold begins to oscillate coherently with a frequency that lies in the visible to near infra-red (NIR) region of the electromagnetic spectrum [12, 13]. This results in light absorption by colloidal dispersions of nanosized gold, with the LSPR absorption peak within the visible to NIR range.

This LSPR absorption peak is also tunable within this range, which depends on a number of factors, such as particle size, shape, aggregation/de-aggregation, surface characteristics, and the refractive index of the surrounding medium [14, 15]. Typically, this peak for spherical gold colloids (approximate diameter 5-15 nm) varies close to 520 nm. However, upon changing the shape to rod-shaped ones, two distinct peaks arise, with the transverse peak (accounting for the cross-sectional diameter of the rods) around 520-530 nm, and the longitudinal peak (accounting for the length of the rods) towards red and NIR regions (within 600 to 1200 nm) [16]. Therefore, by varying the aspect ratio (length/diameter) of the rods, gold nanorods with multiple transverse and longitudinal peaks can be prepared, which will have immense applications in multiplexed diagnostics.

There are several other features of nanosized gold particles which can be exploited in imaging and diagnosis of biological samples. For example, the high electron density and light scattering associated with gold nanoparticles make them useful contrast agents in live cells using electron microscopic and dark-field imaging, respectively. Gold nanoparticles can also be used for signal enhancement of small, Raman-active molecules for surface-enhanced Raman scattering

(SERS)-based detection. In the realm of *in vivo* imaging gold nanoparticles have potential applications in two-photon optical, optical coherence tomographic (OCT) and photoacoustic tomographic imaging, etc. Finally, owing to their ability to convert optical absorption into heat energy via nonradiative electron relaxation dynamics, gold nanoparticles are presently being used for photothermal therapy of cancer *in vivo* and as drug carriers for targeted drug delivery.

Biomedical applications of gold nanoparticles

Nanosized gold has potential applications in every sphere of medicine, whether it is in the identification of unique biochemical signature of a disease or a diseased cell circulating in the blood, or in the immunization of an individual as a safeguard against invasion by a pathogen such as a virus, or in the early diagnosis and treatment of cancer. Simply put, these applications can be broken down into diagnostics, prophylaxis, therapeutics, and theranostics. Among them, *in vitro* bioanalytical techniques such as biosensing have better potential for clinical translation as here the nanoparticles are not introduced in the body, and hence, concerns about their *in vivo* toxicity and long-term persistence in the body are absent. In addition, despite these potential toxicity concerns, gold nanoparticles are showing considerable promise in therapeutics such as photothermal therapy and siRNA therapy, with a few products being in the active clinical pipeline. A number of such applications are presented in the following few sections.

Analytical and diagnostic techniques

As stated earlier, the unique optical properties of nanosized gold has been extensively exploited in developing numerous assays for the detection of a myriad of biomolecules *in vitro*, such as nucleic acids (DNA, RNA, other oligonucleotides), proteins, peptides, lipids, other small biomolecules. These assays are colorimetric, electronic, scanometric, and Raman-based detection strategies, some of which have recently been commercialized and approved by the American Food and Drug Administration [17]. Such detection has significant implications in the early diagnosis of diseases, environmental monitoring for the presence of pathogens and harmful chemicals, genetic or proteomic profiling of biospecimens and organisms, etc. In addition to these *in vitro* diagnostic assays, gold nanoparticles are also being used for *in vivo* diagnosis, particularly towards the advanced detection of cancer. These diagnostic techniques are summarized below, with representative examples.

Colorimetric biosensing

Colorimetric assays take advantage of analyte-induced aggregation events that result in measurable changes and shifts of nanoparticle surface plasmon absorption bands. One of the earliest examples of gold nanoparticle-based colorimetric detection of specific nucleic acid sequences was provided in the laboratory of Chad Mirkin in 1996. Here, using 13-nm gold particles tagged with a complementary polynucleotide sequence, it was shown that the color of the solution changed from red to blue upon analyte-directed aggregation in the presence of only the target polynucleotide sequence [18]. This color change is a consequence of interacting LSPRs and aggregate scattering properties. The introduction of nanoparticles into some well-studied DNA assays results in improved sensitivity. For example, surface plasmon resonance (SPR) is used to detect and probe real-time DNA hybridization surfaces with detection limits of 150 nM target [19].

Rapidly increasing demand of disease diagnostics is significant due to the fast and accurate detection of DNA. In this study detection of hepatitis B virus (HBV) DNA hybridization was carried out in homogenous solution. This is a highly specific electrochemical and ultrasensitive sensing technique in which magnetic nanoparticles (MNPs) were synthesized and further modified with β-CD. Herein to ensure the hybridization a stem-loop like DNA was used as a probe and was labeled with dabcyl at thé 5 -end and Au NPS at' 3 -end where former act as a guest and later as an electrochemical tag. Thus, initially, the DNA remains in a stem-loop configuration which prevents the docking with β-CD/MNP in solution due to steric repulsions. Further the stem-loop DNA Probe was dissociated in the presence of complementary target DNA and the formation of double-stranded DNA (dsDNA) takes place because of hybridization. Then β-CD/MNP was linked with ds DNA due to the host–guest recognition between β-CD and dabcyl. Thus, the electrochemical signals provided by Au nanoparticles could be sensitively transduced by these hybridization events. Then an external magnetic field has been applied to capture the Au NPs through electrode. Thus, the designed sensor is capable for discrimination between the single-nucleotide polymorphism (SNP)-containing sequences and healthy sequences [20].

Gold nanoparticle-based colorimetric biosensing has also been used for the detection of proteins and peptides, relying on the specific interactions between nanoparticle-bound antibodies with the target protein [21]. Halas and co-workers have used antibodies conjugated to the surface of gold nanoshells to detect proteins in saline, serum, and whole blood [22]. Upon interaction with the target protein, the antibody functionalized nanoshells aggregate, resulting in a corresponding broadening of the nanoshell extinction peak at 720 nm. More information on colorimetric biosensing using gold nanostructures is available in several excellent reviews [2-23].

Molecular beacons

Another technique of detection of nucleic acids is using molecular beacons, which relies on the interaction of a fluorescent probe with a quencher. Gold nanoparticles have been extensively used as quenchers since here the quenching is much more efficient than molecular quenchers, resulting in a more sensitive probe [24]. In an interesting example, Nie and co-workers have designed a molecular beacon with oligonucleotides functionalized on one end with gold nanoparticles and the other end with a molecular fluorophore. The 'loop' structure is formed with the fluorophore nonspecifically binding to the gold surface, along with quenching of fluorescence. Upon binding to the 'target' oligonucleotide sequence the 'loop' breaks, resulting in separation of the fluorophore and the gold nanoparticle (quencher), leading to fluorescence signal [25]. In another study, to measure the trypsin in biological samples a selective fluorometric platform has been constructed with the help of Au-peptide-FITC beacon. FITC, which is already a fluorophore, was quenched by gold nanoparticles owing to FRET mechanism and further it was recovered after selective cleaving of beacon peptide by trypsin. The concentration range of 10-100 nmol/l with a correlation coefficient and limit detection 0.995 and 5 nmol/l respectively was used for the calibration plot for trypsin. In addition trypsin in blood plasma can also be used by using present approach [26].

Photoluminescence

There is a growing excitement in the use of plasmonic properties of nanosized good for enhancing the photophysical processes resulting in luminescence. The weak photoemission of gold can be enhanced by many orders of magnitude when coupled with an appropriate plasmon excitation [4]. A gold nanoparticle possesses both linear and non-linear (two-photon) photoluminescence, the later manifesting at pulsed laser excitation. The photoluminescence arising from nanosized gold has several advantages over that of organic dye-based fluorophores, which includes higher absorption cross-section and resistance to photo bleaching. Two-photon luminescence is particularly exciting as it enables excitation and emission at the near infra-red (NIR) window with maximum light permeability through biological tissues, emission far removed from the cellular/tissue auto fluorescence window, and requirement of low-power density during imaging thus ensuring minimum damage to biological specimens.

Several reports exist in literature that has demonstrated the potential of nanosized gold particles in cellular (*in vitro*) and intravital (*in vivo*) luminescence imaging. For example, using two-photon luminescence imaging, Tong *et al.* showed that gold nanorods conjugated with folic acid could be efficiently targeted to KB cells (which over express folic acid receptor), but not to NIH/3T3 fibroblast cells (which does not overexpress folic acid receptor). The internalized gold nanorods exhibited high signal-to-noise luminescence, which also enabled single-particle tracking within the cells to study the intracellular nanoparticle-dynamics [25]. The *in*

vitro two-photon luminescence imaging using bioconjugated gold nanoparticles has also been extended to cell supported tissue-phantoms. For example, anti-EGFR conjugated gold nanorods could be imaged with a penetration depth of 75 nm in epithelial tissue phantoms consisting of collagen-supported EGFR-overexpressing A431 skin cancer cells, with minimum damage to the tissue [27-29]. In another study a thin film of gold on a polyethene terephthalate substrate was induced by low energy Ar ion along with other thermodynamic interpretations and corresponding photo-luminescence (PL) properties and specific surface plasmon response (SPR). These properties observed are associated due to the absorption of methyl orange (MO) dye molecules on the surface and its surface-enhanced Raman scattering (SERS) effect. The spherical nanoparticles formation take place due to the dwetting of the film because of sputtering and ion induced thermal spike. Further thermodynamic driving forces played a key role in embedding the nanoparticles in modified PET [30].

In vivo intravital two-photon luminescence imaging using gold, nanorods were first demonstrated by wang *et al.*, who have monitored flow dynamics of CTAB-coated nanorods through the blood vessels in a mouse earlobe, following their tail vein injection [31]. The luminescence signals were three times higher than the background autofluorescence. However, the authors did not observe any signal 30 min after injection, indicating fast clearance of these nanorods from the blood. This blood-circulation time, however, could be significantly increased following coating these nanorods with a layer of polyethylene glycol (PEG) [32].

Optical coherence tomography (OCT)

Optical coherence tomography (OCT) is an interferometric imaging modality where backscattered NIR light is detected. This technique is particularly promising for biomedical imaging applications because it provides noninvasive imaging of living tissues with 10–25 times greater spatial resolution than that produced by ultrasound imaging and up to 100 times better than magnetic resonance imaging or computed tomography [4]. NIR-active contrast agents such as gold NRs can significantly enhance the OCT contrast based on modulations in optical absorption or scattering. Using polyacrylamide-based tissue phantoms, Troutman *et al.* have demonstrated OCT image distribution of gold nanorods with appropriate scattering and anisotropy coefficients , along with an estimated detection limit as low as 30 p. p. m. [33]. Furthermore, gold nanorod mediated OCT contrast enhancement has also been demonstrated *in vivo* in an excised sample of human breast invasive ductal carcinoma [34]. Recently, using a sensitive temperature variation of OCT, known as photothermal OCT (PT-OCT), the time-dependent imaging and distribution of gold nanorods (GNRs) in the sentinel lymph node (SLN) of mice *in vivo* was shown [35].

Photoacoustic tomography (PT)

Photoacoustic tomography (PAT) is a noninvasive imaging technique based on NIR-induced photoacoustic effects, with a depth penetration of several centimeters in biological tissues [4]. Owing to photoinduced cavitation effects produced by NIR-absorbing gold nanorods, they serve as excellent contrast agents in PT imaging. For example, gold nanorods significantly enhanced PT images in nude mice when subcutaneously injected in picomolar concentrations [35]. Gold nanorods have also been used as PT contrast agents for quantitative flow analysis in biological tissues [36]. Owing to their potential for *in vivo* imaging, gold nanoparticles as contrast agents for OCT and PT imaging are being actively investigated in various laboratories at present.

Electron microscopic and dark field imaging

In vitro imaging based investigations of cellular composition and processes can yield rich information about the nature, differentiation pattern and stimuli-sensitivity of various cells, such as immune cells, cancer cells, stem cells, etc. Fluorescence imaging based investigations have been extensively carried out in this regard, where organic dyes, and more recently bio conjugated quantum dots, have been used as the fluorescent probes. However, the photo bleaching tendency and wide emission bands of organic dyes, as well as the inherent toxicity and blinking associated with quantum dots, pose significant challenges towards successful fluorescence-based cellular imaging, particularly when prolonged imaging to probe a dynamic cellular process is in question.

Optical scattering based imaging techniques such as darkfield imaging using bioconjugated gold nanoprobes can effectively remedy this issue as such probes are non-toxic and are resistant to photobleaching. Several groups have demonstrated the use of bioconjugated nanoparticles and nanorods in dark field imaging of cells and tissues, including dynamic molecular and macromolecular investigations within them [37-38]. Hu *et al.* have exploited the tunability of longitudinal plasmonic bands of gold nanorods to obtain wavelength-selective plasmon-enhanced scattering from multicolored gold nanorods for targeted imaging of cancer cells. Moreover, they have demonstrated the combined dark field and electron microscopic imaging of cancer cells treated with bioconjugated gold and silver nanoparticles in a multiplexed manner [39].

Surface-enhanced raman scattering (SERS)

SERS is a promising surface-sensitive technique where scattering signal from Raman-active molecules are enhanced enormously, upto a factor of 10[15] when they are adsorbed on the surface of metallic nanoparticles. The exact mechanism of this enhancement is not yet clearly understood, with two parallel theories being put forward. The electromagnetic theory is based on the enhancement of electric field on the nanoparticle surface mediated by localized surface plasmons, whereas the chemical theory proposes the formation of charge-transfer complexes between the nanoparticle and the Raman-active molecules. Nevertheless, owing to the biocompatibility of metallic nanoparticles, SERS is fast gaining popularity as a bioanalytical technique capable of ultrasensitive detection of molecules to a single molecule level [40-41].

For example, Kneipp and coworkers used p-mercaptobenzoicacid (p-MBA)-conjugated Au nano aggregates for SERSimaging of pH in living NIH 3T3 cells [42]. Recently, Nie and coworkers have used malachite green as a Raman probe attached to gold nanoparticles, co-conjugated with an antibody specific to EGF receptors, to target and image EGF-receptor positive cancer cells *in vitro* and *in vivo*. As contrasted to non-targeted nanoparticle controls, the targeted nanoparticles were efficiently localized at the tumor site *in vivo* and detected by specific SERS bands of malachite green [43]. Several other examples of SERS-based Bioimaging using nanosized gold can be found in excellent reviews written on this subject [44-46].

Therapeutic applications of gold nanoparticles

In addition to their various diagnostic and imaging capabilities as illustrated in the previous sections, owing to the biocompatibility and facile surface chemistry of nanosized gold, they have been used as carriers of therapeutic agents for targeted delivery to desired cells/tissues. These therapeutic agents can be traditional drugs such as doxorubicin and paclitaxel, or new generation therapeutics such as antisense and short-interfering (si) RNA. In addition, the unique light-to-heat conversion capability of gold nanoparticles is being increasingly exploited in the photothermal therapy (PTT) of cancer, with immense potential for clinical translation. Such applications are summarized below:

Delivery of chemotherapeutics

Among all the clinical cancer therapeutic treatments available till date, chemotherapy still looms large and gold nanoparticles have advanced into drug delivery applications because of its low inherent toxicity and rich surface chemistry. As opposed to 'soft' polymer-nanoparticles where drugs are often simply encapsulated from where they release in a sustained manner, in 'hard' nanoparticles such as gold drugs are either chemically conjugated or electrostatically complexed on the surface. The surface chemistry of the gold nanoparticles plays an immense role in formulating an efficient drug delivery platform. Surface modification of the gold nanoparticles not only provides an increased circulation time and thus slows down the RES uptake, when modified with PEG molecules, but also can be easily targeted to the diseased site when coupled with specific biomolecules [47]. The therapeutic efficacy of

the drug usually depends on upon the method used for the drug delivery and thus the availability of the drug at the target site. The release of the drug from the nanoparticles can be modulated either by internal stimuli such as pH and enzymes, or external stimuli such as light [48-49].

Non-covalent complexation of the drug with the gold nanoparticles provides a relatively simple platform in which drug can be incorporated with the gold nanoparticles without being chemically modified into a prodrug, or any other functional derivative. The use of appropriate ligands on the surface of gold nanoparticles provides the hydrophobic pockets where the hydrophobic drugs can be partitioned. Although non-covalent complexation of the drug with the gold nanoparticles can provide a relatively simple drug delivery platform, the covalent conjugation of the drug on the nanoparticles surface is far stronger and much stable [45]. However, the high stability of the covalent conjugation may result in an inefficient release of the drug at the target site [50]. Non-covalent complexation thus provides a slightly better platform for drug delivery using gold nanoparticles. Here, the surface of the gold nanoparticles can be modified with different polyelectrolytes such as poly (diallyl dimethyl ammonium chloride), poly (sodium-4 styrene sulfonate), poly (allylamine hydrochloride), which provides a net charge on the surfaces for non-covalent complexation with a countercharged therapeutic agent [51-52].

There have been a number of reports on the use of gold nanoparticles for drug delivery via non-covalent interactions. An example of drug delivery using PEG-modified gold nanoparticles is provided by Paciotti *et al.*, who have used PEG-modified gold nanoparticles for delivery of the anti-cancer drug Paclitaxel [53]. They have shown that the 26 nm gold nanoparticles coated with a mixture of tumor necrosis factor and PEG-thiol can be targeted to the tumor cells by extravasation and provide an efficient delivery of paclitaxel. Tom *et al.* have shown that the release kinetics of ciprofloxacin functionalized gold nanoparticles is greatly influenced by the size of the functionalized nanoparticles and the type of release medium [54]. Rotello *et al.* have used 2.5 nm gold nanoparticles functionalized with a hydrophobic alkanethiol interior and a hydrophilic shell of tetra (ethylene glycol) for entrapping the drug into the hydrophobic monolayer [55].

Several reports on the covalent conjugation of the drug on the gold nanoparticle surface suggest a better efficiency of the drug in comparison to the same amount of free drugs. Chen *et al.* have reported a seven-fold increase in the efficiency of methotrexate conjugated to gold nanoparticles. The authors have conjugated the gold nanoparticles to the carboxylic group of the methotrexate and studied the cytotoxicity of the drug in the *in vitro* and *in vivo* Lewis lung carcinoma models [56]. In another report, PEG-coated gold nanoparticles were conjugated to TNF-α to maximize the tumor damage and minimize the systemic toxicity of TNF-α. The authors observed an enhanced therapeutic efficacy when gold nanoparticles conjugated TNF-α was injected intravenously in conjunction with local heating. This conjugate, now known as CYT-6091, is currently in Phase 1 clinical trials for pharmacokinetic and clinical efficacy [57].

To modulate the release kinetics of the covalently conjugated drug from the gold nanoparticles, several different approaches involving the use of an external stimulus have been explored. In one such report, Aryal *et al.* have conjugated the anti-cancer drug doxorubicin on the surface of the gold nanoparticles via a pH-sensitive hydrazone bond [58]. The acidic endosomal pH in the tumor cells resulted in the release of the doxorubicin from the gold nanoparticles, whereas at physiological pH the conjugate showed almost negligible release of the drug. Apart from pH, light has also been used as external stimuli for the conjugated drug release. Nakanishi *et al.* have reported photoresponsive gold nanoparticles conjugated histamine as a cell-signaling agent. The succinimidyl ester can be easily photocleaved using UV-irradiation [59]. Rotello *et al.* have used a photocleavable ligand to conjugate 5-fluorouracil with 2 nm gold nanoparticles [60]. The photocleavable ligand contains o-nitrobenzyl ester group which dissociates upon UV-light irradiation (365 nm) and releases the drug at the target site. Also, an enzyme (phosphodiesterases) based cleaving of the phosphate bond linking the anti-cancer drug paclitaxel to gold and iron oxide nanoparticles have been shown [61].

Photodynamic therapy (PDT)

PDT is a clinically approved and successful cancer therapy for various neoplastic and non-malignant diseases. In PDT, the therapeutic compounds known as photo sensitizers (PS) are activated with a specific wavelength of light, which results in a photochemical reaction leading to the generation of reactive oxygen species (ROS) such as free radicals and singlet oxygen ($^1O_2$) [10]. These species can rapidly cause significant toxicity leading to cell death via apoptosis or necrosis. The efficiency of PDT also depends on the concentration of PS accumulated in the target tissue and the associated wavelength of light used for excitation. Nanoparticles provide an important platform in increasing the efficiency of the PDT by delivering the PS to the target site, acting as energy transducers and as PS as such. There have been numerous approaches proposed for the selective delivery of the PS to the target site, which includes targeting the PS by conjugating to integrin antagonist/specific biomolecules, high payload PS delivery via various polymeric nanoparticles, liposomes, and micelles.

Gold nanoparticles have been explored in the field of PDT for the delivery of PS's like phthalocyanines to the target site [62]. The delivery of PS by using gold nanoparticles can be carried out either by covalent conjugation of the PS to the nanoparticle surface or by non-covalent complexation. The advantage of using gold nanoparticles relies on the fact that gold nanoparticles can easily be targeted to the diseased site by using specific biomarkers. Also, the surface of the gold nanoparticles can be easily modified with PEG molecules to avoid the opsonization process, which leads to the clearance of nanoparticles from the systemic circulation and accumulation in the liver and spleen. Stuchinskaya *et al.* have reported the use of a 4-component system of PS-gold nanoparticle conjugate for targeted *in vitro* PDT in breast cancer cells [63]. Burda and coworkers have shown non-covalent complexation of PEGylated gold nanoparticles with phthalocyanine 4 (Pc 4), a FDA-approved PS, as a delivery vehicle for the PS in a glioma cancer model [64]. The authors suggested that the non-covalent complexation of PS with nanoparticles provided fast and target-specific drug accumulation at the disease site, in comparison to free drug and covalent conjugate counterparts.

Gene delivery

Gold nanoparticles and nanorods, following appropriate surface modification, has been extensively employed for the delivery of oligonucleotide-based therapeutics such as antisense RNA, siRNA, microRNA, etc [65-66]. In the free form, these therapeutic RNA molecules have a very short half-life in physiological conditions, owing to their vulnerability for degradation by endogenous nucleases. Nanosized gold particles serve as macromolecular carriers that not only protect them from degradation in the biological milieu, but also steer them to desired cells/tissues and facilitate their cellular entry. Also, since the formation of the nanoparticle-oligonucleotide complex (the nanoplex) often involved, simple electrostatic interactions between conically-modified nanoparticle and the inherently anionic RNA molecules (owing to the presence of phosphate backbone), the chances of any chemical modification-mediated loss of functionality of the RNA molecule are negligible. Moreover, by exploiting the LSPR phenomenon associated with GNPs/GNRs, their complexation with genetic materials and subsequently their delivery and distribution within target tissues can be optically monitored [67].

Chad Mirkin's group has extensively demonstrated the non-covalent or covalent binding of various RNA molecules to gold nanospheres, and their applications in nucleic acid detection assays, from within the cells and outside [17]. Recently, Paras Prasad's group has demonstrated the use of gold nanorods electrostatically with therapeutic siRNA that plays a role in modulating the key components of the dopaminergic signaling pathway in brain cells *in vitro*, with efficiency greater than that of commercially available reagent (siPORT) [67]. As this pathway is implicated in opiate addiction, such nanotechnology mediated gene silencing strategies are expected to play a critical role in the treatment of drug addiction. The authors also showed that these nanoplexes displayed significantly higher transmigration efficiency across an *in vitro*

model of the blood-brain barrier (BBB) over that of free siRNA, without compromising the functional integrity of the barrier. Therefore, these nano plexes appear to be ideally suited for brain-specific delivery of appropriate siRNA for therapy of drug addiction and other brain diseases. The same group also showed this technology to induce an immune response using single-stranded RNA (5′-PPP-ssRNA) molecules attached to gold nanorods in pulmonary cells [68].

Radiation therapy

There has been a recent thrust in exploiting the physical properties of the gold nanoparticles to enhance the efficacy and specificity of radiation therapy. Clinical radiation therapy for cancer utilizes high energy (MeV) photons or electrons (by the external beam) or low energy (keV) photons (by internally place radioisotopes). Metal-enhanced radiation therapy involves high atomic number (Z) materials like gold to increase absorption of low kilovoltage x-rays in the diseased site as compared to the normal tissue [69]. The interaction of x-rays with gold nanoparticles results in the generation of multiple low energy Auger electrons which usually short-lived species. The therapeutic efficacy of the radiation therapy heavily depends on the ratio of radiation dose to cancer cells to the dose imparted to normal tissues. Gold nanoparticles play an important role in enhancing the effective radiation dose to the diseased sites. In the presence of gold nanoparticles, *in vitro* studies have been performed to quantify the dose enhancement [70].

The experimental results have shown that lower energy sources produced the highest effects. Interestingly, the dose enhancement from megavoltage irradiation was much higher than expected (>25%) due to the proximity of the nanoparticles to the cell nuclei. In few studies, application of gold nanoparticles in RT has been further expanded in small animals as well. Hainfield *et al.* have reported a significant anti-tumor effect with an overall increase in survival from 20% to 86% in case of mice receiving gold nanoparticle-assisted RT, versus RT alone, in a mammary carcinoma mouse model [71]. The mice were injected intravenously with 2.7g/kg of 1.9 nm gold nanoparticles using 250 kVp x-rays. The results of the early theoretical and experimental studies have shown great potential for gold nanoparticle-aided radiation therapy to improve cancer treatments substantially.

Photothermal therapy (PTT)

Photothermal therapy (PTT) is a branch of light-induced thermal therapy in which electromagnetic near infrared (NIR) or infrared (IR) light is used for generating localized heating (up to 113 F) in the tumor tissues, with minimal invasiveness [72]. Thermal therapies cause necrosis of the cells through lysis and rupture of membranes and release of digestive enzymes. In addition, thermal therapeutic procedures are relatively simple to perform and therefore have the potential of improving recovery times, reducing complication rates and hospital stays.

There have been many reports of different platforms of gold nanoparticles being used as photothermal sensitizers, which vary from spherical clusters of gold nanoparticles to gold nanorods, nanoshells, nanocages, etc [73-75]. The metallic gold nanoparticles are the strongest absorbers and their absorption coefficient, due to profound plasmon resonance, is at least three orders-of-magnitude greater when compared to other organic photosensitizers. The tunability of the gold surface plasmon peak to NIR region offers irradiation in the 700-900 nm spectral range which allows deeper penetration of the laser radiation into the biotissues. The basic principle for the use of gold nanoparticles in PTT relies on the absorption of the incident NIR or IR light by gold nanoparticles which results in the excitation of the free electrons at the surface of gold nanoparticles [76]. The excitation at the plasmon resonance frequency causes a coherent collective oscillation of the free electrons, and when there is an interaction between these excited electrons and the crystal lattice of the gold nanoparticles, the electrons relaxes, and the thermal energy is transferred to the lattice. Subsequently, the heat from the gold particles is dissipated into the surrounding environment. When this photo excitation is carried out *in vivo* at cancer sites, it leads to irreparable and localized damage in the tumor tissues. In the following section, we have categorized the various gold nano platform used for the PTT in various cancer models.

Gold nanoshells

Gold nanoshells are by far the most exploited nano platform for PTT applications, specifically in breast adenocarcinoma tumor models. The nanoshells are composed of a dielectric silica core covered with a thin metallic gold shell. The size of the nanoshells varies from 100-300 nm. By tuning the relative dimensions of the core to the shell, the optical resonances can be turned over a wide range of wavelength, up to NIR region [7]. Also, the biocompatible gold nanoshell surface provides an ideal platform for chemical conjugation to various biomolecules for active targeting to the specific diseased sites and stealth polymers like PEG for enhanced circulation.

The research groups of N. Halas and J. West at Rice University have a done a tremendous amount of work in using gold nanoshells as a potential candidate for the PTT. Hirsch *et al.* have shown that human breast carcinoma cells incubated with gold nanoshells, when exposed to NIR light, induced cell cytotoxicity due to the photothermal effect [77]. Also, they have shown the successful *in vivo* irreversible thermal tissue damage in the breast adenocarcinoma tumor mice model using NIR-absorbing, non-bleaching nanoshells, monitored by real-time MR thermal imaging. The histological experiments with the damaged tissue sections confirmed the photothermal induced morbidity. Apart from these, Stern *et al.* have also reported tumor ablation by the NIR laser using PEGylated gold nanoshells injected intravenously into tumored mice [78]. The control mice group without the nanoshells injections showed a continued tumor growth as opposed to treated mice with no tumor for several months. Stern *et al.* have followed it up with a dose dependent study of the nanoshells in tumored animals [79]. They have shown that a slightly lower nanoshells dose (7 µl/g) only resulted in tumor growth arrest at 21 d, but not tumor ablation, which was otherwise observed with high dose (8.5 µl/g) with 93% tumor necrosis and regression. The reason why such a subtle difference in nanoshell dose could cause dramatically, different therapeutic efficacy deserves careful investigation. One explanation could be that most of these nanoshell-mediated PTT is based on the tumor accumulation of the nanoshells by passive uptake via the EPR effect. A careful investigation including the active targeting using the disease specific ligands and dose escalation studies would definitely provide insight for a better PTT efficiency.

These demonstrations of the enormous potential of nanoshells in efficient tumor kill,coupled with the provision of real-time thermal MR monitoring of the therapeutic phase,paved the way for nanoshell-mediated PTT to knock the door of clinicians. In fact, Nanospectra Biosciences, which holds the license for medical use of Rice University's nanoshell technology, began the first human clinical trial of nanoshell PTT in 2008. They have come up with the commercial name for the nanoshells as AuroLase® Therapy. The company is currently conducting a pilot study in patients with refractory head-and-neck cancer under an open Investigational Device Exemption (IDE), and plans to commence additional clinical studies in other cancers soon.

Gold nanorods

The absorption spectrum of gold nanorods can be easily tuned to the NIR region by changing their aspect ratio. One of the better-explored applications of nanorods is in the field of PTT. El-Sayed *et al.* have demonstrated the potential use of anti-EGFR targeted gold nanorods for selective photothermal therapy of cancer cells using a near-infrared low-energy continuous-wave laser [80]. The use of pulsed laser mode provides the flexibility for localized cellular damage in case of individual metastatic or residual cells. Takahashi *et al.* have shown that by using the multi-pulse near-infrared lasers selective cell damage is achievable in cells treated with gold nanorods [81]. The effect of high energy lasers on nanorods and nanospheres has been studied to evaluate the PT properties of the nanorods before the nanorods disintegrate under the laser power. Goodrich *et al.*

have used shown a significant amount of nanorods accumulation in 24 h in the murine colon carcinoma tumor after a systemic infusion of gold nanorods [82]. They concluded that the combination of nanorod dose and laser exposure level was sufficient for ablative effects in all treated animals and a complete ablation in>40% of the treated animals. However, further investigation of dose accumulations and laser dosimetry may lead to a highly effective therapy. In another report, Huang *et al.* have shown that appropriate surface modification of the CTAB coated gold nanorods with polyelectrolytes play an important role in stabilization of the optical response and in turn a reliable PT response [83].

Gold spheres/clusters

Spherical gold nanoparticles usually vary in size from few nanometers to 100 nm. A typical 13 nm citrate stabilized spherical nanoparticles show 520 nm plasmon peak, which lies in the visible range of the light spectrum. Zharov *et al.* have shown that since the absorbance wavelength of the small-sized nanoparticles lies in the visible range, which is suboptimal for *in vivo* applications, the assembly of gold nanoclusters on the cell membrane showed a significant increase in local absorption and red shifting. This led to higher cell morbidity, compared to the cells that did not have nanoclusters [84]. However, there have been reports in which small sized spherical gold nanoparticles have been used in PTT [85]. Recently, branched gold nanoparticles have been reported as potential PT agents because of large absorption cross section in the NIR window [86]. These branched nanoparticles were actively targeted with the antibody antigen-binding fragments against the HER2 antigen, which is overexpressed in breast and ovarian cancer cells.

For PTT using gold-based nanoplatforms there have been two fundamental issues, (i) the local accumulation of the gold nanostructures in the target tissues should exceed a threshold concentration at which the nanostructures show an efficient thermal ablation, and (ii) the excitation light should be able to penetrate to deeper tissues. The first issue can be explored by an efficient active targeting with specific biomolecular ligands aimed at a particular disease, or by a localized delivery of the nanoparticles. However, an equal emphasis should be devoted for maximizing the tissue penetration of the excitation light, by optimizing the wavelength of light used, laser power, pulse sequences, etc., assisted with imaging modalities for real-time monitoring of the PTT efficacy. Improved fiber-optic based delivery of light in internal body locations is another avenue that can aid the advancement of PTT in the clinic.

CONCLUSION

In conclusion, it can be envisioned that the already rich applications of gold in medicine will further expand, with visible benefits towards the treatment of several diseases. Healthcare for the next century will heavily look forward towards the success of nanomedicine, with theranostics and personalized medicine being the visions to be realized. Facile surface chemistry and exquisite tunability of optical properties have led to the emergence of several bioconjugated gold nanoparticle-based bioanalytical assays for ultrasensitive, multiplexed and high-throughput detection of biomolecules. Also, owing to their both inherent and externally-incorporated diagnostic and therapeutic capabilities, gold nanostructures have already shown promise in theranostic medicine. In parallel, a key area the needs a thorough investigation would be the potential toxicity of gold nanostructures in humans, particularly when their long-term persistence in the body is a concern. A better understanding of the potential toxicity, dynamics, and route of expulsion from the body will portray a realistic picture of the promise of gold nanostructures in the human healthcare, thus clarifying several speculations that date back to ancient times.

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CONFLICT OF INTERESTS

Declared none

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