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Gold Nanoparticles: A Revival in Precious Metal Administration to Patients

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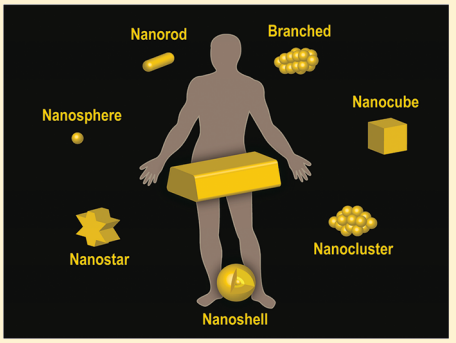
ABSTRACT: Gold has been used as a therapeutic agent to treat a wide variety of rheumatic diseases including psoriatic arthritis, juvenile arthritis, and discoid lupus erythematosus. Although the use of gold has been largely superseded by newer drugs, gold nanoparticles are being used effectively in laboratory based clinical diagnostic methods while concurrently showing great promise in vivo either as a diagnostic imaging agent or a therapeutic agent. For these reasons, gold nanoparticles are therefore well placed to enter mainstream clinical practice in the near future. Hence, the present review summarizes the chemistry, pharmacokinetics, biodistribution, metabolism, and toxicity of bulk gold in humans based on decades of clinical observation and experiments in which gold was used to treat patients with rheumatoid arthritis. The beneficial attributes of gold nanoparticles, such as their ease of synthesis, functionalization, and shape control are also highlighted demonstrating why gold nanoparticles are an attractive target for further development and optimization. The importance of controlling the size and shape of gold nanoparticles to minimize any potential toxic side effects is also discussed.



KEYWORDS: Nanoparticles, gold, toxicity, humans, applications

One of the most significant developments in recent years has been the development of new materials in the nanometer scale called nanoparticles. Nanoparticles are expected to form the basis of many of the technological and biological innovations of this century, exhibiting distinct advantageous physical, chemical, and biological properties. They also have the potential to help establish specific beneficial processes and achieve selectivity within biological settings. To date, a large number of nanoparticles have been synthesized, especially those made from noble metals such as gold. Gold nanoparticles can be manufactured into a variety of shapes including gold nanospheres, nanorods, nanobelts, nanocages, nanoprisms, and nanostars.[1] The chemical, optical, and electromagnetic properties of gold nanoparticles are strongly influenced by their size and shape. For example, in comparison to metallic gold which is golden yellow, spherical gold nanoparticles have a visible red wine color while gold nanorods are blue (aspect ratio 2−3) or black (aspect ratio 3) in solution.[2] The ease of synthesis and the unique properties of gold nanoparticles make them ideal candidates for translation from the laboratory setting into the clinical arena for use in humans. Additional enthusiasm for the use of gold nanoparticles in patients stems from gold's previous clinical use in treating several diseases, most notably rheumatoid arthritis (RA), with minimal biological side effects. Although gold may have fallen out of favor as a mainstream therapeutic agent, its use in nanoparticles is set to revive its application in medical care in both patient diagnosis and treatment. We review the chemistry, biology, pharmacokinetics, and toxicology of gold and consider its new use as a clinically applicable nanoparticle, thereby potentially seeing the resurgence of gold use in everyday clinical practice in the near future.

The ease of synthesis and the unique properties of gold nanoparticles make them ideal candidates for translation from the laboratory setting into the clinical arena for use in humans.

The History of Gold. The use of gold for medicinal purposes dates back to 2500 BC to the ancient Chinese and Egyptians.[3,4] In medieval Europe, numerous recipes for gold elixirs existed and in the 17th and 19th century gold was used to treat fevers and syphilis, respectively.[5] The use of gold in modern medicine began in 1890 when the German bacteriologist Robert Koch discovered that gold cyanide was bacteriostatic to the tubercle bacillus in vitro.[3] This subsequently led to the treatment of tuberculosis with gold in the early 20th century. As RA was initially thought to be an atypical form of tuberculosis,[6] Laude used gold to treat RA

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Table 1. Examples of the Use of Bulk Gold in Clinical Practice

Disease	Gold Form	Name	Role	Mechanism	Time Period	Side Effects
Oral Cavity	Gold Alloy	Jensen Foil	Cavity Filling	Dental Support	16th Century – Present	Rare Allergic Reaction
Rheumatoid Arthritis	Gold Sodium Thiomalate (IM)	Aurolate	Anti-inflammatory	Mitochrondrial inactivation and apoptosis	1930s – present	Nausea, Vomiting, GI Distress
Rheumatoid Arthritis	Aurothioglucose (IM)	Auranofin	Anti-inflammatory	Unclear	1930s – Present	Dermatologic (Dermatitis, pruritus, rash)
Psoriatic Arthritis	Gold Sodium Thiomalate (IM)	Aurolate	Anti-inflammatory	Mitochrondrial inactivation and apoptosis	1930s – present	Nausea, Vomiting, GI Distress
Discoid Lupus Erythematosus	Aurothioglucose (PO)	Auranofin	Lesion Reduction	Unclear	1980s – 1990s	GI Distress

in 1927. Although gold therapy proved to be ineffective for tuberculosis, a study by the Empire Rheumatism Council confirmed gold to be effective in RA, with Forestier showing beneficial results in RA patients in 1935.[5,7] Gold has since been used as a therapeutic agent to treat a wide variety of rheumatic diseases including psoriatic arthritis,[8] juvenile arthritis, and discoid lupus erythematosus;[9,10] however, its use has been largely superseded by newer drugs. Gold has also been used in several other areas of medicine including prostheses in dentistry[11] and ophthalmology,[12] gene delivery,[13] and gold coated coronary[14] and renal[15] stents, to name a few (Table 1).

The Chemistry of Gold. Gold is a noble metal found in group 1B of the periodic table with an atomic number of 197. Gold can exist in a number of oxidation states: (−I), (0), (I), (II), (III), (IV), and (V), however, only gold(0), (I), and (III) are stable in aqueous solution. Hence, in vivo, gold exists in equilibrium between its metallic ground state (gold(0)) and its oxidized states (gold(I) or gold(III)).[6] Metallic gold does not oxidize or burn in air, even when heated, and has been shown to be inert to strong alkalis and acids thereby making it one of the least chemically reactive metals known to man.[11] In contrast, gold(I) and (III) are unstable with respect to gold(0), with gold(III) being a strong oxidizing agent which is reduced to gold(I) by biologically occurring reductants such as thiols.[5] As gold(I) preferentially reacts with S-donors, rather than O- and N- donors, it can be stabilized by thiolate ligands. These resulting gold thiol compounds then undergo biological ligand exchange reactions which account, in part, for their pharmacological activity.[5]

Gold in Humans. Humans contain a mean of 0.35 μg of gold (0) per gram of dry tissue weight[16] which, according to calculations by Merchant, equates to 2.45 mg of gold in an average 70 kg man.[6] Blood gold concentrations in healthy human subjects have also been reported to be around 0−0.001 ppm,[17] with additional studies reporting small quantities of gold in hair (0.3 μg/g), skin (0.03 μg/g), and nails (0.17 μg/g).[18−20] Up to 0.8 μg of gold per dry weight has also been measured in fingers beneath gold wedding rings of normal individuals.[21] Interestingly, gold is also sometimes used in food in very minute quantities in pastries, chocolates, and even alcoholic beverages.[22]

Gold Therapy. On the basis of the chemistry of gold, gold(I) is used as the main therapeutic agent as it is water soluble, is less reactive than gold(III) and is easily stabilized in a complex by the addition of ligands. Gold can be delivered to patients intravenously, intramuscularly, or orally with gold preparations specifically designed for each particular route of administration. Accordingly, gold taken orally needs to be lipid soluble for it to be absorbed within the gastrointestinal tract and will therefore have different physiochemical, pharmacokinetic, and toxicological properties compared to water soluble gold that is injected.[9,23] This is supported by experiments demonstrating only 1% of injectable gold is absorbed when given orally compared to 100% when given intramuscularly.[24] Although gold has been recently used as an anticancer and antimicrobial agent,[5] most of the studies on the efficacy, toxicity, and pharmacokinetics of gold preparations were previously performed in patients with RA who were treated with gold in the late 20th century. However, despite gold being used in clinical practice for several decades there is still considerable debate as to whether injectable or oral gold preparations are better for patients.[25] Initially, oral gold treatments that were developed in the 1980s, such as Auranofin, were thought to have improved pharmacokinetic profiles with less tissue retention, less toxicity, and reduced serum gold levels that were maintained for longer.[5] However, their clinical side effect profile and fear of long-term immune suppression have resulted in injectable compounds, such as gold sodium thiomalate, remaining the preferred gold drugs for RA treatment.[25]

Gold Pharmacokinetics and Biodistribution. The bioavailability of gold in patients very much depends on the route of administration. While injectable gold compounds are fully absorbed with maximum levels attained after about 2 h,[26] only 20−25% of oral gold is absorbed.[23,24,27] Furthermore, intermittent dosing regimens of injectable gold result in fluctuating blood gold levels with high peak and low trough concentrations.[28] In contrast, oral gold preparations can be taken regularly and made with prolonged blood half-life preparations, resulting in a nearly constant concentration of gold for the duration of a patient's treatment. However, with chronic daily oral administration the serum gold concentrations reaches a plateau, and in some cases,

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despite constant dosing, gradually starts to decline.[29] Various mechanisms have been put forward to explain this phenomenon including insufficient drug compliance, increased drug clearance, or an increased distribution volume (i.e., a shift from protein-bound gold to cell-bound gold).[29]

Following absorption of gold, either from tissues or the gastrointestinal tract, approximately 95% is bound to albumin and/or globulin where it can remain within the plasma for several months.[4,30] Gold has also been found within the cellular compartment of blood, primarily in the erythrocyte fraction.[31,32] Here, gold has been shown to be within or attached to the membranes of red blood cells (RBCs),[24,33] with uptake dependent on either the amount of gold available for red cell precursors in the bone marrow or the gold binding capacity of plasma proteins.[32] Indeed, it has been shown that uptake into RBCs ceases after 48 h even though there is still considerable gold in the plasma, presumably as all the gold by this time is tightly bound to plasma proteins. As gold uptake into RBCs differs among people, being more pronounced in smokers,[34] this could explain the large variability in gold distribution seen among patients. Gold is widely distributed throughout the body with organs of the reticuloendothelial system, especially the lymph nodes, having the greatest affinity for this heavy metal.[16] The liver and bone marrow have each been shown to account for 25% of the total body gold burden with the skin and bone each accounting for 20%.[23] Furthermore, the exact form of gold in these locations remains unknown although it appears to be inactive as it remains detectable in tissue samples taken from patients who had been treated with gold years earlier.[35] In general, gold has little affinity for keratinous tissue,[18] but it can accumulate in the skin dermis[36,37] during intravenous administration, with negligible levels recorded when gold is given orally.[28] At very high levels of intravenous administration, gold has also been shown to deposit in the cornea as detected by slit lamp examination.[38]

Gold Metabolism and Excretion. Gold is primarily excreted in the urine and feces and although the rate of excretion varies considerably from patient to patient, the basic pattern remains the same.[30,36] Following intramuscular injection of gold, it has been shown that urinary excretion was greatest during the first day postinjection while fecal excretion was greatest during the middle of the week.[30] Although, the amount of gold excreted in the urine and feces increases as the amount of injected gold increases, the excretion rate was not directly proportional to the amount injected.[30] The high binding capacity of albumin for gold may explain the slow rate of gold clearance throughout the week following gold injection. When gold is given orally, 85−95% is excreted in feces and the remaining 5−15% in urine, regardless of dose.[27,28] The majority of gold recovered in the feces represents nonabsorbed gold, gold breakdown products, gold shed from mucosal cells to which it was adsorbed and a minor contribution from the biliary tract.[24,39] Once gold treatment is established, a dynamic equilibrium is set up in the body with gold moving between the blood, body stores, urine, and feces.

Gold Toxicity. Any toxicity associated with gold depends on its oxidation state when given to patients. Metallic gold (gold(0)) is an extremely inert metal which is widely used throughout the world in both jewellery and prostheses. Indeed, most of the human population has had prolonged dermal contact with gold(0) in the form of jewelry, with only exceptionally rare cases of adverse reactions or allergic contact dermatitis. Furthermore, approximately half of the 1 billion people in the modern industrialized world carry dental prostheses made of gold with relatively few cases of oral lesions being reported despite the close prolonged contact of the metal with the oral mucosa.[6] However, gold(0) can, in very minute amounts, be converted to gold(I) by amino acids contained in sweat and saliva which can then be absorbed through the skin or gingival mucosa and later enter phagocytic and antigen presenting cells.[40] That notwithstanding, the metabolic impact of this is usually insufficient to evoke clinical symptoms.[6] Moreover, as gold(0) is readily available, has a very low toxicity profile, and can be made into a consistently small size and shape, it has been used as a delivery vehicle for gene therapy.[41] Indeed, microprojectile bombardment of cells with DNA on gold particles has been developed as an effective method of high frequency gene transfer with minimal damage to living cells.[42] Experiments injecting naked gold beads into the epidermis of pigs, whose skin is an excellent model for human skin, concluded that apart from acute impact physical effects, which resulted in mild transient dermal irritation, there were no direct toxicities or adverse effects on health, survival, clinical chemistry, or hematology values related to the gold beads.[6]

Gold(I) is normally used as therapeutic agent in both injectable and oral preparations. The toxicities surrounding gold(I) have been primarily understood by examining patients treated with gold for RA; however frustratingly, serum and urine levels of gold have been of no value in predicting impending toxicity in patients. The most common toxicity associated with gold treatment is skin and mucous membrane hypersensitivity reactions, with nonspecific pruritic erythematous, macular, and papular rashes appearing first. Other rarer skin reactions include cheilitis, eosinophilia, chronic papular eruptions, contact sensitivity, erythema nodosum, allergic contact purpura, exfoliative dermatitis, and pityriasis rosea.[6] This diverse range of dermal reactions appears not to depend on the gold concentrations in the skin and rarely occur in patients who receive less than 250 mg of gold salts.[11] In fact, it is generally regarded that these reactions represent the balance between the total body burden of gold salts and the patient's genetic and metabolic makeup. Management involves the cessation of gold therapy with most cases resolving within 3 months of onset depending on their extent and severity. The most common form of gold-induced dermatitis is nonallergic, since following clearance of the original eruption, patients can be restarted on gold treatment without developing further dermatitis.[43] In contrast, allergic contact dermatitis occurs at a lower incidence and represents an immune reactivity to gold which usually necessitates total cessation of gold therapy.[44] Diarrhea is also frequently associated with administration of gold complexes, but with a greater incidence when patients use oral gold preparations.[45] Less frequently, gold has been associated with nephrotoxicity as demonstrated by minor and transient proteinuria in people treated with injectable gold complexes.[3,46] Occasionally, this may progress to glomerulonephritis with nephritic syndrome although patients usually recover fully within a few months.[9,47] Hematological abnormalities can also be sometimes produced by gold complexes and include eosinophilia, thrombocytopenia,[9] and rarely aplastic anemia probably as a result of a direct inhibition of myelopoiesis.[48] Several other reports have also mentioned the rare consequences of using gold complexes including entercolitis with bloody diarrhea,[49,50] diffuse inflammatory lung reactions,[51] and neurotoxicity.[52] Gold therapy is not recommended during pregnancy,[53] as animal studies have shown it to be teratogenic.[54] Caution is also advised in the puerperium, despite conflicting reports as to whether significant absorption occurs in the infant, since gold can also be found in breast milk.[55]

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Gold(III) is rarely used as a primary therapeutic agent as it is a strong oxidizing agent and thus very reactive. However, gold(I) can transform into gold(III) within phagolysosomes, which may account, in part, for some of its toxic effects. In brief, gold(I) is oxidized to gold(III) via a redox system involving myeloperoxidase and other lysosomal enzymes within phagolysosomes containing gold (i.e., aurosomes). Gold(III) then diffuses away from its site of generation where it can interact with and denature "self-proteins" surrounding proteins, thereby possibly explaining why autoimmunity occurs during a few cases of gold therapy.

Gold Nanoparticle Synthesis. Gold nanoparticles can be synthesized into a variety of different sizes and shapes (Figure 1) by different strategies;[56,57] however, the most common method is by chemical or electrochemical reduction of a gold(III) precursor. Control over shape and size is achieved through careful experimental conditions including the specific reducing agent, reaction time, temperature, and use of a capping agent, the latter binds to select nanoparticle faces and blocks growth beyond a certain nanometer range.[58] The most common method to prepare gold spherical nanoparticles is a single-phase water-based reduction method using citrate reduction as described by Turkevich[59] and Frens.[60] By variation of the concentration of citrate and gold for the thermal reduction, spherical particles of different sizes can be made.[61] Newer methods, such as UV initiated particle growth, have also recently been introduced to improve the size distribution and spherical shape of larger sized gold nanoparticles (i.e., 9−120 nm).[61] As spherical gold nanoparticles have absorption peaks near 540 nm, the nanoparticle size and shape can be modulated to bring this peak closer to the optical window of tissue which is between 700 and 800 nm. Furthermore, the surface plasmon resonance (SPR) peaks of gold nanoparticles can also now be optimally tuned,[62] thereby allowing multiplexing of gold nanoparticles. Recently, significant progress has been made in synthesizing nonspherical gold nanoparticles using seed-mediated growth.[56,63] For gold nanorods, a gold spherical nanoparticle seed (i.e., diameter of 1−5 nm) is first synthesized. Next, a solution containing more gold ions, cetyl tetrammonium bromide (CTAB), and ascorbic acid (a mild reducing agent) is added to selectively reduce gold(III) to gold(I). A seed solution containing citrate-capped, penta-twinned gold nanoparticles then catalyzes the reduction of gold(I) ions on their surface with a careful choice of experimental conditions enabling the seed to grow and elongate into a nanorod.[56] The aspect ratio is controlled by the concentration of silver nitrate in the growth solution. Branched gold nanoparticles are more difficult to synthesize reproducibly; however, their sharp edges and corresponding high localization of SPR modes make them excellent candidates for biological applications.[56] Gold nanostars with a magnetic core are able to couple polarized resonance with spatial control.[64] When gold nanostars are placed in an external magnetic field, the orientation of the points which make up the star shape (and hence field enhancement) is controlled. Like spheres, gold nanoprisms[65] maintain an aspect ratio near unity, yet have red-shifted absorption resonance peaks. Nanoshells are created by coating a silica or polymeric core with a thin gold layer,[66] the thickness of which controls the optical properties of the nanoparticle which can be subsequently tuned for efficient heating when irradiated with a near-infrared (NIR) laser. A final class of gold nanoparticle is the gold nanocluster, which contain hundreds of gold atoms (e.g., Au102) and behave as intermediates of nanoparticles and molecular gold.[67] As gold nanoparticles can be easily functionalized and thus targeted, they are therefore ideal particles for in vivo gene delivery, biological imaging, diagnostics and disease treatment.

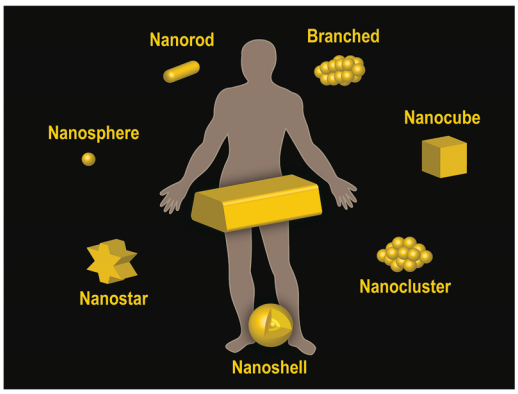

Figure 1. Schematic representations of gold nanoparticles used in clinical practice.

Gold Nanoparticle Toxicity. The toxicity of nanoparticles has also been suggested to differ dramatically from their corresponding bulk material. The small size of nanoparticles will affect their mode of endocytosis and cellular processing.[68] In addition, their high surface area to volume ratio can dramatically alter their chemical and physical properties resulting in them possessing unexpected toxicities and biological interactions. Since nanoparticles will also have a greater amount of their surface in direct contact with the body, they are therefore more reactive to both themselves and their surrounding environment.[69] The main molecular mechanism by which nanoparticles incur toxicity has been hypothesized to be from an increase in oxidative stress as a result of free radical formation.[68] These reactive species are exceedingly toxic in vivo, especially within intracellular compartments, resulting in the oxidation and damage of lipids, proteins, and DNA. While the slow clearance and tissue accumulation of these free radical producing nanoparticles makes organs of the reticuloendothelial system (i.e., the liver and spleen) targets for toxicity, the high blood flow through organs such as the kidney and lungs also place these organs at high risk of oxidative damage. When nanoparticles are introduced into the systemic circulation, they can also interact with blood components to cause hemolysis and thrombosis[69] and with the immune system to cause immunotoxicity.[70] Furthermore, nanoparticle aggregation following systemic administration not only leads to a loss of nanoparticle function but also can cause end organ damage from capillary occlusion.

Studies by Chithrani and Chan have shown gold nanoparticles enter cells via a receptor-mediated clathrin-dependent endocytosis pathway, with 50 nm nanoparticles taken up at a faster rate and higher concentration compared to other nanoparticle sizes.[71,72] In general, they demonstrated that the rate of uptake of gold nanoparticles into cells is lower with an increasing aspect ratio. In addition, gold spherical nanoparticles have a greater efficiency of uptake compared to gold nanorods due to the thermodynamic driving forces for membrane wrapping and receptor diffusion kinetics.[71] Despite this, gold nanorods have been shown to be more toxic compared to spherical particles.[73] One possible explanation for this could lie in their method of synthesis with the cationic surfactant CTAB; however, reports regarding this have been conflicting.[69] As the size of the gold nanoparticles decreases, their rate of exocytosis from cells

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Table 2. Examples of the Use of Gold Nanoparticles in Clinical Practice

Type of Gold Nanoparticle	Nanoparticle Size (nm)	Role	Disease State	Sponsor/Lab
Nanosphere	13	siRNA delivery	Unspecified	Mirkin
Nanorod	10 x 40	Photothermal ablation; CT contrast and thermal imaging	Unspecified	Bhatia
Gold-silica Nanosphere	60/140	Raman Imaging	Colon Cancer	Gambhir
Gold Nanoshell (Aurolase™)	150	Photothermal therapy	Head and Neck cancer	NanoSpectra NCT00848042
Gold Colloidal Nanosphere (Aurimune™)	27	Stimulate immune response to tumor growth	Solid Tumors	NCI NCT00436410

dramatically and linearly increases, with 14 nm nanoparticles leaving cells twice as fast compared to particles of 100 nm size.[71] Furthermore the fraction of gold nanorods exocytosed was higher than spherical-shaped nanostructures. Studies by Pan and colleagues have also shown that the cytotoxicity of gold nanoparticles primarily depends on their size, with particles 1−2 nm in diameter being toxic whereas larger 15 nm gold particles are comparatively nontoxic, irrespective of the cell type tested.[74] Furthermore, particles of 1.4 nm were found to be highly toxic as they irreversibly bind to the major grooves of B-DNA, an effect not observed with larger or smaller particles due to steric reasons. Although there are only a limited number of in vivo studies investigating the systemic effects of gold nanoparticles, 13 nm PEG-coated gold nanoparticles have been shown to have long circulating times, eventually accumulating in the liver where they can induce acute inflammation and apoptosis.[75] The surface charge of gold nanoparticles has also been shown to be important in determining particle toxicity, with cationic gold nanoparticles exhibiting moderate toxicity owing to the electrostatic binding of the particles to the negatively charged cell membrane. In contrast, anionic particles have no toxicity as they are repelled from the membrane.[76]

Taken together, the size, shape, and surface charge of gold nanoparticles need to be carefully considered when designing gold nanoparticles for human use in order to optimize their therapeutic function, while concurrently decreasing their toxicity profile by minimizing their cellular uptake and interactions. One way to reduce any potential toxicity from gold nanoparticles is by the addition of surface poly(ethylene glycol) (PEG). PEG is a coiled polymer of repeating ethylene ether units with dynamic conformations which are inexpensive, versatile, and FDA approved.[77] In both drug delivery and imaging applications, the addition of PEG to nanoparticles reduces uptake by the reticuloendothelial system and increases circulation time versus uncoated counterparts.[78] Recent studies have also shown that PEGylated nanoparticles generally have lower accumulation in the liver compared to non-PEGylated nanoparticles and higher tumor accumulation versus background.[79] Aggregation of nanoparticles also decrease following the addition of PEG due to passivation of the nanoparticle surface and the reduction in the coating of serum and tissue proteins, resulting in so-called "stealth" behavior. PEG also increases the solubility of nanoparticles in buffer and serum due to the hydrophilic ethylene glycol repeats and the enhanced permeability and retention (EPR) effect.[80,81] Alternative passivating polymers which can be added to gold nanoparticles besides PEG include chitosan, dextran, poly(vinylpyrrolidone) (PVP) as well as the copolymer polylacticcoglycolic acid (PLGA).

Clinical Use of Gold Nanoparticles. Over the past few years, gold nanoparticles have been used effectively in laboratory based clinical diagnostic methodologies (Table 2). In particular, DNA-functionalized gold nanoparticles can detect specific DNA and RNA sequences by rapidly binding to nucleotide sequences within a sample with high sensitivity.[82] Furthermore, arrays using gold nanoparticles with specific chemical functionalities are currently being developed for biomarker platforms to detect, identify, and quantify protein targets used for clinical diagnosis.[58,83] However, the main excitement concerning gold nanoparticles is their potential to cross over into clinical practice for use in humans.

From an imaging perspective, gold nanoparticles have shown great promise for their use in computed tomography, Raman spectroscopy, and photoacoustic imaging. Raman spectroscopy is an optically based technique which allows the molecular interrogation of tissues based on the inelastic scattering of light.[84] However, to date this imaging modality has not crossed into mainstream clinical practice due to the limited depth penetration of the optical beam used to carry the Raman signal and the weak intrinsic signal generated by pathological tissues. The latter of these problems has recently been overcome by taking advantage of the phenomenon known as surface enhanced Raman scattering (SERS). SERS is a plasmonic effect where molecules adsorbed onto a nanoroughened noble metal surface experience a dramatic increase in the incident electromagnetic field, thereby resulting in high Raman intensities.[85] Nanoparticles have therefore been created with a gold nanocore surrounded by a Raman organic molecule. This arrangement dramatically increases the incident electromagnetic field of the Raman organic molecule via SERS, thereby dramatically amplifying the intensity of the Raman signal. As the Raman organic molecules have a unique and narrow spectral signature, which can be changed between nanoparticles, this allows multiple

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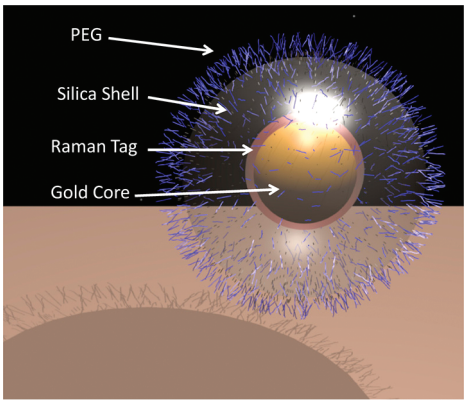

Figure 2. A 3D representation of the Raman-active silica−gold nanoparticle.

nanoparticles to be independently detected simultaneously in vivo in a process known as multiplexing.[86] The entire nanoparticle is encapsulated in a silica shell to hold the Raman organic molecule on the gold nanocore (Figure 2). This exciting discovery means that functionalized/targeted Raman gold nanoparticles may offer a noninvasive technique to detect early disease, especially in circumstances where the Raman probe can be applied closely to the target tissue. One example could be the potential use of functionalized Raman gold nanoparticles to detect early dysplastic lesions in the colon using a Raman based colonoscope. Furthermore, these Raman gold nanoparticles have been shown in human cell culture to cause negligible toxicity at low concentrations with only minimal cytotoxicty and oxidative stress was observed after prolonged exposure at high concentrations.[87] Studies examining the fate of these nanoparticles in living animals have also shown that following *intravenous* administration, nanoparticles were removed from the circulation by marcophages in the liver and spleen with only a mild acute inflammatory response and an increase in oxidative stress in the liver.[88] No evidence of significant toxicity was observed by clinical, histological, biochemical, or cardiovascular parameters after 2 weeks. In addition, *intrarectal* administration of these nanoparticles demonstrates no significant bowel or systemic toxicity with no evidence that these nanoparticles cross the bowel lumen. Although additional studies are required to investigate the long-term effects of these Raman gold nanoparticles, these initial results support the idea that they can be safely used in living subjects, especially when administered *rectally*, thereby supporting their clinical translation. Photoacoustic imaging is another imaging modality which allows deeper tissues to be imaged with high spatial resolution.[89] In this technique, subjects are illuminated with short laser pulses, and as the light photons propagate through tissue, they are absorbed and converted into ultrasound waves which can be detected externally. However, as with Raman spectroscopy, many diseases do not exhibit a natural photoacoustic contrast and hence it is necessary to administer a photoacoustic contrast agent. One such agent showing great promise is the gold nanorod, which has a higher optical cross section than nanospheres in addition to a robust photoacoustic signature.[90]

From a therapeutic perspective, gold nanoparticles have shown promising results in the treatment of a variety of diseases. Gold nanoparticle−oligonucleotide complexes have been used as intracellular gene regulation agents for the control of protein expression in cells[41] while gold nanoparticles coupled to recombinant tumor necrosis factor alpha have been used with promising results in the systemic treatment of nonresectable cancers.[91] Furthermore, gold nanoparticles have been shown to have intrinsic antiangiogenic properties by inhibiting vascular endothelial growth factor induced proliferation of endothelial cells through an interaction with the heparin-binding domain.[92,93] This has led to subsequent work showing the ability of gold nanoparticles to treat cancers in which VEGF plays a major role in disease progression. Gold nanoparticles have also been shown to reduce ascites accumulation in vivo in a mouse ovarian cancer model,[92] inhibit proliferation of multiple myeloma cells by up-regulating p21 and p27 which causes cell cycle arrest,[94] and induce apoptosis in B-chronic lymphocytic leukemia.[95] In addition, as angiogenesis plays a part in the pathogenesis of RA, preliminary studies have also begun to investigate the effect of gold nanoparticles in treating RA with promising results.[96] Gold nanoparticles, especially gold nanorods and nanoshells which have a resonance absorption in the NIR spectrum, can also be used for photothermal therapy.[97,98] Here, nanoparticles are first immobilized at the site of interest either via targeting ligands or by the EPR effect. Next, laser pulses heat the nanoparticle for tumor ablation. Gold nanoshells measuring ca. 120 nm, under the brand name Aurolase, are currently undergoing clinical trials in the treatment of refractory tumors of the head and neck (Clinical Trials gov. Identifier: NCT00848042). In small animal studies, these nanoshells have been used to ablate tumors by heating nanoparticles which have accumulated in tumor tissue with laser irradiation at 808 nm which causes a temperature increase of ca. 20 °C.[66,99] Advantages of photoablative treatment include the ability to customize the treatment with the location and duration of the light pulse. One limitation is that deeper tissue may not receive the same thermal dose as superficial tissue and that the location of a tumor needs to identified prior to the initiation of treatment.

Finally, the diagnostic gold nanoparticle molecular imaging agents previously described can also be used for therapeutic applications, in a combined "theranostic" approach to patient care. We are currently investigating approaches that couple gold nanoparticle imaging agents with different energy pulses (i.e., radiofrequency) to heat and destroy targeted tissues. In addition, gold nanoparticles could also be coupled to different chemotherapeutic agents to deliver high concentrations of chemotherapy to specific targeted cells.

Advances in the synthesis and functionalization of gold nanoparticles have generated excitement and a certain expectation that gold could be returning to use in humans, but in a different guise.

Conclusion. Although the use of gold in clinical practice has declined significantly over the past decade, parallel technological advances in the synthesis and functionalization of gold nanoparticles have generated excitement and a certain expectation that gold could be returning to use in humans, but in a different guise. Gold has been shown to be extremely biocompatible in humans on a bulk level; however, the consequences of its use as a nanoparticle

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may be determined by alternative chemical and biological properties. Thus, gold nanoparticles for use in humans should be designed based on data from both bulk gold treatment in humans and in vivo gold nanoparticle validation experiments. Taken together, nanoparticles made of metallic gold (gold(0)) which are spherical in shape, anionic, and of a size greater than 20 nm would be expected to have the least toxicity in humans. Furthermore, the gold nanoparticle preparation should be optimized depending on its method of delivery (i.e., intravenous vs oral vs intrarectal) to decrease systemic absorption and distribution while increasing urinary and fecal excretion. Future research will need to determine the optimal gold nanoparticles for each potential human application, and inevitably, trade-offs will have to be made regarding some of their diagnostic and therapeutic properties vis-a-vis their associated toxicity profile. Overall, gold nanoparticles are ideally placed to make the transition from the laboratory benchtop to the clinical bedside in the very near future.

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