Gold nanostructures in medicine: past, present and future

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Abstract

Since ancient times it has been assumed that gold has healthy properties, and even today, many biomedical applications have been discovered. The number of these discoveries will increase due to the characteristics of gold as manipulable size, shape, composition and conjugation with molecules, functional groups and / or therapeutic agents. In the present review, an overview of the past, present and future about the synthesis methods and applications of gold nanostructures is discussed according to the revision of major scientific papers and the most recent published patents.

Keywords: Biocompatible gold nanoparticles; Chemical properties; Conjugated nanoparticles; Contrast agents; Diagnosis; Localized heating nanodevices; Nanocages; Nanomedicine, Nanoparticles; Nanorods; Nanoshells; Nanospheres; Optical properties; Synthesis methods; Theranostic nanoparticles; Therapeutic agents; Treatment

1. Introduction

Nanostructured gold has advantageous optical, chemical and physical properties which make it suitable for novel biomedical applications [1]. The biocompatibility, resistance to the oxidation, photo-bleaching immunity and high-contrast properties of nanogold have been used to diagnose and treat diseases [2,3]. The applications of gold nanostructures in medicine are preferably accompanied with organic ligands attached to its surface to obtain novel imaging, diagnostic and therapeutic properties. The attachment or conjugation of gold nanostructures produces highly stable nanostructures [4], and also provides a platform to transport and deliver drugs selectively. The optical properties of gold based nanostructures are very sensitive to their size, composition, morphology, surrounding environment properties, inter-particle distance and surface properties [1]. Absorption and scattering properties of gold nanostructures can be mediated from the visible to infrared region; providing useful nanomaterials for biomedical applications. Surface plasmon resonance of gold based nanostructures is very sensitive to physicochemical changes, so they can be monitored by inexpensive equipment or even by the naked eye. The optical and high contrast properties of conjugated nanogold have been successfully used for imaging and diagnosis of major diseases using standard clinical modalities such as X-ray, computed tomography and magnetic resonance imaging [5,6]. Gold based nanostructures with optical properties between 600 nm and 1000 nm have been used as localized heating tools and contrast agents in living organisms [7]. Cancer is one of the major diseases affecting around the world and many methods to detect, diagnose and treat it have been developed using gold nanostructures.

The synthesis and conjugation of gold nanostructures have been mainly focused in the development of well-defined shape, monodispersed, biocompatible, stable, environmentally friendly gold nanostructures with superior properties. Innovations in novel gold conjugates are mainly focused on development of nucleation sensitive and selective gold nanoconjugates to diagnose and treat diseases, and more recently, design theranostic gold nanostructures.

In the present review, a revision of the major scientific papers and an emphasis on the recent patents related to the past, present and future methods for synthesis and biomedical applications of gold based nanostructures is presented.

2. Synthesis of gold-based nanostructures

At the present time there are many subtypes of gold nanostructures based on the size, shape, and physical properties. The common gold nanostructures with potential biological applications are nanospheres, nanorods, nanoshells and nanocages (see Figure 1). Almost all methods for synthesis of gold nanostructures start with the reduction of commercial gold salts, such as tetrachloroauric acid ($HAuCl_4$) and the presence of reducing agents for instance sodium citrate, sodium borohydride, ascorbic acid or formaldehyde. The reducing agents many times also stabilize and prevent the agglomeration along with control of growth and shape. The

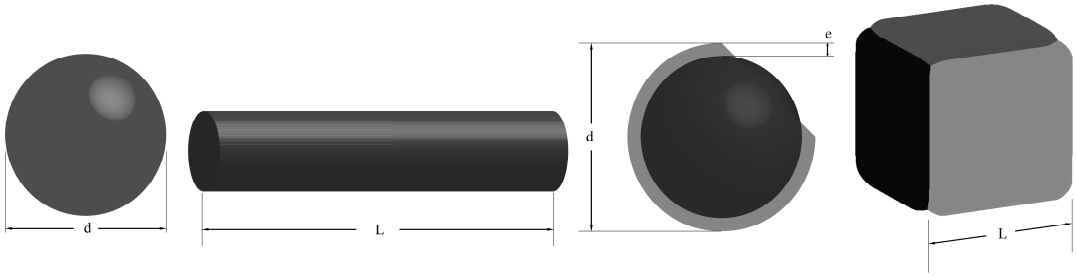

Figure 1. Representative gold nanostructures. From left to right: solid gold nanosphere, nanorod, nanoshell and nanocage.

patents related to the synthesis of gold-based nanostructures; almost all of them make improvements in monodispersity, surface modification, stability under hard conditions and novel conjugates.

2.1. Solid gold nanospheres

Several ways to synthesize gold-based nanostructures have been developed since ancient China, Egypt and Roman times where colloidal gold was first used for therapeutic and decorative purposes [3,8]. Ancient colloidal gold is supposed to be prepared using gold salts and all kinds of organic substances including urine as reducing agent [9]. Despite the long time since gold nanoparticles (AuNPs) were used, the modern era of AuNPs synthesis began with M. Faraday in 1850s, who reported the first scientific article explaining the color to the colloidal nature of AuNPs [10] along with G. Mie who provided a theoretical treatment of the optical properties of gold colloids [11]. Faraday showed that gold chloride can be reduced by heat alone or by reaction with many different reagents including organic matter, phosphorus, tartaric acid, and others. For approximately fifty years, the scientific community working with colloidal solutions was unconscious of Faraday´s work. It was not until Zsigmondy´s studies which took care of Faraday´s procedures for colloidal solutions [12,13]. Zsigmondy formulated a method for preparing colloidal gold by using formaldehyde as reducer and combining his method with phosphorous reduction of Faraday he developed the "nuclear method" or seed-mediated synthesis and invented the ultramicroscope which allowed to visualize the colloidal gold nanoparticles [14].

Svedberg, a pioneer in the research of electrochemical methods for the synthesis of gold nanoparticles, used every conceivable reducing agent available in his time, like hydrogen, hydrogen peroxide, hydrogen sulphide, carbon monoxide, carbon disulphide, nitric oxide, phosphorus, phosphorus tertoxide, hypophosphoric acid, sulphur disoxide, sodium thiosulphate, sodium bisulphate, ferrous sulphate, tin, stannous chloride, acetylene, terpenes, alcohols, glycerine, aldehydes, acrolein, oxalic acid and oxalates, tartaric acid, sugar, starches, phenols, hydroxide acids, hydrochinones, hydrazines, hydroxylamines, protalbic acid, electric sparks, alpha, beta, gamma-rays, etc [15-17]. Svedberg constructed his ultracentrifuge and pioneered in ultramicroscopic research to study mainly particles size, particle size distribution and sedimentation [18].

In 1917 W. Ostwald in his publication on colloid science showed several principles useful for the theoretical aspects and synthesis of gold colloids [9]. Ostwald used gold chloride as a precursor and sodium bicarbonate as a pH controlling agent, and also he said that it was needed to introduce his finger into the solution when it becomes stained with bluish violet by the colloid gold produced through the reducing actions of the organic substances contained in the skin. Ostwald´s observations showed the importance of particle size in keeping particles dispersed, the acidity of the solutions, the spontaneous productions of nuclei and the velocity of the growth of particles.

Turkevich and co-workers in 1951 introduced the citrate reduction of Au^{III} to Au^{0} in water to produce gold nanoparticles [19]. Turkevich and his group investigated the process of nucleation and growth in gold colloids and making use of the electron microscope, they were able to make an extensive study of the shape, mean size and size distribution and the factors than govern these properties.

Frens in his publication in 1973 studied the effects of the concentrations of sodium citrate during the nucleation of the particles obtaining gold particles ranging from 12 to 160 nm [20]. He demonstrated that only by changing the citrate concentration, different diameters of monodispersed gold nanoparticles can be obtained and came to the conclusion that the final particle size in the suspension is governed by the number of nuclei which form and grow into particles.

In 1990´s Brust reported one step method for the synthesis of hydrophobic small gold nanoparticles bearing a surface coating of thiol, using two-phase (water-toluene) reduction of $AuCl_4$- by sodium borohydride in the presence of an alkanethiol [21]. The great advantage with the Brust method is the possibility to obtain gold nanoparticles ranging from 1-3 nm and behaving like simple chemical compounds. The nanoparticles can be precipitated, redissolved and chromatographed without any apparent change in properties.

In 2003 Bishop and coinventors patented the application of novel thiol stabilized gold nanoparticles for decorative uses [22]. Their invention claims to produce gold nanoparticles with a number of advantages over thiol derivatives gold nanoparticles prepared by previous researchers; like significant increase in solubility.

The latest revisiting of the Turkevich method has allowed to finely control the particle size, size distribution, shape, stability, physicochemical properties and subsequently conjugation of the nanostructures [23-25]. In 2007 Zhong and coinventors patented a method of synthesizing highly monodispersed gold nanoparticles ranging from 30 to 100 nm, using seed nanoparticles under controlled conditions of pH, temperature and time [26].

Almost any application of gold-based nanostructures in medicine has to be surface modified with ligands containing functional groups such as thiols, phospines, and amines, which

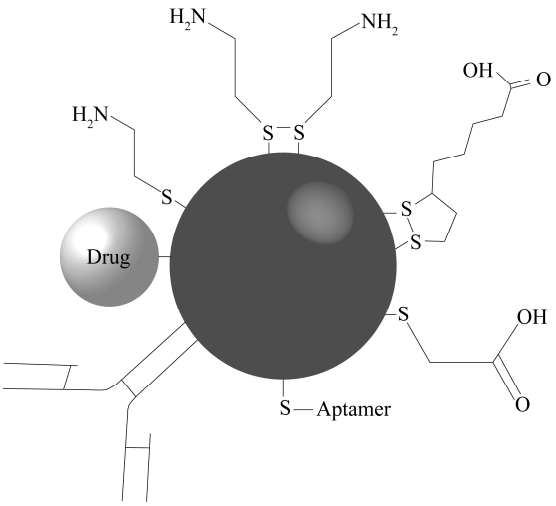

Figure 2. Illustrative gold nanostructure conjugated with 2-Mercaptoethylamine, Cystamine, Lipoic acid, Thioglycolic acid, an aptamer, an antibody and loaded with a drug.

exhibit affinity for gold nanostructures (see Figure 2). These functional groups can be used to bond proteins, oligonucleotides, aptamers and antibodies. Preparing stable gold conjugates basically depends on three interactions: a) the electronic attraction between the negatively charged gold nanoparticles and the abundant positively charged sites on the protein molecule, b) an adsorption phenomena involving hydrophobic pockets on the protein binding to the metal surface, and c) the potential for covalent binding of gold to free sulfhydryl groups forming covalent bonds [27]. A considerable number of methods for the synthesis of gold nanospheres conjugates have been patented. Baschi and coinventors in 2007 patented the synthesis of carbohydrate antigen-nanoparticle conjugates [28]. The inventors claim that the conjugates can be used to inhibit metastasis of tumor cell in mammals. More recently, Belmares and coinventors patented methods for conjugating gold nanoparticles with biomolecules, including proteins, antibodies and also methods of detecting a target by contacting the target with a gold-conjugate biomolecule; the inventors claim that their invention method provides superior gold conjugated biomolecules with higher sensitivity than those made from conventional gold conjugation methods [29].

Hainfeld patented functional associative coatings for nanoparticles. His method claims to produce gold nanoparticles stable even in 1 M NaCl and free of particles aggregates after multiple repeated washing cycles by centrifugation. He basically employed dodecanethiol and Tween® 20 to coat gold nanoparticles between 2 and 40 nm [4].

2.2. Gold nanorods

Nanorods are one dimensional nanoparticles. Unlike nanospheres, the optical properties, hydrodynamic behavior as well as phase behavior of gold nanorods are influenced by their shape anisotropy [30]. The absorption profile of gold nanorods includes two absorption bands: one due to light absorbed along the short axis and the other due to absorption along the long axis. As a result of this optical control and sensitivity to changes in local environment; gold nanorods are useful materials for sensing, photothermal therapy, and imaging applications [31].

Predominantly, there are three methods for the synthesis of gold nanorods through wet chemistry, a) template method, b) electrochemical method, and c) seeded growth method. Wet chemical methods are characterized for reduction of an aqueous solution of chloroauric acid where reduced gold atoms initially can form a sub-nanometer cluster particle in the first nucleation stage, leading to growth. Particle aggregation is prevented through vigorous stirring and by adding appropriate stabilizing agents [30]. Recent improvements in the synthesis of gold nanorods have allowed better uniformity, higher production, and simple production processes [32,33].

Template method is considered to be the initial method for the synthesis of gold nanorods [34]. It was introduced by Martin and co-workers in 1994 [35-37]. Their method is based on the electrochemical deposition of gold within the pores of nanoporous polycarbonate or alumina template membranes. The diameter of the nanorod is determined by the pore diameter of the membrane, while the length can be controlled through the amount of gold deposited within the pores of the membrane.

The electrochemical process for gold nanorods production was introduced by Wang´s group [34,38]. The method provides a synthetic route for preparing high yields of Au nanorods. The synthesis is conducted within a simple two-electrode type electrochemical cell. A gold metal plate is used as a sacrificial anode while the cathode is platinum plate. Both electrodes are immersed in an electrolytic solution containing a cationic surfactant such as, hexadecyltrimethylammonium bromide (CTAB). The anode is initially consumed forming $AuBr_4$-. These anions are complexed to cationic surfactants and migrate to the cathode where reduction occurs. An important factor for controlling the dimensions of gold nanorods according to Wang´s group is the presence of a silver plate inside the electrolytic solution. The redox reaction between gold ions generated from the anode and the silver metal leads to the formation of silver ions. The concentration of silver ions and their release rate determined the length of the nanorods.

Seeded growth method was initially used to synthesize small seed gold nanoparticles, mainly to make more monodisperse colloids [39,40]. Brown´s group produced gold nanoparticles with diameters between 20 to 100 nm with improved monodispersity using hydroxylamine as surface catalyzer and sodium citrate as a reducing agent [41-43]. Brown´s group underlined that iterative hydroxylamine seeding leads to the formation of gold nanorods along with a small population of other gold nanostructures (5-10%).

Jana and his group investigated a step-by-step particle enlargement employing seed-mediated method [33,44,45]. They observed the presence of additional seeds in the successive growth and were able to inhibit it by carefully controlling the growth conditions by using ascorbic acid, which cannot reduce the gold salt in the presence of the micelles if the seed is not present. Jana´s group produced gold nanorods applying their borohydride-ascorbic method over a wide range of CTAB concentrations. They concluded that the formation of anisotropic nanoparticles was dependent on both, the nucleation rate as well as surfactant concentration, and also that their method is suitable for gram-scale synthesis of gold nanorods.

Nikoobakht and El-Sayed [46] introduced improvements to the seed-mediated method by using a co-surfactant mixture of CTAB and benzyldimethyl hexadecyllammonium chloride (BDAC), and concluded that the use of binary surfactant results in nanorods of fairly good uniformity, higher yield, and yet fewer byproducts.

Additionally to the methods mentioned above for the manufacturing of gold nanorods, gold nanorods have also been synthesized by Kim and coworkers using photo-reduction [47]. They were able to synthesize gold nanorods with controlled aspect ratio in the presence of silver ions. As a growth solution, they used an aqueous solution of CTAB along with tetradodecylammonium bromide, and hydrogen tetrachloroaurate was added to the solution as the precursor of gold, and acetone along with cyclohexane was added to loosen the micellar structure. Finally the solution was irradiated with UV light for about 30 h. This method is characterized by generation of gold nanorods without producing seed particles and with excellent uniform configuration.

Niidome et al. patented a method for manufacturing metal nanorods [48]. Their invention particularly relates to the technology for suppressing a generation of spherical metal nanoparticles, together with technology for controlling a configuration of the metal nanorods, so as to design its spectral characteristics. Their method includes: a step of adding a reducing agent to a metallic salt solution; a step of radiating light into the metallic salt solution containing the reducing agent; and a step of leaving the light-radiated metallic salt solution containing the reducing agent stationary in a dark place so as to grow metal nanorods. The method has the advantages of the photo-reduction and chemical reduction methods, making the process easier in a short time.

2.3. Gold nanoshells

Gold nanoshells are spherical gold nanostructures; they are composed of a dielectric core covered by a thin gold shell. Gold nanoshells have large optical absorption and scattering cross-sections along with novel chemical and physical properties, which make them faultless candidates for applications in medicine [49].

Halas along with her group developed a general approach to make metal nanoshells based on molecular self-assembly and colloid reduction chemistry [50]. They used silica nanoparticles synthesized by Söber method [51] as dielectric cores and then organosilane molecules were absorbed onto these particles. The organosilane molecules bound to the silica extend their amine groups outward as a new termination surface. Subsequently small gold nanoparticles (1-3 nm) are covalently bound to the organosilane linkage molecules via the amine group. The gold decorated silica nanoparticles are used as nucleation sites for the reduction of an aged mixture of chloroauric acid with potassium carbonate in the presence of a reducing agent, obtaining silica particles covered by a thin gold shell. This procedure with similar procedures for preparing metal nanoshells were patented by Halas [52-54]. Several improvements in the synthesis of gold nanoshells were proposed since then [55]. Susuki and Kawaguchi synthesized gold nanoshells via in situ gold nanoparticles formation using thermosensitive core-shell particles as the template [56]. According to their results, the use of microgel interiors offer significantly reduced particle aggregation, as well as thickness control of the gold nanoshells using electroless gold plating.

Lugwing and coinventors patented a method of forming nanoshells on polymeric materials, in particular biodegradable polymeric materials [57]. Their invention follows the established methods for the synthesis of gold nanoshells described in the US006699724B1, US006685986B2, US006660381B2 and others patents.

Kah and coworkers proposed a single step deposition-precipitation process, as a feasible alternative route to seed gold hydroxide nanoparticles onto silica core to subsequently produce gold nanoshells [58]. They concluded that their deposition process has shown to be cost effective.

Nowadays the synthesis of gold nanoshells for medical applications is accompanied by conjugates which make them biocompatible along with additional characteristics required for their effective application [59,60].

2.4. Gold nanocages

Noble-metal nanocages comprise a novel class of nanostructures possessing hollow interiors and porous walls [61]. Gold nanocages with controllable pores on the surface have been synthesized via galvanic replacement reaction between Ag nanocubes and $HAuCl_4$ in water [62,63]. Ag nanocubes bearing truncated corners react with $HAuCl_4$ in water. The pore size is mainly determined by the molar ratio of chloroauric acid to silver. Silver nanostructures with controlled morphologies can be produced through polyol reduction, where $AgNO_3$ is reduced by ethylene glycol to generate silver atoms and then nanocrystals or seeds. Subsequent addition of silver atoms to the seeds produces the desired nanostructures through controlling the silver seed crystalline structures in the presence of the protection of poly(vinylpyrrolidone), a polymer that is capable of selectively binding to the (100) surface. The silver nanostructures, used as a sacrificial template, can then be transformed into gold nanostructures with hollow interiors via the galvanic replacement [63,64]. The hollow interiors and wall thickness of the resultant gold nanocages could be readily controlled, to very high precision, by adjusting the molar ratio of silver to $HAuCl_4$.

Chung et al. patented a method for the preparation of gold nanocages containing magnetic nanoparticles [65]. These gold nanocages have an optical property of strongly absorbing or scattering light in the near-infrared region. The invention claim to overcome the fundamental optical limitations of using metal nanostructures; particularly overcome the limitation related to the penetration depth of the light by using strong magnetic nanostructures and biomaterials bound to the nanocages.

Gold nanocages to be useful in biomedical applications such as cancer diagnosis and treatment must have long body circulation times and accumulate at sites of interest. Their convenient compact size, relative bioinertness and appropriate bioconjugation makes them ideal for nanomedicine applications [61,66].

3. Biomedical applications

One of the most important areas of research in the general field of nanotechnology is the development of nanomedicines, which refer to highly specific medical intervention at the molecular scale for diagnosis, prevention, and treatment of diseases [67].

The promising present and future applications of gold based nanostructures for the diagnosis and treatment of human

diseases mainly rely on the capacity to make them non-toxic, non-immunogenic, biocompatible, environmentally friendly, and stable under harsh conditions. Furthermore, high control over particle geometry, suitable properties for conjugation with a huge amount of biomaterials, cellular and subcellular targeting and efficient-programmable clearance from the body are of great importance for their successful application. All these desired characteristics and even more had been and will be the challenges for the nano research groups around the world.

The present and upcoming applications of gold nanostructures in medicine are extremely broad. Gold nanostructures have been proposed for use in diagnostics, prevention, and treatment of diseases. One of the most exciting areas of nanomedicine is the development of nanodevices for theranostics, which refers to a combination of diagnostic and therapeutic properties in single nanoparticles. Theranostics nanodevices have been described as the next generation nanomedicines and have the potential to dramatically improve the therapeutic outcome of drug therapy and lead to the development of personalized medicine, where the device may be tailored for treatment of individual patients on the basis of their genetic profiles [67].

3.1. Diagnostics (Imaging)

Metallic nanoparticles present highly tunable optical properties that can be adjusted to desirable wavelengths by altering the shape and composition of the nanoparticles [68]. Therefore, metallic nanoparticles are widely used as enhancer agents in imaging techniques, from tracking or imaging of cells to *in situ* diagnostics of cancer [69,70]. Furthermore, by using the correct types of encapsulating agents and by modifying the surface with antibodies or proteins, metallic nanoparticles can be used for simultaneous actuation of detection and treatment *in vivo* of certain illnesses [71].

In many biological tissue components, light absorption is minimized in the near infrared (NIR) region, so gold nanostructures can be designed to be activated in this region for *in vivo* imaging and hyperthermia treatments [68]. Metallic nanoparticle probes can also overcome many limitations of conventional NIR organic dyes, such as poor hydrophilicity and photostability, low quantum yield and detection sensitivity and an insufficient stability in biological systems [68]. Zhang et al. developed fluorescent metal nanoshells as a molecular imaging agent to detect single microRNA (miRNA) molecules in lung cancer cells. The metal nanoshells were composed of silica spheres with encapsulated $Ru(bpy)_3^{2+}$ as cores and thin silver layers as shells. Such metal nanoshells displayed an enhanced emission intensity (up to 6-fold), longer lifetime emissions and an extended photostability (up to 2-fold). Such stronger emission and longer lifetime allowed for the nanoshells to be isolated distinctly from the cellular autofluorescence on the cell images. By measuring the changes in the miRNA expression levels, it may provide a reference to lung cancer early diagnosis as well as other diseases [72].

Apart from their use in imaging techniques requiring NIR wavelengths, gold nanoparticles are also employed in Surface-Enhanced Raman Scattering (SERS), or as contrast agents for computed tomography, magnetic resonance imaging, optical coherence tomography and photoacustic imaging. Wang et al. used photoacoustic imaging and tomography to track the distribution of poly(ethylene glycol) coated gold-silica nanoshells circulating in the vasculature of a rat brain and found that such nanoshells enhanced the optical absorption in the brain vessels by up to 63%, which allowed for a more detailed image of the vascular structure at greater depths [73]. SERS using gold or silver nanoparticles with an attached reporter molecule with a specific Raman signature can be explored to highlight cellular structures and provide molecular structural information on the cellular environment in live cells [68,74]. Matschulat et al. used SERS gold and silver nanoaggregates to image live cells in a duplex imaging approach, and by using different cluster analyses, they were able to image the positions of different types of SERS probes along with the spectral information from cellular constituents [75].

3.2. Drug delivery

One of the problems faced during delivery of any drug, is that the vast majority of drugs are not tissue specific, meaning they usually become evenly distributed within the body, and they exhibit a short half-life in the blood stream as well as a high overall clearance rate. In fact, only small amounts of the drug administrated reach the target site, which can be a bigger problem for certain diseases such as cancer [68]. Furthermore, the even distribution of the drug in the body can lead to severe side effects. In order to solve such problems, much research has already been done on the use of nanoparticles as vectors for drug delivery. In the case of metallic nanoparticles, their performance can be easily tuned to control the drug release rate and particle disintegration by changing the size and surface functionalization of such nanoparticles. So far, studies on the use of metallic nanoparticles for drug delivery have shown that such systems can offer delivery of unstable drugs, as well as a more targeted distribution and capability to evade or bypass biological barriers. Nonetheless, their nanometric size means they can easily enter various cells, making it harder to be tissue specific. In order to solve this problem, metallic nanoparticles have been conjugated with various biomolecules and ligands (such as peptides, biopolymers, DNA, RNA, antibodies and the like) generating new strategies for targeted drug delivery [76]. From the metallic nanoparticles used, gold nanoparticles are the most chosen due to their ease of synthesis and functionalization, and also to their reduced toxicity. Gold nanoparticles for drug delivery include delivery of anticancer drugs such as paclitaxel or platinum based drugs, as well as 5-fluorouracil, an anti-leukemic drug which has also been tested in gold nanoparticles for antibacterial and antifungal activities [77]. Furthermore, the use of metallic nanoparticles for drug delivery allows for an external control of the drug delivery [76]. Sershen et al. developed optically active gold-gold sulfide nanoshells coated by temperature-sensitive hydrogels for photothermally modulated drug delivery, such polymer-nanoshell composites strongly absorbed NIR light, and in response to such irradiation, released multiple bursts of any soluble material that was held within the hydrogel matrix [78]. Radt and co-workers used gold nanoparticles coated with polymers, and were able to release the contents of the nanoparticles by shining a laser on such loaded particles [79]. Such intelligent delivery systems have been used successfully for the release of encapsulated enzymes on demand with a single nanosecond laser pulse. Such controlled systems can become interesting not only for drug release , but they can be

used for the delivery of other substances such as genes, pesticides, cosmetics and chemicals used in food industry [76].

3.3. Gene therapy

Gold nanostructures have shown potential as intracellular delivery vehicles for antisense oligonucleotides and for therapeutic siRNA by providing protection against RNAses and ease of functionalization for selective targeting [68]. Glijohann *et al*, synthesized and characterized polyvalent RNA-gold nanoparticle conjugates which effectively knocked down the production of luciferase on luciferase transfected HeLa cells. In addition, they showed that the resultant conjugates had a life time six times longer than dsRNA, readily entered cells without the use of transfection agents and had a higher gene knockdown capability, in their cell model [80]. Lee *et al.* developed gold nanoparticles which were first modified with the polymer PEG and with siRNA and then coated with poly(β-aminoester)s; which are known polymers that facilitate DNA delivery. In their study, they showed that developed nanoparticle formulations facilitated high levels of *in vitro* siRNA delivery in a model of luciferase knockdown in HeLa cells. In addition, they showed that their nanoparticles facilitated the siRNA delivery as good as or better than the commercially available lipid reagent Lipofectamine 2000 [81]. Concerning the temporal and spatial control of gene delivery, Braun *et al.* developed a gold nanoshell functionalized with a TAT-lipid layer for transfection and selective release of siRNA, where the TAT-lipid coating mediated the cellular uptake of the nanomaterial, whilst the release of the siRNA was dependent on near infrared (NIR) laser pulses [82]. Moreover, in gene therapy, other researchers have shown a cytoplasmic siRNA delivery system and efficient gene silencing using gold nanoparticles [83-85]. In other studies that do not focus on mammalian cells, gold nanorods were functionalized with ssRNA that decreased the replication of H1N1 influenza viruses, producing a locally therapeutic response [86].

3.4. Hyperthermia

Hyperthermia is based on increasing the temperature on living cells to produce cell death. It is commonly accepted that above 42 °C cell viability is strongly reduced. Therefore, the use of hyperthermia as a destructive therapy is to cause the immediate, irreversible destruction of malignant or dysfunctional tissues, such as cancerous tumors [68]. Depending on the combination of times and temperatures used, hyperthermia can cause different effects from reduction of tumor metabolism and cell apoptosis to immediate physical cell destruction. Due to the properties of metals, metallic nanoparticles can be used to heat up the cancerous cells beyond their temperature tolerance limits, and kill them selectively if the nanoparticles are functionalized to target the tumor cells specifically. The heating of the nanoparticles is usually achieved by exposing the entire patient or the targeted area to an alternating current magnetic field, an intense light source or radiofrequencies which will cause the nanoparticles to heat up and induce thermal ablation of the tumor [68].

The first clinical (Phase II trial) application of interstitial hyperthermia using magnetic nanoparticles, was carried out by Johannsen *et al.* in Germany by injecting magnetite nanoparticles into patients with locally recurring prostate cancer [87]. They obtained successful results using minimally invasive ablation of the tumor, and were able to repeat heat treatments due to nanoparticle retention in the prostate, using an AC magnetic field (100 kHz).

Nevertheless, one of the current challenges when designing metallic nanoparticles for hyperthermia treatment there are still various problems to deal with such as the use of high frequencies of the oscillating magnetic fields employed, which range in the KHz and MHz. Furthermore, the majority of the nanoparticles used for treatment are directly injected into the tumors, meaning they may not be as effective for treatment of tumors located far inside the body. With respect to the former, Mohammad *et al.* developed gold-coated superparamagnetic iron oxide nanoparticles (SPIONS) that showed a 4 to 5 fold increase in the amount of heat released on application of low frequency oscillating magnetic fields. In addition, they were able to show that such SPIONS did not cause much of a cytotoxic response in MCF-7 breast carcinoma cells as well as H9c2 cardiomyioblasts [88]. Continuing with the latter problem, in 2011 Maier-Hauff carried out the first clinical trial utilizing magnetic nanoparticles (SPIONS) for hyperthermia treatment of brain cancer. Patients with recurrent glioblastoma multiforme received neuronavigationally controlled intratumoral instillation of an aqueous dispersion of iron-oxide (magnetite) nanoparticles and subsequent heating of the particles in an alternating magnetic field. Hyperthermia using such nanoparticles in conjunction with a reduced radiation dose was found to be safe and effective, and lead to longer overall survival following diagnosis of first tumor recurrence, compared to conventional therapies in the current treatment of recurrent glioblastoma [89].

With respect to hyperthermia treatment *in vivo*, metal nanoparticles have been thoroughly used as photothermal agents due to their ability to absorb a wide spectral range of 650-900 nm, as well as convert such radiation into heat in picoseconds [68].

4. Gold nanostructures for diagnostic, therapeutic and theranostic applications: an overview of the patents

Patents related to gold nanoparticles with potential applications in medicine are mainly focused on the development of gold based nanostructures with superior properties which overcome their present drawbacks in nanomedicine, and make them suitable for their clinical translation. Recent inventions claim to develop novel gold nanostructures and methods not only for diagnostic or therapeutic applications, almost all the patents describe methods for producing targeted modified gold nanostructures with theranostic properties.

Patented gold nanostructures are potential tools for applications in the diagnosis and treatment of cancer and other diseases. Major types of gold nanostructures like spheres, nanorods and nanoshells have been used in laboratory experiments to diagnose and treat cancer. Gold nanostructures are principally used as enhanced contrast agents owing to their properties to produce very high contrast in optical imaging, optical coherence tomography, X-ray, computed tomography (CT), magnetic resonance imaging, positron emission tomography, and ultrasound techniques.

A goal of the nanodiagnostics is to identify diseases at the earliest stage, particularly at the molecular level [90]. Gold based nanoparticles are excellent candidates for biological sensing and medical imaging applications. Their strong signal,

resistance to photobleaching, chemical stability, ease of synthesis, simplicity of conjugation chemistry and biocompatibility make them an attractive contrast agent for imaging of cells and tissues [91].

Standard clinical imaging modalities such as X-ray computer tomography, magnetic resonance imaging, and ultrasound are not efficient in detecting tumors and metastases that are smaller than 0.5 cm, and they can barely distinguish between benign and cancerous tumors [90].

Patents related to the diagnostics based on gold nanostructures, claim to provide new gold nanoconjugates that overcome the limitations of the clinical imaging modalities, enhance the contrast agents and the biocompatibility; set platforms for cellular tracking, target diagnostic studies, and image monitored therapies.

In order to develop novel tools with molecular resolution, Aras et al. [90] patented a method for nanoparticles-based molecular imaging. His invention describes methods for producing and using drug labeled gold nanoparticles; specifically lisinopril-coated gold nanoparticles. Using the drug labeled nanoparticles as CT tracers of angiotensin-converting enzyme (ACE), Aras and coinventors were able to visualize the abdominal aorta as well as the cardiac blood pool activity. ACE has been associated with a number of pathophysiologies, including those associated with cancer and the cardiovascular system. According to the invention, targeted imaging of ACE is of crucial importance for monitoring tissue ACE activity as well as treatment efficacy. With the purpose of achieve cellular uptake and selectively, gold nanostructures have been modified with many organic ligands. Chen et al. [92] patented an invention of modified gold nanoparticles that enables effective targeting of the nanoparticles to a desired tissue for the provision of early diagnosis, imaging and treatment. Chen designed cysteamine and/or cysteamine/thioglucose gold nanoparticles. Cysteamine gold nanoparticles are strongly positive and selectively bind onto the cell´s surface; thioglucose gold nanoparticles target the cell cytoplasm and take advantage of the fact that cancer cells have an increased requirement for glucose. Once the modified gold nanoparticles reach the target, they significantly enhance conventional treatment modalities at the cellular level. Thus, their invention claims to be useful for diagnosis and treatment of cancer providing a non-invasive, real-time, targeted cancer imaging-therapeutic in one step.

For the diagnosis and treatment of cancer, electromagnetic radiation with wavelength between 600 nm and 1000 nm has been exploited together with gold nanostructures with optical properties in the infrared region. For the purpose, gold nanoshells, nanorods and nanocages are the principal agents used. Patents recently published claim to develop new gold nanostructures with superior properties to gold nanoshells, nanorods and nanocages. Hosomi et al. [7] described a method to make spherical gold nanoparticles conjugated with organic ligand molecules like 2,2-bis(3-aminophenyl)hexafluoropropane (33-6FD). Using organic molecules such as 33-6FD attached to gold nanoparticles the inventors were able to produce small gold nanoparticles (1-10 nm) with at least one plasmon absorption peak in the wavelength range of 700-800 nm. Moreover, the invention of Gobin et al. [93] formulated diagnostic and therapeutic nanoparticles by creating hybrid gold/gold sulfide nanoparticles (GGS) within an iodine-containing chitosan matrix surrounding the metallic nanoparticles. GGS nanoparticles have dual capabilities of absorbing near infrared energy to act as a therapeutic agent by generating heat energy effective for cell ablation, or for release of therapeutic compounds embedded in the chitosan matrix, and creating diagnostic benefit by the incorporation of X-ray or MRI contrast agents.

The Hasonomi and Gobin patents argue that they can produce gold nanostructures with superior properties to be used in the diagnosis and treatment of cancer than gold nanospheres, nanorods and nanoshells. Hasonomi and Gobin gold nanostructures are smaller than gold nanoshells and gold nanorods, making them useful for their transport to malignant tumors through holes of approximately 100 nm formed at the connection branch points between the preexisting blood vessels and the new blood vessels produced by those malignant tumors. Furthermore, the spherical form facilitates their bloodstream circulation, and as the density of these gold nanostructures are closer in density to that of pure gold, they are better contrast agents than nanoshells and gold nanocages. In addition to these advantages, their synthesis is easier than the fabrication of gold nanoshells and gold nanocages. Finally, Gobin nanostructures incorporated iodine which together with the gold nanostructure enhance the X-ray opacity, CT contrast, reduce the toxicity of iodine and also Gobin claim a method for using theranostic hybrid nanoparticles.

Gold nanostructures are known to be highly reactive but biocompatible; ancient colloidal gold was supposed to possess healthy properties. Tamarkin et al. [94] recently patented a new property of colloidal gold, in the form of Au^{3+}. They discovered that colloidal gold can significantly reduce or eliminate the toxicity of biologically-active factors, such as cytokines, growth factors, chemotherapeutic agents, nucleic acids therapeutic agents, and other immune products while maintaining their therapeutic effectiveness. They also claim a method for treatment of diseases by administration of one or more biologically-active factors bound to colloidal metal nanoparticles and their release over a longer period of time resulting in reduced toxicity and fewer side-effects [94].

5. Conclusions and perspectives

Gold based nanostructures have proved to be useful in the development of novel tools for medicine. Future challenges of the nanomedicine will address the design of highly sensitive and selective theranostics agents for the treatment of many diseases. Innovation in the synthesis and conjugation of nanostructured gold conjugates should provide gold nanostructures through uncomplicated synthesis methods or even one step fabrication. Gold nanostructures with size, shape, and surface chemistry must be carefully defined in terms of their biological properties, including absorption, distribution, metabolism, excretion [95], and also a platform for observing and tracing gold nanostructures together with targeted cells.

Mainly up-to-date, applications of gold based nanostructures to treat major diseases like cancer depend on the leaky vasculature of the tumors [93]. To address specific and efficient theranostic treatments, novel gold conjugates should be designed to provide non-invasive and molecular clinical treatments. Furthermore, the size and the overall properties of the nanostructures should be fine controlled to reach desired tissues or cells, overcoming the limitations of biological barriers and also diagnose and treat deep targets.

The transport and delivery of therapeutic agents with gold nanostructures should provide synergetic properties, finely controlled release, non-side effects, neutralization of the drug toxicity while maintaining the therapeutic effectiveness as well as high selectivity. Furthermore, the theranostic gold nanoparticles should provide enhanced benefits with smaller quantities of biologically active factors and also within minimum nanoparticles concentrations. Finally, besides the theranostics properties of gold nanostructured conjugates, the next generation of the nanomedicine is to address personalized medicine, where the nanodevices may be tailored for treatment of individual patients based on their genetic profiles, and for vaccinating humans or animals against biologically active factors and diseases.

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