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EVOLUTION OF GOLD NANOPARTICLES: PHARMACOKINETICS, TOXICOLOGY AND PATENT OVERVIEW.

Jessy Shaji*[1] and Seema Darade[1]

[1]Dept. of Pharmaceutics, Prin. K. M. Kundnani College of Pharmacy, Cuffe Parade, Mumbai 400005, India.

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***Corresponding Author**
Dr. Jessy Shaji
Dept. of Pharmaceutics,
Prin. K. M. Kundnani
College of Pharmacy, Cuffe
Parade, Mumbai 400005,
India.

ABSTRACT

Gold nanoparticles (AuNPs) have been used as a preferred therapeutic agent to treat a wide variety of diseases mainly cancers. Although the use of gold has been largely suspended by newer drugs, gold nanoparticles are still being used effectively in laboratory based clinical diagnostic methods concurrently showing great effectiveness *in vivo* as a theronostic agent. Also recent advances in nanocarriers for therapeutic use with Au-containing drugs is improving the beneficial actions and reducing toxic properties of these agents. For these reasons, gold nanoparticles are well placed to enter mainstream clinical practice in the near future. The present review summarizes the chemistry, Therapeutics, route of administration, synthesis methods, pharmacokinetics, bio-distribution, and toxicity of bulk gold nanoparticles based on clinical observation and experiments in which gold nanoparticles were used to treat patients with various diseases. The beneficial attributes of gold nanoparticles, such as their ease of synthesis, biocompatibility, functionalization and shape control are also highlighted demonstrating why gold nanoparticles are an attractive target for further development and optimization. The biomedical applications and current discoveries in the field of gold nanoparticles were also highlighted via patent and clinical trial overview.

KEYWORDS: Gold nanoparticles, biodistribution, metabolism, toxicity.

1. INTRODUCTION

Gold has been exploited for its putative medical applications throughout the history of civilisation. The use of gold for medicinal purposes started back to 2,500 BC by ancient

Chinese and Egyptians. In medieval Europe, numerous recipes for gold elixirs (aurum potabile) existed. In the 17th century gold was used to treat fevers whereas in 19 th century mixture of gold chloride and sodium chloride was applied for treatment of syphilis.[1-3] 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. This subsequently led to the treatment of tuberculosis with gold in the early 20 th century. As rheumatoid arthritis was initially thought to be an atypical form of tuberculosis, gold was used to treat RA 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. Gold has since been used as a therapeutic agent to treat a wide variety of rheumatic diseases including psoriatic arthritis, juvenile arthritis and discoid lupus erythematous.[10]

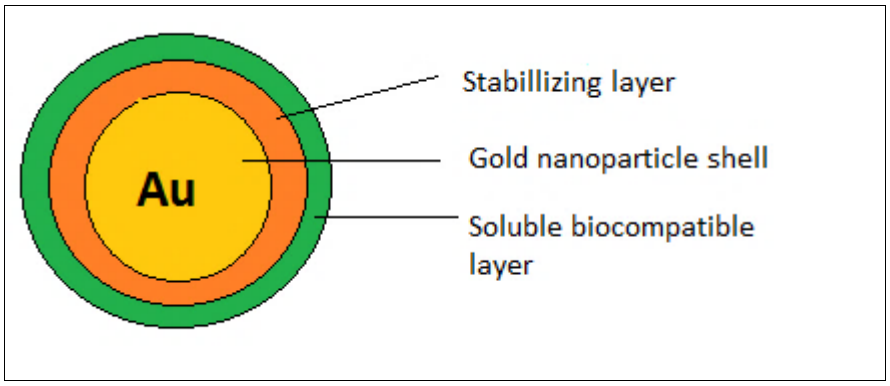

Fig.1. Gold Nanoparticle (AuNPs)

AuNPs has gained much attention and emerged as an attractive carrier for delivery in the field of nanotechnology. AuNPs as described in fig.1 are hybrid materials featuring an inorganic gold core possessing negative charge surrounded by organic monolayer which depicts the reactivity and solubility of the nanoparticles. AuNPs have numerous applications in biomedical sciences including drug delivery, tissue /organ imaging, photothermal therapy and identification of pathogens in clinical specimens strongly influenced by their shape and size. For example, in comparison to metallic gold which is golden yellow, spherical gold nanoparticles have a visible red wine color whilst gold nanorods are blue (aspect ratio 2–3) or black (aspect ratio 3) in solution. AuNPs have the advantage of ease of synthesis, ready functionalization through thiol (sulfur moiety) and amine linkages, enhanced permeability and retention (EPR effect), high stability and modulation of drug release at remote place which defines the versatility of AuNPs as drug delivery carriers in treating several diseases, most notably rheumatoid arthritis (RA) and cancers, with minimal biological side effects.[4-6]

AuNPs can be easily synthesized by most common method of chemical synthesis like citrate reduction as described by Turkevich and Frens, Out Brust synthesis and physical irradiation method which are quite expensive with byproducts toxic to the environment.[20-24] The safe and economical alternative method for the synthesis of AuNPs is by green chemistry using various plant antioxidants.[34] AuNPs can be manufactured into a variety of shapes including gold nanospheres, nanorods, nanobelts, nanocages, nanoprisms and nanostars. [26] 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. The present review highlights the chemistry, biology, pharmacokinetics and toxicology of gold and considers its new use as a clinically applicable nanoparticle. This review also gives the brief account of the recent findings in this field such as nanoformultions, patents and clinical trials related to AuNPs.

Advantages

- Ease of synthesis and fast bio conjugation
- Bio inert
- Controlled dispersity
- Less invasive
- High surface area which provides dense drug loading
- Provides increased contrast for diagnosis
- Unique optical, physical and chemical properties due to their size and shape
- Biocompatible and are readily available for conjugation with small biomolecules such as proteins, enzymes, carboxylic acid, DNA, and amino acids
- Surface easily modified to incorporate an array of ligands for multifuntionality such as targeted delivery
- Due to small size and uniform dispersion it can easily reach to the targeted site with blood flow.

Disadvantages

- Stability depends strongly on the charge of the targeting groups.
- Control of structure is limited by requirement for self-assembly.
- Their high surface area to volume ratio leads to acute or chronic toxicity.
- Reticuloendothelial system (RES) gets affected in presence of gold nanoparticles.

2. CHEMISTRY OF GOLD NANOPARTICLE FABRICATION

Gold always exists in equilibrium between its metallic ground state (Au) and its oxidized states (Au^{+}) or (Au^{3+}). Metallic gold not only found to be inert to strong alkalis and acids but also does not undergo oxidation in presence of air or heat, thereby making it chemically inert metal, whereas Au^{3+} being a strong oxidant reduced to Au^{+} by biologically occurring reducing agents such as thiols.[3] Au^{+} preferentially reacts with sulfur donors (citrates, thiols, or other adsorbed ligands) rather than O- and N- donors, to form gold nanoparticles which can be stabilized by thiolate ligands. These resulting gold thiol compounds undergoes biological ligand exchange reactions which account for the pharmacological activity of mixed monolayer-protected AuNPs.[5] Molecules bind to AuNPs via non-covalent conjugation which includes different interactions such as through specific binding affinity, electrostatic interactions and hydrophobic interactions. These non-covalent interactions are widely utilized in drug delivery and diagnosing areas due to their reversible and easy release. Alternatively, covalent conjugation of molecules to AuNPs fabricates a stabilized conjugates, which is more preferable when stable constructs are required.[6,7]

3. THERAPEUTICS AND ROUTE OF ADMINISTRATION OF GOLD NANOPARTICLES

According to the chemistry of gold nanoparticles, Au^{+} is used as the main therapeutic agent as it is water soluble and easily stabilized in a conjugate by the addition of capping agent such as citrate, tannic acid or Polyvinyl pyrrolidone (PVP).[8,9] AuNPs can be delivered to the patients intravenously, intramuscularly, topically or orally using specific dosage form designed for each route of administration. AuNPs administered orally should be lipid soluble for its absorption within the gastrointestinal tract and therefore will have different pharmacokinetic and toxicological properties compared to water soluble AuNPs that is injected. The above hypothesis is supported by experiments showing only 1% of injectable AuNPs get absorbed when given orally compared to 100% when given intramuscularly. Gold nanoparticles have been evaluated for it's efficacy, toxicity and pharmacokinetics by performing studies in patients with RA and were treated successfully.[6,10]

4. PHARMACOKINETICS OF GOLD NANOPARTICLES

For gold nanoparticles to be efficient and promising candidate in therapeutics, a thorough understanding of their pharmacokinetics is necessary which can be depicted by proper characterization of the AuNPs and a good in-vivo model with an appropriate sample size and

robust statistical methods. Injectable AuNPs are fully absorbed with C_{max} attained after about 2 hours whereas only 20–25% of gold is absorbed when administered orally. This might be due to the insufficient drug compliance, increased clearance or increased distribution volume (V_d). Furthermore intermittent dosing regimens of injectable AuNPs showed fluctuating blood gold levels with high peak and low trough concentrations. In contrast, oral AuNPs formulations with prolonged $t_{1/2}$ resulted in a nearly constant concentration of AuNPs during the whole treatment. After the absorption of AuNPs with its active ligand, either from tissues or the gastrointestinal tract, approximately 95% of gold bounds to albumin and or globulin present in plasma and remain for several months.[11] AuNPs has also been found within the blood cell compartment, primarily in the erythrocyte fraction attached to the membranes of red blood cells (RBCs) with uptake dependent on either the plasma protein binding capacity of AuNPs (ceases the uptake after 48 hours) or concentration of gold present for RBCs. AuNPs is widely distributed throughout the system prominently with organs of the RES, especially the lymph nodes, having higher affinity for metal particles. The liver and bone marrow have each been shown to account for 25% of the total body gold burden.[12]

The biodistribution and bioavailability of AuNPs in the patient very much depends on multiple factors, such as route of administration, size, surface modification, surface charge, hydrodynamic radius and opsonization including protein binding. To support the above statement various researches has been carried out. According to Makino group, particle size deemed to play important role in *in vivo* permeation. Studies of Citrate capped gold nanoparticles of variable sizes (d = 15 nm, 100 nm, 200 nm) showed that the 15 nm particle had the highest permeation coefficient and permeated deeply in the skin layers. Whereas the larger particles (100 nm and 200 nm) showed a lag time of about six hours, and remained on the skin surface.[12] Furthemore the *in vivo* study with ICP analysis of the various organs and blood of mice revealed that the majority of the gold, regardless of size, was present in the liver, lung, and spleen.[13] The 15 nm particle discovered to have accumulated the most in all the tissues including blood, liver, lung, spleen, kidney, brain, heart, and stomach and able to cross the blood brain barrier whereas the 200 nm particle showed a very minute presence in the organs including blood, brain, stomach and pancreas.[14] In an experiment by Khan and coworkers, five types of dendrimer encapsulated gold nanoparticles (d = 5–22 nm) having positive, negative or neural surface charges were injected into mice. After sacrifice, the various organs, blood, and excrements were analyzed for gold content. The researchers concluded that the smallest positive particles accumulate in the kidneys and larger ones

accumulating in the spleen, liver, lungs and heart.[7,15] Brandau lab systematically demonstrated the effect of size and ionic ligands of monodisperse 1.4 nm and 18 nm AuNPs administration through 2 routes: intratracheal instillation into the lungs (IT) and intravenous injection into the tail vein of rats. The results indicated that the 1.4 nm particles translocated through the respiratory tract compared to the 18 nm particle. However after i.v. injection, the accumulation in other organs revealed a different pattern.[16]

To increase biocompatibility, incorporation of natural or synthetic polymers that masks the hydrophobic nature and passivate the surface of monolayer of nanoparticles such as poly(ethylene glycol) (PEG) or peptides are extensively used which also avoids the nonspecific interaction especially of biomolecules. Cho et al. studied the compartmental pharmacokinetics of 13nm PEG coated AuNPs in mice after intravenous (i.v.) injection of 0.85 or 4.26 mg/kg at various time points up to 7 days.[17] Plasma Cmax and Area Under Curve(AUC) were dose-dependent; terminal elimination T1/2, Mean Residence Time (MRT), total plasma clearance (Cl), and volume of distribution (Vd) were not affected by dosage. The blood T1/2 of the 4.26 and 0.85 mg/kg dose groups were 32.65 and 28.50 h, respectively.

The degree of metabolism and degradability of bio conjugated nanoparticles is very crucial to prevent bioaccumulation and enhance elimination. Smaller AuNPs are primarily excreted in the urine and feces however the rate varies from patient to patient and independent of amount injected or administered. An intramuscular injection of gold nanoparticles resulted in excretion through urine during the first day whilst through feces in middle of the week. [14] The high binding capacity of albumin for AuNPs may results in the slow rate of clearance during the treatment post AuNPs injection. When gold nanoparticle formulation is administered orally, 85–95% is excreted in feces containing non-absorbed AuNPs, their breakdown products, gold shed from mucosal cells and little fraction from biliary tract and the remaining 15–5% in urine, independent of dose. According to De Jong et al, the majority of studied AuNPs are larger than the renal filtration cutoff i.e. nanoparticles with final hydrodynamic diameters ≤ 5.5, they were instead found to be eliminated from the blood by the RES followed by accumulation in the spleen and liver.[18]

5. METHOD OF GOLD NANOPARTICLE SYNTHESIS

AuNPs can be readily synthesized into a variety of dimensions (different size and shapes) by an array of methods such as chemical, physical and biological methods which mainly are based on the reduction of chloroauric acid in the presence of a stabilizing agent.[19] However

the most common method for synthesis of AuNPs is by chemical or electrochemical reduction of gold (III) precursor.

5.1 Chemical Methods

In chemical methods AuNPs are generally produced by reduction of Hydrochloroauric acid ($HAuCl_4$) using significant reducing agent. This causes Au^{3+} ions to be reduced to neutral gold ions. 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 later binds to select nanoparticle faces and blocks growth beyond a certain nanometer range.[20]

The Turkevich method pioneered by Turkevich J. et al in 1951 and refined by Frens G. in 1970s which is also referred as citrate mediated reduction, is the most simple, single-phase water based method to prepare spherical gold nanoparticles.[21,22] Generally, it is used for synthesizing monodisperse spherical AuNPs by varying the concentration of reducing agent and gold. It involves the reaction of small amounts of hot $HAuCl_4$ in the presence of reducing agents like citrate, amino acids or ascorbic acid as depicted in Fig.2. The network of gold nanowires formed during the evolution of gold nanoparticles acts as a transient intermediate responsible for the dark appearance of the reaction solution before it turns ruby-red. The colloidal gold will form because the citrate ions act as both a reducing agent and a capping agent. The larger particle size can also be attained by controlling the experimental conditions like temperature, stirring rate and rate of addition of reactants however this can affect the monodispersity and shape of AuNPs.[23] To produce larger particles, sodium citrate amount should be less (down to 0.05%). The reduction in the amount of sodium citrate will reduce the amount of the citrate ions in solution, which is available for stabilizing the nanoparticles, causing the small particles to aggregate into larger ones, until the total surface area of all particles becomes small enough to be covered by the existing citrate ions in solution.[24]

The citrate-stabilized AuNPs can undergo irreversible aggregation during functionalization process with thiolate ligands. To conquer this problem several strategies have been developed including using a surfactant, Tween 20, prior to the modification to prevent aggregation or using thioctic acid as an intermediate via a twostep functionalization. However, the high dilution step makes the large scale production challenging.

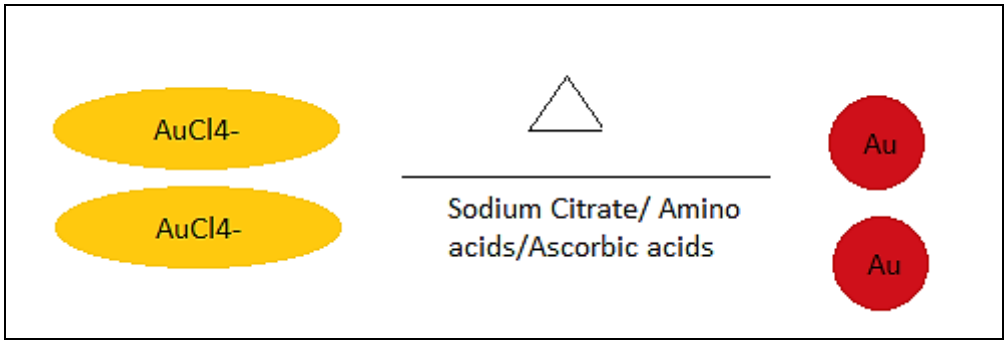

Fig. 2: Chemical synthesis via Turkevich method (citrate reduction).

Brust and Schiffrin in early 1990s made a breakthrough discovery known as Brust method, which can be used to fabricate organic soluble AuNPs stabilized by alkanethiols. It involves biphasic reduction of $HAuCl_4$ solution with a phase transfer agent such as tetra octylammonium bromide (TOAB) in toluene and sodium borohydride ($NaBH_4$) as a reducing agent, respectively resulting in a color change of solution from orange to brown as shown in Fig.3. This method produces AuNPs with particle size 2 to 6 nm by varying the reaction conditions such as gold-to-thiol ratio, reduction rate, and reaction temperature.[25] These alkanethiol-protected AuNPs bears control over particle diameter and grain-size distribution alongwith higher stability due to the synergic effect of the strong gold-thiol linkage and van der Waals forces between the neighboring ligands. These AuNPs can be dried and readily redispersed in solution without any aggregation making them outstanding precursors for further conjugation.[26] Furthermore purification of AuNPs stabilized with dodecanethiol from TOAB was reported by Schriffin.[27]

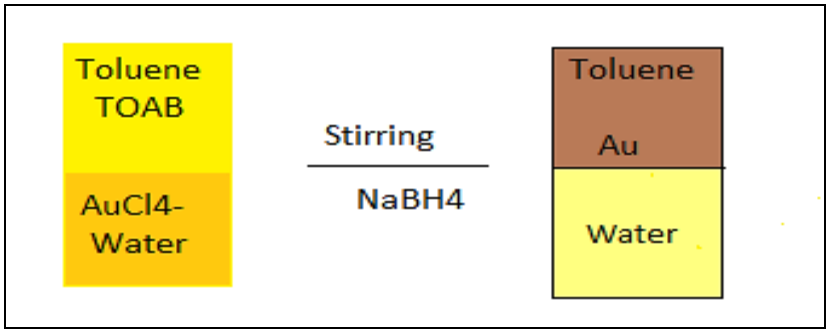

Fig 3: Out-Brust synthesis.

5.2 Physical methods

γ- Irradiation was known to be the finest method for the synthesis of AuNPs with controllable size (~ 2 - 40 nm) and high purity. In this method natural polysaccharide alginate solution was used as stabilizer.[28] Akhavan A. et al offered a unit step γ-irradiation method to

fabricate AuNPs of size 2 - 7 nm by using bovine serum albumin protein as stabilizer.[29] AuNPs are also synthesized by means of photochemical reduction method involving $HAuCl_4$ and aqueous glycine solution exposed to UV irradiation.[30, 31] Microwave irradiation method was embraced to synthesize AuNPs by using citric acid as a reducing agent and cetyltrimethyl ammonium bromide (CTAB) as a binding agent.[32] In addition, other utilized physical technique such as ultrasonic waves, laser ablation, solvothermal method and electrochemical methods are available in literature for synthesis of AuNPs.[33]

5.3 Green synthesis

In recent years, plant mediated biosynthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Hence an attempt has been made for green synthesis of AuNPs by chemical reduction technique using plant extract. Advantages of green synthesis over the conventionally synthesized AuNPs i.e. citrate capped AuNPs are; Good blood biocompatibility, Physiological stability, low cost and long term stability. Prerequisite for green synthesis is that the reducing agent derived from plant extract should have active amine or thiol group in their structure.[34]

According to Li et al in producing AuNPs using plant extracts, the extract is simply mixed with a solution of the gold salt at room temperature at constant stirring; the reaction is complete within minutes depicted by color change from yellow to ruby red.[35] Dwivedi et al studied that the nature and concentration of the plant extract, the concentration of the gold salt, the pH, temperature, stirring time, speed and contact time are known to affect the rate of synthesis of the AuNPs, their quantity and other characteristics.[36]

Table No.1 Examples of plant extract used for green synthesis of gold nanoparticles.

Sr.No	Authors	Plant extract used	Nanoparticles/ Key features/ Application era	Reference
1	Ali et al	*Mentha piperita* (peppermint)	Silver and gold nanoparticles synthesis having Antibacterial activity against clinically isolated human pathogens such as E. coli and S. aureus.	[37]
2	Kumar et al	*Cassia auriculata*	Synthesis of spherical and triangular gold nanoparticles (15–25 nm)	[38]
3.	Parida et al	*Allium cepa*	Gold nanoparticles with average size of 100 nm internalized by MCF-7 breast cancer cells via endocytosis	[39]

4.	Edison et al	*Terminalia chebula*	Gold nanoparticles with sizes ranging from 6 to 60 nm. Active against both Gram-positive S. aureus and Gram-negative E. coli.	[40]
5.	Boruah et al	*Camellia sinesis*	Green Synthesis Of Gold Nanoparticles Using Camellia Sinensis And Kinetics Of The Reaction.	[41]

6. GOLD NANOPARTICLE TOXICITY

Over Last decade, various methods for synthesis of AuNPs of controlled size and shape have been employed of which nanorods are now commercially available with various companies. Knowledge of the origin of nanoparticle toxicity allows chemists to design solutions to mitigate the toxicity. Hence assessment of AuNPs toxicity in-vitro (in cell culture) using viability assays, which depicts the dose dependent toxicity is highly essential. Alkilany et al. stated that the characterization of AuNPs components such as gold core, surface conjugated stabilizing agents and leftovers from synthesis is crucial to understand the origin of toxicity. The chief molecular mechanism by which nanoparticles incur toxicity has been assumed to be from oxidative stress generated by free radicals which are extremely toxic *in vivo*, resulting in the oxidation and damage of lipids, proteins and DNA.[42] Toxicity of gold nanoparticles after administration can be aggrevated by the small particle size that will impact the endocytosis mode, cellular processing and high surface area to volume ratio which alters the physical and chemical properties of the surface conjugated ligands and gold core.[43] Chan et al studied that AuNPs enter the cells via a receptor- mediated clathrin-dependent endocytosis pathway, with uptake of 50 nm nanoparticles at faster rate compared to other nanoparticle sizes.[44] Studies by Pan et al have shown that the cytotoxicity of AuNPs mainly depends on their size, with particles of 1.4 nm in diameter being toxic, triggering necrosis, mitochondrial damage and oxidative stress whereas there was no cellular damage for 15 nm gold nanospheres on all examined cell lines emphasizing the size dependent toxicity.[45] Goodmann et al concluded that the surface charge of AuNPs has also been important in determining particle toxicity with cationic AuNPs of small size exhibiting moderate toxicity owing to the electrostatic binding to the negatively charged cell membrane compare to anionic particles which are nontoxic due to repulsion from cell membrane irrespective of the cell type.[46] Additionally, gold nanospheres have a greater efficiency of uptake compared to gold nanorods due to the thermodynamic driving forces for membrane binding and receptor diffusion kinetics. Despite this, gold nanorods have been shown to be more toxic compared to

gold nanospheres, this could be due to their method of synthesis with the cationic surfactant CTAB.[47] Taken together, the size, shape and surface charge of AuNPs need to be carefully considered when designing AuNPs for human use in order to optimize their therapeutic function, whilst concurrently decreasing their toxicity profile by minimizing their cellular uptake and interactions. AuNPs introduced into the systemic circulation can also interact with blood components to cause hemolysis and thrombosis. According to Dobrovolskaia et al the size range of AuNPs matches with the proteins or even small viruses, hence immune system might also react strongly to it's presence in the body resulting in induced immunotoxicity.[48] RES is part of the immune system with complex components which identify, capture, and filter foreign antigens and particulates. Any potential toxicity arising from AuNPs can be reduced by it's uptake via the RES as well as increase in the circulation time. The slow clearance and tissue accumulation of AuNPs targets liver and spleen (organs of the RES) for toxicity.[49] Furthermore the high blood flow through organs such as the kidney and lungs also place them at high risk of oxidative damage. Cho et al studied the toxicity of 13 nm PEG-coated gold nanoparticles in mice and found that after injection the nanoparticles accumulated in the liver, and induced acute inflammation and cellular damage.[17,50, 51]

7. AN OVERVIEW OF PATENTS

Patents related to AuNPs with possible applications in biomedicine are mainly focused on the development of gold based nanostructures with superior properties which overcome their present limitations in nanomedicine and make them suitable for their clinical translation. Recent inventions claim to develop novel AuNPs and methods not only for diagnostic or therapeutic applications but also for producing targeted modified gold nanoparticles with theranostic properties as explained in Table No.2. The glimpse of earlier patents by eminent scientists in the field of metallic nanoparticles synthesis and formulation are explored and tabulated as follows in Table No. 3.

Table 2: Examples of target specific anti-cancer nanoformulations.

Target moiety	Nanoformulation	Active compound	Indication	Therapy	Reference
Epidermal growth factor receptor	Peptide-targeted gold nanoparticles	Pc 4	Brain cancer	Photodynamic therapy	[52]
LHRH receptor	Gold nanorods	Goserelin	Prostate cancer	Radiotherapy	[53]
Transferrin receptors	PEGylated gold nanoparticles	AuNPs	Mouse neuroblastoma	Chemotherapy	[54]

Table 3: Overview of Patents in the field of gold nanoparticle synthesis and its theronostic applications.

Patent No./Patent Application No.	Assignee	Description	Reference
US2009/007524354B2	Research Foundation of State University of New York, Binghamton, NY (US)	Controlled Synthesis Of Highly Monodispersed Gold Nanoparticles	[55]
US2012/008257670B1	Western Kentucky University Research Foundation, Bowling Green, KY (US)	Monodisperse Gold Nanoparticles And Facile, Environmentally Favorable Process For Their Manufacture. Such as green synthesis, using dextrose as reduing and capping agent and Eischericha coli bacterium etc.	[56]
US2012/008323694B2	Nanoprobes, Inc., Yaphank, NY (US)	Gold Nanoparticles For Selective IR Heating Huang	[57]
US2014/008697129B2	IMRA America, Inc., AnnArbor, MI (Us)	Stable Colloidal Gold Nanoparticles With Controllable Surface Modification And Functionalization	[58]
US2014/008759054B2	Council of Scientific & Industrial Research, New Delhi (IN)	DNA Loaded Supported Gold Nanoparticles embedded in sharp carbonaceous carriers, Process For The Preparation And Use Thereof	[59]
US2006/ 0021468 A1	Ah et al, STAMFORD (US)	Gold Nanoparticles And Method Of Synthesizing The Same	[60]
US 2010/0034735 A1	Jie Chen, Wilson Roa Edmonton (CA)	Targeted gold Nanoparticles For Cancer Diagnosis And Treatment	[61]
US 2010/0172997 A1	University Of North Texas , Denton, TX (US)	Gold, Silver, And Copper Nanoparticles Stabilized In Biocompatible Aqueous Media	[62]
US 2011/0111002 A1	Calin Viorel Pop, Brooksville, FL (Us)	Transport And Delivery Of Glutathione Into Human Cells Using Gold Nanoparticles	[63]
US 2011/0318415 A1	Chun Li, Missouri City, TX (US); Jian You, Houston, TX (US)	Hollow Gold Nanospheres (Haunss) And Haunss-Loaded Microspheres Useful In Drug Delivery	[64]

US 2012/0235095 A1	Leonid Vigderman, Eugene R Zubarev (US)Houston, TX (US)	High-Yield Synthesis Of Gold Nanorods With Optical Absorption At Wavelengths Greater Than 1000nm Using Hydroquinone	[65]
US 2012/0302940 A1	Jackson State University; Jackson; Ms (US)	Popcorn Shape Gold Nanoparticle For Targeted Diagnosis, Photothermal Treatment And G01n In-Situ Monitoring Therapy Response For Cancer And Multiple B82y Drug Resistance Bacteria	[66]
US 2013/0260033 A1	Maiorano et al. Lecce (IT)	Method Of Synthesizing Branched Gold Nanoparticles Having Controlled Size And Branching	[67]
US 2011/0110858 A1	Aras et al. Baltimore, MD (US)	Gold Nanoparticle Imaging Agents And Uses Thereof. lisinopril-coated gold nanoparticles were prepared to provide a new type of probe for targeted molecular imaging of ACE by tuned K-edge computed tomography (CT) imaging.	[68]
WO 2012/118930 A3/ US 2012/0225021 A1	University of California	Stable colloidal gold nanoparticles with controllable surface modification and functionalization	[69, 70]
US 2017/9549998 B2	The curators of University of Missouri	Stabilized gold nanoparticle and contrast agent	[71]
WO 2003/075961 A2	University of California	Gold nanoparticles used for x-rays imaging	[72]
US 2013/8558019 B2	Virginia tech intellectual properties Ltd.	Thiolated paclitaxels for reaction with gold nanoparticles as drug delivery agents	[73]
WO2016/118092 A1	Mehmet Ali Onur	Gold nanoparticles functionalized with semaphorin 3f and preparation thereof	[74]

8. RECENT CLINICAL TRIALS

Gold has been utilized as a nanomedicine in clinical trials due to its unique combination of optical properties, thermal properties, and tunable size, shape, and surface chemistry.[75] Although there are still no gold-based nanomedicines that have been approved to date by the FDA, few examples of AuNPs are being actively investigated in clinical trials for a variety of therapeutic applications specified in Table 4.

Table 4: Recent clinical trials in gold nanoparticle based therapy.

Drug product	Active ingredient	Sponsorer/ organisation	Clinical Trials Gov. identifier	Indications	FDA approved date/ clinical trial status	Reference
Aurimmune (CYT-6091)	TNF-α bound to colloidal Gold Nanoparticle	Cytimmune Sciences	NCT00356980	Head and Neck Cancer	completed	[76,77]
AuroLase®	Silica Gold nanoshells combined with PEG	Nanospectra Biosciences	NCT00848042	Aurolace therapy of cancer	completed	[78]
CYT-6091	(rhTNF) bound colloidal gold	Clinical cancer research	NCT00436410	solid primary and/or metastatic lung tumor treatment	completed	[79]

9. CONCLUSION

Gold nanoparticles have materialized as a promising candidate with incredible versatility for next generation biomedicine application. Gold nanoparticles have been shown to be extremely biocompatible and easily conjugated with biomaterials however, its active use as a therapeutic or diagnostic agent in various cancers, gene therapy and many other diseases may be determined primarily on two key aspects as per recent advances in research technologies: 1) The intrinsic properties of gold core. 2) The ability of surface modification by incorporating an array of ligands. Multifuctionality of the AuNPs is a key advantage over conventional approaches of drug delivery. Accurate mechanism of uptake of AuNPs which is likely differ based on structure of monolayer, size, shape and surface charge should be known to facilitate its design, synthesis and optimization for safe and efficient delivery at the target site. Although AuNPs have been reported to be inherently nontoxic, future research including systematic studies on toxicity, pharmacokinetics, and efficacy need to be carried out and verified under precise conditions prior to its use for clinical trials. This is especially needed for the negatively charged nanoparticles involving the cationic surfactant CTAB.

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