ejpmr, 2016,3(2), 54-67

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 3294-3211

EJPMR

GOLD NANOPARTICLES IN CANCER THERAPY AND DIAGNOSTICS

[1*]Prof. Heyam Saad Ali and [2]Dr. Samrein Ahmed

[1]Medical Institute for Cancer Research, Department of Biochemistry, University of Sharjah, UAE.
[2]Department of Pharmaceutical Sciences, Dubai Pharmacy College, UAE.

***Correspondence for Author: Prof. Heyam Saad Ali**

Medical Institute for Cancer Research, Department of Biochemistry, University of Sharjah, UAE.

Article Received on 30/11/2015 **Article Revised on 21/12/2015** **Article Accepted on 13/01/2016**

ABSTRACT

Cancer is the present leading cause of death worldwide, accountable for approximately one fourth of all deaths in the USA and UK. Nanotechnologies provide incredible opportunities for multimodal, site-specific drug delivery to these disease sites. Gold nanoparticles are evolving as promising agents for cancer therapy and are being explored as drug carriers and diagnostic agents due to their predominantly distinctive set of physical, chemical and photonic properties. This review familiarizes the field of nanotechnology in cancer therapy with a focus on recent research on development of gold nanoparticle for therapeutic and diagnostic applications.

KEYWORDS: gold nanoparticles, cancer therapy, nanomedicine, nanotechnology.

1.INTRODUCTION

Nanotechnology is an advanced and unconventional technique that involves the science, engineering, and technology directed at the nanoscale,.[1-3] It incorporates particles of the order of 1 billionth of a meter (10^{-9} m) or as defined by the National Nanotechnology Initiative (NNI) at sizes of roughly 1-100 nanometers.[4] This technology has many applications specifically in the medical field known as nanomedicine. In recent years, nanomaterials have been the focus of research literature due to their potential impact in therapy and diagnosis of cancer, which postulates promising future for nanotechnology in medical applications.[5] Cancer is one of the prominent causes of death worldwide, occupying the second place in developing countries and showing a growing incidence over time.[6] Cancer is a state of abnormal cell growth known as the malignant tumor or malignant neoplasm, with a potential to invade other cells or organs in the body.[7] Cancer is affecting all age groups and is associated with serious medical, psychological, virtually, economic and social insinuations.[8-10] The most common types of cancers are stomach, lung, breast, colon-rectum, prostate, cervix-uteri, mouth, pharynx, liver and esophagus. Its intricacy relies in being a class or group of combined diseases which makes it extremely challenging to find a single cure and target a specific tissue. Cancer is caused by multiple possible factors; the great majority is due to environmental and inherited genetics. Environmental factors includes the pollutant, lifestyle, tobacco, diets, obesity, infections, radiation, stress, lack of physical activity, economic.[11] Current cancer therapy approaches are based on surgery, radiotherapy and chemotherapy where the latter is the one that shows the greater efficacy for treatment, expressly in advanced stages. The application of nanotechnology research in the field of cancer encompasses multi-interdisciplinary approaches integrating medicine, biology, engineering, chemistry, and physics.[12] Many materials have been used in nanotechnology for cancer diagnosis and therapy. This review article will only focus on different aspects of how gold nanoparticles have been studied as carriers for chemotherapeutic drugs, therapeutic agents in photodynamic, gene, and thermal photothermal cancer tissue ablation therapy and as molecular imaging agents to detect and monitor cancer progression.[13-14]

Physicochemical properties of Gold

Gold is a chemical element with symbol Au (from Latin: aurum) and has an atomic number 79. It can be obtained directly by mining techniques in its pure form or by neutron bombardment of other elements such as mercury and platinum in special nuclear reactors.[15]

Fig 1. Piece of metallic gold showing metallic luster

Metallic gold has unique properties that include electrical and magnetic fields, metallic luster reflection [Figure 1], and Plasmon frequency which is due to the negative reflection to the positive transmitting charges.[16] Gold is existing as a pure metal or combined with other metals in alloys. It is used as metallic support material in dentistry in fabrication of crowns and bridges. Both gold metal and alloys have been also used in the past for the treatment of nervous disorder such as epilepsy, depression, and migraine.[16]

Gold may exist in two oxidation states which immediately precipitate in presence of a reducing agent forming colloidal solutions.[16] Metallic gold is an inert element while its salts and radioisotopes are pharmacologically active, for example anti-inflammatory properties, and may be used in treatment of arthritis. Currently available gold products include sodium aurothiomalate and auranofin which are used as anti-tuberculosis and anti-rheumatoid arthritis agents. Colloidal gold has the ability to absorb certain materials on the surface and act as carriers. This property has many potential applications in the treatment of medical conditions such as restorative dysentery and also in the fields of nuclear medicine, immunology[17] and diagnosis and treatment of cancer and other diseases.[18]

Ingested pure metallic gold is considered biocompatible as it neither causes irritation nor toxicity. However, soluble gold salts such as gold chloride induce toxicity in the liver and kidneys. The common used antidote in gold toxicity is Dimercaprol.[18] According to American Contact Dermatitis, pure gold metal is considered non potent compared to gold-nickel alloys which cause contact allergic dermatitis.[19]

2. Methodology

This review article was written after searching, evaluating and analyzing different review and research articles obtained from Science Direct® and Academic Search Complete® scientific databases available in the Medical Library at the University of Sharjah.

3. Gold nanoparticles (GNPs)

3.1 Structure and size of nanoparticles

The structure of gold nanoparticles is one of the key constituents in their characterization. Study of the structure is very important because it is associated to their properties, which leads to their performance and applications in certain fields. Gold nanoparticles (GNPs) have unique structures, shapes, sizes and properties which allow different functionalities, efficient delivery systems and biomedical applications in oncology field in different aspects. The Diversity in shape, size and surface properties [Figures 2 and tables 1, 2] has influence in interaction, behavior of GNPs with biological systems.[20] The physicochemical properties of GNPs related with functionalities include small size-to-volume-ratio, low toxicity and ease of detection, amenability of synthesis and functionalization exhibited in binding of amine to thiol and high reactivity to the living cells.

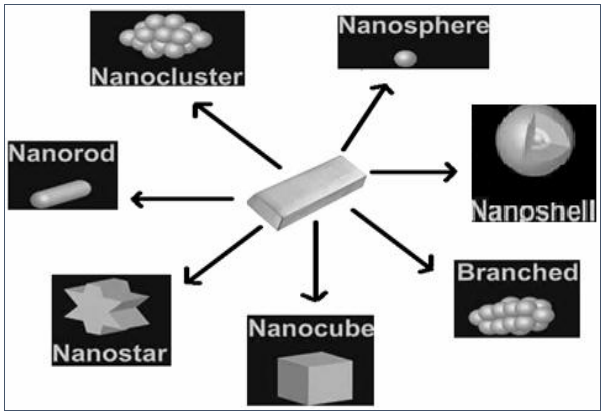

Figure 2. Different shapes of GNPs (21)

Table 1. Shapes of gold nanoparticles and their applications[21]

Shape	Size (nm)	Applications
Nano-rod	2.5	Drug delivery and photothemal
Hollow	25	Catalysis , photo-electronics, & cancer therapy
Triangle	3.85- 7.13	High effective against bacteria such as E.coli
Faceted	50-100	Effective reproducible, and stable large area substrate for NIR Sers near infrared
Nanotube	50	Field enhancement applications and refractive index sensing
Nano cage	50	Effective molecular contrast agent and nonlinear endoscopy
Branched	90 nm	Substrate for SER tested image for kidney cells,
Nano belt	Thickness-80 nm, width 20 um, length 0.15 m	Strain sensor

3.2. Surface Plasmon absorption

The size of the material in nanometer scale has significant influence in changing its electronic motion and hence the properties of this material. For gold in metallic form, the electronic oscillation motion in the conduction band causes great surface electric fields resulting in radiative properties.[22]

The spectrum of Surface Plasmon absorption depends not only on the size but also the shape of the GNPs.

When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, a strong absorption band around 520 nm in the spectrum is detected as shown in Figure 3-4.[23]

When the shape of the nanoparticles changes from nanosphere to nanorods, the surface plasmon absorption spectrum also changes.[24]

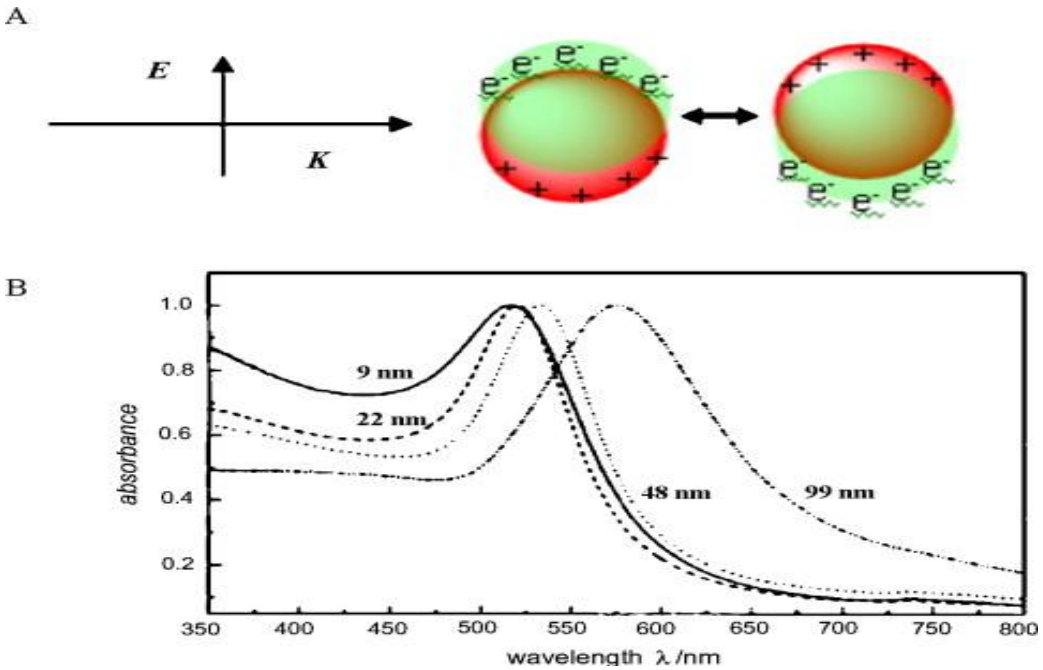

Figure 3. (A). Schematic illustration of surface plasmon resonance in plasmonic nanoparticles. (B). Extinction spectra of gold nanoparticles in different sizes. The electric field of incident light induces coherent collective oscillation of conduction band electrons with respective to the positively charged metallic core. This dipolar oscillation is resonant with the incoming light at a specific frequency that depends on particle size and shape. For gold nanoparticles, the SPR wavelength is around 520 nm depending on the size of the nanoparticles ((B) is reproduced with permission from Ref.[23]

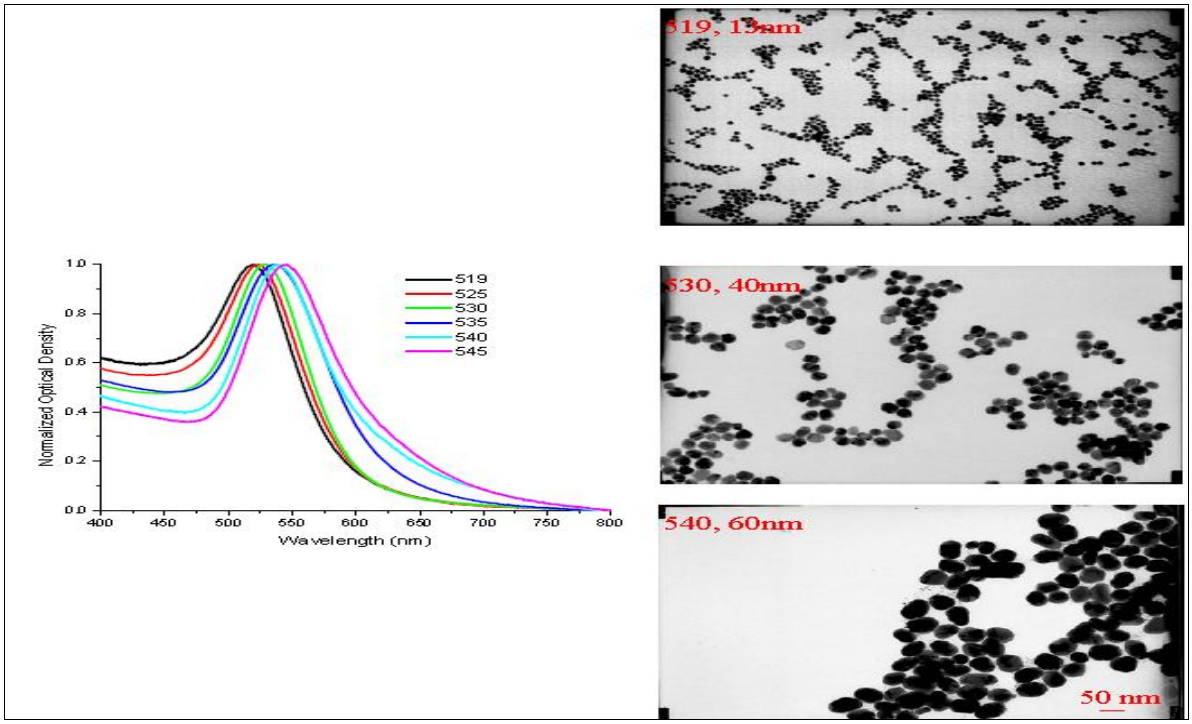

Figure 4. Surface Plasmon absorption spectra (left) and TEM (right) of spherical gold nanoparticles in different sizes. The surface Plasmon absorption maximum is weakly defendant on the size of the Nano spheres , from Ref.[23]

3.3 Surface Plasmon light scattering property of GNPs

When a beam of white light is absorbed towards a GNPs suspension, it will illuminate and scatter a colored light. This scattered light suspension will be similar in its appearance as the fluorescent solutions (Figure 5). This phenomenon depends on the size, shape and composition of GNPs. For instance GNPs of average diameter size 56 nm will scatter green light while those measuring 78 nm will scatter yellow light.[24]

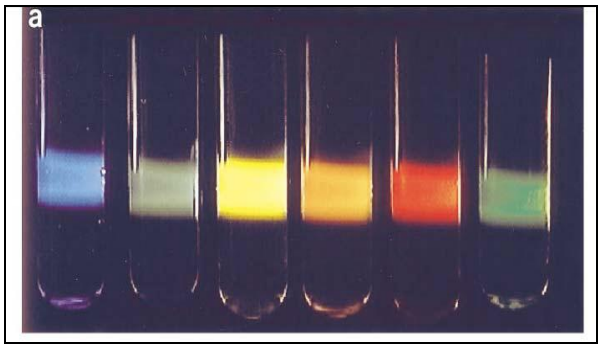

Figure 5. Scattered light is different for size, compositions and shapes of GNPs from Ref.[26]

3.4 Catalytic property of Gold nanoparticles

The large surface-to-volume ratio is a unique property of GNPs compared to bulk or metallic gold. This property attracted the attention of many researchers and gave the opportunity to use GNPs as catalysts for chemical reactions, despite the fact that gold is inert in its metallic form. GNPs catalytic property is attributed to their availability in small size and presence of a large number of active atoms on their surfaces.[25]

GNPs are used in both heterogeneous and homologous catalysis. Examples for the use of GNPs in heterogeneous catalysis include oxidation of carbon oxide at low temperature, propylene epoxidation and hydrogenation reaction.[25] The size and the surface of GNPs property determine the activity of this catalysis reaction. On the other hand, Colloidal GNPs are also used for homologus catalysis such as the use of Citrate-stabilized colloidal GNPs in the active redox catalyst, when using hexacyanoferrate (III) and thiosulfate ions. This reaction depends on the surface and concentration of GNPs and the use of strong stabilizer for capping purpose.[26]

3.5. Optical tuning

The unique electronic optical property of the gold nanorods depends on their shape, size and surface/volume ratio. For instance, changing the shape from sphere to rods the surface Plasmon resonance split into two bands that differ in their sensitivity to the size changes of the GNPs. This optical property is used in experimental detection changes of parameters during the synthesis of GNPs.[27]

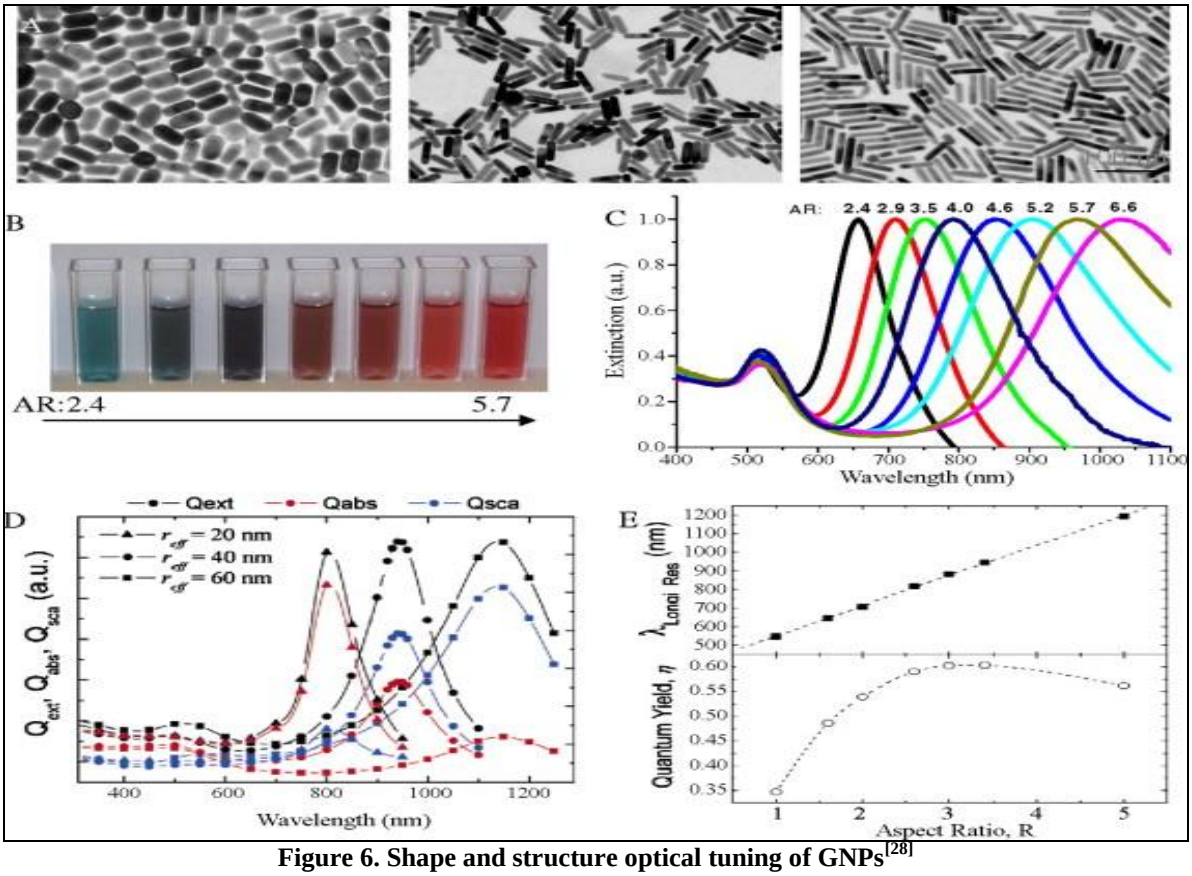

Figure 6. Shape and structure optical tuning of GNPs[28]

3.6 Advantages and disadvantages of Gold Nanoparticles

GNPs have many advantages and disadvantages which affect their potential applications in nano-medicine. They are summarized in Table 2

Table 2. Advantages & disadvantages of Gold Nanoparticles

	Advantages	Disadvantages
GNPs	Large surface area Low hydrodynamic mean size Multimodal applications(targeting, diagnostic, therapy) Scaffold for additional agents Ease of surface modification Stability and biocompatibility	High cost for large-scale production Lack of standard protocols for translation into clinics non-biodegradability

4. Synthesis of gold nanoparticles

4.1 Chemical

GNPs can be obtained by reduction of $HAuCl_4$ using different reducing agents. For example , citrate, thermo-reduction method was used for the synthesis of gold nanoparticles having efficient SERS (surface enhanced Raman spectroscopy) in short reaction time by using inositol hexaphosphate (IP_6) as a reducing agent for HAuCl4.[29] The reduction method has been used for the preparation of dendrimers/gold nanoparticles. These particles were synthesized by the reduction of aqueous solution of $HAuCl_4$ and dilute solution of dendrimers by sodium borohydride.[30] Mathias Ulbricht and co-workers reported the direct and one-step synthesis of water soluble gold nanoparticles of size < 10 nm with two different thiols including 1-mercaptoundec-11-yl-hexa (ethylene glycol) (EG6) and dodecanethiol.[31] GNPs can also be synthesized via seeding growth method where GNPs are grown from gold nanoparticles encapsulated in polyethylene glycol attached with dendrimers and have high near infra-red absorption by using formaldehyde as a reducing agent.[32] GNPs of size range 1.8 - 3.7 nm have been obtained by using peptide-biphenyl hybrids (PBHs) which are good stabilizers for gold preventing aggregation of the formed Au solution as a consequence of attractive Van der Waals, via single-phase system. The size of the obtained GNPs depends upon the structure and type of capping agent being used.[33] The synthesis of gold nanoparticles of size 2 - 20 nm has been reported via reduction of $HAuCl_4$ with sodium borohydride by using ethanol and isopropanol in the presence of tris (2- aminoethyl) amine.[34] Non-seed mediated temperature synthesis method of gold nanoparticles has been reported by the reduction of gold ions in ethylene glycol with NaOH as reducing agent resulting GNPs having mean diameter of 75 + 10 nm.[35] Other non-seeding method involves the reduction of $HAuCl_4$ by sodium borohydride in presence of bovine serum albumin used as capping agent resulting in highly stable gold nanoparticles of size 7.8 + 1.7 nm. Partially functionalized GNPs can be obtained by surfactant assisted method where a bifunctional surface active agent ligand such as hexadecyl trimethyl ammonium bromide (CTAB) act as linker between solid substrate and gold nanoparticles.[36]

4.2 Physical methods

GNPs with controllable size and high purity can be obtained γ-irradiation. This method can result GNPs with size 5 - 40 nm or size 2 - 7 nm using natural polysaccharide alginate solution or bovine serum albumin protein, respectively as stabilizer.[37, 38] Microwave irradiation method can be used to synthesize GNPs by using citric acid as a reducing agent and cetyltrimethyl ammonium bromide (CTAB) as a binding agent.GNPS can also be synthesized using photochemical techniques. In this method gold salt are reduced by radical formation coated with polyethylene glycol diacrylate by UV-process in the presence of photo-initiator 2-hydroxy-2- methyl-1-phenyl-1-propane[39] resulting in gold-polyethylene glycol core-shell nanoparticles with size 10 – 50 nm in water.

4.3 Green methods

Green chemistry synthesis routes are environment friendly and non-toxic. For example Egg shell membrane (ESM) are used for the preparation of GNPs of size 25 + 7 nm by immersing ESM in aqueous solution of HAuCl4 without using any reductant.[40] Another green synthetic approach was developed using high-power ultrasounds and sodium dehydrate[41] to preppare gold sononanoparticles of size 5 - 17 nm. In addition, sun light irradiation has been used to reduce the gold salt synthesize Gold, producing gold nanoparticles which were modified with folic acid and capped by 6-mercaptopurine.[42] A recent green chemistry method for the preparation of GNPs has been reported, in which GNPs were formed in aqueous NaCl solution from the bulk gold substrate by natural chitosan without using any external stabilizer and reductant.[43]

5. Characterization of Gold nanoparticles

Despite many GNPs applications in various fields but still some issues regarding accurate and well resolved characterization remains immature. Currently, various traditional techniques and strategies have been used in characterization of nanoparticles include electron microscopy (EM), atomic force microscopy (AFM), dynamic and static light scattering (DLS and SLS), x-ray photoelectron spectroscopy (XPS), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) etc. figure 7 and table 4 below.(44)

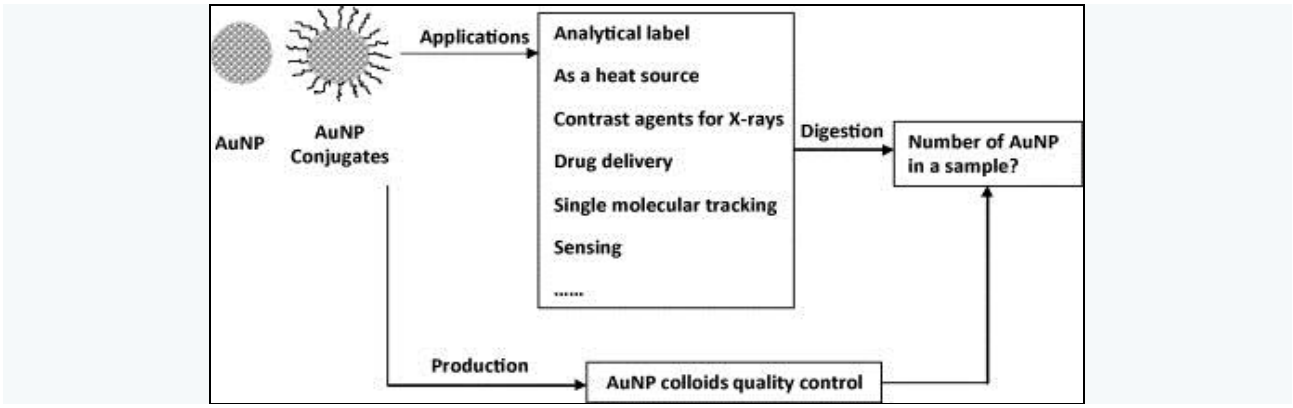

Figure 7. Quantitative analysis of GNP is very important in measuring the quantity

Table 3: List of various techniques used in particles and nanoparticles measurement

Acoustic Attenuation Spectroscopy	Laser Doppler Velocimetry (LDV)
Aerosol Mass Spectroscopy (Aerosol MS)	Laser Doppler Velocimetry (LDV)
Material Sensing	Laser Light Diffraction
Cascade Impaction	Light Microscopy or Optical Imaging
Condensation Nucleus Counter (CNC)	Micro-electrophoresis
Differential Mobility Analysis (DMA)	Differential Mobility Analysis (DMA)
Condensation Nucleus Counter (CNC)	Sedimentation (Gravitational & Centrifugal)
Electrical Zone Sensing (Coulter Counting)	X-ray Diffraction (XRD)
Electro-acoustic Spectroscopy	
Electro-kinetic Sonic Amplitude	

5.1Qualitative Analysis

5.1.1. Microscopic technologies

Microscopic technologies are used in qualitative analysis of GNPs for identifying the morphology of nanoparticles. This technique includes scanning probe Microscopy such as Atomic Force Microscope (AFM) and Scanning Thermal Microscope, and Near- Field Scanning Optical Microscopy such as Scanning Electron Microscope (SEM). There are variations between these techniques regarding the resolution and extent of visualization.[45-46] Fluorescence microscopic techniques are also used to visualize the chemotherapeutic agent or bio-molecule in cellular uptake using fluorescence probes.[47]

5.1.2. Particles measurements

The measurement of particles, size and distribution of the prepared GNPs is conducted using DSL. The measurements of the UV-Vis spectrum always should be repeated for a minimum of five times in this technique.[48]

5.1.3 Surface characterization by SERS and Raman measurements

Surface- Enhanced-Raman Spectroscopy (SERS) spectroscopy yields information about the bio-molecules adsorbed on the gold surface. The vibrational Raman spectra of molecules or molecular moieties in direct contact to the gold in the nanoparticles are strongly enhanced.[49]

5.2. Quantitative analysis

5.2.1 Mass spectroscopy

Mass spectroscopic (MS) methods analyze the mass–charge ratio (m/z) of a charged species with high resolution and large range of "molar" mass.[50]

5.2.2 Direct amperometric methods

In this technique the electrochemical properties of gold nanoparticles when they are small enough to have molecular-like electrochemical voltammograms.[51]

5.2.3 Cathodic linear sweep voltametry

This technique is used when the GNPs are not considered like molecules. Each molecule consist of a number of atoms. For example assuming GNPs are perfect spheres and their densities are identical to bulk gold.[52]

5.2.4 Anodic stripping voltammetry

Stripping analysis is an extremely sensitive electrochemical technique for measuring trace metals. Its higher sensitivity, compared with other amperometric methods, is attributed to the electrochemical pre-concentration. Typical detection limits are as low as 10−9–10−12 M.

The cathodic linear sweep voltammetry and the anodic stripping voltammetry methods are means of Au analysis. In order to obtain the value of population of number of AuNPs, other methods, such as TEM, are required to characterize the average size (diameter) of the AuNP.[53]

5.2.5 ICP–MS methods

Inductively coupled plasma–mass spectroscopy (ICP–MS) offers many benefits to trace metal detections. The limit of detection of element gold can be as low as 1 part per trillion, about 1 pg/mL. AuNP dispersions can be directly analyzed without any previous dissolving.[54]

6. Pharmacokinetics & biodistribution of GNPs

Certain biological barriers have to be overcomed in order for GNPs to be effective as drug delivery systems. Uptake by RES is common in nano drug-delivery system and ensues through engulfment and opsonization. It can be evaded by coating GNPs with hydrophilic polymers and decreasing their size. Cellular targeting is principally essential in nanoparticle drug-delivery systems and can be attained through the functionalization of GNPs with both tumor-targeting ligands and antibodies.[55] Nanoparticle-delivery systems with particle size between 10 and 100 nm are related with a series of pharmacokinetic and biodistribution parameters.[56-57] De Jong and co-workers[58] reported that after IV different sizes of GNPs being administered in males, the highest amount of Au was detected in the blood, liver and spleen and lower amounts in the lungs, kidneys, testis, thymus, heart and brain, 24 h post injection. AuNPs of size 10-nm were the most prevalent in different organs, with the highest Au content in the liver, followed by the spleen (i.e., organs of the RES). When AuNPs were functionalized with PEG, different tendencies are witnessed[59] where blood showed the maximum amount of Au after 24 h. The biodistribution sketches of rod-shaped have demonstrated sustained circulation in the blood of rats over periods as long as 14 days.[60] Increase in RES organs was detected with a plateau in GNP buildup in the liver at 1 day circulation. Retention in the liver for over 28 days was also found, signifying reduced capability of hepatobiliary clearance/excretion. On coating with PEG, Niidome *et al.* detected no accumulation in major organs, with the exemption of the liver, 72 h post injection. PEGylation conveyed intense improvements to both the pharmacokinetics and biodistribution profiles of systemically directed GNPs.[61]

7. Functionalization of GNPs and delivery systems

The most perplexing approach in delivering the chemotherapeutics agents is to target cells and tissues in an appropriate concentration to provide the desired therapeutic outcomes. The problems encounter targeted delivery system involves the unpredictable systemic half-life, incomplete clearance from the body and off-track effects associated with physiologic actions.[62] Conventional cancer therapy including, chemotherapy, radiation, and surgery, however, patients undergoing chemotherapy frequently they suffer the common side effect experienced in chemotherapy, (such as nausea, vomiting, constipation, diarrhea, fatigue etc.) beside the emotionally and psychologically disturbing alopecia.[63-64] Also, the chemotherapeutic deliveries are not as so much effective, they are accompanied by multidrug resistance (MDR) and the poor penetration of the therapeutic agent. Hence, the optimum strategic plan is to specifically using multifunctional targeted drug delivery system for therapeutic, diagnostic and detection for malignant cells.[65] unctionalization facilitates targeted delivery of these nanoparticles to various cell types, bioimaging, gene delivery, drug delivery and other therapeutic and diagnostic applications. For example, hexagon and boot shaped GNPs show different surface enhanced Raman scattering (SERs) which in turn can be used to detect molecules conjugated to GNPs such as avidin, thereby making these functionalized GNPs (fGNPs) useful for biolabelling, bioassay, clinical diagnosis and therapy.[66] Commonly used functionalization strategies are based on the use of any one or a combination of the groups such as oligo- or polyethylene glycol (PEG), bovine serum albumin (BSA), oligo or polypeptides, oligonucleotides, antisense or sense RNA molecules, antibodies, cell surface receptors and other similar molecules as shown in Figure 8.

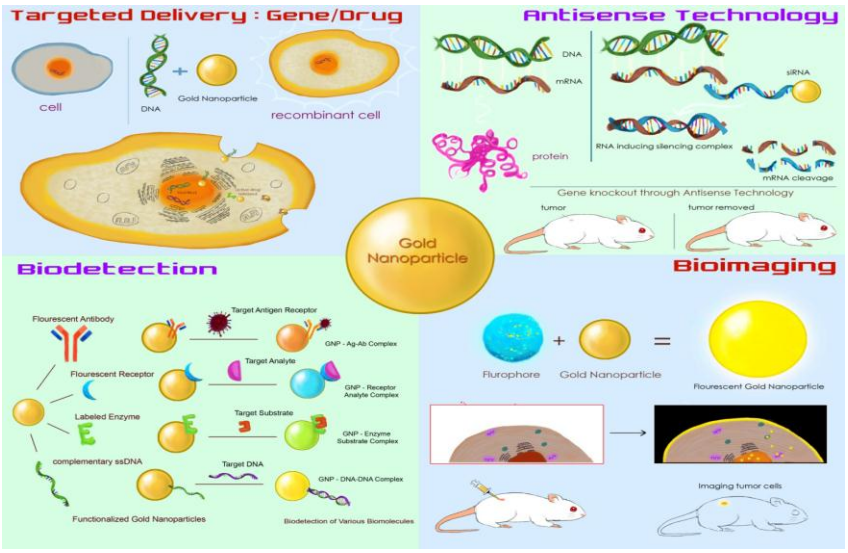

Figure 8- Types of functionalization of gold nanoparticles and their potential biomedical applications.[67]

GNPs are suitable for the delivery of the drugs to cellular destinations due to their ease of synthesis, functionalization and biocompatibility (table 4). GNPS functionalized with targeted specific bio-molecules can effectively destroy cancer cells or bacteria (figure 9).

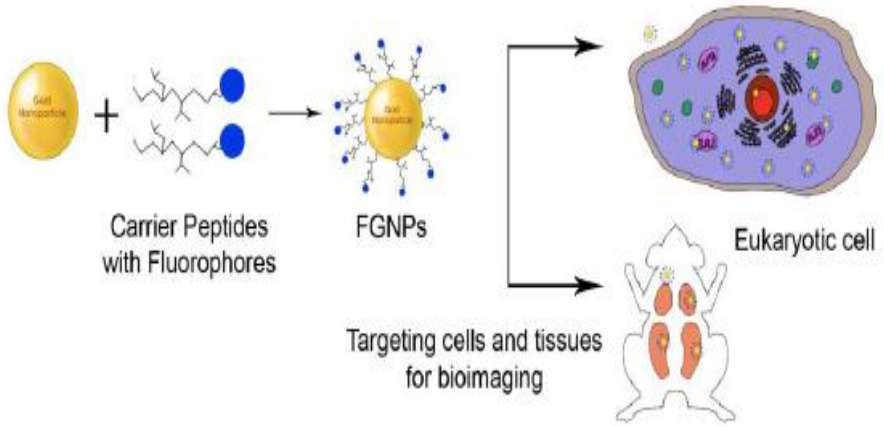

Figure 9 -Functionalized GNPs (fGNPs) for drug delivery: Targeting specific cells with higher loading efficiency, targeted delivery and efficient release of drugs.[67]

Table 4 Post-synthetic functionalization methods used for preparation modified biodiagnostic and therapeutic gold nanoparticles

Biomolecule	Green chemistry	Dendrimers	Polymers (protected)
Peptide	Ionic liquids	Poly(amidoamine) based	Linear and Hyperbranched polymer
Phospholipids	Polysaccharides: chitosan	Other dendrimers	Polymers
Synthetic lipids	Polysaccharides: sucrose		
microorganisms			
Viruses			

7.1 Cellular uptake of gold nanoparticles (GNPs)

The progression in GNPs nanotechnology field necessitates the significance of studying their cellular uptake and intracellular fate. Since GNPs are destined to be for delivering cytotoxic agents to perturbed cells, it has to cross the cell membrane though it has to specifically target the diseased cells. Research has proved that GNPs cross the plasma membrane either via clathrin dependent or independent-mediated endocytosis, which represents the major route, or directly penetrate through the lipid bilayer without facilitation.[68,69] GNPs were also found to predominantly accumulate in the endosomes and small fractions were observed in the cytosol and mitochondria.[70,71] The rate of the GNPs cellular uptake relies on the size, shape, charge of the coating surface molecules.[72-75] Manipulating GNPs coating surface molecules can enhance the cellular uptake of the GNPs. Coating GNPs with cell penetrating peptide (CPP), for instance TAT a viral peptide, was shown to accelerate the cellular uptake rate by six folds.[76] The delivery role of GNPs is not confined to their cellular internalization but also includes their release from the endosomes to the cytosol or other organelle to facilitate drug delivery; this was found to be challenging and hence led to proposing various methods to overcome the endosomal aggregation such as microinjection, gene gun, sonication or by osmotic shock.[68] Remarkably, chemically modifying GNPs with penetrating peptide (Pntn) facilitates their escape from the endosomal sequestration. Furthermore, stabilization of the GNPs with citrate or CLANIN helped in reducing endosomal aggregation of the GNPs; thus helps in their direct cytosolic transportation.[68]

The effect of GNPs on the cellular machinery depends on the type of the cell and GNPs physiochemical properties.[77] GNPs that were taken by some cancer cell lines were shown to induce cell cycle arrest and apoptosis[78], while their uptake by untransformed cells provokes cellular differentiation.[79] or it might yield to the GNPs exocytosis.[80,81] The variations in the effects and fates of the GNPs between different cell types are related to the difference in cellular trafficking to GNPs. In order to add more versatility and specificity to GNPs, coating antibodies can be added to GNPs to certainly bind antigens that are only present on the surface of those cells.[82] In some occasions, targeting a specific intracellular organelle with GNPs is required such as the nucleus; this can be performed following cytosolic conveyance, by adding suface peptides house organelle-localization signal “boarding-pass” to access that certain organelle.[78,83]

8. Applications of GNPs in cancer

8.1. GNPS in Diagnostics

8.1.1 Conventional Cancer diagnosis

Conventionally, cancer is diagnosed by cell pathology such as biopsy, endoscopy, and imaging. In all these

methods the cells are examined under microscope. In Endoscopy the body is looked inside using X ray, CT (computed tomography), ultra sonography and MRI (magnetic resonance imaging).[84]

8.1.2 Surface modified Gold nanoparticles for Diagnosis of Cancer

Gold nanoparticles can be adjusted with PEG and covalently conjugated with monoclonal counter acting agent (MAb), Herceptin (HER), that empowers acknowledgment of bosom growth cells communicating their exceedingly particular tumor-related antigens and PEG to give stealth attributes.[85] A study has demonstrated functionalized gold nanorods inside HER2/neu overexpressing bosom tumors in tumor-bearing bare mice, consequently giving a novel imaging procedure to right on time recognition of disease. Gold nanoparticles having a glutathione top with COOH bunches and folic corrosive notwithstanding a FITC tag were utilized to target carcinoma cells.[86] These fGNP's unequivocally associated just with HeLa cells, because of the statement of the folic corrosive receptors, barring the non-carcinogenic cells, therefore, giving a simple and delicate technique for disease cell recognition.

8.1.3 Cancer diagnosis by Colorimetric Assay:

Direct-representation/location of growth cells utilizing colorimetric measure has been at present advancing quick because of their effortlessness and adaptability.[87] Recently, Lu et al.,[88] have shown a name free, quick and profoundly touchy multifunctional oval-molded gold nanoparticles in view of basic colorimetric and exceptionally delicate two-photon diffusing examine for the specific identification of bosom malignancy. At the point when oval-molded gold nanoparticles were blended with bosom disease SK-BR-3 cell line, an unmistakable shading change happened and two photon scrambling power was expanded by around thirteen times. The system was unmistakably ready to recognize the destructive cells from noncancerous cells furthermore recognized it from other bosom tumor cell lines that express low levels of HER2.

8.1.4 Cancer diagnosis using Immunoassay and Electrochemical Based Method

Gold nanoparticles can be utilized as bearers as a part of optical based protein connected resistant sorbent examine (ELISA) for the examination of vital biomarkers present in blood tests for the treatment of bosom disease. The measure receiving gold nanoparticles as an enhancer brought about higher affectability and shorter examine time when contrasted with traditional technique.[89]

8.1.5 Cancer diagnosis by Imaging and Microscopy Techniques.

In the field of optical imaging methods, Photo-acoustic tomography, Multi-photon Plasmon reverberation microscopy, optical soundness microscopy, and third-consonant microscopy are promising new strategies for the analysis of disease.[90]

8.1.6 New strategies for diagnosis of Cancer

Another measurement has been included the finding of malignancy by Peng and his associates wherein they joined strong stage microextraction with GC/MS for recognizing unstable natural mixes going about as biomarkers for lung disease.[91] The gold nanosensor can quickly recognize the breath of lung malignancy patients from the breath of sound people in an environment of high dampness. Bosom tumor undifferentiated organisms surface marker can be distinguished by applying another methodology for double mode detecting in light of focusing on, utilizing pointer and signal upgrade utilizing surface Plasmon reverberation (SPR) and surface-improved Raman dispersing (SERS).[92] By applying these ideas, it was conceivable to distinguish the cell surface markers antigen, CD44 and CD24, in three bosom tumor cell lines to recognize subpopulation CD44+/CD24− of malignancy undeveloped cells (CSCs).

8.2 GNPS as drug and gene delivery carriers for cancer treatment

8.2.1 GNPS as drug delivery carriers for cancer treatment

GNPs in concentration of 1% with 150-KVp and radiation have been hypothesized to treat prostate cancer.[93] Currently, Brachytherapy utilizing iodine-125 (I-125) or palladium-103 has been utilized to treat Patients with confined prostate tumor.

Besides, another study has been hypothesized utilizing the photoelectron fluence to give neighborhood dosage testimony. Then again, as indicated by MC displaying it is expected that kilovoltage permit the physical measurements improvement paying little heed to high dosages of GNPs.[94]

Kong T, and et al demonstrated a study using thioglucose and cysteamine-capped 10.8-nm with 200-kVp, to detect the cancerous cells from noncancerous cells and also distinguished it from other breast cancer cell lines that express low levels of HER2.[95]

Gold nanoparticles functionalized with fluorescently marked heparin had been as of late utilized for the focused on identification and apoptotic slaughtering of metastatic tumor cells.[96] The basic standard utilized as a part of the study was the over-expression of heparin-debasing catalysts by metastatic malignancy cells. The fGNP's could be helpful for both detection and treatment of tumor cells. Folatemodified PEG ligands were conjugated to doxorubicin to involve the monolayer of their gold nanoparticles. The gold nanoparticles conjugate had expanded cytotoxicity to cells with overexpression of folate-receptors and diminished cytotoxicity to normal cells when contrasted with free doxorubicin.[97]

Functionalized gold nanorods cause photothermal tumor damage in vivo after a single dose treatment. The tumors were irradiated with laser, bringing about a nearby Temperature increment to 70 °C after 5 min of enlightenment. This helpful irradiation was sufficient to completely obliterate all irradiated tumors that were infused with PEG-covered gold nanorods.[97]

Experiments had been performed utilizing both gold nanospheres, which maximally absorb 520 nm light, and gold nanorods. The gold nanorods were hatched with a non-malignant epithelial cell line and with 2 malignant epithelial cell les for examination considers. The immune response conjugated poles specially tie to the dangerous cell lines and when illuminated with a ceaseless 800 nm laser, cell passing came about even at low laser force of 80 Mw.[98] Tumor necrosis factor-alpha (TNF-α), a cytokine, was demonstrated as a brilliant anticancer agents for the therapeutic application because of its dangerous impacts against tumor cells.[90] Later, the delivery system of nanoparticle drug was planned with TNF-α conjugated PEG covered gold nanoparticle, which effectively expanded harms to tumor cells.[99] The mix of temperature and TNF-α conjugated PEG covered gold nanoparticle brought about improved treatment results contrasted with that of the treatment with TNF-α alone. Methotrexate (MTX), an inhibitor to dihydrofolatereductase, was utilized as a chemotherapeutic drug for treating different kindes of tumers.[100] A hybrid material of MTX-gold nanoparticle was readied to analyze the antitumor and poisonous impacts in vivo and in vitro studies. In a relative study, MTX-gold hybrid smothered the tumor development, while level with measure of free MTX did not demonstrate any antitumor impact.[101] Overexpressed folate-receptor was used in growth cells to create focused on delivery system for doxorubicin.[102]

Empty gold nanospheres had been created with double limit of photothermal removal of tumor cells and in addition the arrival of doxorubicin upon irradiation with close infra-red light.[103] Round and hollow gold nanoparticles with fluorescein or doxorubicin had been utilized for medication delivery in tumor cells.[104] Porphyran topped GNPs were used as transporters for anticancer medication (doxorubicin) in human glioma cell line LN-229. The cytotoxicity of this medication was higher upon conjugation to gold nanoparticles when contrasted with the medication alone.[105-107]

8.2.2 GNPS as gene delivery carriers for cancer treatment

Gene Delivery using PEGylated gold nanoparticles:

The most usually utilized nanoparticles for quality conveyance is PEGylated gold where the quality expression was upgraded to around 100-fold .It has been accomplished utilizing transfection impact utilizing a plasmid DNA interceded through electroporation.[108] In this procedure, the transgenes were steady available for use and the DNA was discharged and went through the cell films.

Amino acid gold nanoparticles

Gold nanoparticles functionalized with amino corrosive have likewise been utilized as effective quality conveyance vectors without bringing on cytotoxicity.[109]

So also, productive conveyance of siRNA to the host cells was accomplished utilizing PEGylated gold-poly (β-amino ester) nanoparticles, wherein the poly (β-amino ester) was the key particle in the intracellular focusing of the DNA.[110]

An adenoviral vector based gene delivery system

An adenoviral vector based quality conveyance framework has been contrived by utilizing gold nanoparticles immobilized on the attractive nanoparticles.[111] The issue of viral tropism in the host was maintained a strategic distance from alongside proficient quality conveyance utilizing this system. These fGNP-DNA conjugates were steady and gave critical transgene expression.

9. CONCLUSION

Thus nanotechnology has many applications specifically in the medical field and in recent years, nanomaterials due to their potential impact in therapy and diagnosis of cancer have been the focus of research literature, which postulates promising future for nanotechnology in medical applications.

10. REFERENCES

1. Sanna A, Pala N, Sechi M. Targeted therapy using nanotechnology: focus on cancer. International Journal of Nanomedicine., 2014; 9: 467-483.
2. Drbohlavova J, Chomoucka J, Adam V, Ryvolova M, Eckschlager T, Hubalek J, Kizek R. Nanocarriers for Anticancer Drugs-New Trends in Nanomedicine. Current Drug Metabolism., 2013; 14: 547-564.
3. Silva GA. Introduction to Nanotechnology and Its Applications to Medicine. Surgical Neurology., 2004; 61: 216-220.
4. Nel A, Xia T, Madler L, Li N. Toxic potential of materials at the nanolevel. Science., 2006; 311: 622–7.
5. Service RF. American Chemical Society meeting. Nanomaterials show signs of toxicity. Science., 2003; 300: 243.
6. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: A Cancer Journal for Clinicians., 2011; 61: 69-90.
7. Blanco E, Hsiao A, Mann AP, Landry MG, Meric-Bernstam F, Ferrari M. Nanomedicine in cancer therapy: Innovative trends and prospects. Cancer Science., 2011; 102: 1247-1252.
8. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nature Nanotechnology., 2007; 2: 751-760.

9. Siegel R, Naishadham D, Jemal A. Cancer statistics. CA Cancer J Clin., 2013; 63: 11-30.
10. Anand P, Kunnumakkara AB, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. "Cancer is a preventable disease that requires major lifestyle changes". Pharm. Res., 2008; 25: 97–116.
11. Levy-Nissenbaum E, Radovic-Moreno AF, Wang AZ, Langer R, Farokhzad OC. Nanotechnology and aptamers: applications in drug delivery. Trends Biotechnol., 2008; 26: 442-449.
12. D. M. Parkin, P. Pisani and J. Ferlay. Estimates of the worldwide incidence of eighteen major cancers in 1985. International Journal of Cancer., 1985; 54: 504-606.
13. Brewer, Scott H.; Franzen, Stefan. "Indium Tin Oxide Plasma Frequency Dependence on Sheet Resistance and Surface Adlayers Determined by Reflectance FTIR Spectroscopy". The Journal of Physical Chemistry B., 2002; 106: 12986–12992.
14. Sherr, R.; Bainbridge, K. T. & Anderson, H. H. "Transmutation of Mercury by Fast Neutrons". Physical Review., 1941; 60: 473–479.
15. Lide, D. R., ed. (2005). "Magnetic susceptibility of the elements and inorganic compounds". CRC Handbook of Chemistry and Physics (PDF) (86th ed.). Boca Raton (FL): CRC Press. ISBN 0-8493-0486-5.
16. Bozzola, John J. & Russell, Lonnie Dee (1999). Electron microscopy: principles and techniques for biologists. Jones & Bartlett Learning., 65. ISBN 0-7637-0192-0.
17. Hainfeld, James F.; Dilmanian, F. Avraham; Slatkin, Daniel N.; Smilowitz, Henry M. "Radiotherapy enhancement with gold nanoparticles". Journal of Pharmacy and Pharmacology., 2008; 60: 977–85.
18. Yang, Y., Matsubara, S., Nogami, M., Shi, J., Huang, W.: Onedimensional self-assembly of gold nanoparticles for tunable surface plasmon resonance properties. Nanotechnology., 2006; 17: 2821–2827.
19. Chanda, N.; Kattumuri, V.; Shukla, R.; Zambre, A.; Katti, K.; Kulkarni, R.R.; Kan, P.; Fent, G.M.; Casteel, S.W.; Smith, C.J.; *et al*. Bombesin functionalized gold nanoparticles show *in vitro* and *in vivo* cancer receptor specificity. Proc. Natl. Acad. Sci., 2010; 107: 8760–8765.
20. G. Ali Mansoori, Pirooz Mohazzabi, Percival McCormack, and Siavash Jabbari. Nanotechnology in cancer prevention, detectionand treatment: bright future lies ahead. World Review of Science, Technology and Sustainable Development, 4 Nos. 2/3, 2007.
21. AK Khan , R Rashid, G Murtaza and A Zahra. Gold Nanoparticles: Synthesis and Applications in Drug Delivery. Tropical Journal of Pharmaceutical Research., July 2014; 13(7): 1169-1177.
22. Susie Eustis and Mostafa A. El-Sayed. Why gold nanoparticles are more precious than pretty gold: Noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. Chem. Soc. Rev., 2006; 35: 209–217.
23. Xiaohua Huang, Mostafa A. El-Sayed. Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. Journal of Advanced Research., 2010; 1: 13–28.
24. Stephan Link and Mostafa A. El-Sayed . Optical Properties And Ultrafast Dynamics Of Metallic Nanocrystals. Annual Review of Physical Chemistry., 2003; 54: 331-366.
24. Tunable surface plasmon resonance (SPR),surface-enhanced Raman scattering, electrical, magnetic, thermal conductivity, antibacterial activity, chemical and biostability. Proc. Natl. Acad. Sci., 2006; 107: 8739–8750.
25. 25-A.K. Sinha, S. Seelan, S. Tsubota, M. Haruta. Catalysis by Gold Nanoparticles: Epoxidation of Propene. Topics in Catalysis., 2004; 29: 95-102.
26. Paul L Freund and Michael. Spiro Catalysis by colloidal gold of the reaction between ferricyanide and thiosulphate ions. Journal of the Chemical Society Faraday Transactions., 1986; 82: 720-72.
27. B.T. Draine, J. Goodman. Beyond Clausius–Mossotti: wave propagation on a polarizable point lattice and the discrete dipole approximation. Astrophys J., 1993; 405: 685–697.
28. K.S. Lee, M.A. El-Sayed. Dependence of the enhanced optical scattering efficiency relative to that of absorption for gold metal nanorods on aspect ratio, size, end-cap shape and medium refractive index. J Phys Chem B, 2005; 109(43): 20331–20338.
29. Sun X, Zhang G, Keynton RS, O'Toole MG, Patel D, Gobin AM. Enhanced drug delivery via hyperthermal membrane disruption using targeted gold nanoparticles with PEGylated Protein-G as a cofactor. Nanomed: Nanotechnol Biol Med., 2013; 9: 1214-1222.
30. Heidari Z, Sariri R, Salouti M. Gold nanorods-bombesin conjugate as a potential targeted imaging agent for detection of breast cancer. J Photochem Photobiol B: Biol., 2014; 130: 40-46.
31. Pissuwan D, Niidome T, Cortie MB. The forthcoming applications of gold nanoparticles in drug and gene delivery systems. J Control Release., 2011; 149: 65-71.
32. Bisker G, Yeheskely-Hayon D, Minai L, Yelin D. Controlled release of Rituximab from gold nanoparticles for phototherapy of malignant cells. J Control Release.,, 2012; 162: 303-309.
33. 30. Paino IM, Marangoni VS, de Oliveira Rde C, Antunes LM, Zucolotto V. Cyto and genotoxicity of gold nanoparticles in human hepatocellular carcinoma and peripheral blood mononuclear cells. Toxicology letters., 2012; 215: 119-125.
34. Guo W, Pi Y, Song H, Tang W, Sun J. Layer-by-layer assembled gold nanoparticles modified anode and its application in microbial fuel cells. Colloid

Surfac A: Physicochem Engineer Aspects., 2012; 415: 105-111.
35. Sawada H, Takahashi K. Facile preparation of gold nanoparticles through autoreduction of gold ions in the presence of fluoroalkyl end-capped cooligomeric aggregates: LCST-triggered sol-gel switching behavior of novel thermoresponsive fluoroalkyl endcapped cooligomeric nanocomposite-encapsulated gold nanoparticles. J Colloid Interfac Sci., 2010; 351: 166-170.
36. Khan MS, Vishakante GD, Siddaramaiah H. Gold nanoparticles: A paradigm shift in biomedical applications. Advanc Colloid Interfac Sci., 2013; 199- 200: 44-58.
37. Rezende TS, Andrade GRS, Barreto LS, Costa Jr NB, Gimenez IF, Almeida LE. Facile preparation of catalytically active gold nanoparticles on a thiolated chitosan. Mater Lett., 2010; 64: 882-884.
38. Chen K-S, Hung T-S, Wu H-M, Wu J-Y, Lin M-T, Feng CK. Preparation of thermosensitive gold nanoparticles by plasma pretreatment and UV grafted polymerization. Thin Solid Films., 2010; 518: 7557- 7562.
39. Murawala P, Tirmale A, Shiras A, Prasad BLV. In situ synthesized BSA capped gold nanoparticles: Effective carrier of anticancer drug Methotrexate to MCF-7 breast cancer cells. Mater Sci Engineer C., 2014; 34: 158-167.
40. Giljohann DA, Seferos DS, Daniel WL, Massich MD, Patel PC, Mirkin CA. Gold nanoparticles for biology and medicine. Angewandte Chem., 2010; 49: 3280-3294.
41. Lan M-Y, Hsu Y-B, Hsu C-H, Ho C-Y, Lin J-C, Lee S-W. Induction of apoptosis by high-dose gold nanoparticles in nasopharyngeal carcinoma cells. Auris Nasus Larynx., 2013; 40: 563-568.
42. Namazi H, Fard AMP. Preparation of gold nanoparticles in the presence of citric acid-based dendrimers containing periphery hydroxyl groups. Mater Chem Physic., 2011; 129: 189-194.
43. X-ray Rajasekhar Anumolu1 and Leonard F. Pease III. Rapid Nanoparticle Characterization. Nanotechnology and Nanomaterials » "The Delivery of Nanoparticles", *book edited by Abbass A. Hashim, 2012; (17): 348-379.*
44. Willets KA, Van Duyne RP. Localized surface plasmon resonance spectroscopy and sensing. Annu Rev Phys Chem., 2007; 58: 267-297.
45. Tsai, D. H.; Zangmeister, R. A.; Pease III, L. F.; Zachariah, M. R. & Tarlov, M. J. (2008). Gasphase Ion-mobility Characterization of SAM functionalized Au Nanoparticles. Langmuir., 2008; 24: 8483-8490.
46. Dong W-F, Sukhorukov GB, Moehwald H. Enhanced Raman Imaging and Optical Spectra of Gold Nanoparticle Doped Microcapsules. Phys Chem Chem Phys., 2003; 5: 3003-3012.
47. Fissan, H. J.; Helsper, C. & Thielen, H. J. Determination of Particle-Size Distributions by Means of an Electrostatic Classifier. J. Aerosol Sci., 1983; 14: 354- 357.
48. Smith WE. Practical understanding and use of surface enhanced Raman Scattering /surface enhanced resonance Raman scattering in chemical and biological analysis. Chem Soc Rev., 2008; 37: 955-964.
49. Zhang, Y. , Shuang, S., Dong, C., Lo, C.K., Paau, M.C., Choi, M.M.F. Application of HPLC and MALDI-TOF MS for studying as-synthesized ligand-protected gold nano-clusters products. Analytical Chemistry, 2009; 81: 1676-1685.
50. Kim, S. K.; Ha, T. & Schermann, J. P. (2010). Advances in Mass Spectrometry for Biological Science. Phys. Chem. Chem. Phys., 2010; 12: 13366–13367.
51. Weiwei Yang, Yu Bai, Yancai Li, Changqing Sun. Amperometric nitrite sensor based on hemoglobin/colloidal gold nanoparticles immobilized on a glassy carbon electrode by a titania sol-gel film. Analytical and Bioanalytical Chemistry., 2005; 382: 44-50.
52. J. Wang. Analytical Electrochemistry. Wiley-VCH, Hoboken, New Jersey., 2006; 272.
53. R. Allabashi, A. de la Escosura-Muñiz, L. Liste-Calleja, A. Merkoçi. ICP-MS: A powerful technique for quantitative determination of gold nanoparticles without previous dissolving. Journal of Nanoparticle Research., 2009; 11: 2003–2011.
54. Paciotti GF, Kingston DGI, Tamarkin L. Colloidal gold nanoparticles: a novel nanoparticles platform for developing multifunctional tumor-targeted drug delivery vectors. Drug Dev. Res., 2006; 67: 47–54.
55. Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. Future Med. Nanomed., 2008; 3: 703–717.
56. Akiyama Y, Mori T, Katayama Y, Nidome T. The effects of PEG grafting level and injection dose on gold nanorod biodistribution in the tumor-bearing mice. J. Control. Release., 2009; 139: 81–84.
57. De Jong WH, Hagens WI, Krystek P, Burger MC, Sips AJ, Geertsma RE. Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. Biomaterials., 2008; 29: 1912–1919.
58. Terentyuk GS, Maslyakova GN, Suleymanova LV, et al. Circulation and distribution of gold nanoparticles and induced alterations of tissue morphology at intravenous particle delivery. J. Biophotonics., 2009; 2: 292–302.
59. Perrault SD, Walkey C, Jennings T, Fischer HC, Chan WCW. Mediating tumor targeting efficiency of nanoparticles through design. Nano Lett., 2009; 9: 1909–1915.
60. Oh E, Delehanty JB, Sapsford KE, et al. Cellular uptake and fate of pegylated gold nanoparticles is dependent on both cell-penetration peptides and particle size. ACS Nano., 2011; 5: 6434–6448.

61. Duncan, B.; Kim, C.; Rotello, V.M. Gold nanoparticle platforms as drug and biomacromolecule delivery systems. J. Contr. Release., 2010; 148: 122–127.
62. Scheinberg, D.A.; Villa, C.H.; Escorcia, E.E.; McDevitt, M.R. Conscripts of the infinite armada: Systemic cancer therapy using nanomaterials. Nat. Rev. Clin. Oncol., 2010; 7: 266–276.
63. Kim, D.W.; Kim, J.H.; Park, M.; Yeom, J.H.; Go, H.; Kim, S.; Han, M.S.; Lee, K.; Bae, J.Modulation of biological processes in the nucleus by delivery of DNA oligonucleotides conjugated with gold nanoparticles. Biomaterials., 2011; *32*: 2593–2604.
64. Zhou, X.; Zhang, X.; Yu, X.; Zha, X.; Fu, Q.; Liu, B.; Wang, X.; Chen, Y.; Chen, Y.; Shan, Y.; *et al.* The effect of conjugation to gold nanoparticles on the ability of low molecular weight chitosan to transfer DNA vaccine. Biomaterials., 2008; 29: 111–117.
65. Sajja HK1, East MP, Mao H, Wang YA, Nie S, Yang L. Development of multifunctional nanoparticles for targeted drug delivery and noninvasive imaging of therapeutic effect. Curr Drug Discov Technol., 2009 Mar; 6(1): 43-51.
66. Pooja M. Tiwari, Komal Vig, Vida A. Dennis and Shree R. Singh. Functionalized Gold Nanoparticles and Their Biomedical Applications. *Nanomaterials.*, 2011; 1: 31-63.
67. Nativo P, Prior IA, Brust M: Uptake and intracellular fate of surface-modified gold nanoparticles. *ACS Nano.*, 2008; 2: 1639-1644.
68. Ng CT, Tang FM, Li JJ, Ong C, Yung LL, Bay BH: Clathrin-mediated endocytosis of gold nanoparticles in vitro. *Anat Rec (Hoboken).*, 2015; 298: 418-427.
69. Kim CS, Li X, Jiang Y, Yan B, Tonga GY, Ray M *et al.*: Cellular imaging of endosome entrapped small gold nanoparticles. *MethodsX.*, 2015; 2: 306-315. Karatas OF, Sezgin E, Aydin O, Culha M: Interaction of gold nanoparticles with mitochondria. *Colloids Surf B Biointerfaces.*, 2009; 71: 315-318.
70. Karatas OF, Sezgin E, Aydin O, Culha M: Interaction of gold nanoparticles with mitochondria. *Colloids Surf B Biointerfaces.*, 2009; 71: 315-318.
71. Oh E, Delehanty JB, Sapsford KE, Susumu K, Goswami R, Blanco-Canosa JB *et al.*: Cellular uptake and fate of PEGylated gold nanoparticles is dependent on both cell-penetration peptides and particle size. *ACS Nano.*, 2011; 5: 6434-6448.
72. Liu X, Huang N, Li H, Jin Q, Ji J: Surface and size effects on cell interaction of gold nanoparticles with both phagocytic and nonphagocytic cells. *Langmuir.*, 2013; 29: 9138-9148.
73. Neshatian M, Chung S, Yohan D, Yang C, Chithrani DB: Uptake of Gold Nanoparticles in Breathless (Hypoxic) Cancer Cells. *J Biomed Nanotechnol.*, 2015; 11: 1162-1172.
74. Chithrani BD, Ghazani AA, Chan WC: Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Lett.*, 2006; 6: 662-668.
75. Cesbron Y, Shaheen U, Free P, Levy R: TAT and HA2 facilitate cellular uptake of gold nanoparticles but do not lead to cytosolic localisation. *PLoS One.*, 2015; 10: e0121683.
76. Alkilany AM, Murphy CJ: Toxicity and cellular uptake of gold nanoparticles: what we have learned so far? *J Nanopart Res.*, 2010; 12: 2313-2333.
77. Kang B, Mackey MA, El-Sayed MA: Nuclear targeting of gold nanoparticles in cancer cells induces DNA damage, causing cytokinesis arrest and apoptosis. *J Am Chem Soc.*, 2010; 132: 1517-1519.
78. Choi SY, Song MS, Ryu PD, Lam AT, Joo SW, Lee SY: Gold nanoparticles promote osteogenic differentiation in human adipose-derived mesenchymal stem cells through the Wnt/beta-catenin signaling pathway. *Int J Nanomedicine.*, 2015; 10: 4383-4392.
79. Oh N, Park JH: Endocytosis and exocytosis of nanoparticles in mammalian cells. *Int J Nanomedicine.*, 2014; 9(1): 51-63.
80. Oh N, Park JH: Surface chemistry of gold nanoparticles mediates their exocytosis in macrophages. *ACS Nano.*, 2014; 8: 6232-6241.
81. Cardoso MM, Peca IN, Roque AC: Antibody-conjugated nanoparticles for therapeutic applications. *Curr Med Chem.*, 2012; 19: 3103-3127.
82. Gu YJ, Cheng J, Lin CC, Lam YW, Cheng SH, Wong WT: Nuclear penetration of surface functionalized gold nanoparticles. *Toxicol Appl Pharmacol.*, 2009; 237: 196-204.
83. Oh E, Delehanty JB, Sapsford KE, Susumu K, Goswami R, Blanco-Canosa JB *et al.*: Cellular uptake and fate of PEGylated gold nanoparticles is dependent on both cell-penetration peptides and particle size. *ACS Nano.*, 2011; 5: 6434-6448.
84. M. Eghtedari, A. V. Liopo, J. A. Copland, A. A. Oraevsky, and M. Motamed, Engineering of hetero-functional gold nanorods for the in vivo molecular targeting of breast cancer cells, Nano Letters, 2009; 9: 287–291.
85. Zhang, Z.; Jia, J.; Lai, Y.; Ma, Y.; Weng, J.; Sun, L. Conjugating folic acid to gold nanoparticles through glutathione for targeting and detecting cancer cells. Bioorg. Med. Chem., 2010; 18: 5528– 5534.
86. W. Zhao, M. A. Brook, and Y. Li, Design of gold nanoparticlebased colorimetric biosensing assays.ChemBioChem, 2008; 9: 2363–2371.
87. W. T Lu, S. R. Arumugam, D. Senapati et al.,Multifunctional ovalshaped gold-nanoparticle-based selective detection of breast cancer cells using simple colorimetric and highly sensitive twophoton scattering assay, ACS Nano, 2010; 4: 1739– 1749.
88. Ambrosi, F. Airo, and A. Merkoci, Enhanced gold nanoparticle based ELISA for a breast cancer biomarker,Anal. Chemistry, 2010; 82: 1151–1156.
89. X. G. Shi, S. Wang, S. Meshinchi et al., Dendrimer-entrapped gold nanoparticles as a platform for

cancer-cell targeting and imaging, Small., 2007; 3: 1245–1252.
90. G. Peng, U. Tisch, O. Adams et al., Diagnosing lung cancer in exhaled breath using gold nanoparticles,NatNanotechnol, 2009; 4: 669–673.
91. K. Lee, V. P. Drachev, and J. Irudayaraj, DNA-gold nanoparticle reversible networks grown on cell surface marker sites: Application in diagnostics. ACS Nano., 2011; 5: 2109–2117.
92. Cho SH, Jones BL, Krishnan S. The dosimetric feasibility of gold nanoparticle-aided radiation therapy (GNRT) via brachytherapy using low-energy gamma-/x-ray sources. Phys Med Biol., 2009; 5: 54:68.
93. Noh, S.M.; Kim, W.K.; Kim, S.J.; Kim, J.M.; Baek, K.H.; Oh, Y.K. Enhanced cellular delivery and transfection efficiency of plasmid DNA using positively charged biocompatible colloidal gold nanoparticles. Biochim. Biophys. Acta., 2007; 1770: 747–752.
94. Kong T, Zeng J, Wang X, Yang X, Yang J, McQuarrie S, et al. Enhancement of radiation cytotoxicity in breast-cancer cells by localized attachment of gold nanoparticles. ACS Nano., 2008; 4: 1537–43.
95. Guo, S.; Huang, Y.; Jiang, Q.; Sun, Y.; Deng, L.; Liang, Z.; Du, Q.; Xing, J.; Zhao, Y.; Wang, P.C.; *et al*. Enhanced gene delivery and siRNA silencing by gold nanoparticles coated with charge-reversal polyelectrolyte. ACS NANO., 2010; 4: 5505–5511.
96. -R.S. Norman, J.W. Stone, A. Gole, C.J. Murphy, T.L. SaboAttwood, Targeted photothermallysis of the pathogenic bacteria, Pseudomonas aeruginosa, with gold nanorods, Nano Lett., 2008; 8: 302–306.
97. X.H. Huang, I.H. El-Sayed, W. Qian, M.A. El-Sayed, Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods, J. Am. Chem. Soc., 2006; 128: 2115–2120.
98. Rai, A.; Prabhune, A.; Perry, C.C. Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. J. Mater. Chem., 2010; 20: 6789–6798.
99. Mocellin, S. & Nitti, D. TNF and cancer: the two sides of the coin.Front. Biosci., 2008; 13: 2774 - 2783.
100. Visaria, R. K. et al. Enhancement of tumor thermal therapy using Au nanoparticle-assisted tumor necrosis factor alpha delivery.Mol. Cancer Ther., 2006; 5: 1014- 1020.
101. Huennekens, F. M. The methotrexate story: a paradigm for development of cancer chemotherapeutic agents.Adv. Enzyme. Regul., 1994; 34: 397-419.
102. Chen, Y. H. et al. Methotrexate conjugated to Au nanoparticles inhibits tumor growth in a syngeneic lung tumor model. Mol. Pharm., 2007; 4: 713-722.
103. B. Asadishad, M. Vossoughi, I. Alemzadeh, Folate-ReceptorTargeted Delivery of Doxorubicin Using Polyethylene Glycol Functionalized Gold Nanoparticles. IndEngChem Res., 2010; 49: 1958–1963.
104. You, J.; Zhang, G.; Li, C. Exceptionally high payload of doxorubicin in hollow gold nanospheres for near-infrared light-triggered drug release. ACS NANO., 2010; 4: 1033–1041.
105. Kim, B.; Han, G.; Toley, B.J.; Kim, C.K.; Rotello, V.M.; Forbes, N.S. Tuning payload delivery in tumour cylindroids using gold nanoparticles. Nat. Nanotechnol., 2010; 4: 465–472.
106. Venkatpurwar, V.; Shiras, A.; Pokharkar, V. Porphyran capped gold nanoparticles as a novel carrier for delivery of anticancer drug: In-vitro cytotoxicity study. Int. J. Pharm., 2011; 409: 314–320.
107. Ghosh, P.S.; Kim, C.K.; Han, G.; Forbes, N.S.; Rotello, V.M. Efficient gene delivery vectors by tuning the surface charge density of amino acid-functionalized gold nanoparticles. ACS NANO., 2008; 2: 2213–2218.
108. Lee, J.S.; Green, J.J.; Love, K.T.; Sunshine, J.; Langer, R.; Anderson, D.G. Gold, poly(β-amino ester) nanoparticles for small interfering RNA delivery. Nano Lett., 2009; 2: 2402–2406.
109. Kamei, K.; Mukai, Y.; Kojima, H.; Yoshikawa, T.; Yoshikawa, M.; Kiyohara, G.; Yamamoto, T.A.; Yoshioka, Y.; Okada, N.; Seino, S.; et al. Direct cell entry of gold/iron-oxide magnetic nanoparticles in adenovirus mediated gene delivery. Biomaterials., 2009; 31: 1809–1814.
110. Chakravarthy, K.V.; Bonoiu, A.C.; Davis, W.G.; Ranjan, P.; Ding, H.; Hu, R.; Bowzard, B.J.; Bergey, E.J.; Katz, J.M.; Knight, P.R.; *et al.* Gold nanorod delivery of an ssRNA immune activator inhibits pandemic H1N1 influenza viral replication. *Proc. Natl. Acad. Sci.*, 2010; 104: 10172–10177.
111. Paciotti GF, Myer L, Weinreich D, Goia D, Pavel N, McLaughlin RE, et al. Colloidal gold: a novel nanoparticle vector for tumor directed drug delivery. Drug Deliv., 2004; 11: 169–83.