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GOLD NANOPARTICLE: AS NOVEL CARRIER FOR DRUG DELIVERY

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ABSTRACT

Nanoparticles have successfully come to aid various disease states, but the advances in biomedical imaging depend largely on the shape, size, and selectivity of the nanoparticles to the target. Among nanoparticles, gold nanoparticles demonstrate special advantages in this field due to their unique properties, small size and high surface area-to-volume ratio. These particles have been widely used in various biomedical applications and drug delivery systems due to their inert nature, stability, high dispersity, non-cytotoxicity and biocompatibility. Gold nanoparticles are being used effectively in laboratory based clinical diagnostic methods while concurrently showing great promise in vivo either as a diagnostic imaging agent or a therapeutic agent.Gold Nanoparticles have appeared as an attractive candidate for various drug molecules or considered as extraordinary molecular carrier for the targeting including DNA, RNA, proteins, pepetides,drugs, genes and other molecules of therapeutic significance. This Review focuses on gold therapy and its biodistribution in humans, characteristics, types, synthesis methods, and functionaliztion of gold nanoparticles and its emerging applications various fields.

KEYWORDS: Metallic nanoparticle, Gold nanoparticles, Surface plasmon resonance, Cancer imaging.

INTRODUCTON

Conventional preparations like solution, suspension or emulsion suffer from certain limitations like high dose and low availability, first pass effect, intolerance, instability, and they exhibit fluctuations in plasma drug levels and do not provide sustained effect, therefore there is a need for some novel carriers which could meet ideal requirement of drug delivery system. Recently nanoparticles delivery system has been proposed as

colloidal drug carriers.[1] Nanotechnology can be defined as reaserch for design, synthesis, and manipulation of strctuture of particles with Diamensions smaller than 100 nm.[2]

Nanoparticles (NP) are a type of colloidal drug delivery system comprising particles witha size range from 10 to 1000 nm in diameter. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials.[3] The key advantages of nanoparticles are (1) improved bioavailability by enhancing aqueous solubility, (2) increasing resistance time in the body (increasing half life for clearance/increasing specificity for its cognate receptors and (3) targeting drug to specific location in the body (its site of action). This results in concomitant reduction in quantity of the drug required and dosage toxicity, enabling the safe delivery of toxic therapeutic drugs and protection of non target tissues and cells from severe side effects.[4] It is increasingly used in different applications, including drug carrier systems and to pass organ barriers such as the blood-brain barrier, cell membrane etc.[5] They are based on biocompatible lipid and provide sustained effect by either diffusion or dissolution.[6]

Metallic nanoparticles having size between1 to 100 nm in diameter have different shapes and can be composed of one or more inorganic compounds, such as noble metals, heavy metals, iron,[7] The majority of them exhibit size-related properties that differ significantly from those observed in microparticles or bulk materials.

These properties of noble metal nanoparticles includes.[8]

1. High surface-to-volume ratio, broad optical properties, ease of synthesis, and facile surface chemistry and functionalization hold significant role in the clinical field for cancer Therapeutics.
2. Depending on their size and composition they have properties, such as quantum confinement in semiconductor nanocrystals, surface plasmon resonance in some metal nanoparticles and superparamagnetism in magnetic materials.
3. The highly tunable optical properties of noble metal NPs (e.g., gold, silver, or a combination of both), makes them easily tuned to desirable wavelengths according to their shape (e.g., nanoparticles, nanoshells, nanorods, etc.), size (e.g., 1 to 100 nm), and composition (e.g., core/shell or alloy noble metals), facilitating their imaging and photothermal applications under native tissue.
4. These NPs can have prolong *in vivo* circulation for drug and gene therapy when they

are functionalized with various moieties, such as antibodies, peptides, and/or DNA/RNA to specifically target different cells and with biocompatible polymers (e.g., polyethylene glycol and PEG).

5. Moreover, the efficient conversion of light or radiofrequencies into heat enables thermal ablation of targeted cancer cells.

6. Nanoparticles on induction gets merged into a solid at relatively lower temperatures, often without melting, leads to an improvement and easy-creation of coatings for electronics applications (eg, capacitors).

7. The wavelength of material nanoparticles are below the critical wavelength of light which renders them transparent. Due to their transparent property, they are very useful for applications in cosmetics, coatings, and packaging.

8. The non-destructive easy attachment of metallic nanoparticles with DNA opens up avenue for

medical diagnostic applications.

9. Nanoparticles can traverse through the vasculature and localize any target organ. This potentially can lead to novel therapeutic, imaging, and biomedical applications.

Gold Nanoparticals

Colloidal gold, also known as gold nanoparticles, is a suspension (or colloid) of nanometer-sized particles of gold.[9]

Gold Therapy

On the basis of the chemistry of gold, gold(I) is used as the main therapeutic agent as it is water soluble, is less reactive than gold(III) and is easily stabilized in a complex by the addition of ligands. Gold can be delivered to patients intravenously, intramuscularly, or orally with gold preparations specifically designed for each particular route of administration. Accordingly, gold taken orally needs to be lipid soluble for it to be absorbed within the gastrointestinal tract and will therefore have different physiochemical, pharmacokinetic, and toxicological properties compared to water soluble gold that is injected.[10,11]

Gold Pharmacokinetics and Biodistribution

The bioavailability of gold in patients very much depends on the route of administration. While injectable gold compounds are fully absorbed with maximum levels attained after about 2 h[12]nly 20_ 25% of oral gold is absorbed,[13,14,15] Furthermore, intermittent

dosing regimens of injectable gold result in fluctuating blood gold levels with high peak and low trough concentrations.[16] In contrast, oral gold preparations can be taken regularly and made with prolonged blood half-life preparations, resulting in a nearly constant concentration of gold for the duration of a patient's treatment.[17]

Characteristics Of Gold Nanoparticles.[18,22]

1)Gold nanoparticles are chemically inert. These have greater biological compatibility.
2)Functionalization through thiol linkages.
3)Gold nanoparticles provide microscopic probes for the study of the cancer cell.
4)Gold nanoparticles accumulate in the cancerous cell and show the cytotoxic effect i.e. apoptosis or necrosis of the specific cell and cell specific receptor.
5)These have high stability due to the gold-sulphur bonds.
6)Their photo physical properties can be exploited for drug release at remote place.

Types Of Gold Nanoparticles.[23]

1)Gold Nanorods

These are synthesized by template method. These are prepared by electrochemical deposition of gold within the pores of nanoporous polycarbonate template membranes. Gold nanorods diameter is according to the diameter of pore of the template membrane.

2)Gold Nanoshells

Surface plasmon resonance peaks (ranging from visible to near I.R. region) is used for the designing and fabrication of gold nanoshells. The core of gold nanoshells is made up of silica and outer surface is made up of gold.(Fig 1) Gold controls the thickness of the shell.

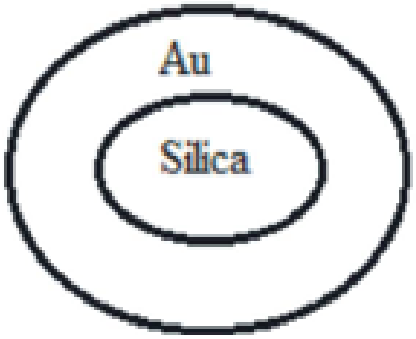

Fig.1-Gold Nanoshells

Gold Nanocages

Through galvanic replacement reaction between truncated silver nanocubes and aquoqes HAuCl4 Gold nanocages is synthesized. Following Figure Shows Shpe of gold Nanocage.

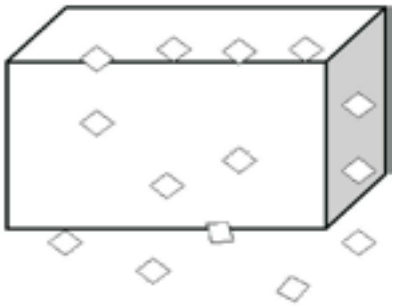

Fig.2-Gold Nanocage

SERS Nanoparticles

SERS is an optical technique like fluroscence and chemiluminescence having better exhibited by gold nanoparticles. sensitivity, high levels of multiplexing, robustness and greater performance in blood and biological.

Solid Nanospheres

These are synthesized by reduction of an Aqueous HAuCL4 by using citrate as reducing agents.

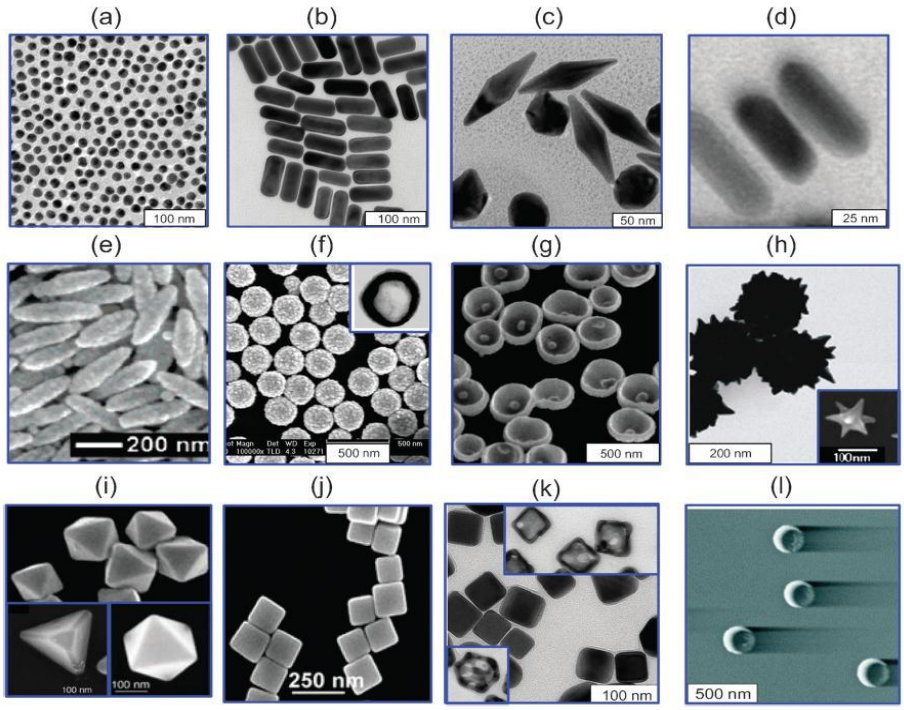

Fig 3-Various shapes of GNPs

Synthesis Of Gold Nanoparticals

1)Turkevich method

The method pioneered by J. Turkevich et al. in 1951 and refined by G. Frens in 1970s, is the simplest one available. Generally, it is used to produce modestly monodisperse spherical gold nanoparticles suspended in water of around 10–20 nm in diameter. Larger particles can be produced, but this comes at the cost of monodispersity and shape.

It involves the reaction of small amounts of hot chlorauric acid with small amounts of sodium citrate solution. The colloidal gold will form because the citrate ions act as both a reducing agent, and a capping agent.

Recently, the evolution of the spherical gold nanoparticles in the Turkevich reaction has been elucidated. Interestingly, extensive networks of gold nanowires are formed as a transient intermediate. These gold nanowires are responsible for the dark appearance of the reaction solution before it turns ruby-red.

To produce larger particles, less sodium citrate should be added (possibly down to 0.05%, after which there simply would not be enough to reduce all the gold). The reduction in the amount of sodium citrate will reduce the amount of the citrate ions available for stabilizing the particles, and this will cause the small particles to aggregate into bigger ones (until the total surface area of all particles becomes small enough to be covered by the existing citrate ions).[24]

2)Brust method

This method was discovered by Brust and Schiffrin in early 1990s, and can be used to produce gold nanoparticles in organic liquids that are normally not miscible with water (like toluene).

It involves the reaction of a chlorauric acid solution with tetraoctylammonium bromide (TOAB) solution in toluene and sodium borohydride as an anti-coagulant and a reducing agent, respectively.

Here in figure 4, the gold nanoparticles will be 2 to 6 nm in diameter. $NaBH_4$ is the reducing agent, and TOAB is both the phase transfer catalyst and the stabilizing agent.[24,25]

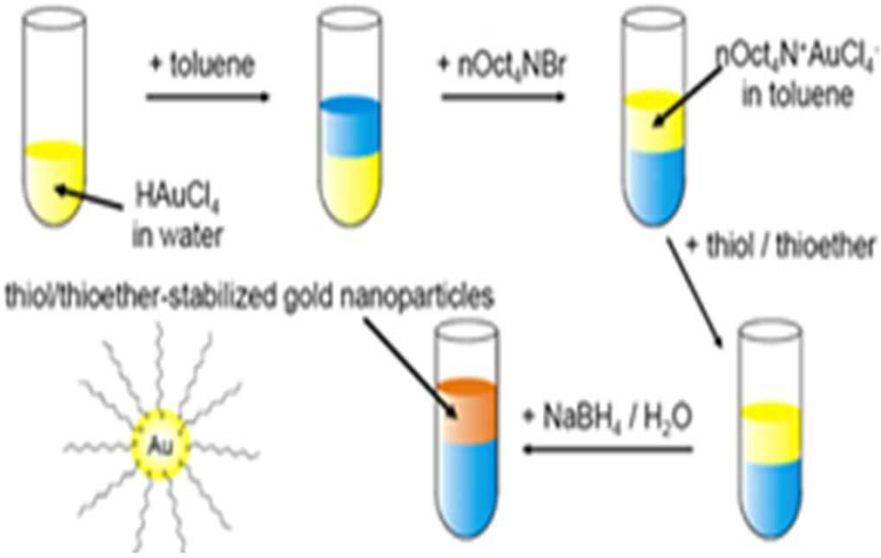

Fig.4 – Synthesis of GNP by Burst Method

3)Martin Method

This method, discovered by the Eah group in 2010, generates –naked‖ gold nanoparticles in water by reducing HAuCl4 with NaBH4. Even without any other stabilizer like citrate, gold nanoparticles are stably dispersed. The size distribution is nearly monodisperse and the diameter can be precisely and reproducibly tunable from 3.2 to 5.2 nm. The key is to stabilize HAuCl4 and NaBH4 in the aqueous stock solutions with HCl and NaOH for >3 months and >3 hours respectively. In addition, the ratio of NaBH4-NaOH ions to HAuCl4-HCl ions must be precisely controlled in the –sweet zone‖.

Naked gold nanoparticles are coated with a monolayer of 1- dodecanethiol and then phase-transferred to hexane simply by shaking a mixture of water, acetone, and hexane for 30 seconds. Since all the reaction byproducts remain in the wateracetone phase, no post-synthesis cleaning is needed for gold nanoparticles in the hexane phase. The amount of 1-dodecanethiol is only 10 % of gold atoms in number. All these synthesis procedures take just <10 minutes. This new synthesis method is simple, cheap, easy-to-adopt, greener, and therefore important for many practical applications and/or beginners by combining the easiness of the Turkevich method with the monolayer-protection of the Brust method. Note that no non-volatile and difficult-to-remove phase-transfer agent such as TOAB in the Brust method is required.

The precise size control in the diameter range of 3.2 to 5.2 nm is critically important for

both the phase-transfer of gold nanoparticles from water to hexane and their 2D selfassembly on a toluene droplet. Each synthesis batch in <10 minutes generates gold nanoparticles of milligrams. Scaling up to the gram scale is to be explored.[24,25]

4)Sonolysis

In process based on ultrasound, the reaction of an aqueous solution of HAuCl4 with glucose, the reducing agents are hydroxyl radicals and sugar pyrolysis radicals (forming at the interfacial region between the collapsing cavities and the bulk water) and the morphology obtained is that of nano ribbons with width 30- 50 nm and length of several micrometers. These ribbons are very flexible and can bend with angles larger than 90^0c (Fig.5). When glucose is replaced by cyclodextrin (a glucose oligomer) only spherical gold particles are obtained suggesting that glucose is essential in directing the morphology towards a ribbon.

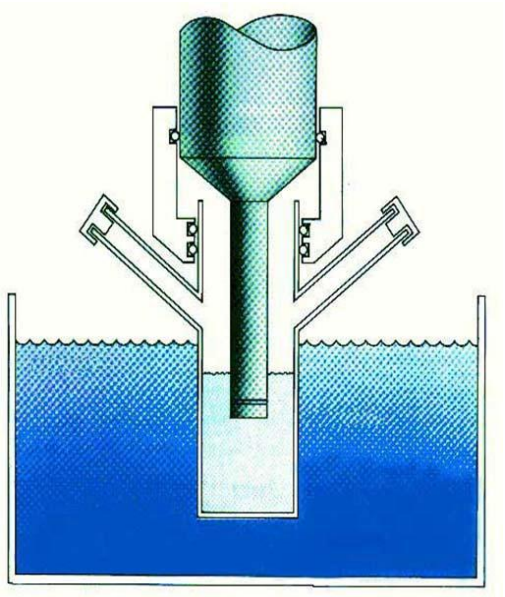

Fig 5- Sonolysis

Properties Of Gold Nanoparticles-1) Surface Plasmon Resonance

The unique optical property of GNPs is one of the reasons that GNPs attract tremendous interests from different fields of science, especially in the development of sensors. The spherical GNP solutions show a range of vibrant colors including red, purple and violet when the particle size increases, and they could be used to stain glass in ancient time. The intense color is caused by the strong absorption and scattering of *ca.* 520 nm light.[26] which is the result of the collectiveoscillation of conduction electrons on the surface of GNPs when they are excited by the incident light. This phenomenon is known as surface

plasmon resonance (SPR), and it depends greatly on particle size and shape. Thus, the SPR peak is tunable by manipulating the size of GNPs, and this property cannot be observed on bulk gold and GNPs with a diameter smaller than 2 nm. The SPR peak is not only sensitive to size and the shape, but also many factors such as protective ligand, refractive index of solvent, and temperature. The interparticle distance particularly shows great influence on SPR. Therefore, the red-shifting and the broadening of the peak are observed when GNPs are aggregated due to analyte binding. The color change of aggregated GNPs from red to blue is the principle of colorimetric sensing. A number of recent researches and reviews provide a detailed discussion on the factors that influence the SPR of GNPs.[27,28,29] The emitting of the SPR can be used for measuring electrical properties. When metal nanoparticles form 1D, 2D or 3D structures, the combining of surface plasmons from the neighbor nanoparticles results in new optical properties which depend on the extent of assembly. According to the work by Creighton *et al.* different metal ions have different SPR band positions in the UV/Vis spectrophotometer. Surface plasmons are surface electromagnetic waves that propagating a direction parallel to the metal/dielectric (or metal/vacuum) interface. Since the wave is on the boundary of the metal and the external medium (air or water for example), these oscillations are very sensitive to any change of this boundary, such as the adsorption of molecules to the metal surface. The simplest way to approach the problem is to treat each material as a homogeneous continuum, described by a frequency-dependent relative permittivity between the external medium and the surface.

This quantity, hereafter referred to as the materials' "dielectric constant (ε)," is complex-valued. The real part of the dielectric constant of the metal must be negative and its magnitude must be greater than that of the dielectric. This condition is met in the IRvisible wavelength region for air/metal and water/metal interfaces (where the real dielectric constant of a metal is negative and that of air or water is positive).

SPR is a prominent spectroscopic feature of noble metal nanoparticles (NPs), which gives rise to a sharp and intense absorption band in the visible range. The physical origin of the absorption is a collective resonant oscillation of the free electrons of the conduction band of the metal. For a spherical nanoparticle that is much smaller than the wavelength of the incident light its response to the oscillating electric field can be described by the so-called dipole approximation of Mie theory.

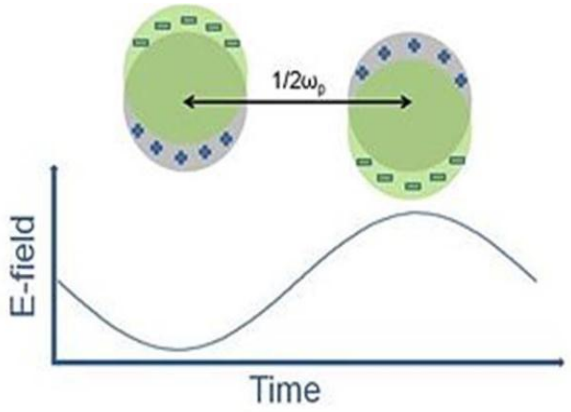

Fig. 6 - Schematic of surface plasmon oscillations induced by an oscillating electric field in a metal.

sphere. The displacement of the conduction electrons (green color) relative to the nuclei (gray color) is shown. The frequency of the surface plasmon resonance is denoted ωp and shown in fig.6.

Mie Theory

Surface Plasmon Resonance

The wavelength-dependent extinction cross section of a single particle, *Cext(λ)*, which defines the energy losses in the direction of propagation of the incident light due to both scattering and absorption by the particle, is described in terms of the dielectric function of the metal, $\varepsilon(\lambda) = \varepsilon'(\lambda) + i\varepsilon(\lambda)$, and the dielectric.

constant of the medium, εm. For gold nanoparticles smaller than 60 nm the scattering cross section is negligible when compared to the absorption cross section.[31] where λ is wavelength of the incident light and R is the particle radius.

Localized surface plasmon polaritons (LSPRs) are collective electron charge oscillations in metallic nanoparticles that are excited by light. They exhibit enhanced near-field amplitude at the resonance wavelength. This field is highly localized at the nanoparticle and decays rapidly away from the nanoparticle/ dieletric interface into the dielectric background, though far-field scattering by the particle is also enhanced by the resonance shown in fig.7. Light intensity enhancement is a very important aspect of LSPRs and localization means the LSPR has very high spatial resolution (subwavelength), limited only by the size of nanoparticles. Because of the enhanced field amplitude, effects that depend on the

amplitude such as magneto-optical effect are also enhanced by LSPRs.

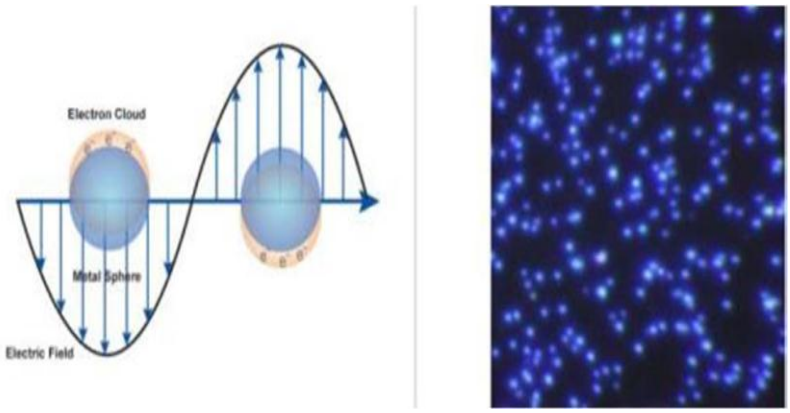

Fig.7 - Localized surface plasmon polaritons

2)Fluorescence properties

Apart from absorption and scattering properties, GNPs also display fluorescence properties which could be utilized in sensor fabrication. Photoluminescence can be generated from GNPs under certain conditions, such as laser.[32] and ultraviolet (UV) excitation.[33] GNPs can enhance or quench the fluorescence of fluorophore depending on the distance between the fluorophore and the particle.[34] Based on radiating plasmon model, Lakowicz suggested the absorption and scattering are related to the enhancing or quenching the fluorescence. As incident energy is dissipated by absorption and far-field radiation is created by scattering, larger GNPs are more likely to enhance fluorescence. Smaller GNPs are more likely to quench fluorescence because the scattering component is dominant over absorption.[35] Fluorescence is enhanced because the far-field radiation from the fluorophore is reflected back on itself. Hence, fluorescence can be quenched by fluorescence resonance energy transfer (FRET)[36] photoinduced electron transfer (PET).[37] or nanosurface energy transfer (NSET) pathaway .[38]

3)Enhancement of Raman scattering

GNPs can enhance Raman scattering, which leads to the development of SERS based sensing. Raman scattering is normally weak. The electromagnetic contribution to SERS is induced by the intense optical frequency field which originates from the plasmon resonance of GNPs, which can be used for single molecule detection.[39] The enhancement of Raman scattering depends on various factors including particle size, shape and aggregation. There are now two mechanisms to illustrate how SERS is enhanced, one is long-range electromagnetic effect and the other is short range chemical effect. The electromagnetic

contribution to SERS is induced by the intense optical frequency field which originates from the plasmon resonances of GNPs, while the chemical mechanism is induced by the electron transfer between molecules and the surface. From electromagnetic, the enhancement factor is about 1011, which can only be obtained at the interstitial sites between two particles or at outside sharp surface protrusions. As for single molecular detection, electromagnetic enhancement is not strongly enough, there should be chemical or other related enhancement to accomplish single molecular detection.[40]

Functionalization Of Gold Nanoparticals

1) PEGylation

PEGylation is one of the most commonly used functionalization methods for GNPs. GNPs are coated with a layer of PEG alone or in conjunction with other molecules such as biotin, peptides or oligonucleotides, thereby helping the internalization of these GNPs to the target cells. Due to their ability to bind the cell membrane, these functionalized GNPs can serve as good drug-carriers. PEGylated GNPs functionalized with biomolecules such as lectin, lactose and biotin have been synthesized.[41-48] PEGylated GNPs are useful in cellular and intracellular targeting of biological materials.

Hetero-bifunctional PEGylated GNPs were synthesized in which GNPs were functionalized with thiol group on one end and coumarin, a fluorescent dye on the other. These fGNPs could make their way into the cells which could be tracked easily because of the attached dye.[49] The stability and functional integrity of PEGylated GNPs is of concern as it is affected by factors such as the molecular weight of PEG, the attached functional groups, the ligand and the size of the GNPs used. The efficacy of one of such group of PEGylated GNPs in the ablation of tumors was tested in mice using thioctic acid anchored PEGylated GNPs.[50] The internalization of these fGNPs was dependent on the size of the nanoparticles, molecular weight of the PEG and the ligands used for PEGylation. Also, the distribution of these GNPs into various cells was dependent on their physiochemical properties.

2. Peptide/Amino Acid Conjugation

Functionalization of nanoparticles with amino acids and peptides has been another effective way to enhance specificity and efficacy of nanoparticle based delivery systems. GNPs functionalized with amino acids such as lysine, polylysine and glycine bind DNA with higher efficiency for gene delivery without toxicity. Primary ammonium groups of these

amino acids contributed to a higher binding capacity to the cationic groups on DNA. Also lysine dendrons were found superior to polylysine for expression of the reporter β galatosidase gene.[51] Likewise, amine functionalized GNPs carrying siRNA-PEG conjugates against human prostate carcinoma cells were shown to be effective in the inhibition of specific cancer genes . Carboxylated GNPs synthesized using glutamic acid were found better in synthesizing fGNPs with proteins as the carboxyl group of amino acid facilitates attachment of proteins through their amine groups. However, conformational changes were observed in the protein after attachment to the GNPs. GNPs functionalized with peptides have also been used as effective cell-targeting agents. The peptide CALNN and its derivative CALNNR8 were used to functionalize GNPs for targeting intracellular components.[52] Distribution of these fGNPs was dependent on the concentration of the peptide as well as on the size of the GNPs. GNPs (30 nm size) were able to cross cell membrane efficiently by endocytosis and micropinocytosis and showed higher affinity for DNA, RNA and endoplasmic reticulum in the cell. When in mixture both CALNN and its derivative CALNNR8 could make their way to the nucleus whereas the CALNNR8 was mostly trapped into the endoplasmic reticulum due to the higher affinity of the ER for arginine rich signal peptides. Thus, with constant nanoparticle-diameter and increasing peptide density the cellular targets shift from nucleus to endoplasmic reticulum, whereas when the density of the peptide was kept constant against the diameter of the nanoparticles endocytosis was reduced. The cell viability could be attributed to the extent of fGNP internalization. Similarly, a sensor for the detection of the interaction between β-amyloid peptide with metallic ions Zn2+ and Ca2+ was designed using GNPs functionalized with β-amyloid peptide-CALNNGK (biotin) G, using standard biotin-streptavidin chemistry.[53] Time dependent study of the interaction between the fGNP was used to suggest the levels of expression of β-amyloid peptide related genes in a simple colorimetric based assay using the optical changes occurring in the absorption spectra of the fGNPs before and after interaction with the peptides. Peptide functionalized GNPs are also known to activate macrophages, holding promise to be used as adjuvants for vaccine delivery. This is possible due to their ability to bind different biomolecules and expose smaller molecules to the immune system, which are otherwise unrecognizable by the macrophages.[54]The GNPs functionalized with an amyloid growth inhibitory peptide (AGIP) associated with Alzheimer‘s disease were found useful for intracellular drug delivery. They can selectively target the β-amyloid fibers and sweet arrow peptide (SAP) which could be recognized by the bone marrow derived

macrophages. The onset of pro-inflammatory immune response was found to be dependent on the sequence and length of the peptides. However, the macrophages were unable to recognize either of AGIP or SAP alone. These fGNPs were recognized by the macrophages due to TLR-4, a pattern recognition receptor. These fGNPs further activated the pro-inflammatory cytokines TNF-α, IL-1β and IL-6; thus, stopping macrophage proliferation. These fGNPs were then internalized by the macrophages and processed. GNPs can therefore be conjugated to adjuvants, cofactors or adaptor proteins for an effective immune response.

Cellular and subcellular targeting of fGNPs depends on the peptide used for conjugation and the type of cells in question.[55] PEGylated GNPs (30 nm) functionalized with Arg-Gly-Asp

(RGD) peptide and a nuclear localization signal peptide lysine-lysine-lysine-arginine-lysine was found to target specifically the nucleus of cancer cells.[56] Likewise, GNPs functionalized with the peptide conantokin-G were internalized by HER293 cells through selective binding to N-methyl-D-aspartate receptors.[57] In another study, GNPs functionalized with protein transduction domains (PTDs) from HIV Tat protein were used to follow their intracellular path. PTDs are peptides that can translocate to cell and nuclear membranes in a temperature and receptor independent manner. fGNPs were shown to make their way either into the nucleus (if nuclear localization signal peptide is used) or to the cytoplasm of the target cells through an endosomal path. Peptide sequence thus regulates the entry of the conjugated GNPs. The HIV Tat peptide conjugated GNPs could not enter the HepG2 cells whereas the GNPs with adenoviral nuclear localization signal and integrin binding domain could enter the nucleus.

Peptide-conjugated GNPs are also being used to devise a protein kinase assay using secondary-ion mass spectrometric imaging. This method uses the change in the mass of the peptide substrate after kinase action.[58] and is much simpler as opposed to traditional methods using radioactive or fluorescent labels. Thus the peptide conjugated nanoparticles hold promise to be used for bioimaging, diagnosis and therapeutic applications. GNPs are also being functionalized using both peptides and oligonucleotides for perinuclear localization for various functions such as cell imaging, target- specific internalization, etc.[59]

Similarly, bioconjugated gold nanorods have been employed as probes for imaging. A

mouse monoclonal antibody specific to human epidermal growth factor receptor 2 (HER2), over-expressed in SKBR3 breast carcinoma cells, was conjugated to either GNPs or nanorods which can be used for biomedical imaging of the carcinoma cells.[60] GNPs functionalized with Bombesin peptides, can be used for imaging of cancer cells as Bombesin has high affinity to gastrin releasing peptides that are over-expressed in cancer cells.[61] GNPs coated with polyelectrolytes were found to restructure the 3D constructs made of collagen and cardiac fibroblasts, reduced contraction and altered the expression of β actin, α-smooth muscle actin and collagen type I, suggesting the potential applications for anti-fibrotic therapies.[62] Likewise, GNPs were also found to enhance cross linking of collagen fibrils as well as sites to deliver signaling compounds that direct self assembly and reduce inflammation.[63]

3. Oligonucleotide Functionalized Nanoparticles

Several research groups have devised methods to functionalize gold and other nanoparticles using oligonucleotides either alone or with some modifications. DNA conjugated nanostructures can be synthesized in a controlled manner, either by attaching a specific number of single stranded DNA molecules through thiol caps or by saturating the surface of the GNPs by single stranded DNA molecules.[64] Kinetic and thermodynamic studies on DNA hybridized to GNPs have shown that ssDNA first adheres to the GNPs and then slowly diffuses on its surface.[65]

Secondary structure of a DNA hairpin inhibits interaction between GNPs and DNA thereby increasing the stability of adhered DNA. Aptamer-GNP conjugation has been exploited to target prostate cancer cells.[66] This was achieved by attaching GNPs with an oligonucleotide complementary to the sequence of the anti-PSMA (prostate specific membrane antigen), thus facilitating the attachment of PSMA-GNPs to anti-PSMA antibody. These results show a promising role of such fGNPs in the detection and imaging of cancerous cells. In another novel study, DNA functionalized GNPs were employed to design a chip based DNA bio bar code sensor to detect target DNA sequences.[67] Here, the bio bar code amplification of the target DNA is assessed using a complementary DNA attached to GNPs and subsequent detection of the amplified DNA instead of the original target DNA.

4. Other Common Functionalization Methods

Apart from DNA and proteins, various other molecules have also been used effectively

for functionalization of GNPs for various applications. GNPs functionalized using goat anti-human IgG were used to formulate a bioassay to detect human IgG in serum samples.[68] GNPs modified with carboxyl and alcoholic groups were functionalized using antibodies for the detection of E. coli O157:H7.[69] GNPs have also been employed in the immobilization of enzymes to offer an inert and biocompatible system.[70] The enzyme glucose oxidase has been immobilized on chitosan-GNPs for the quantitative detection of glucose. This method helps the enzyme retain its activity at higher temperature and extreme conditions. GNPs were used to detect 5-fluorouracil (an anti-leukemic drug) due to the quenching effects of GNPs against the fluorescence of 5-fluorouracil. Also, this conjugate has been shown to have antifungal and antibacterial activity.[71]

APLICATIONS OF GOLD NANOPARTICALS 1)Gold nanoparticles and bio sensors

The primary principle involved in the design of a biosensor based on gold nanoparticles is that the gold nanoparticles are functionalized with a thiolated biomolecule which upon recognizing the perfecting biomolecule brings about change in the optical absorption of gold nanoparticles.[72]

2)**Antimicrobials**

Although silver has a long history of being used as an antimicrobial, in recent years gold has also become a good rival for silver. For example gold nanoparticles can fight against ‗E. coli' bacteria.[73-77]

3)**Gold Nanoparticles in Cancer Diagnosis and Therapy**

The main problem with many currently available cancer treatments is that they cannot be precisely targeted. As it is very hard to get an effective drug, such as paclitaxel, directly to the tumour, large doses are needed in the hope that enough of the drug will reach the diseased cells where it is needed. Recently gold nanoparticles have found a role to deliver drug easily. Cancer therapy has various routes such as chemotherapy, photo-thermal therapy and radiotherapy. Gold nanoparticles have been investigated for potential candidates to aid in photo-thermal therapy and radiotherapy. It is important to understand the difference between normal and cancerous tissue to efficiently improve hybrid nanoparticles in cancer diagnosis and treatment. Optical and electronic properties of gold nanoparticles can be used to improve the contrast in molecular imaging for the detection of cancer at early levels.[73-77]

4)**Needle-free drug delivery**

Gold-based technologies are also provide a unique needle-free delivery system, a technique that used gold nanoparticles and allowed vaccines to be delivered through the skin making use of the fact that small particles can pass through gaps between cells while large ones cannot.[73-77]

5)**Gold nanoparticles against HIV/AIDS**

One of the most efficient usages of gold nano particles in recent years is detecting and fighting against HIV.

6) **fGNPs for Targeted Delivery From Nanomaterials**

fGNPs have been used to target drugs and biomolecules to specific cell types and organelles such as the nucleus or mitochondria. GNPs functionalized with PEG and 3-mercaptopropionic acid was shown to penetrate the nucleus of HeLa cells without causing severe cytotoxicity and hence can be used as a nuclear drug delivery carrier.[78] Similarly, GNPs encapsulated by liposomes have been studied for their cellular targeting and uptake capacity while carrying drugs or other cargos.[79] Intracellular uptake of GNPs as small as 1.4 nm has been shown to enhance internalization by 1000-fold. Such nanoparticles harbor significant potential to be used as gene delivery vehicles, drug-carriers and carriers for other biomolecules.

6.1 Gene Delivery

A PEGylated GNP based delivery system was evaluated for its transfection efficiency using a plasmid DNA mediated through electroporation.[80] Gene expression was enhanced to about 100-fold with DNA-PEGylated GNPs compared to naked DNA after intravenous injection. The transgenes were stable in circulation and the DNA was released and passed through the cellular membranes. Likewise, in another study, plasmid DNA encoding murine interleukin-2 (pVAXmIL-2) was mixed with positively charged colloidal GNPs increasing the transgene expression significantly with reduced toxicit.[81] yGNPs functionalized with amino acid have also been used as efficient gene delivery vectors without causing cytotoxicity.[82] Similarly, efficient delivery of siRNA to the host cells was achieved using PEGylated gold-poly (β-amino ester) nanoparticles, wherein the poly (β-amino ester) was the key molecule in the intracellular targeting of the DNA.[83]

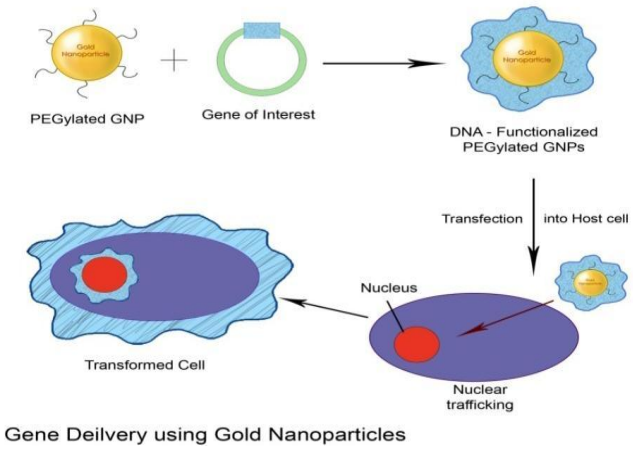

Figure 8. PEGylated gold nanoparticles (GNPs) for gene delivery.

6.2. Drug Delivery

GNPs are suitable for the delivery of the drugs to cellular destinations due to their ease of synthesis, functionalization and biocompatibility. GNPs functionalized with targeted specific biomolecules can effectively destroy cancer cells or bacteria.[84] Large surface to volume ratio of GNPs offer a large number of drug molecules being carried by the GNPs.[85] GNPs have been used for the co administration of protein drugs due to their ability to cross cellular membranes.[86] possibly due to the interaction of GNPs with cell surface lipids.

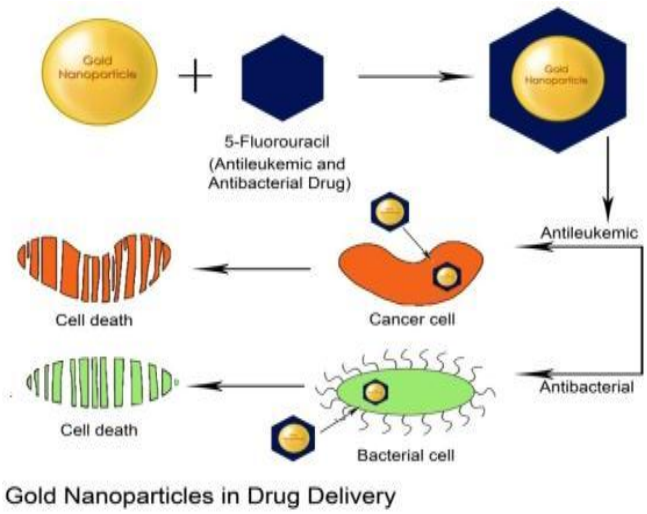

Figure 9. Functionalized GNPs (fGNPs) for drug delivery: Targeting specific cells with highe loading efficiency, targeted delivery and efficient release of drugs.

6.3 Detection

GNPs are also being used for detection of various biological molecules including proteins, enzymes, DNA, antigens and antibodies, etc.

6.3.1. Detection of Biological Molecules

GNPs have been used for the detection of proteins, based on their characteristic surface plasmons .[87] For this, GNPs have been functionalized using bifunctional molecules which were conjugated on one side to the GNPs through their thiol group and on the other side to the electron-rich aromatic side chains of proteins through a diazonium moiety. The model was tested using thrombin as the protein. The vibrations of the diazo-bond formed between the bifunctional molecule and the target protein tends to enhance due to the conjugation of GNPs constituting the Raman marker. After the functionalized GNPs interact with antithrombin as a sensitive recognition element, immobilized on a substrate, thrombin can be detected through surface enhance Raman Spectroscopy. Selectively immobilized oligonucleotide modified GNPs have been used to develop a chip based array through electro-deposition on screen printed GNPs.[88] The method allows a multimodular detection based on the use of multiple oligonucleotides and also excludes the non-specific interactions.

6.3.2. Detection of Microorganisms

Detection of microorganisms can be achieved by several biochemical, microbiological and molecular methods. Recent advances in the field of nanotechnology have made it possible to detect microorganisms by using nanoparticles functionalized with oligonucleotides complementary to the gene tags of the microorganisms. GNPs were used to detect *Salmonella enteritidis* and *Listeria monocytogenes*, where GNPs deposited within the flagella and in the biofilm network.[89] Similarly, GNP–Poly(para-phenyleneethynylene) could efficiently identify both Gram positive and negative bacteria based on the differential response by each bacteria.[90]

A gold nanoparticle based chemiluminescence assay was designed for the detection of *Staphylococcus* enterotoxin B (SEB).[91] Antibody against SEB was bioconjugated to the GNPs through physical adsorption followed by adsorption of the complex on a polycarbonate surface. The SEB was then detected based on sandwich type ELISA and chemiluminescence signal arising from the secondary antibody.

6.4 Other Applications of GNPs

6.4.1. Enzyme Immobilization

GNPs have been used as immobilization matrices for enzymes. GNPs with a carboxyl terminated thiol group were functionalized through the attachment of the enzyme glucose oxidase.[92] The immobilized enzyme was found to be more stable thermally as compared to free enzyme. Such immobilized systems can be very useful in several biotechnological processes in food and environment fields.

6.4.2. immunoassay

Various immunoassays have been designed using GNPs functionalized with antibodies such as human IgG and antibodies against pathogenic bacteria Immunosensors have been recently developed using single chain fragment variable recom.[93,94]binant antibodies (scFv) instead of traditional mono or polyclonal antibodies.

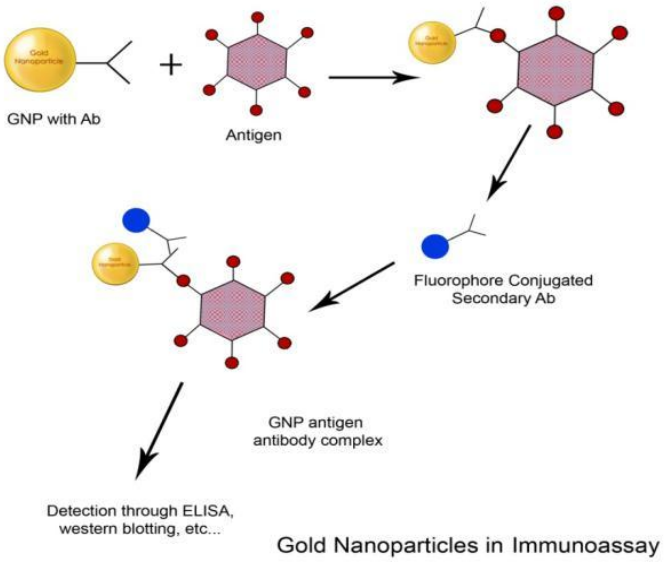

Figure 10. Antibody functionalized GNPs for use in immunoassay.

6.4.3. SNP Detection

Single nucleotide polymorphisms (SNPs) have by far been the most appropriate method for the detection of point mutations or polymorphisms in various genes, which can be easily, detected using complementary single stranded DNA molecules.

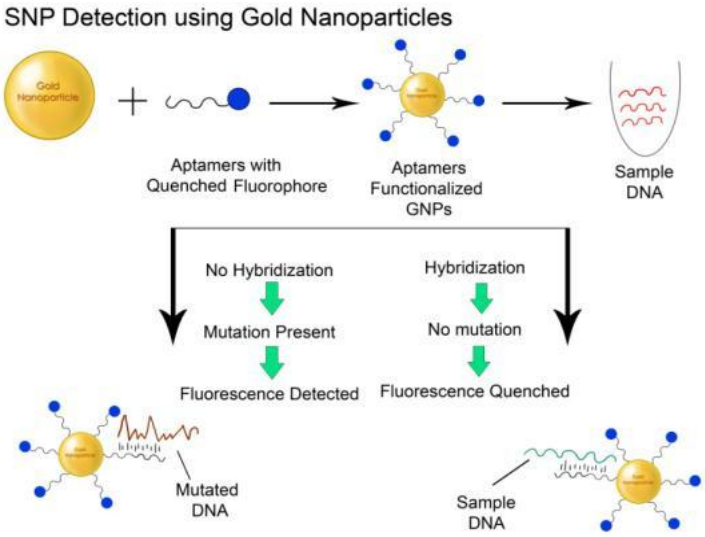

Figure 11. GNPs functionalized with ssDNA for Single nucleotide polymorphism (SNP) detection.

SNPs are often associated with disease detection including diabetes mellitus, β-thalassemia, etc. GNPs functionalized with single-strand-specific-nucleases have been used to detect SNPs.[95]

6.4.5. In Microscopy Functionalized GNPs have found their usage in electron microscopy.[96]

The problem of limited resolution of Cryo-electron microscopy single particle analysis due to poor alignment of samples can be obviated by using two dimensionally arranged protein arrays labeled on GNPs through genetic tag sites on proteins. GNPs functionalized with nickel-nitrilotriacetic acid were used and *Mycobacterium tuberculosis* 20S proteasomes with 6x-histidine tags were assembled into 2D arrays and were used for three-dimensional reconstruction of biological macromolecules.[97]

CONCLUSION

Gold nanoparticles are quite advantageous over traditional drug delivery systems due to their low toxicity, tasteless nature, and ability to deliver biomolecules like DNA‘s very efficiently into desired targets within few minutes. Thus coated gold nanoparticles form an integral and emerging form of novel drug delivery systems Targeted delivery and programmed release of therapeutic drugs to the specific site is achieved by using gold nanoparticles because they can bear high drug load and release it to the specific site

through various administration routes and can interact with cancerous cell. Side effects of conventional drugs have been minimized by conjugation with gold nanoparticles and they increase the quality life of patients.

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