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CHAPTER 1

INTRODUCTION

This chapter is an introduction to the thesis and given a brief overview of metal nanoparticles, especially gold nanoparticles. The different methods used for synthesis of gold nanoparticles, its properties, surface modification and bioconjugation have been discussed. A part of the chapter discusses the various applications of gold nanoparticles, particularly for diagnostic and therapeutic applications in cancer therapy. Finally some previous patents and reports on the synthesis of gold nanoparticles, their biocompatibility, and application in delivery of drugs have been discussed. The chapter finally illustrates the status of nanoparticulate systems in the international market.

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INTRODUCTION

1.1 Nanotechnology

Nanotechnology [1] has become a popular term representing major efforts in science and technology of today. It is unique in the sense that it represents not just one specific area, but a vast variety of disciplines ranging from basic material science to personal care.[2] Nanotechnology, more appropriately called nanoscience, refers to research and development at the nanoscale level. A nanometer (nm) is one thousand millionth of a meter. About four gold atoms lined up side-by-side make up a nanometer. For comparison, a troy ounce of gold contains about 95,090,000,000,000,000,000,000 atoms of gold, a number so big that it's beyond human conception. A single human hair is about 80,000 nm wide; a red blood cell is approximately 7,000 nm wide. A bacterium cell is 1,000 times smaller than a dot, we need to go 1,000 times smaller than even the cell, so small that the nano thing is to a single bacterium cell as the dot is to us.[3]

Researchers are interested in the nanoscale because it is at this scale that the properties of materials can be very different from those at a larger scale. In some way, nanoscience and nanotechnologies are not new. Chemists have been making polymers, which are large molecules made up of nanoscale subunits. For many decades, nanotechnology has been used to create tiny features on computer chips. However, advances in the tools that now allow atoms and molecules to be examined and probed with great precision have enabled the expansion and development of nanoscience and nanotechnologies.

One of the important areas of nanotechnology is "nanomedicine" which, according to the National Institute of Health (NIH), refers to highly specific medical intervention at the molecular scale for diagnosing, treating and preventing disease, using molecular tools and molecular knowledge of the human body.[4] Many of the current "nano" drug delivery systems, however, are remnants of conventional drug delivery systems that happen to be in the nanometer range such as liposomes, dendrimers, emulsions, nanocrystals and other polymeric nanoparticles (Figure 1.1).[5,6] These conventional liposomes, polymeric micelles and nanoparticles are now called "nanovehicles," and this, strictly speaking, is correct only in the size scale. Use of these nanoparticles has already produced exciting results. Liposomes are being used as pharmaceutical carrier for various drug delivery applications including tumors. [7]

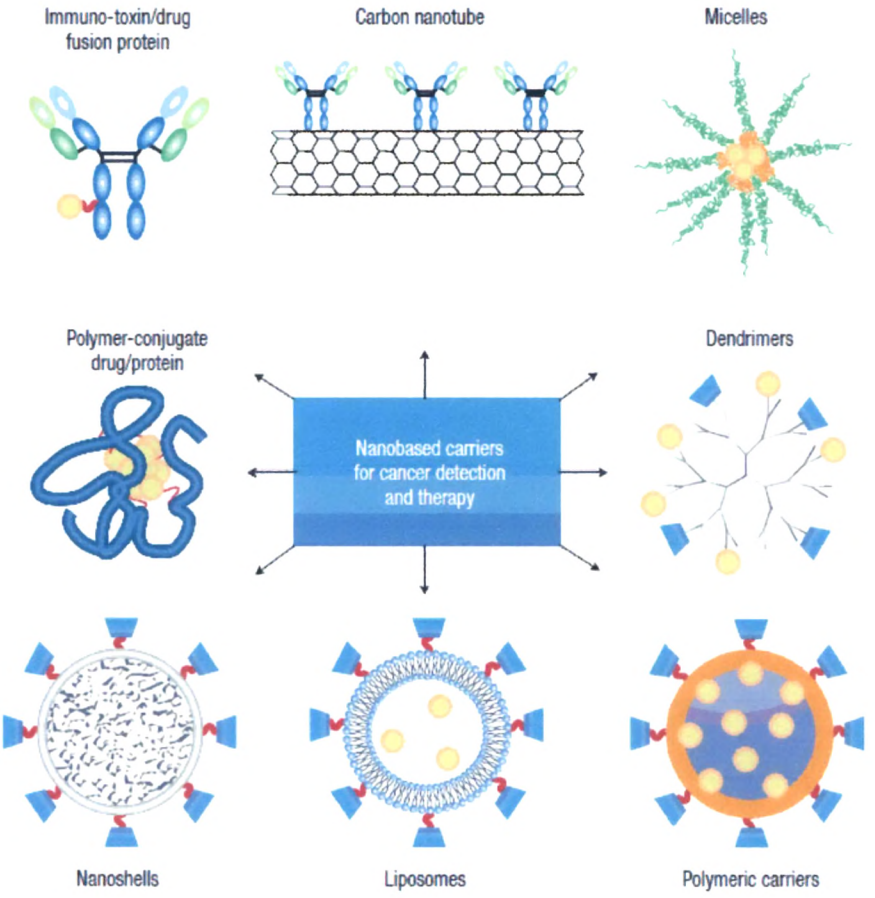


Figure 1.1: Nanoparticulate based drug delivery system. [8]

1.2 Metal nanoparticles

Colloidal metals originally called 'sols' - first generated interest because of their intense colors, which enabled them to be used as pigments in glass or ceramics. The most extensively studied metal as a colloid is "gold" because of its well known optical properties. It infact is very easy to find examples of the use of metal nanoparticles (gold and silver) as decorative pigments since the time of the Romans, such as those contained in the glass of the famous Lycurgus Cup (4th century AD). The cup can still be seen at the British museum [9] and possesses the unique feature of changing color depending upon the light in which it is viewed. It appears green when viewed in reflected light, but looks red when a light is shone from inside and is transmitted through the glass. Analysis of the glass reveals that it contains a very small amount of tiny (~70 nm) colloids of gold and silver. It is the presence of these nanocolloids that gives the Lycurgus cup its special color display (Figure 1.2).

Figure 1.2: Lycurgus cup at the British museum [9]

In 1857, Michael FaradayT an English chemist and physicist reported the preparation of deep red colored solution of gold colloids by the reduction of aqueous chloroaurate ions ($AuCl_4$) using phosphorus in CS_2.[10] He investigated the optical properties of thin films prepared from dried colloidal solutions and observed reversible color changes of the films upon mechanical compression (from bluish- purple to green upon pressurizing) resembling the color of the wine red solutions of the particles.[10] He mentioned in his paper that the particles in their finest state often remain unchanged for many months and have all the appearance of solutions. The solution contained not dissolved, but only diffused gold.[11] This probably was the first rationalized report on the purposeful synthesis of colloidal gold nanoparticles, Soon thereafter, the term "colloid" was coined by Thomas Graham (1861) for suspended particles in liquid medium.[12] During recent developments in this field, the term "colloid" has been replaced by "nanoparticles" to describe particles with size range from 1 to 100 nm.[13] The famous quote of Louis Pasteur "The role of infinitely small is infinitely large" well suits the current trends of nanotechnology research. In the 19th century, a great deal of theoretical and experimental research was conducted on many colloidal metals. In the 21st century, the number of potential applications of these colloidal particles is growing rapidly because of the unique electronic structure of the nanosized metal particles and their extremely large surface areas. [14]

1.3 Properties of metal nanoparticles

At the nanoscale, as the size of the material approaches to the nanometer regime, materials exhibit unique optical, electronic and magnetic properties which are unusual, in the sense that they are not observed in their bulk counterpart. This makes nanostructures attractive and can be explored for a wide range of applications. The combination of these unique properties with the appropriate size scale has motivated the introduction of nanostructures into biology. [15]

1.3.1 Plasmon resonance in metal nanoparticles

The interesting optical attributes of metal nanoparticles, as reflected by their bright intense colors, are due to their unique interaction with light. In the presence of oscillating electromagnetic field of the light, the free electrons of the metal nanoparticle undergo a collective coherent osciliation with respect to the positive metallic lattice. This oscillation is known as the surface plasmon resonance (SPR) [16] and has been explained by Mie in 1908, based on the Maxwell's equation of scattering.[17] Gold, silver and copper nanoparticles possess plasmon resonances in the visible spectrum, which give rise to very intense colors. For application point of view, gold and silver nanoparticles are the metals of choice because of their much higher stability as compared to other metal nanoparticles. Additionally, the optical properties of nanoparticles depend significantly on their size and shape as well as on the dielectric constant of the surrounding medium. For example, in spherical gold nanoparticles, the plasmon absorption red shifts with increasing diameter of the nanoparticle.[18] Likewise, quantum dots (semiconductor nanoparticles such as CdSe and CdTe) exhibit red shift in their band gap (emission) as their size increases.[19,20] Silver nanoparticles of spherical, pentagonal and triangular shape appear blue, green and red respectively under a dark field microscope, suggesting strong correlation between optical property and shape of the nanoparticles.[21]

Applications based on optical properties in the visible region have been receiving considerable scientific interest due to its promising technological development. Since the optical properties of metal nanoparticles are sensitive to surface modifications and environment around it, cross linking of antibody/ antigen/thiolated DNA functionalized silver/gold nanoparticles exhibit dramatic change in the optical properties due to overlap of dipole resonance from neighbouring nanoparticles. This forms the basis for colorimetric technique for following molecular recognition events. On these guidelines, DNA tagged gold nanoparticles were studied extensively for DNA base pair sequence recognition and genetic disorder, which occurs primarily due to base imperfections in DNA molecule.[22]

1.3.2 Scattering property of metal nanoparticles

Gold nanoparticles have the ability to resonantly scatter visible and near-infrared light upon the excitation of their surface plasmon oscillation. The scattering light intensity is extremely sensitive to the size and aggregation state of the particles.[23] Nanoparticles with 58 nm in diameter scatter green light while 78 nm in diameter scatter yellow light, while gold nanorods scatter red light under illumination of a beam of white light.[24] Preliminary studies have reported their use as contrast agents for biomedical imaging using confocal scanning optical microscopy. Sokolov et al., described the scattering of anti-EGFR/Au nanoparticles for cervical cancer when stimulated with a laser at single wavelength,[25] Gold nanoparticles have several advantages for cellular imaging compared to other agents. They scatter light intensely and are much brighter than chemical fluorophores. They do not photo bleach and they can be easily detected in as low as 10"16 M concentration.[26]

1.3.3 Magnetic properties

Ferromagnetic particles form single domains with large magnetic moments at nanoscale. Metal nanoparticles change magnetic properties drastically. Under thermodynamic equilibrium, magnetization behavior of these nanoparticles is similar to that of atomic magnetization but with large magnetic moment. Below a certain size, ferromagnetic particles become super-paramagnetic in nature.[27] These particles do not show hysteresis in magnetization, since there is only one domain per particle.

1.3.4 Melting point

Metal nanoparticles below 100 nm of size have low melting point than in its bulk form. Number of surface atoms increases with decrease in the size of nanoparticles. Since it decreases the co-ordination number of atoms, atoms on the surface can be easily rearranged than those atoms present inside the nanoparticles, thus melting process starts at lower temperature leading to decrease in melting point.[28]

1.3.5 Mechanical properties

Mechanical strength of the material depends on several parameters such as impurities, dislocations etc. More defects in materials lead to less mechanical strength. Thus, due to small cross section and less number of imperfections, nano materials such as nanowires and nanorods show enhanced mechanical strength. Since imperfections are thermodynamically more energetic, small size of nanomaterials eliminates imperfections in the crystal and acquires better mechanical strength.[29]

1.4 Methods for the synthesis of metal nanoparticles

After realization of application and importance of nanomaterials in various regimes and in order to explore novel physical properties and phenomena, different techniques have been developed to generate metal nanoparticles. Generally there are two strategies to obtain materials on the nanoscale. Bottom up method where the atoms (produced from reduction of ions) are assembled to generate nanostructures, or top down method where material is removed from the bulk material, leaving only the desired nanostructures. The important criteria for the synthesis of metal nanoparticles are control over size, shape, surface functionalities and various properties of nanoparticles. However choice of method is mainly selected depending upon the desired application. Simple reduction of metal salts by reducing agents in a controlled fashion generally produces spherical nanoparticles, because spheres are the lowest energy shape. Metal nanoparticles, especially gold, silver and copper, have been extensively investigated over the past decade due to their unique electronic, optical and catalytic properties. These properties are neither those of bulk metal nor those of molecular compounds. The most well known and frequently used method to synthesize spherical gold nanoparticles dispersed in water is the reduction of chloroaurate ions ($AuCl_4$) in a boiling sodium citrate solution, Turkevich method (1951).[30] The formation of uniform gold nanoparticles is revealed by a deep wine red color observed after ~10 minutes. The average particle diameter can be tuned over quite a wide range (~10-100 nm) by varying the concentration ratio between the gold salt and sodium citrate. However, for particles larger than 30 nm, deviation from a spherical shape is observed, as well as a larger polydispersity. The same procedure can be used to reduce silver salt, but particle size control is very limited. Another most impressive procedure that has become extremely popular for gold nanoparticles synthesis is the two-phase reduction method developed by Brust and co-workers in the laboratory of Professor David Schiffrin (1994)[31] for smaller (~2 nm) gold nanoparticles, in which an aqueous solution of gold ions is transferred to an organic phase, mediated by a phase transfer agent, followed by reduction with borohydride. Depending on the ratio of the gold salt and capping agent, the particle size can be tuned to between ~1 nm and ~10 nm. Several refinements of the preparative procedure have been developed, which includes the development of analogous methods for the preparation of silver nanoparticles.[32-34] Slight changes in the spherical geometry strongly affect the optical properties of metal nanoparticles. For this reason, various methods for the synthesis of anisometric nanoparticles in solution (nanorods, nanowires, nanodisks, nanoprisms, etc.) are continuously being reported in literature particular for gold[34,35] and silver.[36] Examples of metal nanoparticles with various shapes and sizes, together with dispersions of varying colors arising from different effects, are shown in figure 1.3. Even though numerous synthesis methods have been employed to fabricate gold nanoparticles, lack of sufficient stability in water under strong electrolyte conditions and pH changes has somehow impeded the development of real world applications of nanomaterials. To overcome this, use of stabilizers have been reported.

1.4.1 Polymer stabilized metal nanoparticles

The use of polymeric stabilizers for metal nanoparticles has inspired the studies of various synthetic routes to link polymers to metal particles and to investigate the properties as well as potential applications in biomedicine and biotechnology.[37] The use of polymers not only enhances long term stability and amphiphilicity of gold nanoparticles, but also the functionalization of gold nanoparticles to promote compatibility and processibility. Polymers provide stabilization for metal nanoparticles not only through the steric bulk of their framework, but also by binding weakly to the nanoparticles surface through heteroatom that play the role of legends. Though an early report dates back to 1718, where starch was reported to stabilize gold particles dispersed in water[39] much effort has recently been put to prepare polymer protected gold nanoparticles and also other metal particles, in particular, inspired from the two-phase reduction method developed by Brust and co-workers as mentioned above.[31] Gold nanoparticles have been stabilized by a variety of other biomolecules, like proteins, peptides and gums.[40, 41]

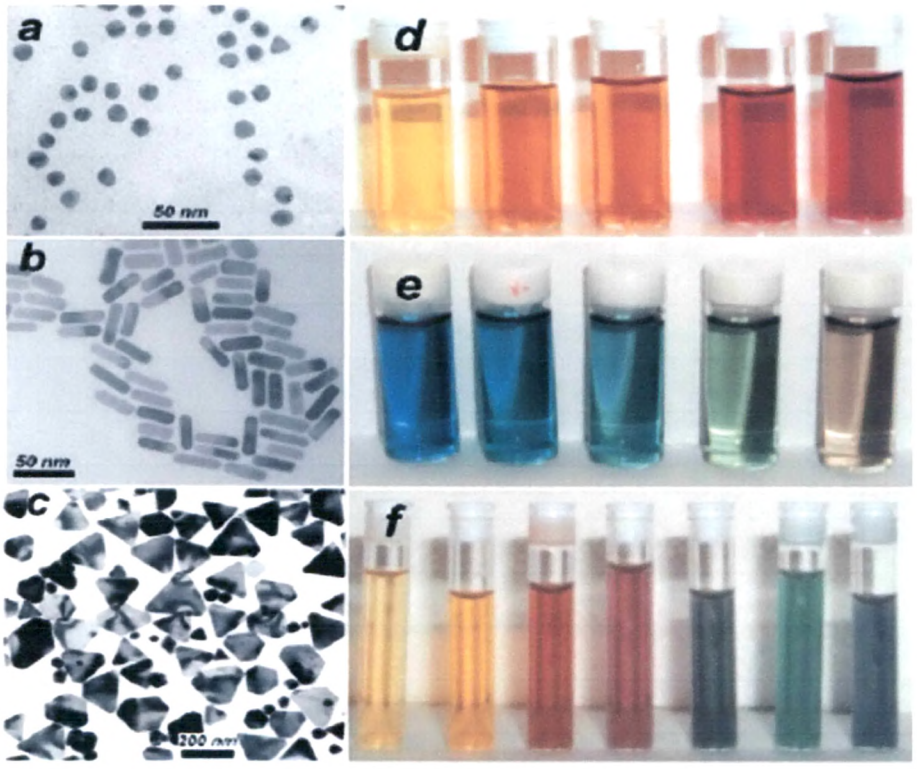


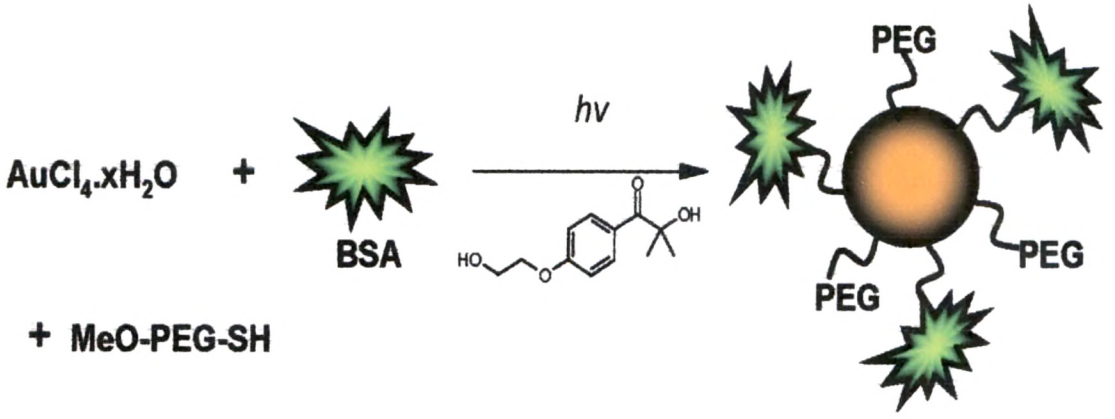
Figure 1.3 Left: Transmission electron micrographs of gold (a) nanospheres (b) nanorods and silver (c) nanoprisms (mostly truncated triangles) formed using citrate reduction, seeded growth, and dimethylformamide reduction, respectively. Right: Photographs of colloidal dispersions of gold/silver alloy nanoparticles with increasing gold concentration (d), gold nanorods of increasing aspect ratio (e) and silver nanoprisms with increasing lateral size (f).[38]

The polymer containing an appropriate functional group can serve both as reducing and stabilizing (capping) agent. A number of homopolymers and functionalized block copolymers are useful for the preparation of functional nanoparticles.[42] Ishii et al., successfully synthesized gold nanoparticles in an aqueous medium with biotinyl-poly(ethylene glycol)-ib-poly[2-(N,N-dimethylamino) ethylmethacrylate.[43] Sakai et al., performed the single step synthesis of gold nanoparticles in an air saturated aqueous solution of poly (ethyiene oxide-b-propylene oxide-b-ethylene oxide).[44] These triblock copolymers can effectively reduce tetrachloroaurate hydrate at room temperature and stabilize the metal particle. In general, water soluble polymers such as poly[2-(N,N-dimethylamino)ethyl methacrylate] (PDMA),[43] poly(2-vinylpyridine) (PVP),[45] polyvinyl alcohol) (PVA),[46] and poly (acrylic acid)[47] are well known for their ability to coordinate with metal particles.

1.5 Surface modification and bioconjugation of metal nanoparticles

1.5.1 Surface modification

Stabilizing molecules maintain a shell around the colloidal nanoparticles. One of the ends of the stabilizing molecules are either adsorbed or chemically linked to the gold surface, while the other end points towards the solution and provides colloidal stability. After synthesis of the nanoparticles, the stabilizer molecules can be replaced by other stabilizer molecules in a ligand exchange reaction. As thiol moieties bind with high affinity to gold surfaces, most frequently thiol-modified ligands are used which bind to the surface of the gold nanoparticles (which are by several groups also called "monolayer-protected clusters") by formation of gold- sulfur bonds.[48] Ligand exchange allows, for example, the transfer of gold nanoparticles from an aqueous to an organic phase (and vice versa) by exchanging hydrophilic surfactants with hydrophobic surfactants (and vice versa).[49] In this way, by choosing the surfactant molecules, it is possible to adjust the surface properties of the particles. For applications in aqueous solution, typically thiol-based surfactants with carboxylic groups at the other end pointing towards the solution are used. These molecules provide colloidal stability due to their negative charges; in addition they can also be used as anchor points for the further attachment of biological molecules. Often poly(ethylene glycol) (PEG) is used as a ligand as PEG reduces nonspecific adsorption of molecules to the particle surface and it provides colloidal stability. The PEG brushes on the surface of nanoparticles that repel each other for steric reasons.[50] The cytotoxicity results of PEG stabilized gold nanoparticles prove the biocompatibility of this molecule. Another approach is the use of mixed monolayer stabilized gold nanoparticles. Scheme 1.1 represent one step synthesis of PEG/BSA stabilized gold nanoparticles by the photochemical process. Mixed monolayer AuNPs are ideal for drug and gene delivery purposes.[51]



Scheme 1.1 ***Photochemical synthesis of mixed monolayer (BSA/PEG) stabilized gold nanoparticles.***[51]

1.5.2 Bioconjugation

Biological molecules can be attached to the metal nanoparticles in several ways. If the biological molecules have a functional group which can bind to the gold surface (like thiols or specific peptide sequences), the biological molecules can replace some of the original stabilizer molecules when they are added directly to the nanoparticle solution. In this way molecule like oligonucleotides, peptides or PEG can be readily linked to gold nanoparticles and subsequent sorting techniques even allow particles with an exactly defined number of attached molecules per particle to be obtained. [52] Alternatively, biological molecules can also be attached to the shell of stabilizer molecules around the gold nanoparticles by bioconjugate chemistry. The most common protocol is the linkage of amino groups on the biological molecules with carboxy groups at the free ends of stabilizer molecules.[53] With related strategies, almost all kinds of biological molecules can be attached to the nanoparticle surface. Though such protocols are relatively well established, bioconjugation of gold nanoparticles still is not trivial and the characterization of synthesized conjugates is necessary, in particular to rule out aggregation effects or unspecific binding during the conjugation reaction. In particular, in many conjugation protocols, the number of attached molecules per gold nanoparticle is only a rather rough estimate, as no standard method for determining the surface coverage of particles modified with molecules has yet been established. [54, 55]

The nanobiology toolkit has been greatly enhanced by noble metal nanostructures, which have proven to be highly versatile and tunable materials for a range of bioapplications including biophysical studies, biological sensing, imaging and medical diagnostics.

1.6 Gold nanoparticles in diagnosis

1.6.1. Biological imaging

Researchers have used various exogeneous agents to visualize key subcellular compartments. Conventional exogeneous imaging agents include lanthanide chelates and organic fluorophores. However, organic fluorophores are prone to photobleaching, low quantum yields and broad emission window. Lanthanide chelates, on the other hand, are prone to nonselective localization in extravascuiar space. The shortcomings of the conventional imaging agents have limited their applications as biomedical diagnostic tools and have stimulated interest in typical nanomaterials, such as magnetic nanoparticles,[56] quantum dots[57] and gold nanoparticles[58] as alternative contrasting agents. These nanomaterials are optimal diagnostic tools since they eliminate most of the vulnerabilities of the conventional imaging agents. Gold nanoparticles are unique exceptions because they are more tolerable and compatible with cellular environment.[59,60] Noble metal nanoparticles have strong surface plasmon resonance (SPR) due to which they scatter light very strongly at the SPR frequency, making them very promising for optical imaging of biological systems.[26] While scattering and absorption are competing processes, the relative contribution of scattering increases rapidly with increase in the nanostructure volume. The scattering from a 10 nm gold nanoparticle is negligible, but an 80 nm gold nanoparticle offers scattering 5 orders of magnitude larger than the typical emission from a dye. Such highly enhanced cross sections offer sensitive and highly contrast imaging[61] allowing use of the much simpler but powerful dark field microscopy. While most imaging techniques require sophisticated and expensive lasers, optical components, detectors and complex image processing, dark field imaging using gold nanoparticles requires a simple optical microscope equipped with a dark field condenser. The latter ensures that the excitation light is incident at high angles such that only light scattered by the sample is collected by the microscope. The gold nanoparticles are excited by a broad white light source, but only light frequencies corresponding to the SPR are strongly scattered. The nanoparticles are seen as bright spots with a color corresponding to the SPR frequency on a dark background. In fact, due to the high scattering cross section, individual nanoparticles can also be imaged.[62] The dark field microscopy technique can be utilized very effectively for the molecular specific imaging of biomolecules by integrating the gold nanoparticles with specific targeting molecules. El-Sayed et al., diagnosed cancer by imaging the cancer biomarker epidermal growth factor receptor (EGFR), present in significantly higher amounts on cancer cells.[61] Gold nanospheres conjugated to

anti-EGFR antibodies specifically target the cancer cells. The cancer cell surface defined by strong SPR scattering from gold nanoparticles bound specifically to the EGFR on the cancer cells. Thus, cancer cells could be easily identified from the healthy cells, in which case the gold nanoparticles were dispersed randomly due to nonspecific binding. Gold nanoparticles can also be conjugated to a range of proteins, antibodies and small molecules.[63] The ligands can be chosen depending on the disease and biomarkers to be targeted.

1.6.2 X-ray computed topography

X-ray computed tomography is another non-invasive diagnostic method that generates three dimensional images of different cells based on a series of two dimensional X-ray images compiled around a single rotating axis. Contrasting agents are often utilized to enhance the contrast between cells because of their affinity to absorb X-rays. These agents have various disadvantages like renal toxicity, vascular permeation and limited imaging interval due to rapid renal excretion. The limitations observed in current computer tomography contrasting agents can be overcome by the use of gold nanoparticles. Gold nanoparticles present several advantages over the current contrasting agents, such as higher X-ray absorption coefficients, versatility in surface modification and regulated control in the size and shape of the gold nanoparticles. Gold nanoparticles can be imaged with high signal-to-noise ratio with X-ray computer tomography. Kim et al., studied computer tomography of gold nanoparticles coated with PEG[64] to test their *in vivo* application as computer tomography contrast agents for angiography and hepatoma detection. X-ray absorption coefficient measurements *in vitro* revealed that the attenuation of PEG coated gold nanoparticles was 5.7 times higher than Ultravist (a contrasting agent) at equal concentration. The PEG coated gold nanoparticles had a longer blood circulation time, approximately 4 hours without apparent loss of contrast in a mice model. Also, a two-fold contrast enhancement was seen between the hepatoma and its surrounding healthy liver cells for up to 24 hours. These results showed the feasibility of gold nanoparticles as a computer tomography contrast agent. Functionalization of gold nanoparticles with biomolecules such as folate (avidly taken up by cancer cells),[65] and even viruses[66] allows for the selective targeting of cancer cells. Unlike organic molecules dyes, multiple functionalities can also be incorporated onto the same nanoparticle, making them superior for targeted drug delivery applications.

1.6.3 Gold nanoparticles as biosensors

Biosensors employ biological molecules such as antibodies, enzymes, carbohydrates and nucleic acids to identify or follow the course of any biological phenomena of interest. Interactions, such as hydrogen bonding and charge-charge transfers between the ligand and receptor molecules, coupled with read-out techniques such as colorimetry, fluorescence, biomagnetic signals, etc, are used for sensing specific biochemical events.[67] Biosensors are finding use in various applications: food processing, environmental monitoring, biowarfare defense; to detect bacteria, viruses and biological toxins.[67] Due to their small size, gold nanoparticle based sensors could have an important impact in diagnostics.[68] Gold nanoparticles having special optical and electronic properties can bind the molecules to the particle surface and can change the plasmon resonance frequency directly, which is observable by their scattered light in dark field microscopy, in particular on the single particle level. On the other hand the plasmon resonance frequency is dramatically changed when the average distance between gold nanoparticles is reduced so that they form small aggregates. This effect of plasmon coupling can be used for colorimetric detection of analytes. The method was pioneered by Mirkin and coworkers [69, 70] and is nowadays maybe the most well known example of a gold based sensor. The original assay was developed for the detection of DNA. Gold nanoparticles are conjugated with oligonucleotides that are complementary to the target sequence which is to be detected. Without the presence of the target sequence the gold nanoparticles are freely dispersed and the colloidal dispersion appears red. In the presence of the target sequence, the gold nanoparticles bind to the target by hybridization of complementary strands of DNA. As each gold nanoparticle is bearing several oligonucleotides, hybridization results in the formation of small aggregates of gold particles, which leads to a change in the plasmon resonance and the colloidal dispersion appears violet/blue color. When the sample is heated, even single sequence mismatches result in a different melting temperature of the aggregates which causes color change. Several DNA assays have been derived from this concept and nowadays the method is established in a way that quantitative detection of DNA sequences of very low concentrations is possible.

A new aggregation phenomenon of DNA-functionalized gold nanoparticles, induced by noncross-linking target DNA hybridization, has been reported. This phenomenon allows simple and rapid colorimetric sensing of DNA hybridization that is sufficiently sensitive to detect terminal single-base-pair mismatches.[71] Based on its simplicity and easy read out, this technique has opened up a new possibility for reliable genetic diagnosis. Another colorimetric hybridization assay that uses unmodified/unfunctionalized gold nanoparticles

for sequence specific detection of DNA has appeared in the literature.[72] Based on the differences in electrostatic properties between single stranded DNA (ssDNA) and double stranded DNA (dsDNA), ssDNA selectively adsorbs on and stabilizes the gold nanoparticles against aggregation in high salt buffers relative to dsDNA. This assay has an added advantage of being completely independent of the detection step while adaptable to sensing single-base-pair mismatches between probe and target. Gold nanoparticles/enzymes-based biosensors that measure cellular glucose levels have also been developed for potential use in diabetes management.[73] These sensors use glucose oxidase immobilized on gold nanoparticles to detect glucose concentrations. Gold nanoparticles immobilization of glucose oxidase resulted in glucose sensors with enhanced sensitivity and stability. Using gold nanoparticles, to which a yeast iso-1 - cytochrome c (Cytc) is covalently attached, could be used as a colorimetric sensor to follow the folding or unfolding of an appended protein molecule.[74] Upon exposure to buffers of different pH, the appended Cytc unfolds at low pH, thus inducing gold nanoparticles aggregation while refolding at high pH, results in the loss of aggregation. These conformational changes caused measurable shifts in the gold nanoparticles color and could be detected by UV/VIS absorption spectroscopy.[74]

1.7 Gold nanoparticles in therapy

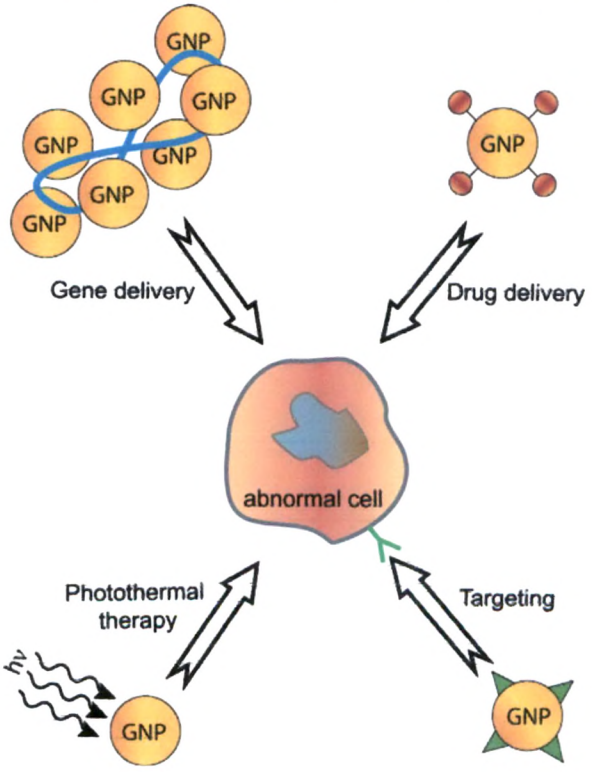
1.7.1 Historic perspective on the use of gold in medicine

Gold has been exploited for its putative medical properties throughout the history of civilization.[75] In medieval Europe aurum potabile (drinkable gold) was invested with the power to cure a diverse range of diseases, but because it was prepared by simply quenching a piece of the heated metal in wine, it was unlikely that it contained much if any gold. It was not until the sixteenth century that European alchemists learned to use aqua regia to dissolve gold and were used in medicinal treatments.[75] Gold cordial could be found in the new pharmacopoeias of the 17th century and was advocated by Nicholas Culpepper for the treatment of ailments caused by a decrease in the vital spirits, such as melancholy, fainting, fevers and falling sickness. Mahdihassan explored the historical use of gold in eastern traditions especially in India and China. In India, cinnabar gold is known as "Makaradhwaja".[76] It was used as a drug for vigour of youth. Gold also has a long history of use in the western world as netvine, a substance that could revitalize people suffering from nervous conditions.[77] The use of gold compounds in modern, twentieth century medicine began with the discovery by the German bacteriologist Robert Koch that gold cyanide $K[Au(CN)_2]$ was bacteriostatic towards the tubercle bacillus.[78] Following the discovery by Robert Koch, gold based therapy for tuberculosis was introduced in 1920s. Gold therapy soon proved to be ineffective for tuberculosis but, clinical study sponsored by the Empire Rheumatism Council confirmed the effectiveness of gold compounds against rheumatoid arthritis.[79] The treatment of rheumatoid arthritis with gold(I) salts was first popularized by Jacques Forestier in the early 1930s.[80] He had reasoned simply that gold salts probably stimulated the body's defense mechanisms against tuberculosis and might induce a similar effect in combating rheumatoid arthritis. Since that time gold drugs have also been used to treat a variety of other rheumatic diseases. The new compound introduced into clinical use in the last 30 years is auranofin, triethylphosphine (2,3,4,6-tetra-0-acetyl-β-1-D-thiopyranosato-S)gold(I), introduced in 1985 for arthritis.[81] The presence of phosphine ligand makes auranofin more lyophilic with better retention in the circulation. The absorption of gold in the kidney was also reduced with significant reduction in the nephrotoxicity. Red colloidal gold is also used in India in the form of Ayurvedic medicine for rejuvenation and revitalization during old age under the name of Swarna Bhasma ("Swarna" meaning gold, "Bhasma" meaning ash).[76] Antitumor activity of cisplatin was discovered in 1969 that prompted the investigation of other metal containing antitumor drugs. Gold has also been included in the search on the basis of three rationales (i) both Pt (II) and Au (III) form analogous square planar complexes with d^8 configuration of the central ions, (ii) analogy to the immunomodulatory effects of gold (I) antiarthritic agents and (iii) complexation of known antitumor agents with gold(I) and gold (III) to produce to

compounds with enhanced activity. This discovery led to the screening of many phosphine containing gold drugs, of particular interest is bis(diphos)gold(I) complexes.[82] This complex showed promising antitumor properties but exhibited cardiovascular toxicity that precluded their use in clinical trials. The mechanisms of action of gold drugs are poorly understood. However it is believed nowadays that under biological conditions gold(I) and gold (III) species are reduced to gold(0), which may be the active species. There is hardly any report that describes the use of gold nanoparticles as an anticancer agent. However, recently there are reports which have shown the antiangiogenic property of gold nanoparticles.[83]

1.7.2 Recent advances on the use of gold nanoparticles in medicine

The historical data on the use of gold in various diseases, combined with present data on the toxicity studies suggest that colloidal gold nanoparticles are relatively inert and biologically compatible carriers.[59] Several investigators have grafted different delivery platforms onto gold nanoparticles surface to attempt cellular selectivity, internalization and localization within heterogeneous population of cancer cells in solid tumors (Scheme 1.2). These delivery platforms generally consist of macromolecules such as proteins and peptides, small molecules such as paclitaxel etc. Several of these platforms have shown very promising results in delivering of gold nanoparticles with drugs into solid tumors.



Scheme 1.2: Various applications of gold nanoparticles in therapy.[84]

1.7.2.1 Gold nanoparticles as a heat source (photothermal therapy)

Photothermal therapy is a less invasive experimental technique that holds great promise for the treatment of cancer.[85] It combines two key components: (i) light source, specifically lasers with a spectral range of 650-900 nm for deep tissue penetration, and (ii) optical absorbing gold nanoparticles which transform the optical irradiation into heat on a picoseconds time scale, thereby inducing photo thermal ablation. Gold nanostructures such as gold nanoshells,[86] gold nanocages[87] and gold nanospheres[88] have demonstrated effective photothermal destruction of cancer cells and tissue. Figure 1.4 shows the gold nanostructures commonly used for photothermal therapy. Gold nanoshells are ideal agents for detecting and imaging cancer - the same nanoparticles can be used as therapeutic agents to treat cancer with photothermal therapy. Unlike nanospheres, nanoshells can be engineered either to scatter NIR radiation for imaging, or to absorb it and efficiently convert it to heat for the selective destruction of targeted tumor cells.[89] Gold nanoparticles conjugated to antibodies can be selectively targeted to cancer cells without significant binding to healthy cells.[90] Irradiation of the cancer cells with selectively labeled nanoparticles with a laser of frequency overlapping with the surface plasmon resonance absorption maximum of the nanoparticles results in selective heating and destruction of cancer cells. This requires much lower laser powers than those required to destroy healthy cells to which nanoparticles do not bind specifically. Gold nanoparticles (10-50 nm) offer 5 or more orders of magnitude compared with conventional dyes; thus much lower laser energies can be used to achieve cell destruction, making therapy minimally invasive.[90]

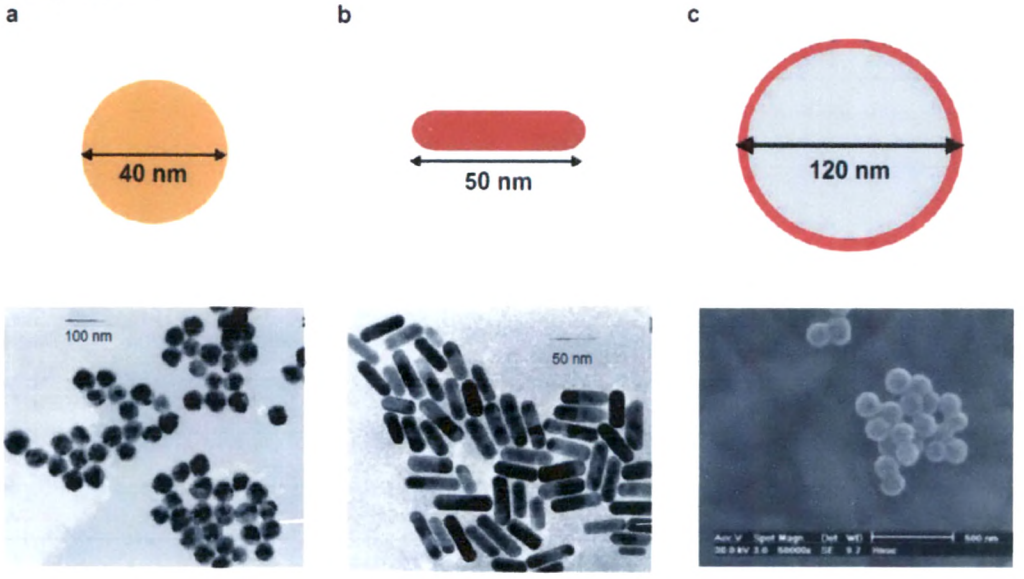


Figure 1.4: TEM images of plasmonic gold nanostructures commonly used for photothermal therapy, a) nanosphere, b) nanorods, c) nanoshell.[89]

Further, the therapy can be combined with dark field imaging, which is a significant advantage over other nanostructures such as quantum dots or carbon nanotubes, which can achieve only one of the two, imaging or therapy. While the use of visible light resonant gold nanospheres can be useful for external skin/surface cancer treatments, for tumors within bodily tissue, it becomes necessary to use NIR light in the biological window. El-Sayed group have demonstrated the use of gold nanorods, which have longitudinal surface plasmon resonance of around 800 nm overlapping with a NIR Ti:sapphire laser.[85] NIR laser irradiation of cancer cells labeled with gold nanorod/anti-EGFR conjugates resulted in selective destruction of the cancer cells. The approach of using noble metal nanoparticles for selective targeting, molecular imaging and selective therapy is general and versatile. Cancer is only one example. The use of plasmonic nanoparticles can be extended to other biological applications, for example, destruction of viruses or bacteria[91] or controlled localized denaturation or cleavage of proteins and nucleic acids, potentially useful for diagnostic or therapeutic goals. The potential of photothermal therapy in disease inten/ention has recently been extended to include parasite infections. Using gold nanorods conjugated with antibody selective for Toxoplasma gondii, reported that plasmonic heating with a 650 nm laser at power density of 51 W/cm2 resulted in more than 80% destruction of T. gondii tachyzoites.[92]

1.7.2.2 Gold nanoparticles for gene therapy

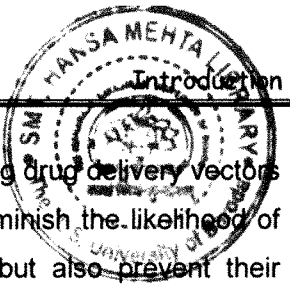
Gene therapy presents an ideal strategy for the treatment of genetic as well as acquired diseases. Viruses provide a logical vehicle for gene therapy and have been shown to be highly efficient but raised many safety concerns from unpredictable cytotoxicity and immune responses. Current non-viral gene delivery systems suffer from less efficiency. The vectors encounter numerous barriers between the site of administration and localization in the cell nucleus. The effective delivery vehicles need to provide efficient protection of nucleic acid from degradation by nucleases, efficient cell entry and release of the nucleic acid in functional form in the nucleus. Gold nanoparticles are attractive candidates for gene delivery. They can be made quite smail to provide a high surface-to-volume ratio, maximizing the payload/carrier ratio. Rotello et al., have shown that gold nanoparticles functionalized with cationic quaternary ammonium groups can bind plasmid DNA through electrostatic interactions and protect DNA from enzymatic digestion.[93] These non-covalent DNA-nanoparticle conjugates provided an effective means of gene delivery in mammalian 293T cells.1941 Same group have also reported the photochemical uncaging of the loaded DNA on surface of the gold nanoparticles.[95] In vitro studies using a T7 RNA polymerase assay demonstrated that DNA transcription was restored upon UV irradiation. Cell culture studies indicated that the particle-DNA complexes were effectively taken up by cells. Release of fluorescein isothiocyanate-labeled DNA upon irradiation was established by observation of bright fluorescence inside the cells. Moreover, a high degree of nuclear localization was observed for the released DNA.

1.7.2.3 Gold nanoparticles for protein and peptide delivery

Proteins such as tumor necrosis factor-a (TNFα) have been successfully grafted onto gold nanoparticles surface and utilized for cancer therapy in conjugation with hyperthermia to kill cancer cells.[96] TNFα is a potent cytokine that induces systemic inflammation. Additionally, TNFα is known to be overexpressed in solid tumors and mediates hemorrhagic necrosis in solid tumors. The later property suggests TNFα may find use in cancer therapy. However, TNFα has low therapeutic index due to nonselective acute toxicity that results from cell exposure. Recent observations on selective uptake of gold nanoparticles by tumors have enabled a re-evaluation of the potential application of TNFα in cancer therapy. TNFα grafted onto gold nanoparticles surface reduced the systemic toxicity compared to the native unconjugated TNFα. More importantly, the TNFα-gold nanoparticles conjugates were able to accumulate preferentially in the tumor vasculature. The selective uptake of the gold nanoparticles into the tumor has been suggested to be due to the leaky vasculature of the tumor blood vessels, which allows gold nanoparticles of sizes ranging between 20 to 100 nm to passively diffuse into the tumor interstitium. A current example of chemotherapy agents based on this technology is CYT-6091 or Aurimune™ developed by CytImmune (Rockville, MD, USA) is into phase II clinical trials for the treatment of melanoma, colorectal cancer and urinary tract cancer. Aurimune™ is a multivalent drug consisting of 33 nm colloidal gold nanoparticles, onto which is grafted TNFα for specific solid tumor targeting and thiol-derivatized poly(ethylene glycol) to bypass reticuloendothelial system (RES)[97] In addition to cancer therapy, recent studies have demonstrated the use of functionalized gold nanoparticles for the delivery of peptide, insulin via oral and nasal route.'981 The particles were stabilized by chitosan, a non toxic biopolymer. Chitosan coated gold nanoparticles strongly adsorb insulin on their surface and were effective fortransmucosal delivery of insulin.

1.7.2.4 Gold nanoparticles for delivery of drugs

The emerging field of nanobiotechnology offers the potential for the development of exquisitely organ/tumor targeted therapies. Nanobiotechnology also holds the promise of increasing the therapeutic index, stability, bioavailability of current cancer therapies, as a prime example.[99] Indeed, the blending of material science and tumor biology is leading to the development of innovative vectors with the potential of achieving the long sought after goal of tumor targeted drug delivery, getting the active molecule(s) solely where it is needed, at the solid tumor. Yet, to successfully achieve these goals, nanoparticle delivery systems must overcome the biological barriers that are naturally present in the body, as well as those that develop during tumor growth and progression. Bionanotechnologists now have available effective strategies to engineer nanoparticle delivery systems to



address the barrier system of the body. For example, tumor targeting drug delivery vectors are now approaching "true nanometer size", which will not only diminish the likelihood of their being opsonized in the blood and taken up by the RES but also prevent their clearance in the narrow confines of the inter-endothelial slits present in the red pulp of the spleen. To bypass RES, antibiofouling agents such as thiol- derivatized poly-ethylene glycol (PEG-SH) have been grafted onto nanoparticles surface as secondary coating. It has been observed that this secondary coating could delay RES clearance to liver from 0.5 hours to 72 hours in a mice model, an approximately 150 fold improvement compared with the unmodified nanoparticles.[100] Once these nanoparticle vectors are free to circulate throughout the body, they may passively as well as actively sequester in and around solid tumor due to the inherent leakiness of the tumor neovasculature and the presence of tumor specific ligands on the surface of these nanoparticle vectors.

Recently gold nanoparticles conjugated with various chemotherapeutic agents have been reported to address various limitation associated with the unconjugated molecules. Gibson et al., described a novel approach that permitted high load functionalization of 2 nm gold nanoparticle with anticancer drug, paclitaxel.[101] The resulting hybrid nanoparticles contained a 67 wt % organic content, which corresponds to ~ 70 molecules of paclitaxel per 1 gold nanoparticle. This approach gives a rare opportunity to prepare hybrid particles with a well defined amount of drug and offers a new alternative for the design of nanosized drug delivery systems. Similarly Hwu et al., reported the conjugation of paclitaxel onto superparamagnetic iron oxide and gold nanoparticles (gold as core) to over come the drawback of paclitaxel of low tumor specificity and low solubility in water.[102] The paclitaxel conjugated Fe_3O_4 nanoparticles. Possessed phosphate moieties for selectively targeting cancer cells. By possessing the PEG-SH spacer and the phosphate joining unit, these new paclitaxel nanomaterials functioned as a prodrug of paclitaxel, which was liberated in the presence of phosphodiesterase. It also possessed magnetic tracking capability and good hydrophilicity. In other study, methotrexate was grafted on the surface of the gold nanoparticles. Methotrexate, an analogue of folic acid, has been reported as an alternative formulation strategy to circumvent tumor cell resistance which was invariably developed upon repeated use of this versatile anticancer drug. It was shown that methotrexate gold nanoparticles conjugates rapidly accumulate in LL2 (Lewis lung carcinoma) cells, inducing higher cytotoxic effects on the tumor compared with free methotrexate which showed no antitumor effects.[103] Paciotti et al., reported development of multifunctional nanotherapeutics on a colloidal gold nanoparticle platform.[104] In this approach, the vector consisted of TNFa, thiolated poly(ethylene glycol) and paclitaxel, which were all bound on the same 26 nm gold nanoparticles. The release of paclitaxel from the vector was

investigated *in vitro* in B16/F10 melanoma tumor cells. It was obseived that the vector remained inactive unless treated with dithiothreitol, suggesting that the vector is acting as a prodrug from which paclitaxel must be released to elicit the desired anticancer effects. In vivo co-administration of cysteamine, an approved therapeutic, with the vector was found to activate paclitaxel release. Compared to unconjugated TNFα and paclitaxel, it was shown that the paclitaxel-gold nanoparticles-TNFα a vector delivers 10 fold more TNFα and paclitaxel to the tumor site.

Gold nanoparticles have also been conjugated with clinically useful anti-leukemic and anti-inflammatory drugs 6-mercaptopurine and its riboside derivative. The resulting conjugates were reported to possess substantially enhanced antiproliferative effects against K-562 leukemia cells compared to the corresponding free forms of these drugs.[105] In addition, Gold nanoparticles-6- mercaptopurine conjugates have shown antibacterial and antifugal activities against various strains of Gram-positive and Gram-negative organisms. This enhanced activity of 6-mercaptopurine gold nanoparticles conjugate may be attributed to the high penetrating power of gold nanoparticles through the microorganism cell wall, their small size and high surface area. Vancomycin capped gold nanoparticles also had enhanced in vitro antibacterial activities. Vancomycin capped gold nanoparticles presumably acts as a rigid polyvalent inhibitor of vancomycin- resistant enterococci.[106] Gold nanoparticles conjugates of fluorescent small molecules, such as coumarin, have also been developed as cellular probes and delivery agents. It was shown that attachment of coumarin, through a carbamate bond, to PEG-functionalized gold nanoparticles caused significant enhancement of emission intensity. Upon incubation with MDA-MB-231 cells (human breast adenocarcinoma cell line), the modified nanoparticles were rapidly internalized in the cells and localized in the perinuclear region as evidenced through intracellular particle tracking.[107]

1.8 Literature survey

1.8.1 Research articles

1. **V. Kattumuri et al.,** reported the synthesis and stabilization of gold nanoparticles in nontoxic phytochemical gum arabic matrix. The results demonstrated the ability of the gum acacia matrix to provide in vitro and in vivo stability and to maintain the nanoparticulate properties of gold nanoparticles intact for several months in aqueous/saline/phosphate buffered solutions as well as in powder form. In vitro analysis and *in vivo* pharmacokinetics of gold nanoparticles was studied in pigs to gain insight in the organ specific localization of gold nanoparticles vector and X-ray CT contrast measurements to know the potential utility of the nanoparticles in molecular imaging. The results demonstrated that naturally occurring gum acacia can be used as a nontoxic phytochemical construct in the production of readily administrable biocompatible gold nanoparticles for diagnostic and therapeutic applications in nanomedicine.[108]

2. **Y. M. Mohan et al.,** have reported a facile green approach for the spontaneous formation of silver nanoparticles in the presence of gum acacia polymer, without the addition of any typical reducing agent under mild conditions. Gum acacia acted as a reducing agent as well as stabilizing agent. Silver nanoparticles were obtained by mixing equal amounts of 0.5 wt % aqueous solutions of acacia and silver nitrate. The formation of silver nanoparticles was confirmed with ultraviolet-visible, fourier transform infrared, X-ray diffraction and X-ray photoelectron spectroscopy analysis. The particle size of the silver nanoparticles obtained from transmission electron microscopy was ~5 nm. Gum acacia polymeric chains promoted the reduction process and acted as good stabilizer. The formed nanoparticles were stable for 5months. The advantage of this methodology was that it was possible to prepare silver nanoparticles without any organic solvents or strong reducing agents.[109]

3. **R. Shukla et al.,** studied the cytotoxicity and immunogenic effects of sodium borohydride reduced and lysine capped gold nanoparticles. The findings indicated the biocompatible properties of gold-Au(0) nanoparticles such as nontoxicity, non-immunogenicity and high tissue permeability without hampering cell functionality. Atomic force microscopy suggests that gold nanoparticles were internalized inside the cell via a mechanism involving pinocytosis, while confocal laser scanning microscopy and transmission electron microscopy studies indicated their internalization in lysosomal bodies arranged in perinuclear. The studies underlined the noncytotoxic, no immunogenic and biocompatible properties of gold nanoparticles with the potential for application in combining cancer imaging and tumor targeted drug delivery in cancer therapy.[59]

4. **W. S. Cho et al.,** reported an in vivo toxicity study using 13 nm sized gold nanoparticles coated with PEG (MW 5000). The studies indicated that the 13 nm s zed PEG-coated gold nanoparticles were seen to induce acute inflammation and apoptosis in the liver of the mouse. These nanoparticles were found to accumulate in the liver and spleen for up to 7 days after injection and had long blood circulation time. In addition, transmission electron microscopy showed that numerous cytoplasmic vesicles and lysosomes of liver Kupffer cells and spleen macrophages contained the PEG-coated gold nanoparticles. It was concluded that cytotoxicity of nanoparticles depends on the physical dimension, surface chemistry and shape of the nanoparticles. The results suggested that careful scrutiny of the in vitro and *in vivo* toxicities of nanoparticles is required even for materials that have been previously shown to have limited or no toxicity at the cellular level.[110]

5. **D. R. Bhumkar et al.,** have demonstrated a novel method for the synthesis of gold nanoparticles using a biocompatible polymer, chitosan with improved surface properties for binding of biomoiecules. The penetration enhancement property of chitosan has been explored for administration of insulin by transmucosal route. The studies indicated that chitosan acted as a reducing as well as stabilizing agent for the synthesis of gold nanoparticles. The studies also indicated that oral and nasal administration of insulin loaded chitosan reduced gold nanoparticles had lead to improved pharmacodynamic activity as evidenced by higher reduction in blood glucose level as compared to the insulin loaded sodium borohydride reduced gold nanoparticles and insulin solutions.[98]

6. **R. T. Tom et al.,** studied the adsorption of ciprofloxacin molecule on gold nanoparticle using different analytical techniques. The antibacterial drug has been used to protect gold nanoparticles of two different mean diameters, 4 and 20 nm. The protection was complete with about 65 and 585 ciprofloxacin molecules covering 4 and 15 nm nanoparticles, respectively. The ciprofloxacin adsorbed particles were stable in the dry state as well as at room temperature. The rate of release of the drug molecule from the nanoparticles was more in the basic medium than in water. The kinetics depended on the size of the particle; faster desorption was seen in smaller particles. The study showed that metal nanoparticles could be useful carriers for ciprofloxacin and other biomolecules. Most of the bound molecules could be released over an extended period of time.[111]

7. **J. R. Hwu et al.,** applied the nanotechnology for solving three problems associated with paclitaxel as an effective anticancer drug that is low solubility in water, low bioavailability for selectively targeting cancer cells and lack of a reliable method for its detection and tracking. They conjugated paclitaxel molecule onto superparamagnetic iron oxide and gold nanoparticles. The gold nanoparticles were synthesized through different methods for the production of both hydrophilic and hydrophobic paclitaxel conjugates. Three paclitaxel conjugated nanoparticles were synthesized by the use of iron oxide (Fe_3O_4) and gold as the core. The paclitaxel-conjugated Fe_3O_4 nanoparticles possessed phosphate moieties for selectively targeting cancer cells. By possessing the PEG-SH spacer and the phosphate joining unit, these new paclitaxel nanomaterials functioned as a prodrug of paclitaxel, which was liberated in the presence of phosphodiesterase. It also possessed magnetic tracking capability and good hydrophilicity. These conjugated nanomaterials constitute a new class of candidates as anticancer drugs.[102]

8. **C. Park et al.,** reported the therapeutic ability of cyclodextrin capped gold nanoparticle as carrier for noncovalent encapsulation of an anticancer drug. The surface of the gold nanoparticles was functionalized with cyclodextrin as a drug pocket, anti-epidermal growth factor receptor antibody as a targeting moiety and poly(ethylene glycol) as an anti-fouling shell. The β-Lapachone, was efficiently encapsulated into the hydrophobic cavity of cyclodextrin on the surface of the gold nanoparticles carrier. The glutathione mediated release of β-lapachone from the surface of gold nanoparticles was demonstrated by an experiment with low and high glutathione. The anti-EGFR antibody onto the gold nanoparticles carriers increased the intracellular uptake of gold nanoparticles carriers as compared with nanoparticles without targeting ligand. The in vitro cytotoxicity study revealed that gold

nanoparticles with anti-EGFR and β-lapachone exhibited a higher apoptosis effect than that caused by gold nanoparticles with only β-laoachone. The work suggested that gold nanoparticles covered with cyclodextrin and tumor targeting ligands may find useful applications for the development of nanoparticles with therapeutic and diagnostic modalities.[112]

9. **K. A. Janes et al.,** described the feasibility of using chitosan nanoparticles as colloidal carriers for doxorubicin hydrochloride. They entrapped the cationic, hydrophilic molecule into nanoparticles by masking the positive charge of doxorubicin by complexing it with the polyanion, dextran sulfate. This modification doubled drug encapsulation efficiency relative to controls. They also investigated the possibility of forming a complex between chitosan and doxorubicin prior to the formation of the particles. The particles demonstrated an initial burst release followed by a very slow release. The evaluation of the activity of doxorubicin loaded nanoparticles in cell cultures indicated that those containing dextran sulfate were able to maintain cytostatic activity relative to free doxorubicin, while doxorubicin complexed to chitosan before nanoparticle formation showed slightly decreased activity. Additionally, confocal studies showed that doxorubicin was not released in the cell culture medium but entered the cells while remaining associated to the nanoparticles.[113]

10. **D. R. Kalaria et al.,** reported doxorubicin loaded biodegradable poly (D,L- lactide-co-glycolide) (PLGA) nanoparticles for oral chemotherapy. Doxorubicin loaded PLGA nanoparticles were prepared by a double emulsion method. The particles displayed good pH dependent stability in the pH range 1.1-7.4. Differential scanning calorimetry and X-ray diffraction studies were carried out in order to ascertain the nature of doxorubicin in formulations in conjunction with accelerated stability studies. Formulations were evaluated in vitro for release behavior in phosphate buffer and *in vivo* in rats for their toxicity profiling and pharmacokinetic behavior. Initial biphasic release (20%) followed by a sustained release (80%) for 24 days was observed under in vitro conditions. The doxorubicin loaded nanoparticles demonstrated superior performance in vivo as evident by enhanced bioavailability and lower toxicity. The doxorubicin loaded nanoparticle formulations (oral administration) showed lower toxicity than the corresponding i.v. doxorubicin (doxorubicin nanoparticles and doxorubicin solution). The reduced toxicity observed could be due to the gradual release of the incorporated doxorubicin from the nanoparticle formulation or as a result of reduced exposure as the doxorubicin loaded nanoparticles were sequestrated in tissues.[114]

1.8.2 Patent status: In the present survey, patents on metal nanoparticles and biologicals are addressed (Table 1.1).

Table 1.1: Patents on metal nanoparticles and biologicals

Sr. No.	Inventors/ Year/Patents no.	Patent Title	Findings
1.	K.K. Katti, R. Kannan and K.K. Katti, 2008 US 20090074674	Stabilized, biocompatible gold nanoparticles and enviro-friendly method for making same.	A process of stabilized, biocompatible gold nanoparticles that are stabilized with material from polyphenols- or flavanoids-rich plant material. The stabilizing agent comprises gum arabic. The embodiment of the invention includes diagnostic and therapeutic application (imaging) of cancer in human.
2.	C.L. Lee and C.C. Wan. 2003 US 6,572,673	Process for prepairing noble metal nanoparticles.	A process for preparing metal nanoparticles by reacting suitable metal salts and anionic surfactant containing an anionic group of carboxylic group (COO^-) as reducing agent. The nanoparticles were uniform in size, no large microparticles were formed, the nanoparticle thus prepared could be dispersed stably in polar and non- polar solvent.
3.	S.G. Boyes, M.D. Rowe and J. Hotchkiss. 2008 US 20090060839	Gold nanoparticles conjugates and uses thereof	It includes gold nanoparticles conjugates, particularly to polymers, and the subsequent conjugation to targeting agents and therapeutic agents and their use in targeting, treating and/or imaging disease states in a patient. Therapeutic agents include any therapeutic compounds that are capable of preventing or treating a disease in a patient.
4.	T. Lawrence and G.F. Paciotti. 2001 US 6, 274, 552	Composition and method for delivery of biologically active factors.	It includes a method of targeted drug delivery for the treatment of disease through the administration of custom complexes containing of one or more biologically active factors bound to a colloidal metal where atleast one of the biological active factor is capable of binding a high affinity receptor on a cell surface.
5.	T. Lawrence and G.F. Paciotti. 2007 US 7,229,841	Colloidal metal compositions and methods.	It includes the vectors which comprise of colloidal metal nanoparticles especially gold nanoparticles covalently attached with PEG (polyethylene glycol) and a therapeutic agent (TNF) useful in detection or treatment of solid tumors. Gold nanoparticles-TNF was less toxic and more effective in reducing tumor burden than native TNF.

6.	Y. Barenholz and A. Gabizon. 1990 US 4, 898, 735	Liposome/ doxorubicin composition and method	The composition consisted of doxorubicin and alpha-tocopherol entrapped in the liposomes and ferrioxamine in the aqueous suspension phase. The liposomes were about 0.2 microns in diameter. The drug/liposomes composition was effective in treating human neoplasms and particularly, primary and metastatic liver tumors and leukemias. The liposomes with entrapped doxorubicin significantly reduced side effects over those produced by free drug administration.
7.	O.J. Eun, L.K. Hyoung P.T. Gwan and N.Y. Sung. 2003 US 6,589,548	Controlled drug delivery system using the conjugation of drug to biodegradable polyester.	The invention relates to a molecular (doxorubicin) sustained controlled release system constructed by the conjugation of molecules to be released with biodegradable polyester polymer via covalent bond. The molecular release rate from the above system can be regulated to be proportional to the chemical degradation rate of the biodegradable polyester polymers, resulting in near zero order kinetics profile of release without showing a burst effect. Moreover, a high loading efficiency of hydrophilic drugs could be achieved.

1.8.3 Status of nanoparticulate systems.[115-116]

Table 1.2: Examples of nano-carrier based drugs in the market.

Sr. No.	Brand name	Type of material	Indication	Company
1	Doxil	PEGylated Doxorubicin liposomes	Metastatic ovarian cancer	OrthoBiotech
2	Abraxane	Albumin-bound paclitaxel particles	Lung cancer, breast cancer	Abraxis Oncology
3	DaunoXome	Liposomes - Daunorubicin	Kaposi's sarcoma	NeXstar Pharmaceutical
4	Myocet	Liposomes - Doxorubicin	Breast cancer nd ovarian cancer	The Liposome Company
5	Oncasper	Polymer protein conjugate- PEG -L-asparaginase	Acute Lymphoblasticleukemia	Enzon
6	Infliximab	Neutralizing TNF antibody	Fungal infections and hepatosplenic T-cell lymphomas	Centocor
7	Neulasta/PEG filgrastim	Polymer protein conjugate-PEG-granulocyte colony-stimulating factor	Prevention of hemotherapy associated neutropenia	Amgen
8	PEG-intron	PEG-a-interferon 2b	Hepatitis C	Schering- Plough
9	AmBisome	Liposomal formulation of amphotericin B	Systemic fungal infections	NeXstar Pharmaceutical

Table 1.3: Nanoparticulate systems in clinical development

Sr. No.	Name	Type of material	Indication	Status
1	Caelyx(R) (SCH 200746)	Pegylated liposomal doxorubicin	Advanced or metastatic breast cancer	Phase IV
2	Nano silver gel (SilvaSorb)	Silver nanoparticle	Antimicrobial	Phase III
3	Abraxane ®	Paclitaxel albumin-stabilized nanoparticle	Ovarian epithelial cancer, Fallopian tube cancer	Phase II
4	Aurimmune ™ (CYT-6091)	TNFa bound colloidal gold nanoparticles	Advanced solid tumors	Phase II
5	Doxorubicin & Temsirolimus	Pegylated liposomal doxorubicin	Resistant solid malignancies	Phase I
6	Carboplatin and Paclitaxel	Albumin stabilized nanoparticle	Advanced or metastatic Solid tumors	Phase I
7	AuroLase	Gold nanoshell	Head and neck Cancer	Phase I
8	AuriTol™ (CYT-21001)	Paclitaxel and TNFa bound colloidal gold nanoparticles	Solid tumors	Preclinical
9	AuriCin™ (CYT-31000)	Pegylated colloidal gold bound TNF with doxorubicin	Solid tumors	Preclinical
10	Bioconjugated nanoparticles	Luminescent quantum dots	Cancer diagnostic	Preclinical

Table 1.4: Companies commercializing nanomaterials for diagnostic and therapeutic applications.

Sr. No.	Company	Major area of activity	Technology
1	Advectus Life Sciences Inc	Brain tumors	Polymeric nanoparticles engineered to carry antitumor drug across the blood brain barrier
2	NanoPharm AG	Drug delivery	Polybutilcyanoacrylate nanoparticles coated with drugs and surfactant to cross blood brain barrier
3	Liplasome Pharma	Drug delivery	Smart lipid based nanocarriers for targeted transport of anticancer drugs
4	NanoCarrier Co., Ltd	Drug delivery	Micellar nanoparticles for encapsulation of drugs, proteins, DNA etc
5	Alnis Biosciences, Inc	Bio-pharmaceutical	Biodegradable polymeric nanoparticles for drug delivery
6	Nanoprobes, Inc	Bio-markers	Gold nanoparticles bioconjugates for TEM and/or fluorescent microscopy
7	Nanoshpere, Inc	Gold biomarkers	DNA barcode attached to each nanoprobe for identification purposes
8	QuantumDot Corporation	Luminescent biomarkers	Bioconjugated semiconductor quantum dots
9	Smith & Nephew	Acticoat bandages	Nanocrystal silver toxic to pathogens
10	PSiVida Ltd	Drugs and gene delivery	Nanostructured porous silicone for drug delivery

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