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Biomedical Applications of Gold Nanoparticles: Opportunity and Challenges

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Abstract: Biocompatible gold nanoparticles have gained considerable attention in recent years for potential applications in nanomedicine due to their interesting size dependent chemical, electronic and optical properties. In particular, the prospective use of gold nanoparticles as contrast enhancement agents in X-ray Computed Tomography (CT) and Photo Acoustic Tomography for early diagnosis of specific tumors is being extensively researched. Additionally, gold nanoparticles show promise in enhancing the effectiveness of various targeted cancer treatments such as radiotherapy and photothermal therapy. For these applications, biocompatible gold nanoparticles labeled with specific tumor targeting biomolecules are needed for site specific delivery. Gold nanoparticles stabilized and labeled with carbohydrate (starch) and glycoprotein (gum arabic) have been generated, characterized and tested for in vitro and in vivo stability. They are found to localize in specific tissues in the animal models. Additionally, gold nanoparticles labeled with a cancer seeking peptide, bombesin, exhibited excellent binding affinity towards prostate and breast cancer cells. The degree of contrast enhancement in cancer imaging or effectiveness of cancer treatments is limited by the number of nanoparticles that can be localized at the target tumor/cancer site. The various biomedical applications of gold nano particles have been discussed.

Key words:

INTRODUCTION

Gold is a rare metallic element with a melting point of 1064°C and a boiling point of 280°C. Several properties of gold such as its excellent conductive properties and its inability to react with water or oxygen, have made it very useful to mankind over time. During the 5th millennium B.C., the extraction of gold started near Varna (Bulgaria) and it is believed that "soluble" gold appeared around the 5th or 4th century B.C. in Egypt and China. The marvellous statue of Touthankamon, which was constructed around that time stands as proof. It was referred with different names such as soluble gold and drinkable gold, before the term "colloid" (from the French word, colle) was coined [1]. Colloidal gold and its beautiful ruby-red colour has fascinated people for many centuries, that can be traced back to ancient times. It was used extensively for cosmetic, decorative as well as for medicinal purposes [2-4]. In the Middle Ages, "Aurum potabile" or "drinkable gold" was used to cure diseases like arthritis and heart problems, venereal diseases, dysentery, epilepsy and tumours and also for the diagnosis of syphilis, a method which remained in use until the 20th century [5-8].The use of colloidal gold as the name of soluble gold for therapeutic purposes was well detailed in a book on soluble gold [9].The authour had briefly described the formation of colloidal gold suspensions and their medical uses, including successful practical cases. By the end of 16th century, colloidal gold was routinely used to make ruby glass and for colouring ceramics, methods that are still in use now. The most famous examples of the use of colloidal gold in ruby glass are the Lycurgus Cup that was manufactured in the 5th to 4th century B.C. and the "Purple of Cassius" [4]. The Lycurgus Cup appears ruby red in transmitted light and turns green in reflected light, due to the presence of gold colloids. The Colloidal gold to dye silk ,Thus it appears that, these kinds of ideas about colloidal gold were common in the 18th century [10].

Multifunctional nanoparticles, which incorporate diagnostic (quantum dots, magnetic, metallic, polymeric and silica nanoparticles) and/or therapeutic (magnetic and metallic nanoparticles) properties, are in the process of

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development. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. The surface of gold nanoparticles can be tailored by ligand functionalization to selectively bind biomarkers. Thiol-linking of DNA and chemical functionalization of gold nanoparticles for specific protein/antibody binding are the most common approaches. Several methods have been utilized for detecting AuNPs such as scanometric, fluorescence, colorimetric, surface-enhanced Raman scattering and electrochemical techniques. These unique aspects have allowed the development of novel AuNP-based assays for clinical diagnostics which promise increased sensitivity and specificity, multiplexing capability and short turnaround times.

Gold nanoparticles represent a new class of biocompatible vectors capable of fulfilling this promise by selective cell and nuclear targeting of which will provide new means for the site- specific diagnosis and treatment of medical conditions. This work outlines the methodology for conjugation of AuNps with target specific biomolecules and details theresults of studies assessing the target specificity and cytotoxicity effects of thus conjugated gold nanoparticles.

Ostwald carried out several studies on metal colloids and subsequently wrote a book titled “The World of Neglected Dimensions”[11]. Nearly half a century later, Feynman visualised the field of nanotechnology quoted that “There’s plenty of room at the bottom” [12]. Since then, with availability of several sophisticated tools, this area of research has shown tremendous progress [13-16]. Being the subject of one of the most ancient themes of investigation in science, gold and its past glory now leads to an exponentially increasing number of applications, especially in the context of emerging nanoscience and nanotechnology. Metallic gold can be reduced to gold nanoparticles by a variety of reducing agents. By definition, nanoparticles can range in size from 1 to 100 nanometers.The nanoparticles have highly interesting optical, electronic and catalytic properties, which are very different from those of the corresponding bulk materials [17]. Colloidal gold nanoparticles present interesting aspects such as, the behavior of the individual particles, size-related electronic and optical properties and their applications to catalysis and biology. The possibility to control and tune these unique optical and electronic properties, can allow these gold nanoparticles to be used as versatile analytical probes. Due to the promises offered by the nanotechnology, these nanoparticles are becoming key materials and building blocks in the 21st century.

Gold Nanoparticles and Their Properties: Gold nanoparticles are defined as stable colloid solutions of clusters of gold atoms with sizes ranging from 1-100 nm (Figure 1). At this nanoscale, AuNps possess different physicochemical characteristics when compared to the bulk gold [18,19], most obvious example being the color change from yellow to ruby red when bulk gold is converted into nanoparticulate gold. This ruby red color of AuNps is explained by a theory called “surface plasmonics”. According to this theory, when the clusters of gold atoms are hit by the electromagnetic field of the incoming light, the surface free electrons (6 electrons in case of AuNps) present in the conduction band of AuNps oscillate back and forth thus, creating a plasmon band which has an absorption peak in the visible region at 530-540 nm [20]. The surface plasmon band (SPB) of AuNps is used as an indicator for formation during the synthesis of AuNps from their precursor salts. The sensitivity of plasmon band absorptivity is the basic detection mechanism involved in the AuNps based bio sensors [21,22]. Physical properties of AuNps in turn depend on the size, shape, particle-particle distance and the nature of the stabilizer used to prevent the agglomeration of nanoparticles [18].

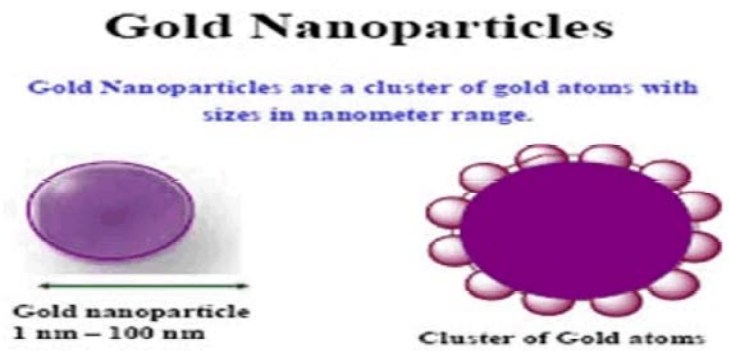


Fig. 1: Colloidal – nanoparticle

According to Mie theory, Surface Plasmon Band (SPB) is absent for AuNps less than 2nm and greater than 500nm [19]. Gold nanorods have two SPB's, one longitudinalwavelength band at 550-600nm and one transverse-wavelength band at 520nm [23,24].The longitudinal-wavelength band is very sensitive and changing the aspect ratio of Gold nanorods changes the absorption region from visible to Near-infra red (NIR) [25]. This unique optical property of Gold nanorods is used in Near-infra red ray therapy [26]; and enhanced Raman scattering of adsorbed biomolecules [27]. Therefore, by changing the size and shape of AuNps, the SPB and scattering may be tuned for application in cellular imaging, drug delivery and therapy. The six free electrons present in the conduction band of nanoparticulate gold makes them potential candidates to bind with thiols and amines [28]. Therefore, AuNps may be easily tagged with various proteins and bio molecules rich in amino acids leading to important biomedical applications including targeted drug delivery [29,30], cellular imaging [31] and biosensing [32]. Further, the free electrons also render AuNps useful as contrast enhancement agents [33]. Imaging studies are based on comparisons of contrast produced by the variations in the electron densities in different tissues. With their high electron densities, AuNps serve as excellent contrast enhancement agents in the detection of tumors.

Synthesis of Gold Nanoparticles: Gold Nanoparticles are traditionally synthesized by reducing metallic gold in +3 state to nanoparticulate gold in +1 state. There are a number of reducing agents reported in the literature for the synthesis of AuNps. Two most important ones are: Tri sodium citrate (Citrate synthesis) discovered by Turkevitch in 1973 [34] and sodium borate (Borate synthesis) introduced by Brust-Schiffrin in 1994 [35]. These two synthesis protocols pose potential problems in case of size control, stability and most importantly toxicity [36]. Therefore, we followed a novel protocol reported that uses a phosphino-amino acid based reducing agent, tris hydroxyl phosphine alanine (THPAL) to synthesize AuNps. THPAL is a water-soluble non-toxic reducing agent. It is reported that swine models can withstand up to 100 mg/kg of body weight of THPAL without showing toxicity, the most important of criteria in the use of nanoparticles for bio medical applications. Due to the strong surface reactivity of free electrons present on AuNps, they easily tend to agglomerate posing stability problems. Naturally occurring, FDA approved non-toxic compounds such as starch; gum arabic and gelatin were used to stabilize AuNps immediately after they are formed [37]. These stabilizers form weak covalent bonds with AuNps so that they easily shed off in the presence of biomolecules with strong electronegative groups with which the AuNps can then react.

Biomolecule-Directed Nanoparticle Organisation - Nanoparticles as Biolabels: The dimensions of the metal nanoparticles are similar to those of biomolecules such as proteins (enzymes, antigens, antibodies) or DNA whose dimensions are in the range of 2-20 nm[38,39,31].Immibilisation of biomolecules onto nanoparticles to yield novel hybrid nanobiomolecules, has been achieved by a variety of techniques including physical adsorption, electrostatic binding, specific recognition and covalent coupling [40]. Under appropriate conditions, noncovalent bonding is a general strategy to bind colloidal gold and macromolecules, with little or no change in the specific activity of the bound macromolecule. This interaction is influenced by a number of factors including ionic concentration, pH conditions (in correlation with the protein pI values) and protein/DNA stabilising levels.

In the case of citrate capped nanoparticles, biomolecules can be linked directly by exchange reactions with stronger binding ligands [31]. For example, the coating of colloidal gold with proteins such as immunoglobulins and serum albumin, which have cysteine residues. If the native proteins doesn't have the cysteine residues, thiol groups can be incorporated by chemical modification [41] by genetic engineering. DNA molecules can also be synthesized with alkylthiol groups at either the 3'- or 5'-end to facilitate binding to gold nanoparticles [41].

By utilising the advantage of specific receptor-ligand interactions, various nanoparticle assemblies have been generated. Analogous to the interactions between the amino acid side chains and the metal atoms in many reaction centres of enzymes, the interaction between biomelcules and the surface of an inorganic nanoparticle provides the way for the coupling of biomolecular recognition systems to generate novel materials. The two sets of nanoparticles are functionalised with individual recognition groups that are either directly complementary to each other, or else are complementary to a molecular linker [31]. The bio-recognition elements such as proteins/enzymes, antigens/antibodies and DNA/oligonucleotides, in conjunction with nanoparticles, have been used for various biotechnological applications

including, affinity separations, biosensing, bioreactors and the construction of biofuel cells.

Dna-Gold Nanoparticles Assemblies and Sensors: Negatively charged DNA was found to substitute citrate ions around gold nanoparticles to form a DNA-nanoparticle probe, which was confirmed by electrophoresis and fluorescence[42]. DNA functionalized gold and semiconductor nanoparticles have been prepared using the n-alkylthiolated DNA and also using DNA containing several adenosyl phosphothioate residues at their ends [31]. Nucleic acids are superior for the fictionalization of nanoparticles, since the possible programmability of DNA base-pairing to organise nanoparticles in space and the range of techniques available for the DNA conjugated to the nanoparticles is able to hybridize with complementary DNA and is thermally reversible [43]. In the presence of complementary strands, the coupled nanoparticles are released at high temperatures due to the "melting" transition of the complementary DNA strand[44] detection of precise DNA sequences [45]. In recent times, the fabrications of DNA-driven assemblies of two-dimensional arrays and three-dimensional networks of gold and silver nanoparticles have indeed attracted considerable interest. The Mirkin group have used DNA as a linker to form macroscopic assemblies of 13-nm gold nanoparticles [45]. DNA as a template to prepare nanocrystal chains consisting of two or three 1.4 nm particles on a single oligonucleotides strand [46].Conjugates of gold nanoparticles-oligonucleotides are of great interest for detection of DNA hybridisation, because of its application in the diagnosis of pathogenic and genetic diseases. Most of the DNA hybridization techniques utilize fluorescent, chemiluminescent, or radioactively labelled probes or requiring special instrumentation or both [47]. A significant enhancement of the shift (40 nm) of the transmission surface plasmon resonance (TSPR) absorption band of the gold nanoislands, was observed, when a self assembled monolayer of a single-stranded DNA deposited onto a glass microscopic slide is hybridised by its complementary DNA functionalised to gold nanoparticles [48]. The sensitivity of SPR biosensing of DNA hybridisation on continuous Au film was greatly enhanced by using Au nanoparticles [49]. Indeed, conductivity changes in gold nanoparticle labelled DNA arrays have recently been employed for selective molecular recognition of targets present in low concentration [50]. The SPB phenomenon has led to the development of a highly selective diagnostic method for DNA, based on the distance-related properties of gold nanoparticles. Aggregation of gold nanoparticles linked by the oligonucleotides mediates a red-toblue colour change (red shift from 520 to 620 nm of the SPB) and this property is utilised in the DNA-sensing method. The effect of the length of the DNA strands that control the interparticle distance has been studied and it was found that the SPB frequency changes are inversely dependent on the oligonucleotides linker length [51]. A new colorimetric technique based on the sensitivity of the surface plasmon band (SPB) has been designed to monitor the sequence specific DNA modifications [52]. Stable, water-soluble, hydroxycapped quantum dots-oligonucleotide conjugates have been used as labels in fluorescence *in situ* hybridization (FISH) studies [53]. Biosensors based on gold nanoparticle-DNA interactions have enabled detection within minutes and quantitative data obtained [54]. Thus, applications in the fields of biosensors, disease diagnosis and gene expression using gold nanoparticle-DNA conjugate probes are clearly called for.

Protein-Based Recognition Systems: Enhanced Immuno Sensing: Biomolecules and inorganic nanoparticles are conjugated by means of various conjugation methods that allow the preparation of well-defined bioconjugate hybrid nanoparticles [31]. Though, a large number of complementary binding pairs are available, nucleic acid based conjugation might offer advantages over protein based assembly, since the physicochemical properties of a single 20-mer oligonucleotide represents 420 different recognition elements [55]. However, it was suggested that extensive use of protein-based assembly may lead to a "factory of the future", directed by multiple highly specific biomolecular recognition elements such as, antibodies that are specific against various antigens [55]. These biomolecule based coupling systems were useful in various diagnostic applications and for generating inorganic nanoparticle networks [31]. The conjugation of proteins on colloidal gold nanoparticles is achieved by the electrostatic interactions between negatively charged citrate on surfaces of gold nanoparticles and positively charged groups of the proteins [56]. The strong interaction between the protein and the colloidal gold nanoparticle surface may increase the surface density of the adsorbed protein and small size of the colloidal gold particles gives the protein molecules more freedom in orientation [57]. Enzymatic activity of fungal protease-gold nanoparticle bioconjugates was reported [58].

Assembly of gold nanoparticles on polyurethane spheres were used to immobilise enzymes such as pepsin and these bioconjugate catalysts were reused as free enzymes [58]. The conjugation of antigens and antibodies on colloidal gold has been used for the development of immunological detection methods[59]. The experimental gold nanoparticle-protein conjugate architectures involves either direct binding of antigen-gold nanoparticle bioconjugates to an antibody modified surface or the exposure of an antibody derived surface to free antigen and then to a secondary antibody-gold nanoparticle conjugate. Recently, a unique, sensitive and highly specific immunoassay system for antibodies using gold nanoparticles has been developed [60]. Biosensors for immunoassays in human serum have been developed [61]. An electrochemical method to monitor biotin-streptavidin (STV) interactions has been established using colloidal gold as an electrochemical label [62]. The Biotin-STV system is a versatile system for developing novel strategies for assembling nanoparticles in suspension or on a substrate and the conjugates form the basis of many diagnostic and analytical tests [31]. The STV - biotin interaction was also used to organize gold colloids that were functionalized by chemisorptive coupling to a disulphide biotin analogue [63]. Niemeyer and Ceyhan prepared biofunctionalised nanoparticles by DNA-directed conjugation of proteins [31]. These studies demonstrate the emergence of a new field of application for colloidal gold in protein immobilisation and biosensing. The specific interaction between antibodies and low molecular weight organic compounds, the so called hapten groups, has been used to cross-link nanoparticles [64]. Gold and silver nanoparticles with the immunoglobulins IgG and IgE, which had a specificity directed against either the d-biotin or the dinitrophenyl (DNP) group respectively [64].

Gold nanoparticles have been immobilised in the gaps of microelectrodes through biospecific interactions and then the silver enhancement of gold nanoparticles has been applied for the electrical sensing of biological binding events [65]. These gold colloids serve as catalytic cores for the reductive deposition of a conducting layer of silver, which short circuits the two electrodes. This ultimately resulted in decrease in ohmic resistance which is used as a positive signal for the sensing of the biospecific interactions [65]. Biosensors for the electrocatalytic detection of hydrogen peroxide were prepared by adsorption of the horse-radish peroxidase enzyme onto electrode-immobilised layers of gold colloids [66]. In another example, conjugates of nanocrystals with IgG molecules were prepared and subjected to immuno-precipitation by using a complementary antibody with binding specificity for the particle-bound proteins. The widespread aggregation observed in this experiment clearly indicated that the nanocrystals were suitable for the sensitive immunoassays and the attachment of

nanocrystals does not interfere with the intrinsic functionality of the biomolecule [67]. In continuation to this work, tagged the micrometre-sized polymer particles with various quantum dots to achieve an optical barcode for biomolecules [68]. The great sensitivity of the surface plasmon band (SPB) by gold nanoparticle adsorption has also led to their use in bioassay applications[69]. Gold nanoparticles were also applied to enhance the detection limits in SPR-based biospecific interaction analysis [70]. The dramatic enhancement of SPR biosensing with colloidal Au was observed in a sandwich immunoassay in which Au nanoparticles were coupled to a secondary antibody , thereby allowing picomolar detection of the antigen [70].

Drug Delivery: Nanoparticles can easily enter cells although the mechanism(s) involved are not well understood. The nanoparticle influx occurs by endocytosis [71,72]. The particles are inserted and diffused through the lipid bilayer of the cell membrane [73]. Furthermore, these nanoparticles were shown to be able to enter the cells even after linkage to proteins such as antibodies [72]. Nanoparticles conjugated with antibodies against exclusive cancer cell surface receptors have been used to specifically bind with cancerous cells [72]. The functionalized nanoparticles have also been used for targeted entry into cells [74]. Phthalocyanine-stabilised gold nanoparticles have been shown to be a potential delivery vehicle for photodynamic therapy [75]. gold nanoparticles with a size of 20 nm have been conjugated to various cellular targeting peptides to provide functional nanoparticles that penetrate the biological membrane and target the nucleus [40]. Various nanoparticles have also applied as targeted biomarkers and drug-delivery agents for diagnosis and medical treatment of cancers [40].

Cytochemical Labels and Other Applications: Colloidal gold nanoparticles prepared in sizes from 1 to 25 nm, are electron dense due to the high atomic number of the gold atoms and, this makes them ideal for electron microscopy. Specific sites in a biological specimen may be visualized by introducing antibody conjugated colloidal gold particles [76,77]. Small gold clusters with a diameter of 0.8 or 1.4 nm, stabilized with arylphosphanes have been routinely used as probes for the site-specific labeling of biological macromolecules in histological applications [78]. Colloidal gold nanoparticles are also used as cytochemical labels for the study of macromolecules with transmission and scanning electron microscopy[79] , light microscopy [80] and freeze-etch electron microscopy [79] and to enhance the signals of both surface enhanced Raman spectroscopy [81] and surface Plasmon resonance [82]. Specific binding of mannose-encapsulated gold nanoparticles to FimH adhesin of bacterial type 1 pili in *Escherichia coli*, have been shown by TEM [83]. The method used in these studies labelled specific proteins on the cell surface using carbohydrate-conjugated gold nanoparticles and the visualisation of the target receptor was easily accomplished with an electron microscope. Colloidal gold, with an indirect digoxigenin-tagged nucleotide and an antidigoxigenin probe, was used for *in situ* hybridisation studies using an electron microscope [84]. Both a gold nanoparticle label and a fluorescein tag are attached to an antibody to yield a single probe for imaging a specimen both by fluorescence and electron microscopy [85]. A further advantage of using the colloidal gold marker is that the colloidal gold nanoparticles can be easily be counted and thus the cytochemical signal may be evaluated quantitatively. Several procedures such as silver enhancement have been developed to amplify the final signal which makes the techniques more sensitive. Separation of acidic and basic proteins was achieved by nanoparticle-filled capillary electrophoresis[86]. gold nanoparticles have been used to manipulate the selectivity between solutes in capillary electrophoresis [87]. Thus, gold nanoparticles serve as large surface area platforms for organo functional groups that interact with the capillary surface, the analytes, or both. The use of gold nanoparticles in conjunction with chip-based capillary electrophoresis to improve the selectivities between solutes and to increase the efficiency of the separation has been reported [88]. In summary, gold nanoparticles that are functionalised with proteins have long been used as tools in the biosciences. Moreover, the synthesis of well defined nanoparticle-biomolecule complexes is particularly important to generate well defined nanoarchitectures. The primary aim of this project is to design and develop a rapid, specific and highly sensitive diagnostic assay for *Neisseria meningitidis* using OMP85 and anti-OMP85 antibody as a model system. In the following chapters, details about the preparation of different target antigens including expression and purification of the recombinant OMP85 antigen are discussed. Polyclonal antibodies were raised against these antigens. Methods were optimised for successful conjugation of both antigens and antibodies to gold nanoparticles. Gold nanoparticles were utilized both as colour reporting agents and also as the signal amplification probes for the detection of the antigen.

Photothermal Cancer Cell Therapy in Nearinfrared Region Using Anti-Egfr Antibody Conjugated Gold Nanorods: Reducing a material's size to the nanometer length scale (which is the length scale of the electronic motion that determines the material's properties) makes it sensitive to further reduction in size or a change in shape. In semiconductor nanoparticles, the property change results from quantum confinement of the electronic motion [89]. In metals the properties of the surface become dominant and give nanoparticles new properties [90]. In noble metals the coherent collective oscillation of electrons in the conduction band induses large surface electric fields which greatly enhance the radiative properties of gold and silver nanoparticles when they interact with resonant electromagnetic radiation [91]. This makes the absorption crossection of these nanoparticles orders in magnitude stronger than the strongest absorbing molecules [92] and the light scattering crossecction orders in magnitude more intense than organic dyes [93]. Thus these particles act as excellent sensors and novel contrast agents for optical detection due to their enhanced absorption and scattering, respectively. In addition, when it is realized that the strong absorbed radiation is converted efficiently into heat on a picoseconds time domain due to electron-phonon and phonon-phonon processes [94], their potential use in photothermal therapy becomes obvious. The use of nanoparticles in medicine is one of the important directions that nanotechnology is taking at this time. Their applications in drug delivery [95-97], cancer cell diagnostics [97-100] and therapeutics [101] have been active fields of research. The scattering properties of gold nanospheres have been used for cancer cell imaging using confocal microscopy [100,102] and simple dark field microscopy [103]. Recently photothermal therapy using the absorption properties of antibody conjugated gold nanoshells [104]and solid gold nanospheres [105] have been demonstrated to selectively kill cancer cells leaving the healthy cells unaffected. In order to use long wavelength laser irradiation that penetrates tissue optimally (can be over 10 cm in penetration depth depending on tissue types) for in vivo photothermal treatment (650-900 nm) [106] the absorption band of the nanoparticles has to be in thtuned by adjusting the ratio of the thickness of the gold shell to the diameter of the silica core (about 120 nm in diameter) and thus enables photothermal therapy in this region. Carbon nanotubes absorb naturally in this region and have recently been proposed as near-infrared therapy agents [108]. It is important to mention that surface plasmon field

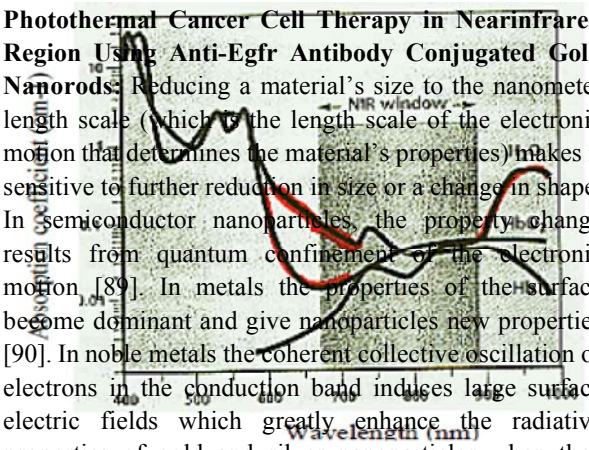
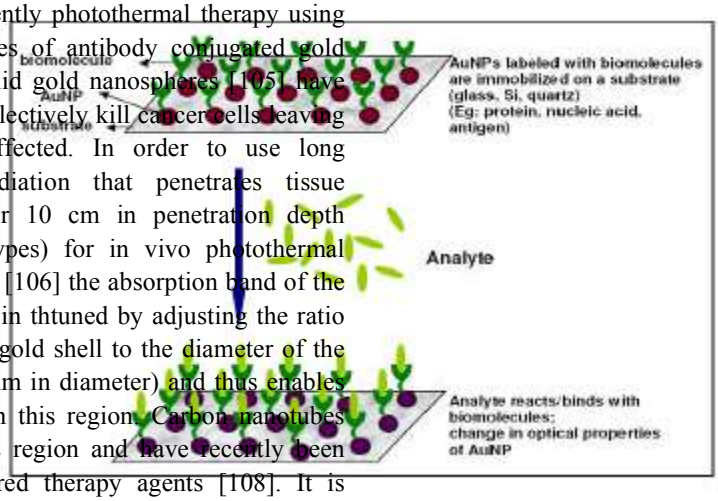


Fig. 2: NIR window

enhancement of the absorption of nanorods is predicted to be the strongest of all the different shapes of gold and silver nanoparticles [109,110]. By changing the shape of gold nanoparticles to gold nanorods, not only can one change the absorption and scatteringe near infrared region. The absorption band of core-shell particles has been wavelength from visible to the NIR region, but also increase their absorption and scattering crossections[107].

In the present work, we demonstrate the potential use of gold nanorods as a novel contrast reagent for selective photothermal therapy of cancer cells using a near infrared low energy cw laser. Solid gold nanorods have several advantages over other photothermal contrast agents. The synthesis of gold nanorods with various aspect ratios which enables tunable absorption wavelength in the near infrared region [92,111,112] is quite simple and well-established. The appropriate size of the nanorods is quite small and is potentially useful in applications such



as drug delivery and gene therapy. In addition, the biosafety of metallic gold is well known and they have been used in vivo since the 1950's [113] and recently the noncytotoxicity of gold nanoparticles in human cells has been studied in detail by Wyatt *et al.* [114].

Gold Nanoparticles in Biosensor Applications: The basic principle involved in the design of a biosensor based on gold nanoparticles is that the AuNPs are functionalized or capped with a thiolated biomolecule which upon identifying the complementary biomolecule causes change in the optical absorption of AuNPs [115]. For example, aptamer functionalized AuNPs specifically binds to thrombin causing aggregation of AuNPs and red shifting the plasmon peak. The specific binding was tested by exposing aptamer functionalized AuNPs to other proteins (BSA or human IgG antibodies) where no AuNP aggregation was observed [116]. Similarly, immunoassays have been based on antigen-antibody interactions. AuNPs functionalized with antigen (antibody) aggregate when matching antibody (antigen) binds causing shift in the plasmon absorption [117,118]. In addition to the above mentioned principle for designing biosensors, Surface Enhanced Raman Scattering (SERS) has emerged as a powerful spectroscopic tool that [119] can be employed in detecting trace amounts of molecules adsorbed on or present near metallic nanostructures along with structural and molecular information of the molecules.SERS now provides a great potential for label-free detection of biomolecules [120,121]. Significant efforts have focused on the binding of the oligonucleosides to metal surfaces and colloids for a variety of applications, including multiplexed DNA detection technology [122], rapid sequencers based on

Fig. 3: Gold Nanoparticles in Biosensor

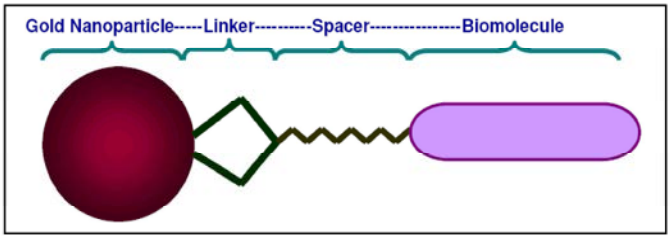


Fig. 4: Design strategy for bioconjugate /hybrid gold nanoparticles

SERS from single DNA bases [120] and real-time DNA detection methodology. For all the above applications new strategies for the synthesis, DNA-nanoparticle assembly methods and development of nanoparticle-based SERS-active substrates are needed. In the present project we have developed a new SERS substrate based on gold nanoparticles in agarose matrix that provides better enhancement in Raman signal of DNA nucleosides than that with commercially available gold nanoparticles. Before moving on to the project outline the following section discusses Raman scattering and SERS.

Bioconjugation of AuNPs: Hybrid gold nanoparticles are produced by the interaction of highly reactive nascent AuNPs with chemical functionalities present on specific molecules of biological interest (including peptides and proteins). The conjugation protocols that are applied for production of radiolabel led bioconjugates, traditionally used for cancer diagnosis and therapy [123-126], can be extended for the labeling nanoparticles of gold and other metals with tumor specific peptides. A hybrid gold nanoparticle has 4 components: (i) AuNP, (ii) Chelating moiety, (iii) Linker/Spacer and (iv) Cancer seeking peptide In the present project, starch stabilized AuNPs are utilized. The nature of bonding between starch and AuNPs is a weak coordination bond between the hydroxyl groups in starch and gold. However in presence of powerful electron donor atoms such as S, this weak coordination bond is expected to break and starch molecules detach from gold (Figure 1.9). The biomolecule chosen for bioconjugation with AuNPs is the seven-amino acid truncated bombesin analogue (BBN8-14) that is known to target gastrin releasing peptide (GRP) receptors that are over expressed on in a variety of neoplasma including small cell lung, prostate, breast, gastric, pancreatic, gastrointestinal carcinoid and colon cancers[127-135]. To impart specificity hybrid AuNP, disulfide moiety is chosen as a chelating moiety. S-S group undergo oxidative addition to AuNP and the reaction is very selective, even in the presence of thiol groups. Thioctic acid, a biological antioxidant [136] and believed to exhibit metal chelating properties [137], contains disulfide and carboxylic acid groups to conjugate to peptide. S-S group acts as a chelating moiety to hold the AuNP and 5 carbon atoms act as a space between S-S and the biomolecule. The thioctic acid modified bombesin is used for AuNP bioconjugation.

Application of Gold Nanoparticles for Immunosensors: Immunosensors are important analytical tools based on the detection of the binding event between antibody and antigen. The recent development of immunoassay techniques focused in most cases on decreasing analysis times, improving assay sensitivity, simplification and automation of the assay procedures, low-volume analysis. Among types of immunosensors, electrochemical immunosensors are attractive tools and have received considerable attention because they are easy and economical to mass production, they are robust and they achieve excellent detection limits with small analyte volumes. Furthermore, the availability of a variety of new materials with unique properties at nanoscale dimension, such as AuNPs, has attracted widespread attention in their utilization for the bioassay, especially for electrochemical detection. Recently, several novel strategies have been proposed to develop electrochemical immunosensors with high sensitivity using AuNPs. A novel and sensitive electrochemical immunoassay for immunoglobulin G (IgG) has been developed by Limoges and co-workers using a colloidal gold label via anodic stripping voltammetry technology. A low detection limit (Concentration as low as 3×10^{-12} M) could be obtained, which was competitive with colorimetric enzyme linked immuno-sorbent assay or with immunoassays based on fluorescent europium chelate labels. Furthermore, Shen's group reported a novel electrochemical immunoassay based on the precipitation of silver on colloidal gold labels. After metal silver dissolution in an acidic solution, the signal was indirectly determined by anodic stripping voltammetry at a glassy carbon electrode.A detection limit as lowas 1 ng mL^{-1} human IgG was achieved. The enhancement in sensitivity for an electrochemical

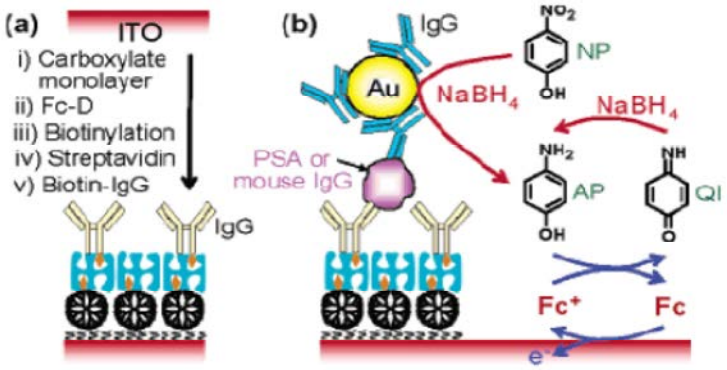


Fig. 5: Gold nanoparticles for immunosensors

immunoassay by the autocatalytic deposition of Au3+ onto AuNPs has been studied by Huang's group. By coupling the autocatalytic deposition with square-wave stripping voltammetry, the rabbit immunoglobulin G analyte could be determined quantitatively. A very low detection limit, 0.25 pgmL^{-1} was obtained, which is three orders of magnitude lower than that obtained by a conventional immunoassay using the same AuNPs labels. Novel enzyme-labeled electrochemical immunosensors were well developed by several groups. For instance, Ju's group reported that a highly hydrophilic and conductive colloidal AuNPs/titania sol–gel composite membrane could be employed as electrochemical sensing interface for horseradish peroxidase-labeled electrochemical immunosensor. Later, a novel electrochemical immunosensor for human chorionic gonadotrophin (hCG) was developed by the same group via the immobilization of hCG on AuNPs doped three-dimensional (3D) sol–gel matrix. The 3D organized composite structure was prepared by assembling AuNPs into a hydrolyzed (3-mercaptopropyl)-trimethoxysilane sol–gel matrix, which showed good biocompatibility. After the interfacial competitive immunoreaction, the formed HRPlabeled immunoconjugate showed good enzymatic activity for the oxidation of ophenylenediamine by H_2O_2. The immunosensor showed good precision, high sensitivity, acceptable stability and reproducibility. Label-free electrochemical immunosensors using AuNPs as enhancing sensing component have been the focus of intense research due to their simplicity, speedy analysis and high sensitivity. The technique is mainly based on the detectionof a change in physical properties as a result of antibody–antigen complex formation. The direct determination of immunospecies by detecting the change of impedance caused by immunoreactions has been demonstrated. A simple and sensitive labelfree electrochemical immunoassay electrode for detection of carcinoembryonic antigen (CEA) has been developed by Yao's group. CEA antibody (CEAAb) was covalently attached on glutathione (GSH) monolayer-modified AuNPs and the resulting CEAAb-AuNPs bioconjugates were immobilized on Au electrode by electrocopolymerization with o-aminophenol (OAP). Electrochemical impedance spectroscopy studies demonstrated that the formation CEA antibody–antigen complexes increased the electron-transfer resistance. The immunosensor could detect the CEA with a detection limit of 0.1 ng mL-1 and a linear range of 0.5–20 ng mL^{-1}.

CONCLUSION

Gold nanoparticles are currently being utilized in several technological applications and are gaining popularity as a form of counter measures against many odds beared through conventional means. As a natural material, gold is known to be safe to man and produce little to no allergic reactions when tested for curing various diseases. Its wide and beneficial applications are becoming more and more demanding. This is sure a very promising element to make our living long lasted gold. Development of environment friendly green methodologies have been fabricated to produce biologically benign gold nanoparticles labeled withbiologically relevant molecules. Furthermore, the gold nanoparticles were evaluated fortheir *in vitro* stability and *in vivo* biodistribution. Additionally, gold nanoparticles were conjugated with cancer seeking peptides to impart target specificity in hybrid gold nanoparticles for their potential applications in cancer imaging and therapy. Gold nanoparticles trapped in an agarose matrix were evaluated for their SERS properties with DNA nucleosides for possible biosensor applications.

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