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APPLICATION OF GOLD NANOPARTICLE IN MEDICAL FIELD

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ABSTRACT

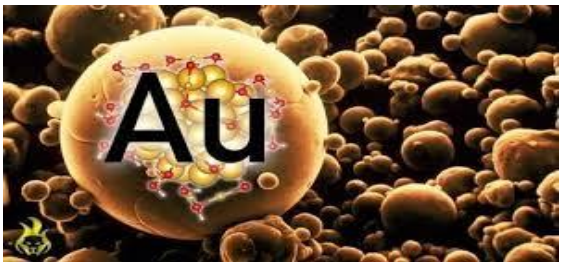
Gold is a Block D, Period 6 element. It is a soft metal that is often alloyed to give it more strength. It is a good conductor of heat and electricity. It is a good reflector of infrared and is chemically inert. The versatile surface chemistry of gold nanoparticles allows them to be coated with small molecules, polymers, and biological recognition molecules, thereby extending their range of application. The morphology of gold nanoparticles is spherical, and they appear as a brown powder. Gold nanoparticles are versatile materials with a broad range of applications in a variety of fields. Researchers have coated gold particles with DNA and injected them into plant embryos or plant cells. This will ensure that some genetic material will enter the cells and transform them. This method enhances plant plastids. The targeted delivery of drugs is one of the most promising and actively developing directions in the medicinal use of GNPs. The options of using GNP conjugated with the following antitumor agents were proposed: paclitaxel, methotrexate, daunorubicine, hemcytabin, 6-mercaptopurine, dodecylcysteine, sulfonamide, 5-fluorouracil, platinum complexes, kahalalide, tamoxifen, herceptin, doxorubicin, prospidin etc. The conjugation was carried out either by simple physical adsorption of the drugs onto GNPs or via the use of alkanethiol linkers. The effect of conjugates was assessed both (chiefly) on in vitro models, using tumor cell cultures, and in vivo, in mice with induced tumors of different natures and localizations (Lewis lung carcinoma, pancreatic adenocarcinoma, etc.).

INTRODUCTION

For centuries gold has captivated mankind and has been considered as a precious metal. Reports state that colloidal gold nanoparticles have been utilized for centuries by artists for their vibrant colors, which are produced by their interaction with visible light. However, only in the 1850s scientists began studying their properties in more detail.

Gold is a Block D, Period 6 element. It is a soft metal that is often alloyed to give it more strength. It is a good conductor of heat and electricity. It is a good reflector of infrared and is chemically inert.

The versatile surface chemistry of gold nanoparticles allows them to be coated with small molecules, polymers, and biological recognition molecules, thereby extending their range of application. The morphology of gold nanoparticles is spherical, and they appear as a brown powder.



Chemical Properties

Chemical symbol	Au
Group	11
Electronic configuration	[Xe] $4f^{142}$ $5d^{10}6s^1$

The chemical properties of gold nanoparticles are outlined -

Physical Properties

The physical properties of gold nanoparticles are given in the following table.

		Imperial
Density	19.30 g/cm^3	0.697 lb/in^3
Molar mass	196.97 g/mol	-

Thermal Properties-

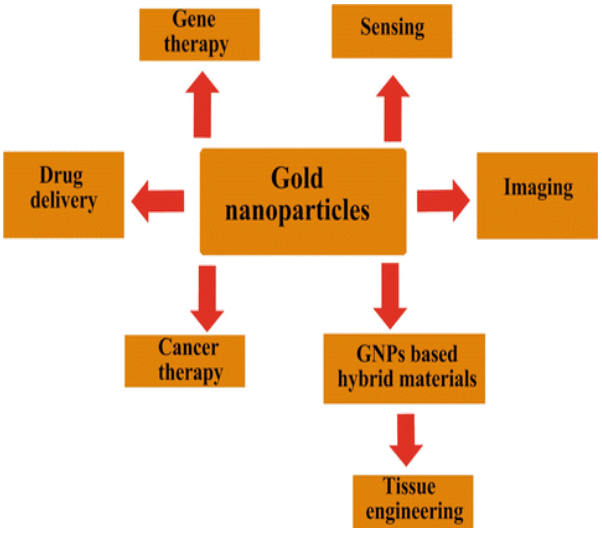
The thermal properties of gold nanoparticles are provided in the table below.

		Imperial
Melting point	1064.43°C	1947.9741°F
Boiling point	2807°C	5084.6°F

Manufacturing Process

Gold nanoparticles are commonly produced in a liquid by reducing chloroauric acid. After dissolving the acid, the solution is rapidly mixed along with a reducing agent. This process then causes Au^{3+} ions to be reduced to neutral gold atoms.

As more of these gold atoms are generated, the solution becomes supersaturated. Gold then begins to precipitate in the form of sub-nanometer particles. If the solution is mixed in a vigorous manner, the particles tend to be uniform in size. A stabilizing agent is sometimes added to prevent the particle from aggregating.



Applications

Gold nanoparticles are versatile materials with a broad range of applications in a variety of fields. Researchers have coated gold particles with DNA and injected them into plant embryos or plant cells. This will ensure that some genetic material will enter the cells and transform them. This method enhances plant plastids.

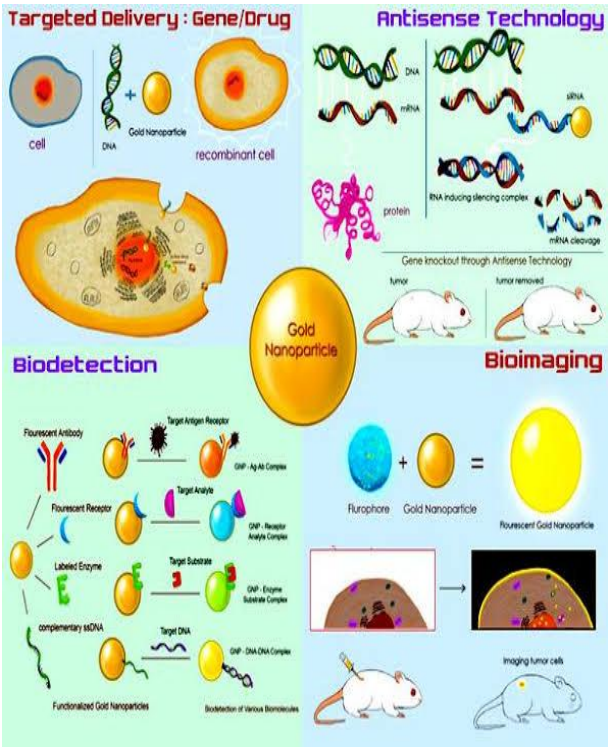
The July 2007 issue of Analytical Chemistry reported that scientists from Purdue University were able to use gold nanoparticles to detect breast cancer. Later it was also discovered that the nanoparticles could detect toxins and pathogens.

The optical-electronics properties of gold nanoparticles are being explored widely for use in high technology applications such as sensory probes, electronic conductors, therapeutic agents, organic photovoltaics, drug delivery in biological and medical applications, and catalysis.

Other applications of gold nanoparticles are listed below:

As an anti-biotic, anti-fungal, and anti-microbial agent when added in plastics, coatings, nanofibers and textiles

- In nanowires and catalyst applications
- In therapeutic agent delivery
- To connect resistors, conductors, and other elements of an electronic chip
- In photodynamic therapy - When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells
- In various sensors, e.g. colorimetric sensor with gold nanoparticles can identify if foods are suitable for consumption
- As substrates to enable the measurement of vibrational energies of chemical bonds in surface enhanced Raman spectroscopy
- The scattered colors of gold nanoparticles are currently used for biological imaging applications
- Gold nanoparticles are quite dense, thus allowing them to be used as probes for transmission electron microscopy
- To detect biomarkers in the diagnosis of cancers, heart diseases, and infectious agents
- As catalysts in a number of chemical reactions
- For fuel cell application.

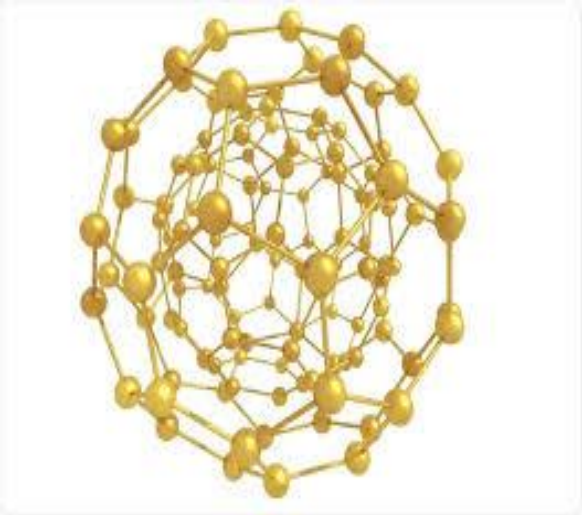


Gold Nano-Particles- Properties and Applications

Introuction

Colloidal gold particles have been utilized for centuries by artists due to the vibrant colors produced by their interaction with visible light. More recently, these unique optoelectronic properties have been researched and utilized in high technology applications such as organic photovoltaics, sensory probes, therapeutic

agents, drug delivery in biological and medical applications, electronic conductors and catalysis. The optical and electronic properties are tunable by changing the size shape, surface chemistry, aggregation state.



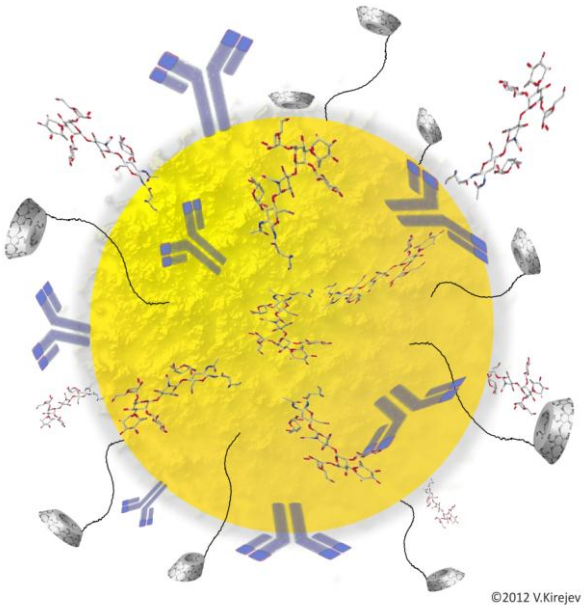
Optical and electronic properties

Gold interact ion with light is strongly dictated by their environment, size and physical dimensions. Oscillating electric fields of a light ray propagating near a colloidal nanoparticle interact with the free electrons causing a concerted oscillation of electron charge that is in resonance with the frequency of visible light. These resonant oscillations are known as surface plasmons. For small (~30nm) monodisperse gold nanoparticles, the surface plasmon resonance phenomenon causes an absorption of light in the blue-green portion of the spectrum (~450 nm) while red light (~700 nm) is reflected, yielding a rich red color.

As particle size increases, the wavelength of surface plasmon resonance related absorption shifts to longer, redder wavelengths. Red light is then absorbed, and blue light is reflected, yielding solutions with a pale blue or purple color.

As particle size continues to increase toward the bulk limit, surface plasmon resonance wavelengths move into the IR portion of the spectrum and most visible wavelengths are reflected, giving the nanoparticles clear or translucent color. The surface plasmon resonance can be tuned by varying the size or shape of the nanoparticles, leading to particles with tailored optical properties for different applications.

This phenomenon is also seen when excess salt is added to the gold solution. The surface charge of the gold nanoparticle becomes neutral, causing nanoparticles to aggregate. As a result, the solution color changes from red to blue. To minimize aggregation, the versatile surface chemistry of gold nanoparticles allows them to be coated with polymers, small molecules, and biological recognition molecules. This surface modification enables gold nanoparticles to be used extensively in chemical, biological, engineering, and medical.



Applications

The range of applications for gold nanoparticles is growing rapidly and includes:

Electronics - Gold nanoparticles are designed for use as conductors from printable inks to electronic chips. As the world of electronics become smaller, nanoparticles are important components in chip design. Nanoscale gold nanoparticles are being used to connect resistors, conductors, and other elements of an electronic chip.

Photodynamic Therapy - Near-IR absorbing gold nanoparticles (including gold nanoshells and nanorods) produce heat when excited by light at wavelengths from 700 to 800 nm. This enables these nanoparticles to eradicate targeted tumors. When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy.

Therapeutic Agent Delivery - Therapeutic agents can also be coated onto the surface of gold nanoparticles.The large surface area-to-volume ratio of gold nanoparticles enables their surface to be coated with hundreds of molecules (including therapeutics, targeting agents, and anti-fouling polymers).

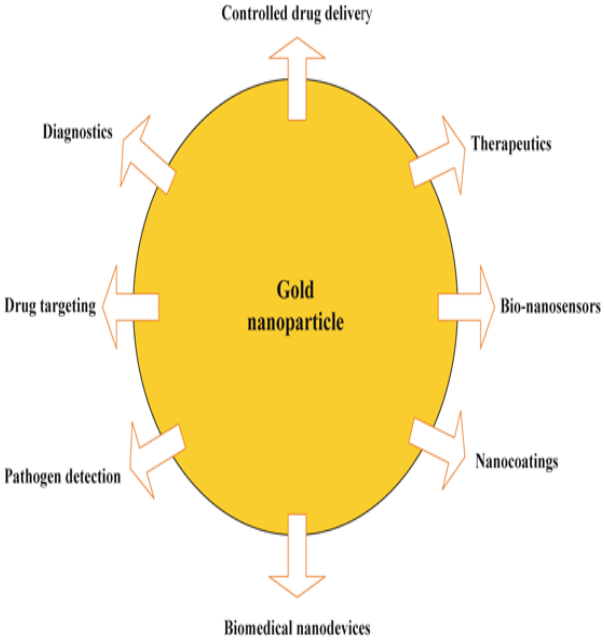
Sensors - Gold nanoparticles are used in a variety of sensors. For example, a colorimetric sensor based on gold nanoparticles can identify if foods are suitable for consumption.Other methods, such as surface enhanced Raman spectroscopy, exploit gold nanoparticles as substrates to enable the measurement of vibrational energies of chemical bonds. This strategy could also be used for the detection of proteins, pollutants, and other molecules label-free.

Probes - Gold nanoparticles also scatter light and can produce an array of interesting colors under dark-field microscopy. The scattered colors of gold nanoparticles are currently used for biological imaging applications.

Also, gold nanoparticles are relatively dense, making them useful as probes for transmission electron microscopy.

Diagnostics - Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents. They are also home pregnancy test.

Catalysis - Gold nanoparticles are used as catalysts in a number of chemical reactions. The surface of a gold nanoparticle can be used for selective oxidation or in certain cases the surface can reduce a nanoparticles are being developed for fuel cell applications. These technologies would be useful in the automotive and display industry application.



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Gold nanoparticles have a broad spectrum of application areas including medicine, food industry, water purification and biological applications.

In particular, gold nanoparticles are applied to drug-delivery, photo-thermal therapy, imaging, sensing, catalysis, and even antimicrobials .The actual list of applications of gold nanoparticles is much longer due to their unique properties.

Gold nanoparticles are known to be biocompatible but conventional reduction methods can leave some toxic chemical species on the surface, which may compromise their advantages.

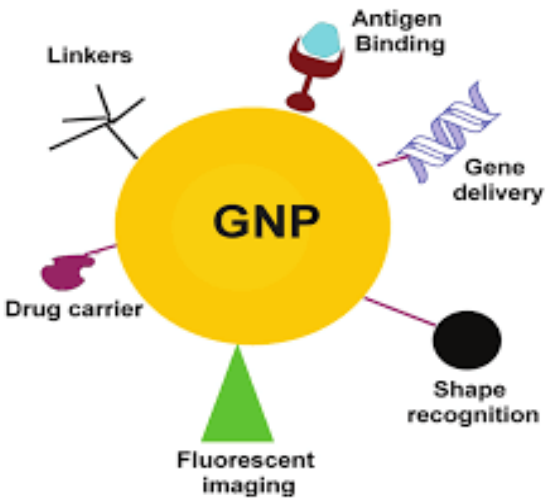
Therefore, greenly synthesized gold nanoparticles have much more potential in different fields.

Gold nanoparticles exhibit natural biocidal properties and although their use as antibacterial agents is not so prominent compared to silver nanoparticles, they still have significant antibacterial effects on several pathogens.

Moreover, green-synthesized gold nanoparticles have also shown their antioxidant and antifungal activity.

Fungi such as eukaryotes possess more resistant cell boundaries compared to bacteria. The efficacy of gold nanoparticles is therefore manifested in their manifold biocidal effects with variable size and shapes have been obtained

Plant mediated synthesis of gold nanoparticles is usually carried out with chloroauric acid ($HAuCl_4$) as a precursor, which is then added to the given plant extract to be reduced into elemental gold.

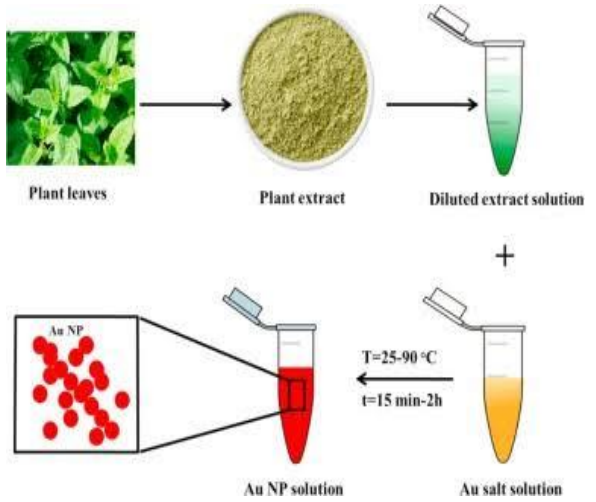


Greenly synthesized gold nanoparticles using plants have low cytotoxicity compared to chemically synthesized gold nanoparticles. These results refer to several shapes and sizes ranging from spherical to triangular and 10 to 300nm respectively shows gold nanoparticles from alkaline pear fruit extract.

Plant extract derived gold nanoparticles are used to coat acrylic glass and window glass and they also have potential as coatings in food packaging materials due to active bacterial mold protection and antioxidant activity.

In the same way, plant mediated gold nanoparticles can be used to cover differen fabrics such as cotton, silk, and leather to achieve antibacterial properties.

Recent reports have shown an enhanced antibacterial activity of green-synthesized gold nanoparticles due to biomolecules originating from plant extracts.



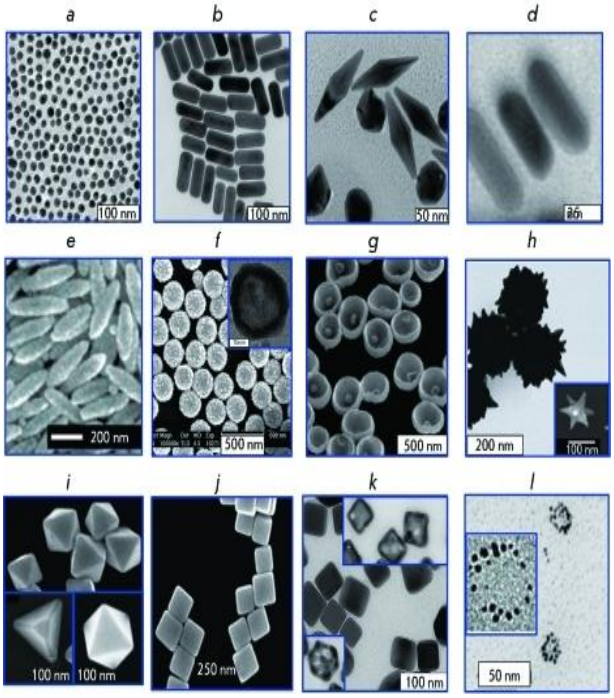
Gold Nano Particles Biological Advances

Functionalized gold nanoparticles with controlled geometrical and optical properties are the subject of intensive studies and biomedical applications, including genomics, biosensorics, immunoassays, clinical chemistry, laser phototherapy of cancer cells and tumors, the targeted delivery of drugs, DNA and antigens, optical bioimaging and the monitoring of cells and tissues with the use of state-of-the-art detection systems. This work will provide an overview of the recent advances and current challenges facing the biomedical application of gold nanoparticles of various sizes, shapes, and structures. The review is focused on the application of gold nanoparticle conjugates in biomedical diagnostics and analytics, photothermal and photodynamic therapies, as a carrier for delivering target molecules, and on the immunological and toxicological properties.

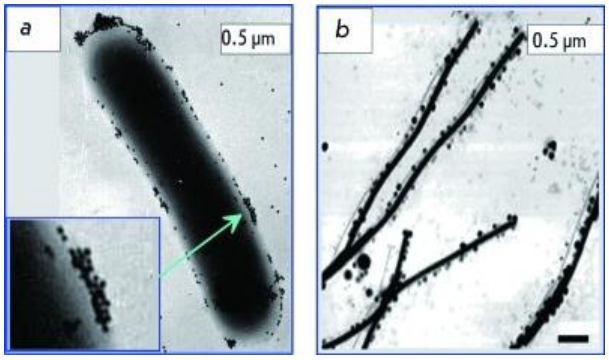
Gold Nano Partcles In Diagnostics

Visualization and bioimaging

Gold nanoparticles have been in active use in the identification of chemical and biological agents. Electron microscopy (predominantly, transmission electron microscopy — TEM) has historically remained the predominant means to detect biospecific interactions using colloidal gold particles (due to their high electron density). It is not by happenstance that the first three-volume publication about the application of colloidal gold was chiefly devoted to TEM using GMP. The use of high-resolution instruments (high-resolution transmission electron microscope – HRTEM) and systems of digital recording and the processing of images are examples of the modern application of electron microscopy equipment. The main practical use of immune electron spectroscopy in modern medico-biological studies is for the identification of causative agents of infectious diseases and their surface antigens. Scanning probe microscopy scanning electron microscopy,, and fluorescence microscopy are frequently used for the same purpose.



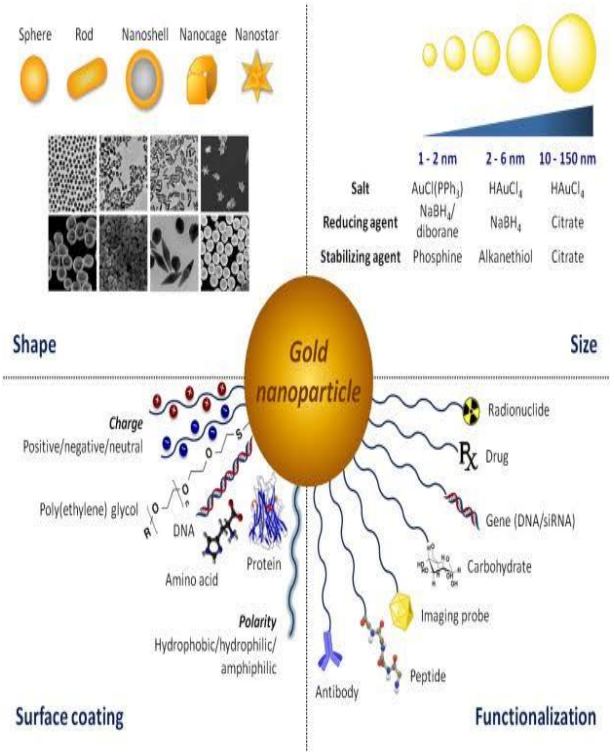
The visualization methods with the use of GNP and optical microscopy in particular, confocal laser microscopy, have gained increasing popularity in medical and biological research. Confocal microscopy is a method for the detection of micro-objects using an optical system, which permits the registering of light radiation only from the objects located in its focal plane; therefore, the scanning of samples along their height can be performed, and their 3D images can be obtained by superposition of scanograms. The use of GNP and antibody–GNP conjugates allows for real-time detection of the penetration of gold into living cells (e.g., cancer cells) at the level of a single particle and even for the estimation of their amount.



The methods for obtaining confocal images include fluorescence detection (confocal fluorescence microscopy) or resonance elastic or two-photon (multiphoton) light scattering by plasmon nanoparticles (resonance scattering confocal microscopy or two-photon luminescence confocal microscopy). These techniques are based on detecting micro-objects using an optical microscope in which the object's luminescence is excited due to the simultaneous absorption of two (or more)

photons; the energy of each of them being lower than that required for fluorescence excitation. The major advantage of this method is that the strong decrease in the background signal results in the contrast being enhanced. The use of two-photon luminescence of gold nanoparticles allows to visualize (amongst other objects) oncomarkers on the surface or inside a cell.

A provides an example of combined bioimaging of a malignant cell using adsorption, fluorescence, and luminescence plasmon resonance labels.

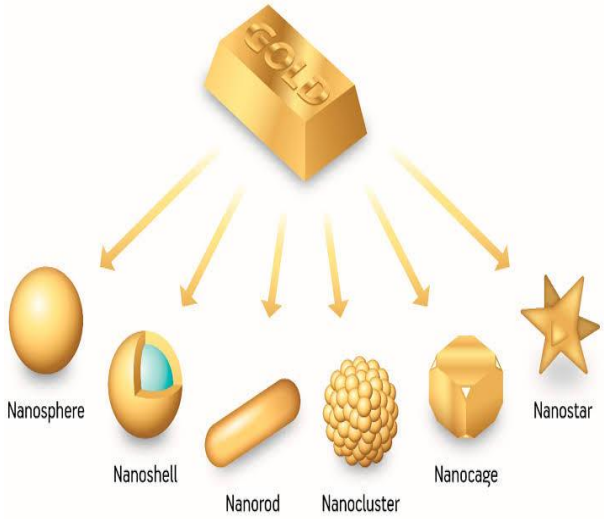


Nanocages belong to a relatively new family of nanoparticles fabricated by galvanic replacement on silver nanocube templates. In this reaction, three silver atoms are replaced by a single gold atom, resulting in the gradual formation of various porous alloy structures of gold and silver, which are called nanoboxes and nanocages .In the formation process of these particles, the plasmon resonance shifts from 430–440 nm for cubes to 700–900 nm for nanocages.

The use of nonspherical and/or heterogeneous particles, as well as self-assembling particle monolayers or island films, opens up new opportunities to enhance sensitivity in detecting biomolecular binding on or near the surface of nanostructures.

The principle of amplification of the biomolecular binding signal is based on inducing strong local electromagnetic fields near particles with sharp regions on their surface or in the narrow (on the order of nanometer or less) gaps between two nanoparticles. It stipulates enhanced sensitivity of plasmon resonance to the local dielectric environment and a high scattering intensity in comparison with spheres of the same volume. Therefore, these nanostructures can be considered as having significant potential for application for biomedical diagnostics purposes using dark-field microscopy.

Gold nanoparticles are used in resonance scattering dark-field microscopy for the detection of microbial cells and their metabolites the bio-imaging of tumor cells and for the detection of receptors on their surface and for the study of endocytosis.



In most biomedical applications, the efficacy of labelling cells with conjugates is assessed at the qualitative level. The method of quantitative assessment of the efficacy of cell labelling with gold nanoparticles that was used for labelling pig embryo kidney cells with gold nanoshell conjugates is one of the few exceptions.

In addition to the aforementioned methods used to detect biospecific interactions using different variants of optical microscopy and GNP, other modern methods for detecting and bio-imaging have recently been in active development; these methods can be combined under the general name “biophotonic methods”.

Biophotonics combines all studies associated with the interaction between light and biological cells and tissues. Biophotonic methods include optical coherence tomography X-ray and magneto-resonance tomography, photoacoustic microscopy and tomography, fluorescence correlation microscopy etc.

Gold nanoparticles of various sizes and shapes are also successfully used in these methods. We believe that biophotonic methods with the use of gold non-spherical nanoparticles may prove to be of considerable promise for *in vivo* bioimaging.

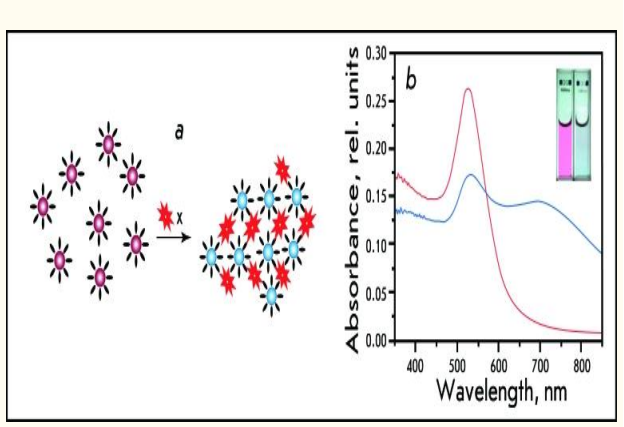
Analytic methods for diagnostics

Homophase methods. Beginning in the 1980s, conjugates of colloidal gold and recognizing biomacromolecules

began to be used in various analytic methods of clinical diagnostics. In 1980, J. Leuvering *et al*. proposed a new method that was called sol particle immunoassay (SPIA). This method is based on two principles:

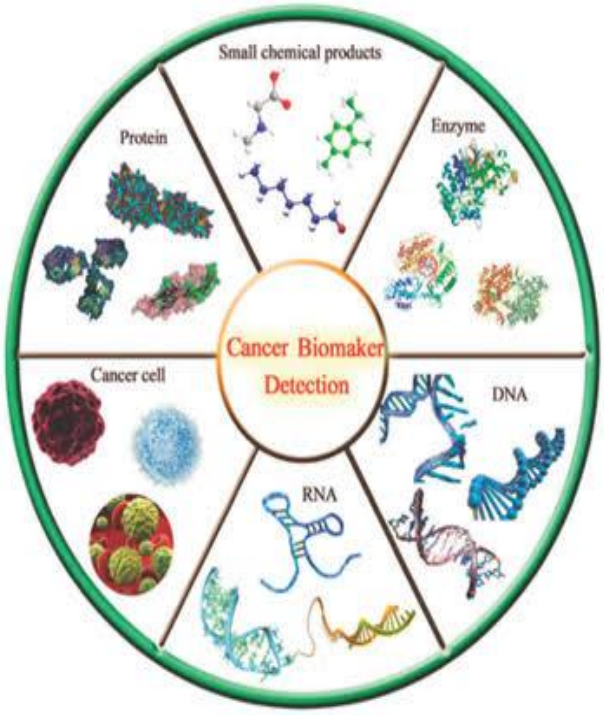
1) The color and absorption spectrum of a sol vary little upon biopolymer adsorption on individual particles
2) When particles approach a distance that is less than one-tenth of their diameter, the sol's red color changes into purpuric; the absorption spectrum broadens and shifts into the red region.

These changes in the absorption spectrum can be easily detected either spectrophotometrically or visually.



An optimized version of this method (using larger gold particles and monoclonal antibodies to various sites of an antigen) was applied to detect chorionic gonadotropin in the urine of pregnant women.

This method was subsequently used for performing immunoassay of the antigens of schistosomes and rubella viruses and for the quantitative determination of immunoglobulins, for determining thrombin (using aptamers) and glucose for the direct detection of cancer cells and leptospira cells in urine, and for determining markers of Alzheimer's disease and protease activity. The simultaneous use of conjugates of gold nanorods and nanospheres with antibodies for detecting tumor antigens.



The data on the determination of the hepatitis B virus in blood using gold nanorods conjugated with specific antibodies.

The implementation of all versions of the SPA method proved to be relatively simple but at the same time both highly sensitive and specific. However, in a number of cases, despite the evident complementarity of a pair, no aggregation took place; the solution's color and the absorption spectra either did not change or changed to an insignificant degree.

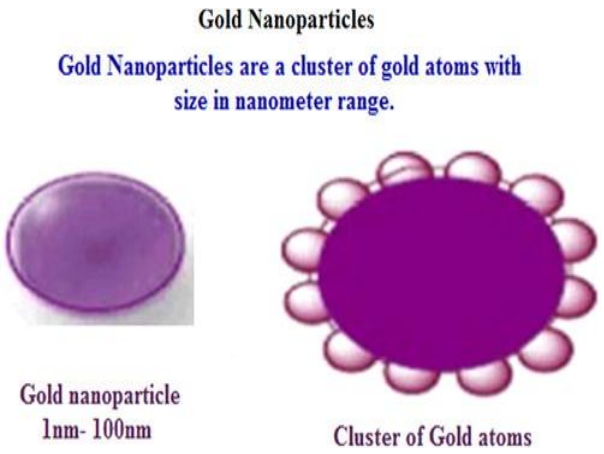
The model of formation of the second protein layer on gold particles without a loss in the aggregate stability of the sol.The changes in spectra caused by adsorption of biopolymers on the surface of gold nanoparticles are relatively small. However, even such negligible changes in absorption spectra resulting from the change in the biopolymer layer structure (or, equivalently, in its average refractive index) near the GNP surface can be recorded and used for a quantitative analysis in biological applications.

Currently, the colorimetric determination of DNA involves two strategies:
(1) The use of GNP conjugated with thiol-modified single-stranded DNA and
(2) The use of nonmodified GNP

The first strategy is based on the aggregation of conjugates of 10–30 nm GNP with thiol-modified single-stranded DNA probes upon introduction of target polynucleotides into the system. In this case, probes of two types are used, which are complementary to two terminal regions of the targets. Hybridization of targets and probes results in the formation of GNP aggregates,

which is accompanied by changes in the absorption spectrum of the solution and can be easily detected visually, photometrically or via dynamic-light scattering

Within the framework of the first strategy, the diagnostic system based on the aggregation of GNP modified by probes of one type upon introduction of DNA targets into the solution under conditions of high ionic strength was used. Meanwhile elaborated a method based on the enhanced stability of conjugates upon the introduction of complementary targets even under conditions of high ionic strength (2 M NaCl), and the aggregation of noncomplementary targets was
observed.



The apparent contradictions between the two approaches were ascribed to the difference in the surface functionalization density.

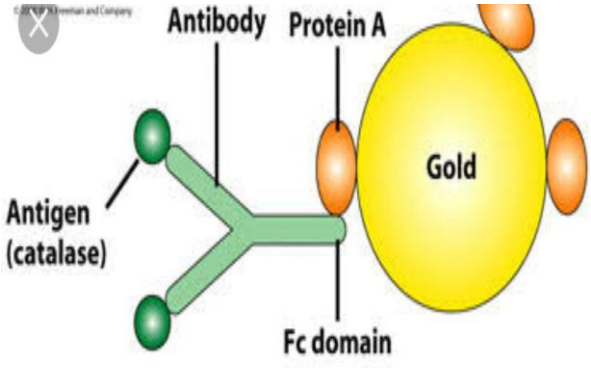
The second strategy is based on the fact that the single-stranded DNA protects unmodified GNP against aggregation upon high ionic strength, while the formation of duplexes upon hybridization cannot stabilize the system. This approach was used to determine the hepatitis C virus.

Recently described a new variant of the second strategy in which single-stranded DNA, unmodified GNP, and cationic polyelectrolyte are used. The same approach turned out to be suitable for determining a wide range of targets, including peptides, amino acids, pesticides, antibiotics, and heavy metals.

Contrary to the procedures with usual GNP, proposed a method for determining HIV-1 U5 viral DNA using nanorods stabilized by cetyltrimethylammonium bromide (CTAB) and the light scattering method with a detection limit of about 100 pM. In the optimized version where absorption spectroscopy is used, the detection limit was reduced to 0.1 pM. It has been recently demonstrated that positively charged GNP coated with CTAB can be used for the detection of DNA targets in combination with spectroscopy and dynamic scattering methods.

The enumerated versions of the method of sol particle aggregation due to the hybridization reaction were used to determine the DNA of micobacteria, staphylococci, streptococci, and chlamydiae in clinical samples.

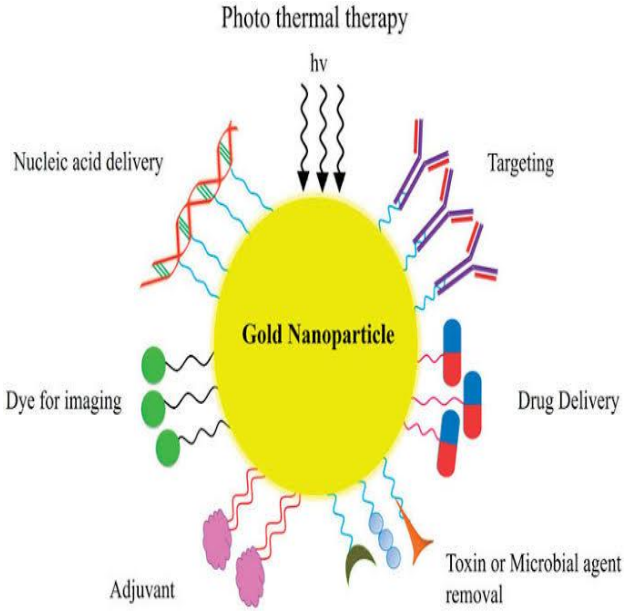
The ability of gold particles to aggregate upon interaction with proteins inducing colour change in the solution served as the basis for the quantitative method of colorimetric determination of proteins.



Gold Nanoparticles In Therapy

Photothermal therapy using gold nanoparticles

Photothermal cell damage is a promising direction in both tumor therapy and the therapy of infectious diseases, which has been intensively developing. The essence of this technique is as follows: gold nanoparticles reach their absorption maximum in the visible or near-infrared region and become hot when irradiated at the corresponding light wavelength. If they are located inside or around the target cells (which can be achieved by conjugation of gold particles with antibodies or other molecules), these cells die.



Thermal exposure has been used in tumor therapy since the 18th century. To do that, both local heating (using

microwave, ultrasound, and radio radiation) and hyperthermia of the entire organism (heating to 41–47°C for 1 h) [were applied. Upon local heating to 70°C, the duration of the procedure can be reduced to 3–4 min. Local and general hyperthermia result in irreversible cell damage caused by the disruption of the cell's membrane permeability and protein denaturation. Healthy tissues are also clearly damaged in this process. All this imposes considerable restrictions on the application of this method.

The revolution in cancer thermotherapy was triggered by the use of laser radiation, which made controlled and directed damaging of tumor tissues possible. The combination of laser radiation with fiber-optic waveguides gave excellent results and was named interstitial laser.

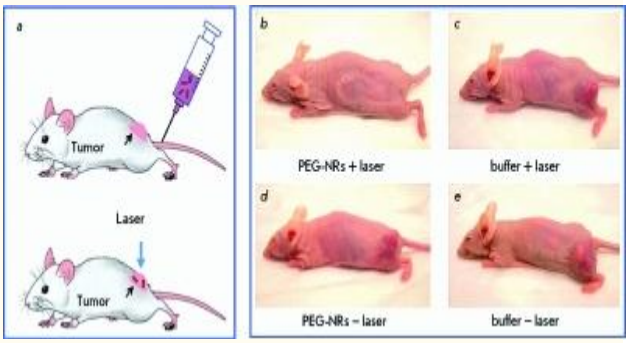
The disadvantages of laser therapy include the low selectivity associated with the necessity of using powerful lasers for the efficient stimulation of tumor cell death.

In 2003, GNP were applied for the first time as agents for photothermal therapy, it was latter proposed to refer to this kind of therapy as plasmonic photothermal therapy (PPTT).

A new method for selective damaging of target cells, which is based on the use of 20–30 nm gold nanospheres radiated by 20 ns laser pulses (532 nm) in order to create local warming-up.. The sandwich technology consisting in labeling T-lymphocytes with GNP conjugates was used for the pulse photothermy in the model experiment. The use of GNP for the photothermal therapy of chemotherapy-resistant types of cancers seems to be the most promising direction. As opposed to photosensitizers (see below), GNP appear unique because the cells retain their optical properties under certain conditions for a significant amount of time. Successive irradiations with several laser pulses allows to control cell inactivation using a method that is not traumatic, while the use of the nanoparticles, properties to simultaneously scatter and absorb radiation makes PPTT possible using optical tomography.

Fig represents an example of the successful therapy of induced tumors in mice. Further development of PPTT and its introduction in clinical practice will depend on how successful scientists will be in solving a host of problems, the most significant ones being

1) selecting nanoparticles with the optimal optic properties; 2) increasing the contrast of nanoparticle accumulation in a tumor and decreasing overall potential toxicity; and 3) elaborating methods for delivering optical radiation to the targets and searching for alternative irradiation sources, which would combine high permeation ability with the possibility of GNP heating.



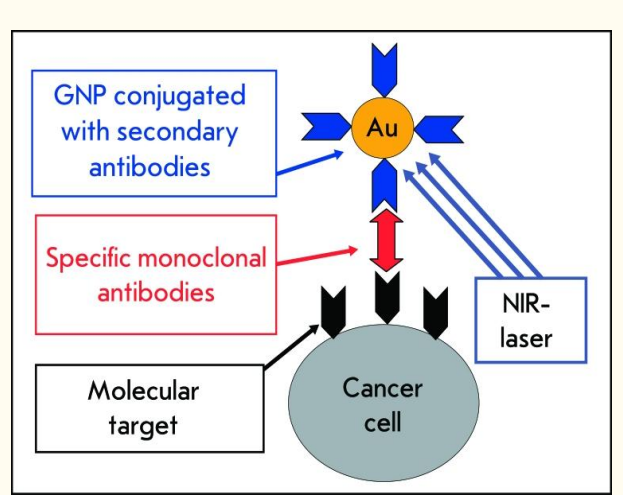
The first requirement is determined by the coincidence of the spectral position of the maximum of the plasmon absorption resonance and the biotissue transparency window in the near-infrared region (700–900 nm). The summarizing theoretical analysis of the photothermal efficiency of GNP depending on their size, shape, structure, and degree of aggregation. It was shown that although gold nanospheres are inefficient in the near-infrared range, their aggregates can be very efficient at appreciably small interatomic distances (below 10% of their diameter). Such clusters form both on a cell' surface and inside cell.

The parameters of gold nanoshells and nanorods that are optimal for PPTT were determined. Today, a number of studies have been published in which the application of gold nanorods, nanoshells, and a relatively new class of particles – gold-silver nanocages – for PPTT is described. The results of a comparison of the efficiency of heating nanorods, nanoshells, and nanocages.

Three fundamental things should be kept in mind in connection with the optimization of the parameters of a particle. First, intrinsic absorption is not the only parameter determining the efficiency of PPTT. The rapid heating of nanoparticles or clusters results in the formation of vapor bubbles, which can cause cavitation cell damage upon irradiation with visible or near-infrared light. The efficiency in the formation of vapor bubbles considerably improves upon the formation of nanoparticle clusters. It is possible that it is this effect, instead of the enhanced absorption, that determines the larger extent of cell damage, other conditions being equal. Finally, irradiation of nanoparticles by high-intensity resonance nanosecond IR pulses may result in the destruction of particles as early as after the first pulse. In a series of studies, attention was on the fact that the heating of GNP and their destruction may result in an abrupt decrease in the photothermal efficacy of "cold" particles tuned to the laser wavelength. The use of femtosecond pulses does not solve this problem because of the low energy supplied; therefore, it is necessary to accurately control the retention of nanoparticles' properties for the selected irradiation mode.
We shall now turn our attention to the second issue connected with the problem of targeted delivery of

nanoparticles into the tumor. This issue has two significant aspects: increasing the contrast in the desired biotarget and decreasing the side effects conditioned by the accumulation of GNP in other organs, primarily in the liver and spleen . Two delivery strategies are typically used. The first strategy is based on GNP conjugation with PEG, and the second one is based on GNP conjugation with antibodies to certain marker proteins of tumor cells. PEG is used to enhance the bioavailability and stability of nanoparticles, resulting in the increase in time of their circulation in blood flow. Citrate-coated gold nanospheres and CTAB-coated nanorods and nanoshells are characterized by low stability in buffer saline solutions. Upon conjugation of nanoparticles with PEG, their stability increases considerably, preventing salt-induced aggregation.

PEGylated nanoparticles are preferentially accumulated *in vivo* due to the enhanced permeability of tumor vessels and are retained in it due to the reduced lymphatic drainage. Moreover, PEGylated nanoparticles possess lower availability for the immune system (stealth technologies). This delivery method is called passive delivery, as opposed to the active method, in which antibodies are used . The active delivery method is more reliable and efficient. Antibodies to tumor markers are used in it. Most frequently, the epidermal growth factor receptor (EGFR) and its varieties (e.g., Her2), and the tumor necrosis factor, (TNF) serve as such markers. The use of GNP conjugated with antibodies simultaneously for diagnostics and photothermal therapy (the so-called theranostics methods) seems to be the most promising. In addition to antibodies, folic acid, ligand of numerous folate receptors of tumor cell, and hormones can be used for active delivery.



The question of the efficacy of targeted delivery of nanoparticles into the tumor has recently resurfaced as the subject of investigation . In experiments with liposomes labeled with anti-Her2-antibodies and GNP labeled with transferrine it was shown that functionalization improves the penetration of nanoparticles into cells; however, the contrast of particle accumulation in the tumor does not improved considerably.

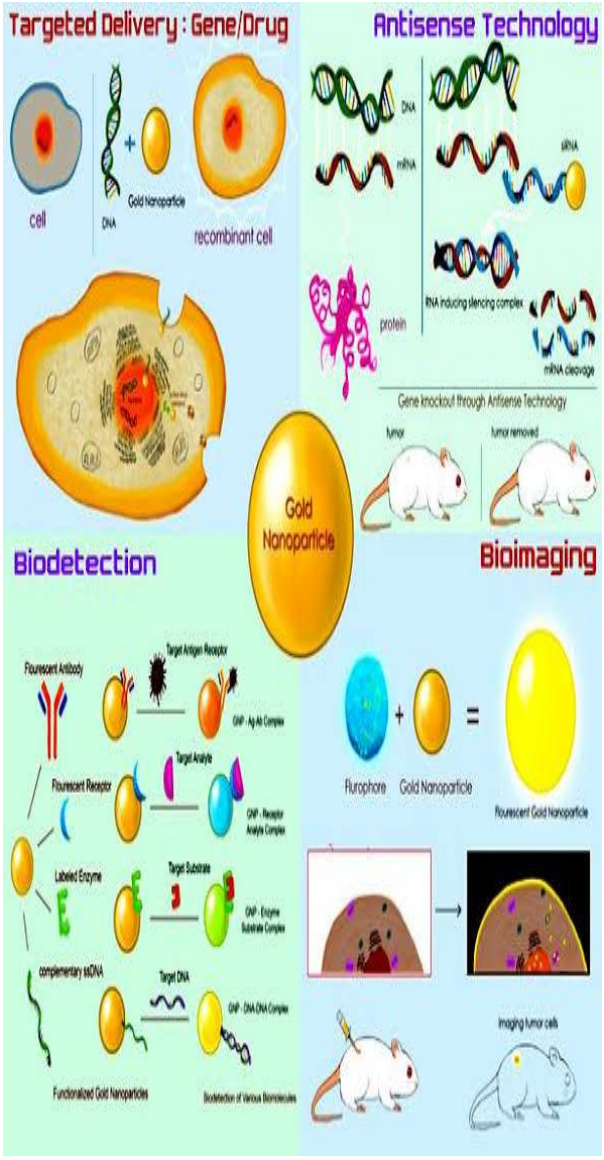
The biodistribution and localization of gold nanorods labeled with three types of probe molecules, including the
(1) scFv-fragment of EGFR antibodies; the (2) N-terminal fragment of the peptide recognizing the urokinase plasminogen activator receptor (uPAR); and the
(3) cyclic RGD-peptide recognizing the $\alpha_v\beta_3$-integrin receptor have been studied . It appears that all three types of ligands fail to significantly improve the contrast of particle accumulation in cell models and in the tumor upon intravenous administration, but they do have a considerable effect on extracellular distribution and intracellular localization. Therefore, a conclusion can be made that in the case of PPTT, the direct introduction of particles into the tumor can be more efficient than intravenous administration.

The last important question associated with modern PPTT has to do with the efficient delivery of radiation to the biotarget. Since the absorption of biotissue chromophores in the visible region is lower by two orders of magnitude than it is in the infrared region, the use of IR radiation dramatically reduces the nontarget thermal dose and increases the deep tissue penetration of the radiation.

Nevertheless, the penetration depth typically does not exceed 5–10 mm therefore, it is necessary to search for alternative solutions. The first approach consists in using impulse (nanoseconds) modes of radiation instead of continuous ones, which allow to increase the intensity of the irradiation without additional side effects.
The second approach consists in using fibre-optic devices for endoscopic delivery of the radiation or delivery inside the tissue.

The advantages and drawbacks of this approach are evident. Finally, radiation with deeper penetration, such as radio radiation, can be used for hyperthermia.

GNP conjugated with antibiotics and antibodies have also been used as photothermal agents to inflict selective damage to protozoa and bacteria.



Photodynamic therapy using gold particles

The photodynamic method is applied in the therapy of oncological diseases, certain dermal or infectious diseases, and is based on the use of light-sensitive agents – photosensitizers (including dyes) and, typically, visible light of a certain wavelength.

Most frequently, the sensitizers is introduced into the organism intravenously; it may also be administered applicatively or perorally. The agents for photodynamic therapy (PDT) can selectively accumulate in the tumor or other target tissues (cells). The affected tissues are radiated with laser light with a wavelength corresponding to the absorption maximum of the dye. In addition to the usual heat release due to absorption, the second mechanism is also significant. It is associated with the photochemical generation of singlet oxygen and the formation of highly active radicals inducing necrosis and apoptosis of tumor cells.

PDT results in tumor malnutrition and death due to the damage inflicted on its microvessels. The major drawback of PDT is that the photosensitizers remain in the organism for a long period of time; as a result, the patient's tissues remain highly sensitive to light. On the other hand, the use of dyes for the selective heating of tissues is characterized by low efficacy due to the small absorption cross-section of chromophores.

It is well-known that metal nanoparticles are efficient fluorescence quenching agents. However, it has been recently demonstrated that the fluorescence intensity can be amplified by a plasmon particle, by locating molecules at optimum distance from the metal. Theoretically, this idea can be used to enhance the efficacy of PDT.

In a number of studies, the proposed method allowed to deliver drugs in polyelectrolyte capsules on GNP that disintegrate under laser radiation and deliver the therapeutic agent to the targets or to use nanoparticles surrounded by a layer of polymer nanogel. Moreover, photoactive agents and peptides facilitating the intracellular penetration are used within conjugates. It has recently been proposed to use composite nanoparticles that, in addition to gold nanoshells, comprise magnetic particles, photodynamic dye, PEG, and antibodies.

Finally, according to the data, nanoparticles conjugated with photodynamic dyes can have a synergetic antimicrobial effect.

Thus, gold nanostructures with plasmon resonance show promise for the selective PPTT of oncological and other diseases. However, it is clear that a number of questions require further study, such as: stability, biocompatibility, chemical interaction between nanoparticle conjugates in physiological environments, blood circulation time, penetration into the tumor, interaction with the immune system, excretion of nanoparticles, etc. We expect that the success in the initial stages in the use of nanoparticles for selective PPTT will be broadened to the clinical stage, provided that the optimal technical parameters are studied further.

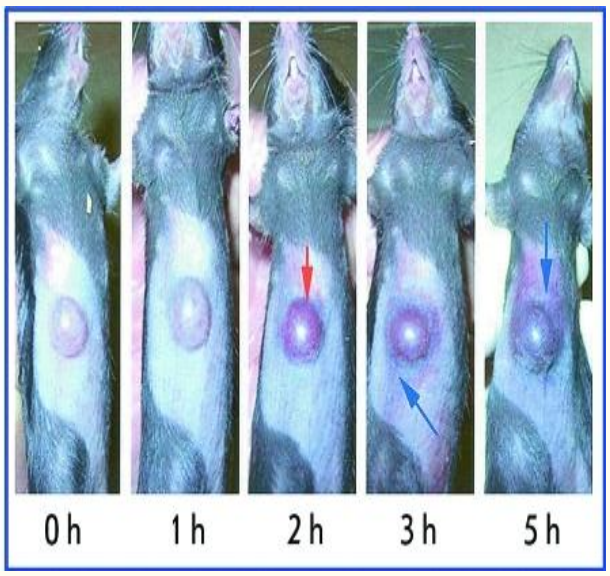
The use of gold nanoparticles as therapeutic agents-

Gold nanoparticles are increasingly actively being used not only in diagnostics and cell photothermolysis experiments, but also for therapeutic purposes. In 1997, the successful application of colloidal gold in a patient with rheumatoid arthritis was first reported.

In 2008, a vast array of data on the ten-year-long clinical trials of the preparation Aurasol for peroral administration upon severe forms of rheumatoid arthritis was published. The positive results achieved upon intra-articular introduction of colloidal gold into rats with collagen-induced arthritis were described. The authors attribute the positive effect to an increase in anti-angiogenic activity due to the binding between GNP and the vascular endothelial growth factor and, therefore, the

decrease in macrophage infiltration and inflammation. Similar results were obtained upon subcutaneous introduction of gold nanoparticles into rats with collagen- and pristan-induced arthritis.

Accumulation of GNP in the tumor is attested by the change in the color of the tumor; the tumor acquires a bright red/purple color (the color typical of colloidal gold and its aggregates), which coincides with the maximum of tumor-specific activity of the TNF. The colloidal gold–TNF vector had lower toxicity and a higher efficacy in reducing tumor size in comparison with the native TNF, since maximum antitumor reaction was attained by using lower doses of the drug.

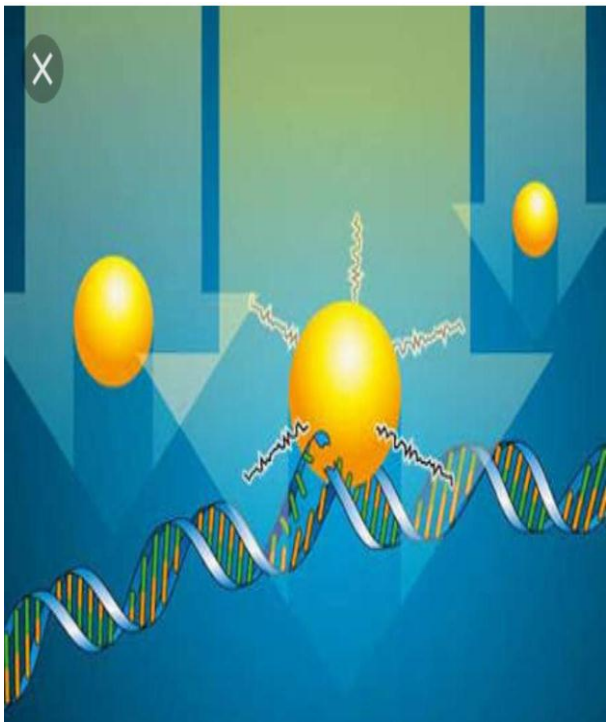


Accumulation of the GNP-TNF conjugates in mice tumors over 5 h after injection. A MC-38 tumor-burdened C57/BL6 mouse was intravenously injected with 15 μg of the GNP-TNF conjugates. The ventral surface of the animal was photographed at the indicated times, showing the color changes of the tumor over 5 hr. Red arrows show tumor uptake in the conjugates; blue arrows show accumulation of the conjugates in the tissues surrounding the tumor

The antiangiogenic properties of GNP were observed *in vitro* and *in vivo*. It turned out that GNP interact with heparin-binding glycoproteins – vascular permeability factors, growth factors of cardiac endothelium and fibroblasts. These agents mediate angiogenesis, including that in tumor tissues; therefore, GNPs inhibit their activity. Since intensive angiogenesis (the process of formation of new blood vessels in organs or tissues) is considered as one of the main tumor growth factors, the existence of antiangiogenic properties in GNPs could make them promising for tumor therapy. It was also demonstrated by the same researchers that gold nanoparticles enhance the apoptosis of the chronic lymphocytic leukemia cells that are stable to programmed death and suppress the proliferation of multiple myeloma cells .

Gold Nanoparticles As Drug Carriers

The targeted delivery of drugs is one of the most promising and actively developing directions in the medicinal use of GNPs. The options of using GNP conjugated with the following antitumor agents were proposed: paclitaxel, methotrexate, daunorubicine, hemcytabin, 6-mercaptopurine, dodecylcysteine, sulfonamide, 5-fluorouracil, platinum complexes, kahalalide, tamoxifen, herceptin, doxorubicin, prospidin etc. The conjugation was carried out either by simple physical adsorption of the drugs onto GNPs or via the use of alkanethiol linkers. The effect of conjugates was assessed both (chiefly) on *in vitro* models, using tumor cell cultures, and *in vivo*, in mice with induced tumors of different natures and localizations (Lewis lung carcinoma, pancreatic adenocarcinoma, etc.) Antitumor agents and antibiotics are the most popular objects of target delivery.



In addition to the active substance, target molecules (e.g., cetuximab) providing better anchoring and penetration of the complex into the target cells were used to design the delivery system. It was also proposed to use multimodal delivery systems, when a gold nanoparticle is loaded with several therapeutic agents (both hydrophilic and hydrophobic) and auxiliary agents, such as target molecules, dyes for photodynamic therapy, etc.

Most researchers note high the efficacy of antitumor agents conjugated with gold nanoparticles.

Antibiotics and other antibacterial agents are also considered as objects that can be delivered by gold nanoparticles. The possibility of producing a stable complex of vancomycin and colloidal gold and the efficacy of such a complex against various

enteropathogenic strains of *Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis* (including vancomycin-resistant strains) have also been demonstrated . Similar results were obtained in.

A complex of ciprofloxacin with gold nanoshells showed high antibacterial activity towards *E. coli* . The anti-leukemia drug 5-fluorouracil, conjugated with colloidal gold, has a noticeable antibacterial and antifungal effect against *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Aspergillus fumigates*, and *A. niger*.

It should be noted that in all of the listed cases, the complexes of drugs with gold nanoparticles were stable, which could be attested by the optical spectra of conjugates.

On the contrary, stable complexes with gold nanoparticles could not be obtained for such antibiotics as ampicillin, streptomycin, kanamycin, hentamycin, neomycin, ciprofloxacin, gatifloxacin, and norfloxacin, which are active against *E. coli*, *M. luteus*, *S. aureus*, and *P. aeruginosa* . Nevertheless, their activity when mixed with colloidal gold was higher by 12–40% than that of the antibiotic when used alone, depending on the antibiotic. On the basis of these data, the authors arrived at the conclusion that the antibacterial activity of antibiotics was enhanced by GNPs. However, the issue of the mechanisms underlying the possible boosting of the antibacterial effect of drugs has remained unsolved. It has been proved experimentally that unbound gentamicin and a mixture of it with gold nanoparticles do not considerably differ in terms of their antimicrobial activity in tests on both dense and liquid nutrient media. It is speculated that stable conjugates of nanoparticles coated with antibiotic molecules are required to enhance the antibacterial activity. Thus, it was proposed to use the antibiotic cefaclor directly in the synthesis of GNPs. As a result, a stable conjugate was obtained. It was characterized by high antibacterial activity against *E. coli* and *S. aureus*.

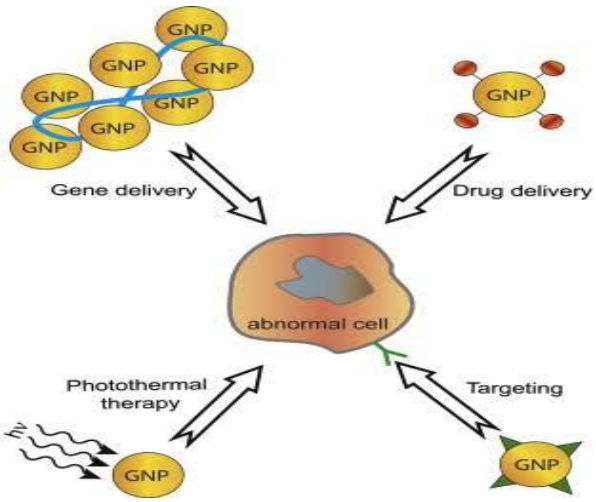
There has been much less data on other drugs conjugated with gold nanoparticles. However, the high anti-oxidant activity of the tocoferol complex with gold nanoparticles should be noted, along with the variants of its potential use that were proposed. Data has been published indicating that, due to high local concentration, GNPs conjugated with the drug TAK-799 manifested a more pronounced activity against the human immunodeficiency virus, as compared with the drug itself. The procedure of per oral and intranasal introduction of insulin conjugated with colloidal gold was elaborated on rat models of diabetes mellitus. A decrease in blood sugar levels comparable with the effect of a subcutaneous introduction of insulin was reliably demonstrated. Finally, the therapeutic effect of the antirheumatic drug etanercept conjugated with gold nanorods.

In the end of this section, we would like to mention gene therapy, which seems to be the ideal strategy concerning genetics, as well as acquired, diseases.

Gene therapy implies an approach based on the introduction of genetic structures into cells and the organism for therapeutic purposes . The desired effect was achieved either due to the expression of the inserted gene or by partial or complete suppression of the function of the damaged or overexpressed gene. Attempts to adjust the structure and function of the ill-functioning (affected) gene were recently made. In this case, gold nanoparticles can act as an efficient agent for delivering the genetic material into the cytoplasm and cell nucleus.

Drug delivery

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Drug delivery technologies are patent protected formulation technologies that modify drug release profile, absorption, distributionand elimination for the benefit of improving product efficacyand safety, as well as patient con- venience and compliance. Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues or other diseased tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation.



Gold nanoparticles in cancer therapy

The rapid advancement of nanotechnology in recent years has fuelled a burgeoning interest in the field of nanoparticle research, in particular, its application in the medical arena. A constantly expanding knowledge based on a better understanding of the properties of gold nanoparticles (AuNPs) coupled with relentless experimentation means that the frontiers of nanotechnology are constantly being challenged. At present, there seems to be heightened interest in the application of AuNPs to the management of cancer,

encompassing diagnosis, monitoring and treatment of the disease. These efforts are undertaken in the hope of revolutionizing current methods of treatment and treatment strategies for a multifactorial disease such as cancer. This review will focus on the current applications of AuNPs in cancer management.

Applications of AuNPs in cancer management-

AuNPs as drug delivery agents targeted to cancer cells

A prominent application of AuNPs is their use as vehicles for delivery of molecules into cells. AuNPs have been described as "promising nanocarriers for therapeutics" owing to their ease of synthesis and functionalization, relative biocompatibility as well as low toxicity in preliminary assays.

However, various factors need to be considered in designing an effective drug delivery system. The properties of AuNPs such as their size, charge and surface chemistry have been shown to affect the uptake of AuNPs into cells as well as their subsequent intracellular fate. In addition, effective drug delivery strategies must take into account the nature of drug-AuNP interaction (covalent/non-covalent binding) as well as the means of drug release following introduction of the drug-AuNP complexes to cells .If AuNPs are used solely as carriers into cells, it is also critical to monitor any toxic effects of residual materials in the cell after delivery; a biodegradable NP vector whose lifespan is limited to the therapeutic window of the drug would be ideal. If the NP vector is cleared from the system once its purpose is reached, it will reduce exposure and limit its toxic effects in the body.

Another issue of concern is the penetration rate of AuNPs into tumors and the specificity of the target sites. Particularly, the epithelial and endothelial barriers are considered to be the main hindrance for the NPs to overcome. Penetration enhancers like metalloproteases against basement membranes and toxins against intracellular tight junctions, may be useful in aiding the uptake of drug-loaded AuNPs into the tumor[44]. Another factor to be considered is the AuNP retention in blood circulation. Some researchers have found that particle retention is also size-dependent and longer circulation time is correlated to higher rate of reaching tumor target. In addition, most studies have only investigated on drug delivery to solid tumors, where it is site specific and easier for quantification of results. It remains to be seen if AuNPs will be effective against non-solid cancers like leukemia where strategies for targeting and treating such cancers can be different from that for solid tumors.

Drug attachment and release from NPs is another challenging area. While the ease of surface modification is what makes AuNP attractive for drug delivery, the strength of drug attachment and timing of the release needs to be suitably controlled to produce the highest therapeutic efficacy. Foremost, the method of release at the tumor site is dependent on how the drug is attached to the AuNP, whether covalently or through non-covalent binding. Generally, drugs in the active form are loaded non-covalently while the covalent-conjugation of the drug to AuNP is in the pro-drug form, thereby requiring a second reaction to release the drug from the attachment as well as to activate it.

Immunologic Properties Of Gold Nanoparticles

Since the 1920s, researchers have shown keen interest in the immunological properties of colloidal metals (gold, in particular). This has been associated mainly with the physicochemical (non-specific) immunity theory proposed by J. Bordet, which postulates that immunogenicity and antigenic specificity depend predominately on the physicochemical properties of the compounds and, first and foremost, on their colloidal state. L.A. Zilber was successful in his attempts to obtain agglutinating sera from colloidal gold.

Moreover, it was shown in a number of studies that the introduction of a rigorous antigen, together with colloidal metals, stimulates the production of antibodies. Furthermore, it was found that certain haptenes adsorbed on colloidal particles can cause the formation of antibodies. In one of the best early reviews, a trove of data on the effect of colloidal gold on nonspecific immune reactions was provided. In particular, it was noted that, 2 h after 5 ml of colloidal gold is introduced intravenously into rabbits, the leukocyte content in 1 ml of blood considerably increases (from 9900 to 19800) against a negligible decrease in mononuclear forms (from 5200 to 4900) and a considerable increase in polynuclear forms (from 4700 to 14900). It should be noted that such effects have not been observed upon the introduction of other colloidal metals. Unfortunately, with the development of immunology and the negation of many postulates in Bordet's theory, interest towards the immunological properties of colloids has abated. However, the data on the amplification of the immune response to antigens adsorbed on colloidal particles has been used in the design of various adjuvants.

It is known that antibody synthesis is induced by agents that have an appreciably developed structure (immunogenicity). They include proteins, polysaccharides, and certain synthetic polymers. On the contrary, a considerable share of biologically active compounds (vitamins, hormones, antibiotics, narcotics, etc.) have a relatively low molecular weight and, therefore, cause a low immune response. In order to overcome this limitation in the standard methods used to produce antibodies *in vivo*, such agents (haptens) are chemically bound to high-molecular-weight carriers (most frequently, to proteins), making it possible to produce specific antisera. However, such antisera usually contain accompanying antibodies to the antigenic structures of the carrier.

In 1986, in a pioneering study by Japanese researchers, information on a successful attempt at producing

antibodies to glutamic acid using colloidal gold particles as a carrier was published. A number of studies were subsequently published, in which this method was applied and developed in order to produce antibodies to the following haptens and rigorous antigens: amino acids, the platelet-activating factor, quinolinic acid, biotin, recombinant peptides, lysophosphatide acid, endostatin, peptides of viral capsid of B and C hepatitis, influenza, murrain, α-amidated peptides, actin, antibiotics, azobenzene, Aβ-peptide, clenbuterol, surface *Yersinia* antigens, transmissible gastroenteritis virus, and tuberculin. In all of the works listed, hapten was directly conjugated with colloidal gold particles and mixed with Freund's complete adjuvant to immunize animals. As a result, sera with a high titre were obtained. The sera required no further purification to remove ballast antibodies**.**

In 1993, it was suggested that hapten (gamma-aminobutyric acid) be bound to the carrier protein before its conjugation with colloidal gold. The proposition was supported in the studies devoted to the production of antibodies to a number of peptides, amino acids, phenyl-β-D-thioglucoronide, and diminazen. The antibodies obtained through this procedure were characterized by both a high specificity to antigens and a higher titre ("extremely high) –from 1 : 250000 to 1 : 1000000, in comparison with those produced using a routine method. The ImmunoSolution company currently offers antibodies to a number of neurotransmitters and amino acids. These antibodies are produced according to the procedure.

In 1996, the possibility of using colloidal gold particles in the antiviral vaccine as the carriers of protein antigen of the capsid of the tick-borne encephalitis virus was first demonstrated. Despite the fact that the vaccine contained no adjuvants, the experimental vaccine had better protective properties as compared with its commercial analogues.
A significant number of studies devoted to the use of GNP in designing DNA vaccines with gene constructions encoding proteins, to which antibodies had to be produced, have been published. In the case of efficient gene expression, these proteins serve as antigens for the development of the immune response. Colloidal gold particles are the most popular examples of nanoparticles–DNA carriers.

The technology used to produce antibodies against various antigens using colloidal gold as a carrier and adjuvant.

In this case, antigens are adsorbed directly at the surface of gold nanoparticles without using any binding agents. It was ascertained that the immunization of animals with an antigen conjugated with colloidal gold (both using Freund's complete adjuvant and without it) results in the obtainment of specific antibodies with a high titre to a wide range of antigens without ballast antibodies. Gold nanoparticles can stimulate antibody synthesis in rabbits, rats, and mice if a lower dose of the antigen is used in comparison with the amount that is required when using a number of conventional adjuvants.

Gold nanoparticles used as antigen carriers were shown to stimulate the phagocytic activity of macrophages and affect the functioning of lymphocytes, which probably is responsible for their immune-modulating effect.

Moreover, gold nanoparticles and their conjugates with low- and high-molecular-weight antigens stimulate the respiratory activity of the cells of the reticulo-endothelial system and the activity of the mitochondrial enzymes of macrophages which may be one of the causal factors behind the adjuvant properties of colloidal gold.

The fact that gold nanoparticles act both as a carrier and an adjuvant (i.e., represent haptens to T-cells) should be considered as the most interesting side of the manifestation of the immune properties of colloidal gold. In particular, gold nanoparticles conjugated with antigens affect T-cell activation: a tenfold increase in the proliferation, as opposed to that upon the addition of a native antigen, was detected. This provides evidence in support of the fact that it is fundamentally possible to act directly on T-cells with the subsequent activation of macrophages and destruction of a pathogen.

However, none of the studies contains data on the mechanisms that underline these properties of gold particles. We consider the discussion in on the preferable macrophage response to corpuscular antigens, as opposed to the soluble ones, to be undoubtedly reasonable. The researchers who study the mechanisms of action of DNA vaccines and use gold nanoparticles to deliver genetic material into the cell also confirm this fact.

The role of Kupfer cells and Langerhans cells in the formation of the immune response was revealed in these studies. The effect of dendrite cells on the formation of the immune response upon the introduction of an antigen conjugated with gold nanoparticles. Moreover, it was noted that when using nanoparticles in medical practice, one should make sure that there are no lipopolysaccharides on their surface. The recent studies were devoted to the interaction between the cells of the immune system and gold nanoparticles.

The penetration of peptide-conjugated GNP into macrophage cytoplasm resulting in their activation was shown by electron microscopy. It was ascertained that after the conjugates interact with the TLR-4 receptors of macrophages, the nanoparticles penetrate into the cell, which is accompanied by the secretion of inflammatory cytokines – TNF, interleukin-1β and interleukin-6–and the inhibition of macrophage proliferation. Upon the introduction of GNP, the amount of macrophages decreases, while their size increases. The level of

interleukin-1 and interleukin-6 and TNF also increases. Another (noninflammatory) mechanism of penetration of gold nanoparticles into macrophages – by interaction with scavenger receptors---is not improbable.The effect on nonconjugated colloidal gold on immune-competent cells *in vivo* was studied.

It was shown that the introduction of GNP into mice results in an increase in the proliferation of lymphocytes and normal killers and an increase in interleukin-2 production.

We believe that the detection of adjuvant properties in GNP creates favorable conditions for the development of a new generation of vaccines.

Biodistribution And Toxicity Of Gold Nanoparticles

All the facts mentioned above are proof that GNP have recently been actively used in different spheres of nanomedicine for diagnostic and therapeutic purposes. Moreover, they are being introduced parenterally into the organism of animals and humans with increasing frequency. The acute questions concerning their biodistribution, blood stream circulation, pharmacokinetics and removal from the organism, as well as possible toxicity at the level of the entire organism or at the level of cyto- and genotoxicity, emerged almost at the same time when GNP started to be used in medicine. It should be noted that data on the biodistribution and toxicity of GNP at the time of writing remained scarce and inconsistent.

It was demonstrated by the analysis of published data that the burst in activity regarding investigations into the biodistribution and toxicity of GNP took place during the past 3–4 years.

Since numerous research groups started their projects independently, there is a vast dispersion in the experimental design, including the size and shape of particles, functionalization methods, types of animals, doses and methods of particle introduction, etc. As a result, there has been serious discrepancies in the data and conclusions on the level and kinetics of biodistribution for toxicity estimations, as well. Yet, certain tentative conclusions can still be made.

Firstly, the organs of the reticuloendothelial system serve as the main target for the accumulation of 10–100 nm GNP; biodistribution homogeneity decreasing with decreasing size. The rapid reduction in particle concentration in blood and their prolonged retention in the organism is associated with the functioning of the hepatobiliary system. Since it takes 3 to 4 months for the accumulated particles to be excreted from the liver and spleen, the question of the doses and possible inflammatory processes is of paramount importance.

Secondly, the available data allows for the reasonable assumption that the effect of nanoparticle penetration via the hematoencephalic barrier depends critically on their size; 5–20 nm being the upper limit. Thirdly, gold nanoparticles 1–2 nm in diameter could be more toxic due to the possibility of irreversible binding to the biopolymers in cells. Also, numerous experiments on cell cultures have revealed no observable toxicity in colloidal particles with a size of 3–100 nm, provided that the threshold dose does not exceed a value of the order of 10^{12} particles/ml.

Data on *in vivo* experiments is scarce and somewhat inconsistent. It can only be assumed that there is no observable toxicity upon the short-term (approximately one week long) introduction of GNP at a daily dose lower than 0.5 mg/kg.

CONCLUSIONS

Thanks to the rapid development in technologies for the chemical synthesis of GNP over the past decade, a great variety of particles with different sizes, shapes, structures, and optical properties are now available to contemporary researchers.

Moreover, the question of the simulation of nanoparticles that would possess the desired physical (optical, thermal, etc.) properties, with subsequent development of the procedures for synthesizing the simulated structures, is now on the agenda.

In terms of applications in medicine, the development of efficient technologies for the functionalization of GNP with different classes of molecules providing stabilization *in vivo* and directed interaction with biological targets is of significance. Today, thiolated derivatives of PEG and other molecules are considered to be the best stabilizing agents. In particular, PEG-coated particles can remain in the blood flow for a longer time and are less susceptible to attacks from the cell components of the immune system.

It is now widely accepted that GNP conjugates are excellent labels for solving the problems of bioimaging, which can be implemented using various optical technologies, including resonance scattering dark-field microscopy, confocal laser microscopy, different variants of two-photon luminescence of GNP, optical coherence tomography, acoustic tomography, etc.

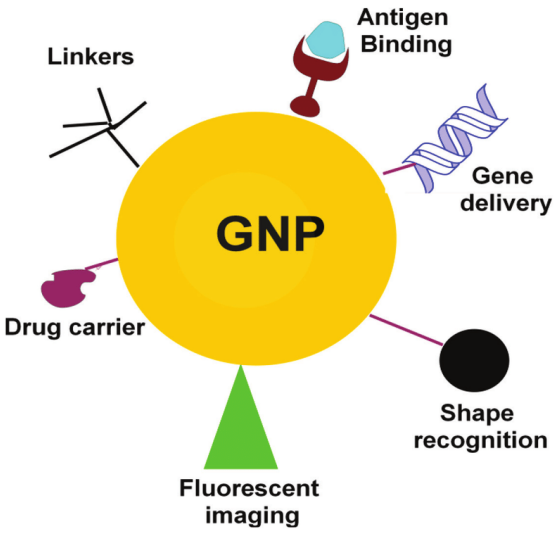
GNP conjugates have found application in analytic studies that can be based both on modern instrumental methods (surface-enhanced Raman spectroscopy, LISNA, IR Fourier spectroscopy, etc.) and on the use of simple solid-phase or homophase procedures (dot analysis, immunochromatography).

Two examples can be given as illustrations: (1) the prostate-specific antigen can be determined using GNP conjugated with antibodies with a sensitivity that is higher than that in the conventional immunoenzyme assay by a factor of 1,000,000.

(2) the strict dependence of color on interparticle distances allows visual detection of mutant DNAs in the so-called "Northwestern spot test" . Along with the examples of clinical diagnostics of cancer, Alzheimer's disease, HIV, hepatitis, tuberculosis, diabetes mellitus, and other diseases, new diagnostic applications for GNP should be expected.

Plasmon photothermal laser therapy of cancer using GNP was first described in 2003 and recently moved into the stage of clinical approval. The actual clinical success of this technology will depend on how quickly several urgent problems can be solved: developing efficient methods for the delivery of radiation to tumors inside the organism using fibre-optic technologies or nonoptical heating methods; (2) elaborating methods for delivering conjugates to tumors, enhancing the contrast and uniformity of accumulation; and (3) developing methods for controlling the *in situ* photothermolysis process.

Targeted delivery of DNA, antigens, and drugs using GNP is one of the most promising directions in biomedicine have revealed the size-dependent possibility of herceptin conjugated GNP into tumor cells with a much higher efficacy in comparison with that of the pure preparation. The recent critical revision of the PPTT concept based on the intravenous-targeted delivery of GNP conjugated with molecular probes to tumor receptors points to the necessity to continue studies in this direction. It seems quite reasonable to use the "non-targeted" PEG-coated gold nanoshells of the SiO



Finally, there is a necessity to continue and broaden studies of the biodistribution and the toxicity of GNP. First of all, a coordinated program is required, which would reveal the correlations between particle parameters (size, shape, functionalization with various molecular probes), experimental parameters (model, doses, method, and administration scheme, observation duration; organs, cells, subcellular structures under study, etc.), and the observed biological effects. Coordinated efforts in the introduction of standards for the particles and methods used for the testing of nanomaterial toxicity are also required.

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