Preparation and application of various nanoparticles in biology and medicine

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Abstract

The present paper considers prospects for application of various nanoparticles in biology and medicine. Here are presented data on preparation of gold and silver nanoparticles, and effects of shape of these nanoparticles on their optical properties. Application of these nanoparticles in diagnostics, for drug delivery and therapy, and preparation of magnetic nanoparticles from iron and cobalt salts are also discussed. Application of these nanoparticles as magnetic resonance imaging (MRI) contrast agents and as vehicles for drug delivery, and preparation of quantum dots and their application as prospective nanoparticles for multiplex analysis and for visualization of cellular processes will be tackled. Finally, prospects for new types of nanocomposites (metallic nano-shells) will be not overlooked.

Introduction

Nanotechnology is a revolutionary direction in modern science that opens new horizons in biology and medicine.[1-7] This direction involves research and development of materials whose sizes are extended form 1 to 100 nm. These sizes are comparable with sizes of molecules and these materials bounded with appropriate molecules can be applied for investigation of molecular processes *in vitro* as well as of processes that take place in live cells without interference of their functions. In the case of metals, when their sizes are decreased to nanoscale range they acquire new unique optical properties that are not inherent in bulk materials. As a consequence of reduction of the sizes of these materials electronic motion is reduced and in this case surface effects begin to dominate. Here the optical properties are determined by a collective oscillation of conduction electrons in resonance with incident electromagnetic radiation. This phenomenon is termed surface plasmon resonance (SPR).[8-9] It is apparent that if in such materials the boundary processes are dominated these bands of surface plasmon resonance will become very sensitive to any change in size and form of these nanomaterials and also to any process that can take place on their surface, in particular absorption and desorption of molecules, aggregation of these particles etc. In this aspect gold and silver nanoparticles have particular interest for scientists. At first, these nanoparticles can be prepared in nanosize range without any problem and at second it is more important that the SPR bands of these nanoparticles lie in visible region.[2] It means that processes that will take place on the surface of these nanoparticles will bring to changes in bands of SPR. Therefore interactions between molecules immobilized on gold nanopaticles with their ligands will bring to changes in optical properties of colloid that can be detected photometrically. Moreover gold nanoparticles have affinity for SH groups and as a result the protein molecules that usually contain such groups can be easily immobilized on their surface.[10] As a rule immobilization on gold nanoparticles does not influence the properties of bounded biomolecules.[11] In addition, gold and silver nanoparticles have high light scattering. The magnitude of light scattering by these nanoparticles can be some orders higher than that of light emission from strongly fluorescing dyes. These unique properties have enabled to employ many important and promising applications of metal nanoparticles in biomedicine such as molecular and cell imaging, biosensing, bioassays, and photothermal therapy.

Another type of nanoparticles of great interest for researchers is magnetic nanoparticles.[12] These nanoparticles are prepared from salt of such metals as iron, nickel, cobalt etc and application of these nanoparticles is also constantly extended. Since these nanoparticles have magnetic properties, they can be manipulated by an external magnetic field. So such nanoparticles can be applied in magnetic resonance imaging for effective detection for instance of cancer tissues. On the other hand, these nanoparticles sensitized with appropriate ligands can be applied for separation of cells of interest such as cancer and fetal stem cells, various classes of immune cells *etc*.[13-15]

Another type of nanoparticles is semiconductor quantum dots (QD): tiny light-emitting particles on the nanometer scale, which are considered as a new class of fluorescent probes for biomolecular and cellular imaging.[16] These nanoparticles were obtained recently and they are nanocomposite materials containing core/shell in various proportions for various materials such as ZnS, ZnSe, CdSe, CdTe, PbS, PbSe etc. In comparison with the known organic dyes and fluorescent proteins, quantum dots have unique optical properties, QDs have size- and composition-tunable fluorescence emission from visible to infrared wavelengths, and one light source can be used to excite multiple colors of fluorescence emission. It permits to prepare multifunctional and multicolored nanoparticles for simultaneous stable observation of various cellular processes under complex conditions *in vivo*.

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Colloidal gold and silver preparation

Colloidal gold and silver spherical nanoparticles can be prepared by reduction of diluted salt solutions of these metals ($HAuCl_4$, $AgNO_3$) by various reducers Usually as such reducers can be applied sodium citrate, ascorbate, white phosphor, tannic acid, glucose, hydroquinone *etc*.[17-22] By variation of the type of reducer, its concentration and concentration of metal salts it is possible to obtain spherical particles with various sizes from 3-140 nm. Optical spectra of these particles are different. Gold particles prepared by these reducers are characterized by SPR absorbance band ranging from 510-540 nm depended on their particle sizes. Small particles are purple whereas large particles are blue. It should be noted that optical spectrum of such spherical gold nanoparticles is changed non-significantly depended on particle sizes. So, spherical gold nanoparticles with sizes of 9 nm have SPR band at 515 nm, whereas particles with sizes of 22 nm have SPR at 520 nm.[23] Therefore only processes that bring to essential aggregation of such particles can be detected. As a result the application of such gold nanoparticles in various recognition processes has limited sensitivity. However, recently were prepared rod-like gold nanoparti-

cles which optical properties are more sensitive to processes that take place on their surface.[24-25] Preparation method employs two steps for the formation of such nanoparticles. In the first step, very small-sized gold nanoparticles are synthesized by a rapid reduction of gold salt solution using a strong reducing agent like $NaBH_4$ in the presence of hexadecyltrimethylammoniumbromide. These particles act as seeds for consequent growth in the presence of a weak reducing agent like ascorbic acid in the second step. Such nanoparticles are characterized by optical spectrum with two bands of SPR. So in this case in addition to band around 520 nm a new resonance band is arisen at 700-800 nm depended on ratio between longitudinal and transversal axes of these nanoparticles. For such type of gold nanoparticles optical changes during any interaction take place more sharply so in this case sensitivity of the noted assays is increased. Silver nanoparticles with sizes ranging from 15-30 nm have absorbance around 395-410 nm. These nanoparticles can be prepared by reduction of silver nitrate salts by such reducers as sodium borohydride, sodium citrate, tannic acid, *etc*. In contrary to gold nanoparticles there are only limited publications about preparation and optical properties of silver nanoparticles with various shapes and sizes.[26-29]

Application of gold and silver nanoparticles

Although gold nanoparticles were applied for a long time as electron dense agents in electron microscopy,[30] their application in situations where their unique optical properties are used has more short history. First investigations on application of spherical gold nanoparticles in immunoagglutination assays were conducted by Leuvering and colleagues.[31] They have sensitized gold nanoparticles by antibodies to human chorionic gonadotropin and then after the addition of appropriate antigen the agglutination of these nanoparticles has been observed photometrically by the changed color of initial solution. The authors have shown that particles with size of 50 nm are more applicable for these purposes. In the presence of appropriate antigen the intensity of SPR band at 520-530 nm is decreased and a new shoulder is appeared at 630-640 nm. In other words the color of purple particles is converted into blue. This approach was further applied by other researchers for detection of other antigens.[32-34]

Sequence-specific methods for detection of polynucleotides are essential for diagnosis of genetic diseases and bacterial infection. Generally these methods are based on hybridization of immobilized target with complementary oligonucleotide probe bounded with reporter molecule. Mirkin laboratory[35-36] have applied immobilization of target oligonucleotides on gold nanoparticles and detected photometrically the changes in optical spectrum as a result of hybridization with complementary oligonucleotide. They have demonstrated that even such a low concentration as 10 fmol of an analyte could be detected by exploiting the agglutination phenomenon of these nanoparticles. There are few successful approaches for very sensitive detection of bacteria. For instance, Storhoff *et al.*[37] were able to detect 50 fM of Staphylococcus DNA only after about 1 h of hybridization. Another rapid and relatively inexpensive method of DNA detection was developed by Baptista and coworkers.[38] Here, gold nanoparticles were functionalized with a specific oligonucleotide complementary to *M. tuberculosis* sequence. The presence of the appropriate bacteria in the system brought to hybridization with its target sequence and as a result to stabilization of gold nanoparticles. Consequent addition of concentrated solution of NaCl does not aggregate these particles with characteristic color changes, and vice versa the absence of bacteria brought to aggregation of gold nanoparticles by the addition of salt solution forming blue color. One of the disadvantages of this approach is the additional step of polymerase chain reaction (PCR) to increase concentration of analyte. Colloidal gold nanoparticles sensitized by some ligands were successfully applied for cancer detection. One of such approaches involves determination of protein kinase activity.[39] It is well known that this process plays an important role in various cellular processes. High levels of phosphorylation are indicators for some diseases in particular for many types of cancer. A procedure for determination of protein kinase activity by gold nanoparticles sensitized by target peptides was developed. Phosphorylation of these peptides on the surface of gold nanoparticles brought to elevation of anionic charges in the phosphorilation sites. As a result the net charge of the peptide is changed and aggregation of gold nanoparticles took place with consequent color changes. Dynamic light scattering of gold nanoparticles was successfully applied for quantitative determination of such a well known cancer marker as prostate specific antigen in free and total forms.[40] In this approach changes in light scattering upon interaction of antigens with their ligands were determined. A similar approach was applied for quantitative determination of human chorionic gonadotropin.[41] Furthermore, gold nanoparticles were applied for investigation of cell-ligand interactions. So, interaction of lectins immobilized on colloidal gold particles with lymphocytes brought to drastic changes in SPR bands. As a result of binding with cells, agglutination of gold-lectin-lymphocyte complex occurred with a decrease of absorbance at 520 nm. This approach permitted to detect the presence of 3000 lymphocytes/ml within 1.5 h without any additional procedure.[42] Hence, lectin sensitized gold nanoparticles were successfully applied for investigation of carbohydrate composition of cells. This approach has demonstrated that sensitized gold nanoparticles can be applied to study any ligand cell interaction with very high sensitivity.

Researches on application of silver nanoparticles in immunoagglutination assays are very limited. There are data about application of silver and gold glyconanoparticles for detection of Concanavalin A (Con A). It was shown that mannose-sensitized silver nanoparticles (16 nm) at a concentration of 3 nM provided an assay for Con A with the largest linear range (between 0.08 and 0.26 mM) in comparison with gold nanoparticles of same sizes. It should be noted, that although these authors have applied in their assays as gold as well silver nanoparticles of same sizes silver nanoparticles have provided more sensitivity.[22] Recently, silver nanoparticles sensitized with antibodies developed against human IgG were applied for determination of human IgG. It was shown that these sensitized silver nanoparticles are able to detect human IgG in concentration range from 25 to 600 ng. Moreover it was shown that silver nanoparticles can be prepared in much more concentrated form than gold nanoparticles.[29] It opens new horizons for application of silver nanoparticles in such assays. Recently, it was reported about synthesis of polycrystalline silver nanoparticles that exhibit both bright luminescence and a large enhancement effect on the Raman scattering signals of proximal molecules. The number of photons emitted from these NPs exceeded that from quantum dots or dye molecules by approximately of 2 and 5 orders of magnitude, respectively. Since fluorescence also can be sensitive to various factors these nanoparticles may be applied effectively in various bioassays.[43]

Selective drug delivery to their targets plays a key role in treatment of diseases. Development of such systems for cancer treatment is very demanded because of high toxicity of these drugs. Drug delivery to cancer tissues can be facilitated by particles sensitized with drugs that are capable of escaping the clearance by the reticuloendothelial system and on the other hands they are selective for tumor tissues. The application of such selective drug delivery systems is able to provide the preferential accumulation of drug in tumor microenvironment. Such approach with gold nanoparticles sensitized with tumor necrosis factor (TNF) was applied for delivery of this drug to tumor tissues. Dose-dependent effects of free and immobilized drug demonstrated that particle delivery system has more efficacy.[44] Additionally, such particle delivery sys-

tems can be applied also for photothermal theraphy (PTT) of tumors. Tumors are selectively destroyed at temperatures range of 41-47°C because of their reduced heat tolerance compared with to normal tissue. The principle of the photothermal therapy is the selective heating and destroying of the local environment. When the PTT agents absorb light, electrons make transitions from the ground state to the excited state. This excitation energy subsequently relaxes through non-radiation decay channels. This brings to the increase of the kinetic energy leading to the overheating of the local environment around the light absorbing species. The heat produced can be employed for destruction of neighboring tissues. From the point of the view of cancer therapeutics, noble metal nanoparticles become very useful as agents for PTT on account of their enhanced absorption cross sections, which are four to five orders of magnitude larger than those offered by conventional photoadsorbing dyes. Moreover, optical properties of these nanoparticles can be tuned and they will absorb light in area where tissue components are relatively transparent and any non specific interference will be absent. The preliminary results on application of gold and gold composites for such therapy were obtained and they demonstrate prospects for such approach.[45,46]

Magnetic nanoparticle preparation

General scheme of preparation of iron oxide magnetic nanoparticles involves the following procedures. These magnetic nanoparticles usually are prepared by chemical co-precipitation method. This procedure involves mixing the appropriate solutions of $FeCl_3$ and $FeCl_2$ and PEG4000. Then concentrated NH_3 aqueous solution is added until the pH value of the solution reached to 9.5. After incubation at 40°C for 30 min, the PEG4000-modified Fe_3O_4 solid precipitation is magnetically separated, washed with water and dried. By this approach it is possible to obtain monodisperse magnetic nanoparticles.[47] However application of such metallic magnetic nanoparticles is limited because of their sensitivity to oxidation. One of the ways to prevent these processes is application of other metals for preparation of magnetic nanoparticles or application of nanocomposites of iron with other metals which will increase their chemical stability.[48-49] So cobalt nanoparticles are more stable and have very satisfactory magnetic properties. Synthesis of Co nanoparticles is conducted via thermal decomposition at 180°C of cobalt carbonyl dissolved in dichlorobenzene containing oleic acid and tri-n-octylphosphine oxide. The nanoparticles of cobalt prepared by this approach have relatively uniform sizes of ~10 nm.[48] Further various nanocomposites of iron with other metals were prepared and their magnetic properties were studied.[50] It is very important that composition, size, morphology and surface chemistry of nanoparticles now can be tailored by various processes not only to improve magnetic properties but also affect to the behavior of nanoparticles *in vivo*.

Application of magnetic nanoparticle

The penetration of magnetic fields through human tissue and the ability to remotely detect or manipulate magnetic materials is very attractive for various area of medicine.

One of the more recent and significant applications of these properties has been the application of magnetic nanoparticles in magnetic resonance imaging (MRI) as a non-invasive imaging system capable of providing high resolution anatomical images. Magnetic nanoparticles have been examined extensively as MRI contrast agents to improve the detection, diagnosis, and therapeutic management of solid tumors.[51-53] As it was shown the particle size influences both the physicochemical and pharmacokinetic properties. Very large particles with mean diameters of 300 nm or micrometers are in use as MRI contrast agent for gastrointestinal tract. Large (150-40 nm) magnetite particles are suitable for imaging liver and spleen, whereas small nanoparticles (40-20 nm) are needed to visualize targeted tissues, *e.g.* tumors.[54] Similarly, these nanoparticles have been proposed as MRI contrast agents for several other clinical applications in cardiovascular medicine including myocardial injury, atherosclerosis, and other vascular diseases.[55,56] The uptake of nanoparticles by macrophages, which have been shown to be marker of unstable athermanous plaques has also been exploited to visualize these lesion prone arterial sites. Clinical studies have demonstrated that MR imaging may be useful in evaluation of the risk of acute ischemic events. Recently investigations were conducted by preparations of various magnetic nanocomposites and were evaluated their effects on MR enhancing signals. In particular authors have tested the ability of these nanoparticles to detect various cancer cell lines. It was shown that Mn containing magnetic nanocomposites sensitized with antibodies to cancer target antigen herceptin are ultrasensitive for cancer detection.[57] Visualization of cancer cells with such nanoparticles supposes that these nanoparticles can be applied also for drug delivery to tissues of interest. However, here there is a problem connected with engulfment of these nanoparticles by reticuloendothelial system of the organism. It is particularly significant for nanoparticles with sizes of more than 100 nm. To avoid these effects nanoparticles are covered by some polymers (dextran, polyethylene glycol, *etc*.). In this case these nanoparticles bounded with chemotherapeutic agents can be delivered to cancer tissues attracted and held in the tumor region by a strong external magnetic field gradient. As it was reported in this case tumor remission is achieved without any negative side-effects which are common after regular cancer chemotherapy.[58-60]

Magnetic nanoparticles sensitized with appropriate biomolecules (antibodies, lectins, oligonucleotides, *etc*.) can be applied effectively for bioseparation of molecules and cells of interest from complex biological systems. In this case nanoparticles sensitized by the noted ligands are incubated in complex mixture of biomolecules and cells. Then after the influence of external magnetic field these particles can be separated from this mixture. After removal of all other components by washing, molecules and cells of interest can be obtained after removal of external magnetic field.[47, 61-63]

Preparation of semiconductor quantum dots

High-qualit y QDs are usually prepared at elevated temperatures in organic solvents such as trinoctylphosphine oxide (TOPO) and hexadecyl amine. These hydrophobic organic molecules serve not only as reaction medium but also coordinate with unsaturated metal atoms on the QD surface to prevent formation of bulk semiconductors. As a result, the nanoparticles are capped with a monolayer of the organic ligands and become soluble only in non polar hydrophobic solvents such as chloroform.[64-65] Therefore for application in biological systems an additional modification of nanoparticle's surface must be done. Recently, methods for preparation of water soluble quantum dots were developed.[66-67] One of these procedures for typical synthesis of CdTe QDs involves the following steps. Fresh solution of NaHTe is prepared by dissolving of Te powder in $NaBH_4$ solution under nitrogen atmosphere at room temperature. Then the NaHTe solution is added into a solution of $CdCl_2$ containing mercaptopropionic acid. The pH value of the reaction solution is brought to 9.5. The solution is then heated to 95°C under nitrogen atmosphere flow. After about 10 hours the solution is cooled naturally to room temperature. Fluorescence quantum yield of these dots is approximately 25%.

Application of quantum dots

Prospects for wide application of quantum dots in medicine is associated with stability of their fluorescence in comparison with of traditional organic fluorophores and with possibilities to prepare multi-colored nanoparticles that are excited by one broad band.[68] It permits to track cellular processes in real time and on the other hand to analyze various parameters simultaneously. Quantum dots have size- and composition-tunable fluorescence from visible to infrared wavelengths, and one light source can be used to excite multiple colors of fluorescence emission. Very large stokes spectral shifts (measured by the distance between the excitation and emission peaks) permit to improve the sensitivity of detection. It is essential since during *in vivo* molecular imaging processes auto-fluorescence from tissue biomolecules takes place creating high background that interferes the label signal. The Stokes shifts of semiconductor QDs can achieve 300-400 nm. It permits to effectively remove the background signals from tissue auto-fluorescence. Moreover, fluorescence is very sensitive to various changes in external factors and in this aspects this type of nanoparticles can be applied for monitoring of these changes. So, since the fluorescence intensity of QDs is found to correlate with the acidity (or alkalinity) of the environment this property was applied for determination of urea. Urease splits urea and during this reaction pH increasing takes place and in this case intensity of fluorescence of QDs is also changed. These changes correlate with urea concentration.[69]

Cellular labeling using organic dyes and fluorescent proteins has great success, and modern instrumentation currently allows simultaneous measurement of up to 13 parameters on individual cells. Nevertheless, traditional fluorophores suffer from several problems, such as photo-bleaching, spectral interference from other molecules and narrow band of excitation. Quantum dots have potential to overcome these problems.[70-73] As it was demonstrated the QD-labeled cells are brighter and more resistant to photo-bleaching. In fact, organic dyes are often photo-bleached and fade by 90% in less than one minute, whereas the QDs are stable for more than 30 minutes under identical experimental conditions. Fluorescence immuno-labeling for detection of different molecules in cells is widely used in cell biology. Owing to their robust optical properties, QDs are ideal probes in this area. Experiments were conducted in which QDs have been used to localize molecules in cells and tissues, both in live and fixed specimens. So, Kaul and colleagues[74] reported immunofluorescence labeling of the heat shock 70 protein, mortalin, using QDs to show different staining patterns in normal and transformed cells. Their study showed that QD labeling had higher sensitivity than similar approaches using conventional dyes. Furthermore, specific QDs probes were developed to elucidate breast cancer cell surface marker Her 2.[75] It was shown that QDs provide more sensitive detection system. QDs were applied successfully for investigation of as intracellular as well as intercellular signal processes.[68,76]

QD-based fluorescence resonance energy transfer (FRET) was applied successfully in various types of bioassays.[70,77,78] So, QDs as acceptors for FRET have been applied in nucleic acid hybridization assays by using Renilla luciferase and QDs labeled with complementary oligonucleotide probes. In the absence of target DNA, the chains hybridized, bringing to close proximity of QDs and luciferase, which resulted in elevation of fluorescent signal of QD as a result of FRET. The emission intensity decreased when the target DNA competed to hybridize with the QD probe.

Investigations directed to multiplexed detection of various antigens simultaneously in EIA like analysis where QDs conjugated antibodies were applied instead of enzyme conjugated antibodies were conducted. Here four various antigens were detected simultaneously but sensitivity of the test was not significantly higher than in EIA.[79,80]

Other nanocomposites

The processes of preparation of novel nanocomposites with new interesting properties still continue. The nanoscale coating of colloid particles with a thin metallic layer to form core-shell nanoparticles or the so-called metallic nanoshells is an active area of research in nanoscience and nanotechnology. Two of the most commonly used metals for the synthesis of metallic nanoshells are gold and silver. As a core for such nanocomposites, there are polystyrene latex nanoparticles, silicone, *etc*. Optical properties of these nanoparticles are determined by SPR of metal nanoshells. Theoretically particles can be prepared where SPR will be shifted to any desired wavelength in the visible and infrared ranges by varying the size ratio of the nanoparticle core and the surrounding metallic shell.[81,82]

Recently, such nanoshell began to be applied in immunoassays of various biological fluids. It was shown that the assay can be conducted in whole blood without any interference from hemoglobin. Such assay is able to detect subnanogram/ml contents of various analytes during 30 min. Interaction of antibody with appropriate antigen shifts plasmon resonance band to infrared.[83] Similar systems were applied by other authors where they have measured surface enhanced Raman scattering for detection of any analytes.[84] Gold nanoshells have also been successfully demonstrated to be a good contrast-enhancing agent for photoacoustic tomography.[85] Nano- or submicron-scale metallic nanoshells are accumulated around tumor sites via passive mechanism referred to the so called *enhanced permeability and retention effect* which is attributed to dysfunctional anatomical conditions such as localized leaky circulatory and lymphatic systems. Although MRI also provides tumor imaging via accumulation of magnetic nanoparticles in tumor tissues here more deep penetration takes place.

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