December 2008 *Volume 2 Issue (2-3)*

Trade Science Inc.

Nano Science and Nano Technology

An Indian Journal

Review

NSNTAIJ, 2(2-3), 2008 [48-52]

Gold nanoparticles: Shining light on cancer treatment

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Received: 13th September, 2007 ; Accepted: 18th September, 2007

ABSTRACT

Many chemicals are continuously being screened as antitumor agents. Advances have been made to envisage site-specific delivery of chemotherapeutic drugs which can effectively reduce problems associated with drug resistance and toxicity. Tremendous progress has been made in the exploitation of the photothermal properties of gold nanoparticles in therapeutics. Nanoparticles of gold, which are in the size range 10-100nm, undergo a plasmon resonance with light. This is a process whereby the electrons of the gold resonate in response to incoming radiation causing them to both absorb and scatter light. This effect can be harnessed to either destroy tissue by local heating or release payload molecules of therapeutic importance. Gold nanoparticles can also be conjugated to biologically active moieties, providing possibilities for targeting to particular tissues. We describe the recent studies regarding synthesis and use of such metal nanoparticles for early cancer detection and treatment.

KEYWORDS

Gold nanoparticles;
Cancer;
Plasmon resonance;
Smart bombs.

INTRODUCTION

One of the most important properties of gold nanoparticles which make them agents of choice in detection and treatment of cancer is their biocompatibility. Since the traditional systems of medicine, colloidal gold has been successfully used for rejuvenation especially in the old age under the name "Swarna Bhasma" (swarna=gold, bhasma=ash). Owing to its non-toxic nature, it is in use in Ayurveda. Gold nanoparticles have a very large surface area to volume ratio. Hence a large amount of drug can be loaded on a single nanoparticle. Surface modifications can be carried out in order to attach organic compounds such as drugs, peptides and antibodies to these nanoparticles. Gold nanoparticles also have anti-angiogenic properties. They inhibit the function of certain heparin binding growth factors. Thus, these nanoparticles alone can also induce apoptosis[1]. Ease of characterization is another advantage that these nanoparticles provide. Characterization can be done using a variety of techniques such as electron microscopy, atomic force microscopy[AFM], dynamic light scattering[DLS], X-ray photoelectron spectroscopy [XPS], powder x-ray diffractometry[XRD], and fourier transform infrared spectroscopy[FTIR].

Synthesis

A major hindrance in achieving stable and feasible

drug delivery systems has been synthesis of colloidal gold and characterization of the particle sizes and shapes. Once an optimum size distribution is obtained, these nanoparticles can be used as stable suspensions owing to their interaction with solvents in spite of differences in the densities. The simple methods involved reduction of an aqueous solution of chloroauric acid ($HAuCl_4$) in the presence of a reducing agent. The mixture consisting of a gold solution and the reducing agent is heated. The solution is then quenched and a stabilizing agent is adding while constantly stirring the mixture. As Au^{3+} ions are reduced (detected by a change in colour), subnano metric particles stabilized electrochemically by adsorption of the stabilizing agent arise in the solution. This was first demonstrated by Turkevich et al in 1951[2] who synthesized monodisperse spherical gold nanoparticles of size range about 10-20nm. Sodium tricitrate was used as the reducing as well as stabilizing agent to prevent agglomeration by causing surface charge repulsion by the negative citrate ions. The diameter of the gold nanoparticles was found to be inversely proportional to the amount of citrate ions.

This basic concept for the synthesis has been maintained but few modifications have been included to overcome certain demerits of the method. Gold nanoparticles used as sensors or targeting agents with ligands have to be biocompatible. This concept was materialized by A.Sugunan[3] and his coworker who found that amino acids with acidic functional groups could be used to synthesize biocompatible nanoparticles. The synthesis was done using monosodium glutamate in varying proportions and the resultant products were characterized using transmission electron microscopy (TEM) and dual beam spectrophotometry (operating between 350-1000nm range). The results indicating a red shift and a blue shift respectively suggested that at molar concentrations of above 3 for the amino acid, large, nonspherical nanoparticles were obtained due rapid crystal growth. On the other hand, anisotropic crystals were obtained at molar concentrations below 3. These nanoparticles ranged from 14.5nm to 24nm for molar ratio of monosodium glutamate to chloroauric acid of 10:2.

Another method developed by Brust and Schiffrin in the early 1990s involves synthesis in a two-phase system of water and immiscible organic solvents. Gold nanoparticles around 5-6nm in size were obtained in a system of water and toluene. Tetraoctylammonium bromide (TOAB) was used as a phase transfer catalyst, while sodium borohydride ($NaBH_4$) was used as a reducing agent. In order to increase the stability of such a suspension, the stabilization by TOAB can be augmented by including alkanethiols. The thiol groups covalently bond with the gold nanoparticle and thus, prevent precipitation. Due to presence of a stronger stabilizing agent (thiol), such a dispersion can be purified further, dried, stored and redispersed whenever required. As a result, suspensions with lower rate of sedimentation can be obtained. The only drawback in the use of a non-aqueous solvent is the application in solution-based diagnostic and therapeutic vehicles.

One of the novel techniques for synthesis developed by A. Sugunan et al.[4] provides the same advantages as that of the non-aqueous systems, but the technique is simpler practically as it involves an aqueous system. The principle remains the same, in that an amino acid is used as a reducing agent, but the mechanism of stabilization is different. Instead of screening of charges on the nanoparticle by adsorption of negatively charged ions, steric hindrance due to adsorption of a bio-polymer called 'chitosan' prevents aggregation. Chitosan is a polycationic polymer and consists of numerous unattached amines. The entire molecule wraps around each gold nanoparticle and hence, prevents agglomeration. Such a suspension can thus, be preserved for months. The use of chitosan as a stabilizing agent increases the rate of reduction and results in polydisperse nanopar ticles of size ~10nm.

Sonochemical reduction[5] involves ultrasound irradiation of an aqueous solution of gold in presence of glucose or cyclodextrin. Surfactants such as sodium dodecyl sulfate or polyethylene glycol monostearate can be included. These act as stabilizing agents and in addition also help in generation of the pyrolysis radicals. This method of synthesis can yield nanoparticles and nanoribbons of width 30-50nm with a uniform size distribution.

Properties

For bulk materials in the microscale, the number of atoms on the surface is negligible compared to those in the bulk of the material. However, this trend changes as one approaches the nanoscale. In the case of nanopar

ticles, surface properties are dominant. For example, gold nanoparticles smaller than 50nm are too hard to exhibit malleability and ductility. The optical properties[6] of the gold nanoparticles which cause them to absorb as well as scatter radiation are most exploited in medical research. The molecular imaging and photothermal therapy using metal nanoparticles is based on the concept of plasmon resonance (a plasmon is a hybrid of the electron plasma (in a metal or semiconductor) and a photon). Plasmon resonance is a collective oscillation of a large number of free electrons at optical frequencies. Below the plasmon resonance frequency, incident light is reflected as the electrons in the metal screen shield the electric field of the light. On the other hand, at higher frequencies the electrons cannot respond fast enough and the incident radiation is transmitted. Thus, a proportion of incident light is scattered and above a threshold of luminescence, considerable amount of heat is generated due to absorption. In case of gold nanospheres, plasmon resonance occurs at a wavelength of 520nm, considerably larger as compared to the particle size. The generated heat is dissipated to the surrounding resulting in a rise in temperature.

A.Govorov and H.Richardson[7] observed that such hyperthermia in optically stimulated gold nanoparticles is influenced by the particle size, particle interactions and density. Nanorods and nanoshells of gold with plasmon frequencies of 650-900nm (red shift) have been developed to reduce absorption by the biological fluids. It can be confirmed that an accumulative effect by addition of heat fluxes of numerous gold nanoparticles generates a higher temperature than expected with individual nanoparticles (1000 times). The proximity of two nanoparticles can also cause an increase or a decrease in heat dissipation as a result of the partial screening of the electric fields inside the nanoparticles.

The application of these principles to molecular imaging and photothermal therapy is pragmatic only when the amount of heat generated by gold nanoparticles can be quantified. This has been done by observations of heating effects like phase transitions in the surroundings, for example, change in chain length of a polymer or melting of ice embedded with such nanoparticles[8].

Targeting of gold nanoparticles

Gold nanoparticles are promising agents in detection and treatment of cancer. One of the major problems in cancer treatment is targeting the drug to the site of action. A disadvantage of many drug based therapies is that the compound does not localize to the target site but is widely dispersed. Treatments based on nanoparticles involves localizing the therapeutic agent by either passive targeting, where the body concentrates inert nanoparticles or by active targeting, where functional modification of the surface of the gold particle enhances the therapeutic delivery system resulting in specific tissue targeting.

Passive targeting

Gold nanoparticles can be designed such that they can pass freely through all other organs in the biological system, but are big enough to be retained by the liver and the spleen. This property can be used in the radio therapeutic treatment of liver cancers. Since, it was proposed that tumor vasculatures are more permeable than that of healthy tissues gold nanoparticles can be accumulated passively[6]. The nanoparticles pass through the tumour vasculature and get deposited in the surrounding tumor tissues. This phenomenon is also called extravasation.

Active targeting

Passive targeting relies on size-specific filtration for concentration of nanoparticles. A more specific approach would be to modify the surface of nanoparticles by conjugating it with an antibody or ligand[6] with an affinity for the desired target. The limitations of this approach would include non-specific binding and potential activation of the host immune system. The interactions with the immune system can be reduced by coating the nanoparticles with a self assembled layer of thiolated PEG (poly-ethylene glycol) or liposome, rendering the surfaces of these particles inert with respect to protein absorption. However, this can also lead to the particle losing its affinity to bind to specific receptors.

Once the gold nanoparticles are concentrated at the desired site, they can be activated by absorption of radiations of a desired wavelength. Depending on the design of the particle, this would lead to localized generation of heat or localized release of chemicals. Additionally, the attached gold nanoparticles can be used to track or image cells.

Cancer detection

Many cancer cells have a protein, known as epidermal growth factor receptor (EFGR), all over their surface, while healthy cells typically do not express this protein strongly. When gold nanoparticles are conjugated with an antibody for the EFGR (anti –EFGR), they can be made to concentrate near the cancer cells[9].

If conjugated nanoparticle solution is added to healthy cells and cancerous cells, the cancer cells can be detected as a shining particle under a simple microscope and thereby, can be differentiated from the healthy cells. It is found that gold nanoparticles have 600 percent greater affinity for cancer cells than for non cancerous cells. This technique has advantages in that it requires only a simple, inexpensive microscope to view the results. Also, the results are instantaneous and the technique is not toxic to human cells.

Cancer treatment

1. Release of payload at a localized tumor tissue

The property of plasmon resonance alongwith the heating effect of metal nanoparticles can be exploited in cancer therapy by first attaching the nanoparticles to a 'biolinker' (for example, an antibody to the epidermal growth factor receptor present on tumor cells alone). Once the gold nanoparticles home-in on the tumor cells, a far infra-red sauna can be used to promote hyperthermia. Owing to their disorganized vasculature, tumor cells face a difficulty in heat dissipation and undergo apoptosis. Since the biolinker is specific to the cancerous cells, the healthy tissues of the body remain unaffected. Even if apoptosis does not occur, the tumor cells can be sensitized to subsequent radiations and chemotherapies.

The therapeutic payload can also be delivered by temperature induced swelling of polymeric nanoparticles. Most of the drugs on distribution into various cellular compartments are entrapped by the endosomes which destroy considerable amount of the drug. Earlier studies involved use of a mixture of poly(ethyleneimine) (PEI) and Pluronic F-127 to deliver genes to the cells[9]. A 40-fold increase in the volume was observed on a temperature drop from 33^0C to 24^0C leading to a rupture of the endosomes and release of the payload. Identical temperature sensitive nanoparticles with gold nanoparticles and Pluronic F-127 are being analyzed for a similar response to a temperature drop. Due to incorporation of gold, stability to the physical turbulence in the blood stream can be obtained. Also, ligands with specific chemistry can be attached to the outer capsule of gold for targeting tumor cells. Another novel delivery technique called 'smart bombs'[10] involves the use of temperature-dependent and pH-dependent nanoparticles. The research focuses on the release of the chemotherapeutic agents without an increase in temperature which could otherwise harm healthy cells also. Gold nanoparticles with a lower consolute temperature (LCST) lower than the body temperature (37^0C) at the physiological pH 7.4 have been formulated. Here, the fact that at acidic pH, there is a further decrease in the LCST was applied. Since, the pH at the cancer cells (site of action) is acidic, precipitation occurs and the nanocapsule breaks apart releasing the payload as a single burst.

2. Increase in bioavailabity of antisense cancer drugs

Antisense drugs are agents which prevent genes from producing harmful proteins, such as those that cause cancer. However, the pace of development of these drugs has been slow. A major challenge has been delivering antisense drugs to cells inside the body while avoiding their breakdown along the way. When multiple strands of antisense- DNA are attached to the surface of a gold nanoparticle, forming a antisense nanoparticle[11], the DNA becomes more stable. It can bind to the target messenger RNA more effectively than the free antisense-DNA alone (like in commercial agents such as Lipofectamine and Cytofectin). Other advantages are that these complexes are less susceptible to degradation, possess less toxicity and are more readily absorbed by the cells, exhibiting greater than 99 % uptake. Once inside the cells the DNA- nanoparticle complexes act as messenger RNA "sponges" that bind to their targets and prevent them from being converted to cancer causing proteins. The gold nanoparticle- DNA complex is also more effective in decreasing cancer gene expression and protein production.

3. Enhanced apoptosis

β-Chronic Lymphocytic Leukemia is primarily characterized by apoptosis resistance. A VEGF (vascular endothelial growth factor) pathway causes apoptosis

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resistance in these cells. This is clearly demonstrated by the fact that addition of VEGF to cells results in increases in anti-apoptotic proteins. Also, recombinant VGEF165 can rescue BCLL cells from spontaneous and drug induced apoptosis. Thus, anti-VEGF antibodies (AbVF) are helpful in causing apoptosis. However, high amount of antibody has only a moderate effect in apoptosis. These antibodies can be conjugated with gold nanoparticles. Preclinical studies were carried out with BCLL cells isolated directly from patients blood[1]. It was found that gold nanoparticle-AbVF conjugation leads to significantly high apoptosis compared to gold or AbVF alone. The reason proposed was this observation was a significant down regulation of anti-apoptotic proteins in gold-AbVF treated cells.

4. Prevention of angiogenesis

Angiogenesis, the formation of new blood vessels is essential for formation and growth of tumors. Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) and basic fibroblast growth factor (bFGF) are two critical cytokines for the induction of angiogenesis. Gold nanoparticles have an intrinsic property whereby they can bind to these factors and inhibit their activity. Gold nanoparticles specifically bind to the vascular permeability factor/vascular endothelial growth factor (VPF/VEGF)-165 and basic fibroblast growth factor, two endothelial cell mitogens and mediators of angiogenesis resulting in inhibition of endothelial/fibroblast cell proliferation *in vitro* and VEGF-induced permeability as well as angiogenesis *in vivo*[12]. In contrast, it was found gold nanoparticles did not inhibit VEGF-121 or epidermal growth factor, two non-heparin binding growth factors, mediated cell proliferation. Gold nanoparticles significantly inhibited VEGF receptor-2 phosphorylation, intracellular calcium release, and migration in *vitro*[13]. Thus, gold nanoparticles have a novel property to bind to heparin binding proteins and thereby inhibit their subsequent signaling events[13].

CONCLUSION

Thus we can conclude that gold nanoparticles can be synthesized in both aqueous and non-aqueous media with a control over their particle size and size distribution. They are biocompatible and when conjugated with suitable biomolecules can be used for targeted drug delivery. The *in vitro* and *in vivo* studies reveal a promising future in research concerning gold nanoparticles.

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