Review Article

ROLE OF GOLD NANOPARTICLES IN THE DETECTION AND TREATMENT OF CANCER

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ABSTRACT

Nanotechnology offers unique approach of controlling variety of biological and medical processes at nanoscale. Recent applications of nanotechnology involve use of nanoparticles for the treatment of variety of diseases like cancer, diabetes, HIV vaccine. Conventional cancer therapy involves a cytotoxic agent that often shows the harmful side effects. Hence, Oncologist nowadays searching for new and advanced methods for early detection and treatment of cancer. By utilizing properties of nanoparticles like optical, magnetic, fluorescent, they can be used in detection, imaging and treatment of cancer. Though the use of different metallic nanoparticles like iron oxide, silver nanoparticles is found to be effective, gold nanoparticles are nontoxic and noble for human beings. Gold nanoparticles have immense potential for cancer diagnosis and therapy on account of their surface plasmon resonance (SPR) enhanced light scattering and absorption. Hence gold nanoparticles can be selectively and widely used. Recent review provides focus on the use of gold nanoparticles as a fundamental tool in cancer treatment.

Keywords: Nanotechnology, Cancer, Gold Nanoparticle, Metallic Nanoparticles, Imaging tumor.

INTRODUCTION[1-3]

Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. Nanoparticles show unique properties at tiny scale hence they can be used in the treatment of various diseases like cancer; diabetes etc. Current cancer therapy includes Surgery, Radiation and Chemotherapy. All three methods are having advantages along with potential limitations. So there is a strong need of finding a new technique for fighting against cancer.

Gold is being chemically inert; it has been used internally in humans for the past 50 years, from its use in teeth to implants to radioactive gold used in cancer treatment. Gold nanoparticles have recently emerged as an attractive candidate in cancer therapy as targeted delivery system. Based on their ability of scattering visible light they have been used as contrast agent. The gold atom has a free electron in the conduction band, which induces a variety of unique and significant optical properties. When Visible IR radiations fall on gold particles, surface properties become dominant. The electric field of the light wave induces coherent oscillation of the free electrons, known as surface plasmon resonance (SPR). This SPR property depends both on the size and on the shape of the gold nanoparticle (GNPs). That is, the color of colloidal gold is due to the above-described interaction between the electric field of light and the free electrons of the gold nanoparticles. Under the resonance condition, the local electromagnetic field around the GNPs surface exhibits a tremendous increase compared with the field of the incident light. This phenomenon has opened up widespread applications of gold nanoparticles.

It is primarily a quantum phenomenon that serves as specialized microscopic probes to study cancer cells, because GNPs selectively accumulate in tumor cells, showing bright scattering. GNPs themselves, being neutral, will not evoke any special cellular response, and the effect will be induced by the conjugated chemical group or biomolecule attached on its surface. So, gold nanoparticles can be used as a targeted drug delivery agent.

SYNTHESIS OF GNP'S[4-6]

GNPs can be prepared by different techniques like photochemical synthesis and biosynthesis. A number of preparation procedures for gold nanospheres have been reported. Of these, the most familiar method is the chemical reduction of chloroauric acid with appropriate reducing agents. Before the reduction process all glasswares were cleaned in aqua regia (3 parts HCl, 1 part HNO_3), rinsed with nanopure H_2O, and then oven dried. Stable colloidal solutions of GNPs are generally prepared in the presence of a stabilizer that prevents aggregation and consequent precipitation of the GNPs.

Majority of synthetic procedures for the GNPs preparation relies on a chemical reduction of gold (III), specifically in the $H[AuCl_4]$ or $[AuCl_4]$form. A suitable reducing agent is added into a reaction mixture, most often citrate or borohydride. In all the cases, an excess of reducing agents or their oxidation products served as the GNPs stabilizer. Generally, two following distinct synthetic protocols according to the environment, in which the GNPs are prepared, can be distinguished: Citrate reduction of gold in water represents undoubtedly the most popular approach of the GNPs preparation. In this method, citrate serves as both a reducing agent and an anionic stabilizer. The method produces almost spherical

particles over a tunable range of sizes covering 5–150 nm by varying stabilizer/gold ratio. Other less frequent reducing agents including carboxylic acids, alkaline borohydrides, hydrazine, and hydoxylamine are used in the same manner as citrate.

PHOTOCHEMICAL SYNTHESIS

In the reaction, the gold precursor used was hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4$ ($3H_2O$). The gold precursor, $HAuCl_4$ absorbs the UV light. It gets excited and generates electronic state. The excited gold precursor is then reduced to gold metal atoms by the solvent, ethylene glycol (EG) and EG serves as solvent as well as the reducing agent. The gold salt is gradually reduced to Au^+. Au^+ then disproportionates to form gold atoms. The gold atoms serve as nucleation sites for further growth of nanoparticles. The surface regulating polymers (e.g. PVP) are expected to bind on the gold surface and insulate the particles from gold ions and further aggregation, thus stopping growth and stabilizing the particles. Further, PVP is believed to have selective interaction between different planes of the gold crystal, thus enhancing the growth along one direction while reducing the growth along another direction. Therefore, with different PVP and gold salt concentrations, we can control the shapes and sizes of the particles.

BIOSYNTHESIS

Biosynthesis of gold nanoparticles involves use of *Terminalia catappa* (almond) leaf extract for the reduction of aqueous chloroaurate ions. The main idea behind the selection of *Terminalia catappa* leaf extract is due to its anticancer, antibacterial and antioxidant activity. This *Terminalia catappa* leaf extract contains microorganisms, its acts as reducing and stabilizing agent. On treating chloroauric acid solutions with *Terminalia catappa* (TC) leaf extract rapid reduction of chloroaurate ions is observed leading to the formation of highly stable gold nanoparticles in solution. The antibacterial and antioxidant properties of biomolecules present in the TC leaf extract have facilitated excellent stability of the nanoparticles. The size of the nanoparticles being in the range 10 - 35 nm with average size of 21.9 nm makes circulation into blood vessels feasible. In addition to the size and stability, the anticancer, antibacterial, antioxidant properties of TC leaf extract could have important application in the use of the biogenic gold nanoparticles in cancer therapy

TYPES OF GNP'S[7-9]

Gold nanoparticles can absorb different frequencies of light, depending on their shape. Rod shaped particles absorb light to near frequencies. This light heats the rods but passes harmlessly through human tissues. Two particular geometries of gold nanoparticles are being studied for use in cancer treatment, namely nanorods and nanocages.

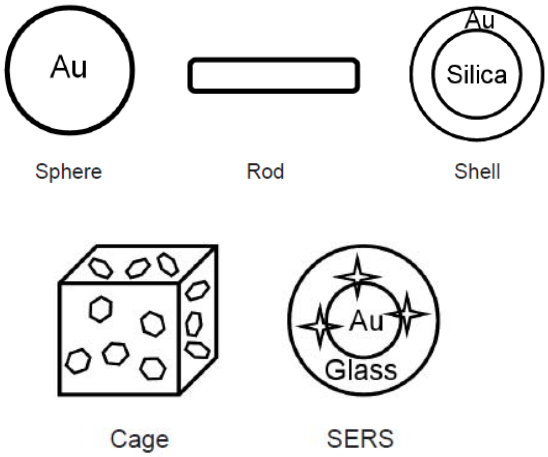

Figure 1: Different shapes of gold nanoparticles.

1. Gold nanorods: They are also called nanowires, are approximately cylindrical with diameters from 10-300nm and lengths from 50-1000nm. These nanorods can be manufactured to be either porous or solid, depending on the methods used to create them. A typical method for creating gold nanorods is by electro deposition of gold within the pores of nonoporous polycarbonate or alumina template membranes. An alumina substrate is initially sputtered with a thin layer of gold to create an electrical contact in order to perform electroplating. The surface of this gold layer is then plated with alternating thin layers of silver and gold, which results in a self-assembly phenomenon which causes the desired nanorod structures. Once the desired number of layers has been deposited, the unwanted silver is chemically etched away, leaving pure gold nanorods.

During cancer research it was found that gold nanorods are effective for diagnosis and killing of cancer cells.

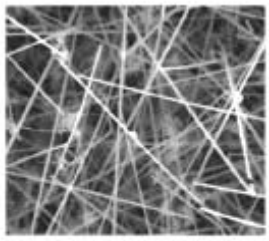

Figure 2: Nanowire

2. Gold nanoshells: These are special class of nanocomposite materials. They consist of concentric particles, in which particles of one material (core) are coated with thin layer of another material (shell) using specialized procedures. The core is typically consisting of dielectric material like silica coated with thin metallic layer of gold (shell). When these nanoshells are inserted in the body, they get attached to diseased cells. Hence they can be imaged. Thickness of nanoshells is about 1-20nm. By manipulating thickness of the layers, the beads can be designed that absorb specific wavelength of light. The most useful nanoshells are those that absorb near IR radiations that are physiologically safe; easily penetrate several centimeters in human tissue. Absorption of light by nanoshells creates an intense heat that is lethal to

cancer cells. In laboratory culture, the heat generated by light absorbing nanoshells has successfully killed cancer cells leaving neighbouring cells intact.

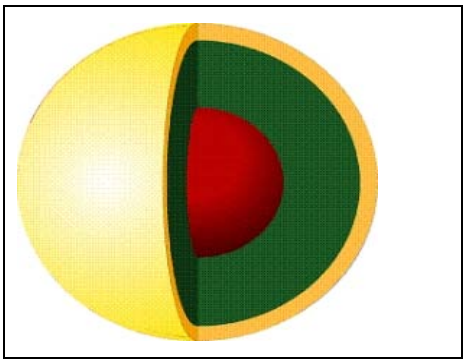

Figure 3: Gold Nanoshell

3. Gold nanocages: Gold nanocages have controllable pores on the surface. They have been synthesized via galvanic replacement reaction between truncated silver Nanotubes and aqueous $HAuCl_4$. Silver nanostructures are generated by polyol reduction. In this reaction silver nitrate is reduced by ethylene glycol to generate silver atoms and then nanocrystals or seeds. Subsequent addition of silver atoms to seeds produces the desired nanostructure through controlling the silver seed crystalline structure in the presence of PVP. PVP (polyvinyl pyrrolidone) is a polymer that is capable of selectively binding to the surface. The silver nanostuctures used template can be transformed into gold nanostructures with hollow interiors via a galvanic replacement. By adjusting the molar ratio of silver to $HAuCl_4$, the dimensions and wall of resultant gold nanocages could be readily controlled.

GOLD NANOPARTICLES FOR CANCER THERAPY[10-11]

Noble metal based nanoparticles can kill cancer cells synergistically with conventionally used radiotherapy or phototherapy. The use of metallic nanoparticles in the treatment of cancer is a promising, relatively recent development in this field. At present, gold is a favoured material for this purpose. This is because gold was found to be noble, nontoxic and stable for human use. Furthermore the synthesis of gold particle is simple and cost effective due to bulk quantity synthesis. When formed into spheres, shells, cages or wires on the scale of ten to one thousand nanometres, gold can be bound to a wide variety of biochemically functional groups and made to target specific types of cells. Gold therapy is called noninvasive radiotherapy because it uses conductive nanomaterial either gold nanoparticles or gold nanoshells. They are then focused using near IR radiations. Both of them exploit their high conductivity both thermally and electrically to allow them to heat up at certain wavelength of light.

Cancerous cells have several times more affinity towards absorption of gold particles than normal healthy tissue. The charactristic features of cancerous cells like leaky blood vessels, surface overexpressed receptors are responsible for this affinity. It was found during study that 50 nm sized nanogold particles are absorbed maximally than larger or smaller sized particles.

The laser intensity and exposure time to kill HSC malignant cells has been found to be $10W/cm^2$ sustained for four minutes. Healthy cells exposed for four minutes required $15\text{-}20W/cm^2$ to be killed. This increased sensitivity of the malignant cells to treatment makes photothermal therapy very desirable, as the effects can be targeted to cause a minimum amount of damage to healthy tissues.

GNP'S as a carriers[12]

Nanoparticles can be used as drug carries for chemotherapeutics to deliver medication directly to the tumor while sparing healthy tissue. Nanocarriers have several advantages over conventional chemotherapy. They can

1) Protect drugs from being degraded in the body before they reach their target
2) Enhance the absorption of drugs into tumors
3) Allow for the better control over the timing and distribution of drugs to tissues, making it easier for oncologist to assess how well they work.
4) Prevent drugs from interacting with normal cells, thus avoiding side effects

Gold nanotechnology relies on the ability of tiny gold nanoparticles to specifically collect in a cancerous tumor by passing through the inherently leaky blood vessels attached to a tumor. So, when injected into a patient, there is a means by which a potent anti-cancer compound attached to a gold nanoparticle, can be directly and accurately delivered to a tumor whilst avoiding healthy body tissue. Such an effective drug delivery mechanism with reduced toxicity is considered to be a major step-forward. Gold has a major advantage in being a very biocompatible metal.

APPROACHES TO DRUG TARGETING[13-16]

An ideal targeted drug delivery approach would not only increase therapeutic efficacy of drugs but also decrease the toxicity associated with drug to allow lower doses of the drug to be used in therapy. To address the challenges of targeting tumours with nanotechnology, it is necessary to combine the rational design of nanocarriers with the fundamental understanding of tumour biology. A vast array of methods, which can further be classified into two key approaches – active and passive have been explored for targeting drugs by means of designing innovative nanosystems. Some of the methods like direct injection, prodrugs or chemical delivery systems have inherent capability to deliver drugs or their appropriate modifications to the specific site of action. However, others require design of a suitable carrier system to be able to deliver the drug to its target site. Following is a description of such approaches to drug targeting.

TARGETING OF DRUG TO TUMOR CELL

1. Passive Targeting
2. Active Targeting

Passive targeting

Passive targeting refers to the accumulation of drug or drug-carrier system at a particular site due to physicochemical or pharmacological factors. Drug or drug carrier nanosystems can be passively targeted making use of the pathophysiological and anatomical opportunities.

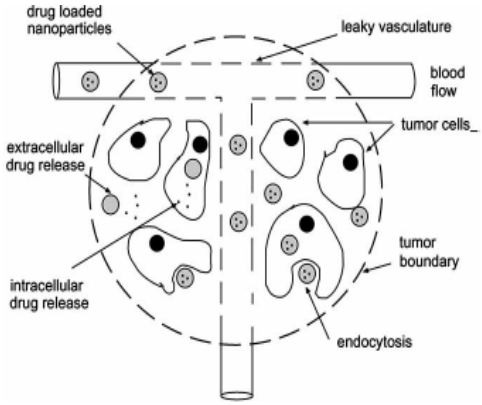

Figure 4: Passive targeting, EPR effect. Tumor cells have leaky vasculature and tend to accumulate the drug inside.

The physiology of diseased tissues may be altered in a variety of pathological conditions, and can be exploited for passively targeting drugs. Release of various chemo tactic factors from the infected/inflamed tissues results in vascular remodeling to enable leukocyte extravasation and hence also increases permeability for particulate drug carriers. Such pathophysiological opportunities include increased vascular permeability in various inflammatory conditions which allows extravasations of the nanosystems and their selective localization in the inflamed tissue.

Similarly, Solid tumors present much more favorable conditions for preferential accumulation of macromolecular drugs and colloidal sized drug delivery systems like polymeric-drug conjugates, liposomes etc. Unlike the tight endothelium of normal blood vessels, the vascular endothelium of angiogenic blood vessels has large gaps (600-800 nm) in between the adjacent endothelial cells. This increased vascular permeability coupled with the impaired lymphatic drainage in tumors allows an enhanced permeability and retention (EPR) effect of the nanosystems in the tumor. A growing tumor must develop its own blood supply to prevent its core from being starved of oxygen and nutrients. But tumor tends to have leaky blood vessels and defective lymphatic drainage. This causes nanoparticles to accumulate in them. They have irregular diameters and abnormal branching patterns, but most importantly, they have thin, leaky walls. Hence cytotoxic drugs accumulate site specifically. For passive tumor accumulation using the EPR effect, the targeted system should have long blood circulation time, should not lose drug activity while in circulation and the drug should stay with the carrier until accumulation of drug at the target is attained. Other factors which may influence tumor accumulation by the EPR effect include the degree of tumor vascularization/angiogenesis and the size of the delivery system. Liposomes, polymeric nanoparticles, micellar systems as well as polymeric-drug conjugates have been successfully used to target drugs to tumor tissues in a passive manner. There are now several nanocarrier based drugs on the market, which rely on passive targeting through a process known as enhanced permeability and retention (EPR effect). It utilizes advantage of inherent size of nanoparticles and tumor vasculture properties. Disadvantage of passive targeting is that some drugs cannot diffuse efficiently within tumor cells. And this is random approach hence this process is difficult to control. This may develop multiple drug resistance.

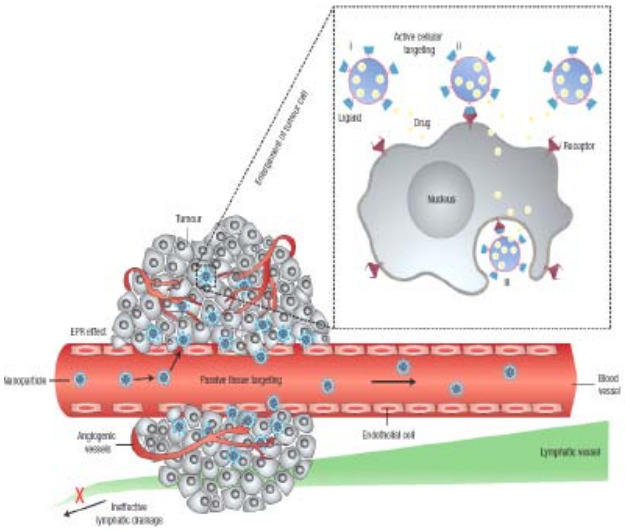

Figure 5: Schematic representation of different mechanisms by which nanocarriers can deliver drugs to tumours. Nanoparticles are shown as representative nanocarriers (circles). Passive tissue targeting is achieved by extravasation of nanoparticles through increased permeability of the tumour vasculature and ineffective lymphatic drainage (EPR effect). Active cellular targeting (inset) can be achieved by functionalizing the surface of nanoparticles with ligands that promote cell-specific recognition and binding. The nanoparticles can (i) release their contents in close proximity to the target cells; (ii) attach to the membrane of the cell and act as an extracellular sustained-release drug depot.

Active targeting

Active targeting employs specific modification of drug/drug carrier nanosystems with “active” agents having selective affinity for recognizing and interacting with a specific cell, tissue or organ in the body. Direct coupling of drugs to targeting ligand, restricts the coupling capacity to a few drug molecules. In contrast, coupling of drug carrier nanosystems to ligands allows import of thousands of drug molecules by means of one receptor targeted ligand. Drug targeting to specific cells has been explored utilizing the presence of various receptors, antigens/proteins on the plasma membrane of cells and also by virtue of the lipid components of the cell

membranes. The receptors and surface bound antigens may be expressed uniquely in diseased cells only or may exhibit differentially higher expression in diseased cells as compared to the normal cells. Active agents, such as ligands for the receptors and antibodies to the surface proteins have been used extensively to target specific cells.

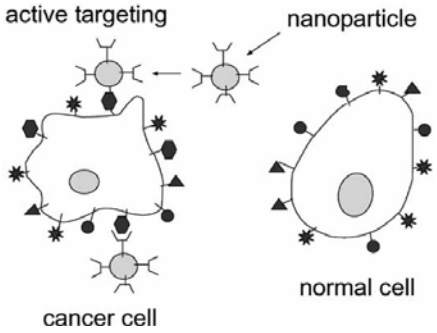

Figure 6: Active targeting: nanoparticles with ligands or molecules attached to their surface can target tumor cells preferentially over healthy cells.

In order to make gold nanoparticles more useful for drug delivery and other biomedical applications (imaging and therapy), they need to be effectively, specifically and reliably directed to specific organ or disease without alteration. For this, surface functionalization of gold nanoparticles is essential in order to target them to specific disease areas and allow them to selectively interact with cells or biomolecules. Surface conjugation of antibodies and other targeting moieties is usually achieved by adsorption of the ligand to the gold surface. A Cancer cell expresses certain cellular proteins on their surface where as normal cell fails. These proteins are called as epithelial growth factor receptors (EGFR). This acts as specific biomarkers and increases specificity of cancer cells. Molecules that bind these cellular receptors (Antibody for EGFR) can be attached to a nanoparticle. So that Nanoparticles can actively target drugs to cancerous cells.

Active targeting can even be used to bring drugs into the cancerous cell, by inducing the cell to absorb the nanocarrier. Active targeting can be combined with passive targeting to further reduce the interaction of carried drugs with healthy tissue. Nanotechnology enabled active and passive targeting can also increase the efficacy of a chemotherapeutic, achieving greater tumor reduction with lower doses of the drug.

DETECTION AND KILLING OF CANCER CELLS[17-20]

I) Imaging

Imaging is an important tool for identification and three-dimensional location of diseased tissue and cells. Imaging can also indicate the location and boundaries of viable diseased cells or tissues during and after certain treatments, particularly in minimally invasive procedure. The most easily and effectively treated tumors are those that are in their earliest stages of development and are small mm in size and localized. Conventional imaging technologies are not sensitive or accurate enough to detect the earliest tumor stages. Furthermore, most conventional techniques represent static images of events rather than a continuous visualization of tumor growth or death. Fortunately, nanoparticles are not only useful in functioning as anticancer therapy delivery vehicles, they can also be engineered to transport contrasting agents or serve as the imaging agents themselves. Nanoparticles can be engineered to serve as intense beacons for imaging purposes by carrying, for example, bioluminescent agents. Nanoparticulate imaging allows for the early detection of tumor and metastases as well as opportunities for real-time monitoring thereby increasing both sensitivity and accuracy of anticancer therapies. Contrast agents can be coupled with various anticancer therapies to monitor treatment successes and failures.

Imaging involves that involve detection of cancer cell. Bio-detection sensitivity of nanomaterials associates intimately with their physical and chemical properties depending on the component, size, and shape. For noninvasive therapy, optical imaging, near IR fluorescence reflectance imaging is commonly used approaches. For imaging of cancer cells, metallic properties of gold are considered. Initially gold nanoparticles are attached to cancer cells. Surface properties of metallic particles become dominant at nanoscale. They absorb much more light than normal. The amount of light that is not absorbed is strongly scattered by them. Hence this optical phenomenon is widely used for cancer imaging.

II) Killing of cancer cell

Thermotherapy

It is a method of utilizing hyperthermia directed towards body tissues for the purpose of damaging proteins and structures within cancerous cells. Sometimes tumor cells directly undergo apoptosis. Gold nanoparticles are being adsorbed by cancerous cells. They are irradiated by near IR radiations. Hence they absorb light and convert it into heat. It causes heating of tumor cells. Healthy cells are capable of surviving exposure to temperatures up to around 46.5 C where as irreversible damage to diseased cell occurs at a temperature in the ranges of 40-46.5°C. During thermo therapy, surrounding cells are more readily able to dissipate the heat and maintains the temperature. Targeted tumor experiences difficulty in dissipating heat due to their disorganized and compact vascular structures.

Photodynamic therapy

This is the form of cancer therapy that utilizes photosensitizing agent and fixed frequency laser. Photosensitizing agent is used here. It is introduced in the blood stream. These are absorbed by both healthy cells and cancerous cells throughout the body. Healthy cells are more efficient in eliminating these. When photosensitizing agent accumulate in sufficient concentration in the cancer cells. Then desired area is

exposed to the light h having wavelength or waveband corresponding to characteristic absorption wavelength or waveband of that photosensitive material. Photo sensitizer absorbs the light and produces singlet oxygen and other reactive free radical species. This leads to number of biological effects including damage to proteins, lipids, cellular components. It often results to cell death. This technique is used to treat tumor below skin or internal organs.

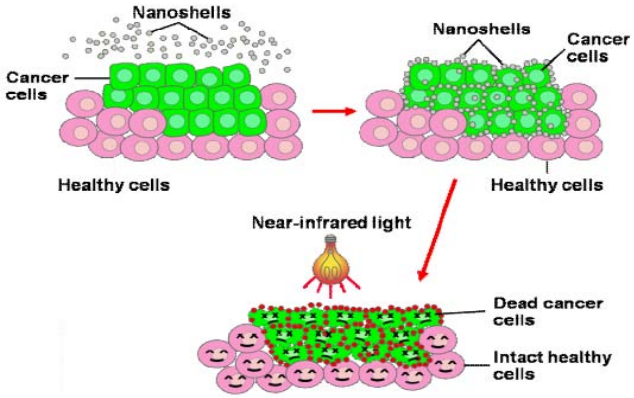

Figure 7: Photo thermal killing of cancer cells

Table 1: Some examples of FDA approved Gold Nanoparticles for cancer treatment

Disease	Nano drug particle	Product name
Breast cancer	Colloidal gold	auroimune
Different cancer	Aluminium bound Paclitaxel nanoparticles	Abraxane
Ovarian cancer	PEGylated liposomes	Doxil
Lung cancer	Liposomal nanoparticles	INGN-401

CONCLUSION

Nanotechnology is prevailing very rapidly in all areas. It was found from experiments that it has wider application in medical field for management of variety of diseases. Attaching the drug to the gold nanoparticles that specifically bind to the antibodies of cancerous proteins proved this technique very effective. In future there is need to deeply study the stability and toxicity of these gold nanoparticles in body. Nanotechnology will effectively control the management of cancer in early stages thus mortality rate due to cancer will be reduced with the further advancement in this practice.

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