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Review Article

NANO GOLD PARTICLES - A NEW MILESTONE IN ONCOLOGY

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ABSTRACT

Gold is used as nano Particles in form of colloidal gold, magnetic nanogold Particles, nanogold sphere, nanoshell, nanorods, gold cluster. Colloidal gold is used in form of gold clusters called nanogold. Nanogold is a 1.4nm gold cluster. Nanogold is actually a coordination compound containing a gold core covalently linked to surface organic groups. The colloidal gold is used to treat experimentally-induced arthritis in rat models. Gold nanoshells are spherical particles with diameters typically ranging in size from 10 to 200 nm. They are composed of a dielectric core covered by a thin gold shell. As novel nanostructures, they possess a remarkable set of optical, chemical and physical properties, which make them ideal candidates for enhancing cancer detection, cancer treatment, cellular imaging and medical biosensing.

Keywords: Colloidal gold, Nanogold Particles, Nanoshell, Nanorods, Cancer detection.

INTRODUCTION

Gold has been used as Swarna bhasma(gold ash), a micro particulate used in traditional Indian medicine has been characterized as globular particles of gold with an average size of 56-57 nm. Gold has been used for immunocytochemistry, when adsorption of antibodies to colloidal gold was discovered by Faulk and Taylor.[1] It is an ideal label for electron microscopy (EM) due to its high atomic number, which scatters electrons efficiently, and the fact that preparative methods have been developed to make uniform particles in the appropriate size range of 5 to 30 nm.[2] Use in light microscopy (LM) generally requires silver enhancement (autometallography; AMG) of these small gold particles. A further advance in this field was the development of Nanogold, a 1.4 nrn gold cluster.[3] A significant difference from colloidal gold is that Nanogold is actually a coordination compound containing a gold core covalently linked to surface organic groups. These in turn may be covalently attached to antibodies. This approach to immunolabeling has several advantages compared to colloidal gold such as vastly better penetration into tissues, generally greater sensitivity, and higher density of labelling.[4, 5] Since Nanogold is covalently coupled to antibodies, it may also be directly coupled to almost any protein, peptide, carbohydrate, or molecule of interest, including molecules which do not adsorb to colloidal gold. This increases the range of probes possible, and expands the applications of gold labeling. Magnetic-NanoParticles materials have attracted such a strong interest because of the physical, electronic, and magnetic properties resulting from their quantum size. The potential for nano-technology is immensely diverse with potential applications in the fields of electronics, biomedical devices, energy applications, military uses, and waste management. Nano-materials could be utilized to design nano-transistors, to develop and deliver medicines for locally treating diseases and ailments within the body, and for the creation new age weapons and armor for military applications.[6] Nanosized gold particles (27 +/- 3 nm) have been proven to be effective in ameliorating the symptoms of mycobacterial-, collagen- and pristane-induced arthritis in rat models. This contrasts with the drug sodium aurothiomalate that was only effective against mycobacterial-induced arthritis but not to the same extent as Au0.[7] Gold nanoshells due to their unique physical characteristics and benign toxicity profile, gold

nanoshells have been at the forefront of a growing number of biomedical applications. They have shown potential as integrated cancer targeting, imaging and therapy agents. As contrast agents, nanoshell bioconjugates have been used to detect and image individual cancer cells *in vitro* and in solid tumors *in vivo*. Outside the realm of cancer treatment, nanoshells have proven their worth in a number of novel applications; for example, as biosensors they have been used for the sensitive detection of biomarkers at the ng/ ml[1] level. Ultimately, nano-technology has the potential to offer new, inexpensive, and more efficient materials for a greater range of applications than achievable by bulk materials today.[8]

NanoGold Background Information

What is nano gold?

Nano Gold is tiny particles of gold that are so small they're measured in nanometers. A Nanometer is a billionth of a meter—smaller than the wavelength of light. When Gold is nano-sized, it has some surprising properties. For example, nano gold can look red, orange, or even blue. The Color depends on the size and shape of the nano particles and the distance between them. The Different colors of nano gold come from a phenomenon called *surface Plasmon resonance*. When Light shines on the surface of a metal, it creates a *surface plasmon*, which is a group of electrons moving back and forth in sync across the surface of the metal. The Electrons "slosh" Back and forth on the metal surface, similar to the way waves of water move in a pond. When the electrons are moving at the same frequency as the light, the Plasmon is said to be in *resonance*. When they're in resonance, the electrons absorb and scatter light, producing the colors you see. Nanoparticles Of gold resonate at frequencies within the visible spectrum of light. Smaller Nano gold particles absorb and resonate with purple, blue, green, and yellow wavelengths of light, so they look red. Larger nanogold particles absorb and resonate with green, yellow, and red wavelengths of light, so they look blue.

How is nano gold used?

NanoGold has been used to create the colors of stained glass since the Middle Ages. Different Sizes of nano gold produce different colors of glass. Particles Around 20Nanometers across produce red glass. Particles Around 30 Nanometers across produce pink glass, and particles around 80 Nanometers across produce orange glass. Today, Gold nano particles can be used as markers to indicate the presence of specific strands of DNA. When Strands of marked DNA combine, the gold nanoparticles come closer together, and the solution changes color.

Gold nanoshells

Future Cancer treatments might use *gold nanoshells* to fight tumors. Gold Nanoshells are tiny spheres of glass covered with a thin layer of gold. In an experimental treatment, gold nanoshells are injected into the body and collect in the tumor. Near-infrared Light is then shined on the tumor. The Light passes safe through healthy tissue, but heats the gold nanoshells and destroys the tumor. Pilot Studies indicate that the treatment is successful, with minimal side effects.[9]

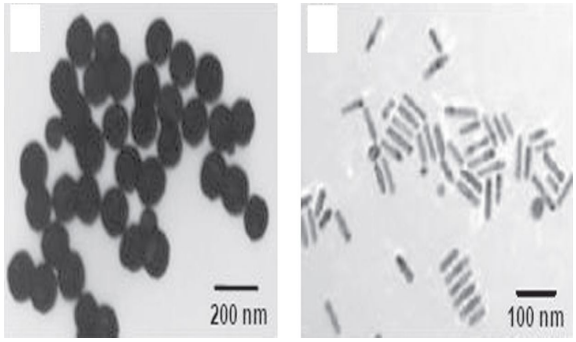

Fig. 1: (a) gold nanoshells and their gold nanoparticle counterparts (b) gold nanorods

SYNTHESIS

Synthesis of magnetic nano-particles

There are many techniques for the synthesis of magnetic nano-particles including microemulsion, reduction of metal-salts, gas-phase reduction of metal complexes, thermolysis of metal-polymer complexes, and thermal decomposition of metal-carbonyl complexes. The thermal decomposition

of metal-carbonyl will be discussed because it is a technique which has successfully been utilized to synthesize magnetic nano-particles. Metal-carbonyl complexes are a commercially available product and have been used in the past for preparation of cobalt and iron based catalysts, making them readily applicable for usage in synthesis of magnetic nano-particles[7].

In this synthesis technique, the fundamental goal is to produce atoms in solution, which quickly and spontaneously formulate into nano-particles. Meanwhile, controlling their size and shape by utilizing a surfactant, which will strongly adsorb onto the surface of the nano-particles, provides a stabilizing cap layer and simultaneously serves as a hindrance for the binding of more atoms onto the nano-particle, hence preventing further growth. The metal-carbonyl pre-cursor serves to provide the metal atoms, the coordinating organic solvent provides a medium for dissolution, and the surfactant serves to control growth and morphology. A metal-carbonyl complex is injected into an organic coordinating mixture at elevated temperatures, which leads to the breakdown of the metal-carbonyl complex into encapsulated metal nano-particles with liquid and gaseous by-products. In the experimental work of Yang et al. cobalt octacarbonyl ($Co_2(CO)_8$) was injected into an organic coordinating mixture made up of dichlorobenzene ($C_6H_4Cl_2$), oleic acid ($C_{18}H_{34}O_2$), and triphenylphosphine ($C_{18}H_{15}P$) at a temperature of 220 °C. In this example, dichlorobenzene is organic coordinating solvent which serves to provide a medium for dissolution of the cobalt octacarbonyl. Once dissolved, nucleation spontaneously occurs and the mixture of triphenylphosphine and oleic acid are used as surfactants which serve to stabilize the nucleated nano-particles. The work of Yang et al. yielded spherical cobalt nano-particles which are 6-8 nm in average size.[7]

Synthesis of gold nanoshell

Silica - core gold nanoshells, 'a new frequency - agile nanoparticle' were first fabricated by Oldenburg, Averitt, Westcott and Halas of Rice University, as described in their 1998 publication, *Nanoengineering of Optical Resonances*.[10] The theory predicting the plasmon resonance - derived optical properties of gold nanoshells had been laid out by Neeves and Birnboii of the Rensselaer Polytechnic Institute in 1989, and further explored by Haus *et al.* in 1992. In this section we describe the synthesis of gold nanoshells employed by Oldenburg *et al.*, along with various techniques for functionalizing their surfaces. A detailed step - by - step synthesis protocol has been provided by Pharm *et al.*[11] The dimensions of the nanoshells (core radius and shell thickness) are controlled by varying the reactant concentrations.

The original synthesis is a four - step process in which first, monodisperse silica nanoparticles are grown using the Stober method to produce the spherical dielectric cores.[12] The Stober method produces spherical silica particles by means of hydrolysis of alkyl silicates and subsequent condensation of silicic acid in alcoholic solution with an ammonia catalyst. In the second step, the surface of the silica nanoparticles is functionalized by the adsorption of an organosilane (3 - aminopropyltriethoxysilane), with its amine tails protruding from the surface. In the third step a solution of gold colloid (~1 – 2 nm in diameter) is added to the solution. The gold colloid is produced separately from reduction of HAuCl 4 by alkaline tetrakis(hydroxymethyl) - phosphonium chloride, according to the method of Duff.[13] The gold particles bond to the organosilane linker via the amine group, producing Silica nanoparticles with a smattered, uneven gold coating.[14] A final reduction Process is used to produce silica nanoparticles with a uniform layer of gold – that is, a gold nanoshell. In the reduction process, the 'seeded' gold particles which are covalently bonded to the silica core serve as nucleation sites where an aged mixture of chloroauric acid and potassium carbonate is reduced in solution in the presence of sodium borohydride. This process forms a highly crystallized gold shell through Oswald ripening.[15] Transmission electron microscopy (TEM) images of the nanoshells during different phases of growth are shown in Figure 2 . UV - visible spectroscopy is used to monitor reaction kinetics, whereby complete nanoshell growth is confirmed by the appearance of characteristic plasmon extinction peak.

Transmission electron microscopy images of nanoshell growth phases from silica core (left) to gold -covered nanoshell (right. When using this method, the polydispersity (standard deviation) of nanoshells is generally close to 10%. Recently, Phonthammachai *et al.* have published an alternative method for nanoshell synthesis, which employs the deposition – precipitation method.[16]

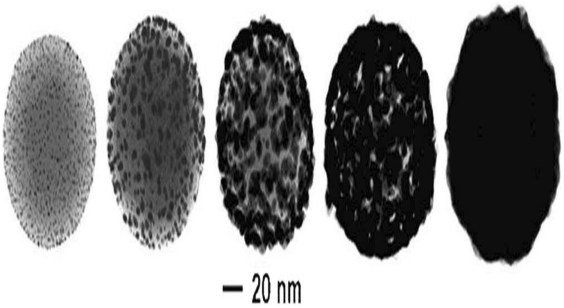

Fig. 2: Transmission electron microscopy (TEM) images of the nanoshells during different phases of growth

BIOCONJUGATION: SMARTER NANOSHELLS

The biologically inert gold surface of nanoshell facilitates bioconjugation with antibodies and other biomarkers, rendering nanoshells capable of selectively binding to *in vivo* targets. The following examples of successful bioconjugation schemes should provide a general idea of the chemistry involved in the production of bioconjugated nanoparticles.

Sokolov *et al.* have synthesized bioconjugates of gold nanospheres with monoclonal antibodies against epidermal growth factor receptor (EGFR), a trans membrane glycoprotein (M^r170000) which is overexpressed in cancers originating from epithelial cells. Colloidal gold of various sizes is prepared using a citrate reduction of $HAuCl_4$.[18] To prepare the bioconjugates, the gold colloid is diluted with 20 m*M* HEPES buffer, and anti - EGFR monoclonal antibodies are reconstituted in the same buffer at 100 $\mu^{g\ ml\ -1}$ and mixed at a 1:1 volume ratio and allowed to interact for 20 min at room temperature. In this environment, gold nanospheres bind noncovalently with anti - EGFR antibodies at their isoelectric point to form stable bioconjugates.[17]

Polyethylene glycol (PEG) is added to the solution up to a final concentration of 0.2 mg ml −1 , after which the solution is centrifuged to remove any unbound antibody. After a second wash, the anti - EGFR gold nanoparticle pellet is resuspended in phosphate - buffered saline (PBS).

Human epidermal growth factor receptor 2 (HER2) is a frequently used breast cancer biomarker, and Loo *et al.* have successfully bioconjugated gold nanoshells with HER2 antibodies to target human mammary adenocarcinoma cells *in vitro.* In the synthesis, *ortho* - pyridyl - disulfi de - *n* - hydroxysuccinimide – PEG polymer (OPSS) is used to tether the antibodies on the surface after which, using NaHCO 3 (100 m *M* , pH 8.5), the OPSS is resuspended in a volume equal to that of the HER2 antibodies. The reaction bonding OPSS to anti - HER2 proceeds on ice for about 12 h, after which any excess OPSS is removed via membrane dialysis. The antibody complex (0.67 mg ml −1) is then allowed to interact with added gold nanoshells (~ 10^{9} nanoshells ml −1) for 1 h, and any unbound antibody is then removed by centrifugation.[19]

The functionalized gold nanoshells pellet is then resuspended in potassium carbonate solution (2 m *M*). Following antibody conjugation, the nanoshell surfaces are further modified with PEG - thiol to prevent any nonspecific adsorption and improve biocompatibility.

Recently, Kumar *et al.* published a complete protocol for conjugating antibodies onto the surface of gold nanoparticles in a highly efficient manner. This novel conjugation strategy employs a heterofunctional linker, hydrazide – PEG – dithiol, to directionally attach the nonbinding (Fc) region of the antibody to the gold surface. This technique enjoys several significant advantages over standard adsorption techniques; mainly that the binding orientation of the antibodies is controlled to ensure maximum functionality and, due to the binding specificity, fewer antibodies are required.[20]

Bioconjugation dramatically enhances the clinical prospects of gold nanoshells by rendering them capable of targeting specific tissues through molecular recognition. Undoubtedly innovative conjugation strategies will play an increasingly important role in the development of gold nanoshells for targeted therapeutics and diagnostics.

Biodistribution, Toxicity Profile and Transport

The dynamic bio-distribution of nanoshells *in vivo* is of considerable clinical interest. Optimal imaging and treatment strategies require achievement of the highest concentration of nanoshells in the target tissue (i.e., a solid tumor), while minimizing concentrations in surrounding healthy tissues. Knowledge of the quantity of nanoshells reaching the target volume is needed to ensure optimal dosing. Ultimately, the *in vivo* transport of nanoshells arises from a complex milieu of physical (pressure gradients, passage through vascular perforations, diffusion), chemical (antibody binding, transient binding) and cellular (endocytosis, vacuolar transport) processes.[21] A number of *in vivo* studies have been carried out to examine the net effect of transport, the differential and time - dependent distribution of nanoshells in

various physiological compartments, namely the targeted tumor, blood, muscle tissue and major organs.[22-25] To date, the vast majority of *in vivo* studies have employed a murine model, whereby nanoshells are delivered intravenously through the tail vein and enter the systemic circulation. The bio-distribution in human patients can likely be predicted based on existing pharmacokinetic modeling techniques, which use well defined scale up laws to estimate transport parameters.[26]

In general, the distribution of nanoshells depends strongly on whether passive or active targeting methods are employed. Passive targeting relies exclusively on the nanoshell's size and a tumor's inherently 'leaky' vasculature to produce accumulation at tumor sites through an enhanced permeability and retention(EPR) effect.[27] In active targeting, the nanoshell's surface is functionalized with antibodies or other bio-molecules, which bind to surface receptors on malignant cells to produce an enhanced accumulation at the tumor site.[28] Both targeting mechanisms are hindered by intrinsic host immune mechanisms, which clear nanoshells from circulation.[29] In both cases, the total circulation time is believed to correlate strongly with accumulation at the tumor site, independent of other anatomical and physiological factors.[30] The surface of gold nanoparticles is now routinely functionalized with PEG (PEGylation) to 'stealth' the nanoparticles from immune surveillance and this have led to a dramatic increase in circulation times.[31]

The following discussion is divided into three parts. First, an overview is provided of the techniques used to quantify the dynamic biodistribution of nanoshells, and the corresponding results. Second, the individual transport mechanisms responsible for the observed biodistribution are explored, based on information obtained from studies examining the transport mechanisms of similar - sized particles. Third, an overview is provided of the benign toxicity profile of nanoshells.

Biodistribution Studies

The current 'gold standard' for quantifying nanoshell concentrations in tissue is neutron activation analysis (NAA). This method requires the tissue excision, dehydration and bombardment of the sample of interest with neutrons in a nuclear reactor. Gold nanoshells absorb the neutrons, undergo a nuclear transition, and emit gamma rays with energies that are characteristic of gold. These gamma rays are then detected and related to the quantity of nanoshells present in the sample. James *et al.* employed this method to measure the concentration of nanoshells (~ 120 nm diameter) in different organs in mice at various time points. Female albino mice with subcutaneous tumors (~ 5 mm) were injected with 100 µ l of an isotonic saline solution of PEGylated nanoshells (2.4×10^{11} nanoshells ml^{-1}, passive targeting) and sacrificed at 1, 4, 24 and 48 h. Blood, liver, lung, spleen, muscle, kidney, bone and brain tissues were then analyzed using NAA. The study yielded several key findings; namely that tumor concentrations peaked at 24 h after injection, and the total tumor accumulation represented approximately 1% of the administered dose. Tumor concentrations were elevated relative to the blood, lungs, brain, bone and kidneys. However, the highest accumulations were found in the liver and spleen, due to involvement of the reticulo endothelial system (RES).[22]

Transport Mechanisms

The transport of molecules and particles in solid tumors is a vast and active area of research because if its potential to illuminate new methods for achieving optimal delivery of therapeutic agents to the tumor site. The following discussion is intended only as an overview of the subject area, outlining the basic characteristics and underlying mechanisms of nanoparticle transport in solid tumors, which can be generalized to nanoshells.

Upon introduction into the host's vasculature, the fate of a typical nanoshell is predictable if the host is tumor - free and the nanoshell surface is not modified by antibodies or other protein - binding ligands. As normal blood vessels are highly impermeable to particles the size of nanoshells[21, 25] , the nanoshells will remain in circulation until they reach the spleen and liver, where they are scavenged by macrophages, such as Kupffer cells, in the host's RES (this accounts for the high concentrations observed in the liver and spleen). Large numbers of nanoshells remain in the RES tissues for many weeks and perhaps longer.[22] However, conflicting data exist with regards to the eventual clearance of nanoshells. Whereas, gold nanoparticles smaller than 10 nm are slowly eliminated from the host's system via renal excretion[29], the case may be different for larger gold nanoparticles. In magnetic resonance imaging (MRI) studies, Choyke *et al.* found that ' virtually no ' contrast agents (gadolinium dendrimers) larger than 11 nm were eliminated through renal excretion[32] ; hence, it stands to

reason that whole gold nanoshells (~ 100 nm) would also be excluded from renal clearance. However, significant amounts of gold and copper have been found in the urine of mice injected with gold - copper nanoshells, which suggests partial renal clearance. Nanoshells are also likely excreted in the feces, similar to quantum dots.[33]

If a tumor is present, then the transport and biodistribution of nanoshells changes significantly. The tumor vasculature is physiologically distinct from normal vasculature, as it lacks a functional lymphatic system[34], exhibits spatial and temporal heterogeneity, structural irregularity, abnormal fluid flow, and hyper permeability to particles with diameters up to 1.2 μ m. The overall result is a series of peculiar transport properties that vary across the tumor type, stage of development and the surrounding microenvironment.

One prominent feature of vascular transport in tumors is the EPR effect, whereby particles with diameters of tens to hundreds of nanometers extravagate through the 'leaky' microvasculature and accumulate in the tumor interstitium. For example, a 400 – 600 nm cut - off has been found for the extravagation of liposomes.[35] Multiple causes of associated tumor 'leakiness' have been identified, including physical openings, cytokine - influenced permeability changes and various cellular transport mechanisms. Hashizume *et al.* showed that, in highly leaky MCa - IV mouse mammary tumors, 14% of the vessel surface was lined with poorly connected, overlapping endothelial cells. Transcellular holes were also present, but these were only 8% as numerous as intercellular openings.[36]

An electron microscopy image showing an open endothelial gap in a tumor blood vessel in a liposome - injected mouse is shown in Figure3, where the liposomes can be seen migrating through the open junction.

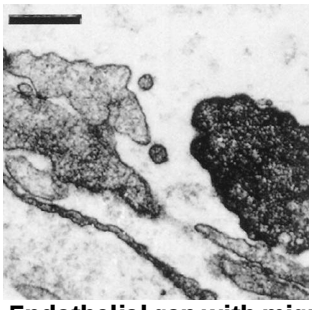

Fig. 3: Endothelial gap with migrating liposomes. Two circular liposomes are seen crossing the gap

Transport also varies across tumor type, microenvironment and stage of development.

For example, Hobbs *et al.* showed delivery to be less efficient in cranial tumors than in subcutaneous tumors, and that delivery was reduced during regression in hormone - dependent tumors during hormonal ablation. Future research aimed at a better understanding of the mechanisms of transport, and how transport varies by tumor type, microenvironment and stage of development, will provide the valuable information required to optimize the therapeutic efficacy of gold nanoshells, gold nanoparticles and other cancer treatments.

Toxicity

Although no comprehensive studies evaluating the long - term (years) toxicity of gold nanoshells have yet been reported, all available evidence indicates that – at physiological doses – gold nanoshells are not cytotoxic and pose no short – term health risks. In fact, the favorable toxicity profile of nanoshells results from the nontoxicity of the shell components. Gold has been used to treat rheumatoid arthritis since the 1930s, and is universally recognized as the most biologically inert of metals.[37] Likewise, silica nanospheres have been shown to be nontoxic in a murine mouse model. The safety of gold nanospheres has been well documented; in experiments performed *in vitro*, gold nanospheres incubated with macrophages were found to be both noncytotoxic and nonimmunogenic. Nanospheres were also found to reduce the production of both reactive oxygen species (ROS) and nitrite radicals, and did not stimulate the secretion of inflammatory cytokines.[38]

Numerous *in vivo* studies conducted in mice have provided the best evidence that nanoshells are not only nontoxic but also safe. In all instances, mice treated with nanoshells exhibited no clinical abnormalities or side effects at months after treatment. It should be noted that, although a prolonged respiratory exposure to high doses of crystalline silica has been linked to lung cancer in epidemiological studies, the carcinogenic potential of gold nanoshells is minimal for the following reasons. The silica used in nanoshells is completely obscured from the host by the gold shell, which is not carcinogenic. Likewise, comparatively lower doses of nanoshells would be necessary for clinical applications, and the intravenous route of administration prevents high concentrations from ever reaching the lungs.

Although gold nanoshells can generally be considered 'safe ', their long - term effects on human health will need to be closely monitored.

BIOMEDICAL APPLICATIONS

Due to their unique physical characteristics and benign toxicity profile, gold nanoshells have been at the forefront of a growing number of biomedical applications. They have shown potential as integrated cancer targeting, imaging and therapy agents. As contrast agents, nanoshell bio-conjugates have been used to detect and image individual cancer cells *in vitro* and in solid tumors *in vivo*. As photothermal agents, nanoshells have successfully been used in animal studies to induce thermal necrosis of tumors. On the laboratory bench, they have been used to potentiate thermal drug delivery in temperature - sensitive hydrogels. Outside the realm of cancer treatment, nanoshells have proven their worth in a number of novel applications; for example, as biosensors they have been used for the sensitive detection of biomarkers at the ng ml $^{-1}$ level.

***In Vitro* Cancer Detection and Imaging**

Detecting cancer in its earliest stages is strongly associated with positive patient outcomes, including reduced morbidity and improved five - year survival rates. As many cancers originate from a small number of malignant epithelial cells, the ability to detect low numbers of malignant or precancerous epithelial cells *in vivo* would represent a giant leap forward in the fight against cancer. Notably, it would facilitate the detection of cancer in its earliest stages, before any significant pathogenesis, tumor formation and metastasis. A number of groups have successfully demonstrated *in vitro* single cancer cell detection, with exceptional contrast and specificity, using bio-conjugated gold nanoparticles as molecular - specific contrast agents. Here, the general detection scheme relies on conjugating nanoparticles to antibodies that target epithelial cell - surface receptors (e.g., EGFR and HER2) which are commonly over expressed in cancer cells. The resultant high concentrations of nanoparticles found on the surface of targeted cancer cells, combined with their high scattering cross - sections, greatly facilitates imaging on the cellular level. Loo *et al.* have used anti - HER2 - conjugated nanoshells to detect and image HER2 - positive SKBr3 breast adenocarcinoma cells using dark - field microscopy *in vitro* (Figure 4).[19,39] In this experiment, both SKBr3 and MCF7 (HER2 - negative) cancer cells were incubated with nanoshells at a concentration of 8 g ml $^{-1}$ for 1 h. Consequently, the SKBr3 cells targeted with molecular - specific anti - HER2 showed a marked (300%) increase in contrast over the nonspecific anti - IgG control group, whereas no appreciable differences in contrast were noted between HER2 - negative control cell groups, indicating that nanoshells targeted the HER2 receptor on SKBr3 cells with high specificity. Other types of gold nanoparticle, such as nanospheres and nanorods, have also been successfully employed to detect and image cancer cells *in vitro*. Durr *et al.* have used anti - EGFR - conjugated nanorods to detect and image A431 skin cancer cells embedded in a 3 - D tissue scaffold using two - photon luminescence (TPL) microscopy.[40] The nanorods produced a TPL signal enhancement of more than three orders of magnitude over the intrinsic fl uorescence of unlabeled cancer cells, which enabled the imaging of cancer cells up to a depth of 75 mm. El - Sayed *et al.* have used anti - EGFR gold nanorods to detect and image two oral squamous carcinoma cell lines, HSC 313 and HOC 3. The nanorods were found to bind specifically and homogeneously to the surface of cancer cells with 600% greater cell affinity than to nonmalignant cells. Dark - field microscopy revealed an intense resonant scattering from the labeled oral cancer cells, whereas scattering from a normal line (HaCaT) was minimal.[41,42]

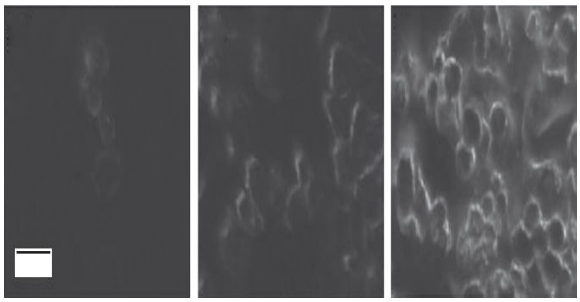

Fig. 4: Dark - field images of SKBr3 cancer cells exposed to (a) no nanoshells, (b) anti - IgG - conjugated nanoshells and (c) anti - HER2 nanoshells. Note the significant difference in contrast between the anti - HER2 nanoshells designed to target breast cancer cells, and the control and anti - IgG nanoshells[19]

***In Vivo* Detection and Imaging**

Recently, progress in the detection and imaging of malignant cells *in vitro* has been followed up by *in vivo* studies, where bioconjugated gold nanoparticles have been used successfully to target and detect tumors in mice. Qian *et al.* have

created specialized gold nanospheres for SERS imaging that are first stabilized with PEG - thiol and then conjugated to a Raman reporter (malachite green) and to anti -EGFR antibodies for active tumor targeting. In the experiment, nude mice with xenografted human head and neck cancer tumors (Tu686) were injected with specialized nanospheres through the tail vein. The tumors were approximately 3 mm in diameter. After 5 h, NIR SERS spectra were obtained using a 785 nm excitation laser on a hand - held Raman system. The SERS spectra measured from an intramuscular tumor located ~1 cm below the surface were distinct from the background spectra, demonstrating the effective detection of small tumors at depths of at least 1 cm. However, based on a favorable signal - to - noise ratio, the authors concluded that the maximum achievable penetration depth for SERS detection was likely in the 1 – 2 cm range.[28]

Gobin *et al.* have demonstrated the role of nanoshells as contrast agents for *in vivo* optical coherence tomography (OCT) imaging. BALBc mice with subcutaneous murine colon carcinoma tumors were injected with PEGylated nanoshells at 20 h before OCT imaging, which was carried out using a commercially available OCT system. Due to their large resonant scattering cross - sections and ability to accumulate at tumor site, the nanoshells were found to signifi cantly enhance the optical contrast of the tumor compared to normal tissue. Thus, in nanoshell - treated mice the integrated scattering intensity was 56% greater in tumor tissue than normal tissue, whereas in control mice the difference was only 16%. It appears that gold nanoshells represent excellent contrast agents, and are suitable for a wide range of imaging techniques.[43]

Integrated Cancer Imaging and Therapy Agents

Gold nanoshells and nanorods are not merely ideal agents for detecting and imaging cancer – the same nanoparticles can be used as therapeutic agents to treat cancer with photothermal therapy. Unlike nanospheres, nanoshells and nanorods can be engineered either to scatter NIR radiation for imaging, or to absorb it and efficiently convert it to heat for the selective destruction of targeted tumor cells. Nanoshells and nanorods are integrated multifunctional nanoparticles, useful for both imaging and therapy. Although the ability of both nanorods and nanoshells to mediate the photothermal destruction of targeted cancer cells *in vitro* has been established, until now only nanoshells have been shown to treat tumors effectively *in vivo*.[39,43]

***In Vitro* Studies**

In an *in vitro* study, Hirsch *et al.* incubated breast carcinoma (SKBr3) cells with unconjugated nanoshells for 1 h, after which the cells were rinsed to remove unbound nanoshells and then exposed to 820 nm laser light with an intensity of 35 W cm^{-2} for 7 min. After treatment, all nanoshell - treated cells within the laser spot were dead, whereas those cells in the control groups remained viable[4] . Others have carried out similar *in vitro* experiments. For example, Stern used unconjugated nanoshells as mediators to photothermally ablate two types (PC – 3 and C4 - 2) of human prostate cancer cell (Figure 5)98[44] , while Loo *et al.* used anti - HER2 - conjugated nanoshells to target and ablate SKBr3 breast carcinoma cells[39] . In another study, El - Sayed *et al.* used anti - EGFR - conjugated gold nanorods to treat two human oral cancer cells types (HSC 313 and HOC 3) and a benign control, human epithelial keratinocytes (HaCat). After having exposed the cells to various intensities of 800 nm lasers light for 4 min, irreversible photothermal injury of nanorod - treated cells was observed for intensities as low as 19 W cm^{-2}. More significantly, photo thermal destruction was observed in malignant cells at less than half the laser power needed to induce destruction in healthy cells, thus permitting the selective destruction of cancer cells. Of particular interest here was the lower laser intensities needed to induce the destruction of malignant cells using nanorods compared to nanoshells. This presumably occurred because nanorods have a larger size - normalized absorption cross - section than do nanoshells. An authoritative study evaluating the effectiveness of both types of nanoparticle under identical experimental conditions is yet to be published, however.[45]

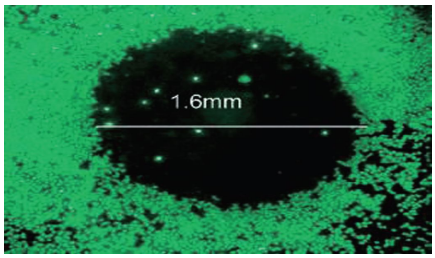

Fig. 5: PC - 3 prostatic cancer cells treated with gold nanoshells and exposed to NIR laser light focused to a spot size of 1.6 mm. Calcein viability staining reveals selective destruction of cells within the laser spot[44]

In Vivo Photothermal Therapy

Multiple *in vivo* studies have demonstrated the efficacy of nanoshells for the noninvasive treatment of tumors through targeted photothermal destruction. Here, the overall concept is straightforward; nanoshells with absorption peaks in the NIR region of the spectrum (~800 nm) accumulate at the tumor site through passive and/or active mechanisms. The 800 nm absorption peak is in the NIR 'optical window' region of the spectrum where tissue absorption is minimal, permitting optimal penetration. When the tumor site is exposed to NIR radiation (which the nanoshells absorb intensely because of plasmon resonance), the absorbed energy is efficiently converted into heat, leading to thermal destruction of the tumor. O' Neal *et al.* have successfully treated mice inoculated with tumors using this technique. In the experiment, albino mice were inoculated subcutaneously with CT26.WT murine colon carcinoma cells in the right dorsal flank, and selected for treatment when the tumors had reached diameters of 3 – 5.5 mm. An aliquot (100 µl) of PEGylated nanoshell solution (2.4 x10 ^{11} nanoshells $_{ml}$ $^{-1}$) was then injected via a tail vein. After allowing a 6 h period for the nanoshells to accumulate, the laser treatment was commenced, with the tumors being exposed to NIR light at 808 nm at 4 W cm $^{-2}$ for 3 min. Measurements revealed a marked increase in surface temperature at the tumor site, to ~50 ° C, and both tumor size and animal survival was monitored for up to 90 days after treatment. In the nanoshell treatment group, a complete resorption of tumors was observed within 10 days, and all mice were healthy and free of tumors at 90 days. By contrast, in the control groups the tumors continued to grow after sham treatment, with a mean survival time of 10.1 days. This dramatic difference in results highlighted the therapeutic potential of nanoshells.[46]

Elliot *et al.* have modeled nanoshell - mediated photothermal therapy using the diffusion approximation to predict spatiotemporal temperature fluctuations in tissue undergoing therapy. The model was validated using results from tissue phantom experiments, and predicted measured temperature values with reasonable accuracy. However, it did not account for those factors present under *in vivo* conditions, such as blood perfusion. However, in looking to the future, a quantitative *in vivo* model of nanoshell - mediated photothermal therapy will surely be very helpful in tailoring individual treatment regimens to human patients.[47]

Drug Delivery

Nanoshells have long shown promise for increasing drug delivery to tumors. Shetty *et al.* have demonstrated enhanced tumor perfusion in mice with xenografted prostate tumors, the perfusion being increased by nanoshell – mediated heating. Mice were injected with nanoshells at 24 h before laser treatment, and perfusion was monitored using MRI. Whereas, heating with low (0.8 W cm $^{-2}$) and high (4 W cm $^{-2}$) laser intensities decreased contrast uptake, heating with an intensity of 2 W cm $^{-2}$ almost doubled the uptake, thus highlighting the potential of nanoshells for improving drug delivery. Nanoshells have also been demonstrated to modulate drug delivery.[48] For example, Sershen *et al.* incorporated nanoshells with an 832 nm resonance into a thermally responsive polymer, *N* - isopropylacrylamide - *co* - acrylamide (NIPAAm), to create a photomediated drug delivery hydrogel composite. Hydrogels based on NIPAAm exhibit a lower critical solution temperature above which the hydrogel undergoes a reversible volume phase change transition. The nanoshells used in the experiment were engineered to have a core radius of 50 nm and shell thickness of 7 nm, in order to maximize absorption. When the composite is illuminated with a diode laser at 832 nm, the nanoshells convert light into heat, inducing a reversible and repeatable light - driven collapse of the composite hydrogel matrix. After 40 min of irradiation at 1.8 W cm $^{-2}$, the hydrogel composite had shrunk to 10% of its initial weight.[49,50] Recently, Bikram *et al.* demonstrated the potential value of nanoshell composites as drug delivery vehicles in specific applications. In this case, hydrogels containing 10 ^{9} nanoshells ml^{-1} were swollen in solutions containing 10 mg ml $^{-1}$ insulin, lysozyme and methylene blue, which was used as a model drug. When the release of each compound was monitored before and after laser irradiation, the release profiles of the embedded drugs upon irradiation were found to depend on their molecular weights. The release of methylene blue (14.1 mg g $^{-1}$ polymer) and insulin (12.9 mg g $^{-1}$) occurred spontaneously, but the release of lysozyme occurred only upon laser irradiation. Moreover, the amounts of insulin and methyleneblue released were approximately doubled on irradiation. Taken together, these results indicate that nanoshell - composite hydrogels have great potential for future drug delivery applications.[51]

Tissue Welding

Nanoshells may represent a rapid means of treating lacerations in an emergency room setting. As an example, Gobin *et al.* have used nanoshells as an exogenous NIR absorber for welding deep tissue wounds. In this study, a nanoshell - based solder (nanoshells + bovine serum albumin (BSA)) was applied to full - thickness incisions made on rats, after which the incisions were irradiated with NIR laser light for several minutes to initiate tissue welding. Notably, the healing results were similar to the suture – treat control group until day 5, after which healing was shown to be better in the suture group.[52]

Biosensors

Nanoshells have several unique properties that are ideal for bio-sensing applications. The position of the plasmon resonance peak and absorbance depended heavily on the refractive index (dielectric constant) of the surrounding medium, which is predicted by Mie theory[53]. As an example, Sun *et al.* have shown that gold nanoshells are more sensitive than nanospheres to changes in the refractive index (*n*) of the surrounding environment, and that the observed peak shift varies linearly with *n.110*[54] In an aqueous medium, a 10% change in *n* corresponds to a substantial peak shift of approximately 50 nm – a finding which is consistent with the results of Tam *et al.* , who measured sensitivities of up to $\Delta\gamma / \Delta n = 555.4$[55] Thus, nanoshells exhibit optical sensitivity to the surrounding environment, a property which may be exploited to detect biomarkers in simple absorbance assays[56,57] .

Nanoshells can also be used to render conventional fluorophores sensitive to the surrounding environment, while protecting them from degradation. Recently, Chen *et al.* observed a fivefold enhancement of the fluorescence efficiency of a tetramethyl rhodamine dye molecule embedded within the silica core of a nanoshell.[58] The peak wavelength of the fluorophore was shifted by over 50 nm when the refractive index of the surrounding medium changed from 1.3 to 1.6. Nanoshells are also capable of immensely amplifying SERS signals by many orders of magnitude ($10^{12} – 10^{15}$) through strong electromagnetic near – field enhancement, thus enabling exquisite detection in the picomolar range.[59] Typically, Raman signal strength scales as the electric field to the fourth power (E^4). The following examples serve to highlight the two primary methods for using nanoshells in bio-sensing schemes, namely absorbance and SERS measurements.

CONCLUDING REMARKS

Because of their unique features and vast potential for a variety of biomedical applications, gold nanoshells and other gold nanoparticles represent a major achievement in nanotechnology. The synergy of ideal chemical, physical and optical properties in a single particle is a resounding affirmation of the promise of nanotechnology in general.

Gold nanoshells have opened new frontiers in medicine. Because they are biocompatible, optically tunable, strongly photoluminescent and bind to antibodies, nanoshells are highly suitable for *in vivo* imaging studies. Likewise, because they accumulate within tumors due to passive and active mechanisms, they hold great promise for revolutionizing cancer detection. Their success in multiple animal studies has confirmed a great potential as agents for photothermal cancer therapy, with the added benefit of serving as contrast agents for cancer detection. Clinical trials, which are currently under way, will most likely establish their efficacy for the treatment of human forms of cancer. However, there are several pressing research problems, which are yet to be investigated. For example, what is the largest size tumor that nanoshells can effectively treat? And, can patient - specific antibodies be targeted to enhance their efficacy? It is hoped that, in the near future, these questions will be addressed and the novel properties of nanoshells will continue to be exploited in a growing number of applications. Clearly, it will be very exciting to see many existing applications make the successful transition from the laboratory bench to the clinic.

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