GOLD NANOSHELLS: A REVIEW ON ITS PREPARATION
CHARACTERISATION AND APPLICATIONS

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ABSTRACT
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. Gold nanoshells are unique in that they combine many ideal features in a single particle. As a direct result of nanoscale resonance phenomena, gold nanoshells have very large optical absorption and scattering cross-sections, which render them highly suitable as contrast agents for imaging.

KEYWORDS: Gold nanoshells, Self assembled monolayers (SAMs), surface plasmon resonance (SPR), Alkanethiol futionization.

1. INTRODUCTION
Gold nanoshells are a novel type of composite spherical nanoparticle consisting of a dielectric core covered by a thin metallic shell which is gold. They are a class of nanoparticles with tunable optical resonances. The diameter of these nanoparticles typically ranges from size 10 to 200 nm.

Gold nanoshells possess highly favorable optical and chemical properties for biomedical imaging and therapeutic applications. By varying the relative dimensions of the core and the shell, the optical resonance of these nanoparticles can be precisely and systematically varied.
over a broad region ranging from the near-ultra violet (UV) to the mid-infrared (IR). Gold nanoshells have a core of silica and a metallic outer layer as shown in figure (1.1)

Fig 1.1: Silica core covered with thin layer of gold.

They can be tuned to preferentially absorb or scatter light at specific wavelengths in the visible and near-infrared (NIR) regions of the spectrum. Metal colloids are well-known for their surface plasmon resonance (SPR) properties, which originate from collective oscillation of their conduction electrons in response to optical excitation. \(^{[1-4]}\) From all the metal colloids gold nanoshells have shown tremendous promise for systematic engineering of SPR. They consist of a dielectric core coated with a few nanometers to a few tens of nanometers of gold. \(^{[5]}\) The SPR of these nanoparticles can be varied over hundreds of nanometers in wavelength, across the visible and into the infrared region of the spectrum, by varying the relative dimensions of the core and the shell. The SPR frequency of a particular metal nanoparticle sample is different from that of the corresponding metal film and has been shown to depend on particle size \(^{[6-8]}\), shape \(^{[9-10]}\), and dielectric properties \(^{[11]}\), aggregate morphology \(^{[12]}\), surface modification \(^{[13]}\), and refractive index of the surrounding medium. \(^{[14]}\) Using the Gustav Mie scattering theory and controllable colloidal growth chemistry, the optical resonance of a core–shell composite nanoshell can be “designed”.

2. PREPARATION

2.1. Preparation of silica nanoparticles \(^{[15]}\)

A mixture of 3ml ammonia (30%) and 50ml of absolute ethanol is stirred vigorously, and 1.5ml, 6.7mmol tetraethyl orthosilicate [Si(OC\(_2\)H\(_5\))\(_4\)] (TEOS) is added drop wise. The reaction completes with the formation silicon dioxide (SiO\(_2\)) nucleus from the TEOS
monomer. \cite{16} The time consumed by the reaction is judged by the change of the solution from clear to opaque white. \cite{17} The concentration of the resultant silica nanoparticles should be approx $7 \times 10^{12}$ particles/ml. Analysis is done by transmission electron microscopy (TEM) which should indicate the silica nanoparticles so formed are spherical in shape with approx 100 nm diameters.

2.2. Functionalization of silica nanoparticle surfaces with 3 aminopropyltrimethoxysilane (APTMS)

With the approximate concentration and surface area of the silica nanoparticles known, the amount of APTMS needed for surface functionalization can be estimated. \cite{17,18} Accordingly, with a small excess of APTMS (approx 50 $\mu$l, 0.28 mmol) should be added to a 100 ml of the vigorously stirred silica nanoparticle solution which is allowed to react for 2 hrs. The functionalization reaction is monitored visually by ceasing stirring and observing the separation of the solution into two layers. The APTMS-coated silica nanoparticles precipitate at the bottom, leaving a clear ethanolic solution at the top. To enhance covalent bonding of the APTMS groups to the silica nanoparticle surface \cite{18,19}, the solution is gently refluxed for 1 additional hour. The APTMS-coated silica nanoparticles are purified by centrifuging and re dispersing in ethanol. Analysis of the purified nanoparticles is done by TEM which should show no discernible difference between pre and post functionalization with APTMS.

2.3. Preparation of colloidal gold nanoparticles \cite{20}

A mixture of 45 ml of HPLC grade water, 0.5ml NaOH(1M) and 1 ml of tetrakis(hydroxymethyl) phosphonium chloride (THPC) solution (prepared by adding 12 $\mu$l (0.067 mmol) of 80% THPC in water to 1ml of HPLC grade water). The reaction mixture is stirred for 5 min with a strong vortex in the reaction flask. After the allotted time, 2.0 ml (27 mmol) of 1% HAuCl$_4$ in water is added quickly to the stirred solution. By variation of the volume of 1% HAuCl$_4$ added, the size of the gold colloid particles could be varied. For example, a change from 2.0 mL to 1.5 mL led to a reduction in the diameter of the nanoparticles from 2-3 nm to 1-2 nm. The latter particles are to be near the detection limit of TEM. Dried samples of the gold nanoparticles are dark brown in color, similar to that of Au$_{55}$ clusters. \cite{20} Using the above procedure colloidal gold particles of size 2-3 nm can be obtained which can be used for further steps.
2.4. Attachment of colloidal gold nanoparticles to APTMS functionalized silica cores [17]
An aliquot of APTMS-functionalized silica nanoparticles dispersed in ethanol (0.5 mL, approx $7\times10^{12}$ particles/mL) is placed in a centrifuge tube along with an excess of gold nanoparticles (5 mL of gold colloid solution, approx $7\times10^{14}$ particles/mL). [17] The centrifuge tube is shaken gently for a couple of minutes and then allowed to sit for 2 hrs. The mixture is then centrifuged at 2000 revolutions/min, and a red-colored pellet is observed to settle at the bottom of the tube. The supernatant is decanted, leaving a slightly red-colored pellet, which is redispersed and sonicated in HPLC grade water. The purified Au/APTMS/silica nanoparticles are then redispersed in 5 mL of HPLC grade water which can then be used for further steps.

2.5. Growth of gold nanoshells
To grow the gold over layer on the Au/APTMS/silica nanoparticles, first a suitable solution containing a reducible gold salt should be prepare. [21] In a reaction flask, 25 mg (0.18 mmol) of potassium carbonate ($K_2CO_3$) is dissolved in 100mL of HPLC grade water. After 10 min of stirring, 1.5mL (20 mmol) of a solution of 1% HAuCl$_4$ in water is added. The solution initially appears transparent yellow colour and slowly becomes colorless over the course of 30 min. To a vigorously stirred 4 ml of the colorless solution, a solution containing (200µl) Au/APTMS/silica nanoparticles is injected, to this 10 µl (0.36 mmol) formaldehyde is added. Over the course of 2-4 min, the solution changes from colorless to blue. This is characteristic for the formation of nanoshells. The nanoshells so formed should be centrifuged and redispersed in HPLC grade water until use.

2.6. Adsorption of normal alkanethiols onto gold nanoshells
In a typical procedure, the gold nanoshells are centrifuged and redispersed in ethanol five times prior to exposure to solutions containing the selected alkanethiols. Solutions of three separate alkanethiols in ethanol (2mmol) are prepared: dodecanethiol, hexadecanethiol, and octadecanethiol ($C_nSH$, $n=12, 16, and 18$, respectively). 25 mL of each of the thiol solutions are placed in separate flasks and vigorously stirred. To each of the thiol solutions is added a 25 mL ($3.5\times10^{11}$ particles/mL) of bare gold nanoshells dispersed in ethanol. Each flask is covered and allowed to stir overnight. The individual samples of $C_nSH$ -functionalized nanoshells are then centrifuged and redispersed five times in ethanol to remove any unreacted reagents.
3. CHARACTERISATION

3.1: Imaging of gold nanoshells by TEM
As described above, small colloidal particles of gold are attached to an APTMS-functionalized silica nanoparticle core and then use the attached gold particles to template the growth of a gold overlayer. The gold nanoshells so formed can be easily analysed for their morphology also it can provide important information like continuity of gold layer over the core, diameter of the nanoparticle, thickness of the gold over the core of silica.

3.2. Solubility of (Self assembled monolayer) SAM-coated gold nanoshells
The solubility of metal nanoparticles in various solvents can be used to get an information about the formation of SAMs on their surfaces. [22] On gold nanoparticles, the alkyl chains of alkanethiol based SAMs extend outward from the nanoparticles, enhancing their solubility in nonpolar aprotic solvents (e.g., hexane) but not in water; in contrast, bare gold nanoparticles are soluble in water but insoluble in nonpolar aprotic solvents. Samples of alkanethiol-treated nanoshells are subjected to water, centrifuged, solvent is decanted, and attempted to redispense them in the following solvents: pentane, hexane, benzene, CCl₄, CH₂Cl₂, THF, ethanol, and water. The solubility test is positive only if: First, the alkanethiol treated nanoparticles do not redispersed in water. Second, even if they redispense in CCl₄ and in ethanol, after a course of few hours the nanoshells should precipitate from these solvents. Third, if the alkanethiol-treated nanoshells are redispersed in pentane, hexane, benzene, CH₂Cl₂, and THF, they should remain dissolved in solution even after 48 hrs.

3.3. X-ray photoelectron spectroscopy (XPS) analysis of SAM-coated gold nanoshells
X-ray photoelectron spectroscopy is a method well-suited for the analysis of SAMs because it provides insight regarding the atomic composition of the SAM and the underlying substrate as well as information regarding the nature of the S-Au interactions. \cite{23-25} Survey spectra should show only the presence of C, S, and Au. In particular, no silicon or oxygen should be detected, which indicates complete coverage of the silica core by the overlying gold shell. Another focus of concern regarding the functionalized nanoshells centered on evaluating the presence of free thiols versus bound thiols on the gold nanoshell surfaces. \cite{25}

3.4. Fourier transform infrared (FTIR) analysis of alkanethiol-functionalized gold nanoshells

Infrared spectroscopy offers a wealth of information regarding the structure of SAMs on flat surfaces \cite{26,27} and on the surfaces of nanoparticles. \cite{28,22,29,30,31,32} In particular, IR spectroscopy affords insight into the order and packing of the alkyl chains extending away from the surface. There are, for example, characteristic band positions and intensities for the C-H symmetric and antisymmetric stretches, which can offer insight into the conformational order and orientation of the alkyl chains of SAMs. \cite{26,27} Polyethylene serves as a useful liquid and/or crystalline model for the methylene backbones of hydrocarbon SAMs. \cite{33} When dissolved in solution, the antisymmetric \{-\-(CH\_2\)-\} bands and the symmetric \{-\-(CH\_2)\}- bands appear at 2928 cm\(^{-1}\) and 2856 cm\(^{-1}\), respectively. In crystalline form, however, the antisymmetric and symmetric bands appear at \{-\-(CH\_2)\}- 2920 cm\(^{-1}\) and \{-\-(CH\_2)\}- 2850 cm\(^{-1}\) respectively.

3.5. Raman analysis of alkanethiol-functionalized gold nanoshells

As described in the previous section, analysis of the \{-\-(CH\_2)\}- bands by FTIR spectroscopy provides insight regarding the conformational order of the alkyl chains of SAMs. Since, however, the symmetric (C-C) bands are weak or almost undetectable by FTIR, little information can be gleaned by IR analysis of the C-C region. \cite{34} In contrast, Raman spectroscopy can readily detect symmetrical bonds, such as for those found in the C-C backbones of SAMs. Furthermore, for alkanethiol SAMs, analysis of the C-S and S-H bands by Raman spectroscopy provides structural and chemical insight into the interaction between the adsorbate and the underlying metal substrate. \cite{21,34,35} Upon chemisorption to gold, the S-H bond of alkanethiols is cleaved. \cite{34} Thus, by examining the relative intensity of the symmetric (S-H) band in free thiol and thiol nanoshell samples, the adsorption step can be monitored.
3.6. UV-VIS spectra of alkanethiol-functionalized gold nanoshells

Theoretical calculations have shown that the plasmon resonance of noble metal nanoshells can vary over hundreds of nanometers. The position of the resonance is dictated by both the shell thickness and the size of the dielectric core. As a rule of thumb, thick shells around small cores give rise to resonances in the visible region, while thin shells around large cores give rise to resonances in the infrared region. Given that the Plasmon resonance of noble metals is further sensitive to the medium in contact with the surface of the metal. UV-vis spectroscopy can be used at wavelengths ranging from 300nm to 1100 nm to analyze the optical absorbances of bare and SAM-coated nanoshells.

4. 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 bioconjugates have been used to detect and image individual cancer cells in vitro and in solid tumours in vivo. As Photo thermal 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 level.

4.1. Cell and phantom imaging

Nowadays, the most imaging studies using gold nanoparticles were carried out in cell culture. The versatile optical properties of gold nanoparticles have enabled optical imaging of cells and phantoms with a wide variety of contrast mechanisms. Subsequently, many other studies have been reported which employed Photo thermal interference contrast.

4.2. 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, tumour formation and metastasis.

4.3. Cancer therapy
Conventional strategies for cancer intervention include surgery, chemotherapy, and radiation therapy. Taking advantage of their unique properties, most studies of gold nanoparticle–based cancer therapy have used photothermal therapy for the destruction of cancer cells or tumour tissue, which may be potentially useful in the clinical setting. When irradiated with focused laser pulses of suitable wavelength, targeted gold nanospheres, nanorods, nanoshells and nanocages can kill bacteria and cancer cells. \[^{[41-43]}\] It was estimated that 70–80°C was achieved through light absorption by the gold nanoparticles and up to 150 antibodies can be conjugated to a nanoshell through a bifunctional PEG linker. \[^{[42, 43]}\] One intriguing observation is that most of these studies targeted either epidermal growth hormone receptor (EGFR) or human epidermal growth factor receptor 2 (HER2), obviously due to the ready availability of monoclonal antibodies (already approved by the Food and Drug Administration [FDA] for cancer therapy) that recognize these two proteins.

4.4 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. \[^{[42, 44]}\] 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.

4.5 Biosensors
Nanoshells have several unique properties that are ideal for biosensing applications. The position of the Plasmon resonance peak and absorbance depended heavily on the refractive index (dielectric constant) of the surrounding medium.

4.6 Drug delivery
Several studies have reported the use of gold nanoparticle as drug delivery vehicles. Tumour necrosis factor– alpha (TNF–α), a cytokine with excellent anticancer efficacy, is systemically
toxic which severely limited its therapeutic applications. A nanoparticle delivery system, consisting of PEG coated gold nanoparticle loaded with TNF–α, was constructed to maximize the tumour damage and minimize the systemic toxicity of TNF–α. Combination of local heating and nanoparticle–based delivery of TNF–α resulted in enhanced therapeutic efficacy than either treatment alone. Thermally induced tumour growth delay was enhanced by pre–treatment with the nanoparticle, when given intravenously at the proper dosage and timing. Tumor blood flow suppression, as well as tumour perfusion defects, suggested vascular damage mediated tumour cell killing. Surprisingly, following intravenous administration, little to no accumulation in the reticuloendothelial system (RES) (eg. liver and spleen) or other healthy organs of the animals was observed.

5. CONCLUSION AND FUTURE PROSPECTIVE
Combining advances in biophotonics and nanotechnology offers the opportunity to significantly impact future strategies towards the detection and therapy of cancer. Today, cancer is typically diagnosed many years after it has developed usually after the discovery of either a palpable mass or based on relatively low resolution imaging of smaller but still significant masses. In the future, it is likely that contrast agents targeted to molecular markers of disease will routinely provide molecular information that enables characterization of disease susceptibility long before pathologic changes occur at the anatomic level. Currently, the ability to develop molecular contrast agents is at times constrained by limitations in the understanding of the earliest molecular signatures of specific cancers. Although the process of identifying appropriate targets for detection and therapy is ongoing, there is a strong need to develop the technologies which will allow imaging these molecular targets in vivo as they are elucidated.

More extensive in vivo animal studies for both cancer imaging and therapy applications are currently underway in order to investigate both the potential and limitations of nanoshell technologies. Additional studies are in progress to more thoroughly assess the biodistribution and biocompatibility of nanoshells used in in vivo imaging and therapy applications. There is tremendous potential for synergy between the rapidly developing fields of biophotonics and nanotechnology. Combining the tools of both fields – together with the latest advances in understanding the molecular origins of cancer may provide a fundamentally new approach to detection and treatment of cancer, as per the data on cancer mortality displayed on worldometers published by the World Health Organization (WHO) it is the disease
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responsible for death of 7.6 million individuals per year calculated in 2005, which is estimated to rise up to 9 million by 2015 and 11.4 million by 2030.

REFERENCES


