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# Gold Nanoshell: The Advancing Nanotechnology to Fight Against Cancer

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### ABSTRACT

It has been almost 40 years since the “cancer war” had been declared. It is now generally believed that personalized medicine is the future for cancer patient management. Gold nanoparticles, nanospheres, nanorods, nanoshells will be discussed in detail regarding their uses in *in-vitro* assays, *ex-vivo* and *in-vivo* imaging, cancer therapy and drug delivery. Multifunctionality is the key feature of nanoparticle-based agents. Targeting ligands, imaging labels, therapeutic drugs, and other functionalities can all be integrated to allow for targeted molecular imaging and molecular therapy of cancer. Gold nanoshells, mainly composed of the silica and gold metal. The antibodies of Anti-Epidermal Growth Factor Receptor is chief component of Gold Nanoshells therapy used to target the cancer cells and to “guide” the Gold nanoshells to detect the cancer cells visually by microscope. The future looks brighter than every at many hurdles remain to be conquered. A multifunctional platform based on gold nanoparticles, with multiple receptor retargeting, multimodality imaging and multiple therapeutic entities, holds the promise for a “magic gold bullet” against cancer. In this review, we will summarize the current state-of-the-art of gold nanoshells with mechanism in biomedical applications targeting cancer.

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## Introduction

There is a convenient and specific clinical need for novel methods for detection and treatment of cancer which offer improved sensitivity, specificity and cost-effectiveness. In recent years, a number of groups have demonstrated that photonics-based technologies are valuable in addressing this need. Optical technologies promise high resolution, non invasive functional imaging of tissue at competitive costs. However, in many cases, these technologies are limited by the inherently weak optical signals of endogenous chromophores and the subtle spectral differences of normal and diseased tissue. Over the past several years, there has been increasing interest in combining emerging optical technologies with the development of novel exogenous contrast agents, designed to probe the molecular specific signatures of cancer, to improve the detection limits and clinical effectiveness of optical imaging. Several scientists has been demonstrated the use and application of gold nanoshells. Recently, interest has developed in the creation of nanotechnology-based platform technologies which couple molecular specific early detection strategies with appropriate therapeutic intervention and monitoring capabilities.

The discovery of the nanoshells was made by Professor Naomi J. Halas and her team at Rice University in 2003. In 2003 Halas was awarded for Best Discovery of 2003 by Nanotechnology.

### Composition of Gold Nanoshells

Metal nanoshells (Figure–1) area new type of nanoparticle composed of a dielectric core such as silica coated with an ultrathin metallic layer, which is typically gold.<sup>1,2</sup> Gold nanoshells possess physical properties similar to gold colloid, in particular, a strong optical absorption due to the collective electronic response of the metal to light. The optical absorption of gold colloid yields a brilliant

red colour which has been of considerable utility in consumer-related medical products, such as home pregnancy tests. In contrast, the optical response of gold nanoshells depends dramatically on the relative size of the nanoparticle core and the thickness of the gold shell. By varying the relative core and shell thicknesses, the colour of gold nanoshells can be varied across a broad range of the optical spectrum that spans the visible and the near infrared spectral regions.

### Types of Gold Nano Particles

There are many subtypes of gold nanoparticles based on the size, shape and physical properties (Figure–2). The earliest studied gold nanoparticles are gold nanospheres (although not exactly spherical in a strict sense). Subsequently, nanorods, nanoshells, and nanocages have all been reported. Another type of gold based nanoparticles, with excellent surface-enhanced Raman scattering properties (termed “SERS nanoparticles”), will also be discussed in this review.<sup>3,4</sup> In the following text, the term “gold nanoparticle(s)” will refer to a collection of all these subtypes and the subtype of gold nanoparticles used in each study will be specified whenever possible. With continued development in the synthesis techniques over the last two decades, most of these gold nanoparticles can now be produced with well controlled size distribution, sometimes with stunning precision (e.g., nanocages).<sup>5</sup>

### Gold Nanoshells

Optical imaging, include those that uses gold nanoparticles as the contrast agents, has very limited applications in human studies. However, in the near-infrared region (NIR; 700–900 nm), the absorbance of all bio molecules reaches minimum which provides a relatively clear window for optical imaging.<sup>6</sup> By varying the composition and dimensions of the layers, gold nanoshells can be designed

and fabricated with surface plasmon resonance (SPR) peaks ranging from the visible to the NIR region.<sup>7</sup> For a given composition of gold nanoshell, the SPR peak can be tuned by changing the ratio of the core size to its shell thickness.

Gold nanoshells with SPR peaks in the NIR region can be prepared by coating silica or polymer beads with gold shells of variable thickness.<sup>8,9</sup> Silica cores are grown using the Stöber process, the basic reduction of tetraethyl orthosilicate in ethanol. To coat the silica nanoparticles with gold in an aqueous environment, a seeded growth technique is typically used. Small gold nanospheres (2–4 nm in diameter) can be attached to the silica core using an amine-terminated silane as a linker molecule, allowing additional gold to be reduced until the seed particles coalesced into a complete shell.<sup>10</sup> The diameter of the gold nanoshell is largely determined by the diameter of the silica core and the shell thickness can be controlled through the amount of gold deposited on the surface of the core.

Gold nanoshells have also been synthesized via *in-situ* gold nanoparticle formation using thermo sensitive core-shell particles as the template.<sup>11,12</sup> The use of microgel as the core offers significantly reduced particle aggregation, as well as thickness control of the gold nanoshells using electroless gold plating. In one study, a virus scaffold has been used to assemble gold nanoshells.<sup>13</sup> This approach may potentially provide cores with a narrower size distribution and smaller diameters (80 nm) than those of silica. (Figure–3 & 4).

### Synthesis and Bioconjugation

The synthetic protocol developed for the fabrication of gold nanoshells is very simple in concept:<sup>14</sup>

**I.** Grow or obtain silica nanoparticles dispersed in solution,

**II.** Attach very small (1–2 nm) metal “seed” colloid to the surface of the nanoparticles via molecular linkages; these seed colloids cover the dielectric nanoparticle surfaces with a discontinuous metal colloid layer,

**III.** Grow additional metal onto the “seed” metal colloid adsorbates via chemical reduction in solution.

This approach has been successfully used to grow both gold and silver metallic shells onto silica nanoparticles. Various stages in the growth of a gold metallic shell onto a functionalized silica nanoparticle are shown in Figure–5.

### Type–1. Synthesis of Gold nano Particles

The original synthesis is a four-step process in which first, mono dispersed silica nanoparticles are grown using the Stober method to produce the spherical dielectric cores. 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-amino propyl triethoxysilane), 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  $\text{HAuCl}_4$  by alkaline tetrakis (hydroxymethyl)–phosphonium chloride, according to the method of Duff.<sup>15</sup> The gold particles bond to the organosilane linker via the amine group, producing silica nanoparticles with a smattered, uneven gold coating.<sup>16</sup> A final reduction process is used to produce silica nanoparticles with a uniform layer of gold that is, a gold nanoshells. 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.<sup>17</sup> Transmission electron microscopy (TEM) images of the nanoshells during different phases of growth are shown in Figure-6.<sup>18</sup>

### Type-2: Bioconjugation: Smarter Nanoshells

The biologically inert gold surface of nanoshells facilitates bio conjugation with antibodies and other biomarkers, rendering nanoshells capable of selectively binding to *in-vivo* targets.<sup>19-21</sup> The following examples of successful bioconjugation schemes should provide a general idea of the chemistry involved in the production of bio conjugated nanoparticles.

Sokolov *et al.* have synthesized bioconjugates of gold nanospheres with monoclonal antibodies against epidermal growth factor receptor (EGFR), a transmembrane glycoprotein (Mr 170000) which is over expressed in cancers originating from epithelial cells.<sup>20</sup> Colloidal gold of various sizes is prepared using a citrate reduction of HAuCl<sub>4</sub>.

To prepare the bioconjugates, the gold colloid is diluted with 20mm HEPES buffer and anti-EGFR monoclonal antibodies are reconstituted in the same buffer at 100g/ml and mixed at a 1:1 volume ratio and allowed to interact for 20 min at room temperature. In this environment, gold Nano spheres bind noncovalently with anti-EGFR antibodies at their isoelectric point to form stable bio conjugates. Polyethylene glycol (PEG) is added to the solution up to a final concentration of 0.2mg/ml, after which the solution is centrifuged to remove any unbound antibody. After a second wash, the anti-EGFR gold nanoparticle pellet is re-suspended 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 bio conjugated gold nanoshells with HER2 antibodies to target human mammary adeno carcinoma cells *in-vitro*.<sup>19,20,24</sup> In the synthesis, orthopyridyl-disulfide-n-hydroxysuccinimide-PEG polymer (OPSS) is used to tether the antibodies on the surface after which, using NaHCO<sub>3</sub> (100 mm, 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) is then allowed to interact with added gold nanoshells for 1 h and any unbound antibody is then removed by centrifugation. The functionalized gold nanoshells pellet is then re-suspended in potassium carbonate solution (2 mM). Following antibody conjugation, the nano shell surfaces are further modified with PEG-thiol to prevent any nonspecific adsorption and improve biocompatibility.

### Materials and Methods Used in Preparation of Gold Nanoshells

#### 1. Gold Nanoshell Fabrication

Cores of silica nanoparticles are fabricated as described by Stober *et al.* in which tetraethyl orthosilicate is reduced in NH<sub>4</sub>OH in ethanol particles are sized with a scanning electron microscope.<sup>14,22</sup> Polydispersity of <10% is considered acceptable. Next, the silica surface is aminated by reaction with aminopropyltriethoxysilane in ethanol. Gold shells are grown using the method of Duff *et al.*<sup>15,23</sup> Briefly, small gold colloid (1-3nm) was adsorbed onto the aminated silica nanoparticle surface. More gold is then reduced onto these colloid nucleation sites using potassium carbonate and HAuCl<sub>4</sub> in the

presence of formaldehyde. Gold nanoshell formation and dimensions are assessed with a UV-VIS spectrophotometer and scanning electron microscopy (SEM).

## 2. Antibody Conjugation

Ortho-pyridyl-disulfide-n-hydroxyl succinimide polyethylene glycol polymer (OPSS-PEG-NHS, MW=2000) is used to tether antibodies onto the surfaces of gold nanoshells. Using NaHCO<sub>3</sub> (100mM, pH 8.5) OPSS-PEG-NHS is re-suspended to a volume equal to that of either HER<sub>2</sub> (specific) or IgG (non-specific) antibodies. At this concentration, the concentration of polymer is in molar excess to the amount of HER<sub>2</sub> or IgG antibody used. The reaction is allowed to proceed on ice overnight. Unbound antibody is removed by centrifugation at 650 G, supernatant removal and resuspension in potassium carbonate (2mM). Following antibody conjugation, nanoshells surfaces were further modified with PEG-thiol (MW=5000, 1μM) to block non-specific adsorption sites and to enhance biocompatibility.<sup>24</sup>

## 3. Cell Culture

HER2-positive SKBr<sub>3</sub> human mammary adenocarcinoma cells are cultured in McCoy's 5A modified medium supplemented with 10% FBS and antibiotics. Cells were maintained at 37°C and 5% CO<sub>2</sub>.

## 4. Molecular Imaging, Cytotoxicity and Silver Staining

SKBR<sub>3</sub> cells are exposed to 8μg/mL of bio conjugated nanoshells for 1 hr, washed with phosphate-buffered saline and observed under darkfield microscopy, a form of microscopy sensitive only to scattered light. The calcein-AM live stain (Molecular Probes, 1 μM) is used to assess cell viability after nanoshell targeting. A silver enhancement stain (Amersham Pharmacia), a qualitative stain capable of detecting the

presence of gold on cell surfaces, is used to assess cellular nanoshell binding. Cells incubated with targeted nanoshells are fixed with 2.5% glutaraldehyde, and exposed to silver stain for 15 minutes. Silver growth is monitored under phase-contrast, with further silver enhancement blocked by immersion in 2.5% sodium thiosulfate.

## 5. Optical Coherence Tomography (OCT)

Optical coherent tomography (OCT) is a state-of-the-art imaging technique which produces high resolution (typically 10–15 μm), real-time, cross-sectional images through biological tissues. The method is often described as an optical analog to ultrasound. OCT detects the reflections of a low coherence light source directed into a tissue and determines at what depth the reflections occurred. By employing a heterodyne optical detection scheme, OCT is able to detect very faint reflections relative to the incident power delivered to the tissue. In OCT imaging out of focus light is strongly rejected due to the coherence grating inherent to the approach. This permits deeper imaging using OCT than is possible using alternative methods such as reflectance confocal microscopy where the out of focus rejection achievable is far lower. Each image required approximately 20 seconds to acquire. System parameters remained the same throughout the experiment.<sup>25</sup>

## 6. *In-vitro* Photo Thermal Nano Shell Therapy

The SKBr<sub>3</sub> breast cancer cells were cultured in 24-well plates until fully confluent. Cells were then divided into two treatment groups: nanoshells + NIR-laser and NIR-laser alone. Cells exposed to nanoshells alone or cells receiving neither nanoshells nor laser were used as controls. Nanoshells were prepared in FBS-free medium (2×10<sup>9</sup> Nanoshells/mL). Cells were then irradiated under a laser emitting light at 820 nm at a

power density of  $\sim 35\text{W}/\text{cm}^2$  for 7 minutes with or without Nanoshells. After NIR–light exposure, cells were replenished with FBS–containing media and were incubated for an additional hour at  $37^\circ\text{C}$ . Cells were then exposed to the calcein–AM live stain for 45 minutes in order to measure cell viability. The calcein dye causes viable cells to fluoresce green. Fluorescence was visualized with a Zeiss Axiovert 135 fluorescence microscope equipped with a filter set specific for excitation and emission wavelengths at 480 and 535 nm, respectively. Membrane damage was assessed using an aldehyde–fixable fluorescein dextran dye. Cells were incubated for 30 min with the fluorescent dextran, rinsed and immediately fixed with 5% glutaraldehyde. Photothermal destruction of cells was attributed to hyperthermia induced via Nanoshell absorption of NIR light.<sup>26</sup>

### Mechanism of Gold Nanoshells on Cancer Cells

**STEP 1:** Gold nanoshells and Antibodies of EGFR (Epidermal Growth Factor Receptor) solution treated with IR Radiation. So, antibodies of EGFR joined with gold nanoshells with the help of amine group. Here, amine group works as a catalyst. (Figure–7)

**STEP 2:** Now, antibodies of EGFR are the keys to unlock the cancer cells for target. So it matches with cancer cells' specific receptor sites. (Figure–8)

**STEP 3:** Then, nanoshells are already in IR region, heated up. At that time pass laser light for specific time. (It depends on size of cancer tumour) which melts the gold nanoshells which are already present around cancer tumour. (Figure–9)

**STEP 4:** It is the final process, in which laser light melts all gold nanoshells, it burns the all targeted cancer cells by the heat and it doesn't affect on normal body cells because there are

no any antibodies of EGFR nor gold shells. So, it is safe. (Figure–10)

### Other Useful Applications of Gold Nanoparticles

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  $\text{ng ml}^{-1}$  level.

**1. Cell and Phantom Imaging:** Now a days, 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. Functional cellular imaging around single molecules has been reported, taking advantage of the enhanced second harmonic signal by antibody conjugated gold nanospheres.<sup>27</sup> Subsequently, many other studies have been reported which employed Photo thermal interference contrast.<sup>28</sup>

**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.<sup>29</sup> As many

cancers originate from a small number of malignant epithelial cells, the ability to detect low numbers of malignant or precancerous epithelial cells.<sup>30</sup> *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.

**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.<sup>31–33</sup> 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.<sup>32,33</sup> One intriguing observation is that most of these studies targeted either 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. 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.<sup>32,34</sup> 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.

**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.

**6. Drug Delivery:** Several studies have reported the use of gold nanoparticle as drug delivery vehicles. Tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), a cytokine with excellent anticancer efficacy, is systemically toxic which severely limited its therapeutic applications.<sup>35,36</sup> A nanoparticle delivery system, consisting of PEG coated gold nanoparticle loaded with TNF- $\alpha$ , was constructed to maximize the tumour damage and minimize the systemic toxicity of TNF- $\alpha$ .<sup>37</sup> Combination of local heating and nanoparticle-based delivery of TNF- $\alpha$  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 RES (eg. liver and spleen) or other healthy organs of the animals was observed.<sup>37</sup>

## Conclusion

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 photo luminescent 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.

In this way, the title would be perfect for gold nanoshells as it's work like a soldier against cancer tumour cells. All other treatments also can be provided for that. Other vast applications in nanotechnology, gold nanoshells would definitely new era for the treatment against non-curable diseases.

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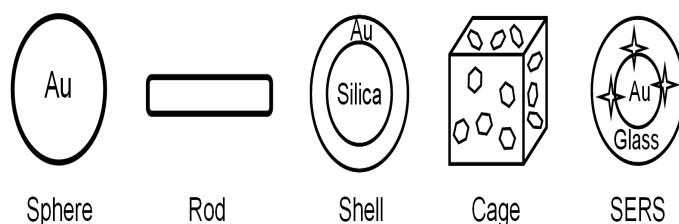
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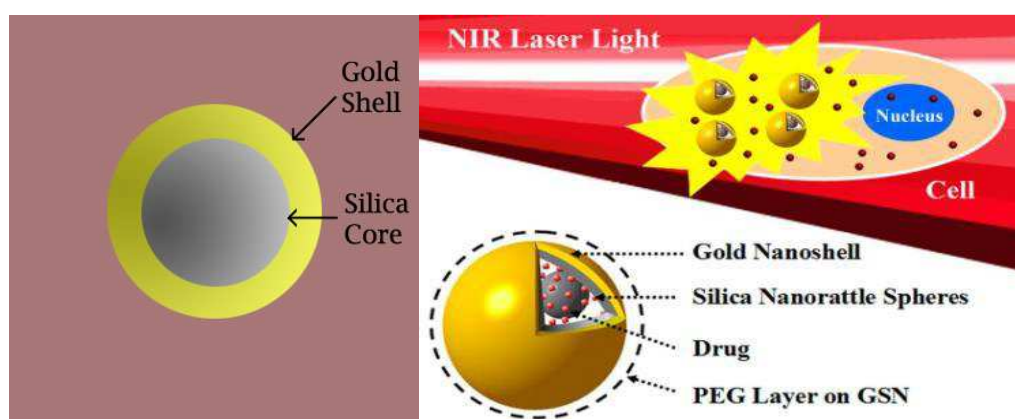
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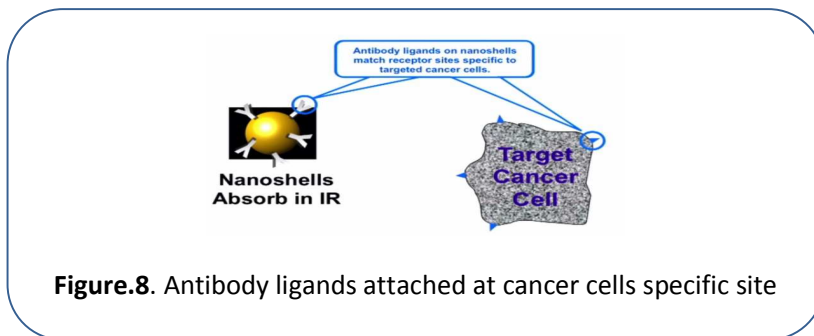
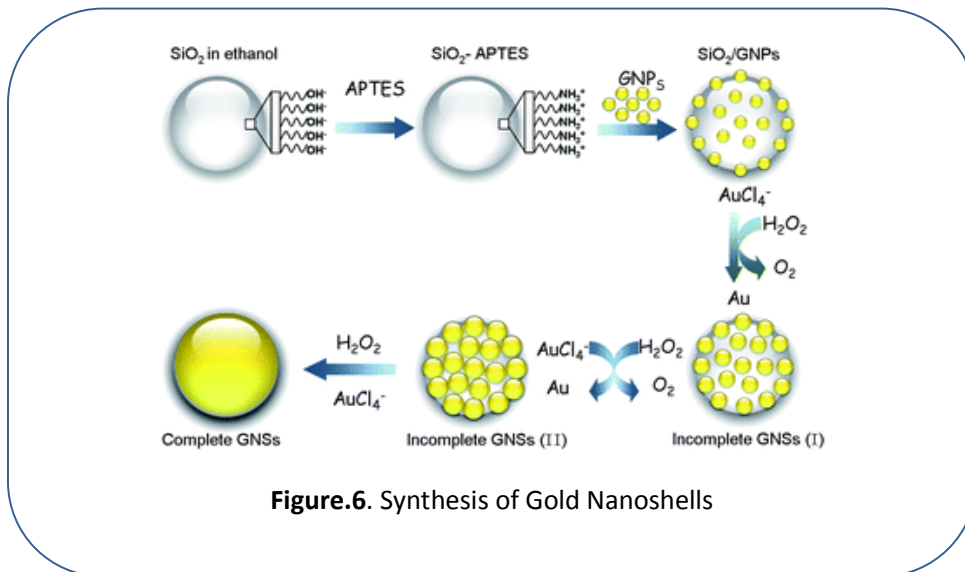
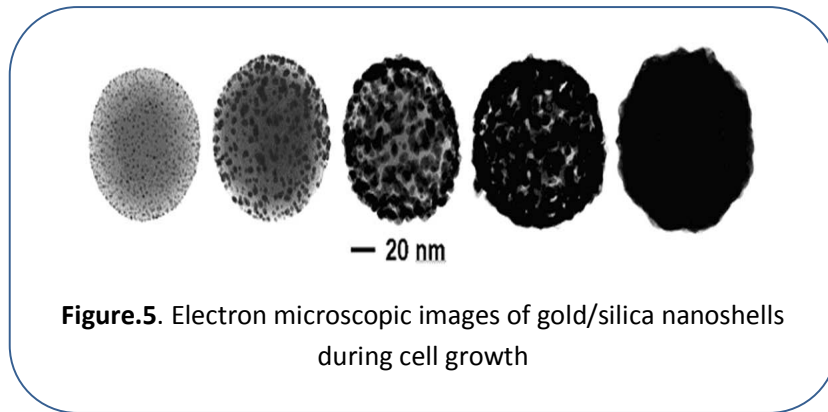
**Figure.1.** Visual Demonstration of Turbidity of Gold nanoshells



**Figure.2.** Different types of Gold Nanoparticles



**Figure.3 & 4.** Preparation of Gold Nanoshells



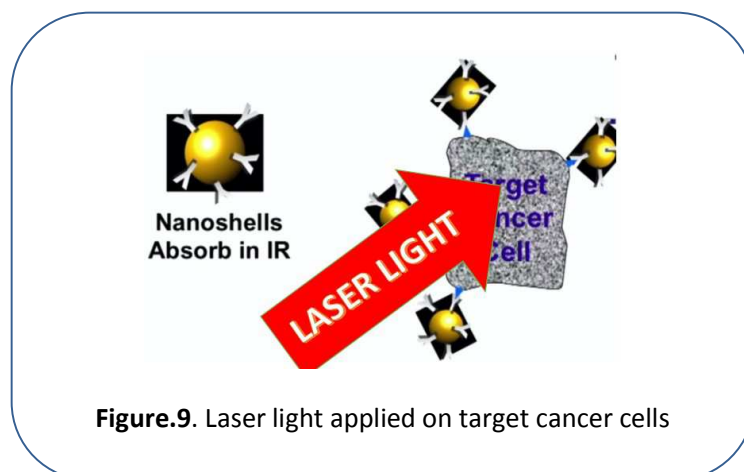


Figure.9. Laser light applied on target cancer cells

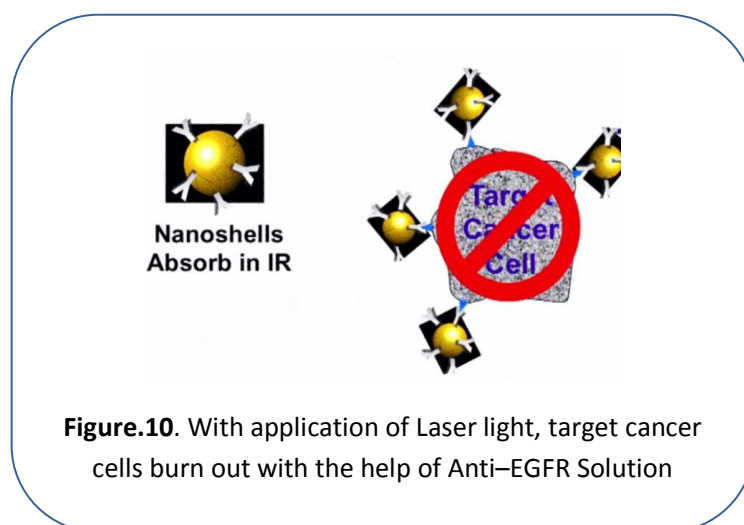


Figure.10. With application of Laser light, target cancer cells burn out with the help of Anti-EGFR Solution