



Enhanced Anticancer Effect of Green Synthesized Nanogold On Different Cell Lines

Dr. Anna H. Faid

Department of laser science and interaction, National Institute for Laser Enhanced Science (NILES) Cairo University, Egypt.

Dr. Marwa Sharaky

Cancer Biology Department, National Cancer Institute (NCI), Cairo University, Cairo, Egypt.

Dr. Marwa A. Ramadan

Department of laser application in metrology, photochemistry and agriculture, National Institute for Laser Enhanced Science (NILES) Cairo University (CU), Egypt.

Abstract:

Cancer is important cause of mortality, due to difficulty in finding safe, economical and specific effective therapeutic targets. The aim was to investigate the cytotoxicity of hybrid gold-chitosan nanospheres (Au@CsNs) on different cell lines using chitosan. Au@CsNs were analyzed by Zeta potential, UV-Visible spectroscopy, TEM and FTIR. Absorbance at 522 nm correlated to surface plasmon resonance (SPR) of AuNPs. TEM images show that Au@CsNs are spherical, with an average size of 12 ± 2 nm. Results of DPSS (diode pumped solid-state) laser irradiation show the photothermal laser stability of Au@CsNs. Au@CsNs showed anticancer effect using SRP assay on different cancer cell lines HCT, FADU, H460, and HEPG2. Our results suggest that Au@CsNs have an antiproliferative effect as there were a significant decrease in cell viability but this depends on the type of cancer cell line. In conclusion, Au@CsNs exhibited great anticancer activity subsequently it will be used in drug delivery and photothermal therapy in future work.

Keywords: Green Synthesis; Chitosan-coated Gold Nanoparticles; Cancer cells and Laser photostability.

Introduction

Cancer is the leading reasons of death, killing a predictable 7.6 million people each year, accounting for 13% of all deaths. Cancer-related deaths are projected to rise to 13.1 million by 2030.^[1] Metastatic cancer mortality rates in Africa and Asia are 7.3% and 57.3%, respectively. Several cancers, including lung, breast, and colon, are the three most common cancers.^[2] One of the anticipated benefits of nanotechnology for biomedical applications^[3-7] specially cancer therapy is tumor targeting.^[8-10] AuNPs are popular for their many advantages, such as B. high target loading efficiency, improved

ability to overcome various physiological barriers, and low systemic side effects an equally important biomedical tool for cancer researchers.^[11, 12] AuNPs are a novel class of anticancer drugs with aggregation and size-dependent cytotoxic activity against various cancer cells.^[13, 14] Utmost nanostructures need widespread post-processing and biofunctionalization to reduce cytotoxicity by means of polysaccharide chitosan for nano reduction and stabilization.^[15] Using a range of biological bases, for example plant extracts, microbes, enzymes and starches, researchers created nanoparticles in a more environmentally friendly way. The creation of nanoparticles from marine resources has garnered a lot of interest lately.^[16] The growth of nanoscience in the development of aquatic resources is in its beginning, despite the fact that the majority of aquatic resources were widely used for a variety of purposes. Shells, pearls, fish bones, and other biologically active materials are employed as sources. These materials have all been used extensively.^[17] Biopolymers can be used to stabilize synthetic metal nanoparticles and prevent particle aggregation. Chitin/chitosan is a typical biopolymer. Chitin/chitosan is a typical biopolymer. After cellulose, chitin is the most prevalent natural biopolymer. Due to its capacity to interact with metal nanoparticles, chitosan, a form of chitosan, is mostly utilized in the creation of nanocomposites.^[18] Chitin, a naturally occurring polysaccharide made up of -(1,4)-linked N-acetylglucosamine units, is the source of a group of polymers known collectively as chitosan (Cs).^[19, 20] Chitosan is soluble in weak acids because of the protonation of its free amine groups,^[21] which is directly related to how much deacetylation it has undergone. Chitosan's varied pharmacological and biological qualities are also influenced by its amine groups.^[22] Good biocompatibility, low immunogenicity, and simple in vivo biodegradation are all benefits of chitosan. This approach has the benefits of being secure, precise, quick, and efficient. The use of nanoscience to physiologically active natural items is highly alluring and is now progressing very quickly. When utilized to provide natural products to treat different types of cancer or other disorders, it has a number of benefits.^[23] Due to their unique capacities for promoting antineoplastic and antibacterial agents, natural substances have been thoroughly investigated for the treatment of numerous diseases.^[8, 24] Among polymer nanoparticles, those made with the natural polymer chitosan are the most effective, affordable, and environmentally beneficial.^[25, 26] According to reports, soluble CS and CS microspheres exhibit some level of toxicity towards specific cell lines, indicating the potential for their use as anticancer medications.^[27] Because of the surface plasmonic molecule, biocompatible Cs-metal nanoparticles make effective photo-absorbers for photothermal therapy.^[28] In this paper, we describe the environmentally friendly production of AuNPs using chitosan. Additionally, research was done on the cytotoxic effects of Au@CsNs on several cell lines.

Material and Methods

synthesis of Au@CsNs

A wine-colored, crimson solution was produced by heating a concentrated 20 μl of 0.125M chloroauric acid (HAuCl_4) solution for 20 minutes in a 20 ml 0.1% chitosan solution to create Au@CsNs.^[29]

Laser Photostability of Au@CsNs

Diod Pumped Solid State (DPSS) laser 532 nm with 150 mW was used to irradiate for 2, 4, 6 and 8 min to test for Au@CsNs photostability then the absorption spectra were observed before and after exposure.

Characterization techniques

SPR of Au@CsNs was observed (PG Instrument, T80+, UK, double beam spectrophotometer). In order to record the absorbance within the required scan range (350nm to 700nm), 400 μl of the solution were added to 4 ml water. The morphology of the prepared Au@CsNs was examined using TEM at the Agriculture Research Center's (ARC) Nanotechnology and Advanced Material Central Lab. Dutch company FEI is its name. Super twin, double tilt, Tecnai G20 Magnification series of up to 1,000,000 X, 200 kV applied voltage, and LaB6 Gun type. A drop from an extremely diluted solution was applied on a copper grid coated with amorphous carbon to form a monolayer, and identified by TEM. DLS analysis were used to determine the surface charges of Au@CsNs. Based on photon correlation spectroscopy, the zeta potential was calculated using the Zetasizer 300 HAS (Malvern Instruments, Malvern, UK). The average zeta potential was found after a 60-s analysis. Without dilution, the zeta potential of AuNPs was calculated. FTIR measurements were made between 500 and 4500 cm^{-1} using an FT-IRs (4100 Jasco-Japan). Using a lyophilizer, Au@CsNs were freeze dried. Following dilution with a potassium bromide (KBr) pellet then the IR spectra were examined.

Cytotoxicity assay

Different concentrations of Au@CsNs were used. Human lung (H460), human colon (HCT116), human liver (HEPG2) and human hypopharyngeal (FaDu) cancer cell line were used. The American Type Culture Collection (ATCC, Minnesota, USA) offer it for use. The National Cancer Institute (NCI), Cairo, Egypt, has kept the tumour cell line. Sulphorhodamine-B (SRB) assay was applied to test Au@CsNs' antitumor effects on all examined cell lines.^[30] In 96-well microtiter plates, cells were cultured at a concentration of 3×10^3 cells/well. They were allowed to attach for 24 hours. For (H460), (HCT116), (HEPG2), and (FaDu) cells, different concentrations of Au@CsNs (200, 400, and 600 $\mu\text{g/ml}$) were then applied to the cells. Three wells were used for each concentration, and the

incubation process went on for another 48 hours. DMSO (1% v/v) was applied as a control. Then tainted with 0.4% SRB dye after being fixed with 20% trichloroacetic acid at the conclusion of incubation. An ELISA microplate reader (TECAN sunrise TM, Germany) was used to spectrophotometrically quantify the optical density (O.D.) of each well at 570 nm. The subsequent formulation was used to get the mean survival fraction at each medication concentration: O.D. of the treated cells compared to the O.D. of control cells. Each drug's IC50 value was analyzed by sigmoidal dose response curve-fitting models in order to achieve 50% suppression of cell growth.

Statistical analysis

The data was presented as mean \pm SD. Graph Pad Software Prism v5 was used to do the statistical analysis. The Tukey multiple comparison test was used for the statistical analysis. When $p \leq 0.05$, differences were statistically significant.

Results and discussion

Numerous chemicals reducing agents, for example trisodium citrate, thiocyanate, and even alcohols, are used to prepare different metallic nanoparticles.^[5, 31-33] Chitosan assists as a capping and reducing agent. Owing to its advantageous organic properties, low toxicity, and high sensitivity to biodegradation, chitosan is used in this situation. The production of nanoparticles is fueled by the electrostatic attraction interactions between the negatively charged AuCl^{-4} ion and the positively charged amino group of chitosan. The size also depends on the reaction temperature. ^[34] Au@CsNs shows surface Plasmon resonance SPR at 523nm as a result of collective oscillations of the electron at exterior of Au@CsNs that is related with the electromagnetic field of the inward light.^[35] CS and AuNPs absorption bands were revealed in Figure (1a) , Since CS display a peak at 300 nm, its nonappearance shows that all of its amino groups are actively produced during the reduction process and that there are no remnants of blank CS in the AuNPs chitosan suspension. Due to the great affinity of AuNPs for interacting with oxidised amine groups, the generated Au@CsNs significantly adsorbs to CS after reduction. In CS suspension, the produced Au@CsNs exhibit exceptional stability.^[36] The usage of Au@CsNs as a cancer treatment agent highlights the significance of photostability. Cells exposed to laser light when these particles are utilized as photothermal agents in photothermal treatment. As shown in figure (1c) upon laser irradiation has no alteration in the absorption peak confirming the stability of Au@CsNs . In figure (1b) TEM image of Au@CsNs have sphere-shaped and size variety from 12 ± 2 nm.

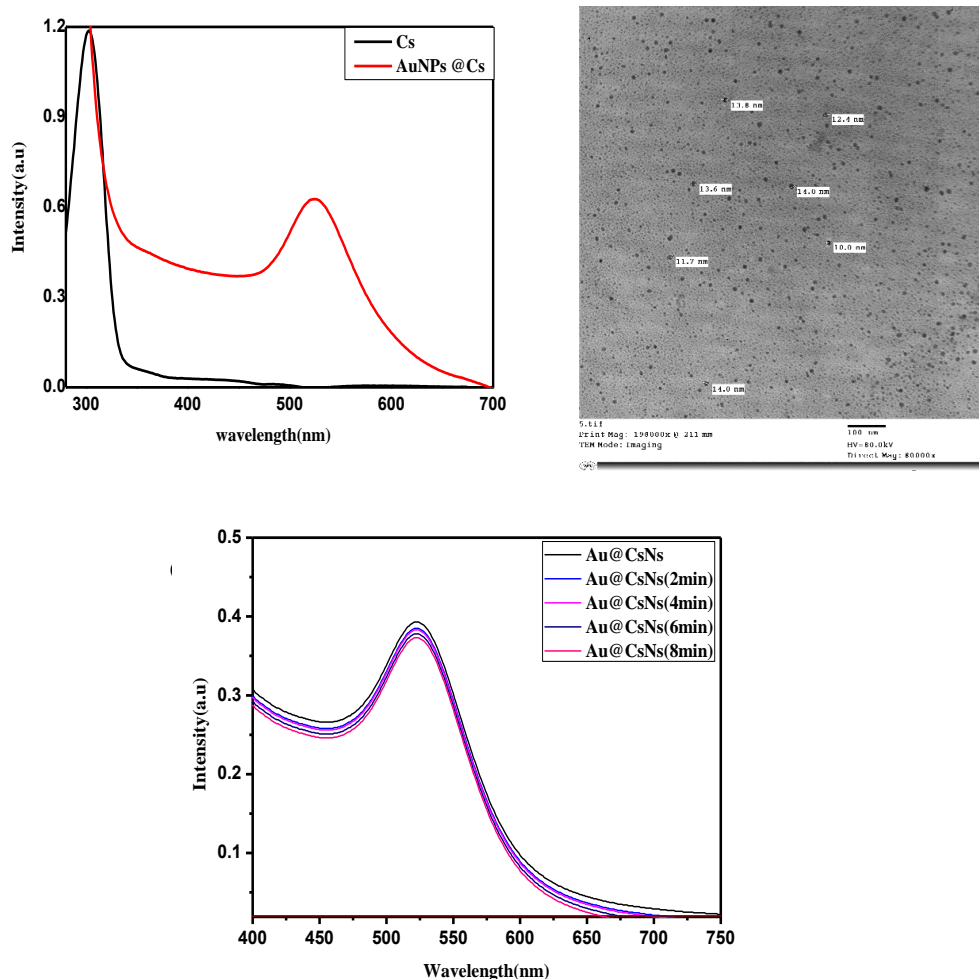


Figure (1): a) UV -Visible absorption of Au@CsNs and CS polymer, b) TEM image of Au@CsNs (magnification 100nm) and c) laser photostability of Au@CsNs

Zeta potential was tested to estimate the superficial charges and constancy of Au@CsNs; as zeta potential was high the nanoparticles are more stable as a result of the larger attractive force among them. Additionally, nanoparticle charge density plays a significant influence in how well they adhere to the negatively charged cancer cell membrane.^[37] Accordingly positive charged nanoparticles are favoured for the treatment of cancer. Usually, particle aggregation is a lesser amount of existence when zeta potential $\geq \pm 30$ mV due to electrostatic repulsions.^[38] The zeta potential and size of the prepared Au@CsNs is 36mV and 120 nm which show its high stability as exposed in figure (2a,2b).

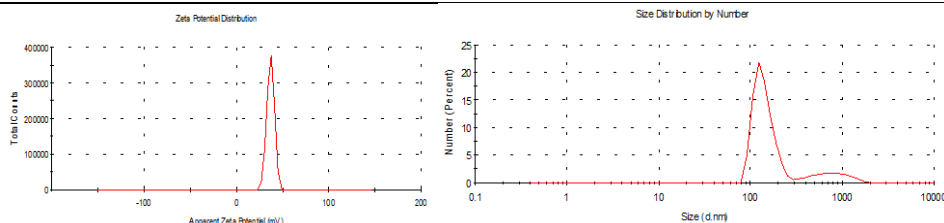


Figure (2): Au@CsNs (a) Zeta potential and (b) particle size.

The prepared Au@CsNs were investigated by FT-IR spectra. Figure (3) shows the two representatives presenting of CS and AuNPs. The principal vibrational band of CS is 3435 cm^{-1} , which due to hydrogen-bonded O-H stretching vibration. The bands of N-H stretching from primary amine and type I amide overlap in identical area^[38], and becomes sharper with a shift to 3430 cm^{-1} in AuNPs. Band at 1628 cm^{-1} for AuNPs was discovered to be displaced to 1653 cm^{-1} for amide I band characteristic to C=O stretching of N-acetyl group, thus demonstrating that AuNPs are entirely confined with CS through affinity contact with amino group, band at 1423 cm^{-1} attributed to bending vibration of OH group was shifted to 1413 cm^{-1} in AuNPs while at 1382 cm^{-1} assigned to the symmetric deformation vibration mode of CH₃, 1322 cm^{-1} equivalent to the CH₂ wagging vibration mode in primary alcohol, and 1258 cm^{-1} (corresponding to the vibration mode of the amide III due to the mixture of N-H deformation and C-N stretching) are not affected by occurrence of a metal surface.^[39] Findings demonstrate that the hydroxyl and primary amino groups of CS were involved in the reduction and stabilisation of Au@CsNs. These characteristics matched those of Cs-AuNPs with cytotoxic activities in tumoral and leukemic cell lines that had been previously reported.^[40, 41]

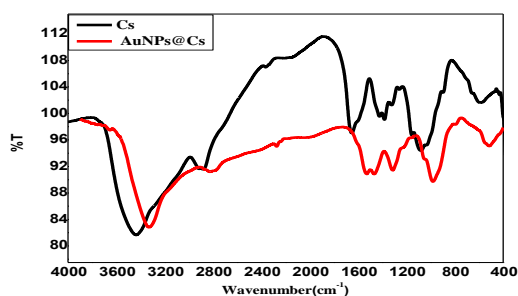


Figure (3): FTIRs of CS polymer and Au@CsNs.

Dose-dependent inhibition effect of Au@CsNs on different cancer cell lines

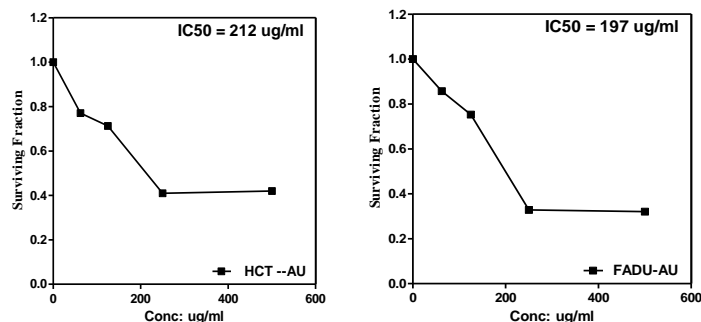
Using the SRP assay, cytotoxicity against various cancer cell lines (H460), (HCT116), (HEPG2), and (FaDu) cells was assessed after 48 hours to determine the effect of Au@CsNs as anticancer nanomaterials. Results showed that the different concentrations of Au@CsNs utilised in this investigation (200, 400, and 600 g/ml) had an impact on all of the evaluated cell lines. According to Figure (4) and Table 1, increasing the concentration of Au@CsNs reduced cell viability and had a distinct value of IC₅₀ on the investigated cell lines. This indicates that the concentration of Au@CsNs has a direct correlation with the anticancer

efficacy of Au@CsNs against cell lines. Compared to the untreated Au@CsNs control group. Cytotoxicity is influenced by NP disparity, which may be connected to rise in cellular endocytosis and ROS.^[42] Furthermore, it was demonstrated that the cytotoxicity was determined by the interaction between negatively charged plasma membrane and cationic nanoparticles.^[43, 44]

Table [1] The IC₅₀ of cancer cell lines after 48h incubation with Au@CsNs.

Sample name	HEPG2		HCT		FADU		H460	
	IC ₅₀	SD	IC ₅₀	SD	IC ₅₀	SD	IC ₅₀	SD
Au	--	--	212	8.00	197	6.00	420	6.00

Numerous cancer cell lines, including PA1 (a human ovarian cancer cell line), MCF7 (a breast cancer cell line), A549 (a lung cancer cell line), and HepG2 have been reported to be cytotoxic to AuNPs.^[45] AuNPs are absorbed more readily by cells as they get smaller. This increased absorption raises the concentration of AuNPs inside the cell, increasing their cytotoxicity toward cells.^[46] Numerous cell types produced ROS as a result of AuNPs. Both caspase-dependent and caspase-independent cell death have been linked to ROS.^[22] Furthermore, a prior study found that Chitosan-AuNPs increased the generation of ROS in lung cancer cells.^[47] Despite the fact that several reports have revealed that AuNPs and chitosan require on caspases to cause cell death, some forms of AuNPs can also cause caspase-independent cell death; this change may be caused by diverse agents used to synthesize AuNPs.^[48] Additionally, in our investigation, we discovered that these variations could be attributed to the properties of each cell line, for instance the nonappearance of caspase-3 in MCF-7 cells, even when utilizing the same Au@CsNs.^[49] According to testimony, AuNPs exhibit growth inhibition of HepG2 cells at concentrations of 50 to 250 mg/l.^[50] According to Lopez-Chaves et al.^[51] the harmful outcome of AuNPs on HepG2 cells was caused by the generation of reactive oxygen species (ROS) and DNA damage. Scientists similarly highlighted HepG2 cell line may be more susceptible to cytotoxicity due to AuNPs' smaller size. Our findings are fairly consistent with those found in earlier articles.^[52]



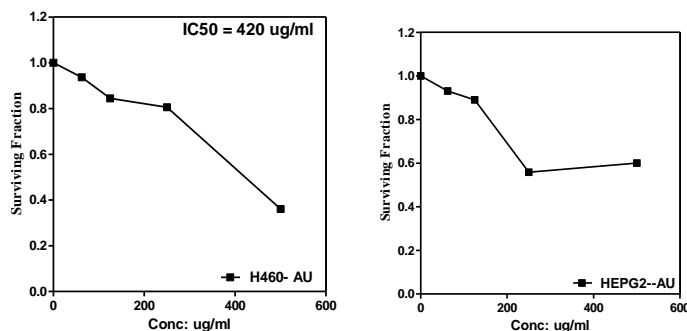


Figure (4): cytotoxicity test at diverse concentrations ($\mu\text{g/ml}$) of Au@CsNs on (HCT, FADU, H460, and HEPG2) cell lines after 48 h.

Conclusions

The goal of this research was to provide an easy-to-use, non-toxic, and ecologically responsible approach for the production of Au@CsNs. The prepared Au@CsNs had an average particle size of 12 nm and was spherically formed. Using the SRP assay, Au@CsNs green produced after 48 hours shown superior anticancer efficacy against several cancer cell lines (H460), (HCT116), (HEBG2), and (FaDu) cells at various doses. In photothermal therapy, Au@CsNs showed greater anticancer activity and holds great promise. Future research is being done to examine the beneficial effects of combined therapy using the produced nanoparticle as an anticancer drug carrier cancer treatment.

Authors' contributions Conception/design:

Marwa A Ramadan and Amna H. Faid considered and achieved the experiments, examined the data. Marwa sharaky perform cytotoxicity experiment. Marwa ali ramadan and amna Hussein faid wrote the manuscript in discussion with Marwa sharaky. All authors read and accepted the last manuscript.

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Declarations Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Competing interests:

All of the authors declare that they have no competing interests.

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