Gold-Curcumin Nanostructure in Photo-thermal Therapy on Breast Cancer Cell Line: 650 and 808 nm Diode Lasers as Light Sources

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ABSTRACT

Background: Au nanoparticles (AuNPs) exhibit very unique physiochemical and optical properties, which now are extensively studied in a range of medical diagnostic and therapeutic applications. AuNPs can be used for cancer clinical treatment with minimal invasion. On the other hand, curcumin is a polyphenol derived from turmeric which is used for medical purposes due to its anti-cancer, anti-microbial, anti-oxidant and anti-inflammatory properties. Despite these potential properties of curcumin, its usage is limited in medicine due to low solubility in water. Conjugation of curcumin to AuNPs (Au-Cur nanostructure) can be increasing its solubility. Photo-thermal therapy (PTT) is a novel kind of cancer treatment which involves two major components: laser and photo-thermoconversion agents.

Materials and Methods: Here, diode lasers emitting 808 nm and 650 nm were utilized as light sources, and synthesized Au-Cur nanostructure was applied as a photo-thermo conversion agent. UV-vis absorbance spectroscopy and dynamic light scattering (DLS) were applied to study the maximum absorption of particles, size stability of the samples and their zeta potential. The synthesized Au-Cur nanostructure under irradiation of laser is used for PPT on 4T1 cells. The cytotoxicity activity of Au-Cur nanostructure and laser irradiation on 4T1 cells was evaluated by MTT assay.

Results: Synthesized Au-Cur nanostructure showed λ_{max} at 540 nm and a mean hydrodynamic diameter of 25.8 nm. 4T1 cells were exposed to an 808 nm diode laser (1.5 W cm^2, 10 min) in the presence of different concentrations of Au-Cur nanostructure. Next, 4T1 cells with Au-Vur nanostructure were exposed to diode laser beam (650 nm, 1.5 W cm^2) for 10 min. The results revealed that Au-Cur nanostructure under laser irradiation of 808 nm more decreased cell viability of 4T1 cells compared to laser irradiation of 650 nm.

Conclusion: It was concluded that combining an 808-nm laser at a power density of 1.5W/cm^2 with Au-Cur nanostructure has a destruction effect on 4T1 breast cancer cells in vitro experiments compared to laser irradiation of 650 nm.

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Keywords

Curcumin ● Lasers ● Semiconductor ● Nanostructures

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Introduction

Breast cancer is a significant public health concern among women [1]. The conventional treatments that most patients receive are surgery, chemotherapy and radiotherapy. Researchers have re-

ported almost 50% of cancer patients die as a result of inaccurate diagnosis or incomplete treatment [2, 3]. During the last decade, efforts were made to develop novel approaches to treat cancer. Hyperthermia delivers adequate external energy to create heat in target tissues. Nowadays, hyperthermia is used to destroy tumors. In this method, tumor's temperature is raised up to 39.5-43°C [4, 5]. Various sources have been developed to raise tumor temperature, such as radiofrequency, microwave, laser and ultrasound [6]. Recently, the development and utilization of nanotechnology in medicine has offered numerous possibilities in diagnosis and treatment [7]. Metal nanoparticles are viable for hyperthermia treatment due to their physical and chemical properties. The surface of these nanoparticles can be easily modified by combining with drugs, genes and targeting ligands [2]. Among nanoparticles, applications of Au nanoparticles (AuNPs) have rapidly increased due to their hereditary and unique features, such as drug delivery, tunable surface plasmon resonance (SPR), biocompatibility, high surface reactivity and oxidation resistance. They also have promising therapeutic opportunities in nanomedicine and cancer therapy [8]. AuNPs have the ability to generate heat as well as their SPR effect, when exposed to light (such as laser), especially in the range of near-infrared (NIR) [9]. The applications of nanomedicine in laser assistant treatment include two categories: photothermal therapy (PTT) and photodynamic therapy (PDT) which have been investigated. In the last decade, laser-based therapeutic approaches included PTT and PDT that have attracted much attention [10]. PTT is a technique to treat cancer in which NIR (650-950 nm) light deeply penetrates the tissue. Consequently, it can destroy tumor cells with minimal damage to surrounding normal tissues [11]. Using PTT, cancer cells are selected locally; hence, the adverse side effects are minimized. In PTT method, radiation can cause intercellular interventions by affecting DNA and protein denaturation [12, 13]. AuNPs with their optical properties are the main PTT agents [8]. Recently, many researchers have focused on properties and applications of curcumin in cancer therapy, and it is shown that it has anti-cancer, anti-oxidant, anti-inflammatory, anti-bacterial and antidiabetic properties. curcumin is mainly cultivated in India and Southeast Asian countries [14, 15]. In *vitro* studies demonstrated that curcumin has more efficacy in tumor cells in comparison to traditional drugs such as 5-fluorouracil (5-FU) and doxorubicin [16]. However, due to its significant hydrophobicity, instability and poor pharmacokinetics, its application remains limited in medicine. Hence, to boost treatment outcome and solubility of curcumin as a drug, it has to be synthesized with various polymers and nanomaterials [17]. In this study, we synthesized AuNPs with curcumin (Au-Cur nanostructure), and reported their therapeutic effects on 4T1 breast cancer.

Materials and Methods

Materials

All chemicals were obtained from Sigma Chemicals Co. (USA), Scharlau Chemie Co. (Spain) or Merck Co. (Germany) and used without further purification.

Preparation of Au-Cur Nanostructure

Generally, Au nanoparticles are synthesized by a chemical method. The conjugation was synthesized as follows; the preparation of Au-Cur nanostructure was made by dissolving 0.034 g of $HAuCl_4$ in 500 µL of DI water. 0.04 g of curcumin in 1 mL of polyethylene glycol (PEG_{600}) was added drop-wise to 1.5 mL of the Au solution under a heater. The mixture was stirred well at 225 °C for about 15 min. Finally, Au-Cur nanostructure was cooled to room temperature and kept under refrigeration.

UV-Vis Absorbance Spectroscopy

UV-vis spectroscopy for both dispersion of Au-Cur nanostructure and curcumin solutions

were evaluated by Rayleigh UV2601 double beam UV-vis spectrophotometer. Spectra from 400 nm to 800 nm were recorded using 10 mm quartz cuvette cells.

Dynamic Light Scattering (DLS) Measurement

The hydrodynamic size and zeta potential of Au-Cur nanostructure dispersion were measured by SZ 100 (Horiba, Japan) instrument with diode laser beam at a wavelength of 532 nm and a power density of 10 mW. For the dispersion of Au-Cur nanostructure in distilled water, this solution was sonicated for 5 min. Brownian motion of nanoparticles leads to light scattering [18]. Zeta potential represents the charge of a nanoparticle in relation to the surrounding conditions. Actually, it is a measurement of electric double-layer produced by the surrounding ions in a solution, and it is a widely used characterization method of nanometer-sized objects in liquids such as pharmaceuticals, inks, foams, liposomes and exosomes [19, 20].

Laser Light Source

Diode lasers, from DAJ Co. (Iran), which emitted 808 nm and 650 nm beams were used as light sources. The output power of the diode laser was calibrated by using Lambda optical power meter (Australia). The protocol used for exposure conditions was to limit the beam diameter to 1 cm and the power density to 1.5 W/cm^2.

Cell Culture

4T1 cell line was obtained from Pasteur Institute (Iran). 4T1 cells were cultured in Roswell Park Memorial Institute -1640 (RPMI) containing 10% FBS (Fetal Bovine Serum) and supplemented 1% penicillin/streptomycin under the condition of 37 °C and 5% CO_2 atmosphere incubator.

4T1 Cells upon Laser Irradiation

In order to compare the cytotoxicity effect of laser irradiation with wavelengths of 650 nm and 808 nm on 4T1 cells, cells were divided into two separate groups including the 808 nm treated group and the 650 nm treated group. After 24 h of incubation of 4T1 cells in 96-well plates (20×10^3 cells per well), the cells were exposed to 808 nm diode laser with power density of 1.5 W/cm^2 for 10 min. In another group, cells were irradiated with a 650 nm diode laser with power density of 1.5W/cm^2 for 10 min. After 24 h, the culture medium was removed and 100 µL of MTT (thiazolyl blue tetrazolium bromide) solution (0.5 mg/mL in PBS), in an amount equal to 10% of the culture volume, was added under sterile conditions. Afterwards, the mixture was incubated at 5% atmosphere for 4 h. Then, after removal of the supernatant, 4T1 cells were centrifuged (1800 rpm, 10 min) and 100 µL of DMSO was added to each well. 4T1 cells not exposed to laser light were used as control group in this experiment. The absorbance of the resulting solution was determined immediately spectrophotometrically using a wavelength of 570 nm with a microplate reader (Polar star omega by BMG LABTECH, Germany). Cell viability was calculated in reference to the cells incubated with culture medium alone. The viability of the cells was assessed by the absorbance ratio of sample to the control group. The experiments presented are from three replicates.

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \times 100$$

Cytotoxicity of Au-Cur Nanostructure

The cytotoxicity effect of Au-Cur nanostructure was measured by MTT assay on 4T1 cells. First, 4T1 cells were seeded into 96-well culture plates. 4T1 cells were treated with three different concentrations of Au-Cur nanostructure (0.1, 0.5 and 1.0 mg/mL) and incubated overnight in 5% atmosphere. Then, cells were washed with PBS three times and

100 µL of MTT (0.5 mg/mL PBS) solution was added to all wells. The control group was incubated in the same condition without the presence of Au-Cur nanostructure.

Photo-thermal Effect of Au-Cur Nanostructure

To assess the photo-thermal effects of Au-Cur nanostructure, 4T1 cells were divided into two groups including N+ L^{808} nm (treated with different concentrations of Au-Cur nanostructure and irradiated with 808 nm diode laser) and N+L^{650} nm (treated with different concentrations of Au-Cur nanostructure and irradiated with 650 nm diode laser). Within two groups, 4T1 cells were incubated with 10 µL from different concentrations of Au-Cur nanostructure (0.1, 0.5 and 1.0 mg/mL). After 4 h incubation, 4T1 cells were irradiated by an 808 nm laser at a power density of 1.5 W/cm^2 for 10 min in the N+ L^{808} nm treated group and incubated overnight. Similarly, in another plate, for the N+ L^{650} nm group, after adding a different concentration of Au-Cur nanostructure (0.1, 0.5 and 1.0 mg/mL) and incubation for 4 h, cells were treated with 650 nm laser at a power density of 1.5 W/cm^2 for 10 min and incubated overnight. MTT assay was performed to investigate the viability of these cells after treatments.

Statistical Analysis

All results are presented as mean±standard deviation (SD). Differences between groups were determined by two-way ANOVA (n=3). A statistical analysis was performed using GraphPad Prism 6.01 software (GraphPad software, San Diego, CA, USA). Significant differences were considered by * $p < 0.05$.

Results

Characterization of Au-Cur Nanostructure

We devised a simple method through which by using Au nanoparticles, the solubility of curcumin in the synthesis was enhanced. Following the synthesis of Au-Cur nanostructure, a UV-vis spectrum of the solution was recorded. Figure 1a shows the UV-vis spectra of an Au-Cur nanostructure solution in comparison with curcumin. Figure 1a reveals that Au-Cur nanostructure and curcumin have peaks at wavelengths of 540 nm and 420 nm, respectively. Based on the absorption spectrum of the synthesized nanoparticle in the range 400 nm to 800 nm, we chose 808 nm and 650 nm for the wavelengths of the laser for evaluation photo-thermal effects of Au-Cur nanostructure in 4T1 breast cancer cells. The particle size of the Au-Cur nanostructure in aqueous solution was measured using the DLS spectrum, and the average calculated hydrodynamic diameter was around 25.8 nm as shown in Figure 1b. For NPs < 50 nm, DLS provides better information than laser diffraction from hydrodynamic size [19]. The zeta potential of Au-Cur nanostructure that represents the mean surface charge is + 2.9 mV (Figure 1c). This result indicates that these NPs have good stability. Charge surface of nanoparticles in the range of -30 mV to +30 mV represents their high stability in drug delivery [19].

Evaluation of Cytotoxicity Effect of Laser Irradiation

To investigate the cytotoxicity effects of laser irradiation on 4T1 cells, cells were irradiated with 650 and 808 nm diode lasers for 10 min, then, the cell viability was measured using MTT assay. Figure 2 shows the cell viability in 808 nm-treated group and 650 nm-treated group. The results demonstrate the cell viability in 808 nm-treated group was lower than the cell viability of 650 nm-treated group. As shown in Figure 2, lights of 808 nm laser induce 12% more killing in cells compared to 650 nm lights, indicating higher cytotoxicity effect on 4T1 cells. The purpose of this test was to compare the killing efficiency of 808 nm and 650 nm laser light on the 4T1 breast cancer cells.

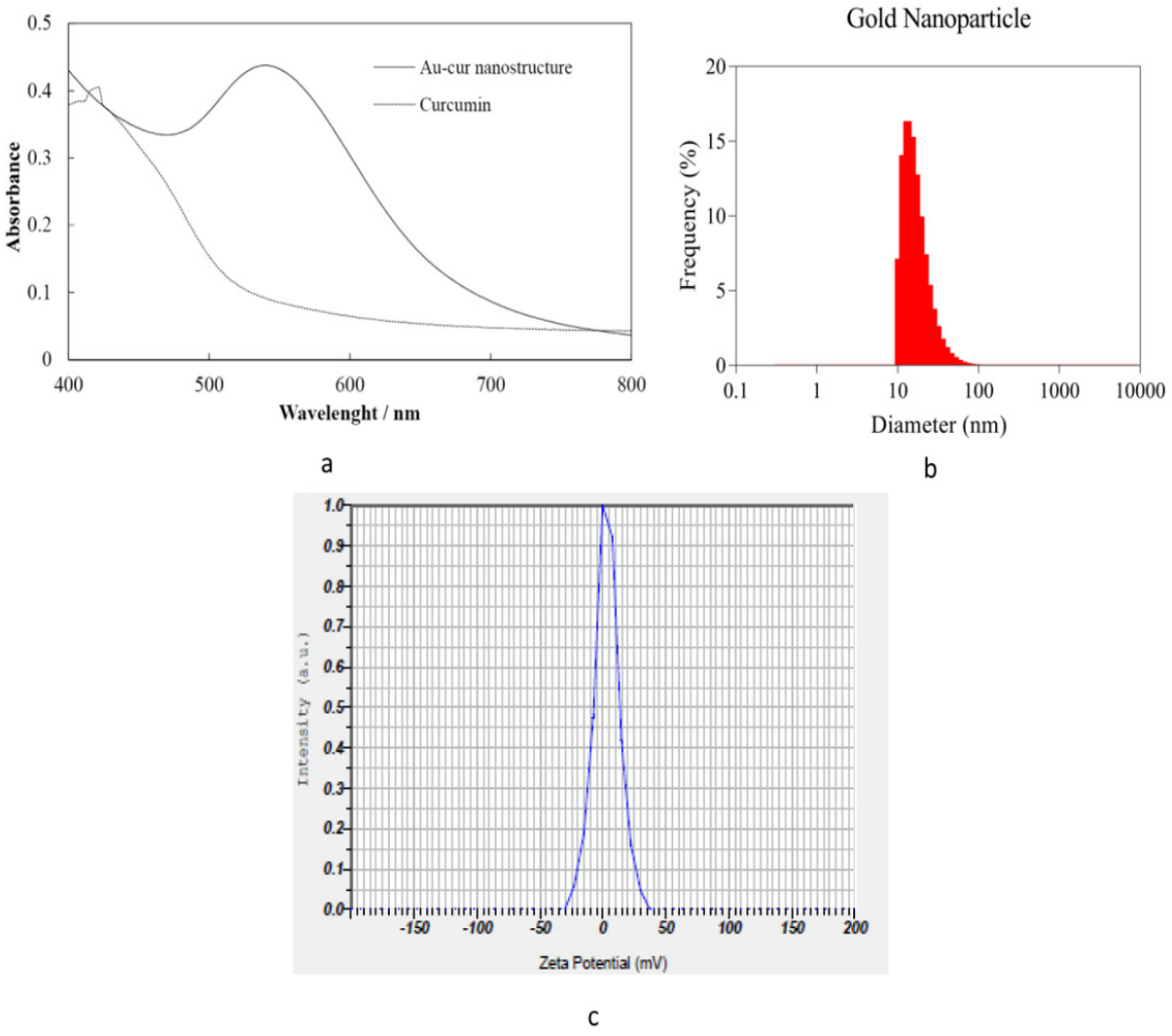

Figure 1: a) UV-vis spectra of Au-Cur nanostructure (----) and curcumin (- - -), b) the particle size diagram, and c) zeta potential values of the Au-cur nanostructure

Evaluation of Cytotoxicity Effect of Au-Cur Nanostructure

The cytotoxicity of three different concentrations (0.1, 0.5 and 1.0 mg/mL) of Au-Cur nanostructure on 4T1 cancer cells were measured by MTT assay. Figure 3 shows the percent of cell viability of 4T1 cells in the presence of different concentrations of Au-Cur nanostructure. Results reveal the percent of viability in the presence of 0.1, 0.5 and 1.0 mg/mL of Au-Cur nanostructure reduce to 92%, 89% and 86%, respectively. Au-Cur nanostructure represents a slight dose-dependent toxicity in 4T1 cells.

Photo-thermal Effect of Au-Cur Nanostructure

Photo-thermal effect of Au-Cur nanostructure was obtained in two groups including N+ L^{808} nm and N+ L^{650} nm. For this purpose, 4T1 cells after incubation with different concentrations of Au-Cur nanostructure for 4 h,

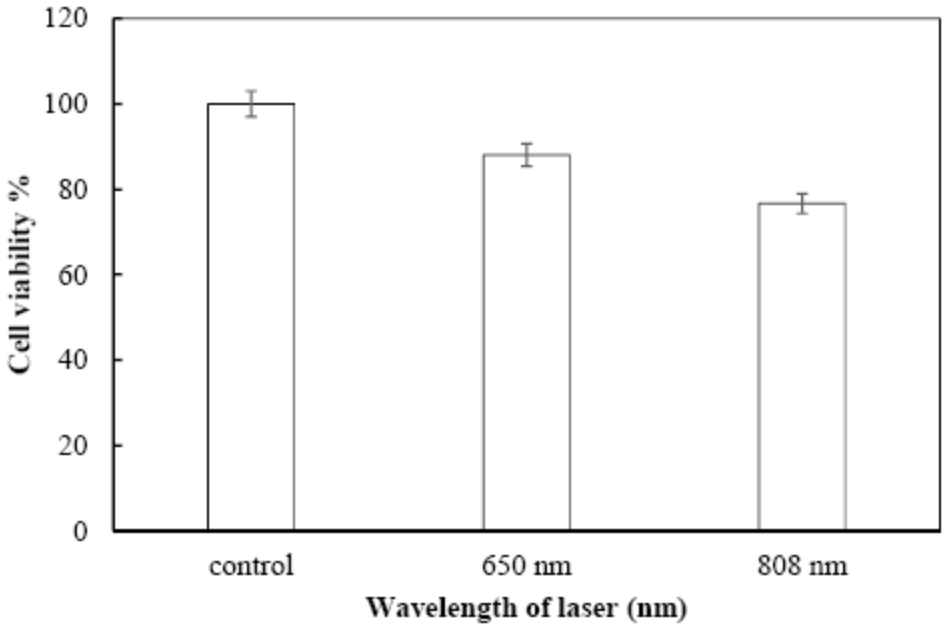

Figure 2: Percent of viability of 4T1 cells in control group (untreated cells), 808 nm-treated group and 650 nm-treated group. The cells irradiated with a 1.5 W/cm^2 power density of lasers for 10 min

were irradiated with 808 nm diode laser at a power density of 1.5 W/cm^2 for 10 min in N+ L^{808} nm. In another plate, 4T1 cells were treated with different concentrations of Au-Cur nanostructure for 4 h and then irradiated with 650 nm diode laser at a power density of 1.5 W/cm^2 for 10 min in N+L^{650} nm. Figure 4 shows the percent of viability in N+ L^{808} nm and N+ L^{650} nm groups. Comparing the viability in the presence of equal amounts of Au-Cur nanostructure, results exhibit the cells exposed to 808nm laser irradiation confront more cytotoxicity than the cells irradiated with 650 nm laser light. With the increase in the concentration of Au-Cur nanostructure, the percentage of killing cells upon irradiation of 808 nm increased. These results suggest that Au-Cur nanostructure might have great promise as a photo-thermal system for cancer therapy under 808 nm laser irradiation.

Discussion

curcumin, a neutrally pigment from the rhizome of turmeric (curcuma longa) has low water solubility under acidic or neutral media, and it decays in alkaline medium [19]. Here, the solubility of curcumin increased and Au-Cur nanostructure formed a clear solution. Recently, curcumin is introduced as a novel natural anti-cancer compound [21] and studies show phototherapy improves the therapeutic property of curcumin [22-24]. curcumin represents anti-tumor ability with apoptotic mechanism and the effects on the expression of Bcl-2, Bax, caspase genes [25]. Phototherapy study showed that curcumin indicated a dose-dependent cytotoxicity on breast cancer cell lines after exposed to 435.8 nm irradiation [22]. Here, Curcumin was applied as a stabilizer and a reducing agent for Au ions in synthesized Au-Cur nanostructure. Au nano-

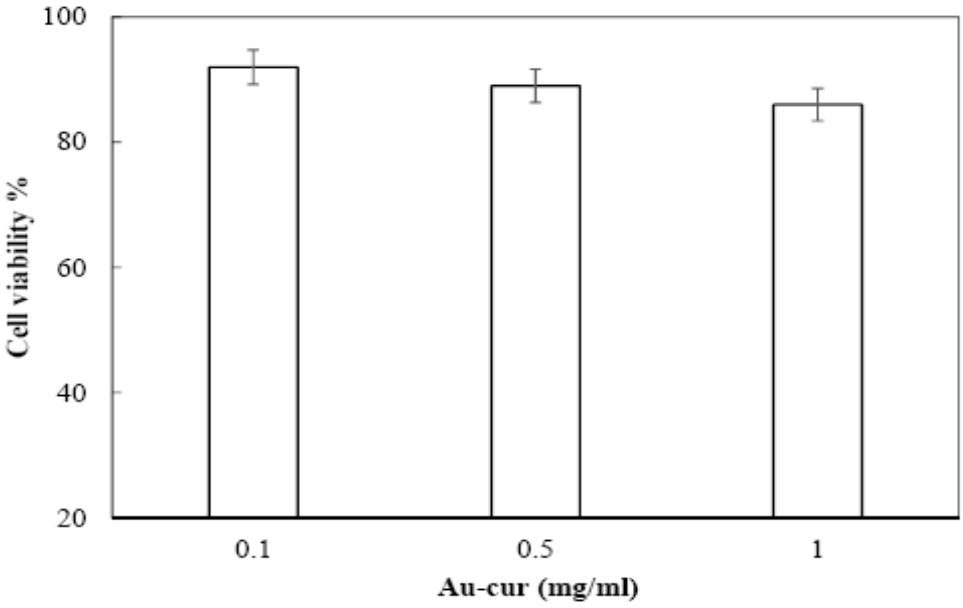

Figure 3: Percent of viability in 4T1 cells after incubation with 0.1, 0.5, 1 mg/ml of Au-Cur nanostructure for 24 h

structures absorb light and convert to heat due to surface plasmon resonance (SPR) behavior [26]. The synthesized Au-Cur nanostructure has maximum absorption at 540 nm, and also absorbs lights in NIR range. The results exhibit Au-Cur nanostructure has more cytotoxicity effect on breast cancer cell lines under the irradiation of 808nm laser. The use of infrared lights in the range of 700-1000 nm in phototherapy is preferable to other lights because of minimal absorption of proteins and DNA, and also deep penetration of lights in tissues [13].

PTT method, similar to other sources of hyperthermia, does not represent complete treatment efficacy [27-29]. For complete ablation of tumor via PTT, proper modification of photo-absorber and the proper light source was needed. Time of irradiation and power density of laser source must be optimized properly. Moreover, the limitation of penetration depth of NIR light is eliminated through internal fiber optic or intraoperative irradiation [30].

Conclusion

In the present study, we successfully synthesized and applied Au-Cur nanostructure as a PTT agent on 4T1 breast cancer cells. Au-Cur nanostructures were characterized, using UV-vis spectroscopy, and DLS. UV-vis absorbance spectroscopy revealed that the maximum peak of Au-Cur nanostructure was at 540 nm, which is related to AuNPs SPR property. The measured hydrodynamic size of Au-Cur nanostructure was 25.8 nm. The zeta potential mean (average) of Au-Cur nanostructure was +2.9 mV. The phototoxicity of Au-Cur nanostructure was examined by MTT assay on breast cancer cells. The results from this study indicate that Au-Cur nanostructure under 808nm laser light radiation was more cytotox-

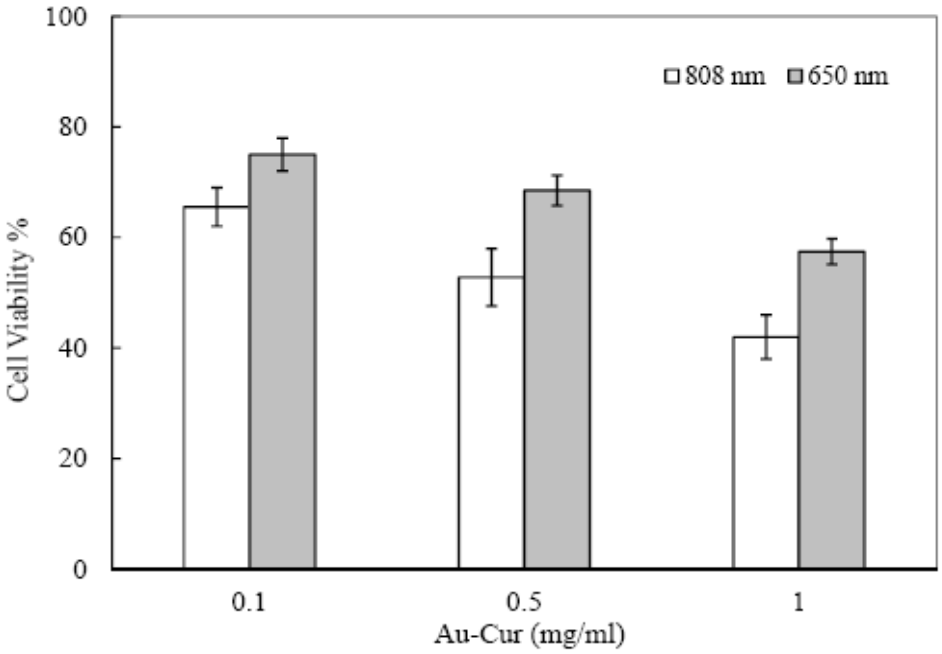

Figure 4: Percent of viability in 4T1 cells after incubation with the different concentrations of Au-Cur nanostructure (0.1, 0.5 and 1.0 mg/mL) for 4 h and then irradiated with diode lasers of 808 nm or 650 nm at a power density of 1.5 W/cm^2 power density for 10 min

ic in comparison to 650 nm laser light. Only 40% of cells were alive after PTT treatment by 1mg/mL of Au-Cur nanostructure under 808 nm laser irradiation, this shows that Au-Cur nanostructure can serve as an effective photo-thermal agent. Therefore, the newly developed nanoparticle can be utilized as a photo-thermal agent to treat 4T1 breast cancer cells.

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Conflict of Interest

The authors declare no conflict of interest

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