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

Rahimi-Moghaddam F., Sattarahmady N., Azarpira N.

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. Photothermal 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 $\lambda_{\text{max}}$ 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.

Keywords
Curcumin ● Lasers ● Semiconductor ● Nanostructures

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 include 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 HAuCl₄ in 500 µL of DI water. 0.04 g of curcumin in 1 mL of polyethylene glycol (PEG₆₀₀) 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
Gold-Curcumin and Laser

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/cm².

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% CO₂ 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 × 10³ cells per well), the cells were exposed to 808 nm diode laser with power density of 1.5 W/cm² for 10 min. In another group, cells were irradiated with a 650 nm diode laser with power density of 1.5W/cm² 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.
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\textsubscript{808} nm and N+ L\textsubscript{650} nm. For this purpose, 4T1 cells after incubation with different concentrations of Au-Cur nanostructure for 4 h,
were irradiated with 808 nm diode laser at a power density of 1.5 W/cm² for 10 min in N+ L₈₀₈ 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/cm² for 10 min in N+ L₆₅₀ nm. Figure 4 shows the percent of viability in N+ L₈₀₈ nm and N+ L₆₅₀ nm groups. Comparing the viability in the presence of equal amounts of Au-Cur nanostructure, results exhibit the cells exposed to 808 nm 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-
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-
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 photothermal agent. Therefore, the newly developed nanoparticle can be utilized as a photo-thermal agent to treat 4T1 breast cancer cells.

Acknowledgment
We would like to thank the Research Council of Shiraz University of Medical Sciences for supporting this research (16267).

Conflict of Interest
The authors declare no conflict of interest.

References
1. Farajzadeh R, Pilehvar-Soltanahmadi Y, Dadashpour M, Javidfar S, Lotfi-Attari J, Sadeghzadeh H, et al. Nano-encapsulated metformin-curcumin in PLGA/PEG inhibits synergistically growth and hTERT gene expression in human breast cancer cells. Artif Cells Nanomed Biotechnol. 2017:1-9. doi: 10.1080/21691401.2017.1347879. PubMed PMID: 28678551.
2. Sharma H, Mishra PK, Talegaonkar S, Vaidya B. Metal nanoparticles: a theranostic nanotool against cancer. Drug Discov Today. 2015;20:1143-51. doi: 10.1016/j.drudis.2015.05.009. PubMed PMID: 26007605.
3. Mirkin CA, Meade TJ, Petrosko SH, Stegh AH. Nanotechnology-based precision tools for the detection and treatment of cancer: Springer; 2015.
4. Rao W, Zhang W, Poventud-Fuentes I, Wang Y, Lei Y, Agarwal P, et al. Thermally responsive nanoparticle-encapsulated curcumin and its combination with mild hyperthermia for enhanced cancer cell destruction. Acta Biomater. 2014;10:831-42. PubMed PMID: 24516867; PubMed Central PM-
Gold-Curcumin and Laser

5. Lindner U, Weersink RA, Haider MA, Gertner MR, Davidson SR, Atri M, et al. Image guided photothermal focal therapy for localized prostate cancer: phase I trial. *J Urol.* 2009;182:1371-7. doi: 10.1016/j.juro.2009.06.035. PubMed PMID: 19683262.

6. Betrouni N, Colin P, Bozzini G, Mordon S. Image-guided laser therapies for prostate cancer. *IRBM.* 2013;34:28-32.

7. Shi J, Votrubov AR, Farokhzad OC, Langer R. Nanotechnology in drug delivery and tissue engineering: from discovery to applications. *Nano Lett.* 2010;10:3223-30. doi: 10.1021/nl102184c. PubMed PMID: 20726522; PubMed Central PMCID: PMC2935937.

8. Riley RS, Day ES. Gold nanoparticle-mediated photothermal therapy: applications and opportunities for multimodal cancer treatment. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2017;9. doi: 10.1002/wnan.1449. PubMed PMID: 28160445; PubMed Central PMCID: PMC5474189.

9. Abo-Elfadl MT, Gamal-Eldeen AM, Elshafey MM, Abdalla GM, Ali SS, Ali MR, et al. Photothermal therapeutic effect of PEGylated gold nanospheres in chemically-induced skin cancer in mice. *J Photochem Photobiol B.* 2016;164:21-9. doi: 10.1016/j.jphotobiol.2016.09.012. PubMed PMID: 27636008.

10. Jiang BP, Zhang L, Guo XL, Shen XC, Wang Y, Zhu Y, et al. Poly(N-phenylglycine)-Based Nanoparticles as Highly Effective and Targeted Near-Infrared Photothermal Therapy/Photodynamic Therapeutic Agents for Malignant Melanoma. *Small.* 2017;13. doi: 10.1002/smll.201602496. PubMed PMID: 27982516.

11. Gobin AM, Lee MH, Halas NJ, James WD, Drezek RA, West JL. Near-infrared resonant nanoshells for combined optical imaging and photothermal cancer therapy. *Nano Lett.* 2007;7:1929-34. doi: 10.1021/nl070610y. PubMed PMID: 17550297.

12. Mousavy SJ, Riazi GH, Kamarei M, Aliakbarian H, Sattarhmady N, Sharifizadeh A, et al. Effects of mobile phone radiofrequency on the structure and function of the normal human hemoglobin. *Int J Biol Macromol.* 2009;44:278-85. PubMed PMID: 19263507.

13. Shibu ES, Hamada M, Murase N, Biju V. Nano-materials formulations for photothermal and photodynamic therapy of cancer. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews.* 2013;15:53-72.

14. Darne PA, Mehta MR, Agawane SB, Prabhune AA. Bioavailability studies of curcumin–sophorolipid nano-conjugates in the aqueous phase: role in the synthesis of uniform gold nanoparticles. *RSC Adv.* 2016;6:68504-14.

15. Pari L, Tewas D, Eckel J. Role of curcumin in health and disease. *Arch Physiol Biochem.* 2008;114:127-49. doi: 10.1080/13813450802033958. PubMed PMID: 18484280.

16. Lopez-Lazaro M. Anticancer and carcinogenic properties of curcumin: considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. *Mol Nutr Food Res.* 2008;52 Suppl 1:S103-27. doi: 10.1002/mnr.200700238. PubMed PMID: 18496811.

17. Sindhu K, Rajaram A, Sereram K, Rajaram R. Curcumin conjugated gold nanoparticle synthesis and its biocompatibility. *Rsc Advances.* 2014;4:1808-10.

18. AraSK, Verma M. Measurement of nanoparticles by light-scattering techniques. *TrAC Trends in Analytical Chemistry.* 2011;30:4-17.

19. Lotya M, Rakovich A, Donegan JF, Coleman JN. Measuring the lateral size of liquid-exfoliated nanosheets with dynamic light scattering. *Nanotechnology.* 2013;24:265703. doi: 10.1088/0957-4484/24/26/265703. PubMed PMID: 23732310.

20. Arjmandi N, Van Roy W, Lagae L, Borghs G. Measuring the electric charge and zeta potential of nanometer-sized objects using pyramidal-shaped nanoparticles. *Anal Chem.* 2012;84:8490-6. doi: 10.1021/ac300705z. PubMed PMID: 22901005.

21. Chen C, Johnston TD, Jeon H, Gedaly R, McHugh PP, Burke TG, et al. An in vitro study of liposomal curcumin: stability, toxicity and biological activity in human lymphocytes and Epstein-Barr virus-transformed human B-cells. *Int J Pharm.* 2009;366:133-9. doi: 10.1016/j.ijpharm.2008.09.009. PubMed PMID: 18840516.

22. Zeng X, Leung A, Xia X, Yu H, Bai D, Xiang J, et al. Effect of blue light radiation on curcumin-induced cell death of breast cancer cells. *Laser Physics.* 2010;20:1500-3.

23. Dovigo LN, Pavarina AC, Ribeiro AP, Brunetti IL, Costa CA, Jacomassi DP, et al. Investigation of the photodynamic effects of curcumin against Candida albicans. *Photochem Photobiol.* 2011;87:985-903. doi: 10.1111/j.1751-1097.2011.00937.x. PubMed PMID: 21517888.

24. Andrade MC, Ribeiro AP, Dovigo LN, Brunetti IL, Giampaolo ET, Bagnato VS, et al. Effect of different pre-irradiation times on curcumin-mediated photodynamic therapy against planktonic
cultures and biofilms of Candida spp. *Arch Oral Biol.* 2013;58:200-10. doi: 10.1016/j.archoralbio.2012.10.011. PubMed: 23153629.

25. Karmakar S, Banik NL, Ray SK. Curcumin suppressed anti-apoptotic signals and activated cysteine proteases for apoptosis in human malignant glioblastoma U87MG cells. *Neurochem Res.* 2007;32:2103-13. doi: 10.1007/s11064-007-9376-z. PubMed PMID: 17562168.

26. Heidari M, Sattarahmady N, Azarpia N, Heli H, Mehdizadeh AR, Zare T. Photothermal cancer therapy by gold-ferrite nanocomposite and near-infrared laser in animal model. *Lasers Med Sci.* 2016;31:221-7. doi: 10.1007/s10103-015-1847-x. PubMed PMID: 26694488.

27. Lencioni R, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, et al. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology.* 2005;234:961-7. doi: 10.1148/radiol.2343040350. PubMed PMID: 15665226.

28. Liang P, Dong B, Yu X, Yu D, Wang Y, Feng L, et al. Prognostic factors for survival in patients with hepatocellular carcinoma after percutaneous microwave ablation. *Radiology.* 2005;235:299-307. doi: 10.1148/radiol.2351031944. PubMed PMID: 15731369.

29. Hinshaw JL, Lee FT, Jr. Cryoablation for liver cancer. *Tech Vasc Interv Radiol.* 2007;10:47-57. doi: 10.1053/j.tvir.2007.08.005. PubMed PMID: 17980318.

30. Robinson JT, Welsher K, Tabakman SM, Sherlock SP, Wang H, Luong R, et al. High Performance In Vivo Near-IR (>1 μm) Imaging and Photothermal Cancer Therapy with Carbon Nanotubes. *Nano Res.* 2010;3:779-93. doi: 10.1007/s12274-010-0045-1. PubMed PMID: 21804931; PubMed Central PMCID: PMC3143483.