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Investigation Of The Potential Of Hollow Gold Nanoparticles As Photothermal Agents For Cancer Therapy: A Study On The Caco-2 Cell Line

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Abstract:
The study aims to investigate the photothermal efficiency of hollow gold nanoparticles (HAuNPs) and their cytotoxic effects on colorectal adenocarcinoma (Caco-2) cell lines. HAuNPs have garnered significant interest as potential agents for photothermal therapy in cancer treatment due to their unique properties. In this research, HAuNPs were synthesized and characterized using various UV-Vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDX). The photothermal efficiency of the HAuNPs was evaluated by irradiating them with 808 nm near-infrared (NIR) laser light, and the resulting temperature rise was monitored using both a thermocouple and an infrared thermal camera. The experimental results demonstrated that the HAuNPs exhibited excellent photothermal properties, efficiently converting NIR light into heat and leading to a significant increase in temperature. Subsequently, the cytotoxic effects of the HAuNPs on Caco-2 cell lines were assessed through in vitro experiments, including cell viability assays, and morphological observations. The study revealed that the HAuNPs displayed excellent photothermal efficiency, resulting in significant therapeutic effects on the Caco-2 cell lines. The photothermal properties of the nanoparticles allowed for effective heating under laser irradiation, leading to a notable reduction in cell viability. These findings highlight the promising potential of HAuNPs as efficient photothermal agents for cancer therapy.

Key Word*: Hollow gold nanoparticle; photothermal agent; photothermal therapy; cancer; caco-2.*

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I. Introduction

Nanotechnology has created new opportunities for biomedical research, presenting creative methods for cancer treatment[1]. A good example of this is the creation of hollow gold nanoparticles (HAuNPs), which have drawn a lot of interest because of their distinctive hollow structure and potential for use in photothermal therapy[2,3]. These nanoparticles' hollow structures offer several benefits, such as greater surface area and improved light-matter interactions, which make them excellent candidates for a range of medicinal applications[4]. This study will examine the intriguing characteristics of HAuNPs and how they might be used to photothermally treat the Caco-2 cell line, a widely studied model for colorectal cancer research.

Hollow gold nanoparticles are nanoscale objects with a hollow interior and a gold shell. This hollow construction has various benefits, including a higher surface area-to-volume ratio, which allows for better light absorption and more efficient heat generation. The surface plasmon resonance (SPR) effect is enabled by gold's plasmonic characteristics, which allow nanoparticles to absorb light at specific wavelengths[5,6,7]. This feature can be used in photothermal therapy, making the particle a promising photothermal agent which converts absorbed light energy into heat, resulting in targeted thermal ablation of cancer cells[8].

Photothermal therapy uses nanoparticles' photothermal conversion characteristics to selectively heat and destroy cancer cells. When exposed to specific wavelengths of light, HAuNPs efficiently absorb the light energy, causing the gold shell to rapidly heat up[9]. This localized heating action can cause overheating in the surrounding tumor tissue, resulting in cancer cell death[10]. Photothermal therapy has shown potential as a minimally invasive treatment method, providing excellent precision while causing minimal damage to healthy tissues[11].

The aim of the study is to examine the potential of hollow gold nanoparticles (HAuNPs) as photothermal agents for targeted cancer therapy employing a human colorectal adenocarcinoma cell line (Caco-2). We hope to assess the efficacy and usability of HAuNPs in cancer treatment by analyzing the photothermal effects, cytotoxicity, and cell viability of Caco-2 cells loaded with HAuNPs under laser irradiation. The characteristics of HAuNPs make them a promising tool for targeted therapy, and this finding may enhance their medicinal uses.

II. Material And Methods

Materials: Acetone (C_2H_6O, ≥ 99%), and ethanol (C_2H_5OH ≥ 99.8%), were purchased from Sigma-Aldrich. Cobalt (II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$, 98%), sodium borohydride ($NaBH_4$, 99%), and gold (III) chloride hydrate ($HAuCl_4 \cdot$ aq, 99.995%), were purchased from Merck.
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's Modified Eagle's Medium High Glucose (DMEM-H), Dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS) tablets, Calcein-acetoxymethyl Ester (Calcein AM) and Ethidium Homodimer-1 (EthD-1) were also obtained from Merck.

Synthesis of Hollow Gold Nanoparticles: Xia et al. have produced hollow structured nanoparticles by using galvanic replacement[12] and recent studies have started to develop and create new techniques. Cobalt salt has great stability and taking advantage of this feature, the reduction cannot be done by citrate alone, and a stronger reducing agent is required. In this case, sodium borohydride is used to reduce the salt and citrate is present only as a capping agent. It is possible to tune the peak of the surface plasmon band absorption between 550 and 820 nm[13]. The preparation of hollow gold nanoparticles involves the synthesis of cobalt nanoparticles as a template, followed by the synthesis of hollow gold nanoparticles. While synthesizing particles the equipment must be clean and air free medium is required. In a round bottom flask, 100 mL of water, 400 µL of 0.1 M sodium citrate, and 100 µL of 0.4 M cobalt chloride were placed. Under nitrogen medium, the solution was mixed with magnetic stirring and then, 100 µL amount of 1 M sodium borohydride was added. The change from pink to dark brown colour showed that cobalt nanoparticles had formed[14]. The solution mixture was purged with nitrogen for 20 minutes to provide a complete hydrolyze of the remaining sodium borohydride. In a beaker, 10 mL of water and 50 µL of 0.1 M chloroauric acid were stirred together. 30 mL of cobalt nanoparticles were transferred under a nitrogen medium and were quickly added at once to the vortexing chloroauric acid solution. The formation of hollow gold nanoparticles can be observed by changing the colour solution to blue[15].

Cytotoxicity assay: The colon carcinoma cells (Caco-2 cell line) grown in DMEM-H were tested for the cytotoxicity of hollow gold nanoparticles using the MTT assay.

The cells were treated with serum-free media containing 10% MTT solution for 3 hours at 37°C under %5 CO_2 to conduct the MTT analysis. A 400 µL DMSO was used to dissolve the formazan crystals for each well after incubation, and 200 µL of the solution was then transferred to 96-well cell culture plates. At a wavelength of 590 nm, the optical densities of the solutions were measured.

For the live-dead assay, cells were rinsed with Ca^{2+} and Mg^{2+} containing PBS+ and then incubated with 1µM Calcein AM and 1µM EthD-1 containing PBS+ at room temperature for 30 minutes. After incubation, the cells are washed once more with PBS+ to remove any remaining dye and examined with a fluorescence microscope.

Photothermal Efficiency: Photothermal efficiencies of synthesized nanoparticles were found by using NIR, 808 nm, at an output power of 0.5 watts.
In vitro photothermal ablation of Caco-2 cells was illuminated by NIR, 808 nm, at an output power of 0.5 watts. Cell viability was measured after illumination by NIR laser using MTT assay.

Characterization: UV-vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDX), FLIR 5 thermal camera, an 808 nm laser source, and an Olympus fluorescence microscope were used.

III. Result and Discussion

Characterization of particles: The synthesized particles were characterized using UV-vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDX) to analyze their optical properties, surface morphology, and elemental composition, respectively.

Initially, the characterization of cobalt nanoparticles was performed, and the results are given in Figure 1.

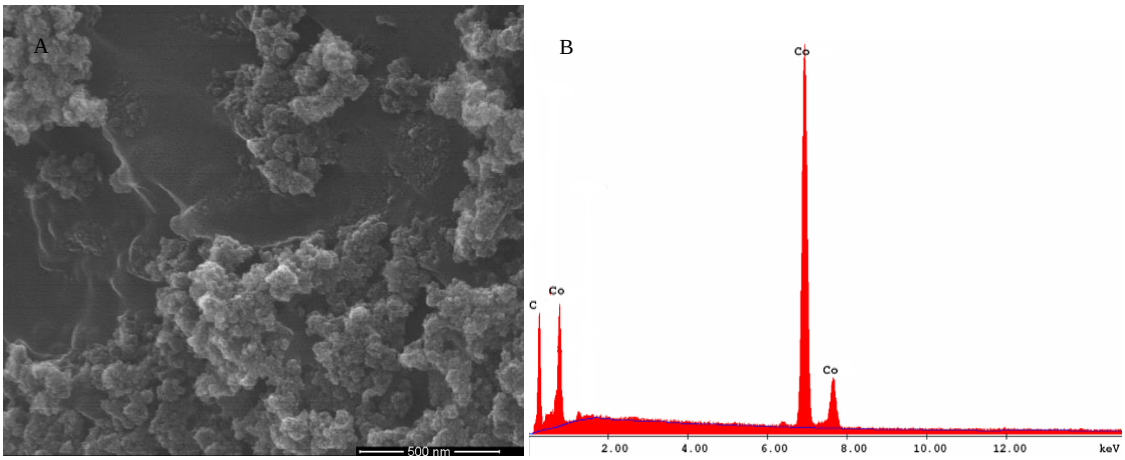


Figure 1: A) SEM image and B) EDX spectrum of Cobalt template for preparation of hollow gold nanoparticles (HAuNPs)

The SEM image and EDX results given in Figure 1., clearly show that cobalt nanoparticles were successfully synthesized, displaying well-defined morphology, and confirming cobalt's dominance as the main elemental component. Following the synthesis of cobalt nanoparticles, they were used as a template for the fabrication of hollow gold nanoparticles, and a thorough characterization of these hollow gold nanoparticles is provided. The characterization of synthesized hollow gold nanoparticles was performed with UV-vis spectroscopy, TEM, and EDX. The characterization results of hollow gold nanoparticles are given in Figure 2 and Figure 3 respectively.

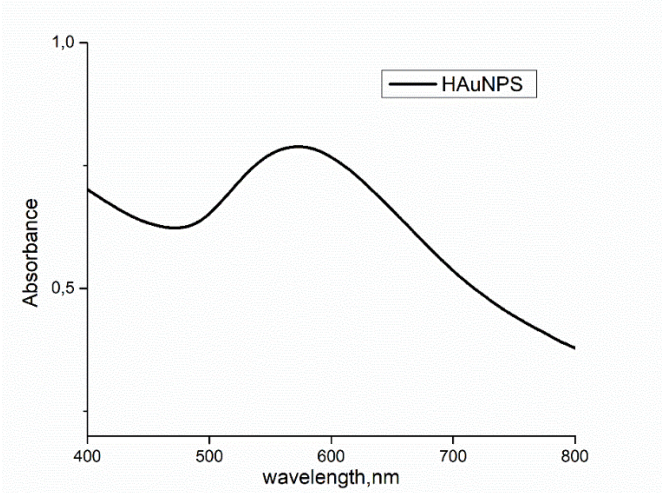


Figure 2: UV-vis spectra of hollow gold nanoparticles (HAuNPs)

UV-vis characterization of gold nanoparticles involves studying how they interact with ultraviolet and visible light, with the absorbance patterns providing unique information about each type of gold particle. In Figure 2, from the ultraviolet-visible spectra of the hollow gold nanoparticle, the absorbance at 575 nm overlaps with the literature respectively [13,15]. The morphological and compositional characterization of HAuNPs results are given in Figure 3.

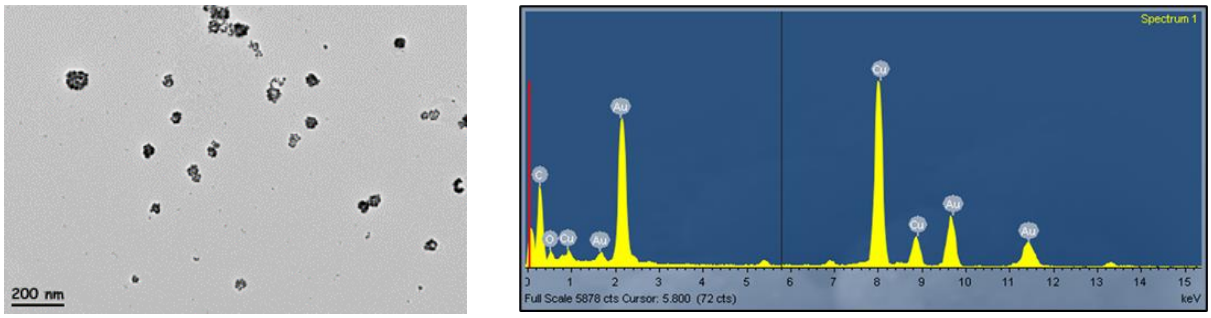


Figure 3: TEM image and EDX spectra of hollow gold nanoparticles (HAuNPs) respectively

Figure 3 illustrates TEM image along with their matching EDX results, which show the structure and elemental composition of the particles being studied. TEM image also showed well-defined particle shapes and sizes, confirming the planned particle synthesis. TEM pictures and UV-vis spectra revealed the produced particles' structural hollow shape and optical characteristics[15]. Moreover, the composition of the material was determined using energy-dispersive X-ray spectroscopy (EDX) analysis, clearly revealing the presence of gold in the synthesized nanoparticles.

Photothermal Efficiencies of the Nanoparticles by Using 0.5-Watt Laser

The photothermal efficiency of the synthesized particle was measured using an 808 nm, 0.5-Watt laser and used for 100 µg/mL particle solution. The measurement results are given in Figure 4. Since nanoparticles are synthesized in aquatic environments, purified water is used as a reference solution.

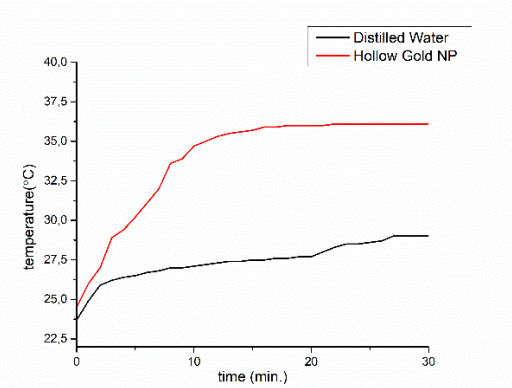
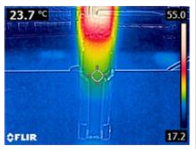
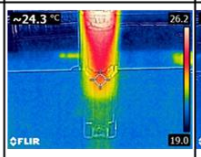
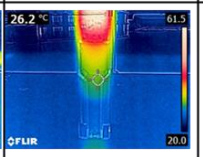
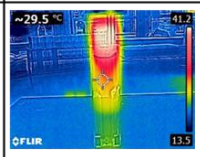
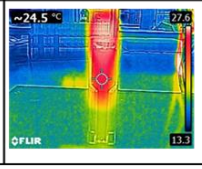
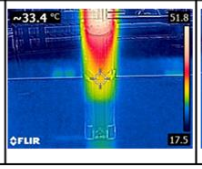
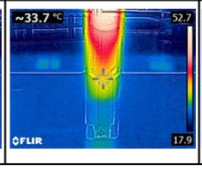
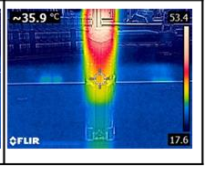


Figure 4: Photothermal efficiency measurement of hollow gold nanoparticles (HAuNPs) by using a 0.5-Watt laser

According to the results given in Figure 4 when a laser is used, as expected, the temperature results increase[16]. For synthesized particles (HAuNPs) with the application of a light source thermal equilibrium was reached at the end of approximately 10 minutes by using a 0.5-Watt laser. The graph in Figure 4 shows that when a laser is used, the temperatures rise quickly at first, but then the rate of rise slows down. In the use of 0.5 W laser measurements, the temperature is from 24.5 °C. to 34.9 °C and the difference ΔT (T_f-T_i) is calculated as 10.4 °C. The temperature change was also followed with a thermal camera, but it was determined that the values obtained differed from the values obtained with the thermocouple because of the environment. For this reason, it was decided that the results obtained with the thermocouple are more reliable. Therefore, both thermocouple measurement images were taken with a thermal camera in all measurements. In Table 1 the thermal images of particles were given. The images were taken every 10 minutes during measurement. A similar increment tendency of the temperature is also seen in images.

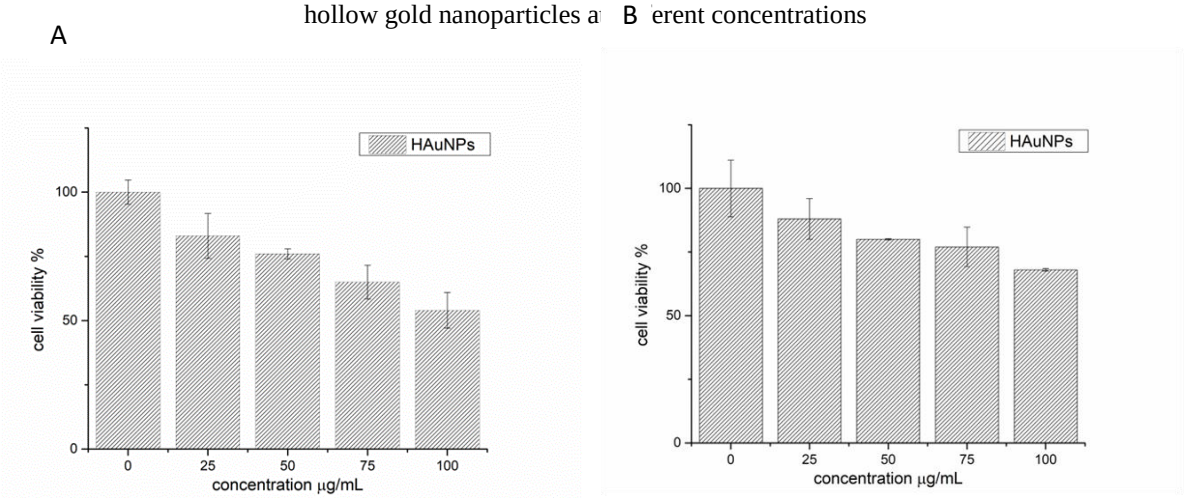
Table no 1 Photothermal camera images of gold nanoparticles while applying 808 nm, 0.5-Watt Laser

Sample/ time (min.)	0	10	20	30
DI H_2O				
HAuNPs				

Toxic Effect of Hollow Gold Nanoparticle on Caco-2 Cell Line and Photothermal Activities Under NIR:

Nanoparticle solutions with concentrations of 0, 25, 50, 75, and 100 µg/mL were used for 24 and 72 hours to determine the nanoparticle concentration and interaction time required for NIR application in the studies conducted with the nanoparticle. To establish the proper maximum safe dose of hollow gold nanoparticles on cell lines, HAuNPs at concentrations of 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL of were introduced to the cells, and MTT assay was performed after 24 hours and 72 hours. All analyses were performed as triplicates (n=3). After 24 and 72 hours of interaction among cells and varied concentrations of hollow gold nanoparticles, MTT analysis was performed to determine cytotoxicity. The results are given in Figure 5.

Figure 5. The MTT analysis result of Caco-2 cell lines at A) 24 and B) 72 hours after interaction with the hollow gold nanoparticles at different concentrations



When looking at Figure 5 in detail, the Caco-2 cell line's viability was seen to diminish at high concentrations of HAuNPs, and the safest concentration that may be employed with sustaining 76% viability was found to be 50 µg/mL.

According to this determined dose, photothermal effect studies were performed. The samples were analyzed using an optical/fluorescence microscope over the course of the first 24 hours, and it was discovered that the nanoparticle solutions entered the cells within the first 2 hours of their addition (Figure 6A). Therefore, it was determined to apply NIR after two hours of the interaction of particles with cells. After 2 hours of incubation at the dose indicated by MTT analysis during cytotoxicity analysis, which is 50 µg/mL, the cell line was exposed to an 808 nm, 0.5 W NIR laser for 10 minutes to observe the particle's photothermal effect. The overall results for this photothermal study of HAuNPs on the Caco-2 cell line, MTT analysis and live-dead assay results are given in Figure 6.

The effect of the treatment on the ratio of alive to not viable cells was qualitatively studied using microscopic imaging techniques. This was accomplished with the use of Calcein AM and EthD-1. In live cells, Calcein AM is hydrolyzed by enzymes to produce calcein, which then fluoresces green. After the application of NIR, fluorescence microscopy was used to detect and see dead cells by staining them with EthD-1 which works by giving characteristic red color after transferring through the cell membrane of dead cells. Images of the cells

were taken before and after a 10-minute laser application without the inclusion of nanoparticles to serve as a comparative standard. In Figures 6A and 6B both the cell viability and morphology were assessed. The results showed that there was no significant difference in cell viability between the control and laser-applied cells, indicating that the laser treatment did not affect the cells' viability.

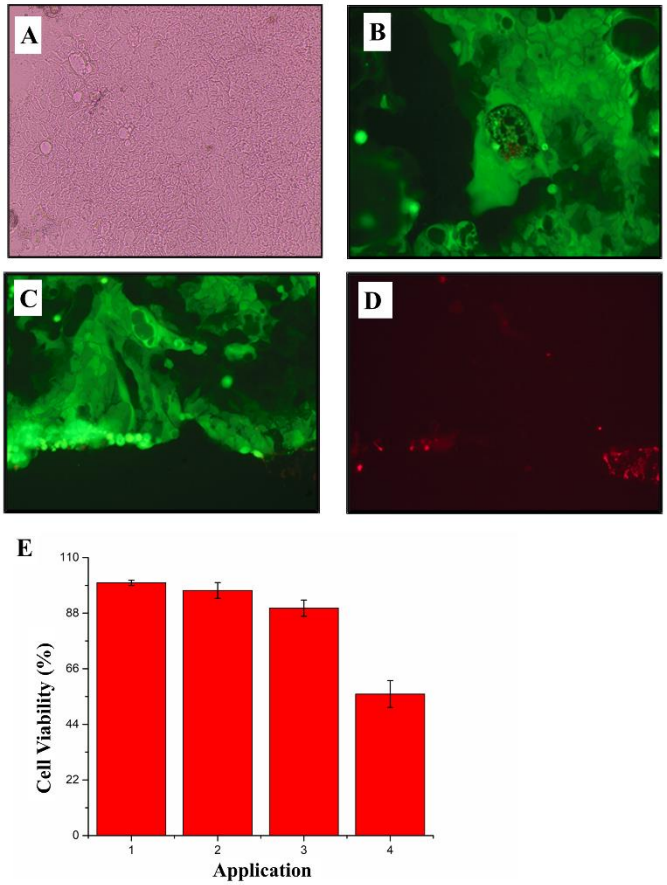


Figure 6: Caco-2 cell line interactions with HAuNPs (50 µg/mL), A) light microscopy, B) Calcein AM staining before laser application, C) Calcein AM staining after laser application, D) EthD-1 staining after laser application, and E) comparative MTT analysis results for different applications; 1. Caco-2 cell line before laser application, 2.Caco-2 cell line after laser application, 3. Caco-2 cell line after incubation with 50 µg/mL HAuNPs, 4.Caco-2 cell line after incubation with 50 µg/mL HAuNPs and laser application

Through the obtained images (6A and 6B) it is continued with Calcein AM and EthD-1 staining with both particle and NIR application on the cell line. The live-dead staining fluorescence microscope observations were given in Figures 6C and 6D. Figure 6C represents the viable cells after NIR application while Figure 6D gives information about the fatal effect of NIR application after treatment of cells with HAuNPs. The MTT results (Figure 6E) also prove the decrease in cell viability which is demonstrated in Figure 6D. According to the MTT findings, the percentage of cell viability at maximum safe doses achieved after a two-hour incubation was determined to be relatively higher or close to the values obtained from the cytotoxicity study (after 24 and 72 hours). When these results were considered through the MTT results in Figure 5, it can be concluded that there was a reduction in cell viability compared to the control group. As shown in Figure 6E, the cell viability reduced from 90% to 56% when HAuNPs (50 µg/mL) were utilized with an NIR source. Overall, the data presented in Figure 6 demonstrates that the use of HAuNPs at a concentration of 50 µg/mL as a photothermal agent resulted in a significant decrease in cell viability from 90% to 56%. This 34% reduction of viability suggests that HAuNPs may be a promising photothermal agent for future applications

IV. Conclusion

The hollow gold nanoparticles were synthesized by a galvanic replacement method, and cobalt nanoparticles were used as a template for the hollow structure. According to the characterization results the particle has absorbance at 575 nm and around 24 nm according to the TEM images. Following the synthesis, the hollow gold nanoparticles were then utilized for photothermal applications, where the 0.5-watt laser was used to selectively heat the nanoparticles, enabling their photothermal efficiency to be evaluated. The results of this photothermal application indicate a temperature increase of 10.4 °C. After getting this effective temperature increase, the hollow gold nanoparticle was studied as a photothermal agent in vitro study with the Caco-2 cell line. The study reveals that using HAuNPs at a specific concentration of 50 µg/mL, as determined by the cytotoxicity analysis, decreases Caco-2 cell viability from 90% to 56% when exposed to near-infrared (NIR) radiation. This 34% reduction of viability result demonstrates the promise of HAuNPs as a photothermal agent for further cancer treatment. The efficacy of these agents in different cell lines and the enhancement of their photothermal performance for other purposes, including cancer treatment, require additional study.

References

[1]. Shukla, R., Bansal, V., Chaudhary, M., Basu, A., Bhonde, R. R., & Sastry, M. (2005). Biocompatibility of gold nanoparticles and their endocytotic fate inside the cellular compartment: A microscopic overview. Langmuir, 21(23), 10644–10654. https://doi.org/10.1021/la0513712

[2]. Ren, Q.-Q. et al. (2015) 'Preparation, modification, and application of Hollow Gold Nanospheres', Journal of Nanomaterials, 2015, pp. 1–7. doi:10.1155/2015/534070

[3]. Rahaman, M., Moras, S., He, L., Madeira, T. I., & Zahn, D. R. (2020). Fine-tuning of localized surface plasmon resonance of metal nanostructures from near infrared to blue prepared by nanosphere lithography. Journal of Applied Physics, 128(23), 233104. https://doi.org/10.1063/5.0027139

[4]. Huang, X., Jain, P. K., El-Sayed, I. H., & El-Sayed, M. A. (2007). Plasmonic photothermal therapy (PPTT) using gold nanoparticles. Lasers in Medical Science, 23(3), 217–228. https://doi.org/10.1007/s10103-007-0470-x

[5]. Liu, S., Pan, X., & Liu, H. (2020). Two-dimensional nanomaterials for photothermal therapy. Angewandte Chemie International Edition, 59(15), 5890–5900. https://doi.org/10.1002/anie.201911477

[6]. Govorov, A. O., & Richardson, H. H. (2007). Generating heat with metal nanoparticles. Nano Today, 2(1), 30–38. https://doi.org/10.1016/s1748-0132(07)70017-8

[7]. Jiang, L., Han, J., Li, J., Jia, W., Tian, Y., Yao, L., & Li, X. (2014). Photothermal therapy of cancer cells using novel hollow gold nanoflowers. International Journal of Nanomedicine, 517. https://doi.org/10.2147/ijn.s55800

[8]. Liu, Y., Crawford, B. M., & Vo-Dinh, T. (2018). Gold nanoparticles-mediated photothermal therapy and immunotherapy. Immunotherapy, 10(13), 1175–1188. https://doi.org/10.2217/imt-2018-0029

[9]. Yang, W., Liang, H., Ma, S., Wang, D., & Huang, J. (2019). Gold nanoparticle based Photothermal Therapy: Development and application for effective cancer treatment. Sustainable Materials and Technologies, 22. https://doi.org/10.1016/j.susmat.2019.e00109

[10]. Lopatynskyi, A. M., Malymon, Y. O., Lytvyn, V. K., Mogylnyi, I. V., Rachkov, A. E., Soldatkin, A. P., & Chegel, V. I. (2017). Solid and hollow gold nanostructures for nanomedicine: Comparison of Photothermal Properties. Plasmonics, 13(5), 1659–1669. https://doi.org/10.1007/s11468-017-0675-1

[11]. Dung, N. T., Linh, N. T., Chi, D. L., Hoa, N. T., Hung, N. P., Ha, N. T., Nam, P. H., Phuc, N. X., Tam, L. T., & Lu, L. T. (2021). Optical properties and stability of small hollow gold nanoparticles. RSC Advances, 11(22), 13458–13465. https://doi.org/10.1039/d0ra09417j

[12]. Chen, J., Wiley, B., McLellan, J., Xiong, Y., Li, Z.-Y., & Xia, Y. (2005). Optical properties of pd−ag and pt−ag nanoboxes synthesized via galvanic replacement reactions. Nano Letters, 5(10), 2058–2062. https://doi.org/10.1021/nl051652u

[13]. Kimling, J., Maier, M., Okenve, B., Kotaidis, V., Ballot, H., & Plech, A. (2006). Turkevich method for Gold Nanoparticle Synthesis Revisited. The Journal of Physical Chemistry B, 110(32), 15700–15707. https://doi.org/10.1021/jp061667w

[14]. Preciado-Flores, S., Wang, D., Wheeler, D. A., Newhouse, R., Hensel, J. K., Schwartzberg, A., Wang, L., Zhu, J., Barboza-Flores, M., & Zhang, J. Z. (2011). Highly reproducible synthesis of hollow gold nanospheres with near infrared surface plasmon absorption using PVP as stabilizing agent. J. Mater. Chem., 21(7), 2344–2350. https://doi.org/10.1039/c0jm03690k

[15]. Schwartzberg, A. M., Olson, T. Y., Talley, C. E., & Zhang, J. Z. (2006). Synthesis, characterization, and tunable optical properties of Hollow Gold Nanospheres. The Journal of Physical Chemistry B, 110(40), 19935–19944. https://doi.org/10.1021/jp062136a