ECB 2021

sciforum

Proceedings

A Green Method to Synthesis the Size-Controllable Gold Nanostars for Photothermal Therapy and Photoacoustic Imaging†

Thi Tuong Vy Phan [1,2,*]

[1] Center for Advanced Chemistry, Institute of Research and Development, Duy Tan University, 03 Quang Trung, Hai Chau, Danang 550000, Vietnam
[2] Faculty of Environmental and Chemical Engineering, Duy Tan University, 03 Quang Trung, Hai Chau, Danang 550000, Vietnam
* Correspondence: phanttuongvy4@duytan.edu.vn
† Presented at the 1st International Electronic Conference on Biomedicine, 1–26 March 2021; Available online: https://ecb2021.sciforum.net.

Published: 31 May 2021

Abstract: Photothermal therapy (PTT) is a novel therapy for cancer treatment which bases on the conversion of photon energy into heat (>43°C), and photoacoustic imaging (PAI) is a new bioimaging method for diagnostic and monitoring cancer. To enhance the impact depth of PTT and the signal of PAI, the near-infrared (NIR)-absorbing photothermal agents have usually been used. The development of novel NIR-absorbing photothermal agents with excellent properties such as good stability under long-term temperature, good absorption in the NIR range, and excellent biocompatibility is needed in modern biomedicine. Previous researches have approved that gold nanostar (AuNS) has promising potential applications in photo-based therapies owing to the good absorption in the NIR range and good photothermal effect. However, the reported methods to synthesis AuNS are complicated and toxic, which can limit its practical application. In this work, we proposed a new environmental strategy to synthesis AuNS by using chitosan and vitamin C. Chitosan plays a multi-role including stabilizing, shape-directing, and size-controllable agents in this method for the first time. The obtained AuNS have good NIR absorption, biocompatibility toward non-cancerous and cancerous cell lines. The in vitro tests proved the high efficiency of obtained AuNS on both PTT and PAI.

Keywords: green synthesis; gold nanostars; chitosan; photothermal therapy; photoacoustic imaging

---

1. Introduction

Photoacoustic imaging (PAI) [1,2] and photothermal therapy (PTT) have been focused on research by many groups recently [3,4]. In most cases, the external agents have been utilized to enhance the performance of PTT and PAI. AuNPs are the most frequently used as photothermal agents owing to their unique surface plasmon resonance characteristics [5]. AuNS is a good candidate for PTT owing to its high absorption in NIR region (700 to 1870 nm) [6]. Additionally, AuNS can work as a photoacoustic agent for PAI [7]. To overcome the disadvantages of traditional methods (i.e., complicated [8,9] and toxicity [8]) to synthesize AuNS, we proposed an environment method for the synthesis of AuNS by using non-toxic materials. We used vitamin C for reducing the Au(III) to Au(0) and chitosan (CS) for stabilizing the AuNS. The size of the resulted nanoparticles ranged from 111 to 250 nm. The in vitro test evidenced that AuNS are good agents for both PTT and PAI.

2. Materials and methods

2.1. Materials

CS (50 to 190 kDa, 75%–85% deacetylation), gold chloride (HAuCl4), hydrochloric acid (HCl, 37%), L-ascorbic acid (vitamin C), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The cell culture materials including Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), antibiotics, trypsin, and phosphate-buffered saline (PBS) were bought from HyClone (South Logan, UT, USA).

2.2. Synthesis of AuNS

To obtain $HAuCl_4$ 0.01 M solution, 157.5 mg HAuCl4.3H2O was added to 25 mL DW. The difference amount of CS was added to distilled water to obtained a range concentration of CS. Then, 1 mL $HAuCl_4$ 0.01 M was added to the CS-vitamin C solution. The AuNS mass was collected by centrifugation, then washing them with distilled water.

2.3. Characterization

The shape of AuNS was visualized under a field-emission transmission electron microscopy (FETEM, JEOL JEM-2010 microscope, Japan). The UV-Vis absorption of AuNS solution was recorded by using a UV-Vis spectroscopy (Thermo Biomate 5 Spectrophotometer). The size distribution of samples was studied by an electrophoretic light scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan). An 808 nm NIR laser with continuous-wave (CW) pulse from Hi-Tech Optoelectronics Co. (Beijing, China) was used on all photothermal-related experiments.

2.4. In vitro PTT

A 6-well plate was prepared with a density of 6×10^5 cells/well. Four groups of the cell are named as control (no treatment), NIR laser, AuNS, and AuNS + NIR laser. Then, 60 μg/mL AuNS solution was added to cells in groups III and IV and incubated further for 4 h. The photothermal treatment experiments were conducted on groups II and IV with NIR laser (1.5 W/cm^2, 5 min). The AO/PI staining was applied to visualize the live/dead cells after the photothermal experiment.

2.5. In vitro PAI

The solutions of AuNS with a range of concentrations (30, 15, 7.5, 3.75, 1.87, and 0.93 μg/mL) were loaded to the PTFE tube and the phantoms were placed in a degas water chamber for PAI [10].

3. Results and Discussion

3.1. TEM and UV-Vis Absorption Spectra

The TEM images were shown in Figure 2. The AuNS which were obtained from experiments with 0.05% to 0.4% (m/v) CS had elongated and sharp tips, meanwhile, AuNS from 0.7% to 0.9% (m/v) CS had short and unsharp tips. The nanoparticle sizes of AuNS ranged from 111 to 225 nm. As shown in Figure 2, the AuNS solution from 0.05% to 0.4% (m/v) CS experiments had absorption peaks from 685 to 750 nm and strong broadband absorption in the NIR region. Meanwhile, the AuNS from 0.7% to 0.9% (m/v) CS experiments had an absorption peak at 605 nm and weak absorption in the NIR region.

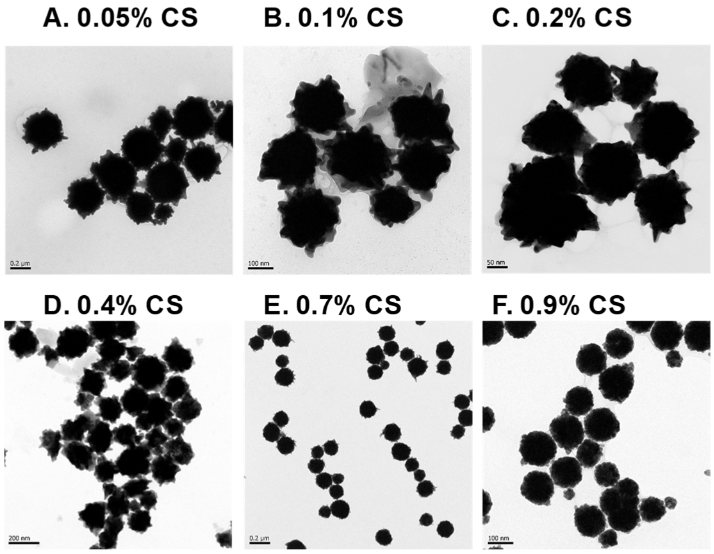

Figure 1. TEM of AuNS.

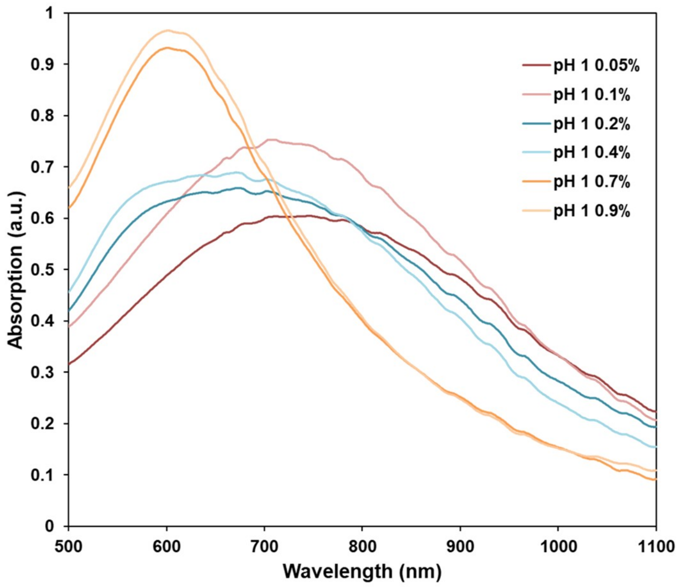

Figure 2. UV-Vis absorption of AuNS.

3.2. *In vitro PTT*

MDA-MB 231 cells were divided into four groups: control, NIR laser, AuNS, and AuNS + NIR laser. The trypan blue staining method was used to discriminate the dead and damaged cells. As shown in Figure 3, only the cells in group AuNS+NIR were blue which indicated the dead and damaged cells. A NIR laser or AuNS alone did not cause damage to the cells.

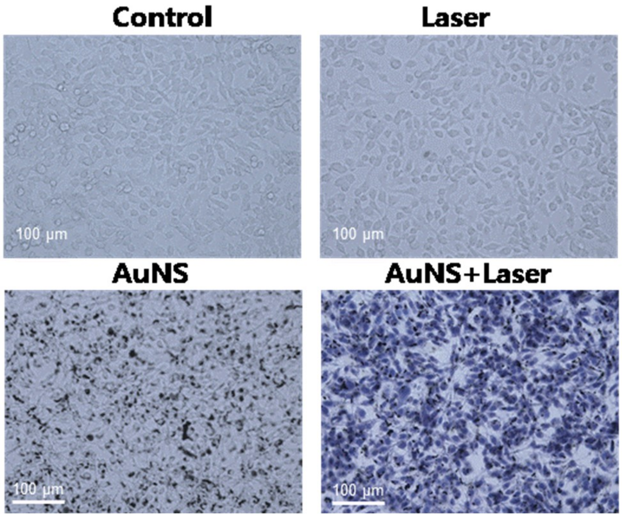

Figure 3. Trypan blue staining of MDA-MB-231 cells of the control group and treated groups: NIR laser only (1.5 W/cm^2, 5 min), 60 μg/mL AuNS, and 60 μg/mL AuNS plus NIR laser (1.5 W/cm^2, 5 min).

3.3. In vitro PAI

AuNS were loaded in the PTFE tubes. The amplitude of the signal was increased when AuNS concentration; meanwhile, the control tube with PBS did not show any PA signals (Figure 4). The PAI with AuNS has potential in diagnosis and monitoring tumors.

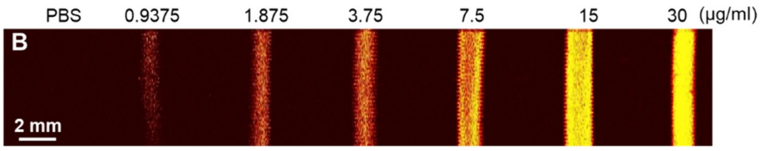

Figure 4. The PA signals from the AuNS with different concentration.

4. Conclusion

We reported the novel and green strategy to prepare AuNS; in this method, vitamin C was used as a green reducing agent and CS was used as a shape directing agent. The AuNS have a size from 111 to 250 nm with peak absorptions in NIR range. AuNS plus NIR laser effectively killed MDA-MB-231 cancer cells, proved their ability for PTT. Furthermore, the good PA signals of the AuNS also confirmed the potential of AuNS–based PAI.

References

1. Huang, X.; El-Sayed, M.A. Plasmonic photo-thermal therapy (PPTT). *Alex. J. Med.* **2011**, *47*, 1–9, doi:10.1016/j.ajme.2011.01.001.
2. Phan, T.T.V.; Bui, N.Q.; Cho, S.-W.; Bharathiraja, S.; Manivasagan, P.; Moorthy, M.S.; Mondal, S.; Kim, C.-S.; Oh, J. Photoacoustic Imaging-Guided Photothermal Therapy with Tumor-Targeting HA-FeOOH@PPy Nanorods. *Sci. Rep.* **2018**, *8*, 1–13, doi:10.1038/s41598-018-27204-8.
3. Weber, J.; Beard, P.C.; E Bohndiek, J.W.S. Contrast agents for molecular photoacoustic imaging. *Nat. Methods* **2016**, *13*, 639–650, doi:10.1038/nmeth.3929.
4. Weber, J.; Beard, P.C.; E Bohndiek, J.W.S. Contrast agents for molecular photoacoustic imaging. *Nat. Methods* **2016**, *13*, 639–650, doi:10.1038/nmeth.3929.
5. Phan, T.T.V.; Bharathiraja, S.; Nguyen, V.T.; Moorthy, M.S.; Manivasagan, P.; Lee, K.D.; Oh, J. Polypyrrole–methylene blue nanoparticles as a single multifunctional nanoplatform for near-infrared photo-induced therapy and photoacoustic imaging. *RSC Adv.* **2017**, *7*, 35027–35037, doi:10.1039/c7ra02140b.
6. Huang, X.; El-Sayed, M.A. Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. *J. Adv. Res.* **2010**, *1*, 13–28, doi:10.1016/j.jare.2010.02.002.

7. Khoury, C.G.; Vo-Dinh, T. Gold Nanostars For Surface-Enhanced Raman Scattering: Synthesis, Characterization and Optimization. *J. Phys. Chem. C* **2008**, *112*, 18849–18859, doi:10.1021/jp8054747.
8. Moorthy, M.S.; Bharathiraja, S.; Manivasagan, P.; Oh, Y.; Phan, T.T.V.; Mondal, S.; Kim, H.; Lee, K.D.; Oh, J. Synthesis of Fe3O4 modified mesoporous silica hybrid for pH-responsive drug delivery and magnetic hyperthermia applications. *J. Porous Mater.* **2017**, *25*, 1251–1264, doi:10.1007/s10934-017-0536-5.
9. Wu, H.-Y.; Liu, M.; Huang, M.H. Direct Synthesis of Branched Gold Nanocrystals and Their Transformation into Spherical Nanoparticles. *J. Phys. Chem. B* **2006**, *110*, 19291–19294, doi:10.1021/jp063711d.
10. Bakr, O.M.; Wunsch, B.H.; Stellacci, F. High-Yield Synthesis of Multi-Branched Urchin-Like Gold Nanoparticles. *Chem. Mater.* **2006**, *18*, 3297–3301, doi:10.1021/cm060681i.
11. Bui, N.Q.; Cho, S.-W.; Moorthy, M.S.; Park, S.M.; Piao, Z.; Nam, S.Y.; Kang, H.W.; Kim, C.-S.; Oh, J. In vivo photoacoustic monitoring using 700-nm region Raman source for targeting Prussian blue nanoparticles in mouse tumor model. *Sci. Rep.* **2018**, *8*, 1–9, doi:10.1038/s41598-018-20139-0.

© 2021 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).