Gold-coated iron oxide nanoparticles as a potential photothermal therapy agent to enhance eradication of breast cancer cells

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Abstract. Iron oxide nanoparticles (Fe3O4 NPs) possess unique physicochemical properties which make them great for biomedical applications. This study reports the development of gold (Au) coated Fe3O4 (Au-Fe3O4 NPs) for photothermal therapy to eradicate breast cancer cells (MCF-7). The spherical shape of monodisperse Au-Fe3O4 NPs with an average size of 20.8 nm was confirmed by TEM. The cell viability evaluation of Au-Fe3O4 NPs showed negligible toxicity toward MCF-7 cells after 24 h. Significant cell reduction was observed for MCF-7 (73.9%) cells following photothermal therapy at highest concentration of NPs (50 μgFe/ml) for 10 minutes illumination when compared with other intervention groups. It can be concluded that, the synthesized Au-Fe3O4 NPs is an effective and promising photothermal therapy agent for breast cancer treatment.

1. Introduction

Recently, Fe3O4 NPs have increasingly garnered interest owing to their extensive biomedical applications such as drug delivery, cancer treatment, hyperthermia therapy and magnetic resonance imaging (MRI) [1]. Fe3O4 NPs possess excellent properties that involve non-toxicity, biocompatibility, superparamagnetic behavior and good magnetic susceptibility [2]. Also, Au NPs display the most potential to form a shell on the Fe3O4 NPs surface due to its, chemically stability, biocompatible, resistant to oxidation and intrinsic optical properties [3]. The Au displays intense surface plasmon resonance absorption in near-infrared (NIR) region allowing it uses for photothermal therapy and thermal imaging [4]. For that reason, the productions of the biocompatible Au-Fe3O4 NPs have emerged as a promising strategy over the recent years [5]. Regarding to these reports, the introduction of efficient photothermal therapy agent integrating magnetic Fe3O4 core and Au shell NPs would be of great significance. Based on the literature, there are many works have done for photothermal therapy by using Au NPs [6] and silver (Ag) NPs [7]. However, there is a lack of study to see how the effect of Fe3O4(core) and Au (shell) as a photothermal therapy agent. In this study, we examined the efficacy of...
Au-Fe₃O₄ NPs through illumination of NIR light for 1, 5 and 10 min. To achieve this goal, MCF-7 cells were treated by Au-Fe₃O₄ NPs and number of cells after illumination to NIR laser were investigated. Fe₃O₄ NPs was synthesized via co-precipitation method and modified their surface with Au by sonication method.

2. Materials and Methods

2.1 Synthesis Au-Fe₃O₄ NPs
The preparation of Au shell coated Fe₃O₄ NPs involved the synthesis of Fe₃O₄ NPs as seeds and following by reduction of HAuCl₄ in the presence of seeds using the sonication method. At 40 kHz (ultrasonic frequency), 5 mg of Fe₃O₄ was ultrasonically dispersed in 20 ml of sodium citrate for 15 min. Afterwards, a freshly prepared HAuCl₄ solution (10 ml, 0.1 M) was added to reduce HAuCl₄ and form the shell located on Fe₃O₄ surface. Sonicated process was then continued for 15 min. Au-Fe₃O₄ NPs were collected by means of a permanent magnet and thoroughly rinsed three times with distilled water and re-dispersed in distilled water.

2.2 Cytotoxicity assay
The cytotoxicity of Au-Fe₃O₄ NPs on human breast carcinomas (MCF-7 cell line) cultured in DMEM were determined by WST-1 assay.

2.3 In vitro photothermal ablation of MCF-7 cells
Cells were illuminated by NIR (808 nm, at an output power 200 mW) for 1, 5 and 10 min. Cell viability was measured after illumination by NIR laser using WST-1 assay.

3. Results and Discussion
Figure 1(a) shows the XRD pattern of Fe₃O₄ (i), and Au-Fe₃O₄ NPs (ii) are compared. The diffraction peaks of Au-Fe₃O₄ NPs 38.2°, 44.4°, 64.6° and 77.7° can be assigned to (111), (200), (220) and (311) are different to Fe₃O₄ NPs peaks but similar to Au NPs peaks [8]. The absence of Fe₃O₄ peaks has been reported by others [9]. This is may be due to the heavy atom effect from Au [10] thus, the Fe₃O₄ core was completely covered by the Au shell. More specific, the core signal was shielded by the Au shell and thus disappeared in the X-ray diffraction pattern.

Figure 1(b) shows a typical UV-visible of Au and Au-Fe₃O₄ NPs. The Au NPs as-prepared shows a plasmon resonance band at 520 nm in the visible region. In absorption spectrum of Au-Fe₃O₄ NPs a peak at 538 nm is observed. This is indicating successful formation of an Au shell on Fe₃O₄ surface by the mentioned method (refer to part 2.1). This redshift (compared to that of Au NPs (520 nm)) can be attributed to formation of Au shell around Fe₃O₄ NPs.

The sodium citrate on the surface of Fe₃O₄ NPs reduced the HAuCl₄ ions in solution and caused them to form homogenous nanoparticles. Figure 1(c, d) displays TEM images and size distribution histograms of Fe₃O₄ (a) and Au-Fe₃O₄ NPs (b). It is seen in Figure (c) The original Fe₃O₄ NPs have a
spherical shape and homogenous characteristic size of about 7.5 nm. After the modification of Fe₃O₄ with Au shell, the resulted Au-Fe₃O₄ NPs retained the spherical shape with the average diameter of 20.8 nm Figure (d). The thickness of the Au shell was estimated to be 13.3 nm. the shell thickness could be changed by varying experimental parameters. To assess the biocompatibility and treatment efficacy in cancer therapy of Au-Fe₃O₄ NPs (50, 25, 12.5 and 6.25 µgFe/ml), WST-1 cell assay was carried out on human breast carcinoma (MCF-7 cell lines), as shown in Figure 1(a). The cell viability evaluation of Au-Fe₃O₄ NPs showed negligible toxicity toward MCF-7 cells lines after 24 h, even at their highest concentrations, which confirms their applicability for biomedical purposes.

In this experimental study, the treated MCF-7 cells with Au-Fe₃O₄ NPs (50, 25, 12.5 and 6.25 µgFe/ml) overnight, were irradiated to NIR (808 nm, at a power of 200 mW, and spot size 2mm) for 1, 5 and 15 minutes times' period. Moreover, Control groups were determined as cells incubated with same concentrations of NPs without NIR irradiation. After NIR irradiation, reduction of MCF-7 cells treated with Au-Fe₃O₄ NPs was observed more in compared to the control groups, a fact which resulted from photothermal effect of Au-Fe₃O₄ NPs.

| Concentration of Fe based on µgFe/ml | 6.25 | 12.5 | 25 | 50 |
|--------------------------------------|------|------|----|----|
| Before irradiation                   | 149.027 ± 2.10 § | 143.774 ± 8.839 | 138.715 ± 5.264 | 152.917 ± 11.868 | 0.220 |
| 1 minute after irradiation           | 107.211 ± 1.602 | 106.973 ± 1.342 | 110.131 ± 1.706 | 78.069 ± 18.189 | 0.008 * |
| 5 minutes after irradiation          | 97.496 ± 4.229 | 107.215 ± 6.172 | 101.193 ± 10.559 | 73.584 ± 6.363 | 0.002 * |
| 10 minutes after irradiation         | 81.384 ± 9.711 | 84.004 ± 7.966 | 81.821 ± 7.987 | 39.882 ± 2.037 | <0.001 * |

Table 1. Comparing cell viability between different time points before and after irradiation and different concentrations of Fe.

Figure 2. (a) The biocompatibility test of Au-Fe₃O₄ NPs against the (MCF-7) cell line for 24 h. (b) Comparison of the cell viability among different concentrations of nanoparticle (6.25, 12.5, 25 and 50 µgFe/ml) in each group of study (before irradiation, and 1, 5, and 10 minutes after irradiation). The groups with similar English letters are not significantly different from each other at a level of 5%.

Table 1 contains mean values of cell viability ± standard deviations (STDs) in the different time durations of irradiation and different concentrations of nanoparticle. The results of the Kruskal-Wallis test showed that there were no statistically significant differences in cell viability after incubation with different concentrations of nanoparticle in control group (p-value > 0.05). But different concentrations of Fe significantly effect cell viability after one, five and ten minutes of irradiation (p-value<0.01 for all). From Table 1, in study groups (one, five and ten minutes after irradiation) the lowest cell viability percentage was observed in concentration of 50 µgFe/ml of nanoparticle (78.069±18.189,
73.584±6.363 and 39.882 ± 2.037 %, respectively). Moreover, Mann-Whitney test was used as the post hoc test for pairwise comparison between different concentrations of nanoparticle in each time duration of irradiation groups. The results of these analyses were presented in Figure 2(b) by the English letters. The mean values in groups with a similar English letter were not statistically significant from each other at p-value <0.05.

4. Conclusions
Au-Fe₃O₄ NPs were successfully synthesized with an average diameter of 20.8 nm and a spherical shape via the sonication method. The cell viability evaluation of Au-Fe₃O₄ NPs showed negligible toxicity toward MCF-7 cells lines after 24 h, even at highest concentration of NPs. MCF-7 cells treated with Au-Fe₃O₄ NPs was greatly decreased (73.9%) at 50 μgFe/ml viability after illumination by 808 nm laser (200 mW, 10 min). In this work, findings agree that the synthesized Au-Fe₃O₄ nanoparticles that are impose any danger to human health, have potential to be employed as photothermal therapy agent to enhance treatment of breast cancer.

5. References
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