RESEARCH PAPER

Green synthesis of glucose-coated gold nanoparticles for improving radiosensitivity in human U87 glioblastoma cell line

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ABSTRACT

Objective(s): Surgery and radiation therapy are the most important known treatments for glioblastoma, which is known as the most malignant tumor of the central nervous system. Numerous studies have proven the effect of different gold nanoparticles in improving radiation sensitivity. But there is still a need for nanoparticles with suitable size and higher sensitivity. Hence, the present study aimed to prepare optimized glucose-coated gold nanoparticles (Glu-GNPs) for improving radiosensitivity against U87 glioblastoma cells.

Materials and Methods: Firstly, Glu-GNPs were synthesized and then their physiochemical characterizations were assessed using dynamic light scattering (DLS). The cytotoxicity of Glu-GNPs was evaluated by MTT assay in U87 and NIH-3T3 cell lines. Additionally, the colony formation assay, which is known as the gold standard test, was used to evaluate the radiosensitivity effect of Glu-GNPs on U87 cells.

Results: The characterization results showed that Glu-GNPs had a size of 50.3 nm and negative zeta potential of -13.8 mV. Cytotoxicity results revealed that treatment with Glu-GNPs significantly inhibited the proliferation of U87 cells. We found that Glu-GNPs at a concentration of 10 µg/ml were not-toxic for U87 cells. Moreover, the colony formation assay results showed that Glu-GNPs significantly increased the effect of radiation and caused U87 cancer cell death at a non-toxic concentration of 10 µg/ml.

Conclusion: Taken together, the Glu-GNPs, with a size of 50.3 nm, increased radiosensitivity and caused cell death at a concentration of 10 µg/ml in U87 glioblastoma cells and deserve further *in vitro* and *in vivo* investigations.

Keywords: *β-D-glucose, Gold nanoparticle, Radiosensitizer, U87 glioblastoma cell*

How to cite this article
Bemidinezhad A, Mirzavi F, Gholamhosseinian F, Gheybi F, Soukhtanloo M. Green synthesis of glucose-coated gold nanoparticles for improving radiosensitivity in human U87 glioblastoma cell line. Nanomed J. 2022; 9(4): 328-333. DOI: 10.22038/NMJ.2022.67425.1714

INTRODUCTION

Glioblastoma is known as the most common primary malignant tumor of the central nervous system [1]. Despite the advances in recent years, the causes of glioblastoma are still largely unknown. However, people with rare genetic features are more likely to develop glioblastoma as a result of mutations in a specific gene that creates many of its characteristic features [2]. A combination of surgery, radiation therapy, and chemotherapy are used for glioblastoma treatment [3, 4]. Radiation therapy has attracted the attention of many researchers due to its non-invasive nature [5]. Ionizing radiation causes cell death by interacting with biomolecules such as proteins, lipids, and DNA [6]. Radiation sensitizers are used to increase the effect of radiation therapy. In the presence of radiosensitizers, radiation causes the most deaths in cancer cells and causes the least damage to healthy cells around the tumor tissue [7, 8]. Metals that have a high atomic number (high-Z) have a good potential for radiosensitization [9]. These metals, including gold (Z=79), bismuth (Z = 83), platinum (Z = 78), silver (47), etc., have

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Note. This manuscript was submitted on July 21, 2022; approved on September 20, 2022

a high surface area for photoelectric beam absorption due to their large atomic radius [10]. Gold metal has attracted the attention of many researchers in different fields due to its properties such as biocompatibility and non-toxicity [11]. Nanomedicine has several advantages compared with conventional cancer treatment such as efficient drug delivery, controlled drug release, and reduced unwanted side effects [12]. Today, gold nanoparticles are used in various fields of medicine and many studies have shown their applications as efficient tools in gene therapy, drug delivery, and radiotherapy for cancer treatment [13]. Many studies have proven the anti-tumor and anti-angiogenic effects of gold nanoparticles [11, 14, 15]. The effectiveness of radiation sensitivity of these nanoparticles depends on their uptake by the cancer cells. Several factors such as the size of the nanoparticle, type of cell line, and surface coating of the nanoparticle affect the rate of cellular uptake of nanoparticles [16-18]. On the other hand, it has been demonstrated that cancer cells absorb more glucose than healthy cells [19]. This unique metabolic feature of cancer cells can be used to design nanoparticles for targeted delivery in cancer therapy [19-22]. Therefore, the best strategy for the synthesis of Glu-GNPs with optimal size and efficient radio-sensitization is very effective in increasing the radiosensitizing power of high-Z metal nanoparticles in cancer treatment. Hence, this study aims to use a suitable method for the synthesis of Glu-GNPs, in order to investigate the cytotoxicity and radiosensitivity effects of these nanoparticles on the U87 glioblastoma cancer cell line.

MATERIALS AND METHODS

Materials

$HAuCl_4.3H_2O$, β-D-glucose, Giemsa stain, and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Germany). Dulbecco's Modified Eagle's Medium (DMEM), fetal calf serum (FCS), and penicillin/streptomycin were purchased from Gibco (UK). Dimethyl sulfoxide (DMSO) was provided by Dr. Mojallali Co. (Iran).

Glu-GNP synthesis

Glu-GNP synthesis was performed according to the green synthesis method described by Juncheng Liu *et al* [23]. Briefly, by reducing the gold ions in $HAuCl4.3H_2O$ solution in the medium containing β-D-glucose, gold nanoparticles with glucose coating were made. In the first step, 200 µl of HAuCl4.3H2O (0.05 M) solution was added to 50 ml of β-D-Glucose (0.03 M) solution. This solution was stirred for more than 30 min and no color change was observed, which indicates the lack of reduction of Au+ ions as a result of the combination of these two aqueous solutions. Next, the NaOH (0.05 M) solution was added dropwise and gradually to the combination of the previous two solutions, this gradual addition is not enough until it causes a significant color change in the solution. The addition of NaOH was continued until the pH reached from 3.3 to 10.5, and during this period, the pH was monitored by a pH meter, and simultaneously the synthesis of nanoparticles was regularly monitored using a Jasco V-570 UV/V spectrophotometer.

Characterization of Glu-GNPs

Dynamic light scattering (DLS) (Malvern Instruments, UK) was used to determine the size, polydispersity index (PDI), and surface charge of Glu-GNPs. The morphology of nanoparticles was determined by the images obtained from Transmission Electron Microscopy (TEM) (ZEISS, LEO 912 ab, Germany). Using X-ray diffraction analysis (XRD), the crystal structure of the nanoparticle was determined in the range of 2Θ = 20°–70°. Fourier Transform Infrared Spectrometer (FTIR) in the range of 4000–500 cm^{-1} was used to confirm the binding of glucose to the gold nanoparticle surface.

Cell culture

The human U87 glioblastoma cancer cell line and NIH-3T3 normal fibroblast cell line were purchased from Pasteur Institute (Tehran, Iran). DMEM media containing 10% FCS and 1% penicillin/streptomycin was used to culture these cell lines. The cells were incubated in 5% CO_2 and 37 °C incubators.

Cytotoxicity assay of Glu-GNPs

U87 and NIH-3T3 cells (8×10^3 cells/well) were cultured in each well of a 96-well plate. After 24 hours, the medium was replaced with a culture medium without FCS containing different concentrations of Glu-GNPs (2.5, 5, 10, 13, 17, and 20 µg/ml) and incubated for 3 hours. After that, the cells were washed with PBS, and 24 hours later, 100 µl of MTT solution (0.5 mg/ml) was

added to each well and the plate was incubated for 3 hours. Then the medium was removed from the wells and 200 µl of DMSO was added to each well. Finally, the wavelength of absorbed light was read in the range of 545 nm versus 630 nm using a Stat Fax 2100 microplate reader (Awareness Technology, Inc., Palm City, FL, USA).

Colony formation assay for radiosensitivity measurement

For this purpose, different numbers of U87 cells were cultured in 6-well plates for each radiation dose (no radiation = 500 cells, 2 Gy dose = 750 cells, 4 Gy dose = 1000 cells, and 6 Gy dose = 1250 cells). After 24 hours, the cells were treated with Glu-GNPs (10 µg/ml) for three hours in a DMEM medium without FCS. Afterward, the medium containing the Glu-GNPs was removed from the wells and 2 ml of 10% DMEM was replaced. After 2 hours, the plates were subjected to radiation doses of 0, 2, 4, and 6 Gy of 6MV photon beam. After 2 weeks, the cells were fixed and stained with Giemsa, and plate efficiency (PE) and the survival fraction (SF) were calculated as follows:

$$PE=\frac{\text{Number of colonies formed}}{\text{Number of cells seeded}}\times 100 \qquad SF=\frac{\text{PE of sample}}{\text{PE of control}}\times 100$$

Statistical analysis

Statistical data analysis was done using GraphPad Prism 9 software (San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to calculate statistical differences among groups. The *P*-value ≤ 0.05 was used as significant difference between groups.

RESULTS

Physicochemical characteristics of Glu-GNPs

Results from DLS showed that the dispersion size of Glu-GNPs by intensity was 62.6 nm, by volume it was 53.6 nm, and by the number it was 48.4 nm. Therefore, the mean size of Glu-GNPs obtained was 50.3 nm with a negative zeta potential of -13.8 mV and a PDI value of 0.214 (Fig. 1A and 1B). The results from TEM showed that the nanoparticle has a spherical morphology (Fig. 1C). XRD was used to obtain the crystal structure of Glu-GNPs in the range of 2Θ = 20°–70° and the peaks obtained from this nanoparticle were consistent with the standard peaks of gold NPs (JCPDS 04-0784) (Fig. 1D). These peaks were at the angles of 27.09, 38.53, 44.37, and 69.02, and the most intense of these peaks was at the angle of 38.53. To ensure the interaction between gold nanoparticles and glucose, FTIR spectroscopy was employed. The FTIR spectra of glucose and Glu-GNPs shown in Fig. 1E confirm the presence of glucose on gold nanoparticles. FTIR spectrum shows a very strong absorption peak at 3409 cm^{-1}, indicating the presence of glucose as a surface capping of the Glu-GNPs [23]. It can be noticed that the spectrum of glucose exhibited a characteristic peak at 3413 cm^{-1} attributed to the stretching vibration of -OH, which is confirmed to -OH absorbed by gold nanoparticles [24]. On the other hand, the presence of well-defined peaks

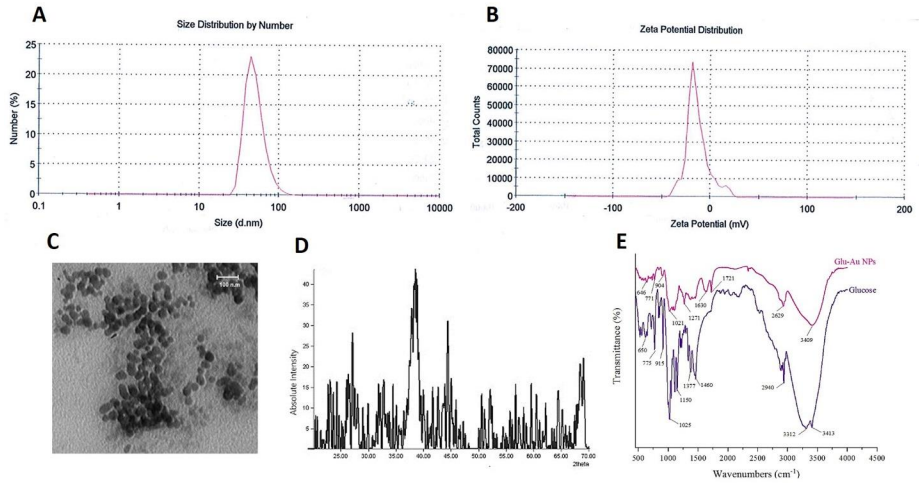

Fig. 1. Physicochemical characteristics of Glu-GNPs. (A) Size distribution and (B) zeta potential of Glu-GNPs were measured using DLS. (C) TEM image of Glu-GNPs. (D) Crystal structure of Glu-GNPs was investigated using XRD. (E) FTIR absorption spectra for both glucose and Glu-GNPs

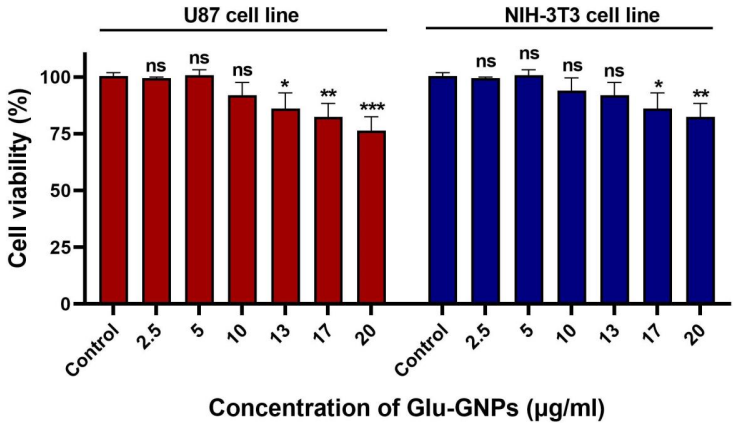

Fig. 2. In vitro toxicity of Glu-GNPs. Cytotoxicity of Glu-GNPs at different concentrations (2.5, 5, 10, 13, 17, and 20 µg/ml) was evaluated on U87 and NIH-3T3 cell lines using the MTT assay. All experiments were performed in triplicates and results are shown as the mean ± SD. Statistical significance was determined at (*P≤0.05, **P≤0.01, ***P≤0.001) compared with the control group

in the 1021–1460 cm^{-1} region in both spectra corresponds to the skeleton vibration of absorbed glucose molecules on the gold surface [25]. Moreover, the prominent region from 650–1460 cm^{-1} and the region from 646–1460 cm^{-1} on the Glu-GNPs spectrum are assigned to C-O and C-C bonds of carbohydrates in glucose as established by earlier studies [26].

In vitro toxicity of Glu-GNPs

The cytotoxicity of Glu-GNP was evaluated *in vitro* on U87 and NIH-3T3 cell lines by the MTT method. As shown in Fig. 2, Glu-GNPs at a concentration of 10 µg/ml were not toxic for U87 cells, and at a concentration of 13 µg/ml were not toxic for the normal NIH-3T3 cells. So a concentration of 10 µg/ml was used for further radiosensitivity investigations.

Radio-sensitization of Glu-GNPs

A colony formation assay was used to determine the radio-sensitization of Glu-GNPs on U87 cancer cells. As shown in Fig. 3, the survival curves show the increasing effect of 6MV radiation at doses of 2, 4, and 6 Gy with Glu-GNPs. The survival fraction decreases with increasing doses of radiation. At each dose, there was a significant difference between the Glu-GNPs group and the non-radiation group (P<0.05). However, the most significant difference was observed with a radiation dose of 6 Gy compared with the control group (P<0.01).

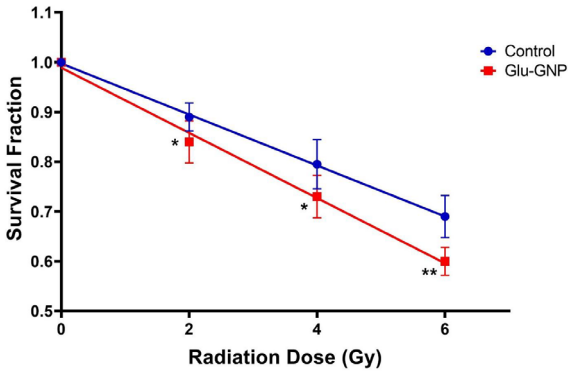

Fig. 3. Survival fraction curves for 2, 4, and 6 Gy radiation doses with 6 MV photon beam power. A colony formation assay was used to determine the radio-sensitization effect of Glu-GNPs on U87 cancer cells. All experiments were carried out 3 times and results are shown as the mean ± SD. Statistical significance was determined at (*P≤0.05 and **P≤0.01) compared with the control group

DISCUSSION

It has been demonstrated that radiation therapy's effectiveness depends on factors such as the size of nanoparticles, type of cell line, concentration of nanoparticles, irradiation energy, and duration of treatment [27]. In the present study, we used the green synthesis method to synthesize Glu-GNPs. Various chemical and physical methods are used for the synthesis of metal nanoparticles, but there are concerns about the use of organic solvents because they are expensive on an industrial scale and also highly toxic for living organisms [28]. Because of this, the use of non-toxic and cost-effective chemicals has been developed in the field of nanomaterials science and is considered a "green" synthetic strategy. Glucose is a biologically compatible "green" chemical, thus nanoparticles coated with glucose can be used as effective tools in cancer

therapy [23]. It has been demonstrated that cancer cells absorb more glucose than healthy cells, which can be used to design nanoparticles for targeted delivery in cancer treatment [19]. In the study conducted by Wang *et al*., Glu-GNPs were synthesized using the classical method, in which sodium citrate was used to reduce gold ions. They found that Glu-GNPs have little cytotoxicity against MDA-MB-231 breast cancer cells with a concentration below 20 nM after 24 hours of treatment [20]. In our study, we also modified gold nanoparticles with glucose to target the high metabolism of cancer cells. Results showed that Glu-GNPs at a concentration of 10 µg/ml, were not-toxic for U87 cells. We also found that Glu-GNPs synthesized by green synthesis are less toxic to NIH-3T3 normal fibroblast cells. More importantly, in the present study, which used the green synthesis method, more glucose was coated on the surface of gold nanoparticles [23]. As a result, Glu-GNPs become less toxic and uptake by the cancerous cells improves.

Nanoparticles can have their best performance when they are made in the best possible size because the size of nanoparticles is the most important factor that plays a role in the penetration of nanoparticles into the target cells [29]. According to previous studies, 50 nm is the optimal size for metal nanoparticles [20, 30]. Nanoparticles larger than 50 nm have difficulty passing through capillaries and entering the cancer cells, and nanoparticles smaller than 50 nm have lower coating strength due to a reduction in the surface area [30, 31]. In this regard, results from DLS showed that the mean size of Glu-GNPs was 50.3 nm with a negative zeta potential of -13.8 mV. In addition, the PDI value of Glu-GNPs was 0.214, which showed that the nanoparticle sizes were homogeneously distributed. Chithrani *et al*. evaluated three shapes of nanoparticles, including, spherical, rod, and cube nanoparticles, and found that nanoparticles in spherical morphology are more absorbed by cells than other morphologies [16]. Our results also showed that the Glu-GNPs made by the green synthesis method had a spherical morphology.

The study conducted by Cuihong Wang *et al.* showed that Glu-GNPs combined with 6-MV X-ray were more sensitive and caused more deaths in the MDA-MB-231 breast cancer cell line [20]. Therefore, in this study, a 6 MV beam was used to optimize the effects of Glu-GNPs. It has been shown that Glu-GNPs increase radiation sensitivity in cancer cells by causing changes in the cell cycle and increasing ROS (reactive oxygen species) generation [32]. In this study, by irradiating a 6 MV photon beam to U87 cells, due to the interactions between the photon beam and gold nanoparticles, a large amount of electrons and photoelectrons are produced. As a result, ROS production increases and causes damage to DNA [19, 32], and finally, the cells treated with Glu-GNPs had more cell death and lower survival percentage than the control group.

CONCLUSION

In the present study, we have prepared optimized Glu-GNPs by the green synthesis method to improve the radiosensitivity against U87 cells. In summary, we found that these nanoparticles, at a non-toxic concentration of 10 µg/ml, have the ability to significantly increase the radiosensitizing properties in the U87 cells and merit further *in vitro* and *in vivo* investigation.

ACKNOWLEDGMENTS

The authors appreciate Mashhad University of Medical Sciences (MUMS) for their financial support (Grant number: 4000774).

CONFLICTS OF INTEREST

The authors reported no potential conflicts of interest.

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