Impact of Gold nanoparticles sizes and concentrations on the Rhabdomyosarcoma cells

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Abstract. The cytotoxic effect of gold nanoparticles (GNPs) on Rhabdomyosarcoma cells (RMS or RD cell line) has been studied by using Methyl Thiazolyl tetrazolium (MTT) assay. Two sizes of the synthesized GNPs (61nm and 71 nm) functionalized with cetyltrimethylammonium bromide with different concentrations of 3.6 ml of 5 ml CTAB solution were used in the current research. The RD cell line was exposed to these particles for 48 hours. The findings showed that the cytotoxic effects of GNPs appeared to depend on concentration. For both sizes at higher concentration, the inhibition rate of IR absorbance was significantly decreased due to GNPs agglomeration on the cell membrane. Lower concentrations of GNPs internalize the cytoplasmic membrane individually by diffusion. Moreover, average particle diameters of 61nm showed a significant inhibition rate of more than 71nm at a lower concentration.

Keywords: gold, nanoparticles, cytotoxic, cytoplasmic, membrane, Rhabdomyosarcoma, cells.

1. Introduction

In the last years, a special attention was given to employing nanoparticles in medicine in what was called recently as nanomedicine. The importance of nanomedicine comes from its ability to improve therapeutic efficacy and imaging capabilities for diagnostic and follow up purposes[1, 2]. Many researches were focusing on the impact of nanoparticles on Rhabdomyosarcoma. Rhabdomyosarcoma is an active and highly malignant cancer developed from skeletal (striated) muscle cells that have not completely differentiated. As the vast majority of cases occur in those below the age of 18, it is generally considered a childhood illness. The unusual physical properties of Gold nanoparticles made it a great source of interest, primarily due to their sharp plasmon absorption peak at the visible region. Moreover, it exhibited absorbance, which provides a powerful detection tool and shows promises in enhancing the effectiveness of various treatments on cancer targets [3, 4]. GNPs is a relevant material as it is an inert, generally nontoxic [5, 6], unreactive, as well as, it is not sensitive to light or air [3]. The therapeutic value of gold nanoparticles depends on their ability to interact with cells, their unique physical properties, and their ability to cause tumor cell damage. Subsequently, the enhanced
permeability and retention impact (EPR) characteristics of a few cancers encourage GNPs invasion into cancer.[7, 8]

An alternative method was based on GNPs functionalization with cetyltrimethylammonium bromide (CTAB). This surfactant can increment cell layer porousness and conjugate with atomic corrosive, as well as, can harm mitochondria, and at long last, actuate apoptosis [6, 9]. Such GNPs capped with CTAB caused cell death because they accumulated in mitochondria, which eventually induce the collapse of the mitochondrial membrane potential as well as production or induce oxidative stress [10]. Both anionic and cationic GNPs are toxic to cells and either negatively and positively charged GNPs cause protein refolding [11], moreover, based on the comes about of Goodman et al. in 2004, it was uncovered that cationic particles (2nm) are tolerably harmful, though anionic particles are remarkably nontoxic[12, 13]. The charged anionic and cationic GNPs can alter the mitochondrial membrane potential resulting in oxidative stress. Apart from these, the anionic and cationic surface charges of GNPs can stimulate lymphoid cells and phagocytosis to an extent greater than neutral GNPs[14, 15].

Nanoparticles (NPs) can induce nanotoxicity against the cells depending on their shape and ligand of the surface functioned that produce more crucial effect, e.g., CTAB, also known as the cationic surfactant, seem increment cell membrane penetrability and conjugate with nuclear acid, CTAB had golden nano sites (GNS) and golden nanorods (GNRs) capped and is much more toxic than sodium-capped citrate GNSs [16, 17]. CTAB alone would be poisonous to cells at a sub-micromolar dosing regimen. [18]. Insufficient surfactant purification or desorption from the GNR layer produces free CTAB molecules in the solution [19]. It creates a bilayer on the gold nanorod surface and guides the forming of nanorod in one direction. [20]. Besides, the use of CTAB molecules is crucial, and therefore the gold nanorods are "made" with a bound surfactant, which gives the nanorods a high positive charge [20, 21]. Knowledge of the sources of toxicity in nanoparticles helps chemists to develop methods for contamination reduction. Different approaches have been used in the case of CTAB-capped nanoparticles to delay CTAB desorption and remove the free CTAB molecules in nanoparticle solutions[19].

The goal of the current study is to study the cytotoxic effect of gold nanoparticles the cytotoxic effect of gold nanoparticles using Methyl Thiazolyl tetrazolium (MTT) assay.

2. Material and Methods:
Gold nanoparticles (GNPs) were synthesized by using pulsed Nd: YAG laser through Withdrawal of a piece of the gold metal plate (with a purity of 99.99%) placed on the bottom of a test tube containing 3.6 ml of 5 mM CTAB solution. The location width of the laser beam was chosen 1 nm in diameter on the metal plate layer. The ablation was performed by using a focused beam output of pulsed laser at 1064 nm and two laser energies (600 and 1000) mJ respectively, with pulse width of 10 ns and pulse repetition rate (PRR) of 6 Hz per second.

For a range of (300-800) nm, the absorption spectra of colloidal gold nanoparticles are calculated at ambient temperature using an Ultraviolet-visible spectrophotometer measuring tool. Atomic force microscopy (AFM) was used to determine the average dimension, surface roughness, and nanoparticle dispersion at ambient temperature distribution of each specimen.

The concentration of the GNP specimens was measured using the calculation of the atomic absorption spectrophotometer, and it was conducted using AAS for the samples packed.

The cell lines most extensively used in RMS work are the RD cells in which RD cells are extracted from biopsy directly biopsy taken from a 7- years-old female suffering from a pelvic RMS and previously treated with radiation and cyclophosphamide and has identified refractory infection [22, 23]. The photos were taken after 24 h, while the evaluation of cytotoxicity was carried out after exposure of the cells to GNPs solutions for 48 h.

Cell viability, representing the efficacy of extracting cancer cells from GNPs, was calculated by withdrawing the medium after 48 h of treatment, applying 20 μl of 5 mg/ml of MTT solution, and incubating at 37°C for four hours. The remaining crystals in the wells were dissolved by applying 200 μL of Dimethyl Sulphoxide (DMSO) accompanied by 15 minutes of incubation at 37 o C. The
microplate reader determined the absorbance at a wavelength of 620 nm. The inhibiting rate of cell growth was calculated according to Betancur-Galvis et al., in 1999 [24] and Gao et al., in 2003 [25] as follows:

\[
\text{Inhibition Rate} = \frac{\text{Mean of Control} - \text{Mean of Treatment}}{\text{Mean of Control}} \times 100
\]  

(1)

3. Results and Discussion

3.1. Gold Nanoparticles:

As shown in Figure 1 (A and B) the UV spectra of Au samples have a single peak. Laser ablation lasted for 3 min and 33 sec with different laser ablation energies (600 and 1000) mJ, and the solution gradually became colored with increasing the number of laser pulses, that is means the concentration of metal nanoparticles increased in the solution as the ablation process increased.

The AFM pictures and size distributions of gold nanoparticles produced by laser ablation of metal plate immersed in CTAB solution is manifested in Figure 2 (A, and B). The produced nanoparticles have an average diameter of 61.83 and 71.16 nm at the laser energies of 1000 and 600 mJ, respectively.

![Figure 1](image1.png)

**Figure 1.** Absorption spectrum of nanoparticles of colloidal gold at laser energy (A) 1000mJ, (B) 600mJ. (plotted by AFM)
Figure 2. AFM images of gold nanoparticles deposited on the slide and its size distributions prepared by laser energy (A) 1000mJ, (B) 600mJ with $\lambda=1064$ nm, and PRR =6 Hz.

3.2. Influence of Gold Nanoparticles on The Rd Cell Line Viability:

The toxic effect of gold nanoparticles with CTAB capping agents was studied in vitro by using the Rhabdomyosarcomas cell line (RD). The level of toxicity was evaluated depending on the influences of cell viability. The cell lines were examined after 48 hr. of exposure to gold nanoparticles. There were no significant changes ($P<0.05$) between the viability of the treated cells with all concentrations of (61.83 nm) gold nanoparticles except in (8.943) µg/ml, Table 1 but as compared with the control group all the other concentrations manifested a significant difference, so we can choose the lower concentration of nanoparticles which gives a significant rate of inhibition for therapeutic purposes. On the other hand, the lower concentration (2.094 µg/ml) of gold nanoparticles with (71.16 nm) diameter reduced cell growth Importantly ($P\leq0.05$) When opposed to other concentrations and control groups. The inhibitory rate (IR) was: 61.167%, while this IR effect was detected in the first group treated with lower concentration (1.118 µg/ml) of (61.83 nm) GNPs. As well as, all higher concentrations show lower inhibition rate, Table 2. However, these results showed that the IR increased with decreasing concentrations, as shown in Figure 3 and Figure 4 of 61.83 nm and 71.16 nm, CTAB capped gold nanospheres, respectively.
Table 1. Mean values of inhibition rate percentage (IR %) for RD cell line by (61.83 nm) GNPs after 48 hr of exposure.

| Concentrations µg/ml | IR % ± S.E.          |
|----------------------|----------------------|
| 8.943                | 27.500±1.443 b       |
| 4.471                | 58.667±1.540 a       |
| 2.236                | 60.000±0.192 a       |
| 1.118                | 61.167±1.058 a       |

Note: Data is demonstrated as mean ± S.E.; values with various small letters superscript (a, and b) differ in the same column (P≤0.05);

Table 2. Mean values of inhibition rate percentage (IR %) for RD cell line by (71.16 nm) of GNPs after 48 hr of exposure.

| Concentrations µg/ml | IR% ± S.E.          |
|----------------------|---------------------|
| 16.75                | 38.167±2.021 c      |
| 8.375                | 49.000±0.385 b      |
| 4.188                | 52.167±0.096 b      |
| 2.094                | 61.167±1.058 a      |

Note: Data is demonstrated as mean ± S.E.; values with various small letters superscript (a, b, and c) differ in the same column (P≤0.05);

Figure 3. Impact of different concentrations of (61.83 nm) gold nanoparticles on cell viability in the RD cell line. The plotted data is mean, n = 3. *p less than 0.05 vs. control group.
Figure 4. Impact of different concentrations of (71.16 nm) gold nanoparticles on cell viability in the RD cell line. The plotted data is mean, n = 3. *p less than 0.05 vs. control group.

Such findings might be attributed to agglomerates of nanoparticles as their density increases and form an internalized cluster, rather than a single particle, through the cell membrane. Therefore, as shown in Figure 5, the volume of each GNP that forms this cluster becomes rather small, exposing the aggregation of GNPs cluster around the cell membrane.

Mustafa T et al. in 2011 [26, 27] studied the role of the nanoparticles’ concentration on the MC3T3-E1 osteoblastic cells uptake. The particles, even after the period of incubation progression, were found concentrated around the nucleus and there was no apparent visible penetration in the nuclear area. The cause could be due to the structure of the nuclear membrane, which composed of two layers. GNPs also can disperse the plasma-membrane independently at lower concentrations, while cells ingest GNPs by endocytosis at high concentrations, as shown by transmission electron microscopy (TEM) study, the effect of which is that large clusters produced by endocytosis on the apical surface of cell plasma membranes are consumed by the cells. In contrast, the active diameters of the GNPs play an essential part in cell treatment at lower concentrations only. The absorption process of GNPs thus relied on the intensity, which is in line with the current research.

Also, another influencing factor is the surface chemistry of nanoparticles. Surface chemistry strongly affects the aggregation of nanoparticles[28] by charging and properties of GNPs after mixing with the biological solutions due to the absorption of non-specific serum proteins on the surface of nanoparticles[29-31]. The endocytotic pathway of the agglomerated GNPs could by enhance into the cells as a result of the surface attachment of that proteins onto the nanoparticles. Exposing to high concentrations of GNPs leading to internalize these large agglomerates in the cells and rapid agglomeration on the cell surfaces occurs[26].

GNPs have a critical toxic role on the cell, maximum IR occurs when particles size 61 nm at 1 µg/ml which have similar effect when the particles size 71 nm at 2 µg/ml, as mentioned before by Osaki and colleagues[32]. The endocytosis was reported to be highly scalable, with an ideal calculation of about 50 nm.
Figure 5. Effects of GNPs on RD cells morphology. With different concentrations and after 24 h- treatment with the same size (61 nm). (A) Control (untreated) (B) 8.943 µg/ml, (C) 2.236 µg/ml, (D) 1.118 µg/ml.

4. Conclusion
Methyl thiazolyl tetrazolium assay was used to study the cytotoxic effect of gold nanoparticles on rhabdomyosarcoma cells. Current research has used two sizes of synthesized GNPs (61 nm and 71 nm) functionalized with cetyltrimethylammonium bromide at different concentrations of 3.6 ml of 5 ml CTAB solution. the present findings manifest that both the sizes and the concentrations of the nanoparticles have a significant inhibition effect on the cell growth; it is preferable to use a lower concentration of small particle diameter with a significant inhibition role in therapeutic application. According to the agglomeration of GNPs on the cell membrane, the inhibition level of IR absorbance decreased significantly. Higher levels of GNPs internalize independently by diffusion the cytoplasmic membrane. In contrast, an average particle diameter of 61 nm at a lower concentration showed a significant inhibition level of more than 71 nm.

5. References
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