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The Effective Killing Percentages of Hela Cells By Gold Nanoparticles and IR Irradiation

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Abstract:

Cancer photothermal therapy (PTT) depends on the size, and shape of nanoparticles, consequently, on the surface plasmon resonance (SPR) peaks of the gold nanoparticles (GNPs) synthesized by pulsed laser ablation (PLA) method in Dulbecco's Modified Eagle Medium (DMED), found to be located between (549-580) nm. The size of GNPs measured the calculation of SPR spectra, dynamic light scattering (DLS) methods and analysis of the transmission electron microscope (TEM) images were found to be ranged between (59-116) nm. The concentration of GNPs in prepared colloidal solution found ranged between (8-16) ppm. Irradiation of GNPs which incubated with Hela cells for 24 h by R light for (15,30, and 45) minutes, enhanced the killing percentage especially for GNPs diameters (50- 70) nm.

Key words: *pulsed laser ablation, cancer cells, gold nanoparticles, photothermal therapy, IR.*

Introduction:

Photothermal therapy is a cancer treatment method which, like chemotherapy, relies on the selective application or the increasing sensitivity of cancer cells to cytotoxic damage in this case from heat, or hyperthermia(1) or is known photothermal imaging (PTI)(2). This new class of therapeutics for cancer treatment is being developed in recent years based on the applications of nanotechnology for selective targeting of cancer cells(3). This phenomenon exploits the interaction of electromagnetic radiation (microwave, radio, infrared, near infrared light and laser or visible radiation) ,as well as ultrasound, and alternating magnetic with nanoparticles (NPs), which produces a heat in NPs(4) and the heat cytotoxic effect(1).

Phototherapy uses specific wavelengths of light to induce selective photodamage on the cancer cells (1, 3, 5, 6). There are two major types of phototherapy (6).

1- Photothermal therapy.
2- Photodynamic therapy (PDT).

For beat on electromagnetic radiations' obstacles and other effects for tissues hyperthermia, the effects of NPs were studied for enhancement the performance of PTT as conjugation agents in ablation and diagnostic imaging (1, 7).

The medical applications of NPs become one of the most important fields in nanotechnology nowadays(8). In 1999, Lin et al reported about PTT by using the absorbed visible light by NPs (9). In 2000 Link and El-Sayed(10) reported that shape and size dependence of irradiative, non-irradiative and photothermal properties of gold nanocrystals. Ivan H. El-Sayed et al. (2006) was first report about GNPs' usage(11) so other types of NPs were used as CuS nanoparticles(12) , and magnetic fluid(13).

Recently, GNPs' applications entered widely to various biomedical fields (immunoassays, biosensors, genomics, photo thermolysis of cancer cells, microorganisms detection and control, targeted drug delivery, optical imaging, monitoring of biological cells and tissues by exploiting resonance scattering, or invivo photo acoustic techniques)(14).

The use of a photothermal agent, such as GNPs or other types of NPs, allows the generated heat to be localized and intensified, confining damage strictly to the area of interest(15).

When the photons were absorbed from NPs, the electronic states excite and back to ground states (7) by constant periodical time, this process known as plasmonic resonance (5), which is a thermal source, the interaction between light and NPs, can occurs in four stages (16, 17): The light is absorbed by the electronic system, creating nascent non-thermal electrons. A thermal equilibrium is reached corresponding to the Fermi distribution as a result of electron–electron scattering

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with cold conduction-band electrons, after a few 100 fs. Subsequently, the electron energy is transferred to the NP lattice via electron–phonon coupling (< a few ps). The lattice energy is later transferred to the surrounding medium through phonon–medium interaction (<several tens to several hundreds of ps) through heating the medium at the expense of the cooling NP. And the final stage is heat diffusion within the medium as NPs

The selection of irradiation modality for GNPs hyperthermia is a balance of three factors:

Sufficient depth penetration to reach the nanoparticle laden cancerous tissue.

Extraneous heating of healthy tissue due to energy absorption by tissue chromospheres.

Off-target nanoparticles and the properties of the chosen therapeutic nanoparticles.

Gold nanospheres are the simplest form of gold nanoparticles. They are easy to fabricate, which is a factor that contribute to their extensive applications(5). It done at the visible region (500-600) nm Practically, GNPs strongly absorbs visible light irradiation(5, 13). In this study, the enhancement of IR radiation with GNPs on the effective killing percentages of Hela cells line was studied.

Materials & methods:

Synthesis and Physical measurements:

Gold nanoparticles were prepared in 10 ml of free Dulbecco's Modified Eagle Medium (DMED) solutions (pH = 7.2-7.4) by pulsed laser (Nd: YAG) on golden disk target (r= 3,and h= 2) mm with purity 99.9999 %, the liquid thickness upon the surface 0.8 mm, the distance between the target surface and the laser source was 15 cm, the frequency of laser pulses was 6 Hz and the laser duration 10 ns.

The GNPs' colloidal solutions prepared with different laser energies (300, 400, 500 and 600) mJ with 250 and 300 pulses for each case (18). The concentration of GNPs ware measured by the atomic absorption spectroscopy (AAS) type (GBC 933 pulse), the UV-Vis photospectoscopy type (SP8001) was used to measure the SPR's peaks to be found located between (549-580) nm, while the size of GNPs were determined by the TEM images, DLS method and the values of the SPR peaks by the Khlebtsov's relation (19).

Biological testing:

The cultural processes

Hela cells were cultured in 96-well microplates in DMEM with 10% Fetal Calf Serum (FBS) to get a monolayer in an incubator at 37oC with 5% CO2. After getting monolayer of cells, culture medium was removed, the cells were washed twice with phosphate buffer saline (PBS), GNPs colloidal and DMEM mixture with volume ratios (0, 25, 50, and 75) % from the listed concentrations in table.1 were added.

To make sure of entering GNPs inside cells, or attaching them, a solution containing an incubated Hela cells with GNPs for 42h was measured by UV-Vis spectrophotometer. The incubated cells with GNPs were removed using (5 µl) of the PBS solution for each well with a weak shaking for two times. T.V solution (1 µl) has been added. The cells are incubated at 37oC for 4 minutes, and shook to spread them from each other. Then, (5 µl) of PBS are added to each well. Consecutively, the cells were incubated for 10 min at 4oC, and 25oC for four times, and then 3 ml of PBS were added.

After the incubating with 37oC with 5% CO2 for 24h, the GNP solutions were removed, and the cells were washed by PBS as previously. The GNPs, effects on the cells was measured with 3-(4, 5-(3-(dimrthylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) (MTT) assay using microtiter plate reader type (HumaReader HS). MTT with incubating for 2.5 h, then MTT was dispossessed, and cells were washed by PBS, dimethyl sulfoxide (CH3)2SO (DMSO) was added, the plate was placed on the shaker for 15 min, then read the remaining MTT in the cells by Microtiter Plate, which gives ratio of the remaining live cells.

Results and discussion:

The relationship between the concentration of GNPs and both the energies and pulses number of laser is linear as shown in figure.1

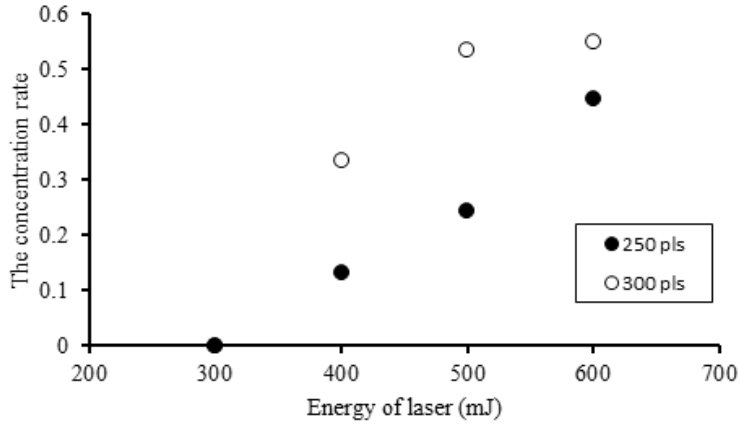

Figure.1: the concentration rate of the prepared GNPs in DMEM by 250 and 300 pulses, with (300, 400, 400, 500, and 600) mJ.

The change of SPR changed with the varying of both energies and pulses number of laser. Shape and high of SPR peaks related with the concentration the aggregation of particles within the surrounding medium (20), as shown in figure.2 and table.1.

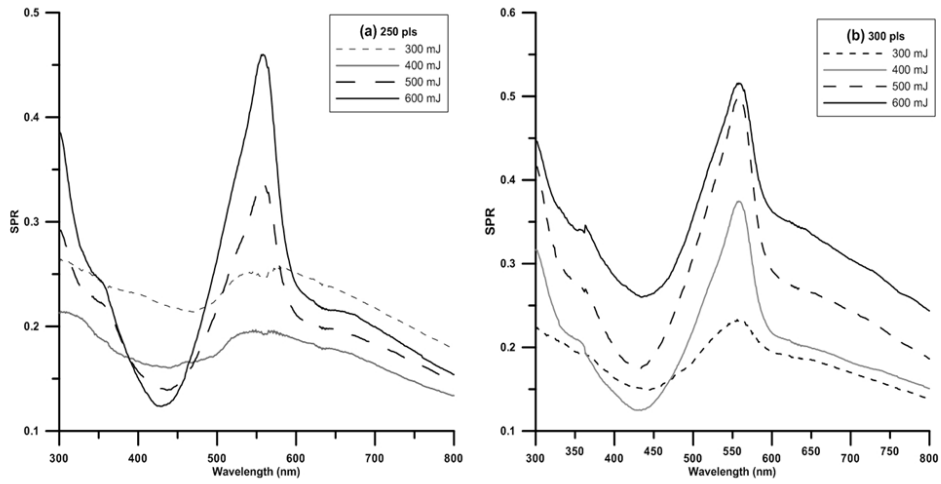

Figure.2: the SPR spectra of the prepared GNPs colloidal liquids by (a) 250 and (b) 300 pulses, and (300, 400, 400, 500, and 600) mJ

Table.1: absorption peaks, Concentration, and size of the prepared GNPs in DMEM

(Energy (mJ	No. of pulses	Peak point	Concentration ((ppm	The Size		
				(by spectra (nm	(by DLS method (nm	(by TEM (nm
300	250	580	8	116	-	-
	300	556	7.33	87	58±٠,٧	59
400	250	549	6.2	72	-	-
	300	568	11	102	103±0.8	88
500	250	558	10.57	92	-	-
	300	560	15.73	93	47±0.06	96.25
600	250	558	14.47	90	-	-
	300	558	16.2	90	135±1.05	71.27

The size of GNPs depends on the number of pulses and energy of the laser, so that; the GNPs' concentration changed the physical properties of the synthesis medium during the synthesis process. Figure.3 represents the TEM images, DLS method of the size distribution of prepared GNPs colloidal liquids using 250, and 300 pulses, power ranged from (300, 400, 400, 500, and 600) mJ.

For fixed laser energy, varying the pulses of the laser leads to varying in the size and number of ablated GNPs. The size dependence on the energy, number of pulses and consequently change in concentration of GNPs will cause a cloud of particles above the target during synthesis process. Hence, the energy of pulse on longer fixed because it will passes through a gaseous cavity (20), in addition, some particles up of the target surface have been obstructed the laser beam as new targets. This leads to decrease the size distribution in the colloidal liquid (21). Aggregation in destabilizes particles electrically in the DMEM solution around the GNPs particle through increasing of the zeta potential -42.31±2.21 in DDDW to -21.04±4.22 in DMEM for diameter of particles 11.25±1.54 nm in DDDW, and 58±0.7 nm in DMEM as the DLS method measurements, which is lead to aggregation in the particles, the red shift in SPR peaks was caused by that, as shown in figure.3. Same results had been reported by Drescher et al (22), and Brewer et al (23). Hence two peaks in SPR spectrum appears in the cases (300 mJ; 250 pulses), and (400 mJ; 300 pulses) as shown in figure.2. The change in the shape of particles as result of aggregation of GNPs has been shown in figure.3.

SPR's spectra of solutions, which have been contained the

incubated Hela with GNPs for 24h. GNPs in (50-70) nm have been shown a highest value of absorption as represented in figure.4. That indicates to a higher concentration of GNPs in the cell or on its cytomembrane. Moreover, all size groups show two absorption peaks in their spectra. First in the visible region, that results by single spherical particles and from the horizontal oscillating of free electrons on the long axis of the ellipse formation. Another peak in the NIR region results by the vertical oscillating that refers to the aggregated cluster formations which are as ellipse forms closed together. The second peak has been demonstrated highest than the first peak. That makes it is more responsive to the photothermal reaction with IR. The cancer cells have epidermal growth factor receptor (EGFR) more than the normal cells (24), this aggregation are as a matrix, which increases the probability of capturing particles via endosome, then, were aggregated in the endocytic vesicle, in the cytosol (13).

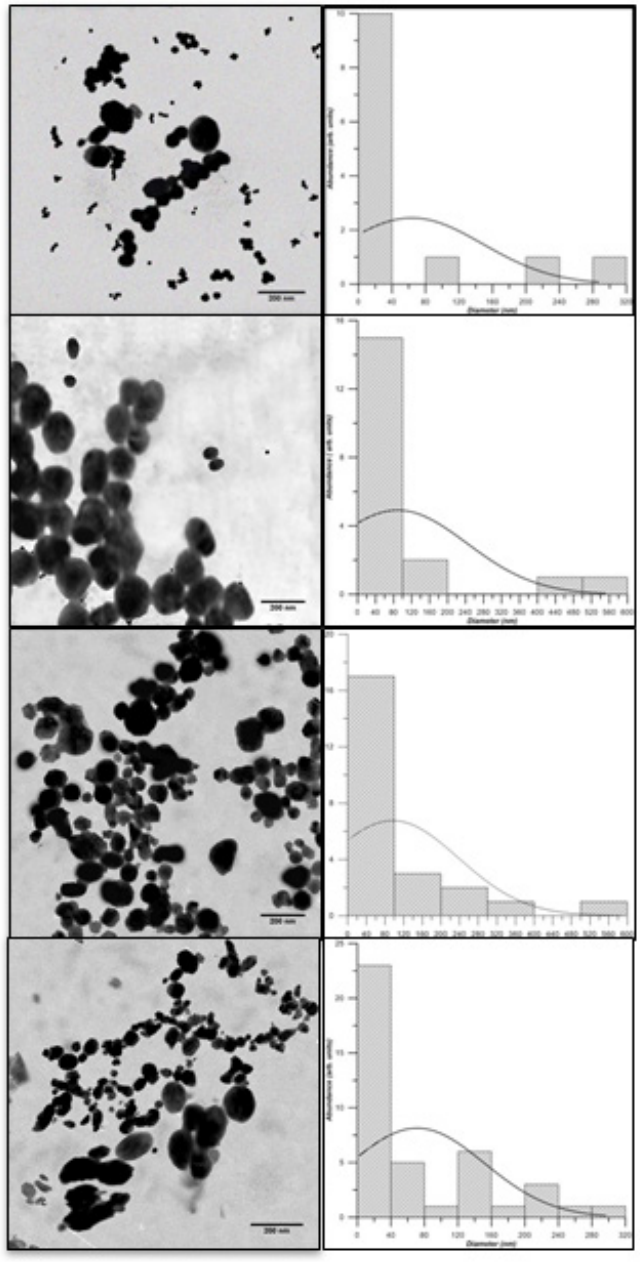

Figure.3: the TEM images and the size distribution of prepared GNPs by deferent laser energy (300, 400, 500 and 600) mJ with 300 pulses in DMEM, scale of images is 200nm.

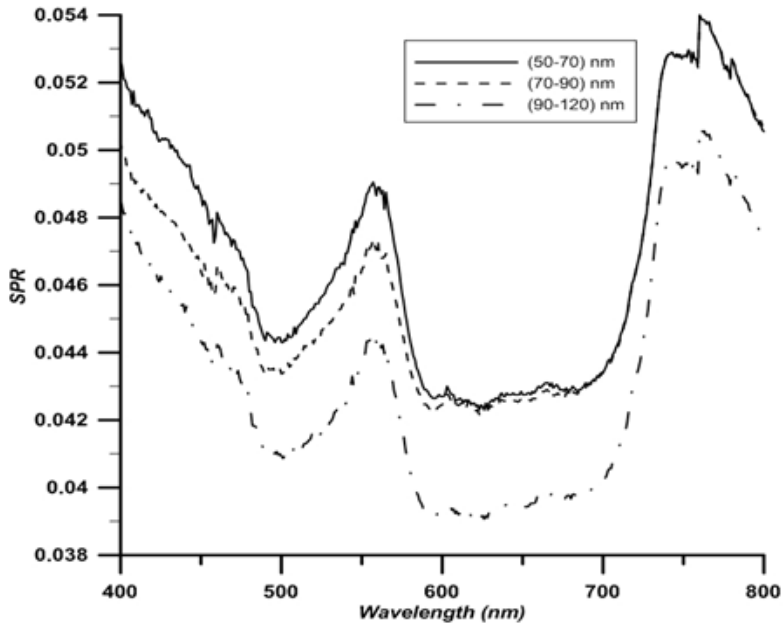

Figure.4: the SPR spectra of the solutions of incubated Hela cells with GNPs in (50-70), (70-90), and (90-120) nm for 24 h.

The statistical values of the active killing percentage of GPNs after incubating it with Hela cells for 24 h are shown in figure .5. With different concentration, GNPs with (50-69) nm diameters have been shown superiority in the cytoxic effect. Highest effect percentage (61.19%) was achieved by (75%) concentration, while in (25and 50) % have been decreased, that can be attributed to the number of GNPs in a matrix on the cytomembrance, because of the decreasing of the particle/cell ratio, thus, the particles that have not able to enter inside of the cell have been decreased.

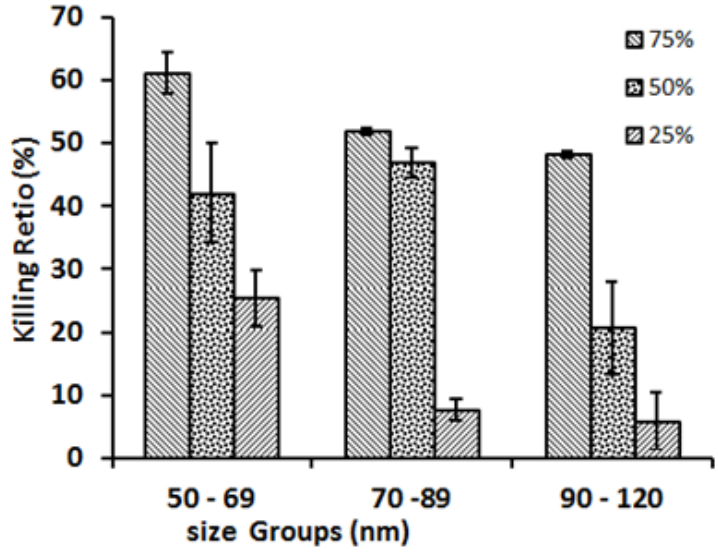

Figure.5: The statistical values of the active killing percentage of GPNs, after incubating it with Hela cells for 24 h

The time effect of IR radiation exposure on the Hela cells with (15, 30, and 45) minutes is represented in figure.6. A linear relation between the killing percentage and the concentration was achieved by the cumulative of effect heat within the cell, and weakness in the cytomembrance of the cell, which lead to mechanically weaken and destroy them. Same interpretation had been given by Kodiha et al (25) Moreover, rising in the killing percentage, may be caused by the thermal inactivation of the cytomembrance, and the mitochondrion(24).

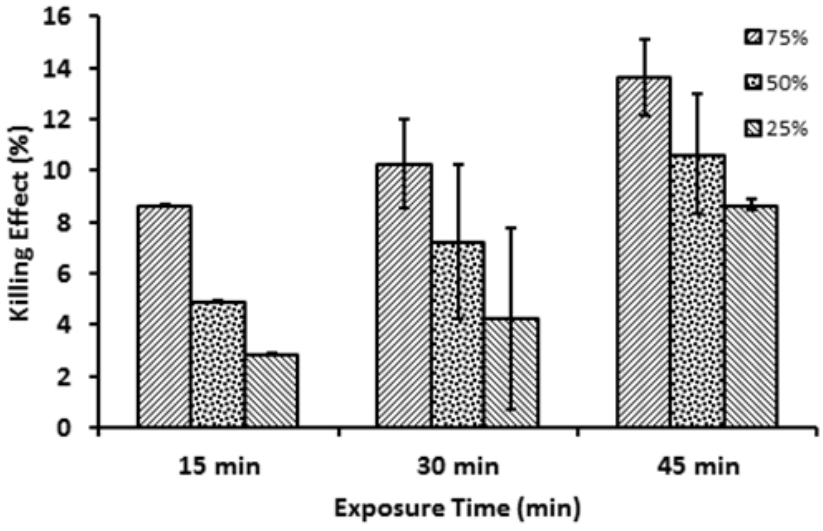

Figure.6: The statistical values of the active killing percentage IR radiation on Hela.

Exposure time with IR has been showing a rise in the effective killing percentage especially for (50-69) nm diameters, as shown in figure.7. The exposure time has not affected on other groups especially with (90-120) nm.

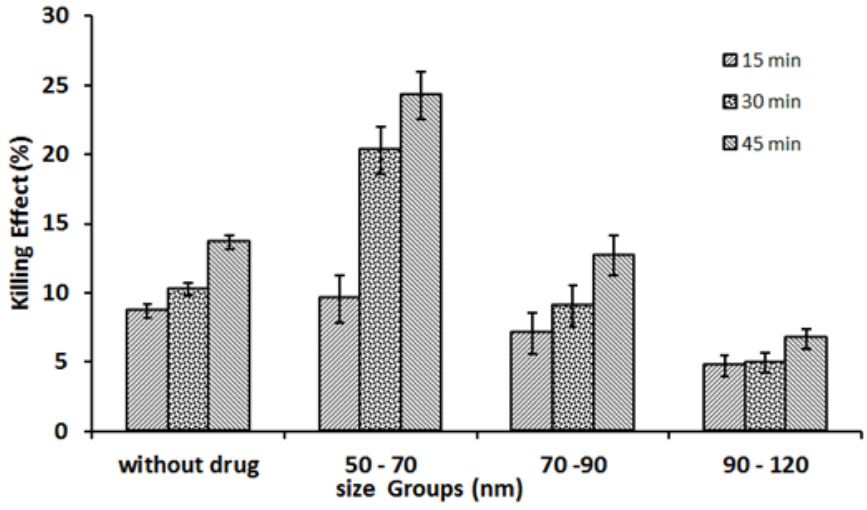

Figure.7: The statistical values of the active killing percentage of GPNs with IR radiation on hela.

Conclusion

Photothermal therapy has become one of the most interesting methods to safely treat of cancer. GNPs ability use as a catalyst with photothermal therapy, which have an ability to apply invivo and exvivo. GNPs have synthesized by many techniques, PLA is one of easier, cheaper, soft and green. The size and concentration of NPs in this method are controlled by many conditions as the laser energy, number of pulses and the nature of the surrounding medium. The synthesis of GNPs in DMEM gives a large, relatively (sometimes over 100 nm). IR exposure that be used in photothermal therapy of Hela cells in exvivo has been produced an acceptable result, but GNPs were used, the results have been increased. Because of GBPs can be coupled with cytomembrance and penetrate the cell. Although, the spherical GNPs have a PSR peaks in the visible region, a red shift achieved when they were synthesized in DMEM. Two peaks have been observed when GNPs penetrated the cell that refers to ellipse formations were found inside the cell. This behavior has given IR an ability to use in the treatment of cancer field for closed area of skin.

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نسب القتل المؤثرة لخلايا Hela باستخدام دقائق الذهب النانوية والتشعيع بالأشعة تحت الحمراء

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الخلاصه:

معالجة السرطان الضوء حرارية (PTT) ترتكز على حجم و شكل الجسيمات النانوية و بناءا على ذلك, على قمم الرنين البلازموني السطحي (SPR) لجسيمات الذهب النانوية (GNPs) المحضرة في الوسط الزرعي (DMEM) بطريقة القلع بالليزر النبضي التي وجد انها تقع بين (548-580)nm. حجم جسيمات الذهب النانوية قيس باستخدام حسابات طيف الرنين السطحي البلازموني و طريقة استطارة الضوء الحركية (DLS) و تحليل صور المجهر الالكتروني النافذ (TEM), وجد انها تتراوح بين (59-116)nm. تركيز جسيمات الذهب النانوية في المحاليل العلائقية تراوح بين (8-16)ppm. تشعيع جسيمات الذهب النانوية المحتضنة مع خلايا Hela لمدة 24 ساعة بواسطة الاشعة تحت الحمراء للمدد الزمنية (45, 30, 15) دقيقة أثر في نسب قتل الخلايا و خصوصا مع الاقطار (50-70)nm.