In Vitro Cancer Cells Therapy with Nano-gold Depending on its Optical Properties

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Abstract

This present study aims to use gold as agent for cancer photo-thermal therapy depending on its optical properties of absorption of light and generation heat due to of local plasmon resonance. In this study we used gold Nano sphere with three different diameters 31 nm, 36 nm and 79 nm which its peak wavelength absorbance at range 532 nm, 535 nm and 546 nm respectively. Then in vitro prepare MDA-MB-231 tumor cell line for exposure to 450nm continuous wavelength laser for 1 minutes with different powers in this procedure MDA-MB-231 cultured divided into 4 groups, group1 incubated with media only and didn't exposure to laser group2 incubated with media only and exposure to laser group 3 incubated with Nano gold and didn't exposure to laser group 4 incubated with Nano gold and exposure to laser. Our results show that it could use Nano gold without cause cytotoxicity to cells and it could use as a good agent for cancer photo-thermal therapy.

Key Words: Gold Nano-sphere, Photo thermal therapy (PPT), Cytotoxicity, Surface Plasmon Resonance (SPR).

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Introduction

According to the World Health Organization (WHO), Cancer is the second leading cause of death globally, accounting for an estimated 10 million deaths, or one in six deaths, in 2020 (*Cancer*, no date). There are many factors that affect the treatment of cancer, like stage level, early detection allow effective treatment also affectivity of drug and method of delivery where effective targeting tumor cells will reduce their effect healthy tissue as well as reduce resistivity of drug etc.,. So for these factors there are wide interest cancer researches one of these branches of research is in nanotechnology research, where the rapid expansion in nanomaterial research increases the future prospect of novel diagnostic methods and treatment of diseases that plague mankind.

Nanotechnology can be defined as manufacture of materials with dimensions in Nano-scale ranging between 1 nm and 100 nm (Liu, Miyoshi and Nakamura, 2007). In these small size of Nano-materials we can obtain unique chemical and physical properties that are differ from their bulk materials (Lanone and Boczkowski, 2006). One of interesting Nano material in cancer research is Nano-gold material, where their increasing interest on their potential as drug delivery, tumor sensors and enhancers in photo-thermal therapy for the destruction of cancers. Apply of Nano gold is getting interesting in these areas of research because it's considered biologically nonreactive and so its suitable for in vivo applications in compared to other materials, the ease in control size and shape of particle during synthesis with easily control their surface chemistry that enables adding functionalized groups on surface and also it's have strong optical properties due to localized surface plasmonic resonance (LSPR), (Lim *et al.*, 2011).

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For gold Nano-sphere, it has a strong absorption in the visible wavelength spectrum when the electromagnetic field frequency is in resonance with the coherent motion of electrons which is known as surface Plasmon resonance absorption (DR, 1983). This surface Plasmon resonance absorption resulting from the dipole oscillations of the free electrons with respect to the gold Nano-spherical ionic core (Link and El-Sayed, 2003). Figure 1 Shows the polarization of the electrons with respect to the Nano gold ionic core as result of a net charges difference shown on the Nano surface when interaction with an electric field and will cause a dipolar oscillation of all the electrons in the same phase.

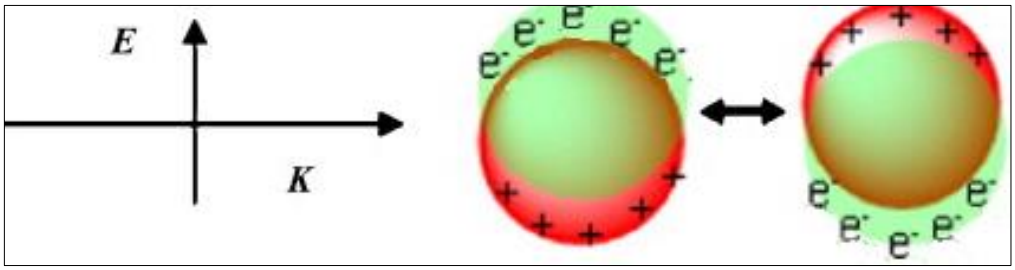

Figure 1. Gold Nano-sphere surface Plasmon absorption show the excitation of the dipole surface Plasmon oscillation (Link and El-Sayed, 2003)

A strong absorption band in visible region of the spectrum is occur when the frequency of the electromagnetic field resonant with the coherent electron motion as shown in figure 2-2, and the result is a brilliant colour of the Nano gold in solution (Huang *et al.*, 2006).

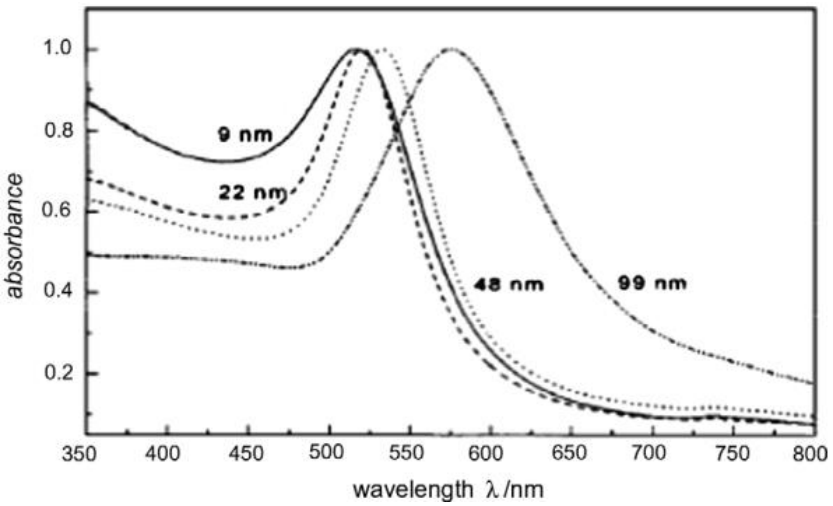

Figure 2. Typical surface Plasmon absorption spectrum of gold Nano-sphere (Huang *et al.*, 2006)

From surface plasmon absorption property of Nano gold we can use it for photo-thermal therapy of cancer, where absorbing light resonate Nano gold and generate local heating use for cancer ablation.

Material and Method

Gold Nano-sphere of different diameter synthesis by chemically according to the method described by by Frens (Frens. P.C Lauterbur, 1973) (Ali and Al-Karam, 2021) and its used directly without further modification in the next procedure, and the synthesised Nano gold characterized by Atomic force microscope to detect its diameter and with UV-visible spectrophotometer to detect its wavelength absorbance and also to calculate Nano gold concentration by using Beer–Lambert low (Navarro and Werts, 2013).

$$A = \varepsilon bc \quad (1)$$

Where A is the absorbance, Ɛ is the molar extinction coefficient (M^{-1} cm^{-1}).
b is the path length of the sample(cm) and c is the concentration of nanoparticles in solution (M).
The extinction coefficient was calculated by Navarro et al equation (Navarro and Werts, 2013).

$$\varepsilon = Ad^{y} \quad (2)$$

Where A = 4.7 * 10^4 M^{-1} cm^{-1}, γ = 3.30 , d ≤ 85 nm
A = 1.6 * 10^8 M-1 cm-1 , γ = 1.47 , d > 85 nm

For preparation Cancer cells for in vitro photo-thermal therapy, the MDA-MB-231 cancer cell line which is previous prepared in laboratory where transferred from falcon flask and cultured in the Microliter plate (96 wells), incubated with Nano gold then exposure to continuous wave laser. The procedure was performed as follow (Mosmann, 1983): first step is seeding, When cells became monolayer in the incubated falcon, the confluent monolayer was trypsinzed incubated for 1 to 2 minutes at 37ºC then examined by light microscope to be sure from getting floating separated cells. After that the floating separated cells were added to the 96 wells microplate by multichannel pipettes and the plate was shaken gently and then each well was feeding with 200 µl media , covered by plate coating and then it was shaken gently and after that the plate was covered by its coating and were incubated in CO2 Incubators at 37ºC for 24-48 hour. Then it was examined by light microscope to check that cells attached to the wells and insure there were no contamination formed. Next step exposure, when we found that cells were in their full activity, media was removed and was replaced by 100µl of new media and 100 µl volume of Nano gold was added to each well of plate and the plate were incubated in CO2 Incubator at 37ºC for 24 hours.

After 24h, diode laser 450nm continuous wave was used , this device work with variable power (0-15)W with power control from(0-100)% and specified by step increment by 10%, Low powers 1.5W (setting 10%), 3W (setting 20%) and 4.5W (setting 30%) was used with exposure 1 minute to each well.

This step of experiment require special environment since that the plate was worked out of incubator within room temperature and properly contamination formation and also need to focus laser on center of each well ,so many wells were use as standard control with three repetition for each sample to reduce mistakes and its prepared as shown in table 1.

Table 1. Preparation of cells in 96 well microplate

Simple	Incubation	Laser exposure
C0	Cells with media only	No
C1	cells incubated with 30nm	No
C2	cells incubated with 36nm	No
C3	cells incubated with 79nm	No
C4	Cells with media only	Yes
S1	cells incubated with 30nm	Yes
S2	cells incubated with 36nm	Yes
S3	cells incubated with 79nm	Yes

Then MTT assay was used for test cells viability, after 24h of exposure the media was removed and then 20 µl of MTT solution was added to each well and the plate was incubated for 4 h at 37°C in CO_2 Incubator, then 200 µl of Dimethyl Sulphoxide (DMSO) was added to each well and then the plate was shaken for 15 minutes in 37°C shaker.

Finally the plate was reading by Eliza reader to determine the optical density of each well and the viability was calculated as follows:

$$Cells\ Viablity = \left[\frac{mean\ of\ treatment}{mean\ of\ control}\right] \times 100 \quad (3)$$

Result and Discussion

The AFM results of gold Nano-sphere diameters are 31.9 nm, 36.19 nm and 79.37 nm, and UV-Visible spectrum show the peak of wavelength absorbance at 532 nm, 535 nm and 546 nm respectively as shown in figure 3, and the result of concentration calculation using equation 1 and equation 2 are shown in table 2.

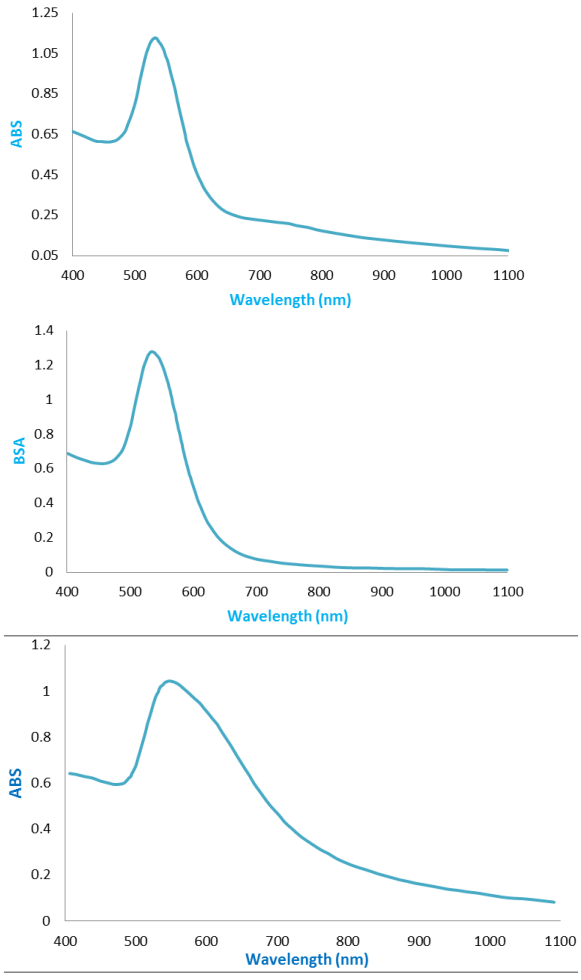

Figure 3. UV-visible spectrophotometer of three samples of gold Nano-sphere

Table 2. Calculated concentration of synthesis Nano-gold

NO	d	A	y	Ɛ	A	b	C
	diameter	constant	constant	molar extinction coefficient	Absorbance	light path	Concentration
	nm			**M-1 cm-1**		**cm**	**nM**
Spherical Sample 1	31.9	4.7*10^4	3.3	4.3*10^9	1.126	1	**0.2611748**
Spherical Sample 2	36.19	4.7*10^4	3.3	6.54*10^9	1.279	1	**0.1956277**
Spherical Sample 3	79.37	4.7*10^4	3.3	87.3*10^9	1.046	1	**0.0119831**

The result cells viability without laser after staining by MTT and read by Eliza are shown in figure 4, this result calculated according to equation 3 where the control samples of Nano gold divided by control without Nano gold and this result are used as control to compare with the result after exposure to laser.

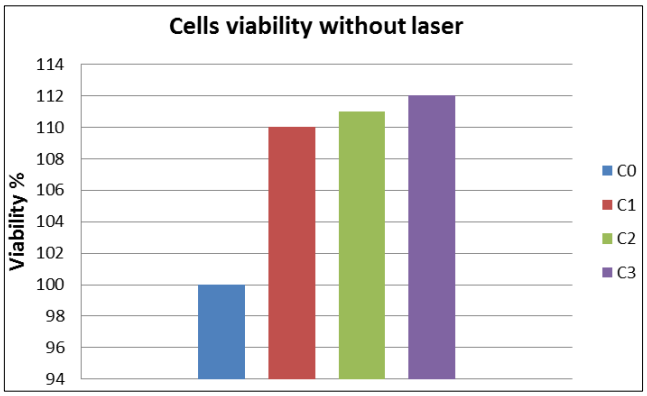

Figure 4. Cells viability without laser

In this result its show the viability slightly increase in cells with Nano-sphere than the cells only. From this inverted result we may can conclude that Nano-sphere provide some heat to cells due to local surface Plasmon resonance of Nano-sphere with the light of room with knowing that the cells ideally need to incubate in 37ºC and our result rum in temperature below this ideal temperature. The cells viability when using laser with output power 1.5 W are shown in figure 5 calculated by equation 3 where mean samples are cells with and without Nano gold after exposure to laser (C5, S1, S2 and S3) and mean control are the cells without exposure to laser (C0) and mean samples are cells with and without Nano gold after exposure to laser.

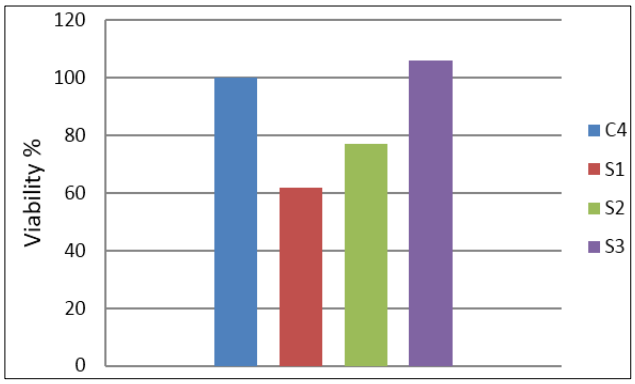

Figure 5. Chart of cell viability with and without Nano-sphere after exposure to 1.5W laser

And if the viability result calculated by use cells with and without Nano gold and without exposure to laser as mean control (C0, C1, C2 and C3) and cells with and without Nano gold and exposure to laser as mean samples (C4, S1, S2 and 3) the result shown in figure 6.

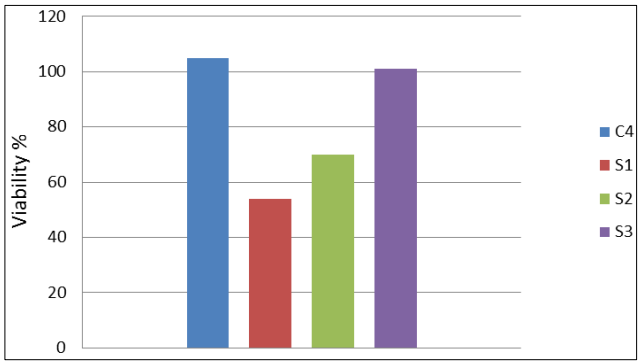

Figure 6. Cell viability with and without Nano-sphere after exposure to 1.5W laser compared with cells with and without Nano-sphere didn't exposure to laser

The result show that there is no thermal damage to cells without Nano-sphere and reduce in viability of cells of samples 1 and 2 and this is due to local surface plasmon resonance of Nano-gold with laser

light and also there is no effect on sample 3 and this is due to miss match between laser wavelength and 79 nm Nano-gold wavelength absorbance that the local surface plasmon resonance is very small effect. When using laser with powers laser it that cause damage to the samples and melting the wells that incubate samples so it cause to read it with Eliza after MTT staining.

Conclusion

The synthesised gold Nano-sphere can targeting cancer cells passively and it's not cause an cytotoxic effect to cells even with increase their diameter, also it can use as an effective photo-thermal agent for cancer therapy due to its optical properties of surface plasmon resonance that generate heat when exposure to specific optical wavelength. Its surface plasmon resonance are in visible range at wavelengths in range of green wavelength so its need to exposure with light wavelength within this wavelength and if there is and mismatch between them no thermal ablation will cause.

References

Ali SM, Al-Karam LQ. Nano Gold Spheres and Rods: Synthesis and Characterization. *In Journal of Physics: Conference Series* 2021; 2114(1).

Cancer.
https://www.who.int/news-room/fact-sheets/detail/cancer

Bohren CF, Huffman DR. *Absorption and scattering of light by small particles.* John Wiley & Sons, 2008.

Frens G. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nature physical science* 1973; 241(105): 20-22.

Huang X, El-Sayed IH, Qian W, El-Sayed MA. Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *Journal of the American Chemical Society* 2006; 128(6): 2115-2120. http://doi.org/10.1021/ja057254a

Lanone S, Boczkowski J. Biomedical applications and potential health risks of nanomaterials: molecular mechanisms. *Current molecular medicine* 2006; 6(6): 651-663. http://doi.org/10.2174/156652406778195026

Lim ZZJ, Li JEJ, Ng CT, Yung LYL, Bay BH. Gold nanoparticles in cancer therapy. *Acta Pharmacologica Sinica* 2011; 32(8): 983-990. http://doi.org/10.1038/aps.2011.82

Link S, El-Sayed MA. Optical properties and ultrafast dynamics of metallic nanocrystals. *Annual review of physical chemistry* 2003; 54(1): 331-366.

Liu Y, Miyoshi H, Nakamura M. Nanomedicine for drug delivery and imaging: a promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. *International journal of cancer* 2007; 120(12): 2527-2537. http://doi.org/10.1002/ijc.22709

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods* 1983; 65(1-2): 55-63. http://doi.org/10.1016/0022-1759(83)90303-4.

Navarro JR, Werts MH. Resonant light scattering spectroscopy of gold, silver and gold–silver alloy nanoparticles and optical detection in microfluidic channels. *Analyst* 2013; 138(2): 583-592. http://doi.org/10.1039/C2AN36135C

Mohammed AA, Alshabander BM, Nasir EM. The role of water absorption on thermal conductivity and mechanical properties for (Recycling hdpe-coal ash) composite. *NeuroQuantology* 2020; 18(4): 11-19.