IMPACT OF PLATINUM NANOPARTICLES AND LASER LIGHTS COMBINATION ON HEP-G2 CANCER CELL LINE

[1*]Sadeq Mohammed Burhan Sahib [1]Neamh Abdulwahid [2]Entidahar Jasim Khamees
1. Dep. of Physics, Faculty of Education for Girls, Uni. of Kufa-Iraq
2. Collage of Medicine, Uni. of Babylon-Iraq

Abstract:
There are many studies on the use of nanoparticles in treated cancer, our work is to treat liver cancer by using platinum nanoparticles in combination with laser lights as an additional method to kill cancer cells by what is known as photodynamic therapy (PDT) based on the Plasmon Resonance Surface (SPR) characteristic of platinum nanoparticles. Three continuous laser dosages were used with wavelengths of 635, 532 and 808 nm. To determine the structural composition of platinum nanoparticles, an X-ray diffraction spectrum was used, Surface Plasmon resonance peaks for platinum nanoparticles were characterized using UV-visible spectroscopy, and the form and scale of the nanoparticles of platinum have been calculated using TEM. The results showed that these particles had a spherical appearance with a diameter of approximately 13 nm. The spectrum showed that the prepared solution contains pure platinum particles with a cubic structure, and this is determined by the boundary tops of the levels (111, 200, 202 and 311) where the spectrum of X-ray diffraction corresponds to the platinum particles. The size of the platinum crystals was also calculated using Scherrer 's equation. Samples have been classified as HEP-G2 cancer cells exposed to platinum nanoparticles and laser irradiation for several minutes (2, 4, 6, 8, 12 and 14 min). The results showed that platinum nanoparticles with laser ablation technology reduced cell viability by 18.8% at a concentration of 2.5 µg / ml. Also, as a result of photodynamic therapy (PDT), the results were obtained when 808 lasers with a capacity of 100 mW were used to irradiate platinum nanoparticles at a concentration of 0.3 µg / ml with a time of 6 minutes, causing cell viability to decline to 18.5 percent.

Keywords: platinum nanoparticles, photodynamic therapy, HEP-G2.

Introduction:

Nanomaterial's having enzymatic activity have the potential to play an essential role in the treatment of many diseases, particularly those concerned with inflammation. Platinum nanoparticle applications (PtNPs) are growing rapidly, as they represent new Nano sized tools for many advanced industries [1, 2] . Platinum nanoparticles are of high importance in many fields especially in medical sciences [3]. In general, the synthesis of NPs is broadly divided into two main classes; Bottom-up approach and top-down approach [1]. Ablative pulsed laser (APL) is an example of a top-down approach. It is an efficient physical technique for nanomaterial synthesis, particularly ablation of solids (including metals, semiconductors, ceramics, and alloys) in liquid environments (pure water or a water solution of a stabilizing agent). This method is much simpler than chemical methods, producing highly purified nanoparticles with weak agglomeration effects [4]. By APL in liquid, synthesis of Pt nanoparticles with various sizes, shapes, and properties is possible [1]. High-purity Pt nanoparticles have been effectively manufactured in pure water by using the APL method utilizing low-power Nd:YAG (1064 nm) laser focused on pure platinum metal. This method produces nanoparticles with approximately zero impurities [5]. It is well known that smaller NPs exhibit greater antibacterial activity than larger NPs. In this regard, Pt NPs are easy to produce in small size (less than 5 nm) making it

powerful antimicrobial agents due to their electrocatalytic properties. PtNPs kill bacteria through the production of reactive oxygen species [6, 7].

Nanoparticles are highly useful in many fields of technological applications, such as therapeutic agents, and in medical fields. In the recent decades it was found that the gold nanoparticles are very useful in treating cancer tumors in vitro and in vivo [8].

The physico-chemical properties of PtNPs apply to a wide range of research applications. Extensive studies have been carried out to establish and develop new PtNP species. Platinum NP applications include electronics, optics, catalysts, and immobilization of enzymes [9].

Platinum NPs are used as catalysts for the exchange of proton membrane fuel cells (PEMFCs), for industrial nitric acid synthesis, for the reduction of vehicle exhaust gasses and as catalytic nucleating agents for the synthesis of magnetic NPs. NPs can act as catalysts in homogeneous colloidal solution or as gas-phase catalysts while supported by solid-state materials. The catalytic activity of the NP depends on the particle's structure, shape and size [10, 11, 12]. Colloidal PtNP is one investigated PtNP type. As a catalyst, Monometallic and bimetallic colloids have been used in a wide variety of organic chemistries, including carbon monoxide oxidation in aqueous solutions, organic and biphasic alkene hydrogenation and organic olefin hydrosilation. Poly-protected colloidal platinum NPs (N-isopropylacrylamide) have been synthesized and the catalytic properties would be measured. When phases were isolated, the solution became more active and inactive because their solubility became inversely proportional to the temperature [9].

Platinum NP has very interesting visual characteristics. In order for the charged particle metal NP to be the same as gold and silver, its frequency-dependent response is mainly regulated by Plasmon 's surface resonance. Photoluminescence occurs when other electrons appear to be exposed to electrical waves on the surface of the metal, which force them to move away from their initial position [13].

Physical experiments have shown that laser light ablation is an effective and efficient analytical tool for high-purity nanomaterials or nanofibers. Generally, the targets used for the synthesis of laser radiation films or nanoparticles appear in bulk and the laser beams are either excimer, Yttrium Aluminum Grenet (YAG) continuous wave or photon lasers. [14]. The introduction in laser fragmentation in liquid has become a well-established technique to produce nanoparticles in 1987 [15]. Limulus amebocyte lysate (LAL) has recently been developed. The aim of developing a variety of nanomaterials with different morphologies, microstructures, phases, and single-stage construction of different functional nanostructures is to develop new application potential in the fields of optics, display, detection, and biology [16]. Laser liquid / solid removal is an effective way of producing a wide range of metallic nano-spheres (NSs) and nanoparticles (NPs). Under sufficiently high laser fluence the surface of the target melts, and the melt is subsequently dispersed into the surrounding liquid under the recoil pressure of its vapor. On the other hand, on a particular surface, only micro-protrusions melt in fluids near the melting threshold with quick laser pulses of less than one nanosecond. These nanoseconds are self-assembled because their time is several orders less than the laser-spot size of 50 to 300 nm, based on the target substance. [17].

The basic of Bio-photonics is the interaction of light with biological matter. This interaction involves the absorption of laser energy by tissue molecules. Photon absorption can only occur if the difference between the two quantified molecular energy conditions is equal to photon energy. [18]. In order to assess their quantitative relationship, the physical properties of the living tissue and the laser beam should be interlinked. The degree of contact as well as the context of the material depends on the properties of the tissue, such as its framework, water holding capacity,

blood flow, accumulation, diffusion, reflective capacity, thermal conductivity, thermal capacity and density [19]. Laser beam criteria include: output power, thermodynamic efficiency, spectra, laser tissue irradiation intensity (interaction time) and laser surface radiation or interaction volume. (effective power density).

The location or origin of tumors, histology or cell analysis and the extent of the disease are indeed the causes of cancer classification. Cancer develops by altering the genetic structure of the cell. This change (mutation) results in uncontrolled growth pattern cells. The major categories of cancer, depending on the embryonic origin of the tissue from which it is derived, are carcinoma (such as breasts, lungs, colon, prostate and bladder) sarcoma (developing bones), leukemia (related to WBCs and bone marrow), lymphoma (related to glands and lymph nodes).

Cell lines are cells that can replicate in vitro and are therefore sustained for longer periods by the serial subculture. This can be divided into cell lines, cell lines and lines of the stem cells. Cell lines are ideal for mechanical experiments because they are simpler than the entire organism. It is easy to isolate certain chemical and compound effects in cell models for certain tissues compared to complete systems with far too numerous control variables for animals and humans. The downside of cell models is that substances in a full living system might not be the same in the absence of hormones, inhibitors and other hormonal factors, and that cell research does not apply directly to humans.

Many researchers have been synthesis a PtNPs in different protocols and studied it properties (e.g. 4, 6, 20) and some of them Synthesized NPs by using energy of lasr (21)

Nanoparticles are used in different applications such as antibacterial, anticancer, and drug delivery transporter, Asharani et.al.(22) Tony used platinum-based cancer therapy when Pt-NPs entered the cells through diffusion, located inside the cytoplasm, and found that Pt-NP would increase cell damage, S-cycle aggregation, and cell death. Helge Gehrke et.al.(23) used Platinum nanoparticles to treated HT29 carcinoma (human colon carcinoma) and they found that PtNPs could cuses DNA strand. This study comes to test the effect of PtNPs, which prepared by LALP technique, and Laser irradiations combination on HEP-G2 cancer cell line:

Material and Methods

In this research, HEP-G2 lines in frozen vials were collected from the Tissue Culture Laboratory at the College of Medicine / University of Babylon (Hepatocellular carcinoma, 15-year-old adolescent boy). The solution of 40 mg / ml of gentamicin was considered to be a stock solution and prepared for use at 4 C˚. The working concentration of gentamycin in the medium is 50 µg/ml (25). A litre of culture medium with sodium bicarbonate 2.2 g was used. Phosphate Buffer Saline (PBS) pH 7.2 was prepared according to Gibco manufacturer manual by dissolving one tablet of PBS in 500 ml deionized distilled water (DDW) with stirring constantly on a magnetic stirrer at room temperature, the pH will be 7.45 and requires no adjustment. Sterilization was done by autoclaving and Hold sterile in a sealed container until used. Fetal Bovine Serum (FBS) has been heat-inactivated, sterile, and added directly to the sterile growth medium. The liquid RPMI-1640 medium was formulated in the Gibco product manual of the powdered RPMI-1640 medium. Cultural identity conditions were optimized: 95 percent air; 5 percent carbon dioxide (CO2) and 37 ° C temperature. The weight of 10.1 gm trypsin-EDTA powder was dissolved in 900 ml DDW in compliance with the Biological Instructions and continuously mixed at room temperature The pH of the medium was set to 7.2 and the volume was finished in a liter. Subsequently, the solution was sterilized using 0.45 and 0.22 µm millipore philters with filtration. The contents have been preserved at-20 ° C).

By using the culture media, two-fold serial dilutions of Platinum-Nano (P.N) from stock solution (50 ppm) was prepared and six concentrations starting from 10 ppm, 5, 2.5, 1.25, 0.6 ,0.3 ppm were used in the present study.

One cell line in the preserved vial was cultivated in a 25 ml specimen culture containing 10% FBS and antibiotics in the growth medium, incubated at 37C as mono-layer. Then the proteolytic trypsin was extracted by us. The crystal violet (CV) method was conducted to test the cell growth optical density of each microtiter plate well that used an ELISA reader [24]. The percentage of inhibition was calculated according to the following equation [25].

$In\% = (OD_A - OD_B/OD_A) \times 100$

In% is Inhibition%,

OD_A : is control optical density,

OD_B : is a test optical density.

According to Freshney (26), the cytotoxicity assays were applied for determination of the effect of serial dillutions of Platnum nano particles on the cell lines (Hep-G2). Six serial dilutions of 10 ppm with a PtN end of 0.3 were tested for 24 hours. Once the growth was as a monolayer before the exponential cycle, the cell monolayer was harvested and resuspended at a concentration of 5X 105 cells / ml with a growth medium and seeded in a 96-well micro-titter plate. As cell growth exceeds 80 per cent, PtN serial dilutions were observed in the wells. The experiment was done on each cell line separately on a microtiter-plates. The result was read by the Elisa reader as optical density by crystal violet cytotoxicity assay.

Three replicates of 96-well plates were seeded with HEP-G2 cells in a concentration of 5*10 and the wells of each plates (except one column from each plates which left without treatment as a control), were exposed to a combination of PtNPs with six serial dilutions (10, 5,2.5,1.25, 0.6 and 0. 3 ppm) and laser with the following parameters : Red laser, λ= 635 nm, laser power 300 mW for 4 minutes for each well. Then the plates were covered with the plastic lid and the first plate incubated for 24 hours. Then the plates washed with 200 µl of a sterile PBS and the effect of the combination on HEP-G2 cell line growth was assessed by C.V cytotoxicity assay in all the experiments. UV-visible Spectroscopy and the lasers source of heating were used in this work.

For statistical analysis, it was done using the Sigma plot version 12 software. Results were expressed as mean± SD with P-value. One way ANOVA was used to compare parameters in different studies groups. P-values ($p \leq 0.001$, $p \leq 0.05$) were considered statistically significant.

Results and Discussion

The characteristics of the colloidal PtNPs, which were prepared by LALP method strongly depend on many parameters. The reduction of colloidal ions formation was confirmed by UV–visible spectrophotometric analysis. A single peak maximum at 295 nm corresponds to the surface Plasmon resonance of keratin nanoparticles was observed in the UV–visible spectrum in the solution (Fig. 1). The size measurements were achieved by the calculation from absorption spectra information of the transmission electron microscope (TEM). Mean particle size and distribution is shown in figure (2). From the shape, it is deduced that smaller grain size of PtNPs was obtained when we used higher pulse number at a wavelength of (1064) nm, pulse energy of 300 mJ with total pulse number of (100) pulse. The laser ablation at the wavelength (1064) nm efficiently produces small granules with a size average of 13 nm. (state the fig. 2-A and B). The diffraction pattern of the X-ray diffraction (Fig.3) shows the division peaks of the nanoparticles between 38.6, 44.4, 64.1 and 77.1540 ° which may be assigned to the diffractions of (111), (200) and (202) planes. The crystalline size was calculated using the Scherer test. The cubic face of the

center (FCC) was the crystal structure of Platinum. All peaks occur abroad, suggesting the development of NPs, which results in comparison to the standard values of card No (04-0802).
In figure 4, a six different concentrations was used (0.3, 0.6, 1.2, 2.5, 5, 10 µg/ml), a size of 10 nm of platinum nanoparticles and lasers (635nm and output power 300mw). From the figure, it can be shown that the concentration 5 µg/ml has a great effect of killing the HEP-G2 cell, a 78.2% was killed (the percentage of cells survivals is 21.8%). In figure 5, a six different concentrations was used (0.3, 0.6, 1.2, 2.5, 5, 10) µg/ml, a size of 10 nm of platinum nanoparticles and lasers (808nm and output power 100mw). From the figure, it can be shown that the concentration 0.3 µg/ml has a great effect of killing the HEP-G2 cell, an 81.5% was killed (the percentage of cells survivals is 18.5%).

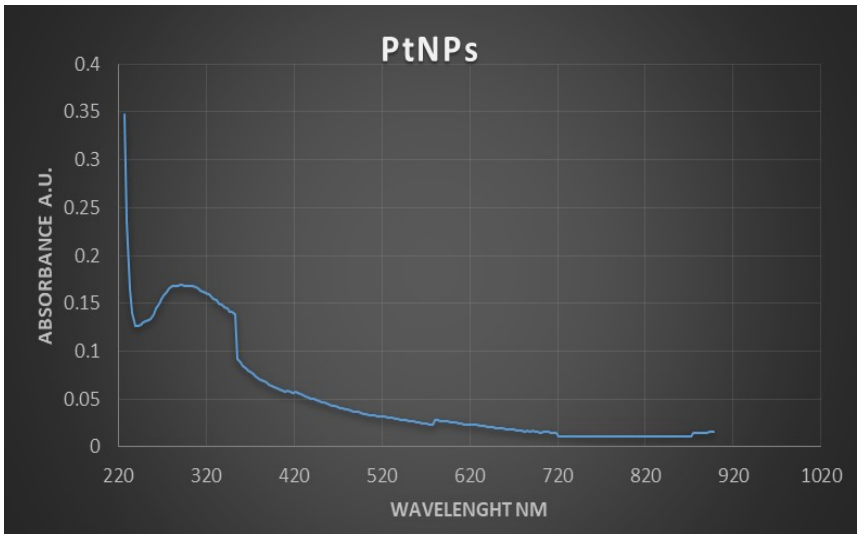

Fig 1: SPR Spectra of PtNPs in DDW

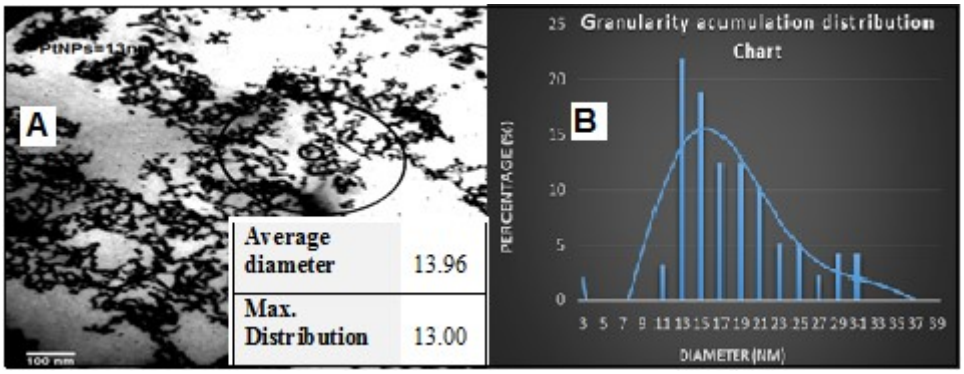

Fig.2: TEM image with ×92000 magnification power and the granulate accumulation distribution chart for PtNps.

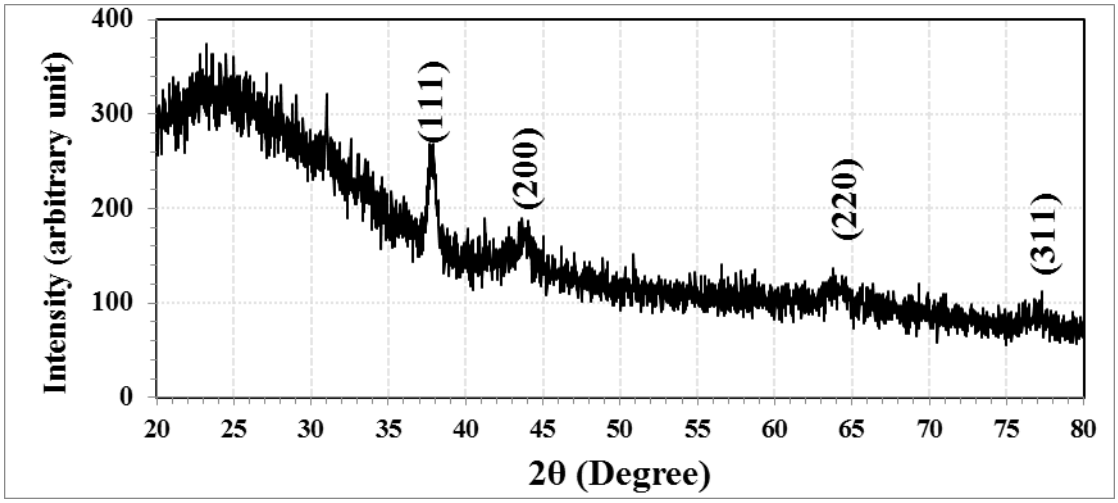

Fig.3: X- Ray diffraction patterns of ptnps prepared by laser ablation technique

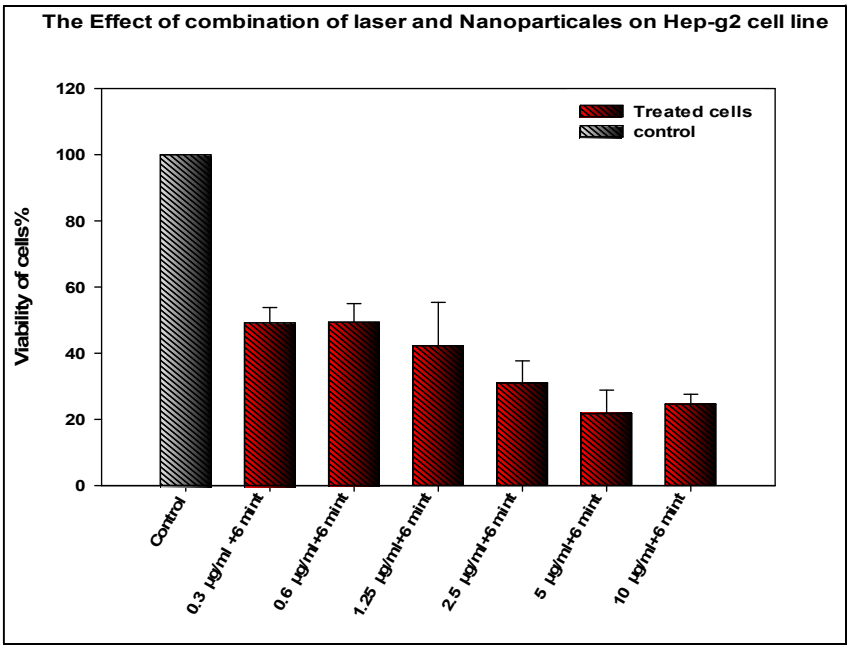

Fig. 4: The Effect Of Combination Of Laser And PtNps On Hep-G2 Cell Line.

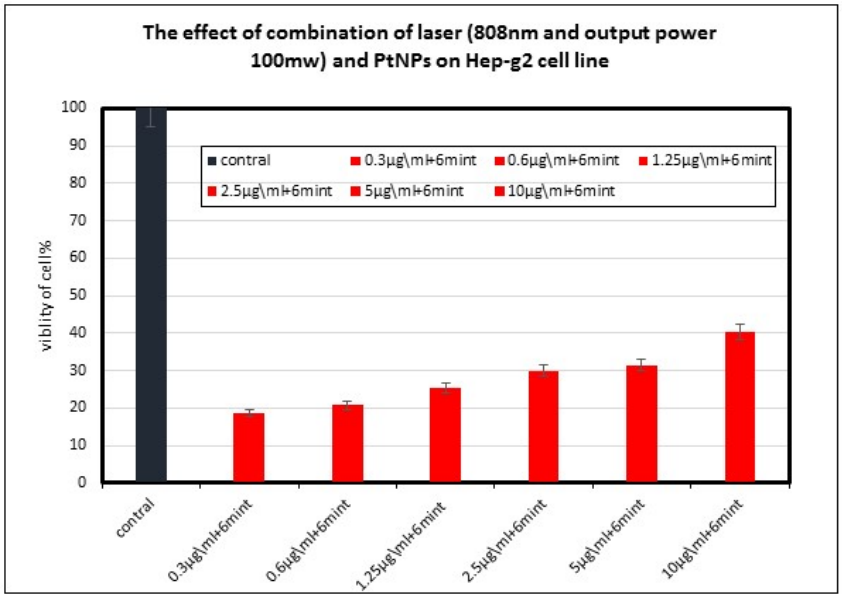

Fig. 3.9 The Effect Of Combination Of Laser (808 nm And Output Power 100 w) and PtNps On Hep-G2 Cell Line.

Conclusion

This study concluded that the high purity, spherical Platinum nanoparticles (PtNPs) with a size of about 10 nm were prepared in double-distilled water using laser ablation technique. Photodynamic effects using Platinum nanoparticles (PtNPs) and 635 nm laser light (300 and 250 mW) showed a decrease in the vitality of the cells to (80.56 and 60 .6 %) respectively.Photodynamic effects using Platinum nanoparticles (PtNPs) and 532 nm laser light (240 mW) showed a decrease in the vitality of the cells to 23.06%.

Acknowledgement

The authors would like to thank the Department of Physics, Faculty of Girls Education, University of Kufa to fund this work. We would also like to thank the University of Babylon for supporting this project.

References

1. Palma MIM, Krishnan B, Castillo GA, Rodriguez, Roy TK Das, Avellaneda DA, et al. Synthesis and Properties of Platinum Nanoparticles by Pulsed Laser Ablation in Liquid. J Nanomater. 2016;43(5):3015–34.
2. Cueto M, Sanz M, Oujja M, Gámez F, Martínez-Haya B, Castillejo M. Platinum nanoparticles prepared by laser ablation in aqueous solutions: Fabrication and application to laser desorption ionization. J Phys Chem C. 2011;115(45):22217–24.

3. Pedone D, Moglianetti M, De Luca E, Bardi G, Pompa PP. Platinum nanoparticles in nanobiomedicine. Chem Soc Rev. 2017;46(16):4951–75. Available from: http://dx.doi.org/10.1039/c7cs00152e
4. Zeng, H., Du, X.-W., S. C. Singh, S. A. Kulinich, S. Yang, J. He and W. Cai, "Nanomaterials via Laser Ablation/Irradiation in Liquid: AReview", Adv. Funct. Mater. J., vol. 22 (7),pp. 1333-53, 2012.
5. Nichols, W.T.; Sasaki, T.; Koshizaki, N. Laser ablation of a platinum target in water. III. Laser-induced reactions. J. Appl. Phys. 2006, 100, 114911.
6. Stepanov, A., A. Golubev, and S. Nikitin, Synthesis and Applications of Platinum Nanoparticles: A Review. 2013. p. 173-199
7. Hashimoto M, Yanagiuchi H, Kitagawa H, Honda Y. Inhibitory effect of platinum nanoparticles on biofilm formation of oral bacteria. Nano Biomed. 2018;9(2):77–82.
8. Mazin Kamil Hamid, "The Effect of Some Physical Parameters of Gold Nanoparticles Produced by Laser Ablation on Antibacterial Effect", Ph.D. thesis, Al-Nahrain University, 2014.
9. Chen, Chun-Wei; Akashi, Mitsuru (November 1997). "Synthesis, Characterization, and Catalytic Properties of Colloidal Platinum Nanoparticles Protected by Poly(N-isopropylacrylamide)". Langmuir. 13 (24): 6465–6472. doi:10.1021/la970634s.
10. Arayanan, R., El-Sayed, M.A. "Shape-Dependent Catalytic Activity of Platinum Nanoparticles in Colloidal Solution". Nano Letters. 4 (7): 1343–1348. Bibcode: 2004. NanoL...4.1343N. doi:10.1021/nl0495256
11. Chaturvedi, S., Dave, P., Shah., N. "Applications of Nano-catalyst in new era." , Journal of Saudi Chemical Society, Vol.16, pp. 307–325, 2012.
12. Marie-Christine Daniel, Didier Astruc, "Gold Nanoparticles Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology", Chem. Rev., Vol. 104, pp. 293-346, 2004
13. Jain, Prashant K; Huang, Xiaohua; El-Sayed, Ivan H; El-Sayed, Mostafa A (2007). "Review of Some Interesting Surface Plasmon Resonance-enhanced Properties of Noble Metal Nanoparticles and Their Applications to Biosystems". Plasmonics. 2 (3): 107. doi:10.1007/s11468-007-9031-1.
14. Stratakis, E., Zorba, V., Barberoglou, M., Fotakis, C., Shafeev, G.A. "Femtosecond laser writing of nanostructures on bulk Al via its ablation in air and liquids", J. of App. Surface Sci., (255),pp. 5346-50, 2009.
15. Alnassar, S., Akman, I.E., Oztoprak, B., Kacar, G., Gundogdu, E.O., Khaleel, A., Demir, A. "Study of The Fragmentation Phenomena of Tio2 Nanoparticles Produced by Femtosecond Laser Ablation in Aqueous Media", J. of Optics & Laser Technology, vol. 51 pp. 17-23, 2013.
16. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arab J Chem. 2017; Available from: http://dx.doi.org/10.1016/j.arabjc.2017.05.011.
17. Zhang, Nan; Han, Chuang; Xu, Yi-Jun; Foley Iv, Jonathan J; Zhang, Dongtang; Codrington, Jason; Gray, Stephen K; Sun, Yugang (2016). "Near-field dielectric scattering promotes optical absorption by platinum nanoparticles". Nature Photonics. 10 (7): 473. Bibcode:2016NaPho..10..473Z. doi:10.1038/nphoton.2016.76.
18. Prasad P. N., "Introduction to biophotonics", book, Edited by .73 John Wiley & Sons, Inc, 2003.

19. Ludwik Pokora. Practical aspects laser in physics and biology, . lasers in medicine surgery and dentistry; part III, Ch. III Centre of Laser Technology in Warsaw, Poland, 2003.
20. Jeyaraj, M., et al., A comprehensive review on the synthesis, characterization, and biomedical application of platinum nanoparticles. Nanomaterials, 2019. 9(12): p. 1719.
21. Kurniawan, G. and A. Khumaeni. Synthesis of iron oxide nanoparticle using pulsed laser ablation method at low laser energy. in AIP Conference Proceedings. 2018. AIP Publishing LLC.
22. Asharani, P., et al., DNA damage and p53-mediated growth arrest in human cells treated with platinum nanoparticles. Nanomedicine, 2010. 5(1): p. 51-64.
23. Gehrke, H., et al., Platinum nanoparticles and their cellular uptake and DNA platination at non-cytotoxic concentrations. Archives of toxicology, 2011. 85(7): p. 799-812.
24. Freshney, R.I. “Culture of Animal Cells: A manual of Basic . Edition, Wiley: New th Technique and Specialized Applications,” 6 York, 2010.
25. Cherbas, L., Gong, L. "cell lines", Methods, 2014.
26. Freshney, I.R. "Basic Principles of Cell Culture", Culture of Cells . for Tissue Engineering, 2006.