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RESEARCH PAPER

Antimicrobial and Cytotoxic Activity of Platinum Nanoparticles Synthesized by Laser Ablation Technique

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ABSTRACT

An ablative pulsed laser is an efficient physical technique for nanomaterial synthesis, particularly ablation of solids in liquid environments. This method is much simpler than chemical methods and produces highly purified nanoparticles with weak agglomeration effects. This study aimed to fabricate new nanoparticles with unique biological activity. Platinum nanoparticles (PtNPs) were prepared striking platinum plate with Nd- YAG (1064 nm) laser pulses in double deionized water (DDW) for a total number of pulses of (100 and 150). NPs samples were characterized using a Transmission Electron Microscope (TEM) and UV-Visible, double beam spectrophotometer. To evaluate the biological activity, three types of pathogenic microorganisms (Pseudomonas aeruginosa, staphylococcus aureus, E. coli, and Candida albicans) and two cell lines (Hepa 1-6) hepatoma and MDCK cells were used. High-purity platinum nanoparticles (PtNPs) with two particle sizes (10 and 20 nm) have been successfully synthesized. The antimicrobial assay showed high anti-pseudomonas activity of these nanoparticles while it showed no effects on other organisms. PtNPs with a particle size of 10 nm showed higher toxicity than PtNPs with a particle size of (20 nm) at the same concentrations used. MTT assay showed that PtNPs have high cytotoxic effects on carcinoma cell lines at low concentrations. As a conclusion, PtNPs showed promising selective antibacterial activity against P. aeruginosa as well as an inhibitory effect on the cancer cell line. These nanoparticles can be used to treat complicated pseudomonas infections.

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INTRODUCTION

Biocompatible nanomaterials 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 nanosized tools for many advanced industries [1][2][3]. Platinum nanoparticles are of high importance in many fields especially in medical sciences [4].

There are two broad classification of the methods used to fabricate NPs; 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

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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 [5]. By APL in liquid, synthesis of Pt nanoparticles with various sizes, shapes, and properties is possible [6].

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 [4].

It is well known that smaller NPs exhibit greater antibacterial activity than larger NPs. In this regard, Platinum NPs are easy to produce in small size (less than 5 nm) making it powerful antimicrobial agents due to their electrocatalytic properties. Pt NPs kill bacteria through the production of reactive oxygen species [7].

Antibiotic resistance is rapidly emerging and spread by horizontal transfer among bacteria [8]. Metals NPs have been widely investigated to fight against Antibiotics resistant bacteria [9][10]

In this study, we tried to investigate the antimicrobial activity of platinum nanoparticles synthesized by pulsed laser ablation technique.

MATERIALS AND METHODS

Preparation of Platinum nanoparticles

Platinum nanoparticles (PtNPs) were prepared using the laser ablation technique. In this method, a piece of high purity (99.999 %) platinum plate was washed with acetone and double deionized water (DDW) in an ultrasonic water bath before using in the preparation of Pt colloidal suspension then immersed in Double Distilled (DDW) and attacked by a pulsed Nd-YAG laser (1064 nm) with a beam diameter of 2.5 mm, pulse duration of 10 nm, and pulse repetition rate of 6 Hz. The total numbers of pulses were (100 and 150).

NPs samples were characterized using Transmission Electron Microscope (TEM) (Model: Hitachi H-7650, Germany), absorption spectra were measured by UV-Visible, double beam spectrophotometer (SP8001, Italy).

Microorganisms

Three types of pathogenic bacteria were used in this study (*Pseudomonas aeruginosa, Staphylococcus aureus*, and *E. coli*) in addition to one opportunistic yeast (*Candida albicans*). *P. aeruginosa* isolated from burn and cultivated on the selective medium (ceramide agar) (Liofilchem, Italy) then transferred into brain heart infusion agar before cytotoxicity testing. *S. aureus* was isolated from the wound and cultivated on mannitol salt agar before transferring to BHI agar (Himedia, India). *E. coli* obtained from a patient suffering from diarrhoea and cultivated on EMB agar (Himedia, India). Candida albicans isolated from vaginal infection and streaked on Sabouraud's dextrose agar (Himedia, India).

Cell line

Hepa 1-6 (Mouse hepatoma) and MDCK cell lines were kindly obtained from (Cancer Research Lab./ College of Medicine/ University of Babylon). Cells were cultured in DMEM supplemented with 5% Fetal Bovine Serum and 1% (penicillin/ streptomycin).

Antimicrobial activity assay

Antimicrobial activity of PtNPs was assisted using the well diffusing method according to Balouiri *et al* [11]. Muller-Hinton agar plates were prepared and incubated overnight before using for detecting the antimicrobial activity. Wells of 0.6 cm diameter were made on the agar plates using sterile gel puncture before culturing. The prepared dilutions of the bacteria and yeast were spread on the plates (100µl). A volume of 50 µl of PtNPs suspension was dispensed into each well using a micropipette. Three concentrations were used (10,20, and 50 µg/ml) in each plate. For evaluating the antifungal activity, the same procedure is used but on Sabouraud's dextrose agar. The plates were incubated at 37C° for 24 h. After incubation, the presence of a growth inhibition zone around the sample loaded well was observed and measured using a measuring scale.

Cytotoxicity assay

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was conducted to evaluate the cytotoxicity of PtNPs on cell lines. The procedure was carried out according to Martin *et al.*[12].

Statistical analysis

All data shown in the results section were stated as the mean ± SD. The data were analysed using a one-way analysis of variance (ANOVA) using sigma plot v12 software.

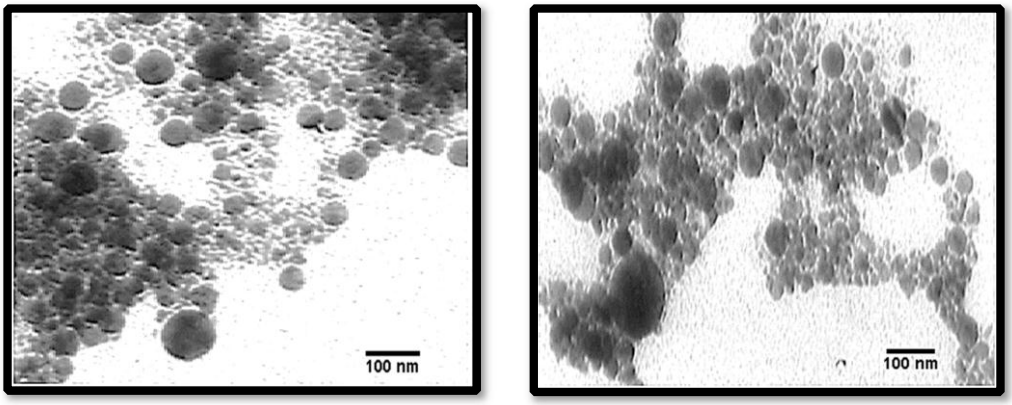

Fig. 1. TEM of prepared platinum NPs by (100,150) pulses in DDW. Larger particles (left) are produced with 100 pulses, smaller NPs (right) produced by increasing total pulses to 150.

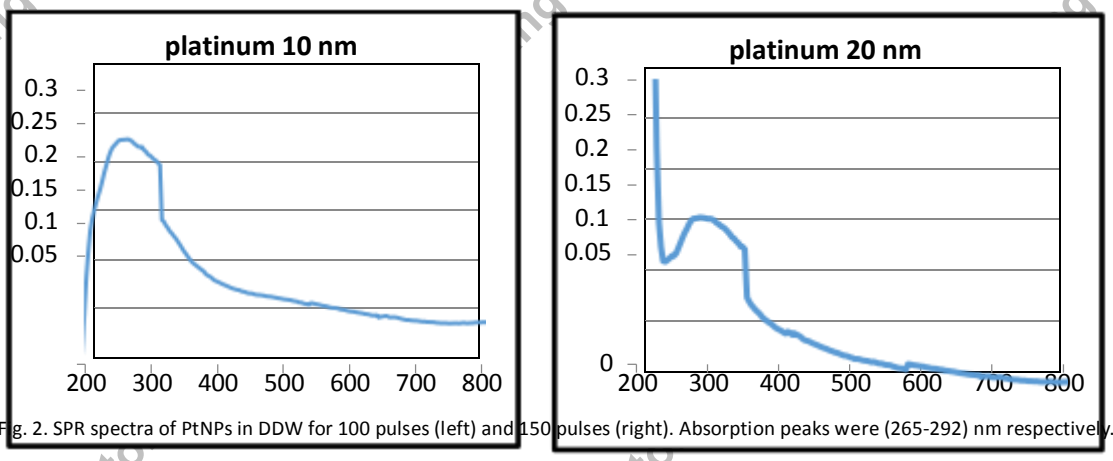

Fig. 2. SPR spectra of PtNPs in DDW for 100 pulses (left) and 150 pulses (right). Absorption peaks were (265-292) nm respectively.

RESULTS AND DISCUSSION

Nanoparticles characterization

Mean particle size and distribution is shown in Fig. 1. 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 or150) pulses. The laser ablation at the wavelength (1064) nm efficiently produces small granules with a size average of (20 and 10 nm).

Surface Plasmon resonance

Fig. 2 shows the surface plasmon resonance (SPR) of PtNPs in DDW. SRP were measured at room temperature, the peaks of absorption were found at (265-292) nm for both PtNPs in DDW. The smaller nanoparticles show a higher amplitude than larger particles.

Antimicrobial activity of PtNPs

Fig. 3 and Table 1 show the antibacterial activity of PtNPs. These NPs showed no toxicity against *E. coli* and *S. aureus* but it showed significant toxicity toward *P. aeruginosa*. Smaller particles show more toxicity.

Fig. 4 and Table 1. show the effect of PtNPs on *candida albicans* growth. There were no inhibitory effects seen for both PtNPs sizes at different concentrations**.**

Cytotoxic effect of PtNPs on Hepa cell line (cancer cells) and MDCK normal cell line are shown in Fig. 5 and Fig. 6 respectively. Both 10 and 20 nm PtNPs showed sever cytotoxicity but 10 nm nanoparticles were the more toxic than 20 nm particles.

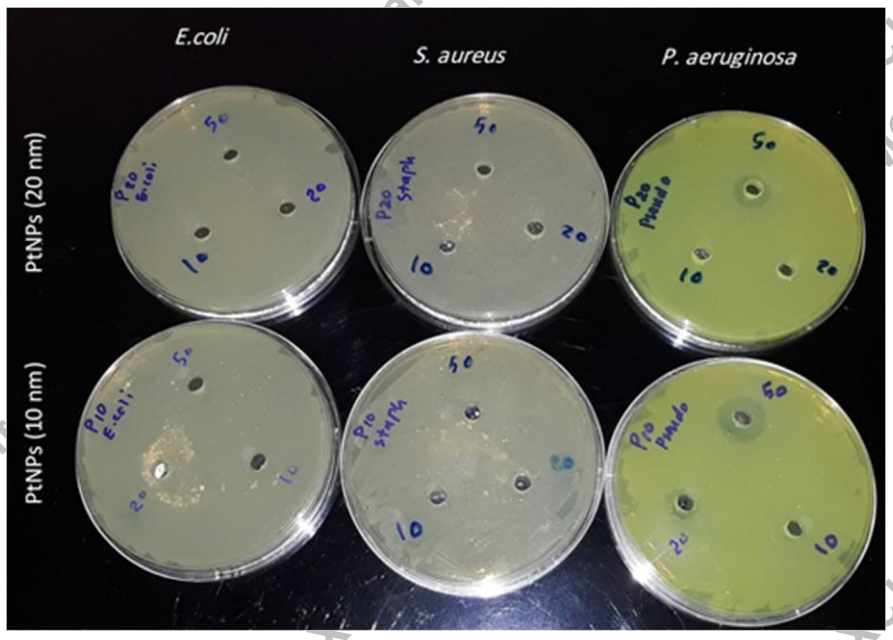

Fig. 3. Antibacterial effect of PtNPs on three different types of bacteria showing the selective anti-pseudomonas effect of PtNPs

Table 1. Antimicrobial activity (expressed as inhibition zones) of PtNPs with sizes of (10 and 20 nm) at different concentrations on tested microorganisms

10 nm				
10	8	-	-	-
20	13	-	-	-
50	17	-	-	-
20 nm				
10	-	-	-	-
20	-	-	-	-
50	13	-	-	-

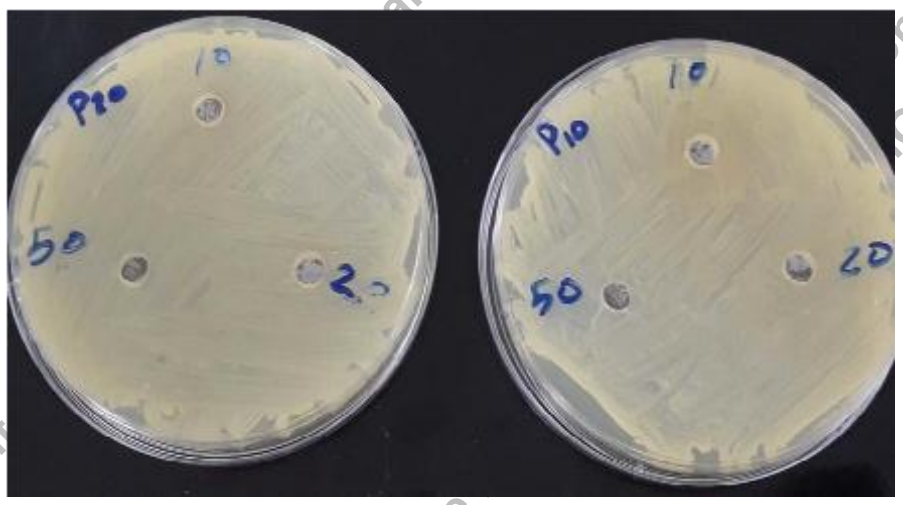

Fig. 4. Effect of PtNPs on *candida albicans* growth

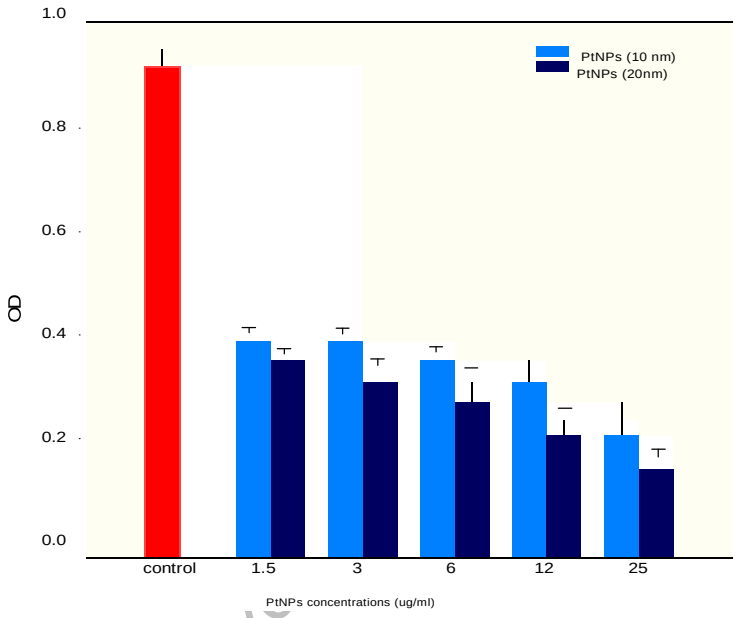

Fig. 5. Cytotoxic effect of PtNPs at different concentrations on Hepa 1-6 (Mouse hepatoma) cell line

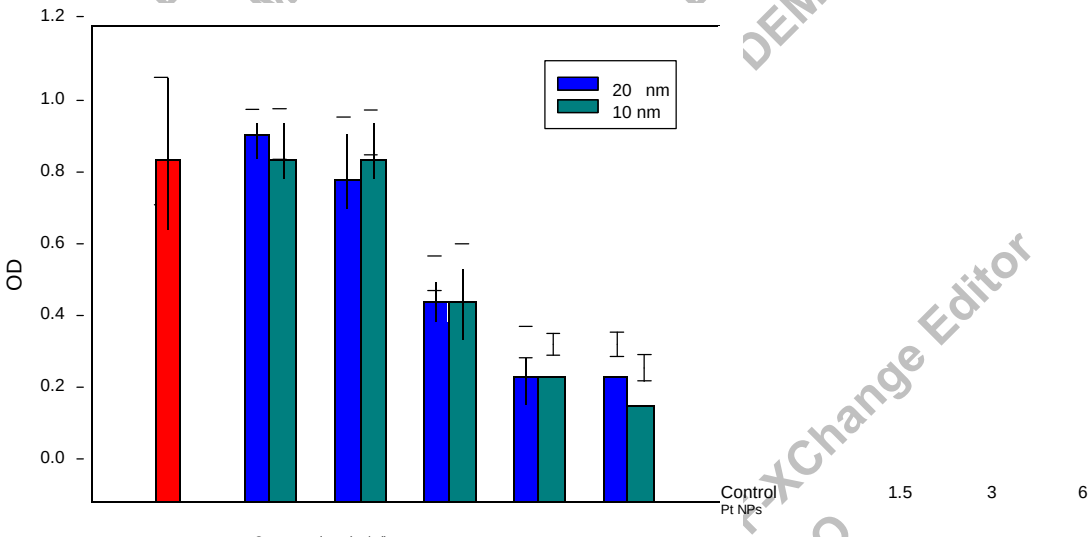

Fig. 6. Cytotoxic effect of PtNPs at different concentrations on MDCK normal cell line

High-purity platinum nanoparticles (PtNPs) with two particle sizes (10 and 20 nm) have been successfully synthesized in pure water by using the laser ablation method. Pt nanoparticles were of spherical shapes, with sizes decreased with increasing pulse numbers. These characteristics are controllable and depend on laser parameters (pulse rate, pulse energy, irradiation time), type of target material, and the nature of the liquid in which the material immersed in [6].

The antibacterial activity of PtNPs was of special interest for a long time. Hashimoto and his team illustrate the inhibitory effect of PtNPs on biofilm formation. They found that the attachment of these particles to bacterial surfaces can induce cell wall disruption or cell membrane lysis. These effects are associated with intercellular ROS generation that results in ROS-associated cellular

damage [7].

PtNPs cytotoxicity increased with decreasing particle size. Inhibitory effect vanishing with the size around 20 nm. Smaller sized PtNPs can enter and interact with the bacterial cells much easier than those of the greater sized particles [13].

A recent study showed that gram-negative bacterial lipopolysaccharide (LPS) which is negatively charged, decreased significantly after its interaction with PtNPs due to the high affinity to NPs and tight binding [14]. This may partially explain the selective cytotoxicity against *P. aeruginosa*, but this effect needs more elucidation. This bacterium contains one of the largest bacterial genomes [15], this may increase the DNA damage possibly caused by free radicals and other genetic attack mechanisms.

The α2-macroglobulin is a structural, wide-spectrum protease inhibitor, and a vital element of innate immunity in humans and mammals. *P. aeruginosa* manufactures a homolog of the human α2-macroglobulin to employ host cells to encourage their survival [16]. In *P. aeruginosa,* α2-macroglobulin found at high percentages in the cell membrane. Once the NPs interact with biological proteins, NP-Protein adsorption immediately arises [17]. This may provide additional explanation to the selected antibacterial activity against *P. aeruginosa* as well as against mice cancer cells.

Many studies reported the possible mechanisms of Pt-NPs entrance and cytotoxicity against cancer cells. It was found Pt NPs cross the cell membrane of the cells through diffusion, and restricted inside the cytoplasm [18][19]. Inside the cells, Pt-NPs cause DNA strand breaks in human colon carcinoma cells via the generation of a high level of ROS in a concentration, time, and size dependency. This DNA damage leads to growth arrest and apoptosis [20]. ROS causes damaging to intracellular macromolecules like proteins and carbohydrates [21]. Resent study was carried on 70 nm PtNPs to evaluate its cytotoxicity on human hepatic cells reported a dose-dependent decrease in cell viability in a dose higher than 25 □□g/mL. They found that PtNPs increase ROS production and actin expression with increasing NP concentration. PtNPs were able to trigger a significant stress response in liver cells, even in the absence of lethal effect [22].

Platinum is the core of some anticancer drugs particularly cisplatin and oxaliplatin[23,24]. These drugs are the drug of choice against various cancers types, including sarcomas, lymphomas, and carcinomas. Its mode of action based on its ability to crosslink with the purines on the DNA; disrupting DNA repair mechanisms, leading to DNA damage, inhibition of gene transcription, and subsequently inducing apoptosis in cancer cells. However, because of the numerous undesirable side effects and the emerging of drug resistance, there is an urgent necessity to test and use different platinum-based drugs [25].

In addition to the genetic effects, PtNPs trigger other toxic effects by decreasing cell metabolism. Although such effects do not affect cell viability or migration. Moreover, smaller PtNPs had a more harmful effect on DNA stability than the larger NPs. Smaller PtNPs caused changes in the activity of caspase 3/7 and caspase 9 [26].

CONCLUSIONS

High-purity spherical shaped ,platinum nanoparticles (PtNPs) with two particle sizes (10 and 20 nm) have been successfully synthesized in pure water by using the laser ablation method. These nanoparticles have similar physical properties with high purity as that produced by chemical methods. These nanoparticles showed promising selective antibacterial activity against one of the most common drug resistance bacteria (*P. aeruginosa*) at lower doses. Cytotoxic test on cancer cell line showed an inhibitory effect on the growth of these cells. These nanoparticles can be used to treat complicated pseudomonas infections. Further studies are needed to evaluate its anticancer activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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