Original Article

An Enhancing Effect of Gold Nanoparticles on the Lethal Action of 2450 *MHz* Electromagnetic Radiation in Microwave Oven

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Abstract

Today, there is an increasing interest in the use of metal nanoparticles in health sciences. Amongst all nanoparticles, the gold nanoparticles have been known to kill the cancer cells under hyperthermic condition by near-infrared frequency electromagnetic waves. On the other hand, although there are different physiochemical methods for disinfection of microbial pollution, however applications of irradiated gold nanoparticles against microorganisms have not yet been investigated. In this study, gold nanoparticles were prepared using D-glucose and characterized (particle size <26 *nm*). In the next step, the enhancing effect of the non toxic level of gold nanoparticles (50 *µg/mL*) on the antimicrobial activity of 2450 *MHz* electromagnetic radiation generated at a microwave oven operated at low power (100 *W*), was investigated by time-kill course assay against *Staphylococcus aureus (S.aureus)* ATCC 29737. The results showed that application of gold nanoparticles can enhance the lethal effect of low power microwave in a very short exposure time (5 *s*).

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Introduction

Researchers demonstrated that electromagnetic waves kill the microorganisms by changing the vibration frequency of the molecules [1]. Although it has been established that high frequency electromagnetic waves have enough power to kill microorganisms, but these electromagnetic waves have many side effects that impact human health [2,3]. Nevertheless, the lower frequencies of electromagnetic waves and lower exposure times can be used to reduce these adverse effects [4].This method has been a candidate as an alternative disinfection process in industries to decrease the number of viable bacteria [5]. Microwaves are categorized as a non-ionizing low frequency electromagnetic wave with frequencies between 300 *MHz* and 300 *GHz* [6]. Many reports have been published on the lethal effects of microwave radiation on microorganisms [7,8]. Mechanisms underlying the lethal effect of microwave radiation on microorganisms are yet to be discovered. Some researchers hypothesized that electromagnetic waves can increase the target temperature and

destroy life. However, non-thermal effects of electromagnetic waves, which are uniquely reported for microwaves, have been reported to be involved in lethal effects of electromagnetic waves [9].

On the other hand, several physicochemical methods have been developed to kill microorganisms [2,10]. Chemicals such as organic and inorganic compounds are widely used for disinfection process [11,12]. Moreover, the antimicrobial activity of different nanomaterials such as gold nanoparticles (AuNPs) has recently been reported [13]. Hyperthermic gold nanoparticles under infrared radiation have been used for destruction of cancer cells [14]. However, to the best of our knowledge, based on literature survey, the antimicrobial activity of AuNPs under electromagnetic radiation has not yet been studied and ought to be recognized.

In this study, AuNPs were synthesized with D-glucose and applied to augment the lethal effect of 2450 *MHz* electromagnetic radiation against *S.aureus*. The results showed that AuNPs significantly enhanced the lethal effect of electromagnetic radiation after a very little incubation time (5 *s*).

Materials and Methods

Synthesis of gold nanoparticles

AuNPs were synthesized by a chemical method as previously has been reported [15]. In this method, anhydrous D-glucose and soluble starch are used as reducing compound and a protecting agent, respectively. Forty μl of 1*mM* $HAuCl_4.3H_2O$ solution (Merck, Germany) and 60 μl of 0.1 *M* D-glucose solution were added to 2 *ml* of 0.2%w pure starch (Sigma, USA) solution. Subsequently, 15 μl of sodium hydroxide (Merck, Germany) solution was added to the aforementioned solution mixed. The reaction mixture was incubated at room temperature for an hour.

In the next step, the prepared AuNPs was characterized by transmission electron microscopy (TEM) (EM 208 Philips), UV-visible spectrophotometry (Labomed Model UVD-2950 UV-VIS Double Beam PC Scanning spectrophotometer operated at a resolution of 2 *min*), and Energy-Dispersive Spectroscopy (EDS) (EM 208 Philips). For TEM experiment, aqueous suspension containing the AuNPs was dispersed ultrasonically, and a drop of suspension was located on carbon-coated copper TEM grids and dried under an infrared lamp. Micrographs were achieved using a TEM (ZIESS 902A, Germany) operated at an accelerating voltage of 80 *kV*.

Finally, prepared AuNPs were separated by centrifugation (20000 $\times g$) for 45 *min* and washed three times with double distilled water. The pellet (0.8 *mg*) was re-dispersed in 1 *ml* distilled water (0.8 *mg/ml*) and homogenized in ultrasonic bath (5 *min*). This colloid was reserved in a screw capped container at 4°*C* for further experiments.

Antibacterial susceptibility test

Liquid serial dilution method using Müller–Hinton Broth (MHB) was used for evaluation of antibacterial activity of AuNPs against *S.aureus* ATCC 29737. MHB was further supplemented with serial concentrations of AuNPs (6.25, 12.5, 25, 50, 100, 400, 800 $\mu g/mL$). The inoculum was prepared from fresh culture of *S.aureus*. For this purpose, a loop full of the culture was suspended in sterile normal saline to 0.08-0.1 optical density (measured at 600 *nm*), which corresponded to about 5×10^7 colony-forming units per milliliter (*Cfu/mL*). An inoculum of approximately 5×10^5 *Cfu/mL* of test strain was inoculated in the prepared MHB as described above. The data are reported as Minimum Inhibitory Concentration (MIC), which was the lowest concentration of AuNPs that inhibited visible growth of test strain after 24 *hr* of incubation at 37°*C* [16].

Time-kill course assay

The antibacterial activity of sub-inhibitory concentration of AuNPs alone (50 $\mu g/ml$) and in combination with 2450 *MHz* electromagnetic radiation was evaluated against *S.aureus* using time-kill course assay. A microwave oven (Samsung M2330DN) operated at a low power (100 *W*) was used in this experiment. A suspension of *S.aureus* was prepared in sterile 0.9% NaCl. Aliquots of this bacterial suspen-

sion (1 *mL*) were dispensed in 8 tubes. Four test tubes containing the bacterial suspension were supplemented with 62.5 *µl* of the above AuNPs stock solution (50 *µg/mL*).

Then 62.5 *µl* of normal saline was added to other test tubes and these vessels were labeled as control tubes. In the next step, a test tube contained AuNPs and its control tube (without AuNPs) were exposed to microwave radiation in the microwave oven (100 *W*) for 5 *s*. This procedure was repeated for the other samples and control tubes for additional times (10, 15 and 20 *s*). The viable counts of bacteria in all the test tubes were determined before and after microwave treatment by standard Most Probable Number (MPN) method [17].

Results

Synthesis and characterization

AuNPs were fabricated using D-glucose in soluble starch solution at room temperature. The appearance of purple color in reaction vessel confirms the formation of AuNPs [18]. The inset in figure 1 shows the reaction mixture before (vessel A) and after reaction with D-glucose for an *hr* at the room temperature (vessel B). The prepared colloid was characterized by UV-visible spectroscopy. The technique outlined above proved to be very useful for the analysis of nanoparticles [18].

As illustrated in figure 1, a strong absorption band with a maxima located at 530 *nm* was observed due to formation of AuNPs produced by the D-glucose. This peak is assigned to a surface plasmon, phenomenon that is well-documented for various metal nanoparticles with sizes ranging from 2 *nm* to 100 *nm* [19,20].

Figure 2A shows the TEM image of the drop-coated film of the AuNPs and figure 2B demonstrates their particle size distribution histogram. Figure 2A shows that small nanoparticles have been prepared with a diameter of less than 28 *nm*. Also, the mode of AuNPs size histogram is under 4 *nm* which is in accordance with 40% of all of the particles (Figure 2B). In the analysis of the AuNPs by EDS, the presence of elemental gold signal was confirmed in the sample (Figure 3). The Au nanocrystallites display an optical absorption band peaking at 2.15 *keV* which is typical

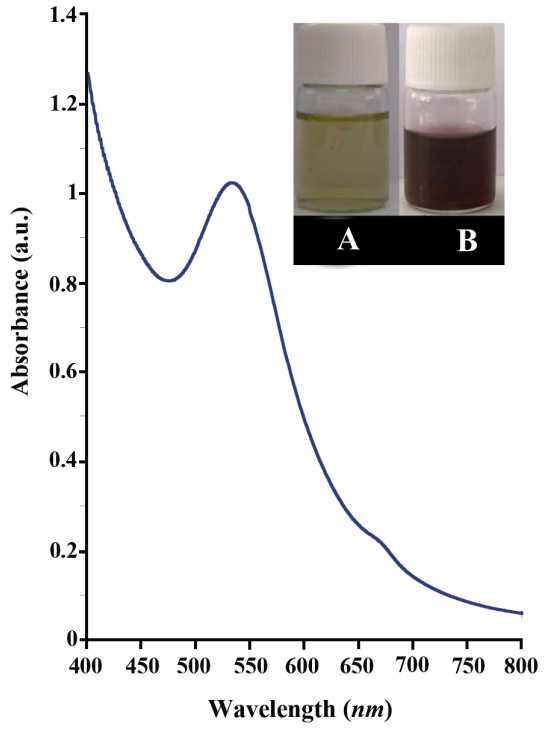

Figure 1. UV-visible absorption spectrum of gold nanoparticles fabricated by D-glucose. The inset in this figure shows the vessels containing the reaction mixture before (A) and after reaction with D-glucose for 1 *hr* at room temperature (B)

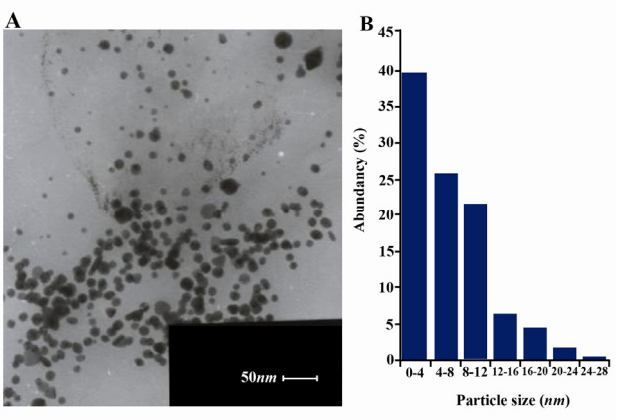

Figure 2. Transmission electron micrograph (A) from a drop-coated film of prepared gold nanoparticles fabricated by D-glucose for 1 *hr*. The transmission electron microscopy image shows that small spherical nanoparticles. The particles size distribution histogram (B) shows that the size of generated nanoparticles was less than 28 *nm* and 40% of these nanoparticles were smaller than 4 *nm*

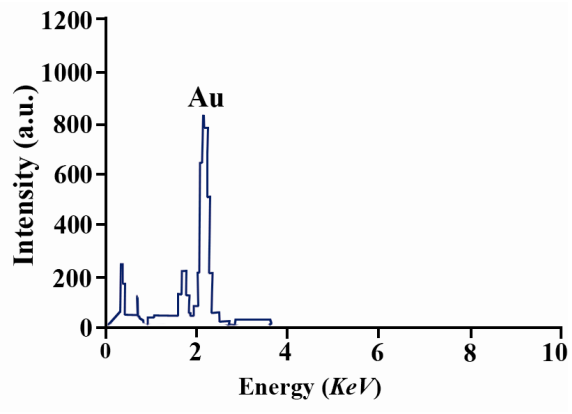

Figure 3. Energy-dispersive spectroscopy spectrum of prepared gold nanoparticles. Gold X-ray emission peak is labeled. Strong signals from the atoms in the nanoparticles are observed in spectrum and confirm the reduction of gold ions to gold nanoparticles

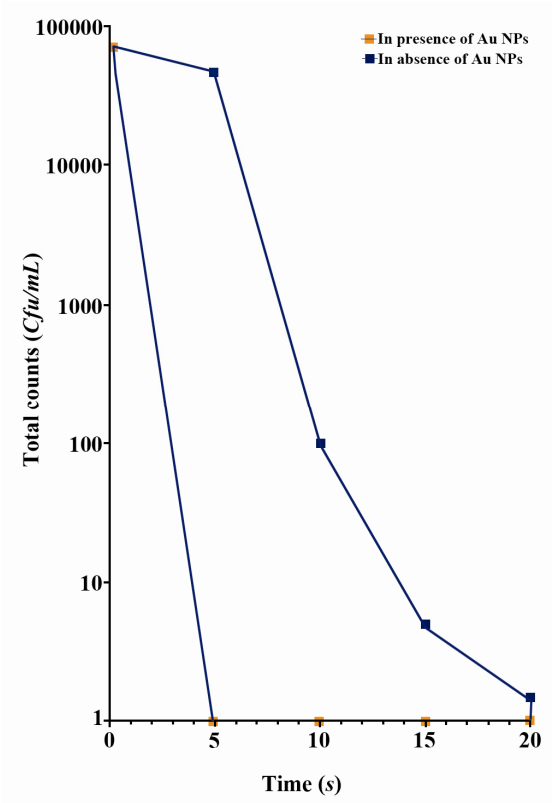

Figure 4. The viability of test stain (*S.aureus*) during treatment with low power microwave in the presence and absence of gold nanoparticles fabricated by D-glucose

of the absorption of metallic gold nanocrystallites [21].

Antibacterial assay

The susceptibility of *S.aureus* ATCC 29737 against AuNPs was determined by serial broth dilution method. The obtained MIC was 100 *μg/mL*. The sub-MIC concentration of AuNPs (50 *μg/ml*) was selected to evaluate its enhancing effect on the lethal effect of certain electromagnetic wave (2450 *MHz*) generated in a microwave oven operated at low power (100 *W*).

Time-killed course study was selected for investigation of antibacterial activity of AuNPs alone and in combination with microwave radiation against *S.aureus*. The result of this experiment is shown in figure 4. Rapid killing rate was observed for test strain in presence of AuNPs (Figure 4). Microbiological examination of the inoculated suspensions which contain AuNPs and treated in microwave oven (100 *W*), showed no viable cells after 5 *s* (Figure 4).

In contrast, we detected considerable numbers of viable cells (about $>10^4$ *Cfu/mL*) in inoculated control tubes (without AuNPs) which incubated under microwave radiation at aforementioned time (5 *s*). This means that adding AuNPs to samples can enhance the antibacterial effect of microwave radiation that generated at microwave oven (100 *W*).

Discussion

It should be pointed out that low oven power (100 *W*) and sub-MIC concentration of AuNPs (50 *μg/ml*) were selected to guarantee that the effect produced in a short time exposure (5 *s*) was due to the combined method and not the effects of each factor alone. On the other hand, the effect observed in this condition could be due to the AuNPs-microwave radiation combination.

In a separate experiment, the temperatures of solutions which contain sub-inhibitory concentration of AuNPs (50 *μg/ml*) and treated by microwave at the same conditions, were also rapidly measured by a thermometer. The obtained temperature were 38°*C*, 39°*C*, 43°*C* and 47°*C* for samples which incubated for 5, 10, 15 and 20 *s* in microwave oven (100 *W*), respectively.

At the concentration tested (50 *μg/ml*), AuNPs significantly improved lethal effect of

microwave radiation against *S.aureus* in 5 *s*. Longer incubation (10, 15, 20 *s*) of control samples (without AuNPs) in a microwave oven operated at 100 *W* led to gradual reduction of viable cells.

Anyhow, comparing the control samples, the application of AuNPs together with microwave radiation can significantly reduce (four times) the time required for complete destruction of test strain (Figure 4). In control tubes, time-kill course assay showed that the numbers of bacteria decreased to half the ratio in 5 *s* under microwave radiation which elevated temperature to 38°*C*. After 10 *s*, the viable counts decreased to about 100 *Cfu/mL* and temperature was 39°*C*. High killing rate was observed in ambient temperatures in microwave oven at 100 *W*. Therefore, non-thermal effects of microwave radiations may be involved in lethal phenomena. However, a marked increase in local temperature can also lead to the denaturation of proteins or disruption of organized biomolecules in the microorganisms. Further investigation should be carried out on the discovery of the possible mechanisms throughout the thermal and/or non-thermal effects in microwave radiation.

Conclusion

This investigation has described the enhancing effect of AuNPs on the lethal action of electromagnetic radiation generated in a microwave oven (100 *W*). Although many studies have been conducted to show the lethal effect of electromagnetic waves, but the antibacterial activity of microwave in the presence of AuNPs nanoparticles has never been investigated. This is a first report on the combined application of AuNPs with electromagnetic radiation for rapid killing of *S. aureus* in a short exposure time (5 *s*).

Electromagnetic radiations have been used as a killing agent [5] and the application of metal nanoparticles together with microwave radiation can reduce the time required to complete deactivation of microorganisms. This combination may be used for removing of contamination in medical devices like gauze or non-metal dentistry devices in short times [22].

Moreover, AuNPs are considered as inert and safe material [23]. Therefore, this method may be selected as a rapid process for sterilization of heat sensitive drug substance or biological products in pharmaceutical industries. Further investigation should be performed in the future works on the stability of different chemicals during short time electromagnetic wave (2450 *MHz*) radiation process using a microwave oven operated at 100 *W*.

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