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Use of cold plasma in the synthesis of gold nanomaterials for parasitic leishmaniasis treatment

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Abstract

The atmospheric air cold plasma has been used to manufacture gold nanomaterials for treating parasitic leishmaniasis. This study experimentally assessed the treatment of *Leishmania* parasites (L. *donovani* and L. *tropica*) by gold nanoparticles. Specifically, atmospheric pressure nonthermal plasma was generated using different diameters (1.0, 2.8, 3.8 and 4.3 mm) of high voltage electrode. Aqueous gold tetrachloride salts ($HAuCl_4 \cdot 4H_2O$) were used as precursor to produce gold nanoparticles. UV–vis spectroscopy and x-ray diffraction were conducted for characterization of the nanoparticles. The optimum condition (a diameter of 1 mm) was chosen to prepare gold nanoparticles, where the grain size was found to be 17 nm. Accordingly, the nanoparticles were synthesised to treat parasitic leishmaniasis using the aforementioned diameter. The effect of nanoparticles on the parasitic lymphoid parasite at 100%, 50% and 25% concentrations were studied after 24 and 48 h of exposure and under the greatest smashing rate. For the *Leishmania* parasites at a molar concentration of 0.4 mM and 4 min treatment in cold plasma, the percentage of L. *donovani* parasites was equal to 55.6% after 48 h of exposure. For the parasites at a molar concentration of 0.2 mM and 4 min exposure in cold plasma, the percentage of L. *tropica* parasites was equal to 69.6% after 48 h of exposure. Results reveal that the atmospheric air cold plasma is a promising technique for producing nanoparticle materials for the treatment of parasitic leishmaniasis that threatens people around the world.

Keywords: Cold plasma, gold nanoparticles, plasma–liquid interactions, Leishmania.

1. Introduction

Plasma is the fourth state of matter, can be considered a quasi-neutral gas, follows the more familiar states of solid, liquid and gas and constitutes more than 99% matter of the universe [1]. Plasma is an ionized gas that usually consists of ions, electrons, neutrals, reactive species, excited species and photons of UV. The plasma that is generated at room temperature is called the atmospheric plasma [2]. Indoor vacuum chambers produce plasmas at low pressures, which are also called low-temperature thermal or quasi-equilibrium plasmas [3] with approximately the same light and heavy species temperatures. At high pressure (such as atmospheric pressure plasma jet discharges), dielectric barrier discharges (DBD) could be created by the ionization of the gas in the ambient environment between two narrow electrodes; therefore, a costly vacuum equipment is unnecessary to generate the plasma, The scheme called floating electrode (FE-DBD) can generate cold plasma from one electrode, and the second electrode can be a living tissue provided that the distance between the electrode and tissue is 3 mm [4, 5]. Interest in producing electrical discharges inside and on the liquid surfaces under atmospheric conditions (called cold atmospheric plasmas here) is growing. The reasons are for the technical use and the further scientific understanding of interactions between plasma and liquid. Stable liquid interfaced plasmas would affect the environmental [5], medical [6–10] and industrial applications [11]. The most common synthesis mode of nanoparticles is wet chemical techniques that cause nucleation with the aid of solution reduction agents [12, 13]. The plasma-assisted synthesis is clean because it provides nucleation without the need for reducing and capping agents; it is also quick and simple. Although different types of plasma sources are available, operating at high pressure (near atmospheric pressure) and room temperatures is useful because these conditions do not cause evaporation of the liquid during the process. Atmospheric pressure plasma is a nonthermal (or nonequilibrium) plasma because the electron temperature is much higher than that of the ions or the gas species and the temperature of gas species remains similar to room temperature. Both types of plasma (thermal and nonthermal) can be used for nanomaterial synthesis. Cold plasmas have been used to synthesize nanomaterials using various gases, such as hydrogen gas, because of their unique characteristics that are related to their bulk equivalents [14, 15].

Nanomaterials have received considerable attention in recent years due to their extremely high surface area and small size. Nanomaterials demonstrate great biological activities inside the human body, which plays crucial roles in biomedicine in a wide range of applications, such as inhibition of cancer, drug delivery [16], cancer treatment [17], applications related to the environment [18] and use of nanomaterials to remove some chemical contaminants from water [19]. Nanomaterials can carry harmful effects to living organisms maybe due to that the traditional chemical approaches to nanomaterial synthesis need to use toxic oxidants or reductants for forming and stabilizing nanoparticles. Thus, an alternative toxic-chemical-free synthesis for biomedical applications is critical for the development of nanotechnology. Plasma technology is recently used as a generation method for nanomaterials due to its distinctive properties compared with the approaches for synthesizing solid, liquid and gas phases. The use of nanomaterials and plasmas in biomedical applications reveals many synergistic effects and a great performance in treatment. Gold-based nanoparticles are the subject of intensive studies and biomedical applications and have attracted considerable attention from the material science and biomedical community owing to their unique optical properties at the nanoscale, biocompatibility and rich surface chemistry. This work primarily aimed to synthesize gold nanomaterials for the treatment of parasitic leishmaniasis.

2. Material and methods

2.1 Linking the system

Normal atmospheric air cold plasma was used in the synthesis of gold nanomaterials. The plasma system consisted of the following five parts. 1) Air compressor, which is a locally manufactured air compressor (Aljawadeen) that is affordable and available in the market. This compressor contains a medium-sized tank and an electric motor works to fill the tank with the air at pressure of (60 atmosphere) and an air outlet containing a regulator to control the speed of exit air. 2) Air flowmeter with a calibrator of 1–10 min L^{-1} to control the air intake. This flowmeter connects to the hollow metal tube. 3) Aqueous gold salts ($HAuCl_4 \cdot 4H_2O$) with a molecular weight of 411.8 g mol^{-1} and dissolved in ionic water at a concentration of 0.4 mM. 4) Hole metal tubes of stainless steel with a length of 10 cm and different internal diameters of 1, 2.8, 3.8 and 4.3 mm. They connect to the cathode of the power supply, equipped with continuous and intermittent high voltage and manufactured for this purpose. The tubes can process voltage of up to 25 kV and cutting of 25 kHz. They have stainless steel conductive length of 7 cm and width of 5 mm and strip ends with a 1 × 1 flat end that connects to the anode. They also have metal tube holder, which carries the glass beaker containing the solution of aqueous gold salts. 5) Metal tube, which is fixed vertically by the catcher. Its upper end is connected by a rubber tube to the air regulator, which in turn connects by a rubber tube to the air compressor. The brine of gold is placed in a small flask (a capacity of 25 mm), and the beaker is placed on a movable holder under the metal tube. The anode (anode) of the voltages is equipped.

2.2 Preparation of the solution

Aqueous tetrachloride salts ($HAuCl_4 \cdot 4H_2O$) were used (a partial weight of 411.8476 g and a purity of 99%) and manufactured by the German company SKMA. A volume of 20 mL of 0.4 mM was prepared, and equation (1) was used to calculate the required concentration (mole L^{-1}).

$$\text{Concentration} = \frac{mass}{(molecular\ weight \times volume)} \tag{1}$$

2.3 Preparation of nanoparticles

To prepare the gold nanoparticles, we followed the steps below: The compressor tank was filled with air, and the metal tube was fixed 1 mm in diameter. After the solution of gold salts of the required concentration and size was prepared, the prepared form was placed on the holder under the metal tube as the mentioned above. The beaker was rounded from the metal tube to the distance between the liquid surface, and the nozzle of the tube was 1 mm. The air in the metal tube was regulated by the flowmeter to control the airflow from the tank. The value of the supplied voltage of the system was gradually increased until the plasma was generated between the tube and the surface of the liquid.

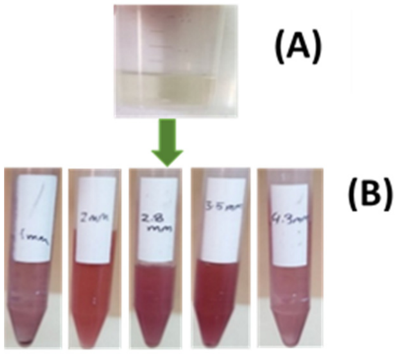

Fig. 1. The gold salt solution (A) before exposure to plasma (B) after 2 minutes exposure to plasma.

2.4 Studying the effect of prepared nanomaterials on the inhibition of leishmaniasis parasites

The study was conducted *in vitro* at the Biotechnology Research Centre of Al-Nahrain University. The study proceeded as follows: *L. tropica* and *L. donovani* parasites were transplanted. The cells, which were supplied by the Biotechnology Research Centre of Al-Nahrain University, were added to the middle cells (PRMI 10%) and distributed in a microplate of 96 wells with 10,000 cells per well. They were incubated at 27 °C [20]. Each plate of 96 wells contained 13 groups for both species of Leishmania. Three groups were exposed to 0.2 mM molar concentration and exposed to plasma for 3 min with nanomaterial dilutions of 100%, 50% and 25%. Three other groups were exposed to 0.2 mM molar concentration and exposed to plasma for 4 min with nanomaterial dilutions of 100%, 50% and 25%. The optimum nanomaterials were obtained when the diameter of the metal tube was 1 mm. are shown in Fig. 1. The last group was considered a standard control group. This group was examined after 24 and 48 h of exposure. The parities were dyed by MTT (methylthiazolyl tetrazolium) and left in the incubator for 2 h and then added with DMSO (dimethyl sulfoxide) to examine the results with the device and compare it with the control. The process was repeated three times, and the growth rate of cells was calculated using equation (2). The statistical implementation of these measurements is shown in Figs. 6 and 7.

$$GI_{\%} = \frac{Vcontrol - Vtreated}{Vcontrol} \times 100\% \tag{2}$$

3. Results and discussion

3.1 Results of nanomaterial tests

In the current research, the gold nanoparticles were prepared using a plasma jet system in atmospheric air rather than using inert gases. The formation of nanoparticles was observed by color-changing of the solutions from transparent to purple (Fig. 1). The presence of gold particles in a color other than aqueous tetrachloride salts is due to surface plasmon resonance. Surface plasmon resonance occurs in metals, such as gold and silver, as a result of their nanometer particles reaching the nanometer scale. Therefore, spectroscopy at visible wavelengths was used to prove the formation of the gold nanoparticles. The optical properties of the prepared gold nanoparticle solutions as a function of the diameter of the plasma system were investigated. Notably, a deviation in the absorption edge as biased in the wavelength from 544 nm to 551 nm for gold nanoparticles indicates a different behavior with an increase in system diameter (Fig. 2). Therefore, the increase in the diameter of the system increases the concentration of the metal ion and then increases the size of the nanoparticles [16].

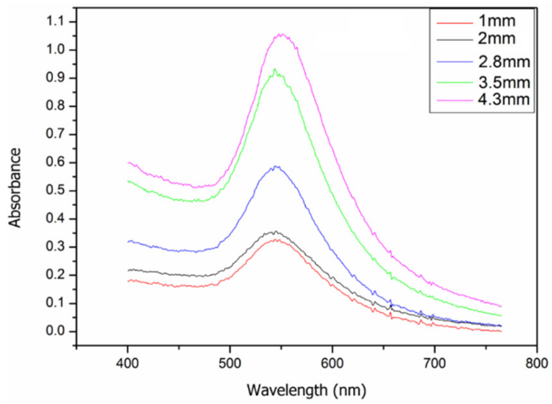

Fig. 2. Visible ultraviolet spectra of gold nanoparticles prepared using jet plasma as a function of system diameter.

Fig. 3 illustrates the X-ray diffraction (XRD) patterns of dried gold particles prepared using plasma jet system as a function of the diameter of the plasma system. The results of XRD pattern tests show a diffraction pattern from gold nanoparticles with an fcc crystalline structure. The peaks of XRD 2θ are observed at 38.18° and 44.44°. They correspond to the 111 and 200 crystalline levels. Notably, the sample is prepared with the diameters of 1, 2 and 2.8 mm with no other peak that could be attributed to impurities. The vertices in the XRD pattern are due to the cubic structure (fcc) centred around the face in Gold. The XRD patterns of gold nanoparticles correspond to the standard gold JCPDS (file 00-004-0784). The peak intensity (111) at 38.18° diffraction is much stronger than the peak (200) at 44.44° due to the presence of ubiquitous specimens at 1 and 2 diameters and the 2.8 mm pattern. For a sample prepared with the diameters of 3.5 and 4.3 mm, the peak is only one for AuNPs (Au nanoparticles) at 2θ of 38.18°. The average volume of AuNPs was calculated using the Debye–Scherrer equation by determining the strongest peak width. The average size of crystals is 17 nm, and the size ranges from 18 nm to 29 nm depending on the diameter of the system.

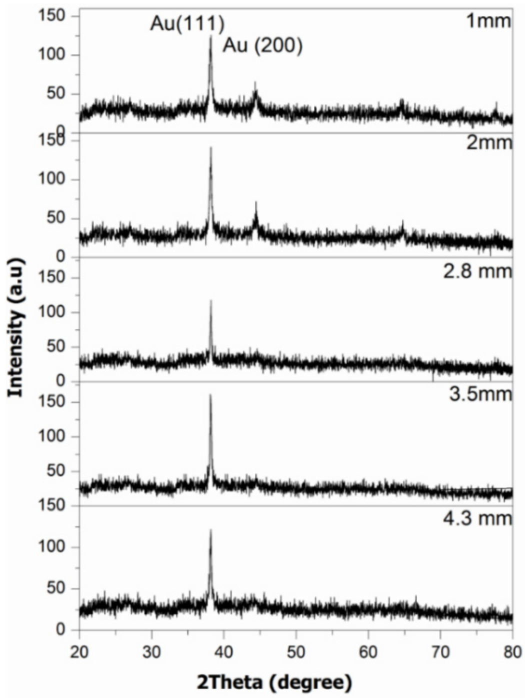

Fig. 3. X-ray pattern of gold nanoparticles prepared using Jet Plasma as a function of system-diameter.

The results of the FESEM (field emission scanning electron microscopy) show that the gold particles produced are irregularly shaped spheres with sizes ranging from 4 nm to 66 nm, which are approximately similar to the values obtained from XRD measurements. The optical and electronic properties of metal particles are greatly influenced by the shape of the nanoparticles. The results confirm that spherical-shaped gold nanoparticles can be produced in diameters within the nanoscale, as shown in Fig. 4.

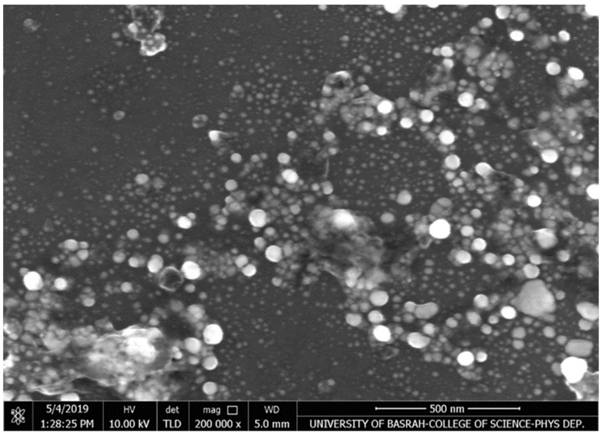

Fig. 4. Images of the FESEM penetrating gold nanoparticles prepared using jet plasma as a function of the diameter of the system (diameter 1 mm).

3.2 Effect of Prepared Nanomaterial on Inhibition of Leishmaniasis *in vitro*

The growth inhibition rate of shamanism (*L. tropica* and *L. donovani*) was calculated at in incubation time of 24 and 48 h to compare the most effective evaluation of treatment. The greatest rate of destruction of *Leishmania* cells was 55.6% after 48 h of exposure to gold nanoparticles (0.4 mM $HAuCl_4{\cdot}4H_2O$ and cold plasma exposure time of 4 min), as shown in Fig. 5. The rate to gold nanoparticles (0.2mM $HAuCl_4{\cdot}4H_2O$ and cold plasma exposure time of 4 min) *L. tropica* cells is 69.6% after 48 h exposure, as shown in Fig. 6.

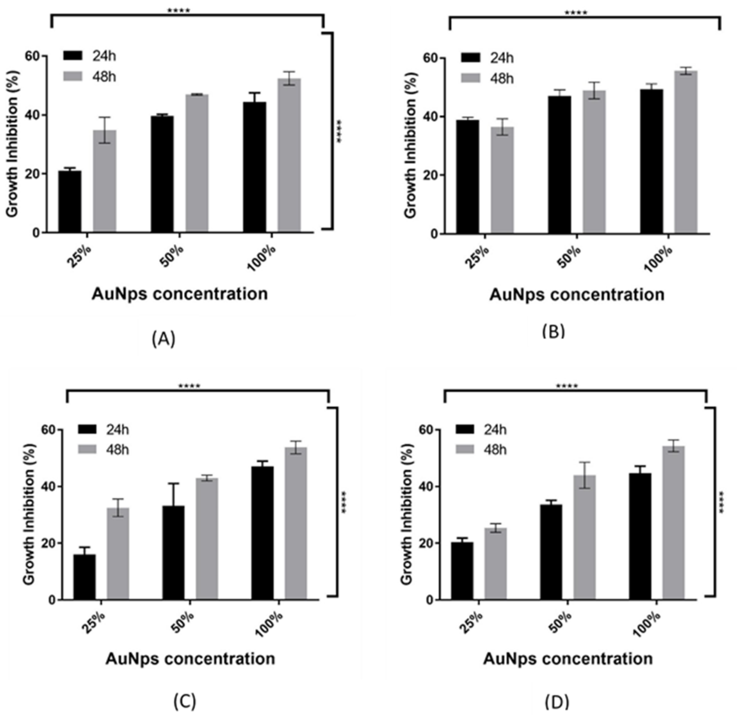

Fig. 5. Inhibition of *Donovani* leishmaniasis cells (A) at 0.4 mM concentration and plasma exposure for 3 min (B) at 0.4 mM molar exposure and plasma exposure for 4 min (C) at 0.2 mM molarity and plasma exposure for 3 Minutes (D) at 0.2 mM molar concentration and subjected to plasma for 4 min.

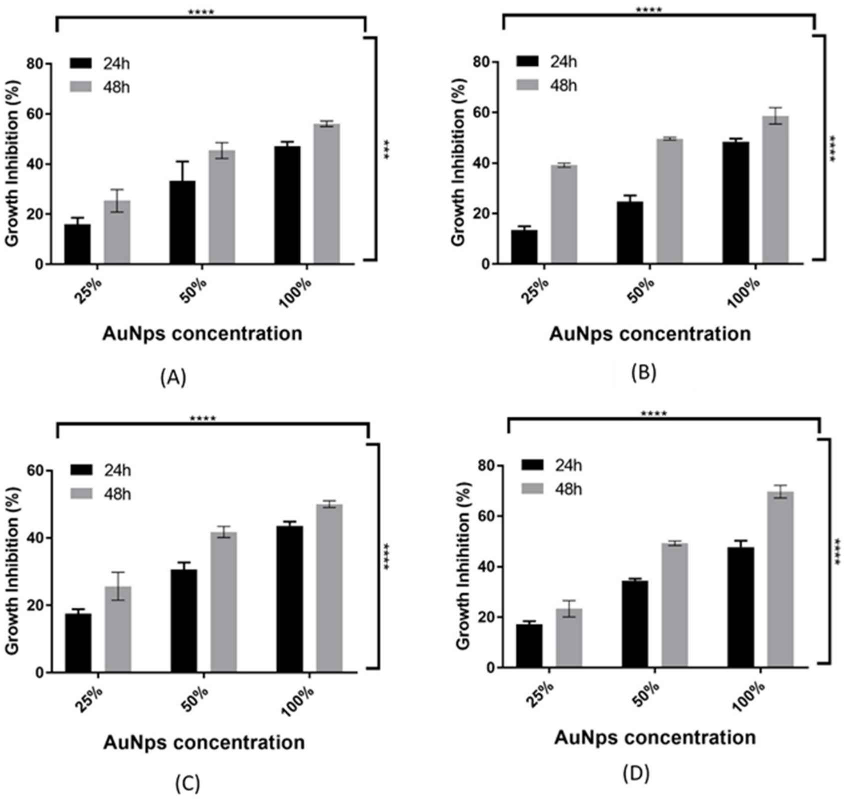

Fig. 6. Inhibition of *Tropica* leishmaniasis (A) at 0.4 mM concentration and plasma exposure for 3 minutes (B) at 0.4 mM molar exposure and plasma exposure for 4 minutes (c) at 0.2 mM concentration and plasma exposure for 3 Minutes (d) at 0.2 mM concentration and exposing to plasma for 4 min.

4. Conclusion

Manufacturing nanomaterials through the plasma jet system using different small diameters is a user-friendly, nontoxic and less time-consuming method. The system has high efficiency to produce gold nanomaterials using aqueous gold tetrachloride salts ($HAuCl_4 \cdot 4H_2O$). Air, instead of other types of gases, is used to feed the plasma jet system. The obtained UV-vis spectra show the absorbance band from 544 nm to 551 nm, which is a characteristic of surface plasmon resonance of AuNPs. The XRD patterns show that the sample is prepared with the diameters of 1, 2 and 2.8 mm contained no other peak that could be attributed to impurities. The peaks in the XRD pattern are due to the cubic structure (fcc) centered around the face in Au. FESEM gives an idea about the morphology of AuNPs. Specifically, the particles are spherical-shaped spheres with sizes ranging from 4 mm to 66 nm, which are approximately similar to the values obtained from XRD measurements. The reported methods in the treatment of dangerous parasites, such as *Leishmania*, require advanced techniques and great efforts to accomplish. By contrast, the nanomaterials manufactured for the treatment of *Leishmania* parasite in this study do not require advanced techniques. Cold plasma technology is comparable to other technologies in terms of its low cost.

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