Photo-extracellular synthesis of gold nanoparticles using Baker’s yeast and their anticancer evaluation against Ehrlich ascites carcinoma cells

Yasser A. Attia,*a Yassmeen E. Farag,b Yasser M. A. Mohamed,c Akaber T. Hussiend and Tareq Youssefb

The chemical methods for the synthesis of gold nanoparticles lead to the formation of some toxic chemicals adsorbed on the surface that may have adverse effects on their medical applications. Hence, the need to develop environmentally benign nanoparticles has attracted growing interest. Gold nanoparticles with 13.0 ± 0.9 nm size have been biosynthesized using an aqueous extract of Baker’s yeast (Saccharomyces cerevisiae) under visible light. The existing components in the aqueous yeast extract were identified for the first time as trimethylsilyl derivatives of butan-2,3-diol, glucose, indole-3-acetic acid and undecanoic acid. This extract acts as a capping and reducing agent for gold nanoparticles. The cytotoxicity of the biosynthesized gold nanoparticles, chemically synthesized gold nanoparticles of 5.0 ± 2.0 nm size and aqueous extract of Baker’s yeast towards Ehrlich ascites carcinoma cancer cells has been studied using the Trypan blue exclusion method. The results show that the killed percentage of Ehrlich ascites carcinoma cells under dark incubation with an aqueous extract of Baker’s yeast, chemically synthesized gold nanoparticles and the photo-biosynthesized gold nanoparticles are 9.7%, 12.5% and 24.6%, respectively. These percentages are increased to 10.63%, 60.6% and 86.5% under visible light incubation, respectively. The killing enhancement of the Ehrlich ascites carcinoma cells under visible light incubation is proposed based on the photothermal properties of the formed plasmonic gold nanoparticles that conjugated with anti-epidermal growth factor receptor antibodies beside the phagocytosis of the excess yeast extract that is present in the photo-extracellular synthesized gold nanoparticles sample. The combination between two different treatment ways can give a third one with high affinity for the treatment of cancer.

Introduction

It is estimated that within the next 30 years, cancer will become the main cause of death. This is a bothersome statistic not because of an increase in the number of incidences of cancer, but because deaths from heart disease could be reduced to nearly half, while the number of cancer-related deaths remains about the same. This emphasizes the need for newer and more effective therapies.5–7 The therapeutic aspects of nanotechnology will be assessed as a new weapon to fight cancer, exploring the advances in areas such as improved bio-imaging, new therapies, and drug delivery.5–7 Gold nanoparticles that have surface plasmon resonance are emerging as promising agents for cancer therapy. The role of gold nanoparticles in cancer treatment is based on their advantages, which arise due to their easy preparation, efficient bioconjugation, potential non-cytotoxicity, and their tunable and enhanced absorption and scattering of electromagnetic radiation that provides an opportunity for their use in cancer cell imaging. Furthermore, their strong absorption provides a possibility for them to be used in efficient cancer diagnostics ultimately coupled with selective photo-thermal therapy.5–11 Gold nanoparticles that have a size less than 50 nm can easily enter the cells and the smaller size than 20 nm can transit out of blood vessels. Most of the medical researchers recognize the use of gold nanoparticles as a new era for cancer treatment.

The chemical methods for the preparation of nanoparticles left some toxic chemicals adsorbed on the surface that may have adverse effects on their applications.12 Hence, the need to
The biological source has been used in nano-manufacturing as a clean and eco-friendly method of preparing nanoparticles (NPs). Intracellular synthesis of magnetite nanocrystallites is an example of naturally synthesized nanomaterials using biological processes. Similarly, intracellular CdS nanocrystallites can be synthesized when a certain type of yeast was challenged with toxic metals such as cadmium. Pooley et al. reported that silver nanoparticles through silver leaching in bacteria lead to the accumulation of silver sulfde within their membrane. The extracellular synthesis of nanoparticles using culture supernatants of microorganisms has been described in several reports. The main features of this type of synthesis are the preparation of nanoparticles in large quantities and easy downstream processing. This led to the development of a variety of synthetic methods for better control of morphology and size. The ability to control various physical properties (shape and size) and to include functionalized groups on the surface of nanoparticles has made metal nanoparticles attractive to search for newer applications. Microorganisms such as Baker's yeast function as many natural anticancer drugs in cancer treatment by the induction of cancer cell apoptosis. Apoptosis is a natural process for the removal of damaged, aged and abnormal cells. The apoptosis process can be triggered by a large number of stimuli like exposure to the chemical and physical agents, or oxidative stress or the removal of growth factors. Research in the previous decade revealed a promising future for apoptosis based cancer therapies. This specific intercellular damage is induced by many therapeutic agents which are seen to involve the chemotherapeutic agents exhibit an indiscriminate killing that involves cancer cells and normal cells. So it is of particular interest to find agents which induce apoptosis of cancer cells with minimal side-effects. In the study performed by Ghoneum et al., it was demonstrated that the phagocytosis of non-pathogenic yeast, S. cerevisiae, induces apoptosis in the breast cancer cells (BCCs). The phenomenon of the induction of the apoptosis process after phagocytosis of certain microorganisms has been studied in the human phagocytic cells. Many studies illustrated an increase in the apoptosis by neutrophils and monocytes/macrophages after the phagocytosis of microorganisms such as Escherichia coli, Mycobacterium tuberculosis, Staphylococcus aureus and Candida albicans.

Many problems with the regulation of apoptosis have been implicated in many diseases. Cancer is a disease that is characterized by too little apoptosis. Moreover, cancer cells typically possess a number of mutations that have allowed these cells to ignore the normal cellular signals regulating their growth and become more proliferative than normal. In addition, under normal circumstances, damaged cells will undergo an apoptosis process, but in the case of cancer, cells mutations may have occurred to prevent cells from undergoing the apoptosis process.

For these reasons, we are trying in this work to combine the apoptosis effect of Baker's yeast and the photothermal effect of gold nanoparticles to a develop a new improved cancer treatment method.

Results and discussion

Gold nanoparticles for cancer therapy

The ease of preparation, potential non-cytotoxicity, possible bioconjugation and surface plasmon resonance are unique properties of gold nanoparticles that have made them of wide interest in biomedical applications. Different methods for the preparation of gold colloids were reported in the last two decades. In the most popular synthesis method, several minutes only are enough for the synthesis of the gold nanoparticles by boiling the auric solution that is reduced by the citrate molecules. The size of gold nanoparticles can be changed easily by changing the citrate concentration. The citrate capped nanoparticles are very stable, where the citrate ion can be replaced and functionalized with different ligands for specific applications. The application of gold nanoparticles in cancer cell diagnostics and therapy includes using the surface plasmon absorption band of the gold nanoparticles and also the strong scattering properties for the diagnosis of the cancer cells from the normal cells. The conversion process of energy by gold nanoparticles from photo-energy to heat energy is used for the photothermal cancer therapy. In Fig. 1, gold nanospheres with uniform size

![Absorption spectra and TEM images of gold nanoparticles that were synthesized by the chemical reduction method using trisodium citrate](image-url)
distribution $\sim 5.0 \pm 2.0 \text{ nm}$ and plasmon band at $\lambda \sim 517 \text{ nm}$ have been prepared by using trisodium citrate as reducing and capping agents (Turkevich method). 33

**Green photosynthesis of gold nanoparticles using an aqueous extract of Baker’s yeast**

One aim of the present study is the development of an improved method for derivatization of metabolites existing in an aqueous extract of Baker’s yeast (*Saccharomyces cerevisiae*). This procedure facilitates identification of metabolites in Baker’s yeast extract and its utility for the synthesis of nanomaterials. The preparation of trimethylsilylenol ethers of metabolites was performed by treatment of the extract with catalytic amounts of trimethylsilylchloride (TMSCl) and trimethylsilylimidazole to produce silylated compounds that were determined using gas chromatography mass spectroscopy. The GC/MS analysis of the culture supernatants of an aqueous extract of *Saccharomyces cerevisiae* shows the analysis of metabolites’ mass library (Fig. 2 and Table 1).

Herein, we developed a highly sensitive and selective method for the identification and quantification of the metabolites in an aqueous extract of Baker’s yeast using chemical derivatization (Scheme 1).

The GC/MS spectrum shows the retention times of 11.20, 11.82, 12.41 and 13.65 min for TMS-derivatives of butane-2,3-diol, glucose and indole-3-acetic acid, respectively, as shown in Fig. 3A and B. Table 2 shows the molecular weight in correlation with the retention time.

When the gold ions (e.g. AuCl$_3$) were added to the aqueous Baker’s yeast extract and then allowed to react under visible light for 30 minutes, it was observed that Au ions were reduced extracellularly to gold nanoparticles (Fig. 4). Au nanoparticles with uniform size distribution $13.0 \pm 0.9 \text{ nm}$ and plasmon band $\lambda = \sim 535 \text{ nm}$ are formed.

The crystalline nature of biosynthesized gold/yeast nanoparticles was further confirmed from XRD analysis. The XRD patterns of the photo-biosynthesized gold nanoparticles are shown in Fig. 5. The four intense diffraction peaks were observed at 2$\theta$ values of 38.30°, 44.43°, 64.67°, and 77.67°, corresponding to the (111), (200), (220), and (311) reflection of the crystalline metallic gold, respectively.

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**Table 1** Identified metabolites in Baker’s yeast extract

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<th>Components</th>
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<th>Compounds</th>
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<tr>
<td>90.08</td>
<td>12.84</td>
<td>Butane-2,3-diol</td>
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<tr>
<td>180.14</td>
<td>18.05</td>
<td>Glucose</td>
</tr>
<tr>
<td>186.21</td>
<td>23.39</td>
<td>Undecanoic acid</td>
</tr>
<tr>
<td>175.16</td>
<td>30.47</td>
<td>Indole-3-acetic acid</td>
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</tbody>
</table>

**Table 2** Identified TMS-metabolites of Baker’s yeast extract

<table>
<thead>
<tr>
<th>Components</th>
<th>RT</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>234.12</td>
<td>11.20</td>
<td>Butane-2,3-diol</td>
</tr>
<tr>
<td>540.24</td>
<td>11.82</td>
<td>Glucose</td>
</tr>
<tr>
<td>258.08</td>
<td>12.41</td>
<td>Undecanoic acid</td>
</tr>
<tr>
<td>319.19</td>
<td>13.65</td>
<td>Indole-3-acetic acid</td>
</tr>
</tbody>
</table>

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**Fig. 2** GC/MS spectrum of the culture supernatants of an aqueous extract of Baker’s yeast.

**Fig. 3** GC-MS chromatogram of TMS-derivatives of metabolites that occurred in *Saccharomyces cerevisiae* aqueous extract.

**Scheme 1** Trimethylsilyl (TMS) derivatisation of metabolites in Baker’s yeast extract.
In our photo reduction study, photosensitization of butane-2,3-diol and indole-3-acetic acid can be carried out.\textsuperscript{34} The yeast extract solution under visible light acts as a reducing and capping agent. The reduction of noble metal ions to nanoparticles is catalyzed through oxidation of glucose to gluconic acid by providing electrons.\textsuperscript{35,36} The formed nanoparticles can be also stabilized by the presence of van der Waals forces between the negatively charged oxygen groups present in the molecular structure of butane-2,3-diol or indole-3-acetic acid, and the positively charged groups that surround the surface of NPs\textsuperscript{35} (see Scheme 2).

Cytotoxicity towards Ehrlich ascites carcinoma cells
(in vitro study)

The cell proliferation or the death is determined by counting viable cells after staining with a vital dye. This is based on the principle that live cells possess intact cell membranes, which exclude certain dyes, such as trypan blue or eosin, while dead cells do not. Trypan blue, a diazo dye, is a vital stain which is used to selectively color dead tissues or cells blue. The live cells or tissues with intact cell membranes are not colored. Since cells are very selective in the compounds which pass through the membrane, in a viable cell, trypan blue is not absorbed; however, it traverses the membrane in the dead cells. So, dead cells are shown with a distinctive blue color under a light microscope and live cells are excluded from this staining.

In Table 3, the cytotoxicity data of aqueous yeast extract, chemically synthesized gold NPs and photo-extracellular synthesized gold NP samples against EAC cells under dark incubation for one hour and under visible light incubation for one hour is shown. The data were expressed as surviving percent compared with untreated control cells. Treatment of EAC cells with aqueous heat killed the yeast extract at increasing concentrations (0.06–1 g dl\textsuperscript{-1}), chemically synthesized Au-nanoparticles (0.06–1 mM) and photo-extracellular synthesized gold NPs (0.06–1 mM) for one hour is shown in Fig. 6. In addition, two control samples were prepared in both dark and visible light incubations.

Fig. 7 shows that the percentage of the surviving cells was decreased by increasing the concentration of the yeast extract and the highest concentration of the aqueous extract (1 g dl\textsuperscript{-1}) induced EAC cell death by 9.7% in dark incubation and by 10.6% in visible light incubation compared with the control samples. Our results are in agreement with Ghoneum et al.\textsuperscript{37} results, which showed that the ratio of cancer cells to yeast of 1 : 10 induced 21.4% of dead cancer cells by making phagocytosis of yeast into the cancer cell and thereby induction of cancer cell apoptosis via a caspase independent mechanism. Earlier studies have been shown which state that breast cancer cells (BCCs) in a culture can phagocytose latex beads and fluorescent Matrigel. That study has confirmed the phagocytic activity by BCCs when using yeast as a test organism.\textsuperscript{40} The results of this study demonstrated that phagocytosis of heat-killed Baker’s yeast induces apoptosis in the highly metastatic cells (MCF-7 and ZR-75-1) and in the non-metastatic cells (HCC70) in a caspase-independent mechanism. Moreover, this observation may have therapeutic implications. It was approved that the apoptosis of cancerous cells by heat-killed Baker’s yeast

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**Fig. 4** Shows absorption spectra and TEM images of gold nanoparticles that were biosynthesized by yeast extract after irradiation to visible light for 30 minutes (A and B); TEM images with scale bar 50 nm.

**Fig. 5** XRD pattern of biosynthesized gold/yeast nanoparticles using yeast extract after irradiation with visible light for 30 minutes.

**Scheme 2** The nature of the interaction between the charged NPs with butane-2,3-diol and indole-3-acetic acid.
is caspase-independent and reactive oxygen intermediate (ROI)-independent,\(^\text{18}\) and showed that phagocytosis of yeast lead to the disruption of the mitochondrial membrane potential. Therefore, it was suggested that the release of some or all of the other mediators such as mitochondrial proteins AIF, a mitochondrial DNA repair enzyme, NADH oxidoreductase, endonuclease G and a serine protease is responsible for the induction of the cancer cell apoptosis by the heat-killed Baker’s yeast.\(^\text{39,40}\) In both of the present bioassays, the effective concentration for 50% cytotoxicity (EC50 value) could not be determined as 50% effect was not observed in any case.\(^\text{41}\)

In the case of the cytotoxicity of the chemically synthesized gold nanoparticles towards EAC cells by using different concentrations from 0.06 to 1 mM, it is found that the percentage of surviving cells decreased by increasing the concentration of Au NPs, and the highest concentration of 1 mM induced EAC cell death by 12.5% in dark incubation and by 60.6% in visible light incubation compared with the control sample. These results are in agreement with the reported results by El-Sayed \textit{et al.}\(^\text{10}\) results which demonstrated that the gold nanoparticles besides Mie scattering have a unique property which is surface plasmon resonance (gold nanoparticles absorb light strongly and transferred to thermal energy). El-Sayed \textit{et al.} demonstrated the photo-thermal therapy for cancer cells \textit{in vitro} by making conjugation between 40 nm gold nanoparticles and anti-epidermal growth factor receptor antibodies (anti-EGFR). After labeling the cancer cells by the antibody conjugated gold nanoparticles, these conjugations were exposed to a continuous wave argon ion laser. The cancer cells need less than half of the laser energy to be killed when compared with normal cells and the normal cells are never affected during this treatment. In our experiment, we have been using 5 nm gold nanoparticles that have higher surface area than that used by El-Sayed \textit{et al.} and the conjugated cells were exposed to a halogen lamp that gave 60.0% death percentage.

However, in the case of the cytotoxicity using photo-extracellular synthesized gold NPs towards EAC cells, the percentage of surviving cells decreased by increasing the concentration from 0.06 to 1 mM. In addition, the highest concentration of 1 mM induced EAC cell death by 24.6% in dark incubation and by 86.5% in visible light incubation compared with the control sample. These results are in agreement with the reported results by El-Sayed \textit{et al.}\(^\text{10}\) and Ghoneum \textit{et al.}\(^\text{37}\) The photo-extracellular synthesized gold NPs enhance the killing of EAC cells \textit{via} photo-thermal treatment after the conjugation between the overproduction EGFR on cancer cells and biosynthesized gold nanoparticles.\(^\text{9–11}\)

In order to confirm that hypothesis, the surface plasmon absorption of the photo-biosynthesized gold nanoparticles was measured using a UV-VIS spectrophotometer with normal cells and cancer cells as shown in Fig. 8. The plasmon band of photoextracellular synthesized gold NPs that is located at 535 nm almost was not changed when added to the noncancerous cells, and cancer cells as shown in Fig. 8. The plasmon band of photo-extracellular synthesized gold NPs that is located at 535 nm almost was not changed when added to the noncancerous cells.
which means the nonbinding between gold NPs and normal cells. However, this band was red shifted (572 nm) when added to the EAC cancerous cells, which confirms the conjugation of gold nanoparticles with the anti-EGFR antibodies after incubation in cell cultures with EAC cells as reported. This homogeneous binding to the surface of the cancer type cells is allowing the enhancement of photothermal treatment of photo-biosynthesized gold NPs and induces the killing of the Ehrlich ascites carcinoma cells. Therefore, due to the chemical components in the yeast extract that work as reducing and capping agents in the cells. Therefore, due to the chemical components in the yeast extract that work as reducing and capping agents in the formation of gold nanoparticles, facilitate the binding of the formed plasmonic gold nanoparticles to the overproduction anti-EGFR antibodies on the cancer cells. In addition to the formation of gold nanoparticles and their photothermal properties, cancer cell death was also induced by the excess of yeast extract in the sample and thereby induction of the cancer cell apoptosis.

It is clear that the combination between two different treatment ways can give a third one with high affinity and a higher effect on the treatment of cancer disease in vitro and in our future work, we plan to use this new cancer treatment for in vivo study.

Conclusions

We prepared and characterized first the aqueous extract of Baker’s yeast extract sample. Then we used the previously prepared aqueous yeast extract sample for the green photosynthesis of gold nanoparticles, and after that we used the cytotoxicity method to determine the effect of the prepared samples on killing the Ehrlich cancer cells in both dark and visible light incubation in comparison with chemically prepared gold nanoparticles. Our results showed that the aqueous yeast extract decreased the Ehrlich ascites carcinoma cells by 9.7% in dark incubation and by 10.6% in visible light incubation. For the chemically synthesized gold nanoparticles, the Ehrlich ascites carcinoma cells decreased by 12.5% in dark incubation and by 60.6% in visible light incubation. However, photo-extracellular synthesized gold nanoparticle samples decreased the surviving percentage of the Ehrlich ascites carcinoma cells by 24.6% in dark incubation and by 86.5% in visible light incubation. A possible mechanism for that behavior has been proposed and confirmed.

Experimental section

Materials

Chlorauric acid (HAuCl₄) and trisodium citrate were purchased from Sigma-Aldrich. Baker’s yeast (Saccharomyces cerevisiae, S.I.LESAFFRE 59703 MARCQ-France) was used for extract preparation. Deionized water was used in all the experiments.

Characterization

UV-Vis spectra were measured using a Perkin Elmer Lambda 40 UV-Visible spectrophotometer using 1 cm path length Hellma quartz cuvettes. Transmission Electron Microscopy (TEM) images were obtained using a Joel JEM-1230 electron microscope operated at 120 kV equipped with a Gatan UltraScan 4000SP 4K × 4K CCD camera. A drop from a diluted sample dispersion was deposited onto an amorphous carbon film on 400 mesh copper grids and left to evaporate at room temperature. XRD measurements were performed using a Philips PW1710 X-ray diffractometer using Cu Kα radiation (λ = 1.54186 Å). The XRD patterns were recorded from 20 to 70° 2θ with a step size of 0.020° 2θ and collecting 10 s per step. A halogen lamp (HALOPAR 20 75 W 230 V 30° GU10, Italy) was used for the extract preparation.

Preparation of Baker’s yeast aqueous extract sample

Dissolve 5 g of active Baker’s yeast in 500 ml distilled water, heat it for 2 h at 90 °C, then filter it using 50 μm pores filter paper. Store the extract at 5 °C for the next preparation procedures.

Chemical derivatization of Baker’s yeast extract

TMS derivatives of metabolites in an aqueous yeast extract were prepared according to literature procedures. 0.05 ml of 1% trimethylsilylchloride (TMSCI) and 0.1 ml of 2% trimethylsilylimidazole (TMSI) were added to 2 ml of an aqueous Baker’s yeast extract. The reaction mixture was allowed to stir under nitrogen for 30 minutes at 60 °C. The resulting mixture was cooled and analyzed directly using GC/MS spectroscopy.

GC-MS analysis

GC-MS images for an aqueous extract of Baker’s yeast and TMS derivative sample were analyzed on a Shimadzu GC-17A. The column temperature was held at 120 °C for 2 min, then increased gradually as 20 °C per min to 270 °C and finally to the temperature reached 300 °C. The helium was used as a carrier gas with a constant flow rate. The GC was directly interfaced to a Shimadzu QP5050Aquadrapole mass spectrometer operated in the electron impact ionization mode (EI) at 70 eV with an interface temperature of 300 °C.

Chemical synthesis of gold nanoparticles (Turkevich method)

Simply, the method is a chemical reduction of gold ions by sodium citrate in aqueous solution. Sodium citrate also serves
as a capping material that prevents aggregation and further growth of the particles. 5 ml of 1% trisodium citrate solution was added to a boiling solution of 40 ml chlorauric acid (HAuCl₄) solution containing 5 mg of gold ions. The solution was boiled for 30 minutes and was then left to cool down to room temperature.

Biosynthesis of gold nanoparticles
50 ml of 1 mM HAuCl₄ solution was added to 50 ml of the yeast solution containing 5 mg of gold ions. The solution was boiled for 30 minutes and was then left to cool down to room temperature.

Cytotoxicity assay
(1) In sterile test tubes the following compounds were added (Table 4):

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<th>Tested compound</th>
<th>Tested compound tubes</th>
<th>Control tubes</th>
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</thead>
<tbody>
<tr>
<td>EAC cells</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
<tr>
<td>RPMI medium</td>
<td>800 µl</td>
<td>900 µl</td>
</tr>
<tr>
<td>Tested compound</td>
<td>100 µl (contain different concentrations)</td>
<td>—</td>
</tr>
</tbody>
</table>

(2) Cell viability was counted by trypan blue exclusion using the haemocytometer as mentioned above.

(3) Cell survival fraction = (T/C): where, T and C represent the number of viable cells in a unit volume and C is the number of total (viable + dead) cells in the same unit volume.

Cytotoxicity method with exposure to visible light:
The previous steps are repeated, but after step 4 expose the samples to a visible light lamp for 1 hour before incubating at 37 °C.

All the experiments were performed in compliance with relevant laws and the institutional guidelines were followed. The experiments were approved by the institutional committee in Higher Education Ministry – Egypt and no human subjects have been used.

Acknowledgements
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References
4 D. Hanaham and R. Weinberg, Cell, 2000, 100, 57–70.