

Gold Nanoparticles: Synthesis and Effect on Viability of Human Non-Small Lung Cancer Cells

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Abstract

Gold nanoparticles recently showed great interest for many uses including food, drug and medical applications. The algae *Undaria sp.* well known as wakame in South Asia are considered to be large edible brown algae. It provides nutritious source of dietary fiber, vitamin Bs and mineral. The present study aimed to investigate the use of *Undaria sp.* for green synthesis of metallic gold nanoparticles. The synthesized nanoparticles were characterized for physicochemical properties including size measurement and tested *in vitro* for their effect on viability of human non-small lung cancer H-460 cell line using the MTT assay. From the results, brown algae were able to chemically form nanoparticles with chloroauric acid solution possibly due to the sulphated polysaccharides found in algae. The particle sizes were found to be approximately 10 nm. The gold nanoparticles stabilized by the algae could decrease the cancer cell viability. However, the properties and biological activity of nanoparticles seemed to depend upon reaction time and temperature. Conclusively, gold nanoparticles synthesized and stabilized by the algae could decrease the cancer cell viability, thus indicating the potential of such nanoparticles for further study for anticancer activity.

Keywords

Brown Algae, *Undaria sp.*, Gold Nanoparticles, MTT Assay, Non-Small Lung Cancer Cells

1. Introduction

Over the past few decades, nanoparticles have been explored to be used in many applications. Encapsulation of nutrients or functional ingredients using nanoparticles is commonly used in food product development [1]. For medical application, nanoparticles are employed as drug carrier especially for drug targeting.

Due to the nanoscale size, nanoparticles provide the advantages in greater penetration in to the cell membrane, thus allowing better absorption and therapeutic effect of drug [2]. For gold nanoparticles, they are considered to be used as carriers for functional ingredients since they are non-toxic and biocompatible. Interestingly, they present anti-angiogenesis activity which could be advantageous for cancer treatment [3]. The synthesis of gold nanoparticles is unlikely to be difficult although some chemicals are required for synthesis. Recently, environmental friendly or green synthesis of gold nanoparticles is of great interest as an alternative to conventional synthesis. The natural ingredients which have been studied for their ability to synthesize the nanoparticles include polysaccharides such as chitosan [4]; however, the optimal condition of synthesis is needed to be investigated in order to ensure the stability of the nanoparticles.

Undaria sp. or wakame (in Japanese) is large edible brown seaweed originated from the north-western Pacific coast. It is rich in dietary fiber, vitamin Bs and minerals. It also contains sulphated polysaccharides which might be useful for eco-friendly synthesis of gold nanoparticles [5]. The objective of this study was to determine the use of *Undaria sp.* for green synthesis of metallic gold nanoparticles. The synthesized nanoparticles were characterized for physicochemical properties and tested *in vitro* for their effect on viability of human non-small lung cancer H-460 cell line.

2. Material and Methods

For the nanoparticle synthesis, chloroauric acid (HAuCl_4) was obtained from Sigma-Aldrich (USA), and brown algae *Undaria sp.* was purchased as a commercial product from Otomegusa Company, Japan. For cell viability test, human non-small lung cancer cells (H460) was obtained from The American Type Culture Collection (ATCC), USA. Roswell Park Memorial Institute (RPMI) medium supplement with 10% fetal bovine serum was from Invitrogen (USA). L-Glutamine and antibiotics were from Invitrogen (USA). The MTT or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and dimethylsulfoxide (DMSO) were supplied from Sigma-Aldrich, USA.

2.1. Gold Nanoparticles Synthesis

A required weight (0.02 - 0.12 g) of pulverized algae was mixed with 50 mL of ultrapure water. The mixture was added with 3.45 μL of 1.4 M HAuCl_4 solution. Gold nanoparticles formation was carried out at different reaction temperature (25°C - 80°C) and time duration (60 - 120 min) with stirring condition. The solution was then filtered using filter paper, Whatman No. 5 to remove algae particles. The final concentration of algae in formulation was 0.04% - 0.24% w/v. The concentration of gold ion in final solution was 0.966 μM (20 ppm).

2.2. Characterization of Gold Nanoparticles

The characteristic peak of gold nanoparticle was observed using a UV-Visible spectrophotometer (UV-160A, Shimadzu, Japan). The morphology of nanopar-

ticles was examined by using JEM-2100 Transmission Electron Microscopy (TEM) (JEOL, Japan). The gold diameter and size distribution were calculated using SemAfore software, JEOL (Japan). Energy-Dispersive X-ray spectroscopy (EDX) measurement was also studied for elemental analysis of the samples. The surface potential of gold nanoparticles was assessed using a ZEN 3600 zetasizer (Malvern Instrument, UK).

2.3. *In vitro* Cell Viability Test

The MTT assay was used to evaluate cell viability of human non-small lung H460 cancer cells. The 2×10^4 H-460 cells were grown in 96-well plate, 100 μ L media was added, and the cells were cultured for 24 hours at 37°C in a humidified 5% CO₂ atmosphere to allow cell growth. The cells were then incubated for 24 hours with either 100 μ L of algae-stabilized gold nanoparticles or 100 μ L of algae solution, while 100 μ L of ultrapure water was used as a control. After incubation, the culture media was discarded and the cells were washed with phosphate buffered saline (PBS). Serum-free media (100 μ L) was added along with 2 μ L of 20 mg/mL of MTT in DMSO and the culture was incubated for another 4 hours at 37°C. The culture media was discarded and the purple formazan was dissolved in 100 μ L DMSO. The UV absorbance of the sample was measured using an Anthos HT II microplate reader (Anthos Labtec Instruments) at the wavelength of 570 nm.

2.4. Statistical Analysis

The percentage of cell viability was expressed as average \pm S.D. ($n = 3$) and statistically significant difference was determined using Student's t-test at p-value ≤ 0.05 .

3. Results

3.1. Gold Nanoparticles Synthesis

The algae were able to reduce the gold solution into gold nanoparticles. The appearance of rather pink solution and the UV absorbance peak of around 530 - 535 nm represented the occurrence of gold nanoparticles [6]. It has been reported that reducing sugars and polysaccharides constituted in algae may facilitate the formation and stabilization of the gold particles [7].

The optimal condition for gold nanoparticle synthesis using wakame algae were studied in terms of algae concentration, reaction time and temperature. It was found that at higher algae concentration resulted in lower absorbance of gold nanoparticles (systems A1-A4 in **Table 1**). The higher in absorbance is related to an increase in a number of nanoparticles synthesized. The effect of reaction time was investigated at constant algae concentration (0.04% w/v) and reaction temperature of 80°C. The result indicated the higher absorbance of gold nanoparticles with increasing time duration of reaction from 60 to 120 min (systems B1-B3 in **Table 1**). Moreover, at 0.04% w/v algae and reaction time of 60 min, the absorbance of nanoparticles was greater if the reaction temperature was raised from 25°C to 80°C (systems C1-C3 in **Table 1**).

Table 1. Variable in algae concentration, reaction time and temperature on gold nanoparticle synthesis using wakame.

Systems	Algae concentration (% w/v)	Reaction time (min)	Reaction temperature (°C)	Absorption peak (nm)	Absorbance
A1	0.04	60	80	533.5	0.195
A2	0.08	60	80	532.0	0.171
A3	0.16	60	80	534.0	0.123
A4	0.24	60	80	533.0	0.118
B1	0.04	60	80	533.5	0.195
B2	0.04	80	80	533.0	0.203
B3	0.04	120	80	533.0	0.207
C1	0.04	60	25	533.0	0.111
C2	0.04	60	60	533.0	0.177
C3	0.04	60	80	533.5	0.195

3.2. Characterization of Gold Nanoparticles Stabilized by Algae

The gold nanoparticles synthesized at 0.04% w/v algae and 80°C were determined for morphology by TEM analysis. The overall shape of gold nanoparticles synthesized was spherical (Figure 1). The average particle size of gold nanoparticles formed in 60 min was 7.85 ± 1.43 nm (Figure 1), while that of 120 min was 10.15 ± 1.96 nm. It was found that when reaction time was increased, larger particles were formed. These results were expected since longer reaction time might allow gold nanoparticles to grow in size. The EDX spectrum of gold nanoparticles synthesized is illustrated in Figure 2. The spectrum possible indicated that the nanoparticles contained might be the gold (Au) element based on 2.8 KeV EDX peak and surface potential. The copper (Cu) spectrum at 7.8 KeV EDX peak and surface potential referred to the copper grid used in the TEM analysis. For the surface potential of nanoparticles, it was found that gold nanoparticles contained negative surface potential indicating that there were negatively charged molecules surround the particles (Table 2). It was likely that sulphated polysaccharides containing negative charges should be around the particle [8]. Hence, nanoparticles are stabilized by electrostatic repulsive force arising from the negative charges on the particle surface [9].

3.3. Cell Viability Test

The effect of gold nanoparticles on viability of human non-small lung H460 cancer cells was determined using MTT assay. The algae-stabilized gold nanoparticles synthesized using 0.04% w/v algae, 80°C and 120-min reaction caused the reduction in cell viability with increasing concentration of the nanoparticles (Figure 3). The toxicity of gold nanoparticles toward the cancer cells apparently was dependent on the synthetic conditions. At the same algae concentration and reaction temperature, an increase in reaction time caused less cellular toxicity (Figure 4(a)). The non-significant differences in cell viability of nanoparticles were found when the reaction temperature was changed from 25°C to 80°C at

120-min reaction and 0.04% w/v (**Figure 4(b)**). However, the nanoparticles seemed to be more toxic to the cancer cells than the corresponding algae solutions. The ability of gold nanoparticles to decrease the cell viability is considered to be arisen from the functional polysaccharides especially the sulfated-based polysaccharides in algae [10].

Table 2. Surface potential of gold nanoparticle synthesized by algae.

System	Algae concentration (% w/v)	Reaction time (min)	Reaction temperature (°C)	Surface potential* (mV)
A1	0.04	60	80	-37.6 ± 1.54
A2	0.08	60	80	-34.6 ± 0.60
A3	0.16	60	80	-33.0 ± 0.36
A4	0.24	60	80	-42.5 ± 1.55

*mean \pm S.D. ($n = 3$).

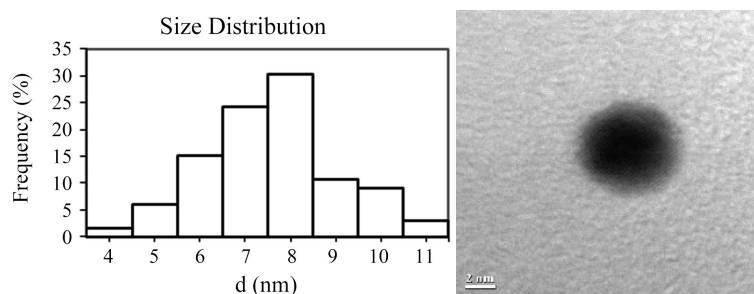


Figure 1. Size distribution (left) and morphology (right, scale bar of 2 nm) of gold nanoparticles synthesized at 0.04% w/v algae, 80°C and 60-min reaction. The average diameter (d) of nanoparticles is 7.85 ± 1.43 nm.

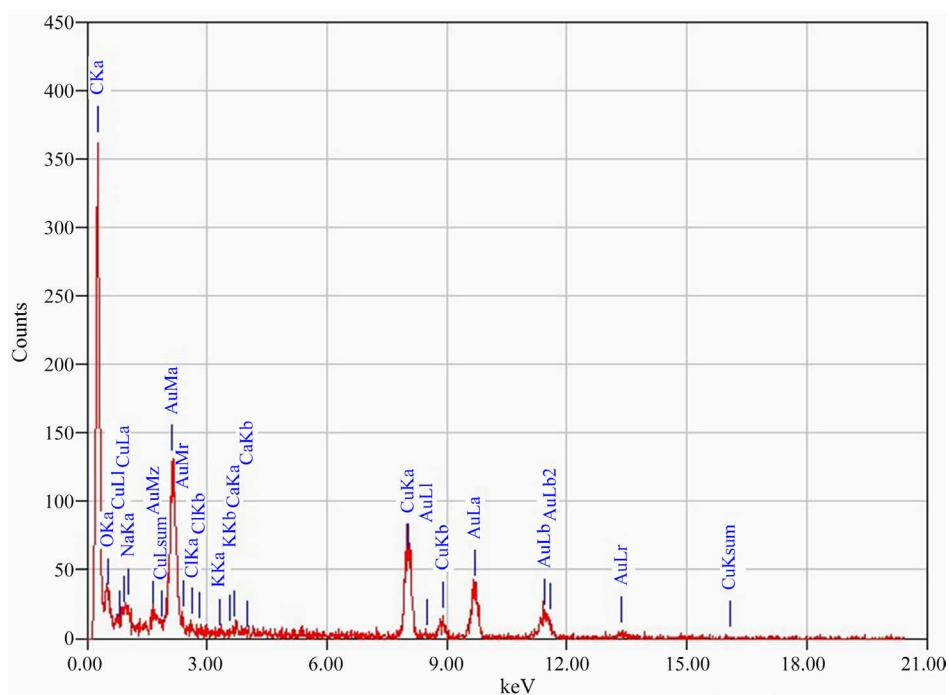


Figure 2. EDX spectrum of gold nanoparticles synthesized at 0.04% w/v algae, 80°C and 120-min reaction.

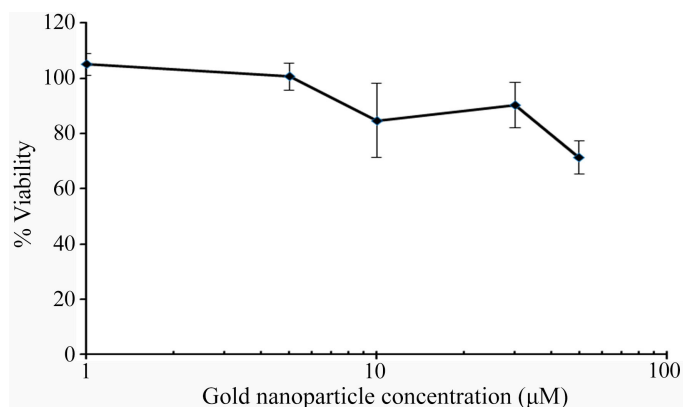


Figure 3. Cell viability after exposure to gold nanoparticles synthesized at 0.04% w/v algae, 80°C and 120-min reaction. The values represent as mean \pm S.D. ($n = 3$).

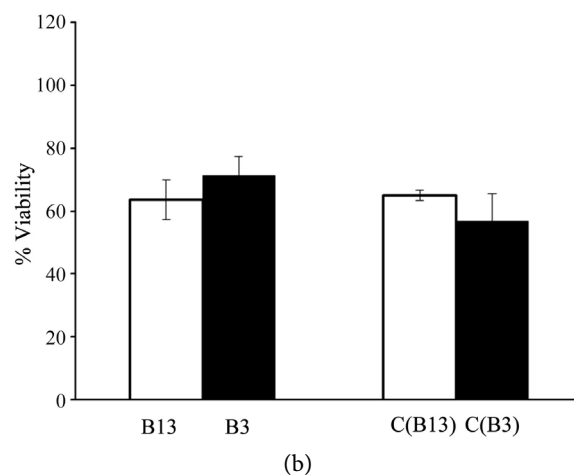
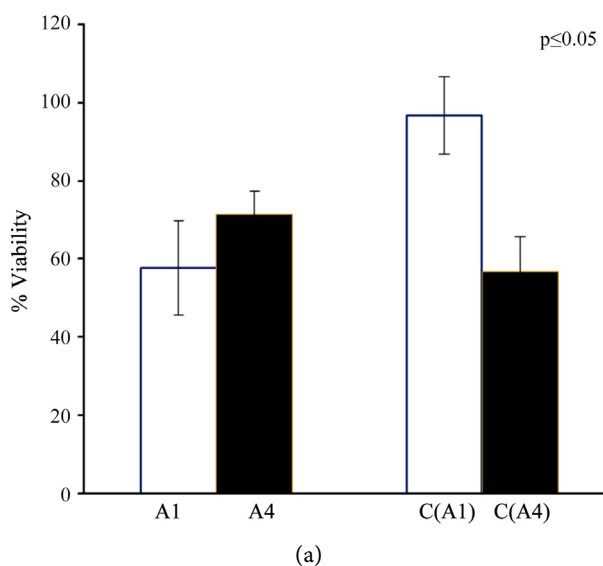


Figure 4. Cell viability (mean \pm S.D. of $n = 3$) after exposure to gold nanoparticles synthesized using (a) different reaction time (A1: 60 min, A4: 120 min) at 0.04% w/v algae, 80°C; and (b) different reaction temperatures (B13: 25°C, B3: 80°C) at 0.04% w/v, 120-min reaction. The corresponding algae solutions were also tested and represent as C(A1), C(A4), C(B13) and C(B3).

4. Discussion

The nanoparticle physical properties and cell toxicity characteristics usually differ and depend on the chemical method used and the biological source of organism to synthesize gold nanoparticles. Due to very slow and limited nanoparticle synthesis, microscopic TEM images with more nanoparticles could not be obtained. In our feasibility experiment, the presence of complex structure of lignan polysaccharides in algae affected the chemical synthesis of nanoparticles and possibly stabilized these gold nanoparticles. However, these claims need more investigations to justify the different morphologies of the nanoparticles synthesized with different algae contents.

The synthesized nanoparticles size was well below 15 nm by TEM analysis and composition was uniformly distributed high contents of gold particles by EDX analysis. Unique negative polysaccharide rich algae offered a great opportunity of negatively charged gold nanoparticle synthesis. The cell viability tests clearly exhibited the chances of gold nanoparticles to cause toxicity to cancer cells in cultures. The increased temperature and more reaction time both enhanced the cell toxicity to cancer cells in culture. However, algae cells stabilized the cell viability.

Recent reports on gold nanoparticle biosynthesis in algae, seaweeds continuously indicated the tremendous potentials with new possibility of these nanoparticles in novel uses including biotechnology, drug delivery and antibacterial potentials. Our major focus is continued use of biosynthesized gold nanoparticles in therapeutic oncology to capitalize them to arrest tumorigenesis, lesions, scars etc. by photodynamic, photothermal therapy based on concept that gold nanoparticles absorb incident photons and convert them to heat to destroy cancer cells [11]-[17]. The synthesis of gold nanoparticles is difficult from algae although selected chemicals initiated the gold nanoparticle synthesis. The major disadvantages of the current methods are that these only give evidence in expansive experiments while large scale gold synthesis from algae is inconclusive.

5. Conclusion

The brown algae *Undaria sp.* was able to use for preparation of gold nanoparticles. The factors influencing the synthesized nanoparticles were the algae concentration, reaction time and temperature. The algae-stabilized nanoparticles were spherical in shape with the negatively charged surface potential. The gold nanoparticles exhibited the inhibitory effect on cell viability which could imply the potential of nanoparticles for further study as carrier for anticancer purpose.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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