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Effect of Glutathione-Stabilized Gold Nanoparticles in 3T3 Fibroblast Cell

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Abstract. Gold nanoparticles (AuNPs) have been used in several biomedical applications such as biomolecule detection, molecular imaging enhancer, drug carrier etc. Their biocompatibility and high surface area ratio are the main advantages of AuNPs to apply *in vivo*without toxicity to the cells. Moreover, glutathione is well-known for its anti-oxidant properties such as cosmetic purpose and reduce cancer progression. In this study we established a combination between gold nanoparticle and glutathione to demonstratepossibility to use glutathione-capped AuNPs that take advantages of both antioxidant and high surface ratio properties. Our results obtained a protocol for generation of highly stable glutathione-stabilized AuNPs that had an average diameter of 9 nm. Reactive oxygen species generated by 3T3 fibroblast cell line after exposure to variousconcentrations of glutathione-capped AuNPs were lower than those of cells treated by non-glutathione AuNPs and glutathione alone. The results suggest thatglutathione-capped AuNP is an effective system to reduce oxidation stress in cells.

Keywords: Glutathione, Gold nanoparticles, oxidative stress, anti-oxidant, nanomedicine,

1. Introduction

Nanotechnology is the study of manage, construct, analyst and application of material that have diameter from 1 to 100 nanometers[1]. Nanotechnology can develop new material such as nanomaterial (nanotube, nanorod and nanoparticles) with different physical, chemical and biological properties. As a result, nanotechnology have been applied in various filed like microchip technology, medical and science research. Gold nanoparticles (AuNPs) is a nanomaterial that normally stay in red colloidal solution[2]. Generally, synthesis of AuNPs use $HAuCl_4$ solution (Au^{3+}) with a reducing agent to convert gold ion into Au^0 , then further be supplied with a stabilizer to prevent aggregation by surrounding the nanogold particles. Sodium citrate is commonly used as both stabilizer and reducing agent [3].

AuNP surface can be modified by various types of biomolecule such as DNA, protein and drugs [4-6]. Moreover, AuNPs is widely used for drug delivery system due to biocompatibility properties and high surface area ratio [7]. Highly surface area increase drug density on the surface allowed higher concentration of drug or multiple drug loaded on single particles [7, 8]. Currently, there are numerous reports of AuNPs application as drug carriers for cancer treatment that yield much better results, compared with the standard anti-cancer drug treatment [9-11].

Glutathione is a tripeptide molecule. It is one of important biological antioxidants, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides [12].

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Glutathione is widely used as a supplement in numerous diseases such as cancer, AIDS, sepsis, trauma for regulation of nitrogen balance that involve in survival rate and disease progression [12-14]. In addition, antioxidant properties of glutathione has been increasingly used for recent cosmetic application [15].

In this study, we propose a new perspective combination of the antioxidant properties from glutathione and the AuNPs as effective biomolecule carriers. Glutathione attaching on the surface of AuNP results in increase of nanoparticle stability compared with citrate-stabilized AuNPs. Serial reduction of oxidative stress in 3TC fibroblast cell line is observed after exposure with glutathione-capped AuNPs.

2. Materials and method

2.1. Glutathione capped - gold nanoparticles (GSH-AuNPs) synthesis

Synthesis of gold nanoparticles was described previously by Low and Bansal [16]. Briefly, 1ml of 0.1M $HAuCl_4$ was added in 500ml ultrapure water and stirring vigorously. 0.06g $NaBH_4$ in 10ml ultrapure water was added using a pipette, 10 mL of $NaBH_4$ solution was transferred dropwise to the flask. The solution in the flask should change from yellow to ruby red in colour. The ruby red colour indicates the formation of gold nanoparticles. After stirring for 30 minutes, 0.1 g of glutathione was added in AuNPs solution. With continually stir for 1 hour, AuNP solution turns to purple indicating the larger size of particles due to glutathione capped. The nanogolds were proceeded to centrifugation at 4900 rpm for 10 minutes, then the supernatant was removed and the precipitant was resuspended in 0.1x PBS.

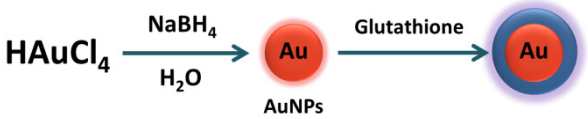

Fig. 1: summary diagram for production of glutathione-coated gold nanoparticles. NaBH4 is used as the reducing agent and glutathione acts as nanoparticle stabilizer.

2.2. Characterized gold nanoparticles

Gold nanoparticles were measured the particle size and zeta potential before being capped with glutathione by zetasizer machine (Malvern). Light absorption measured by UV spectrophotometer at wavelength 400-800nm before and after glutathione capped. Results and discussion

2.3. Reactive oxygen species generated by 3T3 fibroblast cell line

Six isolates of 3T3 fibroblast cell line culture in DMEM media were investigated. Cell culture 3T3 fibroblast cell line was obtained from the American Type Culture Collection (Rockville, MD) and was cultured in DMEM medium supplemented with 10% (v/v) FBS, 100 mg/l of streptomycin and 100,000 U/l of penicillin G at 37◦ C in 5% CO_2 incubator

2.4. Measurement of reactive oxygen species (ROS) generation by 3T3 fibroblast cell line

Intracellular reactive oxygen species (ROS) production was measured in both AuNP-treated and control cells using DCFH-DA (Molecularprobes, USA). Briefly, 10^4cells/well were exposed to citrate-stabilized AuNPs and glutathione-stabilized AuNPs with various concentrations (3.125, 6.25, 12.5 ppm AuNPs) and different incubation times (10, 20, 40, 50 min). After incubation, cells were washed twice with PBS. Treated and control cells were resuspended in 0.5 ml PBS containing 0.1 mM DCFH-DA at 37◦C for 30 min and then incubated with calcium oxalate (as positive control for ROS production) at 37◦C for 30 min. ROS production of cells were subjected to evaluate by luminescence spectrophotometer (Perkin-Elmer, MA).

3. Results and discussion

3.1. Characterization of Gold Nanoparticles

The sodium borohydride-stabilized gold nanoparticles were characterized by UV-visible spectrophotometry method and Zetasizer machine. The result of absorption spectrum of gold nanoparticles is illustrated in figure 2. The plasmon wavelength (λ_{max}) indicates the size distribution of AuNPs that are in

their unique properties. In this study, λ_{max} is approximately 520 nm and shift to 540 nm after being capped by glutathione. These values are in accordance with the colors of AuNP solution observed by naked eyes. These absorption values of gold nanoparticles are similar with other different synthetic methods, reviewed by Daniel [17]. The red shift in λ_{max} is associated with an increase of diameter of gold nanoparticles and size distribution

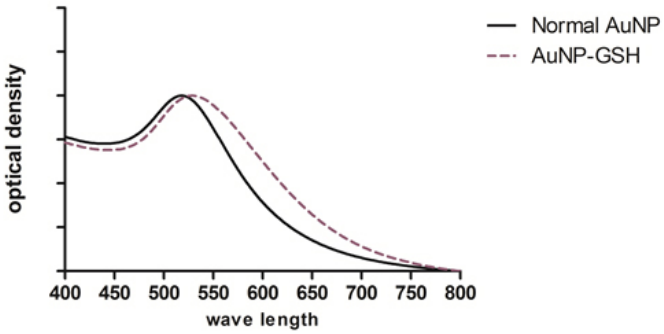

Fig. 2: Spectrophotometry results reveal the shift of maximal absorption peak to 540 nm after surrounding gold nanoparticle surface with glutathione, while the citrate-capped AuNPs show 520 nm maximal absorption peak.

For further characterization of gold nanoparticles, a zeta potential and particles size measurement were done by zetasizer machine. The average gold nanoparticle size is approximately 9.2 nm (Figure 3A) and zeta potential value is -26.8mV (Figure 3B). The zeta potential revealed the stability of colloidal gold in solution. According to the guideline [18], optimum line between stable and unstable colloidal solution is generally taken either more than +30mV or lesser than -30mV. Particles with zeta potentials more positive than +30mV or more negative than -30mV are normally considered stable.

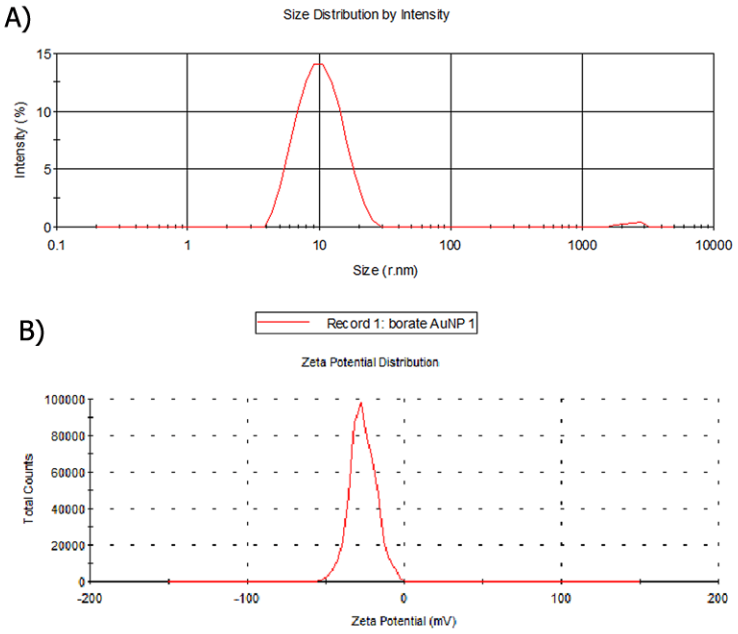

Fig. 3: A) Zetasizer reveals that average size distribution of gold nanoparticles is 9.2 nm. B) zeta potential results showed that the average zeta potential is -26.8mV.

3.2. Reactive oxygen species generated by 3T3 fibroblast cell line after exposure to glutathione-capped AuNPs

The intracellular ROS generation of cells was investigated using the 2',7'-dichlorfluorescein-diacetate (DCFH-DA) (Molecularprobes, USA) as a well-established compound to detect and quantify intracellular

produced H_2O_2. The ability of various concentration of AuNP-treated 3T3 fibroblast cell to generate reactive oxygen species (ROS) was investigated.For all 3T3fibroblast cells, a linear changing fluorescent activity could be observed depending on the incubation time with DCFH-DA and the concentration of treated AuNP. By using a calibration curve, the measured fluorescence signals were converted to % ROS generation compared with the non-AuNP treatment group at different time periods in Figure 4. In glutathione-AuNP treatment (Figure 4A), the % ROS generation showed a linear decrease and invert proportional dependence on exposure time. Interestingly, the results were reproducible on all 3 different selected AuNP concentrations witha fixed incubation period of 10, 20, 40, 50 min at 37°C. Especially at the 3.125 ppm AuNP group, ROS level is significantly lower than those of glutathione treated group. On the other hand, in the citrate-AuNPs treatment group (Figure 4B), The changes of % ROS generation upon the time were inconclusive.

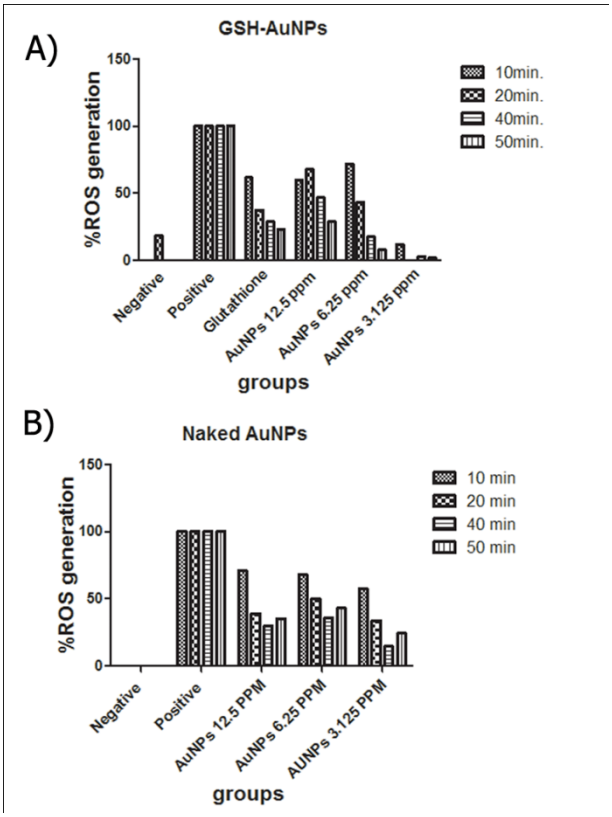

Fig. 4 : A) % ROS generation of glutathione capped gold nanoparticles (GSH-AuNPs). B) % ROS generation of citrate-gold nanoparticles (AuNPs) in 3T3 fibroblast cell line.

In conclusion, we here generated the glutathione-stabilized AuNP at the average diameter 9 nm, the stability of this AuNP reveal optimal value after tested by zeta potential. The % ROS generation in 3T3 fibroblast cell showed that even at as very low concentration as 3.125 ppm of glutathione-AuNP treatment, the ROS generation was considerably decreased compared to both glutathione-alone treated group and non-glutathione AuNP group. This shed the insight of future purpose of this promising AuNPs as a new tool for ROS control in biomedical application.

4. Acknowledgements

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