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INTRODUCTION

Gold nanoparticles (AuNPs) are referred to gold particles in nanoscale size ranging from 1 to 100 nm. AuNPs show the optical properties which are conquered by collective oscillations of electrons (plasma oscillations) that are in resonance with the incident electromagnetic radiation [1]. AuNPs are considered as non-toxic and are attractive to be served as carriers for drug delivery. The surface modification of AuNPs can be designed for tailor-made AuNPs for drug targeting purpose [1]. According to the nanoscale size of AuNPs, they are capable to penetrate into the cells easily [2]. The extent of cellular uptake by AuNPs can be improved by modification of several types of stabilizing ligands surrounding the AuNPs. However, the toxicity of AuNPs might depend upon the types of stabilizers hence cellular toxicity of AuNPs should be determined prior to clinical use [3].

Interestingly, some recent reports revealed that AuNPs combined with sodium hyaluronate were considered to be a candidate for treatment of neurological disorders [4]. AuNPs were able to reduce the oxidative DNA damage, pro-apoptotic markers (cleaved caspase-3, cytochrome c leakage) and tumor necrosis factor in brain injured mice [4]. Accordingly, AuNPs shows the ability to be further developed for prevention or therapeutics for neuronal diseases. In this study, the citrate-stabilized gold nanoparticles (AuCi) and polyethyleneimine-stabilized gold nanoparticles (AuPEI) were synthesized and determined for physicochemical properties. The toxicity of both AuCi and AuPEI in human IMR-32 neuroblastoma cells were evaluated.

MATERIALS AND METHODS

Materials

For nanoparticle synthesis, hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4.3H_2O$), trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) and polyethyleneimine (PEI,-$(CH_2CH_2NH)_n$-, MW = 750 KDa) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Glassware were cleansed with aqua regia ($HCl:HNO_3$ = 3:1, Carlo Erba, Milan, Italy) for the purpose of circumventing gold contamination, rinsed with ultrapure water and oven dried prior to use. For cell line experiment, IMR-32 cells were obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA. Dulbecco's Modified Eagle Medium (DMEM), Ham F-12 medium, fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin antibiotics and PrestoBlue assay kit were all purchased from Gibco (Invitrogen), Carlsbad, CA, USA. Phosphate buffer saline (PBS) was purchased from Sigma-Aldrich, St. Louis, MO, USA.

Synthesis of gold nanoparticles

For synthesis of 200 ppm (1,015 μM) AuCi, the chemical reduction method was used. The thirty-five microliters of 30% (w/w) hydrogen tetrachloroaurate trihydrate were added to 49.5 mL ultrapure water. The sample was then heated up to 90°C in a water bath with stirring. Then, 0.5 mL of 0.4 M trisodium citrate dihydrate was added in to the gold solution and the mixture was continued heating until the color became dark red. In addition, AuPEI at the same concentration of AuCi was synthesized using polyethyleneimine as a polymeric stabilizer. The same procedure as mentioned above was used for synthesis AuPEI except for 0.5 mL of 0.36 M PEI solution was used instead of the citrate solution. The synthesized AuCi and AuPEI were stored at 3-5°C in a light-protected container until use. For *in vitro* toxicity studies, the freshly prepared AuNPs were used.

Characterization of gold nanoparticles

The appearance and color of AuNPs were visually observed. The surface plasmon resonance (SPR) band of AuNPs was characterized by using UV-visible spectrophotometer (Biomate, Thermo Fischer, USA). The UV quartz cuvette with a path length of 10 nm was used and the ultrapure water was a reference standard for measurement. The colloidal surface charge of AuNPs was determined by measurement of zeta potential (Zetasizer NanoZS, Malvern,UK).

Cell line and cell culture

Human IMR-32 neuroblastoma cell line was cultured in a 1:1 mixture of DMEM and Ham's F12 medium with 10% (v/v) fetal bovine serum, 2 mM L-glutamine and antibiotics (50 µg/mL penicillin, 50 µg/mL streptomycin). Cell culture was maintained in an atmosphere with 5% CO_2 at 37°C and medium was refreshed every three or four days with subculturing.

Cytotoxicity test of gold nanoparticles

In order to verify cytotoxicity of AuNPs on neural cells, IMR-32 cells were incubated in the presence of AuCi and AuPEI for different incubation times and determined for cell viability by PrestoBlue assay. The cells were seeded in 96-well tissue culture plate at a density of 1.5 x 10^4 cells per well in 200 µL of assay medium and incubated overnight at 37°C under 5% CO_2 atmosphere. After that, the cells were treated with 10 µL of AuNPs at varying concentrations (0, 0.01, 0.05, 0.1, 0.25, 0.5, 1, 5, and 10 µM) and incubated for 30, 60, 90 min and 24 h. The cells were then washed with PBS and incubated with PrestoBlue reagent in culturing medium for an hour. Finally, cells were subjected to a fluorescence microplate reader to measure signal at emission wavelength of 590 nm and excitation wavelength of 560 nm. The percentage of cell viability was calculated [% cell viability = 100 x (treated cells / untreated cells)] and reported as an average of 3 individual experiments. The treated cells in a 96-well plate were also visualized for cell morphology under the fluorescence microscopy (Nikon TE2000, Japan).

RESULTS

Characterization of gold nanoparticles

The UV absorbance spectra represented the SPR characteristic of AuNPs. Figure 1 shows the peak of maximum absorption wavelength (λ_{max}) of AuPEI and that of AuCi. The λ_{max} of AuPEI and AuCi were in around 528 ± 1 nm and 520 ± 1 nm, respectively. The finding was correspondent to the characteristic peak of appearance of spherical AuNPs [5]. In fact, the absorption peak was in association with the particle size and concentration. The changes in absorbance wavelength could imply the aggregation or the asymmetric shape of the particles.

The surface charges of AuNPs were studied in order to observe the type of charges surrounding the particles. From the results, AuCi presented the zeta potential of -33.22 ± 0.83 mV (n=3). The negative value of zeta potential represents the anionic citrate stabilizer around the particles. In contrast, positive charges from PEI surrounded the nanoparticles yielded the zeta potential of AuPEI to be 25.82 ± 0.49 mV (n=3). Generally, the charged nanoparticles are considered stable owing to the electrostatic repulsive force [6]. The stability of the AuPEI could also result from the steric stabilization of a polymer chain [7].

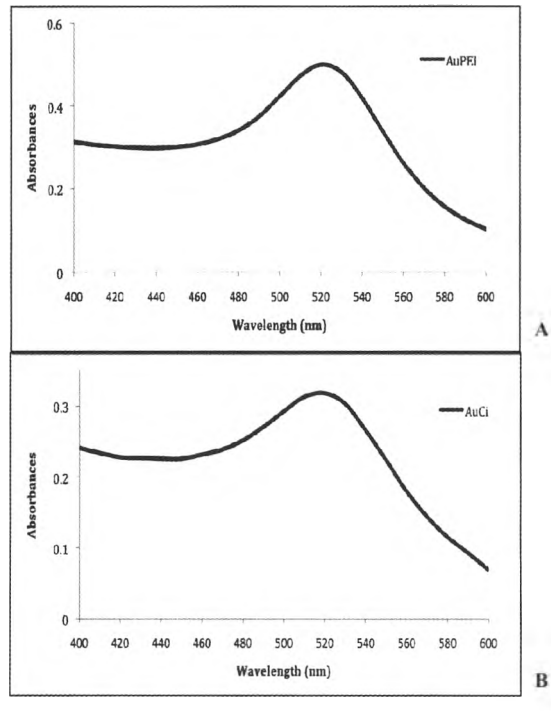

Figure 1: UV-visible absorption spectra of AuPEI (A) and AuCi (B)

Cytotoxicity assay

The cytotoxic effect of AuNPs on human IMR-32 neuroblastoma cells was studied by incubating the cells with a series of AuNP concentrations for different exposure times. The cell viability was determined using fluorescent dye in PrestoBlue assay. From the results, the cell viability seemed to be in dose-dependent and time-dependent manners (Figure 2). The slightly less percentage of cell viability after incubation with AuCi (Figure 2B) was observed when compared to the cells treated with AuPEI (Figure 2A). However, at concentrations lower than 0.5 μM, AuCi seemed not to affect the survival of IMR-32 cells (>80% viable cells). The change in cell morphology after treated with AuNPs was observed and the results were shown in Figure 3. The findings were in agreement with those obtained from cell viability. Only few amount of cells survived when co-cultured with AuCi (Figure 3C) and the cell shrinkage was seen representing the damage of neuronal cell structure as compared to untreated control cells (Figure 3A) and cells treated with AuPEI (Figure 3B).

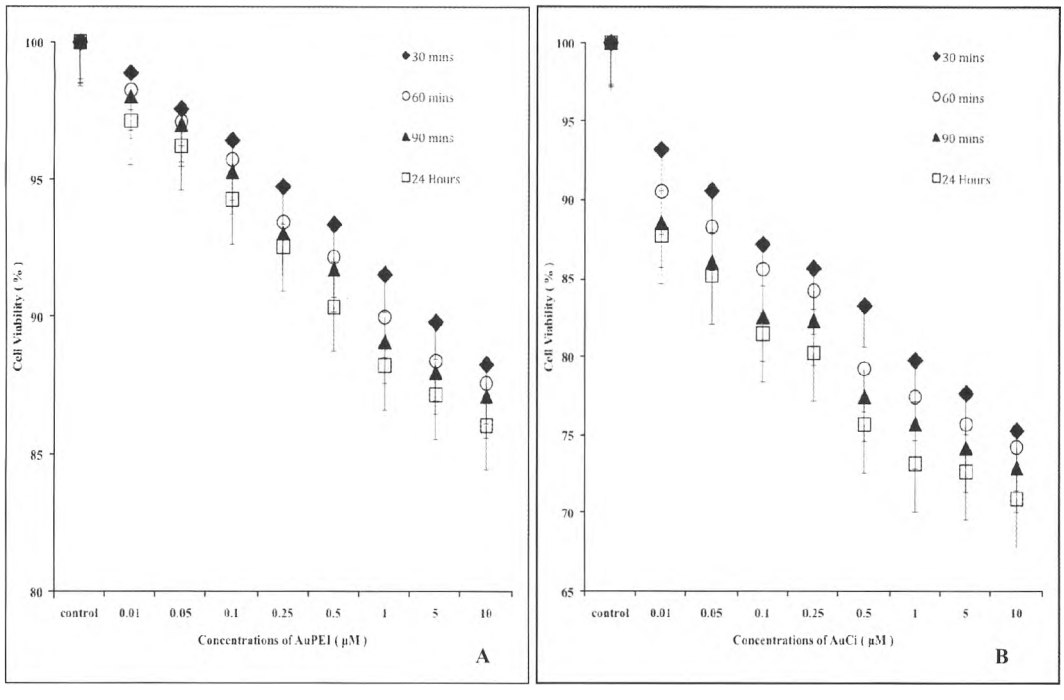

Figure 2: Percentage of cell viability of IMR-32 cells treated with different concentrations of AuPEI (A) and AuCi (B) for incubation times of 30 minutes, 60 minutes, 90 minutes and 24 hours. Results are expressed as mean ± S.D. (n = 3).

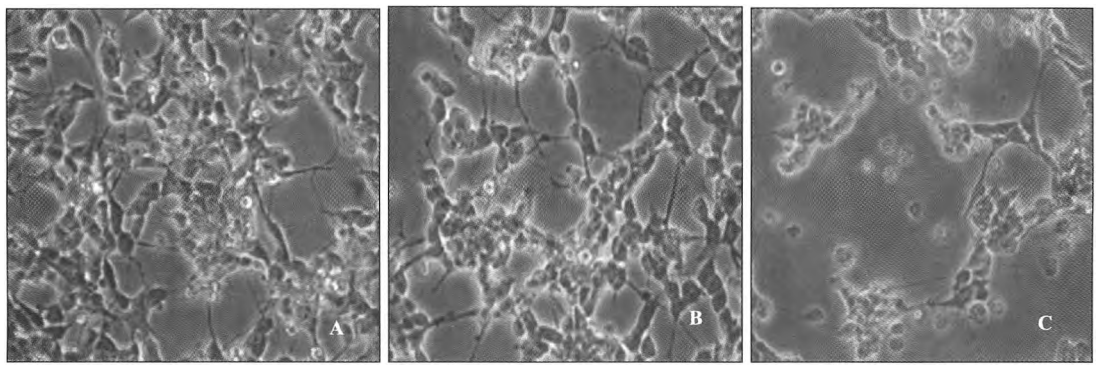

Figure 3: Illustration of cell monolayer of IMR-32 grown in medium (A), co-cultured with 10 μM AuPEI (B) and 10 μM AuCi (C), for 24 hours.

DISCUSSION AND CONCLUSION

In this study, AuNPs stabilized by either citrate or polyethyleneimine were synthesized by chemical reduction method and were evaluated for their toxicity to human IMR-32 neuroblastoma cells. The results indicated that the negatively charged AuCi were more toxic to the neuronal cells than the positively charged AuPEI. Generally, the nanoparticles could efficiently permeate into the cells *via* endocytosis. The charged particles in particular positive charge molecules are prone to readily attach to the cell membrane which is constituted by negatively charged phospholipid bilayer [3]. The finding in this study was unlikely to be in agreement with the explanation based on electrostatic force of interactive molecules. However, the types of cancer cells could possibly cause the differences in cell viability after treatment with AuNPs. The human leukemia cell line (K562) which is considered less fragile than neuroblastoma cells could survive after exposure to AuCi at a high concentration as 250 μM [8]. In addition, there was evident that some specific proteins in the neural system more interacted with the negatively charged AuNPs [9]. Apart from the differences in cancer cells, the properties of synthesized AuNPs cannot be negligible. The size and morphology of the nanoparticles should be considered for cell toxicity and will be further studied.

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