ORIGINAL RESEARCH

Irradiation stability and cytotoxicity of gold nanoparticles for radiotherapy

Xiao-Dong Zhang[1]
Mei-Li Guo[2]
Hong-Ying Wu[1]
Yuan-Ming Sun[1]
Yan-Qiu Ding[1]
Xin Feng[1]
Liang-An Zhang[1]

[1]Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, People's Republic of China; [2]Department of Physics, Tianjin Institute of Urban Construction, Tianjin, People's Republic of China

Abstract: Gold nanoparticles are promising as a kind of novel radiosensitizer in radiotherapy. If gold nanoparticles are shown to have good irradiation stability and biocompatibility, they would play an important role in radiotherapy. In this work, we investigated irradiation effects of gold nanoparticles under 2–10 kR gamma irradiation and cytotoxicity of gold nanoparticles with human K562 cells by using Cell Titre-Glo™ luminescent cell viability assay. The results revealed that gamma irradiation had not induced any obvious instability and size variations in gold nanoparticles. We found that gold nanoparticles showed excellent radiation hardness with an absorbed dose conversation factor of 9.491 rad/R. Meanwhile, the surface plasmon resonance of gold nanoparticles was enhanced obviously after 2–10 kR gamma irradiation. Subsequently, cytotoxicity tests indicated that the extremely high concentration of gold nanoparticles could cause a sharp decrease in K562 cell viability, while the low concentration of gold nanoparticles had no obvious influence on the cell viability. Our results revealed that gold nanoparticles were stable under high-energy ray irradiation and showed concentration-dependent cytotoxicity.

Keywords: gold nanoparticles, gamma ray effects, colloids, cytotoxicity

Introduction

Gold nanoparticles (NPs) have attracted increasing interest in diagnosis and therapy of disease.[1,2] Due to the strong and size-tunable surface plasmon resonance (SPR), fluorescence, and easy-surface functionalization, gold NPs have been widely used in biosensors, cancer cell imaging, photothermal therapy, and drug delivery.[3–9] Today, gold NPs have been conceived as a type of radiosensitizer in radiotherapy because the strong photoelectric absorption and second electron caused by gamma or X-ray irradiation can accelerate DNA strand breaks.[10–12] However, the advanced medical diagnoses instruments, such as X-ray, positron emission tomography, and computed tomography, are always closely related to high energy rays. Thus, the irradiation stability test for gold NPs becomes more and more important. In addition, the further cytotoxicity test for gold NPs is necessary for radiotherapy and drug delivery.

It is well known that gamma irradiation can induce defects of materials, such as color center, which is very useful for the fabrication of laser device and medical thermoluminescence dosimetry.[13–15] However, gamma irradiation can also cause obvious instability and new optical transition of materials.[16,17] Chung and colleagues showed that the Ni/SiC film was seriously unstable and could cause severe reaction in interface by gamma irradiation.[18] In the oxides, gamma irradiation can induce the oxygen-deficient centers, which can influence thermal stability and optical properties of materials.[19,20] In nanomaterials, the influence induced by gamma irradiation is

Correspondence: Xiao-Dong Zhang
Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300192, People's Republic of China
Email xiaodongzhang@yahoo.cn

being reported more often. Recently, Withers and colleagues reported that increasing the gamma irradiation dose induced an 80-fold decrease in photoluminescence in the quantum dots (QDs), which indicated that the QDs were unstable after being exposed to high energy rays.[21] The results indicated that cancer cell imaging and biomarkers of CdSe QDs should be used cautiously before applying the irradiation stability test. A similar concern for gold NPs has emerged because radiotherapy always includes a high energy ray. However, the study of the stability and optical influence of gold NPs induced by high energy gamma ray irradiation has been neglected.

In addition, a key area for nanotechnology will be the assessment of health effects and toxicity of nanomaterials. The increasing toxicity of nanomaterials such as NPs, QDs, nanowires, and nanotubes has been reported.[22] The biosafety of metallic gold is well known and it has been used *in vivo* since the 1950s. However, functionalized gold NPs have shown obvious cytotoxicity.[23] To clarify these problems, the cytotoxicity of gold NPs in human cells has been studied in detail and the results showed that gold NPs were nontoxic up to 250 mM while the ionic gold showed obvious cytotoxicity at 25 mM.[24] Similar results were also reported in the recent radiotherapy of gold NPs *in vitro*.[12] Nevertheless, a further cytotoxicity test at high gold concentrations is still essential because gold concentration in radiotherapy can be as high as 7 mg/mL.[10] Indeed, recent cytotoxicity tests showed that gold NPs were toxic at high concentrations.[25] Thus, the cytotoxicity test will be helpful for further medical applications.

This work focuses on two aspects: whether gold NPs are stable under high energy gamma ray irradiation, and whether high gold concentration can influence the cell viability *in vitro*. Both aspects are related to the application of gold NPs in radiotherapy and health care.

Materials and methods

Gold NPs are fabricated by the classical method introduced by Turkevich.[26] A volume of 100 mL of 0.01% chloroauric acid (HAuCl$_4$ · 4H$_2$O) solution is refluxed and 5 mL of 1% sodium citrate solution added to the boiling solution. The reduction of gold ions by the citrate ions is completed after 5 min. The solution is further boiled for 30 min and is then left to cool to room temperature. This method yields spherical particles with an average diameter of about 15 nm. Although the actual value of the mean size might vary slightly from each preparation, the size distribution is found to be always about 12% of standard deviation. The size and morphology of gold NPs are analyzed by high-resolution field emission transmission electron microscopy (TEM) (Hitachi HF-2000; Hitachi, Guangzhou City, People's Republic of China) operating at 200 kV. Optical absorption spectra in wavelength range of 200–850 nm are measured with DU800 Spectrometer (Beckman Coulter, Fullerton, CA, USA) in a 5 ml glass cuvette.

Gamma irradiation experiments are carried out in the Institute of Radiation Medicine, Chinese Academy of Medical Sciences (CAMS). The gold NPs are separated into four equal parts with 20 mL. Subsequently, they are irradiated by ^{137}Cs with activity of 3600 Ci and photon energy of 662 KeV. In clinical radiotherapy, the exposure dose of gamma ray is around 0.1 to 10 kR. Thus, the irradiated doses of gold NPs are arranged for 2, 4, 6, 8, and 10 kR. After gamma irradiation, the TEM and UV-Vis spectrometer were used to investigate the morphology and optical effect.

Human erythroleukemia cells (K562 cells) are cultured in RPMI-1640 medium (Sigma Aldrich, St. Louis, MO, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics (100 mg/mL streptomycin and 100 U/mL penicillin) at 37 °C in humidified atmosphere with 5% CO$_2$. The cells (in culture medium) are dispensed in 96-well plates (90 mL in each well containing 10^4 cells per well). Gold NPs of 10 μL are dissolved in culture medium and then 100 μL blending are added to each well with different concentrations (18.75–600 μg/mL). The effect of the concentration of gold NPs is assessed using Cell Titre-Glo™ luminescent cell viability assay (Promega, Madison, WI, USA). This assay is a homogenous method of determining the number of viable cells in culture based on the quantitation of adenosine triphosphate (ATP) present, which signals the presence of metabolically active cells. After the treatment, the cells are incubated with 20 μL of Cell Titre-Glo™ reagent and contents are allowed to mix on an orbital shaker in accordance with the assay protocols. This results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is proportional to the number of cells present in culture. The luminescence signal is recorded with a single tube luminometer (TD 20/20, Turner Biosystems Inc., Sunnyvale, CA, USA).

Results and discussions
Irradiation effect on gold NPs
Structural and optical properties of gold NPs under gamma irradiation
Figure 1 shows the surface morphology and size distribution of nonirradiated gold NPs, and 4 kR and 10 kR irradiated
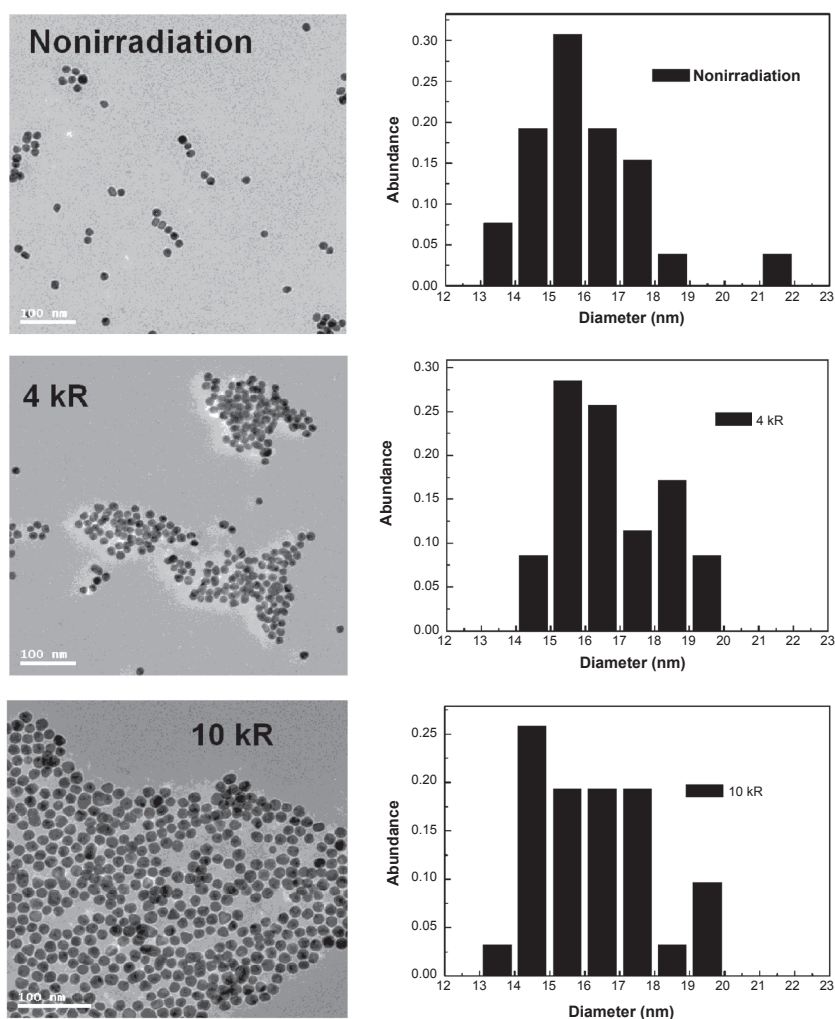
Figure 1 TEM images of the nonirradiated, 4 and 10 kR irradiated gold NPs. The corresponding size histograms are given.
Abbreviations: NPs, nanoparticles; TEM, transmission electron microscopy.

gold NPs. The shape of gold NPs is nearly spherical, and the standard deviation of size distribution is about 12% by statistical analysis, which is very close to the previous work.[27] The average diameters of gold NPs are 15.9, 16.7, and 16.1 nm, which correspond to nonirradiated gold NPs, and 4 kR and 10 kR irradiated gold NPs, respectively, which reveals that gamma irradiation has not induced obvious variation in size. Gachard and colleagues investigated the kinetics properties of KAuCl$_4$ solution with different radiation doses in detail.[28] The results revealed that gold NPs concentration increased with increased irradiation dose. Actually, the gold ions in the solution are reduced to the metallic state by reacting with hydrated electrons produced as a result of radiolysis of water by the incident gamma ray. When the dose is increased, nucleation of the gold NPs increases up to a limiting value corresponding to the total reduction of the solution. Thus, we can deduce that the slight increase in size may originate from

a chemical reaction in rudimental auric solution induced by the irradiation. In addition, we notice that the nonirradiated gold NPs are well dispersed, although slight aggregation is inevitable. Gamma irradiation can induce the aggregation of gold NPs on small scale. Especially, the aggregation of gold NPs irradiated by 10 kR are more obvious than that of 4 kR. However, it is necessary to point out that gold NPs do not turn out to be agglomerated in these dose ranges and the distance between gold NPs become closer. It is very interesting to note that similar aggregation phenomenon in gold NPs has been reported under laser irradiation.[29] Actually, due to the strong photoelectric effect, high energy gamma irradiation can cause lots of surface electrons and charge transfer of gold NPs, which is related to the aggregation of gold NPs. These results also support and explain the recent cell experiment in which unknown aggregation of gold NPs has been observed near the cell membrane after 0.1–1 kR X-ray irradiation.[12]

In radiotherapy, the increasing aggregation of gold NPs can induce the enhancement of optical absorption so that radiosensitization and optical imaging of gold NPs can be more effective.

Figure 2 gives the optical absorption of nonirradiated gold NPs, 2, 4, 6, 8, and 10 kR irradiated gold NPs. The SPR band of 521 nm has been observed in nonirradiated gold NPs, and the peak is not shifted after gamma irradiation. Indeed, the so slight size variation should not induce the obvious shift of SPR peak. Mie theory is the exact solution to Maxwell's electromagnetic field equations for a plane wave interacting with a homogenous sphere of radius with the same dielectric constant as bulk metal.[30] The size of gold NPs is in good agreement with the results calculated by Mie theory. Group 2 is added in order to illustrate the validity and reproducibility of results, in
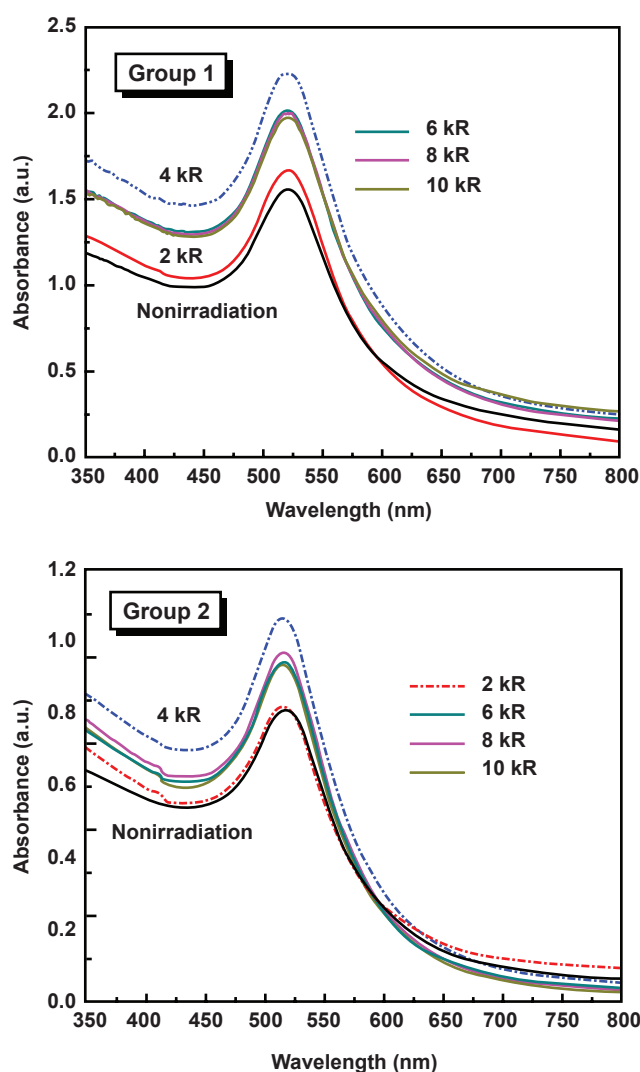
which the concentration of gold NPs is half of group 1. The sole SPR peak in the spectra indicates that gamma irradiation has not caused obvious defects in gold NPs. The full width at half-maximum of the SPR band is about 50 nm, and has no obvious variation after gamma irradiation, which also indicates that gold NPs are very stable after gamma irradiation.

It is worth noting that the SPR peak of gold NPs is enhanced obviously after gamma irradiation. The enhancement of SPR is very obvious at the dose of 4 kR and the enhanced effect is gradually stable above 6 kR. Both groups perform good reproducibility. Besides, we have also investigated the optical effect of sodium citrate solution under 2–10 kR irradiation, which has not shown any absorption in the 500–800 nm. This indicates that the enhanced absorption is from the SPR of gold NPs, not the dissolved citrate. Figure 3 shows the enhanced effect of SPR in group 1 of Figure 2. It can be observed that the absorbance of SPR is increased from 1.56 of nonirradiation to 2.23 of 40 kR irradiation, and stabilized to 2.0 in the range of 6–10 kR. After normalization, the enhancements of SPR intensity are 10.9%, 40%, 29.1%, 25.5%, and 23.1%, which correspond to 2, 4, 6, 8, and 10 kR irradiated doses, respectively. We notice that the high dose irradiations of 6–10 kR have not induced obvious variation in SPR, which indicates that 4 kR may be the optimal energy for SPR modification of gold NPs.

Irradiation mechanism

The interaction between gamma rays and gold NPs can be classified for photoelectric effect, Compton scattering, electron–positron pairs, and high energy excitation (see Figure 4).[31] The photoelectric effect mainly occurs in the 10–500 keV range, while electron–positron pairs caused by photon annihilation dominate above 1.02 MeV. Thus, the photon of 662 keV mainly falls into Compton scattering and excitation. The results of Compton scattering are re-excitation and photoelectric effect. The high energy excitation can induce lots of phonons and less photons because the dominant transition of gold NPs is photon–phonon transition processing. Thus, gamma irradiation can provide strong energy to gold NPs so that it can be transformed to thermal energy and abundant electron. It is well known that the thermal treatment is a kind of common method to boost nucleation of NPs and enhance SPR.[19] Laser irradiation is regarded as a good way to enhance the SPR of gold NPs.[32] By the same mechanism, the thermal effect induced by gamma irradiation can boost nucleation of gold NPs and thus induces enhancement of SPR.[33] While an abundance of free electrons excited by gamma irradiation can lead to production of surface electrons
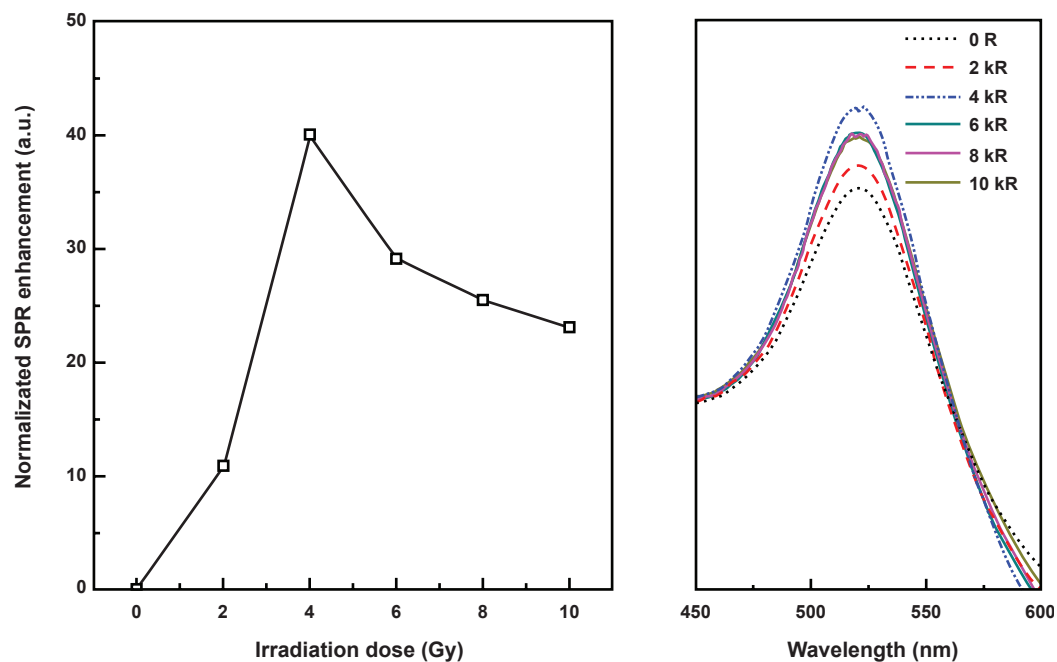

Figure 2 The optical absorption of the nonirradiated, 2, 4, 6, 8, and 10 kR irradiated gold NPs. The gold concentration of group 2 is half of group 1.
Abbreviation: NPs, nanoparticles.

Figure 3 The relative and normalized SPR enhancement of gold NPs dependent on irradiated dose.
Abbreviations: NPs, nanoparticles; SPR, surface plasmon resonance.

and charge transfer between gold NPs, which is responsible for SPR enhancement of gold NPs. Moreover, this detailed charge transfer process, such as electron and hole, has been described by the reaction:[19,28]

$$Au^+ + e^- \rightarrow Au^0 \qquad (1)$$

Gachard and colleagues proposed that the radiation reactions could be classified as three procedures: (1) radiolytic yields of the radicals in solution, (2) nucleation of NPs, and (3) stabilization processing of NPs. Intense gamma rays cause radiolysis of water in aqueous solutions producing primarily species such as H^+, H, HO, OH^- and hydrated electrons.[28] These transient species interact with themselves, water, the solute components, or free radicals generated by irradiation.[34] Lamaestre and colleagues reported that the nucleation of gold NPs was accelerated by the aggregation of charge and the
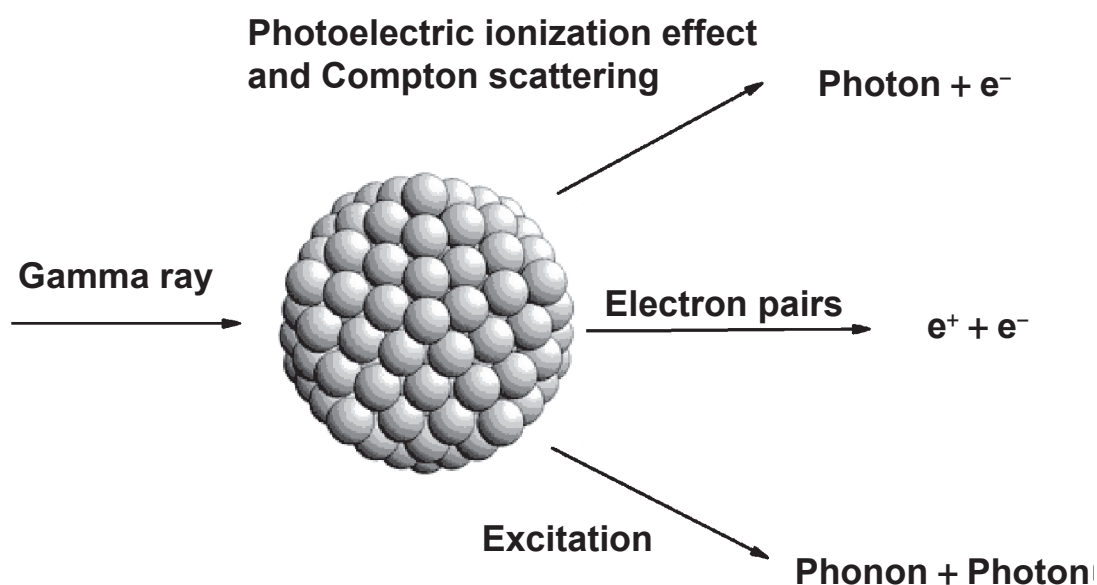

Figure 4 Outline of the interaction between gamma rays and nanoparticles.

environmental redox potential.[35] Therefore, we can conclude that 2–4 kR gamma irradiations induce SPR enhancement. However, with an increasing radiation dose, the residual auric solutions may be used up.[28] Meanwhile, high energy radiation may destroy the chemical bond and cause some damage.[28,34,35] Thus, 6–10 kR irradiations induce the slight decrease of SPR compared with that of 4 kR. Combined with the previous theory and experimental works, we suggest that the high radiation dose may induce partial destruction of the Au–Au chemical bond while the low dose irradiation may be helpful for the nucleation of gold NPs. Gamma irradiation is an efficient means of modifying and controlling the system's redox state via charge transfer and transitions between surface charge states and precursors.

Radiation hardness

The gamma irradiation mechanism of gold NPs is similar to that of QDs. However, the influences of gamma irradiation between QDs compounds and gold NPs seem obviously different. In order to evaluate the irradiation hardness of gold NPs, the exposure dose is converted to the absorbed dose, which is dependent on atomic number (Z) and structure of materials. The absorbed dose D can be described by the following formula:[21,31]

$$D = 0.88 \, [\mu_{en}(hv)/\rho]_{Au}/[\mu_{en}(hv)/\rho]_{air}, \tag{2}$$

where hv is the gamma photon energy and $[\mu_{en}(hv)/\rho]$ is the mass energy absorption coefficient for the subscript material. It is well-known that the energy absorption coefficient at 662 keV is about 2.93×10^{-3} m²/kg for air and 3.16×10^{-2} m²/kg for gold.[36] Calculated from Eq. 2, the absorbed dose conversation factor for gold NPs is found to be 9.491 rad/R. Compared with the previously reported CdSe QDs of 0.899 rad/R, gold NPs have better radiation hardness. The gamma irradiation in the ranges of 2–10 kR can destroy the chemical bond of QDs and lead to the rapid decomposition of QDs,[37] while gold NPs have no similar problems in these dose ranges. Thus, it can be expected that gold NPs show good irradiation stability in radiotherapy.

Cytotoxicvity of gold NPs

The objective of this cell viability study is to assess the cytotoxicity of gold NPs for K562 cells, and further obtain the cytotoxicity curve. Cell Titer-Glo™ luminescent cell viability assay has been used to assess cytotoxicity after culturing in presence of the gold NPs for 48 hours. As is evident in Figure 5, cell viability decreases with increasing gold concentration, which indicates that the cytotoxic
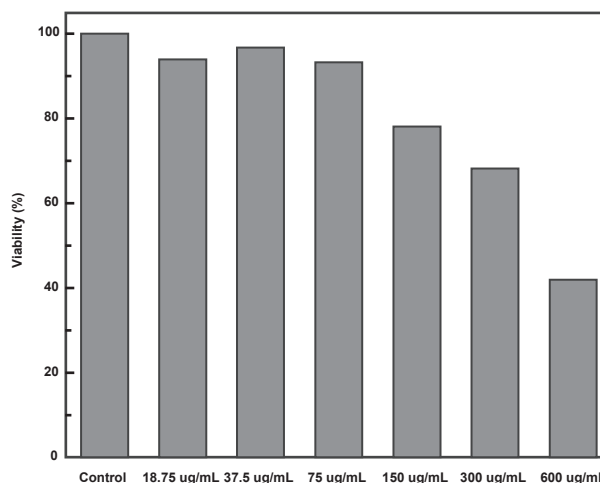

Figure 5 Cytotoxicity of gold NPs with different concentrations and incubation with K562 cells after 48 hours.
Abbreviation: NPs, nanoparticles.

effect of gold NPs increases. It can be observed that the low concentration gold (<75 µg/mL) has not affected the cell viability obviously, and has no obvious cytotoxicity. However, the high concentration gold (>150 mg/mL) can indicate the slight decrease of cell viability. In detail, cell viabilities are 93.9%, 96.7%, 93.3%, 77.5%, 68.8%, and 41.8%, which correspond to 18.75, 37.5, 75, 150, 300, and 600 µg/mL gold, respectively. In addition, the cytotoxicity of phosphate-buffered saline has also been checked in Figure 6, and it has not shown cytotoxicity. The results indicate that gold NPs have obvious cytotoxicity in high concentrations, which is in good agreement with the recent results.[25,38]
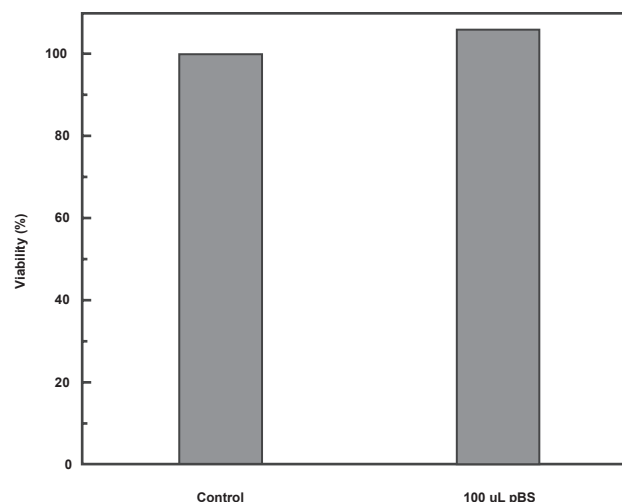

Figure 6 Cytotoxicity of PBS buffer and incubation with K562 cells after 48 hours.
Abbreviation: PBS, phosphate-buffered saline.

Several groups examined the cytotoxicity of gold NPs. The consensus is that gold NPs are safe at low concentrations. However, the results are still controversial and conflicting at high concentrations. Recent reviews highlighted the size, shape, and concentration-dependent cytotoxicity of gold NPs.[38–40] We summarized some recent cytotoxicity tests of gold NPs in Table 1. Connor and colleagues examined the uptake and potential toxicity of a series of gold NPs in human leukemia cells.[24] The results indicated gold NPs were nontoxic and its surface modifiers showed cytotoxicity, which was in good agreement with the results of Goodman[23] and Patra.[41] This cytotoxicity could be related to the electrostatic adsorption between the cationic NPs and the negatively charged cell membranes. In addition, cytotoxicity of gold NPs also depends on the types of cells. For example, gold NPs were found to be noncytotoxic to baby hamster kidney and human hepatocellular liver carcinoma cells, but cytotoxic to a human carcinoma lung cell line at certain concentrations.[38] A similar inconsistency has been found between human–Chinese hamster ovary (CHO) cells and human dermal fibroblast cell lines. Size and shape also play an important role in cytotoxicity of gold NPs. Chan and colleagues examined the uptake of gold NPs of various sizes and shapes into HeLa cells and found that 50 nm spheres were taken up more quickly by the cells than both smaller and larger spheres.[44] Meanwhile, Pan and colleagues reported that 15 nm gold NPs had good biocompatibility while 1.5 nm gold NPs could cause the acute toxicity of fibroblasts, epithelial cells, macrophages, and melanoma cells.[45]

Actually, nearly anything can be toxic at a high enough concentration. Pernodet and colleagues confirmed that a high concentration of gold NPs showed strong decrease of cell proliferation, adhesion, and motility in the human dermal fibroblast cells, which is consistent with our results.[25] We find that gold NPs show concentration-dependent cytotoxicity, although 15 nm gold particles showed good biocompatibility in previous investigations. The cytotoxicity of gold NPs is more important at the potential concentrations where they might be used. In radiotherapy, gold concentration may be as high as 7 mg/mL. Besides, gamma irradiation can induce abundant electrons, which may influence the cellular selectivity of gold NPs. The time-dependent cytotoxicity effect of gold NPs is still not clear, and the toxicity study *in vivo* also becomes more and more important. Thus, the further toxicity of gold NPs should be essential before radiotherapy sensitization and drug delivery.

Conclusions

In summary, the stability test and SPR-enhanced effect of gold NPs exposed in gamma irradiation have been investigated. The results show that gamma irradiation cannot induce obvious size variations in gold NPs under 2–10 kR irradiation. Meanwhile, the SPR of gold NPs was enhanced obviously and gamma irradiation did not cause evident defects. Cytotoxicity test shows that high gold concentrations can cause obvious decrease of cell viability while low gold concentrations have no obvious influence on the cell viability. Gold NPs are a stable material for radiotherapy and drug delivery. However, further cytotoxicity tests *in vivo* is still necessary before high-concentration gold NPs can be used in radiotherapy.

Acknowledgments

Table 1 Summary of the cytotoxicity of gold nanoparticles

Group	Size (nm)	Cell line	Concentrations	Cell viability
Connor[24]	4, 12, 18	K562 human leukemia	25–250 μM	Nontoxicity
Goodman[23]	2	Mammalian cells	0.38–3 μM	Obvious cytotoxicity
Patra[41]	33	BHK21, Hep2G, and A549 human cells	10–120 nM	Selective cytotoxicity
Liu[12]	4–6	Mice CT26 cells	250–500 μM	Nontoxicity
Male[42]	5–6	Human V79 cells	45 μM	Nontoxicity
Zhang[43]	15	Human CHO cells	0.01–100 μg/mL	Nontoxicity
Pernodet[25]	14	Human dermal fibroblast cells	100–800 μg/mL	Serious toxicity
Chithrani[44]	10–100	HeLa cells		
Pan[45]	1.5–15	Fibroblasts, epithelial cells	30–56 μM	Selective cytotoxicity
Our work	15	K562 human leukemia mammalian cells	10–600 μg/mL	Selective cytotoxicity

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