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Effect of size on gold nanoparticles in radiation therapy: Uptake and survival effects

Bindeshwar Sah[1]; Michael P Antosh[1,2]*

[1]Physics Department, University of Rhode Island, USA

[2]Institute for Brain and Neural Systems, Brown University, USA

***Corresponding Author(s): Michael P Antosh**

Physics Department, University of Rhode Island, 2 Lippitt Road, Kingston, RI, 02881, USA

Tel: 401-874-2048; Fax: 401-874-2380,

Email: mantosh@uri.edu

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Abbreviations: GNPs: Gold Nanoparticles; kVp: Kilovolt Peak; MVp: Megavolt Peak; REF: Radiation Enhancement Factor; Gy: Gray; PEG: Polyethylene Glycol; CTAB: Cetyl Trimethyl Ammonium Bromide; CFE: Colony Forming Efficiency; ICP-MS: Inductively Coupled Plasma Mass Spectroscopy; Her-GNPs: Herceptin Conjugated With Gold Nanoparticles.

Abstract

Radiation therapy is one of the most commonly used techniques for the treatment for cancer. A major goal of radiation therapy is to damage cancer cells, while simultaneously imparting as small a radiation dose as possible to nearby healthy cells. Due to a high atomic number and the Auger effect, gold nanoparticles can significantly enhance doses of ionizing radiation. The amount of enhancement due to gold nanoparticles strongly depends upon several parameters, such as cellular uptake of nanoparticles, nanoparticles size, concentration, intracellular location and radiation energy.

Existing literature shows that nanoparticle size can affect the amount of uptake and radio-sensitization. In this review article, we describe the effect of nanoparticle size on the gold nanoparticle-mediated effect, touching on both of these clinically important variables. The results suggest that non-targeted gold nanoparticles see maximum uptake and maximum radiation therapy enhancement around 50 nm.

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Introduction

Cancer is the second leading cause of death in the United States [1]. Using mortality data from the National Center for Health Statistics, Siegel et al. predicted 1,688,780 new cancer cases and 600,920 cancer deaths in the United States for 2017. Radiation therapy is an important technique for the treatment of tumors. X-rays, gamma rays and high energy waves are used for the treatment of almost all types of solid tumors [2]. Radiation can cause cellular damage, inadequately repaired cellular processes, and prevents the cell from surviving or reproducing [3]. However, one difficulty of radiation therapy is that the ionizing radiation may damage healthy cells as well as cancer cells since it must pass through non-cancerous tissue to reach any tumors deeper than skin depth. Additionally, radiation interactions happen in a probabilistic fashion, and it is generally not feasible to have all radiation interactions to happen within the tumor. Since radiation can harm both cancerous and normal tissues, it's important to maximize the radiation dose to cancer cells and to minimize the radiation dose to normal tissues.

Nanoscience is concerned with understanding the unique effects of nanometer-sized materials and their influence on the physical and chemical properties of materials. Nanotechnologies use their unique characteristics to their advantage due to their size. Dowling et al. characterized nanoparticles to be less than 100 nm in size [4]. Nanoparticles have drawn increasing interest from every branch of medicine for their ability to deliver drugs in the optimum dosage range - often resulting in increasing therapeutic efficiency of the drugs, weakened side effects and improved patient compliance [5]. Gold Nanoparticles (GNPs) are an attractive candidate for cell imaging [6-9], targeting drug delivery [10,11], and cancer diagnostics and therapeutic applications [8,9,12-26] due to their size, convenience in preparation and bio-conjugation, strong absorbing and scattering properties as well as their well-known biocompatibility [27].

Gold nanoparticles can enhance tumor radio-sensitization. When x- or γ rays beams pass through a material, they may interact or they may be transmitted through the material without interaction. The interaction of x-rays with gold nanoparticles (a high atomic number material) produces an increase in the absorption of x-rays and release of electrons. The interaction with gold can be via the photoelectric effect, Compton scattering or pair production. After an electron has been ejected from the atom, a vacancy is created in the electron shell structure. This vacancy can be filled by an outer orbital electron in a higher energy state, and that electron jumping between energy states leaves the atom in an excited state. The extra energy of the excited state is released via the emission of either a characteristic x-ray or an Auger electron. This process can repeat, resulting in multiple Auger electrons for one interaction.

Thus, the interaction of radiation with gold nanoparticles results in extra interactions that produce extra electrons. These extra electrons can be used to cause additional damage to tumors, which enhances the effect of radiation. The Auger electrons have a relatively short range [28]. Auger electrons are weakly bound electron and can generate much higher ionization density at a localized area [14]. Gold has a high atomic number, which is approximately 10 times higher than that of soft tissue [29].

Since Auger electrons have a relatively short range, it is important for gold nanoparticles to be close to tumors. This can be aided with the use of tumor-targeting agents [30]. Additionally, it's possible for the nanoparticles themselves to absorb some of the Auger electron's energy through collisions. In this case, the location at which the ionizing events occur in the gold nanoparticles is very important. Most low energy electrons have a short range inside of a nanoparticle, with many being stopped inside of larger particles and only the most energetic and sparsely ionizing electrons to escape [28].

Gold nanoparticles are versatile materials for radiation therapy and drug delivery because they are relatively stable and non-toxic, and because they have unique electronic and optical properties. The size of nanoparticles used for radiosentization affects both how they interact with the biological system and how they interact with radiation [24]. The bio-distribution and route of elimination from the body depend strongly on the size of the nanoparticles. To avoid accumulation of nanoparticles in organs that would cause long-term side effects, such as heart and liver, metal nanoparticles would ideally be removed from the body within a few days, which will still provide a window for radiotherapy with nanoparticles present. The best type of removal is through renal clearance. Renal clearance of nanoparticles is affected by nanoparticle size [24,31-33]. Resident macrophages in the reticuloendothelial system (liver, spleen and lymph nodes) filter out and remove nanoparticles. Nanoparticles of smaller sizes are generally cleared within minutes from systemic circulation via renal excretion after intravenous administration. Resident macrophages in the Reticuloendothelial System (RES) remove larger nanoparticles particularly well, leading to reduced tumor accumulations [34]. Smaller size of nanoparticles can be diffused further into tumor tissue from bloodstream, and therefore present a more even distribution in larger tumors than larger nanoparticles. This effect may balance out the fact that smaller nanoparticles are uptake less and more easily cleared from the body [24,35,36].

This review paper is focused on the effect of gold nanoparticle size on the uptake of gold nanoparticles, and the effect of size on radiation sensitivity enhancement. Aware of the large body of gold nanoparticle literature, in this review the results discussed are from experiments where two or more sizes of gold nanoparticle were directly compared. These experiments will give the most accurate information on gold nanoparticle size effects, as opposed to comparing data from different experiments (with different cell lines, tumor targeting, radiation doses, etc). Each work is discussed in detail, to best understand the differences in methodology; these differences in methodology appear to cause differences in the final results. The experiments discussed in the following two sections are compared and combined in the discussion section.

Effect of size on uptake and toxicity of gold nanoparticles

As mentioned previously, gold nanoparticles enhance radiation using the Auger Effect, which involves short range electrons exiting gold atoms. Thus, since the effect is short range [30], the location of the gold matters. And certainly, so does the amount of gold taken into the tumor (uptake). On the opposite side of effects, toxicity can result if the concentration of gold nanoparticles becomes too high [21]. In this section, we examine the effects of gold nanoparticle size on uptake and toxicity. The papers are examined in detail individually here, and analyzed against each other in the discussion.

Chithrani et al (2006) found that the intracellular uptake is affected by the size and shape of gold nanoparticles. HeLa cells were treated with gold nanoparticles for 6 hours. They

found that 50 nm spherical nanoparticles had greater uptake *in vitro*, compared with 14, 30, 74 and 100 nm. A second shape was examined, in addition to the usual (approximately) spherical shape. Cells took up 500% and 375% more 74 and 14 nm spherical gold nanoparticles (respectively), compared to the uptake of 74 × 14 nm rod-shaped gold nanoparticles. The rod shaped nanoparticles can have larger contact area with the cell membrane receptors compared to spherical nanoparticles, if the interaction is with the longitudinal axis of the rods. The difference in uptake between the spherical and rod-shaped gold nanoparticles were caused by the surface chemistries [37], and the fact that the spherical nanoparticles were stabilized by citric acid ligands. The rod-shaped gold nanoparticles have lower uptake than spherical shaped gold nanoparticles because the protein coating on the surface of the rod-shaped gold nanoparticles may not be homogeneous, and because of the presence of Cetyl Trimethyl Ammonium Bromide (CTAB) on the surface. The ligands on the surface of the rod-shaped gold nanoparticles may not efficiently bond with receptors on the cell's surface.

Huang et al. systematically evaluated the size dependent localization of 2, 6, and 15 nm spherical gold nanoparticles. Three different cancer models were used: monolayer breast cancer cells, an MCF-7 tumor spheroid model, and an in vivo tumor tissue in female Balb/c nude mice. Nanoparticles were targeted using tiopronin. The quantitative analysis by ICP-MS indicated that uptake occurred in a size dependent manner when cells were treated with 1 nM particles for 24 h. Nanoparticles of 2 nm showed higher cellular uptake than 6 and 15 nm nanoparticles, which might be due to their ultra-small structure. Huang et al. found that ultra-small GNPs smaller than 10 nm have a unique advantage over nanoparticles larger than 10 nm in localization to, and passing through, breast cancer cells, multicellular tumor spheroids and tumor in mice. In an in vivo study, the results showed that 2 and 6 nm tiopronin-coated gold nanoparticles were diffused throughout the cytoplasm and nucleus of cancer cells, whereas 15 nm tiopronin-coated with gold nanoparticles were found only in the cytoplasm and had formed aggregates. Tumor bearing mice were intravenously injected with gold nanoparticles at a dose of 5 mg Au/kg. After 24 hours, the amount of gold in tumor tissue was 2.93 μg/g for 2 nm nanoparticles, 0.79 μg/g for 6 nm nanoparticles, and 0.14 μg/g for 15 nm nanoparticles. Compared with 15 nm GNPs, the 2 and 6 nm GNPs were widely distributed in different organs of the body due to their ultra-small structures [38].

Perrault et al. examined the effect of hydrodynamic size of gold nanoparticles, coated with Polyethylene Glycol (PEG), on passive targeting of tumors in vivo. The gold nanoparticles, approximately 20, 40, 60, 80 and 100 nm in size, were coated with PEG were Injected Intravenously (IV) into Athymic nude CD1 mice bearing 1 cm^3 subcutaneous MDA-MB-435 xenograft tumors. Uptake measurements were taken at 1, 4, 8 and 24 h. Uptake of the particles was higher in spleen than liver when normalized per gram of tissue. By using histological measurements, the ability of nanoparticles to penetrate within the tumor is highly dependent on the overall size of the size of the nanoparticle: larger nanoparticles appear to stay near the vasculature, while smaller nanoparticles rapidly diffuse throughout the tumor. This work illustrated that PEGylated gold nanoparticles must be smaller than 100 nm in diameter to move away from the vasculature and throughout the tumor [36].

Arnida et al. evaluated the influence of shape, size, surface properties and concentration on cellular uptake, adsorption of protein and toxicity in human prostate cancer cell line (PC-3). The toxicity and uptake of three types of gold nanoparticles were tested: plain spherical, PEGylated spherical and PEGylated rods. For toxicity, the human prostate cancer PC-3 cells were incubated for 88 hours with 1.5 nM gold nanoparticles. Plain rod GNPs were not used in this study because of the toxicity of the stabilizing agent Cetyl Trimethyl Ammonium Bromide (CTAB). For the uptake studies PC-3 cells were treated with GNPs (30-90 nm) for a 6 hour incubation period. They found that particles 50 nm in diameter had the highest uptake without Polyethylene Glycol (PEG). The surface attachment of PEG reduced cellular uptake. The uptake of gold nanoparticles was size dependent. PEGylation or protein adsorption on the surface of GNPs diminished their interaction with cell membranes, resulting in a drastic reduction in uptake. The size of 50 nm GNPs had highest uptake in comparison to 30 and 90 nm of GNPs, a similar result to Chithrani et al. 2006 [39].

Coating gold nanoparticles with antibodies can regulate the process of membrane receptor internalization. The binding and activation of membrane receptors and subsequent protein expression strongly depends on nanoparticle size. The number of allowable Herceptin binding sites on nanoparticles is dependent on the surface area, which increases with particle size. Using gold nanoparticles conjugated with Herceptin (Her-GNPs) on human breast cancer SK-BR-3 cells, Jiang et al. 2008 showed that 40 nm and 50 nm nanoparticles have greater uptake than 2 nm Her-gold nanoparticles because of higher binding avidity human breast cancer SK-BR-3 cells Larger Her-GNPs can firmly anchor on cell surfaces, resulting in increased receptor binding [40].

Trono et al. (2010) studied the effect of different sizes of gold nanoparticles (5, 10, 20, 30, 40, 50 nm), incubation time and concentration on the uptake by human pancreas cancer cell lines (PK-1, PK-45, Panc-1). Cells were treated for 24 hours with the same concentration of gold nanoparticles (11.8 μM) of different sizes mixed with RPMI-1640. The gold content per cell *versus* different sizes of the nanoparticles showed that the uptake is highly dependent on size. The 5 and 10 nm gold nanoparticles were found to have significant lower uptake than 20 nm. The adsorption of serum proteins on the surface is very important in the internalization of GNPs by the cells. Adsorption of serum proteins is also important to prevent aggregation of GNPs, which can affect the uptake mechanism of the cells. These results confirm that the gold nanoparticle uptake is cell and size dependent [41].

Sonavane et al. used gold nanoparticles of sizes 15, 50, 100 and 200 nm. Gold nanoparticle suspensions (15, 50, 100, and 200 nm) were injected intravenously at a dose of 1 g/kg in Male ddy mice. 24 hours after injections, mice were sacrificed by cervical dislocation and tissues including heart, liver, lung, spleen, kidney, stomach, pancreas and brain were collected. 15 nm GNPs were found to have wide spread concentration of gold in tissues in compared to larger particle size of gold. As the particle size increased, the concentration in spleen increases whereas the concentration in lung decreased. GNPs were mainly accumulated in liver followed by lung, spleen and kidney. Interestingly, 15 and 50 nm GNPs were able to pass through the blood-brain barrier as evident from gold concentration in brain [42]. For 50 nm GNPs, similar to 15 nm, high concentrations of gold were observed in liver, lung and spleen tissues. In the spleen, a larger amount of 50 nm gold nanoparticles were found than 15 nm size of GNPs. A small decrease in brain gold

concentration was observed in 50 nm nanoparticles compared to 15 nm GNPs. Similar to 15 and 50 nm size GNPs, 100 nm size GNPs were found to have large amounts of gold in the liver, lung and spleen. In particular, the concentration of gold in liver and spleen was higher than 50 nm size of GNPs. For 200 nm size of GNPs, a higher concentration of gold was observed in liver followed by spleen, lung and kidney. A small amount of gold was also found observed in the pancreas, brain, stomach and blood.

De Jong et al. performed a kinetic study in vivo tissue distribution of spherical-shaped gold nanoparticles as a function of size. De Jong et al. used nanoparticles of size 10, 50, 100 and, 250 nm, intravenously injected in the tail vein in Male WU Wistar-derived rats. After 24 hours, the rats were sacrificed and blood and various organs were collected for gold determination. The majority of the gold was present in liver and spleen for all gold nanoparticle sizes. The particles of 10 nm were present in various organ system including blood, liver, spleen, kidney, testis, thymus, heart, lung and brain. In contrast, the larger particles were only found to be located in blood, liver and spleen. The tissue distribution of gold nanoparticles is size dependent [43]. The concentration in the liver was found to be the highest for all sizes of GNPs, followed by the spleen. For the injected 10 nm particles, the percentage of the dose, located in the kidneys, brain, reproductive organs, thymus and heart was much higher than for the 50, 100 and 250 nm GNPs. For the lungs, the amount of gold measured after injection of the 50 nm GNPs is relatively high, whereas 100 and 250 nm GNPs hardly were detected in this organ.

Zhang et al. 2011 showed that the size and surface coating of gold nanoparticles play an important role in their bio-distribution in mice. GNPs were coated with PEG and had sizes 5, 10, 30, and 60 nm. Male mice were given an intraperitoneal injection of approximately 200 µL of gold nanoparticles (concentration 4000 µg/kg) over 28 days. The 5 nm particles had a wide distribution in liver, heart, kidney. The 10 nm particles were found to have remained in the liver, and the 30 nm particles were found to have remained in the spleen. The bio-distribution results show that 5 and 10 nm particles were accumulated in the liver and 30 nm particles accumulated in the spleen, while 60 nm have a low distribution in all organs (Heart, Liver, Spleen and Kidney) [44].

Coradeghini et al treated Balb/3T3 mouse fibroblast cells with concentrations of 10-300 µM of 5 and 15 nm gold nanoparticles. Cells were measured for toxicity at the exposure times 2, 24 and 72 hours, using a colony forming efficiency (CFE) assay. The results showed significant toxicity for 5 nm gold nanoparticles at the exposure time 72 h and concentration higher than 50 µM, whereas no significant toxicity was observed for 15 nm GNPs at all concentrations and exposure time [45].

Chen et al injected mice with one of many difference sizes of gold nanoparticles: 3, 5, 8, 12, 17, 37, 50 and 100 nm. Injections were performed intraperitoneally into BALB/C mice at a dose of 8 mg/kg/week. GNPs of 3, 5, 50 and 100 nm were non-toxic into mice, whereas GNPs ranging from 8 to 37 nm caused severe toxicity and death in mice within 3 weeks because the skin had major rashes, bruising, and hemorrhaging. Mice injected with nanoparticles between 8 and 37 nm in size were found to have fatigue, loss of appetite, change of fur color, and weight loss. In contrast, no negative effects were seen after the injection of 5 and 3 nm nanoparticles [46].

Effect of particle size on radiation survival

Gold nanoparticles have been studied for several years as a potential agent for the selective amplification of radiation dose in tumors [22,47-50]. The efficiency of the dose enhancement effect is significantly influenced by the size of gold nanoparticles, both *in vitro* and *in vivo* studies.

Chithrani et al. (2010) treated HeLa cells with concentrations (0.0088 mg/mL) of gold nanoparticle of 14, 50 or 74 nm in diameter and incubated for 24 h. Then cells were irradiated using an orthovoltage unit with dose rate of 4.7 Gy/min at 105 kVp and 2.3 Gy/min at 220 kVp. 660 keV energy photons from a ^{137}Cs source were also used, with a dose rate of 88 cGy/min, and a 6 MV beam from an Elekta Synergy linear accelerator was used with a dose rate of 600 MU/min (1MU is equivalent to 1 cGy at a depth of 1.5 cm in a 10 × 10 cm^2 field). Chithrani et al investigated radio-sensitization as a function of the size of gold nanoparticles and a range of different energies. For energy 220 kVp, they found that nanoparticles of diameter 50 nm showed the highest radiation enhancement. The Radiation Enhancement Factor (REF) was 1.43 for 50 nm particles, compared to 1.20 for 14 nm and 1.26 for 74 nm. Also worth noting: in a one-size radiation energy experiment, the radiation enhancement factor was 1.66 at 105 kVp and 1.17 at 6 MVp for 50 nm particles [17].

Zhang et al. found that the radio-sensitization effects of GNPs, coated with Polyethylene Glycol (PEG), were size dependent both *in vitro* and in vivo. PEG-SH was used as the surface coating, in order to improve mono-dispersity and biocompatibility of gold nanoparticles. In an *in vitro* experiment, HeLa cells were exposed to PEG-coated gold nanoparticles at the concentrations of 0.05 and 0.1 mM 24 h before irradiation. The cells were irradiated by ^{137}Cs (photon energy 662 keV) at the doses of 1, 2, 4, 6 and 8 Gy respectively. GNPs of size 12.1 and 27.3 nm showed a higher radiation enhancement than 4.8 and 46.6 nm size of PEG-coated gold nanoparticles. For all sizes, a significantly decrease in survival rate was seen. In an in vivo experiment, PEG-coated GNPs of size 4.8, 12.1, 27.3 and 46.6 nm (concentration 4 mg/kg) were intraperitoneally injected in female and male BALB/c mice bearing subcutaneous inoculated U14 tumors. The tumors were irradiated with 5 Gy gamma radiations. The results showed that all sizes of the PEG-coated GNPs significantly decreased tumor volume and weight after 5 Gy of irradiation, and that 12.1 and 27.3 nm PEG-coated gold nanoparticles again had greater sensitization effects than 4.8 and 46.6 nm particles. In vivo bio-distribution demonstrated uptake was very higher for 12.1 and 27.3 nm PEG-coated gold nanoparticles than for 4.8 and 46.6 nm nanoparticles. Testing on pathology, immune response and blood biochemistry indicated that the PEG-coated gold nanoparticles did not cause spleen and kidney damage, but did give rise to liver damage and gold accumulation [20].

Conclusion

Nanoparticles have emerged as a significant tool for the potential enhancement of radiation therapy. Gold nanoparticles have a high atomic number (Z), and have the potential to penetrate the tumor vasculature [36]. The enhancement of radiation effects due to GNPs have been tested on various cells lines, concentration of gold, radiation energy, and intracellular localizations *in vitro* and in vivo. Studies on the effects of nanoparticle size on dose enhancement have been done *in vitro* and in vivo.

The *in vitro* and in vivo experiments on uptake and radiation therapy enhancement are summarized in (Tables 1,2). The section on radiation therapy enhancement presents bodies of experimental work. One body of work [17] shows 50 nm to be the optimal size, and a second body of work [20] shows 12-27 nm to be the optimal size. The work presented in the section on uptake and toxicity (summarized in tables 3 and 4) likely hold the key to understanding the radiation therapy results. The work of Sonovane et al., Zhang et al., Perrault et al. and De Jong et al. indicate that smaller nanoparticles move through the body more easily than larger nanoparticles [36, 42-44]. This explains the results from Chen et al. and Coradeghini et al. that the smallest nanoparticles are not as toxic as slightly larger nanoparticles [45,46], and it also explains how Chithrani et al., Jiang et al. and Trono et al. see increased uptake at mid-range sizes compared to smaller sizes [17,40,41]. Perhaps the smallest gold nanoparticles are often too easily moved away from tumors, resulting in lower uptakes and lower toxicities. This would also be consistent with the result of Chithrani et al. where 50 nm gold nanoparticles produced a better radiation enhancement than smaller gold nanoparticles [17]. If more gold nanoparticles are in the tumor, more radiation enhancement is expected.

The dynamics of uptake and size may change, depending on tumor targeting mechanisms. Zhang et al. found nanoparticles in the range of 12-27 nm to work better than nanoparticles smaller or larger than that size [20]. Perhaps the benefits of using Polyethylene Glycol (PEG) allowed the 12 nm nanoparticles to more efficiently reach cells. Similarly, Huang et al. found the smallest size (2 nm) to have the most uptake when using a tumor targeted nanoparticle [38]. As mentioned in the introduction, one theoretical answer for ideal particle size is 'the smaller, the better', based partially on the idea that larger nanoparticles attenuate more kinetic energy from Auger electrons produced inside of them [28]. In the opposite result, Jiang et al. argued that their Her-targeted nanoparticles had more targeting molecules on larger nanoparticles, resulting in better targeting for the larger particles [40].

The evidence presented here suggests that non-targeted gold nanoparticles have an ideal treatment size of 50 nm, but that tumor targeting molecules can change the ideal size, down to very small nanoparticles. Uptake likely follows the same properties, and toxicity is in the inverse since too much uptake leads to toxicity.

Gold nanoparticles have the potential to be a useful tool for the treatment of cancer, but significant work remains to be done before it can be useful for human cancer treatments. Related to this paper, the ideal size must be determined for the full range of treatment situations, including different tumor targeting molecules. Hopefully future work will definitively answer this important question.

Tables

Table 1: Summary of gold nanoparticles radio-sensitization with ionization radiation in vitro.

First Author	GNPs nm	Surface Coating of GNPs	Concentration	Cell lines	Time to RT	Radiation	Dose (Gy)	DEF/Effect
Chithrani [17]	14	Citrate	7 x 109 NPs/ml	HeLa	24 h	220 kVp	0, 2, 4, 6, 8	1.2
	50				24 h	105 kVp		1.66
	50				24 h	220 kVp		1.43
	50				24 h	6 MVp		1.17
	50				24 h	660 keV		1.18
	74				24 h	220 kVp		1.26
Zhang [20]	4.8	PEG	0.05, 0.1 mM	HeLa	24 h	662 keV 137Cs	1, 2, 4, 6, 8	1.41 (4.8 nm)
	12.1							1.65 (12.1 nm)
	27.3							1.58 (27.3 nm)
	46.6							1.42 (46.6 nm)

Table 2: Summary of gold nanoparticles radio-sensitization with ionization radiation in vivo.

First Author	GNP nm	Surface coating of GNPs	Concentration	Cell lines	Animal model	Time to RT	Radiation	Dose (Gy)	DEF/Effect
Zhang [20]	4.8	PEG	4 mg/kg i.v. injection	HeLa	BALB/c mice	As soon as after injction of GNPs	662 keV gamma	5	Tumor growth inhibition
	12								
	27								
	47								

Table 3: Summary of the size dependent of gold nanoparticles in cellular uptake.

First Author	GNPs nm	Surface Coating of GNPS	Concentration	Cell lines	Time	Techniques	Effect
Huang [38]	2	tiopronin	1 nM	MCF-7	24 h	ICP-MS	Higher uptake smaller GNPs; 2 and 6 nm located in cytoplasm, 15 nm only in cytoplasm
	6					TEM	
	15						
Chithrani [37]	14	Citrate	7 x 109 NPs/ml	HeLa	6 h	ICP-MS	50 nm is the highest cellular uptake
	50					TEM	
	74						
Arnida [39]	30	Plain	1.5 nM	PC-3	6 h	ICP-MS	50 nm, maximum uptake
	50	PEG				TEM	
	90						
Jiang [40]	2 -100	Antibodies	10 µg/mL	SK-BR-3		CLSM	40 and 50 nm have greatest effect
Trono [41]	5		11.8 µM	PK-1,	24 h	AAS, TEM	20 nm is higher uptake in comparison than 30, 40 and 50 nm.
	10			PK-45,			
	20			Panc-1			
	30						
	40						
	50						

Abbreviations: ICP-MS: Inductively Coupled Plasma Mass Spectroscopy; CLSM: Confocal Laser Scanning Microscopy; TEM: Transmission Electron Microscopy; AAS: Atomic Absorption Spectroscopy.

Table 4: Summary of gold nanoparticles treated with cancer cells in toxicity.

First Author	GNPs nm	Surface Coating of GNPs	Concentration	Cell lines	Time	Toxicity	Animal Model
Zhang [20]	4.8	PEG	0.005-0.25 mM	HeLa	48 h	IC50 = 0.205 mM	
	12.1					IC50 = 0.477 mM	
	27.3					IC50 = 0.448 mM	
	46.6					IC50 = 0.613 mM	
Arnida [39]	30	Plain	1.5 nM	PC-3	88 h	Cytotoxic	
	50						
	90						
Coradeghini [45]	5	Citrate	≥50 µM	Balb/3T3	72 h	Cytotoxic	
	15			Mouse		Non-toxic	
				fibroblasts			
Zhang [44]	5	PEG	4000 µg/kg		28	Low-toxic	Male mice
	10				days	Cytotoxic	
	30					Low-toxic	
	60					Cytotoxic	
Chen [46]	3					Non-toxic	BALB/C mice
	5					Non-toxic	
	8	Naked	8 mg/kg/week		21 days	Cytotoxic	
	12				21 days	Cytotoxic	
	17				21 days	Cytotoxic	
	37				21 days	Cytotoxic	
	50					Non-toxic	
	100					Non-toxic	

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