Published in final edited form as:

Nanomedicine. 2013 January ; 9(1): 25–27. doi:10.1016/j.nano.2012.09.001.

***In vitro* radiosensitization by gold nanoparticles during continuous low dose rate gamma irradiation with I-125 brachytherapy seeds**

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Abstract

This communication reports the first experimental evidence of gold nanoparticle (AuNP) radiosensitization during continuous low-dose-rate (LDR) gamma irradiation with low-energy brachytherapy sources. HeLa cell cultures incubated with and without AuNP were irradiated with an I-125 seed plaque designed to produce a relatively homogeneous dose distribution in the plane of the cell culture slide. Four sets of irradiation experiments were conducted at low dose rates ranging from 2.1 cGy/h to 4.5 cGy/h. Residual γ H2AX was measured 24 hours after irradiation and used to compare radiation damage to the cells with and without AuNP. The data demonstrate that the biologic effect when irradiating in the presence of 0.2 mg/ml concentration of AuNP is about 70 – 130% greater than without AuNP. Meanwhile, without radiation, the AuNP showed minimal effect on the cancer cells. These findings provide *in vitro* evidence that AuNP may be employed as radiosensitizers during continuous LDR brachytherapy.

Keywords

Gold-nanoparticles; radiosensitizers; brachytherapy; radiotherapy; in vitro

Background

Recent studies have cogently established gold nanoparticle-aided radiation therapy (GNRT) as a promising approach for enhancing radiotherapy treatment efficacy.^{1–5} Following pioneering *in vitro* work by Herold et al.⁶ with gold microspheres using 100 to 240 kVp x-rays, Hainfeld et al. performed *in vivo* experiments using 250 kVp x-rays in mouse models

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to establish proof-of-principle for the therapeutic benefit of gold nanoparticles (AuNP) as adjuvants to radiation therapy.¹ Other *in vitro* studies have provided further corroborating evidence of radiosensitizing effects of AuNP using relatively high kV energy x-rays from x-ray tubes.

While these experimental studies have established AuNP radiosensitization using brief exposures of kV energy x-rays, no study has provided experimental evidence of AuNP radiosensitization during *continuous low dose rate (LDR)* irradiation with brachytherapy sources (like I-125) commonly used in the clinic today.⁹ Such a study is particularly relevant given the growing consensus that GNRT via such LDR brachytherapy sources is more clinically feasible and promising than GNRT using the relatively high energy x-rays from x-ray tubes. In this context, this study specifically demonstrates the *in vitro* radiosensitizing effects of AuNP during continuous LDR irradiation of cancer cells with I-125 brachytherapy seeds. The findings will provide a useful reference for further studies toward the development of GNRT using LDR brachytherapy seeds.

Methods

HeLa cells (American Type Culture Collection, Manassas, VA) were cultured in 8-well chamber slides inside an incubator at 37°C in a humidified atmosphere of 5% CO₂. The cells in four wells of each 8-well chamber slide were incubated with 50 nm AuNP conjugated with methyl polymer (Nanopartz, Inc., Loveland, CO) while the four alternate wells contained cells without AuNP. An AuNP concentration of 0.2 mg/ml was employed to ensure a significant number of internalized AuNP based on previous studies.⁷ Two slides were prepared for each of 4 sets of experiments; one slide was irradiated while the other served as a sham-irradiation control.

Irradiation was done using a custom-built irradiation jig with an I-125 seeds plaque designed to produce a relatively homogeneous dose distribution in the plane of the cell culture slide. The I-125 seeds (0.97 mm diameter × 4.55 mm length, OncoSeed GE Healthcare, Inc., Arlington Heights, USA) have an average photon energy of 28 keV, and had an apparent activity of ca. 2.6 mCi. The jig containing the I-125 seeds plaque is vented, which allows for cell irradiation in an incubator. The height *h* of the cell culture slide above the plaque was varied to produce different dose rates in the plane of the cell culture slide as in previous work.¹¹ Gafchromic EBT2 film calibrated at kV energies was employed to estimate the dose rates at the different heights used for the cell irradiation. Four sets of irradiation experiments were carried out with the cell samples in the jig placed in the incubator. All experiments were conducted with exposure times of 24 hours.

To assess radiation damage, residual γ H2AX foci in cell nuclei, indicative of un-repaired (lethal) DNA damage, were measured by fluorescence imaging as done in previous studies.^{12–14} The cells were kept in the incubator for 24 hours after irradiation before fixation of both irradiated and sham samples in ice-cold methanol. In preparation for fluorescence imaging of the nuclei, cells were rehydrated and 2 μ g of DAPI (4,6-diamidino-2-phenylindole) (Carlsbad, CA) diluted in 200 μ L of mounting media Fluoromount G (Southern Biotech, Birmingham, AL) were added. Merged images of the nuclear staining (DAPI) and Texas Red signals for γ H2AX foci were collected using an Axio imager-Z1 (Carl Zeiss MicroImaging, Inc., Thornwood, NY) fluorescence microscope. The fraction of cells without residual foci (FC, $n > 500$) was determined for each experiment to assess the radiation damage as done in other studies. The radiation damage for cells incubated with and without AuNP was compared by determining the ratio of the FC without gold nanoparticles (FC_W) to the FC with AuNP (FC_{AuNP}). This yielded the residual DNA damage enhancement factor (rDEF):

$$rDEF = \frac{FC_w}{FC_{AuNP}}$$

Results and Discussion

Representative residual γ H2AX fluorescence images are shown in Figure 1 for cells incubated with AuNP (Figure 1A) and without AuNP (Figure 1B). The number of cells with no foci is evidently smaller for the cells incubated with AuNP, indicating increased unrepaired radiation damage.

The $rDEF$ is plotted in Figure 2 for the four irradiation experiments, as well as for the non-irradiated control samples. For the non-irradiated samples (0 Gy/h), the $rDEF$ of 1.04 ± 0.08 is consistent with no biologic effect of AuNP. This highlights the fact that AuNP themselves have minimal effect on the cells in the absence of radiation; this is consistent with previous studies showing AuNP biocompatibility.¹⁶ Meanwhile, results for the irradiated samples (2.1 cGy/h – 4.5 cGy/h) showed $rDEF$ s ranging from 1.7 – 2.3. This indicates appreciably more unrepaired radiation damage (70% to 130% greater) for the cells incubated with AuNP, thus, suggesting significant radiosensitization by the AuNP.

The observed radiosensitization for continuous low-energy LDR sources complements the findings from previous GNRT studies which have shown AuNP radiosensitization using brief exposures with relatively higher kV x-rays.⁴ The current result is particularly relevant given the growing consensus that GNRT with continuous LDR brachytherapy sources like I-125 could more easily meet technical and clinical requirements for implementation than GNRT using relatively higher kV X-ray energies.

Although 0.2 mg/ml AuNP was employed in the current study, the amount of AuNP specifically contributing to the observed radiosensitization depends on the uptake and localization of the AuNP by the cancer cells. This in turn depends on factors such as AuNP size, shape, coating etc. Theoretical studies involving low-energy brachytherapy sources like I-125 predict higher effects of radiosensitization for AuNP localized within or very close to the cell nucleus, due to increased impact from shorter range Auger electrons. Nuclear localization of unsealed Auger electron-emitting isotopes has also been shown to lead to increased biological effects.^{17–19} Such nuclear localization for AuNP may be achievable via active targeting.²⁰ Experiments are planned to further investigate the radiosensitizing effects of AuNP for continuous LDR brachytherapy sources as a function of these different factors.

While all four irradiation experiments showed significant radiosensitization with $rDEF$ s of 1.7 – 2.3, it is not immediately clear why a lower value of 1.7 was obtained for the fourth experiment. This could be due to dose rate, total dose or experimental uncertainties. Extensive studies including extended dose ranges, dose rates, other cell lines and assays, other LDR brachytherapy sources and other factors will be useful in clarifying this and elucidating the physical and biological processes involved, leading to optimization of AuNP radiosensitization.

Overall, the results from this study provide initial experimental evidence showing that significant AuNP radiosensitization is achievable during continuous LDR irradiation with lower kV energy brachytherapy sources like I-125. The results motivate further investigations toward the development of gold nanoparticle radiotherapy using LDR brachytherapy sources widely used in the clinic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of support for research: DOD CDMRP PC110280, NSF-DGE- 0965843,

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Figure 1.
Residual γ H2AX fluorescence images obtained for cells incubated A) with AuNP and B) without AuNP

Figure 2. Comparison of unrepaired (residual) radiation damage for HeLa cells incubated with and without AuNP. The residual DNA damage enhancement factor (rDEF) is shown for 4 sets of irradiation experiments (2.1 cGy/h – 4.5 cGy/h), as well as for no irradiation (0 cGy/h).